School of Molecular and Life Sciences

Adaptive and Phylogeographic Variation in Sympatric Parasitic and Autotrophic Plant Species in Western Australia

Sheree Jaye Walters
0000-0001-6295-8853

This thesis is presented for the Degree of
Doctor of Philosophy
of
Curtin University

January 2021

DECLARATION

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgement has been made.

This thesis contains no material which has been accepted for any other degree or diploma in any university.

SIGNED: Sheree Jaye Walters

DATE: 30 January 2021

ABSTRACT

Understanding spatial patterns of adaptive and neutral genetic variation in plant species is essential for identifying conservation units and designing seed sourcing strategies for restoration. The spatial distribution of genetic variation is determined by life history with these patterns commonly assessed through comparative genetic studies. To date, comparative studies have provided useful information on the effect of numerous life history traits across plant taxa, but few studies have assessed species with different parasitic life histories.

The research within this thesis presents a comparative genetic study assessing patterns of adaptive and phylogeographic variation in two pairs of co-occurring parasitic and autotrophic plant species, and concludes by applying a multidisciplinary approach to delineating provenances for seed sourcing for restoration. Firstly, I investigate the association of adaptive genetic variation with climatic variables and compare patterns for a generalist (Chapter 2) and host-specific parasite (Chapter 3) with respective co-occurring autotrophic species. I then compare patterns of neutral genetic variation and phylogeographic history both within the two pairs of co-occurring species and between the two parasites with different levels of host-specialisation (Chapter 4). In my final data chapter, I delineate and compare the spatial distribution of provenances within and between the two pairs of co-occurring parasitic and autotrophic species, and examine the direction of change under future climate scenarios (Chapter 5).

An analysis of genome-wide markers revealed adaptive genetic variation to associate with different climatic variables within the two pairs of co-occurring parasitic and autotrophic species, suggesting that plant species with different nutrient acquisition strategies may not respond in the same way to selective pressures. Specifically, adaptive genetic variation in the two parasitic species had greater association to temperature variables, which may provide greater selective pressure as parasitic plants rely on higher transpiration rates (which are influenced by temperature) to obtain water and nutrients from host plants. This comparative genetics approach also highlighted some challenges in the application of genotyping-by-sequencing approaches to the study of genetic variation in host-parasite systems (Chapters 2 and 3).

Next, a combination of genome-wide markers and chloroplast sequence data were used to identify patterns of neutral genetic variation and phylogeographic history. I found that patterns of neutral genetic variation differed within the two species pairs, with comparatively lower genetic diversity and stronger genetic structuring in both parasitic species. Genetic diversity was lowest in the host-specific parasite, suggesting that sympatric species with different parasitic life histories can have dissimilar patterns of neutral genetic variation. Phylogeographic analysis also indicated differing patterns of historical diversity and divergence within the two pairs of co-occurring species, although all four species had genetic signals of persistence across the landscape (Chapter 4).

Finally, to compare provenance patterns within and between the two pairs of co-occurring species, I combined the genetic data with a spatial modelling approach to delineate provenances under multiple climate scenarios. This revealed differing provenance patterns within the two pairs of co-occurring species, indicating that seed sourcing approaches will likely differ between parasitic and autotrophic species. Projection to future climate scenarios identified a similar direction of change in provenances within the two pairs of co-occurring species, suggesting that climate change may have a similar effect on seed sourcing in parasitic and autotrophic plants (Chapter 5).

Collectively, the studies presented here illustrate the benefits of comparative research to the study of genetic variation, and the potential for landscape genomics to delineate provenances under multiple climate scenarios.

ACKNOWLEDGEMENTS

This thesis represents a compilation of my work over the past four years that could not have been completed without the guidance, support and encouragement of supervisors, collaborators, colleagues, friends and family. Firstly, I would like to thank my supervisory panel Dr. Paul Nevill, Dr. Todd Robinson, Dr. Margaret Byrne and Dr. Grant Wardell-Johnson, for guidance and support throughout this PhD. I have learnt so much from you about planning and conducting good research, and I take with me a set of invaluable skills that will undoubtedly enhance my future career.

I would like to thank the management team and my colleagues at the Centre for Mine Site Restoration (CMSR) for their support and encouragement over the last few years. I would especially like to thank Prof. Kingsley Dixon for the many discussions on *Nuytsia*, Dr. Renee Young and Vanessa MacDonald for being my contacts during remote fieldtrips, Khiraj Balsing, Todd Buters, Sophie Querido and Mieke van der Heyde for assistance with fieldwork, and colleagues Holly Bradley, Dr. Sophie Cross, Michael Just, Khiraj Balsing, Todd Buters and Dr. Simone Pedrini for company in the office over these years.

The work conducted within this thesis would not have been possible without the support of people at the Department of Biodiversity, Conservation and Attractions (DBCA). Thank you especially to Bronwyn Macdonald for guidance with laboratory work and to Dr. Tony Start for assistance with mistletoe identification.

I would like to thank my family, Bevan, Denise, Kristy, Rachel, Sarah and Chloe, for the many years of support and encouragement that has got me to where I am today.

Finally, to my fiancé and best friend Josh, thank you for your unwavering support, encouragement and inspiration over these past ten years.

The financial support for my PhD came from the Australian Research Council Industrial Transformation Training Centre for Mine Site Restoration (ICI150100041) and I was personally supported by an Australian Government Research Training Program (RTP) Scholarship.

TABLE OF CONTENTS

STATEMENT OF CONTRIBUTIONS

I have obtained permission from the copyright owners to use any third-party copyright material reproduced in this thesis, and to use any of my own published work in which the copyright is held by another party. Signed author attribution statements are included in Appendix I.

Chapter 2: Walters, S. J., T. P. Robinson, M. Byrne, G. W. Wardell-Johnson, and P. Nevill. (2020). Contrasting patterns of local adaptation along climatic gradients between a sympatric parasitic and autotrophic tree species. *Molecular Ecology 29:* 3022-3037. https://www.doi.org/10.1111/mec.15537.

Author Contributions: SJW conducted the study and wrote the manuscript. All authors conceived the ideas and designed the study. Samples were collected and processed by SJW. Data analysis was performed by SJW with input from PN, TPR and MB. The manuscript was edited and approved by all authors.

Chapter 3: Walters, S. J., T. P. Robinson, M. Byrne, G. W. Wardell-Johnson, and P. Nevill. (In press). Association of adaptive genetic variation with climatic variables differs between a host-specific mistletoe and its host. *Evolutionary Applications.*

Author Contributions: SJW conducted the study and wrote the manuscript. All authors conceived the ideas and designed the study. Samples were collected and processed by SJW. Data analysis was performed by SJW with input from PN, TPR and MB. The manuscript was edited and approved by all authors.

Chapter 4: Walters, S. J., M. Byrne, J. R. P. Worth, G. W. Wardell-Johnson, and P. Nevill. [Under review] Nutrient acquisition strategy affects spatial patterns of genetic variation. *Journal of Biogeography.*

Author Contributions: SJW conducted the study and wrote the manuscript. The research and study design was conceived by SJW, PN, MB, and GW. Samples were collected and processed by SJW. Data analysis was performed by SJW with input from PN, JRPW and MB. The manuscript was edited and approved by all authors.

Chapter 5: Walters, S. J., T. P. Robinson, M. Byrne, G. W. Wardell-Johnson, and P. Nevill. Seed sourcing in the genomics era: Multispecies provenance delineation for current and future climates. *In preparation*.

Author Contributions: SJW conducted the study and wrote the manuscript. All authors conceived the ideas and designed the study. Data analysis was performed by SJW with input from PN, TPR and MB. The manuscript was edited and approved by all authors.

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

AMOVA	Analysis of molecular variance
AP	Annual precipitation
BF	Bayes factor
bp	base pair
CMSR	Centre for Mine Site Restoration
cpSSR	Chloroplast simple sequence repeat
CTAB	Cetyl trimethylammonium bromide
DBCA	Department of Biodiversity, Conservation and Attractions
DNA	Deoxyribonucleic acid
EA	Environment association
FDR	False discovery rate
GDA94	Geodetic datum of Australia 1994
GDM	Generalised dissimilarity model
HAP	Haplotype
IBD	Isolation by distance
IT	Isothermality
LFMM	Latent factor mixed model
MAF	Minor allele frequency
$MgCl_2$	Magnesium chloride
MP	Maximum parsimony
MTDQ	Mean temperature of the driest quarter
MTHQ	Mean temperature of the warmest quarter
MTWQ	Mean temperature of the wettest quarter

PC	Principal component
PCA	Principal component analysis
PCoA	Principal coordinate analysis
PCR	Polymerase chain reaction
PD	Population differentiation
PDQ	Precipitation of the driest quarter
PS	Precipitation seasonality
PVP	Polyvinylpyrrolidone
PWQ	Precipitation of the wettest/warmest quarter
RDA	Redundancy analysis
RGB	Red-green-blue
SAMOVA	Spatial analysis of molecular variance
sNMF	Sparse non-negative matrix factorization
SNP	Single nucleotide polymorphism
SSP	Shared socioeconomic pathway
TS	Temperature seasonality

CHAPTER 1

INTRODUCTION

1.1 Genetic diversity in plant species

Genetic diversity is the foundation of biodiversity and determines the evolutionary trajectory of populations, species and ecological communities (Hughes et al., 2008). Genetic diversity within species is driven by the processes of natural selection, mutation, genetic drift and gene flow (Slatkin, 1987). Life history traits are also known to influence genetic diversity and population structure (Loveless & Hamrick, 1984; Hamrick et al., 1992; Hamrick & Godt, 1996; Duminil et al., 2007; Broadhurst et al., 2017). Specifically, patterns of genetic diversity vary with divergent species characteristics such as growth form, range size, pollination syndrome and seed dispersal mechanism (Duminil et al., 2007). Assessments of genetic diversity in nonmodel species have been aided by the recent advancement of next-generation sequencing technology (McCormack et al., 2013; Bragg et al., 2015), which can provide vital genetic information for incorporation into conservation and restoration planning (Funk et al., 2012; Hoffmann et al., 2015; Flanagan et al., 2018; Breed et al., 2019).

Knowledge of genetic variation within plant species is crucial to ensure maintenance of population viability and evolutionary potential (Sgrò et al., 2011; Hoffmann et al., 2015). Overall, genetic variation can be divided into two categories – adaptive and neutral – each shaped by different evolutionary processes with different consequences for the persistence of species (Holderegger et al., 2006). Specifically, adaptive genetic variation is associated with fitness-related traits that are subject to the evolutionary process of natural selection (Conner & Hartl, 2004). In contrast, neutral genetic variation is associated with genes that have no practical effect on fitness and are instead subject to evolutionary processes of gene flow and genetic drift (Conner & Hartl, 2004; Holderegger et al., 2006). Despite the importance of genetic variation in natural populations, rarely has information on both adaptive and neutral genetic diversity been available for plant species.

Adaptive diversity enables plant species to adapt and build evolutionary resilience to environmental conditions; this will be particularly important for plant responses to changing climates (Sgrò et al., 2011). Furthermore, maintaining adequate neutral diversity within populations prevents genetic decline through processes such as inbreeding depression, which impact the long-term survival of species (Spielman et

al., 2004). Accordingly, information on both adaptive and neutral genetic variation should be considered when designing conservation and management strategies for species (Hoffmann et al., 2015; Breed et al., 2019).

1.2 Local adaptation and landscape genetics

Local adaptation is driven by divergent natural selection interacting with gene flow (Kawecki & Ebert, 2004) and results in populations adapting to specific ecological conditions (Williams, 1966; Savolainen et al., 2013). Generally, adaptations within populations occur either through selection on standing genetic variation or new mutations (Hermisson & Pennings, 2005; Barrett & Schluter, 2008), which can facilitate range expansions and ecological speciation (reviewed in Savolainen et al., 2013).

Traditionally, local adaptation in plant species have been studied through reciprocal transplant experiments (reviewed in Hereford, 2009) and common garden experiments (Savolainen et al., 2013). More recently, landscape genetics has enabled the integration of genetic and spatial data to examine interactions between landscape features and evolutionary processes (Manel & Holderegger, 2013; Balkenhol et al., 2017; Storfer et al., 2018); even within nonmodel species (Savolainen et al., 2013; Tiffin & Ross-Ibarra, 2014). Recent methods that investigate patterns of adaptation have been largely correlative approaches; for example outlier detection and environmental association analysis (Storfer et al., 2016; Balkenhol et al., 2017). These genetic studies will be crucial to improving our understanding of local adaptation and responses into environmental pressures (e.g., changing climates; Hoffmann & Sgrò, 2011; Savolainen et al., 2013).

Landscape genetic studies identify local adaptation within species through the association of adaptive genetic diversity with environmental variables (Bragg et al., 2015) and can be used to predict responses of populations to changing climates (Schoville et al., 2012). Advancements in short-read sequencing technology have greatly increased the number of markers sampled across genomes and the ability to detect loci potentially under selection (Stapley et al., 2010), including for nonmodel species that lack reference genomes (Davey et al., 2011). As population history plays a crucial role in determining the nature of natural selection (Siol et al., 2010) and strongly influences the degree of local adaptation (Leimu & Fischer, 2008), it is

imperative that genetic studies consider the effect of neutral genetic diversity on patterns of local adaptation (Savolainen et al., 2013). Recent analytical methods have accounted for neutral processes by incorporating population structure into the statistical models (Rellstab et al., 2015; Balkenhol et al., 2017). The degree of local adaptation is largely independent of life history, habitat heterogeneity and geographical distances (Leimu & Fischer, 2008). Consequently, generalisations may not be possible between different species within the same landscape and studies explicitly comparing local adaptation in co-occurring plant species are rare (but see Yeaman et al., 2016 for an example). Therefore, species-specific information on patterns of adaptive genetic variation is needed to inform species conservation and management (Funk et al., 2012; Flanagan et al., 2018); for example selection of restoration seed sources (Broadhurst et al., 2008; Williams et al., 2014; Hoffmann et al., 2015).

1.3 Gene flow and the influence of historical events

Gene flow between populations is a key determinant of genetic diversity and population structuring within species (Slatkin, 1987). Specifically, high gene flow counteracts genetic drift, reducing population structuring (Frankham et al., 2010) and, over time, restriction of gene flow between populations can ultimately lead to speciation (Bragg et al., 2015). For plant species, gene flow occurs through pollen and seed dispersal. Dispersion mechanisms vary widely between taxa and have been shown to have different consequences for genetic diversity (Loveless & Hamrick, 1984; Hamrick et al., 1992; Hamrick & Godt, 1996; Duminil et al., 2007; Broadhurst et al., 2017). For example, wind pollination typically results in lower genetic differentiation between populations within a species due to the longer-distance gene flow, whereas insect-pollinated species often have higher genetic differentiation between populations due to pollinator dispersal limits (Loveless & Hamrick, 1984). As genetic diversity influences population viability and the long-term survival of species (Spielman et al., 2004; Leimu et al., 2006), understanding the association between species characteristics and gene flow across the landscape is crucial for conservation and management planning (Bragg et al., 2015; Broadhurst et al., 2017).

Identifying barriers to gene flow (e.g., geological, geomorphological, and climatic) has been a key focus of landscape genetics (Manel et al., 2003; Storfer et al., 2007;

Holderegger & Wagner, 2008; Storfer et al., 2010). Neutral genetic variation is used to detect genetic connectivity between populations with genetic discontinuities correlated against ecological and landscapes features (Manel et al., 2003). However, barriers to gene flow can change over time and may influence patterns of genetic diversity within populations. For example, contraction of barriers may result in range expansions, which can lead to allele surfing, population bottlenecks and founder effects (Excoffier et al., 2009). Consequently, the spatial distribution of genetic diversity within species is influenced by a combination of both contemporary processes and historical events (Slatkin, 1987; Bragg et al., 2015), with the latter having profound influence on the current distribution of genetic diversity globally (Hewitt, 2000; Hewitt, 2004).

Phylogeographic studies can provide insight into how historical events and landscape features have influenced contemporary genetic diversity and structure within species (Avise, 2000, 2009). Studies on plant species have been chiefly concentrated on Europe, North America and Asia, and largely under-represented in the southern hemisphere (Beheregaray, 2008; Keppel et al., 2012). Across the same landscape, past geomorphological and climatic events can affect multiple species in a similar manner (Frankham et al., 2010), therefore comparative phylogeography among multiple co-occurring species may provide insight to the influence of past events on regional patterns of biodiversity (Hickerson et al., 2010). For example, elucidating phylogeographic patterns within species and across landscapes can be important for identifying climate refugia (Keppel et al., 2012) and understanding evolutionary history of species across landscapes (Funk et al., 2012).

1.4 Mapping genetic provenances for restoration

Genetic information in plant species can be used to identify conservation units (Funk et al., 2012), predict responses to changing climatic conditions (Bragg et al., 2015) and identify genetic provenances to inform seed sourcing for restoration (Broadhurst et al., 2008; Breed et al., 2019). Identifying seed zones should consider the genetic connectivity across the landscape and genetic variation within populations to ensure adequate genetic diversity is captured in seed collections (Hufford & Mazer, 2003). Ideally, seed collection strategies should focus on collecting genetically diverse, quality seed to ensure restored populations can adequately adapt to current and future

climatic conditions (Broadhurst et al., 2008; Breed et al., 2019). While there is a diverse array of seed provenancing strategies for plant species including local provenancing, composite provenancing (Broadhurst et al., 2008), admixture (Breed et al., 2013), and climate-adjusted provenancing (Prober et al., 2015), they all require information on the distribution of genetic variation across the landscape.

Understanding the distribution of genetic provenances within species can enable restoration practitioners to develop strategies that maximise the genetic health of restored populations (Vander Mijnsbrugge et al., 2010). For instance, seed collected from populations with low genetic diversity and/or from distantly related populations with limited gene flow may result in undesirable outcomes for restoration (e.g., outbreeding depression and genetic swapping), which should be avoided (Hufford & Mazer, 2003; Bischoff et al., 2010). Mapping genetic provenances across the landscape can be achieved by combining genetic data with spatial modelling approaches (e.g., Supple et al., 2018; Rossetto et al., 2019). Typically, provenance delineation is mapped under current climatic conditions; yet genetic provenances can also be mapped using future climate projections (e.g., Supple et al., 2018; Ahrens et al., 2020), which could be used to guide seed sourcing under changing climates (Williams et al., 2014; Breed et al., 2019). However, these recent methods have only been applied to a limited number of plant species with a narrow range of life history traits; no study has yet applied this approach to parasitic plants.

1.5 Biology, host-specificity and genetic diversity of parasitic plants

Parasitism is ubiquitous across the tree of life (Musselman & Press, 1995; Poulin & Morand, 2000; Poulin, 2011) and has independently evolved numerous times, making it one of the most successful lifeforms across all living organisms (Poulin & Morand, 2004). Specifically, parasites are organisms that complete one or more stages of life associated with a host individual (Norton & Carpenter, 1998) with transmission mode and life history strategies varying widely between taxa (Barrett et al., 2008; Poulin & Randhawa, 2015). For instance, parasite transmission can occur directly or via a vector (Poulin & Randhawa, 2015) and parasites can be either obligate (i.e., cannot complete its lifecycle without a host) or facultative (i.e., can complete its lifecycle independent from a host).

Parasitic plants are a diverse group that have evolved over time to rely on other plants for nutrients and survival (Kuijt, 1969; Musselman & Press, 1995). They attach to host plants via a haustorium – a specialised structure that penetrates into either roots or stems – and form a connection with the host's transport tissue. Despite accounting for just 1% of angiosperm species (Kuijt, 1969), parasitic plants occur widely from tropical rainforests to arctic environments (Musselman & Press, 1995; Press & Phoenix, 2005; Heide-Jørgenson, 2008) and are considered keystone species within many systems (Press & Graves, 1995; Watson, 2001; Press & Phoenix, 2005). Life forms differ greatly between species, which are predominantly classified based by the attachment site on the host (i.e., root or shoot parasite) and the occurrence of photosynthesis (Musselman & Press, 1995). Hemiparasites (e.g., *Rhinanthus*, *Viscum* spp) photosynthesize to produce their own carbon, but obtain water and nutrients from the host, while holoparasites (e.g., *Pilostyles*, *Orobanche* spp) rely on hosts for water, nutrients and carbon. Additionally, parasitic plants have varying levels of host specialisation ranging from host generalists to host specific species (Heide-Jørgenson, 2008; Thorogood & Hiscock, 2013; Okubamichael et al., 2016).

Host specialisation provides parasites the opportunity to interact more profitably with a frequently encountered host species by specifically adapting to the hosts' defences (Norton & Carpenter, 1998). Most parasitic plants are generalists that can parasitise a range of host species (Press & Phoenix, 2005; Heide-Jørgenson, 2008). However, some species are highly host-specific and can only parasitise a few host species, often within the same genus (Norton & Carpenter, 1998; Press & Phoenix, 2005). Specialisation comes at the expense of the ability to interaction with other host species (Norton & Carpenter, 1998) and can influence parasite evolution through host-switching (recently reviewed by Thines, 2019) or co-speciation events (i.e., host and parasite evolve simultaneously), which may ultimately lead to speciation (Norton & Carpenter, 1998; de Vienne et al., 2013). Furthermore, the level of host-specialisation influences genetic diversity between parasitic species (Barrett et al., 2008), although relatively few studies have focused on this area.

Genetic diversity and structure in parasitic plants is influenced by life history traits of both the parasite and host (Nadler, 1995; Criscione et al., 2005; Barrett et al., 2008). For instance, genetic diversity in parasitic plants is influenced by the same life history factors as other plant species (see Duminil et al., 2007) in addition to characteristics

such host-specificity and host longevity (Barrett et al., 2008). Genetic studies in parasitic plants have provided insight into the effect of habitat fragmentation (Stanton et al., 2009), climate (Ramírez-Barahona et al., 2017), host specificity (Thorogood et al., 2008) and historical events (Amico & Nickrent, 2009; Zuber & Widmer, 2009). Few studies have compared genetic diversity and structure between parasitic plants and hosts (but see Jerome & Ford, 2002 for an example) and we have little knowledge of adaptation of parasitic plants to climatic conditions.

1.6 Parasitic and autotrophic plant species in Western Australia

A rich and diverse flora is found within the southwest of Western Australia with over 7,300 vascular plant species currently recorded, of which ~49% are endemic (Hopper & Gioia, 2004). The region is an ancient, isolated landscape on a geologically ancient craton with low relief topology and the occasional granite-domed inselberg (Anand & Paine, 2002), and it has remained unglaciated since the Paleozoic Era (Hopper & Gioia, 2004). Increasing aridity across the Miocene and Pliocene resulted in speciation, while subsequent climatic oscillations of the Pleistocene led to diversity, divergence and persistence within refugial populations (Byrne, 2008; Byrne et al., 2014). Due to the enormous diversity of plant life, and the threat from a range of contemporary factors (e.g., habitat fragmentation and climate change), the southwest of Western Australia has been identified as a global biodiversity hotspot (Myers et al., 2000; Mittermeier et al., 2005).

Genetic studies on plant species in the southwest of Western Australia have predominately focussed on species associated with specific geological formations such as granite outcrops and low elevation on banded ironstone ranges (Byrne & Hopper, 2008; Byrne et al., 2019; Krauss & Anthony, 2019), or addressing questions around the effect of range disjunctions (Llorens et al., 2017; Bradbury et al., 2019) and life history traits in shrubs with differing longevity and seed dispersal mechanisms (Millar et al., 2016, 2017). Despite the interest in understanding patterns of genetic variation within plant communities, genetic data is only available for one parasitic species, root hemiparasite *Santalum spicatum* (Byrne, Macdonald, & Brand, 2003; Byrne, MacDonald, Broadhurst, et al., 2003). These studies used restriction fragment length polymorphism analysis on nuclear and chloroplast genomes to detect two genetic clusters, each with different evolutionary histories. Furthermore, genetic research in

this region has predominantly examined neutral genetic diversity and phylogeographic patterns with very few studies addressing questions on adaptive genetic variation (but see Hopley & Byrne, 2019 for an example).

Similar to global statistics, parasitic plants comprise just 1% of plant species across the southwest of Western Australia, with around 72 species across seven plant families (Groom & Lamont, 2015). Many parasitic species across this landscape have ecological and cultural importance. For example, mistletoes contribute to nutrient cycling processes (March & Watson, 2007, 2010; Watson & Herring, 2012) and provide food and habitat resources for bird species (Turner, 1991). Moreover, parasitic species can also have cultural significance to local Indigenous Australians, the Noongar people, with a notable example being root parasite *Nuytsia floribunda* (Hopper, 2010; Watson, 2019). Despite the importance of parasitic plants locally, we know little about the how patterns of climate adaptation and genetic variation compare with other plant species across the landscape.

1.7 Study area and species

The research presented in this thesis was conducted across south-western Australia. Climate in the region varies across a gradient from high-rainfall Mediterranean (>1,200 mm) in the south-west to low rainfall semi-arid in the north-east (<200 mm; Bureau of Meteorology, http://www.bom.gov.au/climate/data/). Vegetation varies across the study area with tall *Eucalyptus* forests in the high rainfall zone of the south-west, *Banksia* woodlands and mallee-heath along coastal and central areas, and *Acacia* scrublands in the semi-arid north-east (Beard, 1990).

Two pairs of sympatric parasitic and autotrophic plant species were selected for this research. The first species pair comprised the root hemiparasite *Nuytsia floribunda* (Labill.) R.Br. ex G.Don (Loranthaceae) and sympatric autotroph *Melaleuca rhaphiophylla* Schauer (Myrtaceae). *Nuytsia floribunda* is a generalist parasite that can parasitise numerous host species simultaneously, including *M. rhaphiophylla* (Calladine et al., 2000). The second species pair comprised shoot mistletoe *Amyema gibberula* var. *tatei* (Blakely) Barlow (Loranthaceae) and host species *Hakea recurva* Meisn. subsp. *recurva* (Proteaceae). *Amyema gibberula* var. *tatei* is a host-specific parasitic that only parasitise *Hakea* species, and exists almost entirely on *Hakea recurva* subsp. *recurva* (Start, 2015).

1.7.1 Description

Nuytsia floribunda is root parasitic tree/shrub that grows to 10 m high with rough, grey-brown bark (Figure 1.1A; Western Australian Herbarium, 1998–2021). The leaves are blueish green, linear and stalkless that are either opposite or scattered (Wheeler et al., 2002). Inflorescence is compound with clusters of stalkless yellow-orange flowers (Figure 1.2A) occurring from October to January (Western Australian Herbarium, 1998–2021; Wheeler et al., 2002). Fruits are dry and enclosed in a three-winged calyx.

Melaleuca rhaphiophylla is a bushy-crowned tree/shrub that grows to 10 m high with papery white-grey bark that strips off in chartaceous sheets (Figure 1.1B; Marchant et al., 1987; Western Australian Herbarium, 1998–2021). Leaves are grey-green to green, alternate, long and needle-like tapering to a hooked point (Wheeler et al., 2002; Holliday, 2008). Flower spikes are white-cream and terminal (Figure 1.2B), typically flowering from September to December (Western Australian Herbarium, 1998–2021), producing numerous fruits (Wheeler et al., 2002).

Amyema gibberula var. *tatei* is a vertical and often spreading aerial hemiparasitic shrub (Figure 1.1C; Flora of Australia, 1984; Western Australian Herbarium, 1998–2021). Leaves are terete, glabrous and fleshy (Flora of Australia, 1984; Watson, 2019), and have vegetative resemblance to *Hakea* hosts (Barlow & Wiens, 1977). The inflorescence is densely white-tomentose, coloured green, red and pink (Figure 1.2C) and flowers from September to November (Western Australian Herbarium, 1998–2021). Fruit are berry-like, globular and fleshy (Flora of Australia, 1984; Watson, 2019).

Hakea recurva subsp. *recurva* is a multi-stemmed tree/shrub that grows to 6 m high with spreading branches and smooth grey bark that gets rough as the plant ages (Figure 1.1D; Western Australian Herbarium, 1998–2021). The alternate leaves are thick, terete and rigid with a downwards curve ending in a sharp point (Figure 1.2D). Inflorescences are axillary, white-cream and sweetly-scented with flowering occurring from June to September (Western Australian Herbarium, 1998–2021; Young, 2006). The winged seed is encased in follicles that open when ripe (Holliday, 2005; Young, 2006).

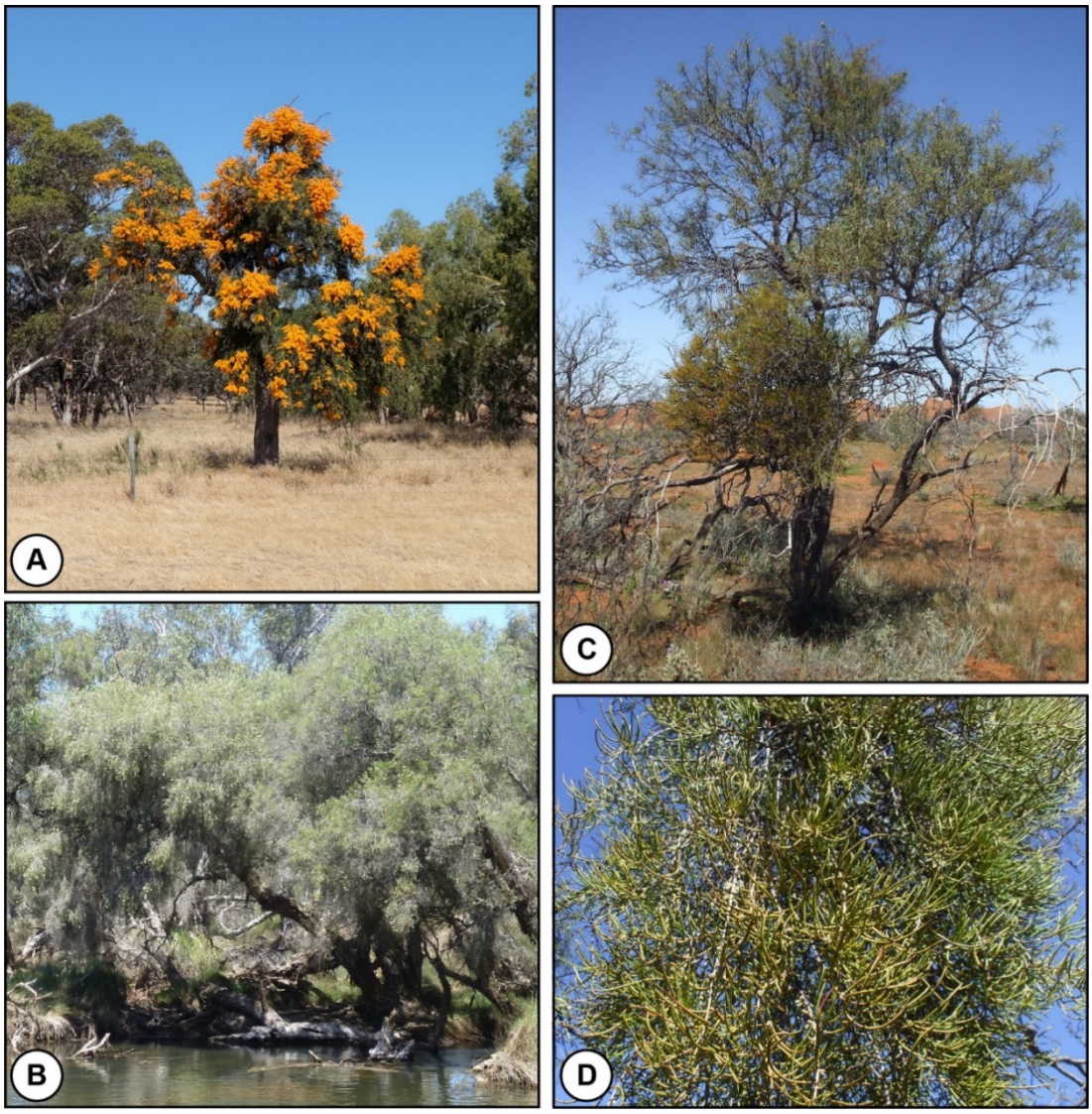


Figure 1.1 Study species (A) *Nuytsia floribunda*, (B) *Melaleuca rhaphiophylla*, (C) *Amyema gibberula* var. *tatei* mistletoe on *Hakea recurva* subsp. *recurva* host plant, and (D) *Amyema gibberula* var. *tatei* mistletoe up close.

1.7.2 Taxonomy and naming

Nuytsia floribunda was first described by Labillardière (1805) as *Loranthus floribunda* but due to distinct differences in form and fruit from other *Loranthus* species, Brown (1831) later changed the name to *Nuytsia floribunda*, which was formally described by Don (1834). The species is monotypic and ancestral within the Loranthaceae family (Vidal-Russell & Nickrent, 2008). The genus *Nuytsia* is named after the Dutch explorer Peter Nuyts who visited the south-western coast of Australia from 1626-1627 (Flora of Australia, 1984; Sharr, 2019) and the species name *floribunda* is Latin for "flowering profusely" (Sharr, 2019).

Figure 1.2 Study species in flower: (A) *Nuytsia floribunda*, (B) *Melaleuca rhaphiophylla*, (C) *Amyema gibberula* var. *tatei*, and (D) *Hakea recurva* subsp. *recurva*.

Melaleuca rhaphiophylla was described by Schauer (1844) and has had no taxonomic changes since. Although the species commonly occurs with other *Melaleuca* species, hybridisation with other species is rare (M. Hislop, pers. comm.). The genus and species names are both Greek, *Melaleuca* meaning "black" and "white", which is named after Asian species in this genus, and *rhaphiophylla* meaning "needle leaved" (Sharr, 2019).

Amyema gibberula var. *tatei* was described by Blakely (1922) as *Loranthus gibberulus* var. *tateii* and the genus was later changed to *Amyema* by Danser (1929). This species has leaves strictly glabrous, which differs from earlier described *Amyema gibberula* var. *gibberula* where the entire plant is densely white-tomentose (Barlow, 1992).

Hybridisation between species is not recorded and they have complementary flowering, which may limit pollen transfer (Watson, 2019). *Amyema* is Greek for "those not yet initiated" and refers to the name being used prior to genus being fully described (Sharr, 2019). The species name *gibberula* is Latin refers to the gibbous structure of the calyx-tube (Sharr, 2019), and *tatei* is named in honour of botanist Professor Ralph Tate (Blakely, 1922; Sharr, 2019).

Hakea recurva was described by Meisner (1856) and a review of *Hakea* taxa later reduced the species to a subspecies and grouped it with *Hakea recurva* subsp. *arida*, which has a similar form but with shorter, thinner leaves (Flora of Australia, 1999). Hybridisation among *Hakea* species is uncommon (M. Hislop, pers. comm.), although some individuals of *Hakea recurva* are purported to be intermediary between subspecies (Flora of Australia, 1999). The genus *Hakea* is named after Baron von Hake, a German patron of botany (Flora of Australia, 1999; Sharr, 2019) and the species and subspecies name *recurva* refers to the curved leaves that bend curve backwards (Sharr, 2019).

1.7.3 Distribution and habitat

Nuytsia floribunda ranges from Kalbarri to Israelite Bay on south-western coast (Marchant et al., 1987; Flora of Australia, 1999; Wheeler et al., 2002). The species is continuously distributed across the kwongan, heath and woodlands of the Geraldton Sandplains, Swan Coastal Plain and Esperance Plains but occurs in more scattered localities throughout the Jarrah Forest and Warren bioregions (Figure 1.3A; Wheeler et al., 2002). Isolated populations exist in the Avon Wheatbelt and are usually associated with granite rocks (Erickson et al., 1973). Populations generally occur on nutrient-poor sandplains, sandy swamps and around rocky outcrops (Flora of Australia, 1984; Western Australian Herbarium, 1998–2021).

Melaleuca rhaphiophylla ranges from Kalbarri to Fitzgerald River National Park and across to the south-western coast spanning the Geraldton Sandplains, Swan Coastal Plain, Jarrah Forest, Warren and Avon Wheatbelt (Figure 1.3B; Marchant et al., 1987; Wheeler et al., 2002). Within these regions, the species is predominantly distributed along watercourses such as inlets, lakes and rivers, and permanent swamps (Marchant et al., 1987; Wheeler et al., 2002), with populations occurring along sandy waterways,

saltmarshes and swamps in white or grey sand, clay soils and limestone (Western Australian Herbarium, 1998–2021).

Amyema gibberula var. *tatei* occurs on *Hakea* hosts, predominantly *H. recurva* subsp. *recurva* (Start, 2015) and occurs sparsely across the range from Yorkrakine Rock in the Avon Wheatbelt into the Goldfields and Murchison bioregions (Figure 1.3C; Flora of Australia, 1984; Western Australian Herbarium, 1998–2021). Specimens have been previously recorded further inland but none of these could be located alive for this thesis. In the south-west of the range, populations are often restricted to habitat associated with granite outcrops due to the widespread clearing for agriculture (Watson, 2019).

Hakea recurva subsp. *recurva* is widespread from the Murchison River to the Avon Wheatbelt and into the Great Victorian Desert (Figure 1.3D; Flora of Australia, 1999). The species is usually associated with granite outcrops, rocky ridges and rocky sandplain slopes, growing in laterite, red sand or loam, and in sandy clay over granite (Western Australian Herbarium, 1998–2021). Populations can be almost continuous across some regions and occur in more scattered localities in others, particularly on the edge of the species range (Western Australian Herbarium, 1998–2021).

1.7.4 Ecology

Nuytsia floribunda is insect pollinated with wind dispersal of the winged seed (Lamont, 1985) and seedlings are known to survive for up to four years without a host (Calladine et al., 2000). The honey-scented flowers attract a variety of bees, wasps, ants and other nectar-feeding insects, and provides a food source for a variety of bird species (Hopper, 2010; Watson, 2019). The species will parasitise most plants and can have underground stems extending up to 110 m from the parent plants (Herbert, 1919). After fire, regeneration can occur through epicormics growth or through lateral suckers that arise from the underground network of roots and rhizomes (Hocking & Fineran, 1983) and they flower more prolifically after fire (Flora of Australia, 1984).

Melaleuca rhaphiophylla is insect pollinated and has gravity dispersed seed (Brophy et al., 2013). The plants provide habitat for birds that nest in the branches and hollows during spring when the surrounding area floods, protecting the birds from predators (Powell, 1990). Fire can kill the species, or damage plants such that they will not produce seed for ~5 years (Powell, 1990).

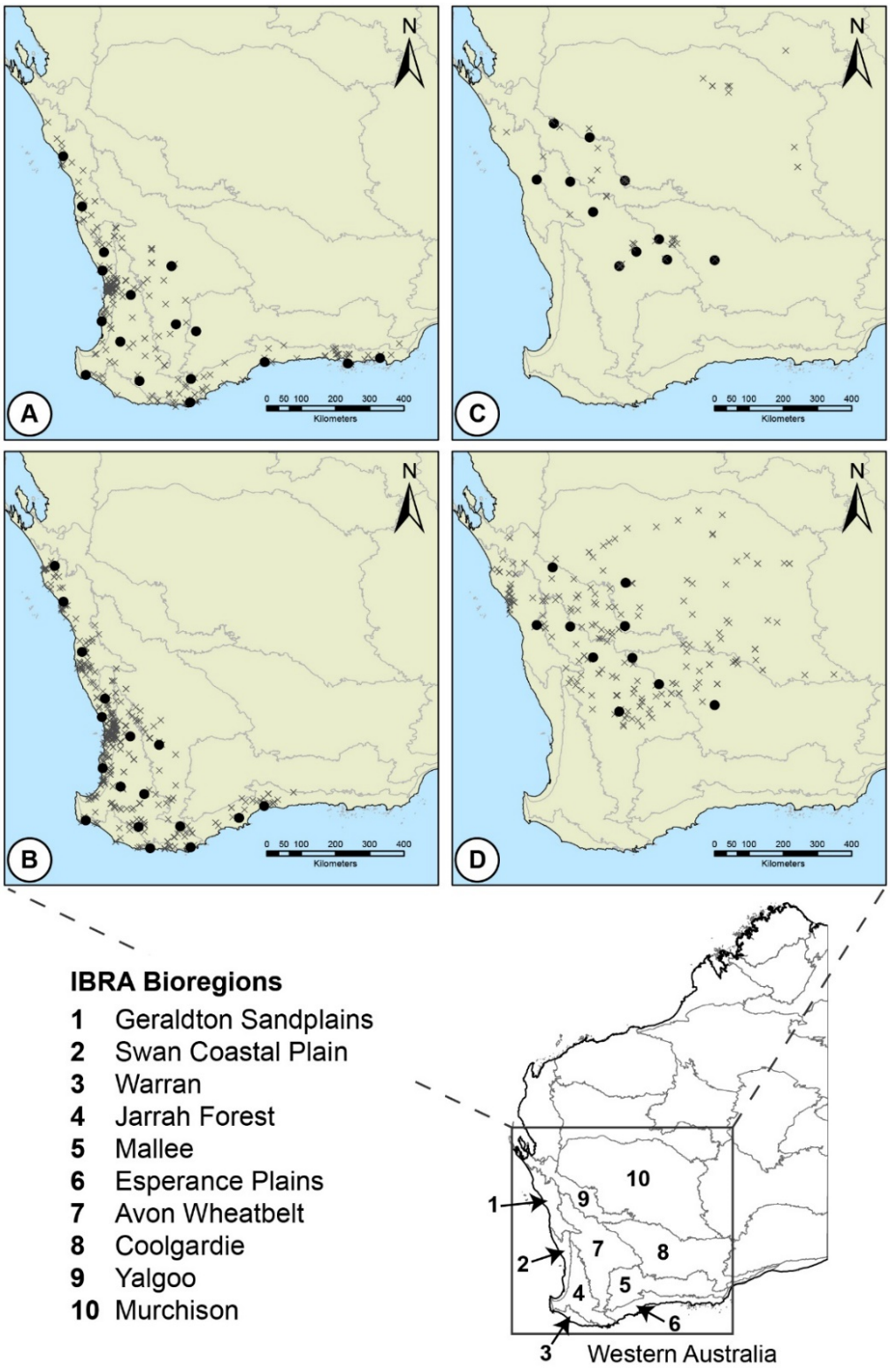


Figure 1.3 Distribution maps for (A) *Nuytsia floribunda*, (B) *Melaleuca rhaphiophylla*, (C) *Amyema gibberula* var. *tatei*, and (D) *Hakea recurva* subsp. *recurva*. Crosses indicate herbarium occurrence records from the Atlas of Living Australia (https://www.ala.org.au/) and circles indicate sites sampled as part of this thesis. The IBRA Bioregions were obtained from the Department of Environment (http://www.environment.gov.au/) and correspond to areas described in the text.

Amyema gibberula var. *tatei* is bird-pollinated and has bird-dispersed seed (Watson, 2019). *Amyema* flowers generally contain large quantities of nectar that, in addition to the ripened fruits, provides food resources for bird species (Turner, 1991; Watson, 2019). The species also provides habitat for *Ogyris amaryllis* (Watson, 2019) and various bird species (Turner, 1991). Plants are killed by fire although fire avoidance may be possible in some habitat types, for example granite outcrops (Start, 2015).

Hakea recurva subsp. *recurva* is generally insect pollinated, with nectar from flowers providing a food source for pollinators, and has gravity/wind dispersed seed (Flora of Australia, 1999). Plants are tolerant of prolonged dry periods and frost, generally requiring well-drained soil and full sun (Holliday, 2005). While there is a paucity of information on the exact fire response of this species, it is likely that the semi-arid habitat occupied is less prone to fire and flooding rains create suitable conditions for seed establishment, similar to other closely related species (Flora of Australia, 1999).

1.7.5 Significance, conservation and uses

Nuytsia floribunda, also known as *moodgar*, is significant to the Noongar people who used the bark for shields and collected roots, suckers and gum as a food source, although this varied between regions (Hopper, 2010; Hansen & Horsfall, 2019; Watson, 2019). When land was cleared for agriculture, trees of *N. floribunda* were left behind as shade trees or for ornamental reasons (Hocking & Fineran, 1983). *Nuytsia floribunda* can be propagated from seed, cuttings and tissue culture (Hopper, 2010), and young seedlings must be placed with a host plant (Ralph, 2009). Despite being parasitic, the species has been previously used in restoration projects through seeding and planting activities, particularly in *Banksia* woodland restoration on the Swan Coastal Plain where *N. floribunda* is a key overstorey species (e.g., Brundrett et al., 2018). The species may also re-colonize restoration areas naturally from neighbouring bushland (e.g., Maher et al., 2008).

Melaleuca rhaphiophylla leaves contain essential oils (Brophy & Lassak, 1992; Brophy et al., 2013) and were used by Noongar people to treat colds and influenza (Hansen & Horsfall, 2019). Noongar people used the flowers as a source of nectar with bark used in food preparation and making shelters (Hansen & Horsfall, 2019). The species forms part of a priority ecological community on the Swan Coastal Plain with the community threatened by urban development and recreation, fire, weed invasion

(e.g., *Typha orientalis*; Powell, 1990), and changes in hydrology (DBCA, 2020). *Melaleuca rhaphiophylla* can be propagated from seed for restoration (Ralph, 2009), and planted around permanently damp areas such as lakes, reservoirs and man-made wetlands (Holliday, 2008) and could be used in shelter belts (Brophy et al., 2013). The species has been previously used in restoration projects on the Swan Coastal Plain through seeding and/or planting activities (e.g., Brundrett et al., 2018), including for post-mining restoration in riparian areas where *M. rhaphiophylla* would naturally dominate the overstorey (e.g., van Etten et al., 2011).

While information on the use of *Amyema gibberula* var. *tatei* is scarce, the berries of more widespread *Amyema* species are known to have been eaten by Noongar people (Meagher, 1974; Hansen & Horsfall, 2019). *Amyema* species can be propagated by removing the outer layer of seed and placing on a suitable host branch (Ralph, 2009). Although mistletoes do not typically feature on species lists for restoration programs, they would naturally disperse into restoration areas via avian disperses, providing that suitable host species are present. While mistletoes are typically shorter-lived that hosts (Start, 2011), threats to mistletoes are similar such as fire and climate change (Start, 2011; Watson, 2019).

Hakea flowers were used as a source of nectar for Noongar people and the gum from these plants was also likely to have been used (Hansen & Horsfall, 2019). The species can be propagated from seed for restoration, usually germinating in 4-5 weeks (Young, 2006; Ralph, 2009). *Hakea recurva* is currently used in mining restoration programs (typically through planting) in the mid-west region of Western Australia (e.g., Commander et al., 2017), where it would naturally occur near granite outcrops and ironstone ridges, and in revegetation programs within the Avon Wheatbelt (e.g., Moore Catchment Council, 2018). The species *Hakea recurva* subsp. *recurva* can also be used as an ornamental plant, as windbreaks or along boundary lines (Holliday, 2005; Young, 2006), although the species can become weedy in some areas (Keighery, 2013).

1.8 Thesis aims and structure

The primary question of this thesis is: "Do sympatric plant species with different nutrient acquisition strategies have similar patterns of genetic variation?" Specifically, this study aims to compare patterns of climate adaptation, genetic diversity and

differentiation, and phylogeographic history between two pairs of parasitic and autotrophic plant species in Western Australia (Figure 1.4). The data chapters in this thesis are written and formatted as four journal articles and include one article that is published (Chapter 2), one that has been accepted (Chapter 3), one currently under review (Chapter 4), and one in preparation (Chapter 5).

As the data chapters of this thesis are intended to be stand-alone pieces of research for publication in scientific journals, each data chapter will consist of an abstract, introduction, materials and methods, results, discussion, conclusion and reference sections. To place the work within the context of the thesis, each chapter begins with a preface that gives a brief description of the contribution that chapters make to the overall thesis. Published papers have been reformatted to maintain the original content but to ensure that referencing style and other formatting is consistent between chapters.

In this thesis, I begin by examining patterns of adaptive genetic variation between a generalist root parasite and a sympatric autotrophic plant species (Chapter 2). The aim of this chapter was to determine whether sympatric plant species with different nutrient acquisition strategies have similar patterns of adaptation to local climatic gradients. These patterns of climate adaptation were investigated by correlating adaptive genetic variation with environmental variables and mapping patterns of genetic composition across the landscape.

In Chapter 3 I examine patterns of adaptive genetic variation and local climate adaptation between a host-specific mistletoe and its co-occurring host species. The aim of this chapter was to compare patterns of adaptive genetic variation in a host-specialised parasite reliant on a single host individual for survival. This builds on the findings of the previous chapter by examining whether patterns observed between parasites and co-occurring autotrophs vary with host specificity.

In Chapter 4 I examine genetic diversity, population differentiation and phylogeographical divergence within the two pairs of parasitic and autotrophic plant species. This chapter aims to assess whether patterns of neutral genetic variation and phylogeographic history were similar within both parasite-autotroph pairs, and between the two parasitic plants with different levels of host-specialisation (generalist versus host-specific). This chapter also seeks to examine and evaluate the evolutionary processes influencing genetic diversity in these species.

In Chapter 5 I combine genetic information on local climate adaptation with spatial modelling to develop provenance maps for each species. The aim of this chapter is to compare the genetic provenances within and between the two pairs of co-occurring species with different life history traits under current and future climate scenarios.

The data chapters are followed by a General Discussion (Chapter 6) that synthesizes the main findings of this thesis and limitations to this work, significance of this thesis, and recommendations for future work.

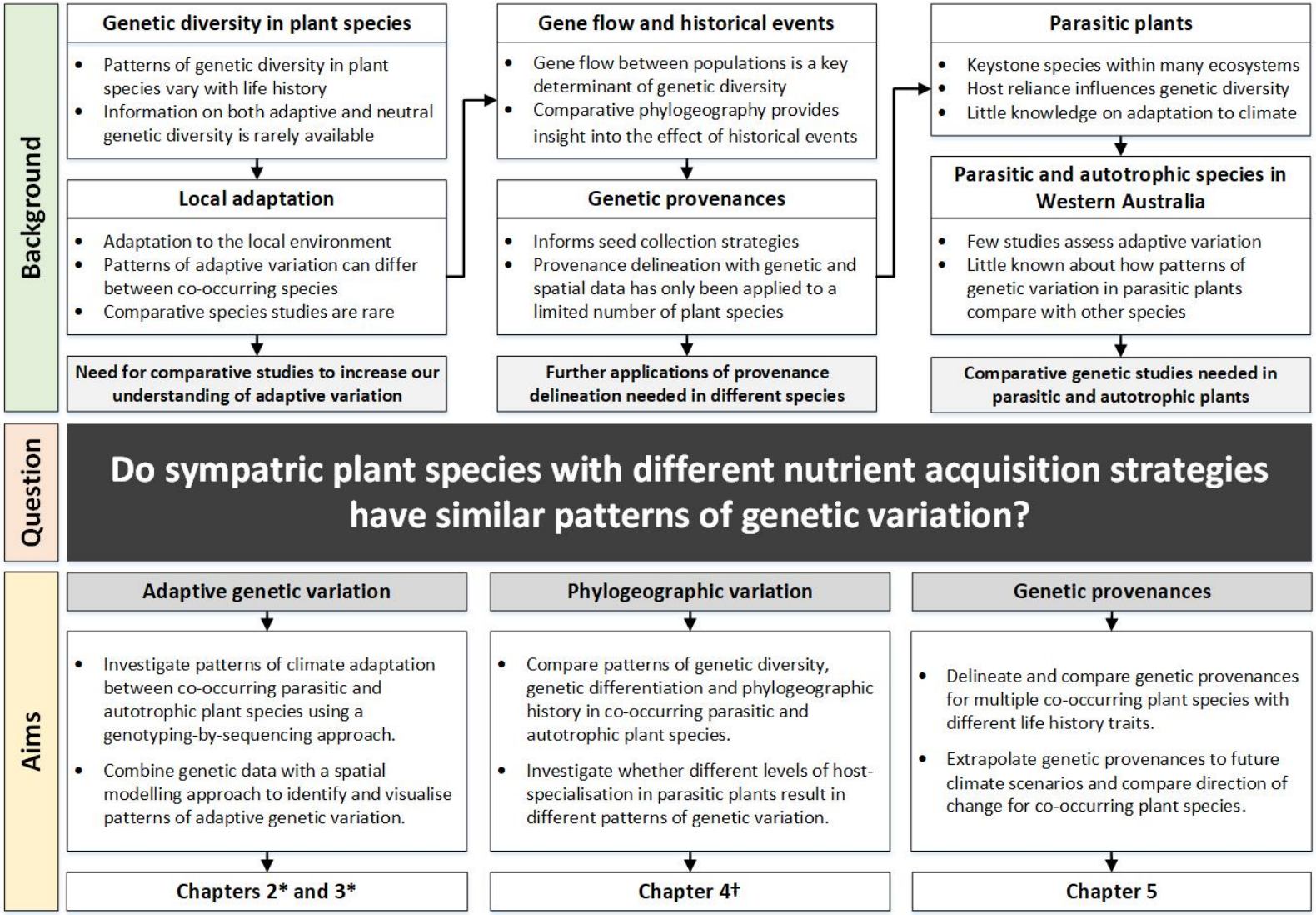


Figure 1.4 Conceptual framework of the research question and aims of this thesis. *Paper published or accepted. †Manuscript under review.

1.9 References

Every reasonable effort has been made to acknowledge the owners of the copyright material. I would be pleased to hear from any copyright owner who has been omitted or incorrectly acknowledged.

Ahrens, C. W., James, E. A., Miller, A. D., Scott, F., Aitken, N. C., Jones, A. W., . . . Rymer, P. D. (2020). Spatial, climate and ploidy factors drive genomic diversity and resilience in the widespread grass *Themeda triandra*. *Molecular Ecology, 29*, 3872-3888. https://doi.org/10.1111/mec.15614

Amico, G. C., & Nickrent, D. L. (2009). Population structure and phylogeography of the mistletoes *Tristerix corymbosus* and *T. aphyllus* (Loranthaceae) using chloroplast DNA sequence variation. *American Journal of Botany, 96*, 1571-1580. https://doi.org/10.3732/ajb.0800302

Anand, R. R., & Paine, M. (2002). Regolith geology of the Yilgarn Craton, Western Australia: Implications for exploration. *Australian Journal of Earth Sciences, 49*(1), 3-162. https://doi.org/10.1046/j.1440-0952.2002.00912.x

Avise, J. C. (2000). *Phylogeography: The history and formation of species*. Cambridge, MA: Harvard University Press.

Avise, J. C. (2009). Phylogeography: Retrospect and prospect. *Journal of Biogeography, 36*, 3-15. https://doi.org/10.1111/j.1365-2699.2008.02032.x

Balkenhol, N., Dudaniec, R. Y., Krutovsky, K. V., Johnson, J. S., Cairns, D. M., Segelbacher, G., . . . Joost, S. (2017). Landscape genomics: Understanding relationships between environmental heterogeneity and genomic characteristics of populations. In O. P. Rajora (Ed.), *Population genomics concepts, approaches and applications* (pp. 261-322). Cham: Springer. https://doi.org/10.1007/978-3-030-04589-0

Barlow, B. A. (1992). Conspectus of the genus *Amyema* Tieghem (Loranthaceae). *Blumea, 36*, 293-381.

Barlow, B. A., & Wiens, D. (1977). Host-parasite resemblance in Australian mistletoes: The case for cryptic mimicry. *Evolution, 31*, 69-84. https://doi.org/10.1111/j.1558-5646.1977.tb00983.x

Barrett, L. G., Thrall, P. H., Burdon, J. J., & Linde, C. C. (2008). Life history determines genetic structure and evolutionary potential of host–parasite interactions. *Trends in Ecology & Evolution, 23*, 678-685. https://doi.org/10.1016/j.tree.2008.06.017

Barrett, R. D. H., & Schluter, D. (2008). Adaptation from standing genetic variation. *Trends in Ecology & Evolution, 23*, 38-44. https://doi.org/10.1016/j.tree.2007.09.008

Beard, J. S. (1990). *Plant life of Western Australia* (A. S. George & N. Gibson Eds. Second ed.). Australia: Rosenberg Publishing Pty Ltd.

Beheregaray, L. B. (2008). Twenty years of phylogeography: The state of the field and the challenges for the Southern Hemisphere. *Molecular Ecology, 17*, 3754-3774. https://doi.org/10.1111/j.1365-294X.2008.03857.x

Bischoff, A., Steinger, T., & Müller-Schärer, H. (2010). The importance of plant provenance and genotypic diversity of seed material used for ecological restoration. *Restoration Ecology, 18*, 338-348. https://doi.org/10.1111/j.1526-100X.2008.00454.x

Blakely, W. F. (1922). *Loranthus gibberulus* var. *tatei*. *Proceedings of the Linnean Society of New South Wales, 47*, 395.

Bradbury, D., Binks, R. M., Coates, D. J., & Byrne, M. (2019). Conservation genomics of range disjunction in a global biodiversity hotspot: A case study of *Banksia biterax* (Proteaceae) in southwestern Australia. *Biological Journal of the Linnean Society, 127*, 390-406. https://doi.org/10.1093/biolinnean/blz050

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

Breed, M. F., Harrison, P. A., Blyth, C., Byrne, M., Gaget, V., Gellie, N. J. C., . . . Mohr, J. J. (2019). The potential of genomics for restoring ecosystems and biodiversity. *Nature Reviews Genetics, 20*, 615-628. https://doi.org/10.1038/s41576-019-0152-0

Breed, M. F., Stead, M. G., Ottewell, K. M., Gardner, M. G., & Lowe, A. J. (2013). Which provenance and where? Seed sourcing strategies for revegetation in a changing environment. *Conservation Genetics, 14*, 1-10. https://doi.org/10.1007/s10592-012-0425-z

Broadhurst, L., Breed, M., Lowe, A., Bragg, J., Catullo, R., Coates, D., . . . Byrne, M. (2017). Genetic diversity and structure of the Australian flora. *Diversity and Distributions, 23*, 41-52. https://doi.org/10.1111/ddi.12505

Broadhurst, L. M., Lowe, A., Coates, D. J., Cunningham, S. A., McDonald, M., Vesk, P. A., & Yates, C. (2008). Seed supply for broadscale restoration: Maximizing evolutionary potential. *Evolutionary Applications, 1*, 587-597. https://doi.org/10.1111/j.1752-4571.2008.00045.x

Brophy, J. J., Craven, L. A., & Doran, J. C. (2013). *Melaleucas. Their botany, essential oils and uses*. Canberra: ACIAR Monograph No. 156. Australian Centre for International Agricultural Research (ACIAR).

Brophy, J. J., & Lassak, E. V. (1992). Steam volatile leaf oils of some *Melaleuca* species from Western Australia. *Flavour and Fragrance Journal, 7*, 27-31. https://doi.org/10.1002/ffj.2730070107

Brown, R. (1831). General view of the botany of the vicinity of Swan River. *The Journal of the Royal Geographical Society of London, 1*, 17-21. https://doi.org/10.2307/1797656

Brundrett, M., Longman, V., Wisolith, A., Jackson, K., Collins, M., & Clarke, K. (2018). *Banksia woodland restoration project: Annual report 6: January – December 2017*. Department of Biodiversity, Conservation and Attractions, Perth.

Byrne, M. (2008). Evidence for multiple refugia at different time scales during Pleistocene climatic oscillations in southern Australia inferred from phylogeography. *Quaternary Science Reviews, 27*, 2576-2585. https://doi.org/10.1016/j.quascirev.2008.08.032

Byrne, M., Coates, D. J., Forest, F., Hopper, S. D., Krauss, S. L., Sniderman, J. M. K., & Thiele, K. R. (2014). A diverse flora: Species and genetic relationships. In H. Lambers (Ed.), *Plant life on the sandplains in southwest Australia: A*

global biodiversity hotspot (pp. 81-99). Crawley, Western Australia: UWA Publishing.

Byrne, M., & Hopper, S. D. (2008). Granite outcrops as ancient islands in old landscapes: evidence from the phylogeography and population genetics of *Eucalyptus caesia* (Myrtaceae) in Western Australia. *Biological Journal of the Linnean Society, 93*, 177-188. https://doi.org/10.1111/j.1095-8312.2007.00946.x

Byrne, M., Krauss, S. L., Millar, M. A., Elliott, C. P., Coates, D. J., Yates, C., . . . Gibson, N. (2019). Persistence and stochasticity are key determinants of genetic diversity in plants associated with banded iron formation inselbergs. *Biological Reviews, 94*, 753-772. https://doi.org/10.1111/brv.12477

Byrne, M., Macdonald, B., & Brand, J. (2003). Phylogeography and divergence in the chloroplast genome of Western Australian Sandalwood (*Santalum spicatum*). *Heredity, 91*, 389-395. https://doi.org/10.1038/sj.hdy.6800346

Byrne, M., MacDonald, B., Broadhurst, L., & Brand, J. (2003). Regional genetic differentiation in Western Australian sandalwood (*Santalum spicatum*) as revealed by nuclear RFLP analysis. *Theoretical and Applied Genetics, 107*, 1208-1214. https://doi.org/10.1007/s00122-003-1365-2

Calladine, A., Pate, J. S., & Dixon, K. W. (2000). Haustorial development and growth benefit to seedlings of the root hemiparasitic tree *Nuytsia floribunda* (Labill.) R.Br. in association with various hosts. *Annals of Botany, 85*, 733-740. https://doi.org/10.1006/anbo.2000.1129

Commander, L., Golos, P., Elliot, C., Merino-Martin, L., Stevens, J., & Miller, B. (2017) *Sinosteel Midwest Corporation: Practitioner restoration manual.* Botanic Gardens and Parks Authority for Sinosteel Midwest Corporation.

Conner, J. K., & Hartl, D. L. (2004). *A primer to ecological genetics*. Massachusetts, USA: Oxford University Press USA.

Criscione, C. D., Poulin, R., & Blouin, M. S. (2005). Molecular ecology of parasites: Elucidating ecological and microevolutionary processes. *Molecular Ecology, 14*, 2247-2257. https://doi.org/10.1111/j.1365-294X.2005.02587.x

Danser, B. H. (1929). On the taxonomy and nomenclature of the Loranthaceae of Asia and Australia. *Bulletin du Jardin Botanique de Buitenzorg, 10*, 291-374.

Davey, J. W., Hohenlohe, P. A., Etter, P. D., Boone, J. Q., Catchen, J. M., & Blaxter, M. L. (2011). Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nature Reviews Genetics, 12*, 499-510. http://doi.org/10.1038/nrg3012

DBCA. (2020). *Priority ecological communities for Western Australia version 30*. Retrieved from

de Vienne, D. M., Refrégier, G., López-Villavicencio, M., Tellier, A., Hood, M. E., & Giraud, T. (2013). Cospeciation vs host-shift speciation: Methods for testing, evidence from natural associations and relation to coevolution. *New Phytologist, 198*, 347-385. https://doi.org/10.1111/nph.12150

Don, G. (1834). *A general history of the dichlamydeous plants* (Vol. 3). London: Rivington.

Duminil, J., Fineschi, S., Hampe, A., Jordano, P., Salvini, D., Vendramin, G. G., & Petit, R. J. (2007). Can population genetic structure be predicted from life-history traits? *The American Naturalist, 169*, 662-672. https://doi.org/10.1086/513490

Erickson, R., George, A. S., Marchant, N. G., & Morcombe, M. K. (1973). *Flowers and plants of Western Australia*. Sydney: Reed.

Excoffier, L., Foll, M., & Petit, R. J. (2009). Genetic consequences of range expansions. *Annual Review of Ecology, Evolution, and Systematics, 40*, 481-501. https://doi.org/10.1146/annurev.ecolsys.39.110707.173414

Flanagan, S. P., Forester, B. R., Latch, E. K., Aitken, S. N., & Hoban, S. (2018). Guidelines for planning genomic assessment and monitoring of locally adaptive variation to inform species conservation. *Evolutionary Applications, 11*, 1035-1052. https://doi.org/10.1111/eva.12569

Flora of Australia. (1984). *Flora of Australia Volume 22, Rhizophorales to Celastrales*. Canberra: Bureau of Flora and Fauna.

Flora of Australia. (1999). *Flora of Australia Volume 17B, Proteaceae 3, Hakea to Dryandra*. Melbourne: ABRS/CSIRO Australia.

Frankham, R., Ballou, J. D., & Briscoe, D. A. (2010). *Introduction to conservation genetics* (Second ed.). Cambridge, UK: Cambridge University Press.

Funk, W. C., McKay, J. K., Hohenlohe, P. A., & Allendorf, F. W. (2012). Harnessing genomics for delineating conservation units. *Trends in Ecology & Evolution, 27*(9), 489-496. https://doi.org/10.1016/j.tree.2012.05.012

Groom, P. K., & Lamont, B. B. (2015). Parasitic plants. In *Plant life of southwestern Australia. Adaptations for survival* (pp. 79-90). Warsaw/Berlin: De Gruyter Open Ltd.

Hamrick, J. L., & Godt, M. J. W. (1996). Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions of the Royal Society B, 351*, 1291-1298. https://doi.org/10.1098/rstb.1996.0112

Hamrick, J. L., Godt, M. J. W., & Sherman-Broyles, S. L. (1992). Factors influencing levels of genetic diversity in woody plant species. *New Forests, 6*, 95-124. https://doi.org/10.1007/BF00120641

Hansen, V., & Horsfall, J. (2019). *Noongar bush tucker*. Crawley, Australia: UWA Publishing.

Heide-Jørgenson, H. S. (2008). *Parasitic flowering plants*. Leiden, The Netherlands: Brill.

Herbert, D. A. (1919). *Nuytsia floribunda* (The Christmas Tree). Its structure and parasitism. *Journal and Proceedings of The Royal Society of Western Australia, 5*, 72-88. Retrieved from https://www.biodiversitylibrary.org/part/238189

Hereford, J. (2009). A quantitative survey of local adaptation and fitness trade-offs. *The American Naturalist, 173*, 579-588. https://doi.org/10.1086/597611

Hermisson, J., & Pennings, P. S. (2005). Soft sweeps: Molecular population genetics of adaptation from standing genetic variation. *Genetics, 169*, 2335–2352. https://doi.org/10.1534/genetics.104.036947

Hewitt, G. (2000). The genetic legacy of the Quaternary ice ages. *Nature, 405*, 907-913. https://doi.org/10.1038/35016000

Hewitt, G. M. (2004). Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society B, 359*, 183-195. https://doi.org/10.1098/rstb.2003.1388

Hickerson, M. J., Carstens, B. C., Cavender-Bares, J., Crandall, K. A., Graham, C. H., Johnson, J. B., . . . Yoder, A. D. (2010). Phylogeography's past, present, and future: 10 years after Avise, 2000. *Molecular Phylogenetics and Evolution, 54*, 291-301. https://doi.org/10.1016/j.ympev.2009.09.016

Hocking, P. J., & Fineran, B. A. (1983). Aspects of the nutrition of root parasitic Loranthaceae. In M. Calder & P. Bernhardt (Eds.), *The biology of mistletoes* (pp. 229-258). Australia: Academic Press.

Hoffmann, A., Griffin, P., Dillon, S., Catullo, R., Rane, R., Byrne, M., . . . Sgrò, C. (2015). A framework for incorporating evolutionary genomics into biodiversity conservation and management. *Climate Change Responses, 2*, 1-23. https://doi.org/10.1186/s40665-014-0009-x

Hoffmann, A. A., & Sgrò, C. M. (2011). Climate change and evolutionary adaptation. *Nature, 470*, 479-485. https://doi.org/10.1038/nature09670

Holderegger, R., Kamm, U., & Gugerli, F. (2006). Adaptive vs. neutral genetic diversity: Implications for landscape genetics. *Landscape Ecology, 21*, 797-807. http://doi.org/10.1007/s10980-005-5245-9

Holderegger, R., & Wagner, H. H. (2008). Landscape genetics. *BioScience, 58*, 199-207. https://doi.org/10.1641/b580306

Holliday, I. (2005). *Hakeas: A field and garden guide*. Sydney, Australia: Reed New Holland Publishers.

Holliday, I. (2008). *Melaleuca rhaphiophylla*. In *Melaleucas: A field and garden guide* (Second ed., pp. 244-245). Sydney, Australia: Reed New Holland Publishers.

Hopley, T., & Byrne, M. (2019). Gene flow and genetic variation explain signatures of selection across a climate gradient in two riparian species. *Genes, 10*, 579. https://doi.org/10.3390/genes10080579

Hopper, S. D. (2010). *Nuytsia floribunda*. *Curtis's Botanical Magazine, 26*, 333-368. https://doi.org/10.1111/j.1467-8748.2009.01671.x

Hopper, S. D., & Gioia, P. (2004). The southwest Australian floristic region: Evolution and conservation of a global hotspot of biodiversity. *Annual Review of Ecology, Evolution and Systematics, 35*, 623-650. https://doi.org/10.1146/annurev.ecolsys.35.112202.130201

Hufford, K. M., & Mazer, S. J. (2003). Plant ecotypes: Genetic differentiation in the age of ecological restoration. *Trends in Ecology & Evolution, 18*, 147-155. http://doi.org/10.1016/S0169-5347(03)00002-8

Hughes, A. R., Inouye, B. D., Johnson, M. T. J., Underwood, N., & Vellend, M. (2008). Ecological consequences of genetic diversity. *Ecology Letters, 11*, 609-623. https://doi.org/10.1111/j.1461-0248.2008.01179.x

Jerome, C. A., & Ford, B. A. (2002). Comparative population structure and genetic diversity of *Arceuthobium americanum* (Viscaceae) and its *Pinus* host species: Insight into host–parasite evolution in parasitic angiosperms. *Molecular Ecology, 11*, 407-420. https://doi.org/10.1046/j.0962-1083.2002.01462.x

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

Keighery, G. (2013). Weedy native plants in Western Australia: An annotated checklist. *Conservation Science Western Australia, 8*, 259-275.

Keppel, G., Van Niel, K. P., Wardell-Johnson, G. W., Yates, C. J., Byrne, M., Mucina, L., . . . Franklin, S. E. (2012). Refugia: Identifying and understanding safe havens for biodiversity under climate change. *Global Ecology and Biogeography, 21*, 393–404. https://doi.org/10.1111/j.1466-8238.2011.00686.x

Krauss, S. L., & Anthony, J. M. (2019). The potential impact of mining on population genetic variation in the Banded Ironstone Formation endemic *Tetratheca erubescens* (Elaeocarpaceae). *Australian Journal of Botany, 67*, 172-182. https://doi.org/10.1071/BT18054

Kuijt, J. (1969). *The biology of parasitic flowering plants*. Berkeley, California: University of California Press.

Labillardière, J. J. (1805). *Novae hollandiae plantarum specimen* (Vol. 1): Huzard, Paris.

Lamont, B. B. (1985). Dispersal of the winged fruits of *Nuytsia floribunda* (Loranthaceae). *Australian Journal of Ecology, 10*, 187-193. https://doi.org/10.1111/j.1442-9993.1985.tb00880.x

Leimu, R., & Fischer, M. (2008). A meta-analysis of local adaptation in plants. *PLoS ONE, 3*, e4010. https://doi.org/10.1371/journal.pone.0004010

Leimu, R., Mutikainen, P., Koricheva, J., & Fischer, M. (2006). How general are positive relationships between plant population size, fitness and genetic variation? *Journal of Ecology, 94*, 942-952. https://doi.org/10.1111/j.1365-2745.2006.01150.x

Llorens, T. M., Tapper, S. L., Coates, D. J., McArthur, S., Hankinson, M., & Byrne, M. (2017). Does population distribution matter? Influence of a patchy versus continuous distribution on genetic patterns in a wind-pollinated shrub. *Journal of Biogeography, 44*, 361-374. https://doi.org/10.1111/jbi.12843

Loveless, M. D., & Hamrick, J. L. (1984). Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics, 15*, 65-95. https://doi.org/10.1146/annurev.es.15.110184.000433

Maher, K., Standish, R., & Hallett, L. (2008). *Restoration of Banksia woodland after the removal of pines at Gnangara: Evaluation of seeding trials. A report to the Department of Environment and Conservation.* Retrieved from https://www.water.wa.gov.au/__data/assets/pdf_file/0004/5584/86707.pdf

Manel, S., & Holderegger, R. (2013). Ten years of landscape genetics. *Trends in Ecology & Evolution, 28*, 614-621. https://doi.org/10.1016/j.tree.2013.05.012

Manel, S., Schwartz, M. K., Luikart, G., & Taberlet, P. (2003). Landscape genetics: Combining landscape ecology and population genetics. *Trends in Ecology & Evolution, 18*, 189-197. https://doi.org/10.1016/S0169-5347(03)00008-9

March, W. A., & Watson, D. M. (2007). Parasites boost productivity: Effects of mistletoe on litterfall dynamics in a temperate Australian forest. *Oecologia, 154*, 339-347. https://doi.org/10.1007/s00442-007-0835-7

March, W. A., & Watson, D. M. (2010). The contribution of mistletoes to nutrient returns: Evidence for a critical role in nutrient cycling. *Austral Ecology, 35*, 713-721. https://doi.org/10.1111/j.1442-9993.2009.02056.x

Marchant, N. G., Wheeler, J. R., Rye, B. L., Bennett, E. M., Lander, N. S., & Macfarlane, T. D. (1987). *Flora of the Perth region*. Perth, Australia: Western Australian Herbarium.

McCormack, J. E., Hird, S. M., Zellmer, A. J., Carstens, B. C., & Brumfield, R. T. (2013). Applications of next-generation sequencing to phylogeography and phylogenetics. *Molecular Phylogenetics and Evolution, 66*, 526-538. http://doi.org/10.1016/j.ympev.2011.12.007

Meagher, S. J. (1974). The food resources of the Aborigines of the south-west of Western Australia. *Records of the Western Australian Museum, 3*, 14-65.

Meisner, C. F. (1856). *Hakea recurva*. In A. L. P. P. de Candolle (Ed.), *Prodromus systematis naturalis regni vegetabilis, sive enumeratio contracta ordinum, generum, specierumque plantarum huc usque cognitarium, juxta methodi naturalis, normas digesta* (Vol. 14, pp. 394). Parisii: Sumptibus Sociorum Treuttel et Würtz.

Millar, M. A., Byrne, M., Coates, D. J., & Roberts, J. D. (2016). Contrasting diversity and demographic signals in sympatric narrow-range endemic shrubs of the south-west Western Australian semi-arid zone. *Biological Journal of the Linnean Society, 118*, 315-329. https://doi.org/10.1111/bij.12736

Millar, M. A., Byrne, M., Coates, D. J., & Roberts, J. D. (2017). Comparative analysis indicates historical persistence and contrasting contemporary structure in sympatric woody perennials of semi-arid south-west Western Australia. *Biological Journal of the Linnean Society, 120*, 771-787. https://doi.org/10.1093/biolinnean/blw005

Mittermeier, R. A., Gil, P. R., Hoffmann, M., Pilgrim, J., Brooks, T., Goettsch-Mittermeier, C., . . . B., d. F. G. A. (2005). *Hotspots revisited: Earth's biologically richest and most threatened terrestrial ecosystems*. Mexico City: CEMEX.

Moore Catchment Council. 2018. *Native revegetation guide for the Moore River catchment* [Brochure]. Western Australia: Author.

Musselman, L. J., & Press, M. C. (1995). Introduction to parasitic plants. In M. C. Press & J. D. Graves (Eds.), *Parasitic plants*. London, UK: Chapman & Hall.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A. B., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature, 403*, 853-858. https://doi.org/10.1038/35002501

Nadler, S. A. (1995). Microevolution and the genetic structure of parasite populations. *The Journal of Parasitology, 81*, 395-403. https://doi.org/10.2307/3283821

Norton, D. A., & Carpenter, M. A. (1998). Mistletoes as parasites: Host specificity and speciation. *Trends in Ecology & Evolution, 13*, 101-105. https://doi.org/10.1016/S0169-5347(97)01243-3

Okubamichael, D. Y., Griffiths, M. E., & Ward, D. (2016). Host specificity in parasitic plants—perspectives from mistletoes. *AoB PLANTS, 8*(plw069). https://doi.org/10.1093/aobpla/plw069

Poulin, R. (2011). The many roads to parasitism: A tale of convergence. *Adv Parasitol, 74*, 1-40. https://doi.org/10.1016/b978-0-12-385897-9.00001-x

Poulin, R., & Morand, S. (2000). The diversity of parasites. *The Quarterly Review of Biology, 75*, 277-293. https://doi.org/10.1086/393500

Poulin, R., & Morand, S. (2004). *Parasite biodiversity*. Washington, D. C.: Smithsonian Institution Press.

Poulin, R., & Randhawa, H. S. (2015). Evolution of parasitism along convergent lines: From ecology to genomics. *Parasitology, 142*, S6-S15. http://doi.org/10.1017/S0031182013001674

Powell, R. (1990). *Leaf and branch - Trees and tall shrubs of Perth*: Department of Conservation and Land Management.

Press, M. C., & Graves, J. D. (1995). *Parasitic Plants*. London, UK: Chapman & Hall.

Press, M. C., & Phoenix, G. K. (2005). Impacts of parasitic plants on natural communities. *New Phytologist, 166*, 737-751. https://doi.org/10.1111/j.1469-8137.2005.01358.x

Prober, S. M., Byrne, M., McLean, E. H., Steane, D. A., Potts, B. M., Vaillancourt, R. E., & Stock, W. D. (2015). Climate-adjusted provenancing: A strategy for climate-resilient ecological restoration. *Frontiers in Ecology and Evolution, 3*(65), 1-5. https://doi.org/10.3389/fevo.2015.00065

Ralph, M. (2009). *Growing Australian native plants from seed: For revegatation tree planting and direct seeding* (Second ed.). Victoria, Australia: Bushland Horticulture.

Ramírez-Barahona, S., González, C., González-Rodríguez, A., & Ornelas, J. F. (2017). The influence of climatic niche preferences on the population genetic structure of a mistletoe species complex. *New Phytologist, 214*, 1751-1761. https://doi.org/10.1111/nph.14471

Rellstab, C., Gugerli, F., Eckert, A. J., Hancock, A. M., & Holderegger, R. (2015). A practical guide to environmental association analysis in landscape genomics. *Molecular Ecology, 24*, 4348-4370. https://doi.org/10.1111/mec.13322

Rossetto, M., Bragg, J., Kilian, A., McPherson, H., van der Merwe, M., & Wilson, P. D. (2019). Restore and Renew: A genomics-era framework for species provenance delimitation. *Restoration Ecology, 27*, 538-548. https://doi.org/10.1111/rec.12898

Savolainen, O., Lascoux, M., & Merilä, J. (2013). Ecological genomics of local adaptation. *Nature Reviews Genetics, 14*, 807-820. https://doi.org/10.1038/nrg3522

Schauer, J. C. (1844). *Melaleuca rhaphiophylla*. In J. G. C. Lehmann (Ed.), *Plantae preissianae sive enumeratio plantarum quas in australasia occidentali et meridionali-occidentali annis 1838-1841* (Vol. 1, pp. 143). Hamburg: Sumptibus Meissneri.

Schoville, S. D., Bonin, A., François, O., Lobreaux, S., Melodelima, C., & Manel, S. (2012). Adaptive genetic variation on the landscape: Methods and cases.

Annual Review of Ecology, Evolution, and Systematics, 43, 23-43. https://doi.org/10.1146/annurev-ecolsys-110411-160248

Sgrò, C. M., Lowe, A. J., & Hoffmann, A. A. (2011). Building evolutionary resilience for conserving biodiversity under climate change. *Evolutionary Applications, 4*, 326-337. https://doi.org/10.1111/j.1752-4571.2010.00157.x

Sharr, F. A. (2019). *Western Australian plant names and their meaning* (A. S. George Ed. Third ed.). Kardinya, Australia: Four Gables Press.

Siol, M., Wright, S. I., & Barrett, S. C. H. (2010). The population genomics of plant adaptation. *New Phytologist, 188*, 313-332. https://doi.org/10.1111/j.1469-8137.2010.03401.x

Slatkin, M. (1987). Gene flow and the geographic structure of natural populations. *Science, 236*, 787-792. Retrieved from http://www.jstor.org/stable/1699930

Spielman, D., Brook, B. W., & Frankham, R. (2004). Most species are not driven to extinction before genetic factors impact them. *Proceedings of the National Academy of Sciences of the United States of America, 101*, 15261-15264. https://doi.org/10.1073/pnas.0403809101

Stanton, S., Honnay, O., Jacquemyn, H., & Roldán-Ruiz, I. (2009). A comparison of the population genetic structure of parasitic *Viscum album* from two landscapes differing in degree of fragmentation. *Plant Systematics and Evolution, 281*, 161-169. https://doi.org/10.1007/s00606-009-0198-0

Stapley, J., Reger, J., Feulner, P. G. D., Smadja, C., Galindo, J., Ekblom, R., . . . Slate, J. (2010). Adaptation genomics: The next generation. *Trends in Ecology & Evolution, 25*, 705-712. https://doi.org/10.1016/j.tree.2010.09.002

Start, A. N. (2011). Fire responses and survival strategies of mistletoes (Loranthaceae) in an arid environment in Western Australia. *Australian Journal of Botany, 59*, 533-542. https://doi.org/10.1071/BT11054

Start, A. N. (2015). The mistletoe flora of southern Western Australia, with a particular reference to host relationships and fire. *Australian Journal of Botany, 63*, 636-646. https://doi.org/10.1071/BT15028

Storfer, A., Murphy, M. A., Evans, J. S., Goldberg, C. S., Robinson, S., Spear, S. F., . . . Waits, L. P. (2007). Putting the 'landscape' in landscape genetics. *Heredity, 98*, 128-142. https://doi.org/10.1038/sj.hdy.6800917

Storfer, A., Murphy, M. A., Spear, S. F., Holderegger, R., & Waits, L. P. (2010). Landscape genetics: Where are we now? *Molecular Ecology, 19*, 3496-3514. https://doi.org/10.1111/j.1365-294X.2010.04691.x

Storfer, A., Antolin, M. F., Manel, S., Epperson, B. K., & Scribner, K. T., (2016) Genomic approaches in landscape genetics. In N. Balkenhol, S. A. Cushman, A. T. Storfer & L P. Waits (Eds.), *Landscape genetics: Concepts, methods and applications* (pp. 149-164). West Sussex, Wiley.

Storfer, A., Patton, A., & Fraik, A. K. (2018). Navigating the interface between landscape genetics and landscape genomics. *Frontiers in Genetics, 9*(68), 1-14. https://doi.org/10.3389/fgene.2018.00068

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

Thines, M. (2019). An evolutionary framework for host shifts – jumping ships for survival. *New Phytologist, 224*, 605-617. https://doi.org/10.1111/nph.16092

Thorogood, C. J., & Hiscock, S. J. (2013). Host specificity and speciation in parasitic plants. In D. S. Rutgers (Ed.), *Phylogeography: Concepts, intraspecific patterns and speciation processes* (pp. 57-79).

Thorogood, C. J., Rumsey, F. J., Harris, S. A., & Hiscock, S. J. (2008). Host-driven divergence in the parasitic plant *Orobanche minor* Sm. (Orobanchaceae). *Molecular Ecology, 17*, 4289-4303. https://doi.org/10.1111/j.1365-294X.2008.03915.x

Tiffin, P., & Ross-Ibarra, J. (2014). Advances and limits of using population genetics to understand local adaptation. *Trends in Ecology & Evolution, 29*, 673-680. https://doi.org/10.1016/j.tree.2014.10.004

Turner, R. J. (1991). Mistletoe in eucalypt forest—a resource for birds. *Australian Forestry, 54*, 226-235. https://doi.org/10.1080/00049158.1991.10674582

van Etten, E. J. B., McCullough, C. D., & Lund, M. A. (2011). Sand mining restoration on the Swan Coastal Plain using topsoil – Learning from monitoring of previous rehabilitation attempts. In the *Eighth International Heavy Minerals Conference 2011, 5 – 6 October 2011* (pp. 323-338). Perth, Western Australia: The Australasian Institute of Mining and Metallurgy.

Vander Mijnsbrugge, K., Bischoff, A., & Smith, B. (2010). A question of origin: Where and how to collect seed for ecological restoration. *Basic and Applied Ecology, 11*, 300-311. https://doi.org/10.1016/j.baae.2009.09.002

Vidal-Russell, R., & Nickrent, D. L. (2008). Evolutionary relationships in the showy mistletoe family (Loranthaceae). *American Journal of Botany, 95*, 1015-1029. https://doi.org/10.3732/ajb.0800085

Watson, D. M. (2001). Mistletoe–a keystone resource in forests and woodlands worldwide. *Annual Review of Ecology and Systematics, 32*, 219-249. https://doi.org/10.1146/annurev.ecolsys.32.081501.114024

Watson, D. M. (2019). *Mistletoes of southern Australia* (Second ed.). Victoria, Australia: CSIRO Publishing.

Watson, D. M., & Herring, M. (2012). Mistletoe as a keystone resource: An experimental test. *Proceedings of the Royal Society B, 279*, 3853-3860. https://doi.org/10.1098/rspb.2012.0856

Western Australian Herbarium. (1998–2021). FloraBase—the Western Australian Flora. Retrieved from https://florabase.dpaw.wa.gov.au/

Wheeler, J., Marchant, N., & Lewington, M. (2002). *Flora of the south west: Bunbury, Augusta, Denmark* (Vol. 2). Crawley, Australia: University of Western Australia Press/Australian Biological Resources Study.

Williams, A. V., Nevill, P. G., & Krauss, S. L. (2014). Next generation restoration genetics: Applications and opportunities. *Trends in Plant Science, 19*, 529-537. https://doi.org/10.1016/j.tplants.2014.03.011

Williams, G. C. (1966). *Adaptation and natural selection*. Princeton, NJ: Princeton University Press.

Yeaman, S., Hodgins, K. A., Lotterhos, K. E., Suren, H., Nadeau, S., Degner, J. C., . . . Aitken, S. N. (2016). Convergent local adaptation to climate in distantly

related conifers. *Science, 353*, 1431-1433. https://doi.org/10.1126/science.aaf7812

Young, J. A. (2006). *Hakeas of Western Australia: A field and identification guide*. Perth, Australia: West Australian National Parks and Reserves Association.

Zuber, D., & Widmer, A. (2009). Phylogeography and host race differentiation in the European mistletoe (*Viscum album* L.). *Molecular Ecology, 18*, 1946-1962. https://doi.org/10.1111/j.1365-294X.2009.04168.x

CHAPTER 2

CONTRASTING PATTERNS OF LOCAL ADAPTATION ALONG CLIMATIC GRADIENTS BETWEEN A SYMPATRIC PARASITIC AND AUTOTROPHIC TREE SPECIES

2.1 Preface

*This chapter consists of a published manuscript titled '**Contrasting patterns of local adaptation along climatic gradients between a sympatric parasitic and autotrophic tree species**' [Molecular Ecology 2020]. The content from section 2.2 onwards is the same as the published manuscript with only minor changes in formatting of references and lettering in figures to ensure consistency within the thesis. Permissions to include this work in this thesis are included in Appendix I.*

The introductory chapter (Chapter 1) of this thesis highlighted the importance of adaptive genetic variation in identifying patterns of adaptation to the local environment, and that patterns of adaptive variation can differ between co-occurring plant species. The introductory chapter also presented the argument that comparative species studies examining adaptive genetic variation are rare, and it is unknown how patterns of adaptive variation in parasitic plants compare to that of autotrophic species. This current data chapter (Chapter 2) presents a comparative genetics study examining adaptive genetic variation in two co-occurring tree species — one a generalist parasite and the other an autotrophic species. Genotyping-by-sequencing was used to obtain genetic data for these two nonmodel plant species, neither of which had genetic information available *a priori*. The study presented in this chapter makes a significant contribution to this thesis as it demonstrates a parasitic plant to have a different pattern of adaptive genetic variation and association to climatic variables compared to a co-occurring autotrophic species, and it increases our knowledge of climate adaptation in plant species more broadly. Here, the parasite examined was a generalist parasite with a wide host range, and this leads into the subsequent chapter (Chapter 3) that examines climate adaptation in a host-specific parasite with a narrower host range.

2.1.1 Acknowledgements

This work was supported by the Australian Government through an Australian Government Research Training Program Scholarship and the Australian Research Council Industrial Transformation Training Centre for Mine Site Restoration (project number ICI150100041).

2.1.2 Data accessibility

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

2.1.3 Author contributions

SJW conducted the study and wrote the manuscript. All authors conceived the ideas and designed the study. Samples were collected and processed by SJW. Data analysis was performed by SJW with input from PN, TPR and MB. The manuscript was edited and approved by all authors.

Walters, S. J., T. P. Robinson, M. Byrne, G. W. Wardell-Johnson, and P. Nevill. (2020). Contrasting patterns of local adaptation along climatic gradients between a sympatric parasitic and autotrophic tree species. *Molecular Ecology 29:* 3022-3037. https://www.doi.org/10.1111/mec.15537.

2.2 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 that 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 hemiparasite *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.

2.3 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 et al., 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 practices (Bragg et al., 2015; Flanagan et al., 2018), particularly given that species respond to

drivers of local adaptation in different ways (Aitken et al., 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 et al., 2010; Hoffmann et al., 2015; Breed et al., 2019).

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 (Kawecki & Ebert, 2004; Blanquart et al., 2013), but more recently using genomic detection methods (Stapley et al., 2010; Savolainen et al., 2013); for example by using genome scans that discover putatively adaptive loci in a reverse-ecology approach (Li et al., 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, 2015). As species within the same landscape can display different signals of selection to climatic variables (e.g., Shryock et al., 2017; Hopley & Byrne, 2019), 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 (Wang & Bradburd, 2014; Bragg et al., 2015). To date, studies on comparative adaptive genomics have focused 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 utilize a variety of host species and inhabit a diverse range of abiotic environments, with many considered keystone resources within ecosystems (Watson, 2001; Press & Phoenix, 2005). Generally, these taxa rely on a

specialized haustorium to penetrate the roots or stems of hosts to obtain water and nutrients (Calder, 1983). As parasitic plants are reliant on the 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 et al., 2016).

Generalist plant parasites utilize multiple host species for survival by parasitising either single individuals at a time (e.g., Barney et al., 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 the 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 et al., 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 (Kaltz & Shykoff, 1998; Gandon & Michalakis, 2002), 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 (Shryock et al., 2017; Steane, McLean, et al., 2017; Supple et al., 2018; Hopley & Byrne, 2019), 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 (Kuijt, 1969; Ehleringer & Marshall, 1995). Therefore, temperature, rather than precipitation, may be a more important driver of adaptation in parasitic plants. Furthermore, 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 et al., 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 southwestern Australia. *Nuytsia floribunda* is a long-lived root hemiparasite with over 100 known host species, including *M. rhaphiophylla* (Calladine et al., 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 geographical 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 utilization 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*). Furthermore, 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.

2.4 Materials and methods

2.4.1 Study species and sample collection

Melaleuca rhaphiophylla Schauer and *Nuytsia floribunda* (Labill.) R.Br. ex G.Don have widespread distributions in southwestern Australia spanning ~700 km north–south and ~600 km east–west (Figure 2.1). 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. Southwestern Australia is an isolated, relatively stable landscape, and phylogeographical 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 were 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 geographical 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 (Table S2.9.1 and Table S2.9.2). 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 device.

2.4.2 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 (Hijmans et al., 2005; Fick & Hijmans, 2017). 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 version 10.7.1 (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 et al., 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 data set. Uncorrelated bioclimatic variables were plotted in the R package RASTER version 3.0-12 (Figure S2.9.1; Hijmans, 2020) and mean values for each population are given in Table S2.9.1 and Table S2.9.2.

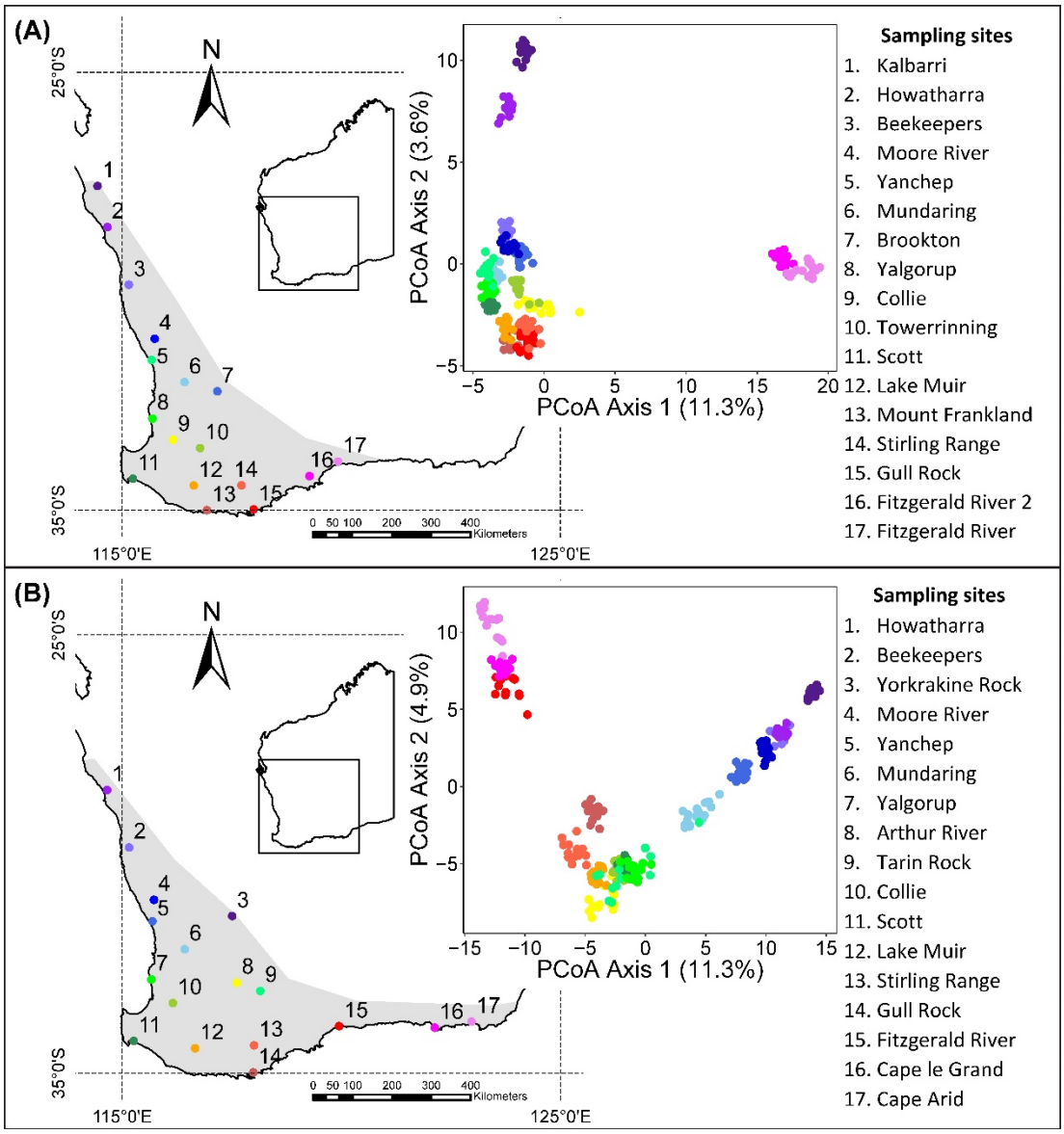


Figure 2.1 Sampling sites and principal coordinate analysis (PCoA) of genomic distance between individuals. Maps show the geographical location of the 17 sampling sites for (A) *Melaleuca rhaphiophylla* and (B) *Nuytsia floribunda* in south-western Australia with species distributions shown in grey (distributional 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.

2.4.3 Genomic data generation and bioinformatics

Approximately 8 mg of silica-dried leaf material for *N. floribunda* individuals and ~10 mg silica-dried leaf material *M. rhaphiophylla* individuals were sent to Diversity Arrays Technology Pty. Ltd 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 (Sansaloni et al., 2011; Kilian et al., 2012).

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 nonmodel 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 were collapsed to obtain ~2.5 million sequences per individual. SNP marker calling was performed in the propriety DARTSOFT14 pipeline with ~25% of samples regenotyped 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 version 1.1.11 (Gruber et al., 2018) in R (R Core Team, 2019) with replicates of individuals first removed from the SNP data sets. We filtered the data set to retain: (a) loci with less than 5% missing data; (b) high reproducibility (DArTseq reproducibility score > 0.98); (c) minor allele frequency (MAF) greater than 5%; and (d) 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 data set by randomly selecting one SNP per fragment to be retained in the data set. Filtered data sets were converted to csv format using the *write.csv* function (R Core Team, 2019).

2.4.4 Population structure

Population differentiation (pairwise F_{ST}) was estimated according to Weir and Cockerham (1984) using the R package HIERFSTAT version 0.04-22 (Goudet, 2005). To investigate population structure for both species, we first analysed the filtered data sets

at an individual level using principal coordinate analysis (PCoA) in the R package ADEGENET version 2.1.1 (Jombart, 2008; Jombart & Ahmed, 2011) and plotted the first two PCoA axes using the R package GGPLOT2 version 3.2.1 (Wickham, 2016).

Second, we estimated the number of ancestral population groups using the sparse nonnegative matrix factorization (sNMF) algorithm in the R package LEA version 2.4 (Frichot & François, 2015), which estimates the most likely number of ancestral genetic clusters (K). We tested values of K between $K = 1$ and $K = 20$ with 10 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 version 2.5-6 (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 geographical distance. Geographical matrices were calculated in the R package FOSSIL version 0.3.7 (Vavrek, 2011) and Mantel tests were run using Pearson's correlation coefficient with 9,999 permutations.

2.4.5 Analytical approaches

2.4.5.1 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 et al., 2017), while the EA approaches used were latent factor mixed

modelling (LFMM; Frichot et al., 2013; Caye et al., 2019), and redundancy analysis (RDA; Forester et al., 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 et al., 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 z-scores do not follow the same distribution as those of the larger data set while using the q-value procedure to control the FDR (Luu et al., 2017).

Analyses were run using the R packages OUTFLANK version 0.2 (Whitlock & Lotterhos, 2015) and PCADAPT version 4.1.0 (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 version 2.18.0 (Storey et al., 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 version 0.0 (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 were 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 (χ^2) distribution (François et al., 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 fit 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 version 2.5-6 (Oksanen et al., 2019) as described by 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 et al., 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 *SD* 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 version 1.6.20 (Chen, 2018). Second, 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 nonadaptive SNPs was significantly associated with species.

2.4.5.2 Patterns of local climatic adaptation

Generalized dissimilarity modelling (GDM; Ferrier, 2002; Ferrier et al., 2002) was used to test whether: (a) genomic variation in the parasite was associated with fewer climatic variables and that the magnitude of allelic turnover would be higher in the

autotrophic plant, using the method described by Fitzpatrick and Keller (2015); and (b) precipitation variables would be relatively less important in predicting allelic turnover than either temperature or geographical distance for the parasitic plant but not for the autotrophic species, using variation partitioning (Borcard et al., 1992). For each species we first estimated pairwise F_{ST} matrices (Weir & Cockerham, 1984) using the R package HIERFSTAT version 0.04-22 (Goudet, 2005). Separate F_{ST} matrices were created for (a) reference SNPs (neutral SNPs not detected by any genomic method) and (b) candidate SNPs, and scaled each F_{ST} matrix to between 0 and 1 prior to running the GDM. SNPs in the candidate data set 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 data set that were identified singularly by one of the EA methods. Population-level climatic data sets were compiled with the geographical 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 version 1.3.11 (Manion et al., 2018), which removed variation attributed to geographical 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 et al., 2007; Fitzpatrick et al., 2013). These procedures were run separately for each data set and only significant bioclimatic variables were retained. The three spline coefficients were summed for each remaining predictor variable (e.g., subset of geographical 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 geographical distance in explaining allelic turnover in the model, we partitioned the deviance resulting from GDMs using geographical distance, all significant temperature variables and/or all significant precipitation variables (Borcard et al., 1992; Yates et al., 2019). The partitioned deviance values were plotted in Venn diagrams using the R package EULERR version 6.0.0 (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 et al., 2013; Fitzpatrick & Keller, 2015). These functions were mapped using GGPLOT2 to visualize the relationship between allelic turnover and climatic variables.

2.4.5.3 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 the autotrophic species, we followed the approach of Fitzpatrick and Keller (2015) to visualize genomic variation. Spatial interpolations of genomic distance were derived for the two data sets (reference SNPs; candidate SNPs) of both species using the fitted GDMs to perform biologically-informed transformations for raster data sets of significant climatic variables into genetic importance values. Geographical 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 data set. 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 data sets (reference SNPs; candidate SNPs) for each species, we ran a PCA on extracted values of the GDM-transformed climatic variables for each data set following Fitzpatrick and Keller (2015) 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 data sets 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.

2.5 Results

2.5.1 SNP generation and population structure

DArTseq technologies produced data sets of 52,450 SNPs for *Melaleuca rhaphiophylla* (n = 272) and 36,881 SNPs for *Nuytsia floribunda* (n = 265). Nine of the 10 biological replicates for *M. rhaphiophylla* had greater than 98% genetic similarity with the tenth replicate having 82.4% similarity. Similarly, 15 of the 16 biological replicates for *N. floribunda* had greater than 95% genetic similarity with the 16th replicate having 88.5% similarity. Following further quality control filtering described in the Methods, the working data sets for each species comprised 5,531 SNPs for *M. rhaphiophylla* and 6,727 SNPs for *N. floribunda* (Walters et al., 2020) with global missing data of 1.30% and 1.01%, respectively. No individuals were removed from the *M. rhaphiophylla* data set, and one individual with a low marker call rate from the Yanchep population was removed from the *N. floribunda* data set.

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 2.1). 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 the presence of statistically significant isolation by distance (IBD) patterns for *M. rhaphiophylla* (p = 0.017) and *N. floribunda* (p < 0.001). Geographical 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 2.1A) were removed. Geographical distance explained 69.0% of

the variation in genetic divergence for the 15 remaining *M. rhaphiophylla* populations ($p < 0.001$).

2.5.2 Genomic signals of selection

The numbers of adaptive and nonadaptive SNPs were significantly associated with species ($\chi^2 = 249.02$, $df = 1$, $p < 0.001$). 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 2.2). 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 2.2), excluding OUTFLANK results still produced a significant association between SNPs and species ($\chi^2 = 48.09$, $df = 1$, $p < 0.001$).

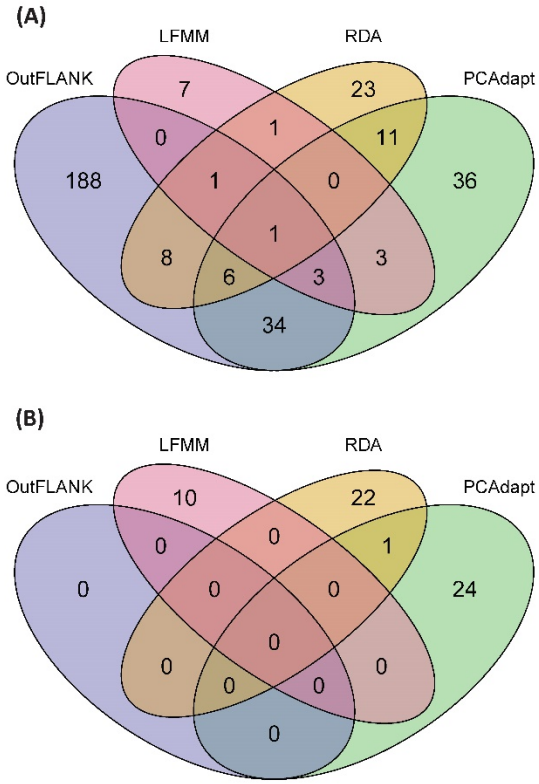


Figure 2.2 Venn diagrams of outlier SNPs for (A) *Melaleuca rhaphiophylla* and (B) *Nuytsia floribunda*. The diagrams show a comparison between the outliers obtained from OUTFLANK, PCADAPT, LFMM and RDA methods. For the univariate environmental association method, LFMM, SNPs that were significant for multiple environmental variables were only included once in the Venn diagram.

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 to 6.29 for *M. rhaphiophylla* and from 4.15 to 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 2.1).

Table 2.1 Number of loci under putative selection across seven climatic variables.

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

Note: Environmental association analyses, LFMM and RDA, were run on *Melaleuca rhaphiophylla* and *Nuytsia floribunda* data sets 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; MTHQ: mean temperature of the warmest quarter; MTWQ: mean temperature of the wettest quarter; PDQ, precipitation of the driest quarter; PS, precipitation seasonality; PWQ, precipitation of the wettest quarter; TS: temperature seasonality).

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 the genomic data. The number of SNPs identified as demonstrating signatures of selection identified also varied across the seven climatic variables for both species (Table 2.1).

2.5.3 Patterns of local climatic adaptation

Candidate SNP data sets 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 SNP data sets. 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 SNP data sets. The best GDM models (i.e., containing only significant predictor variables) explained a similar deviance in turnover in genetic composition for both species (Table 2.2).

Table 2.2 Model fit and relative importance of predictor variables in generalized dissimilarity modelling.

	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 explained	76.69	74.53	75.02	70.86
p-value	0.005	0.000	0.000	0.000
Relative importance				
Geographical 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	-

Note: Reference data sets contained 5,209 neutral SNPs for *Melaleuca rhaphiophylla* and 6,670 neutral SNPs for *Nuytsia floribunda.* Candidate SNP data sets 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: geographical distance; IT: isothermality; MTHQ: mean temperature of the warmest quarter; MTWQ: mean temperature of the wettest quarter; PDQ: precipitation of the driest quarter; PS: precipitation seasonality; PWQ: precipitation of the wettest quarter; TS: temperature seasonality).

Temperature and precipitation variables, together, explained 74.53% of GDM model deviance for the *M. rhaphiophylla* candidate SNP data set, with the majority of allelic turnover explained by both temperature and precipitation (Figure 2.3A). Similarly, temperature and precipitation variables, together, explained 69.28% of GDM model deviance for the *N. floribunda* candidate SNP data set, although 59.6% of allelic turnover was explained by temperature alone (Figure 2.3B). Geographical distance contributed the least to explaining GDM model deviance for both species, and was almost completely nested within the other two variables.

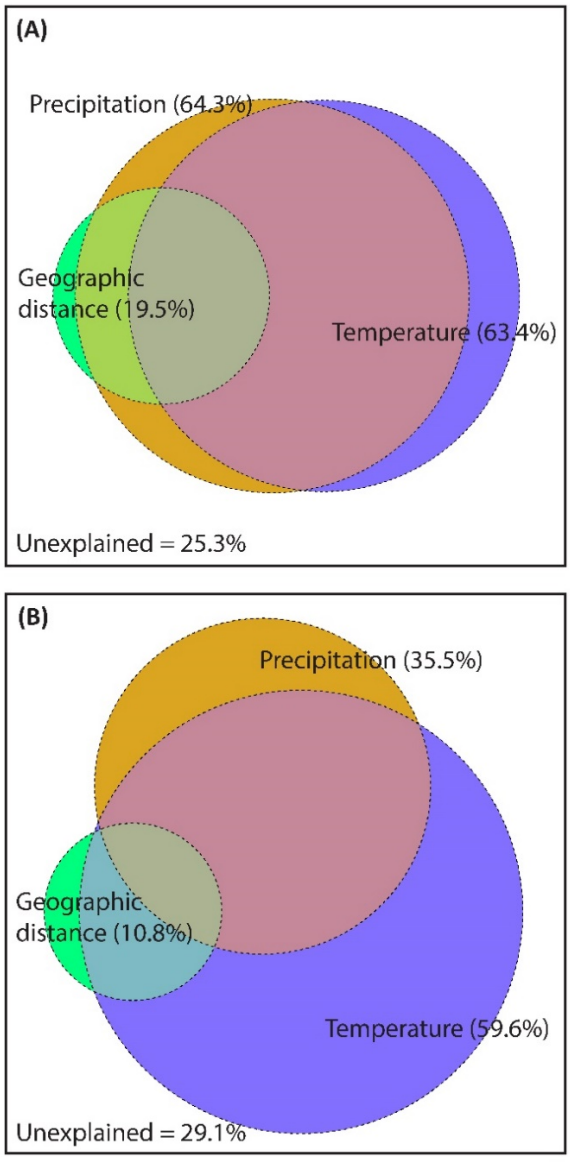


Figure 2.3 Partitioning of generalized dissimilarity model deviance by predictor variables. Three sets of predictor variables were used (geographical distance, temperature and precipitation variables) for (A) *Melaleuca rhaphiophylla*; and (B) *Nuytsia floribunda* adaptive genomic data sets containing only SNPs identified as putatively adaptive in prior genomic analyses.

Patterns of allelic turnover varied by both climatic variable and species (Figure 2.4). The most important predictor variable for both *M. rhaphiophylla* data sets was isothermality with the largest change in allelic turnover predicted to occur above 51% (Figure 2.4A), which corresponds to the southern area of the species range (Figure S2.9.1). In contrast, the most important predictor for the *N. floribunda* reference SNP data set was geographical distance, with a gradual change in allelic turnover across the geographical range (Figure 2.4H). The most important predictor for the *N. floribunda* candidate SNP data set was temperature seasonality (Table 2.2), with the largest change in allelic turnover predicted to occur above 425% (Figure 2.4B), corresponding with inland areas (Figure S2.9.1).

2.5.1 Spatial patterns of local adaptation

The spatial patterns of predicted genetic composition were different for each species and data set (Figure 2.5). Rapid turnover in genetic composition was predicted for *M. rhaphiophylla* in the eastern region of its range and comparatively little elsewhere. In contrast, 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 data sets for *M. rhaphiophylla* were strongest (i.e., Procrustes residuals > 0.7) in isolated areas along the southwestern coast with moderate differences (i.e., Procrustes residuals of 0.4–0.7) detected throughout the western coast region (Figure 2.5C). 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 2.5F).

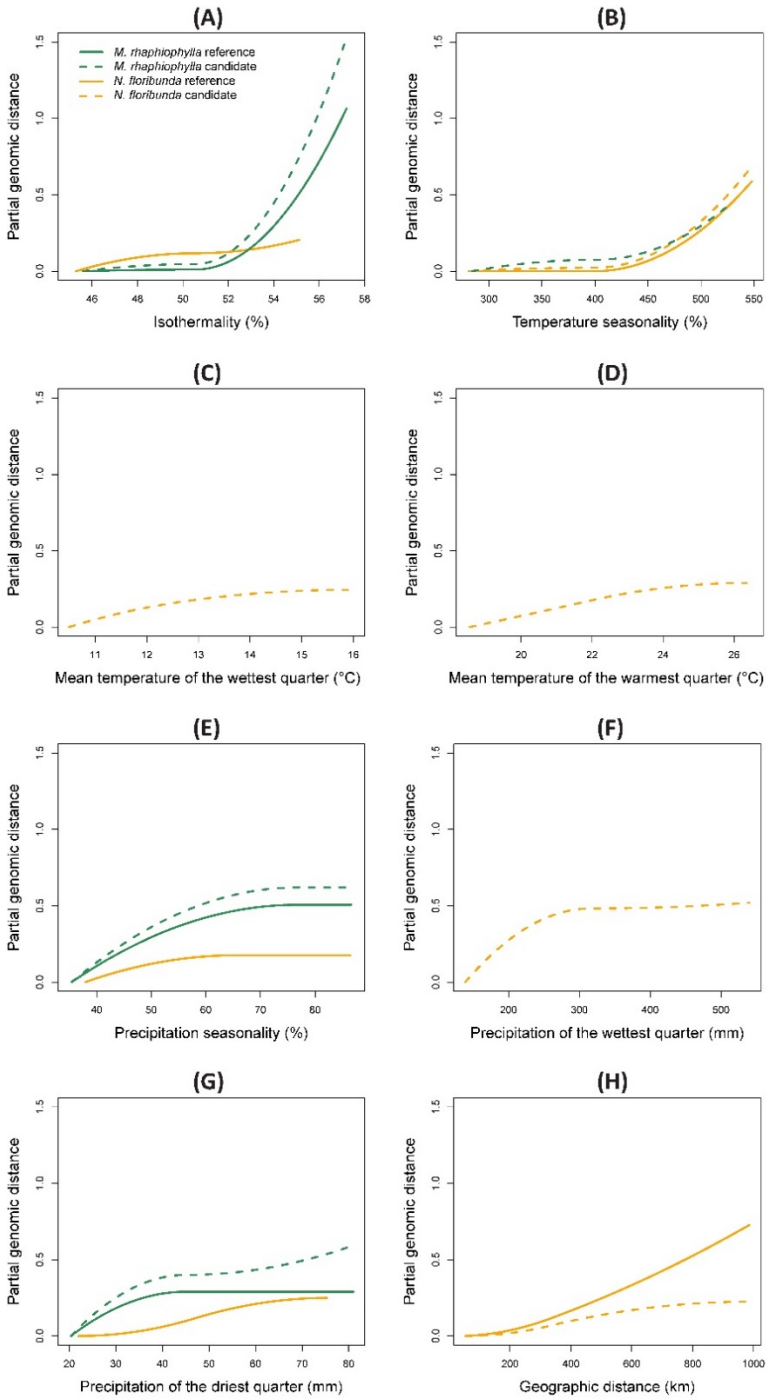


Figure 2.4 Generalized dissimilarity model-fitted I–splines showing allelic turnover across predictor variables. Reference data sets contained neutral SNPs and candidate data sets contained SNPs identified as putatively adaptive in prior genomic analyses. Allelic turnover was plotted only if the data set 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) Geographical 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.

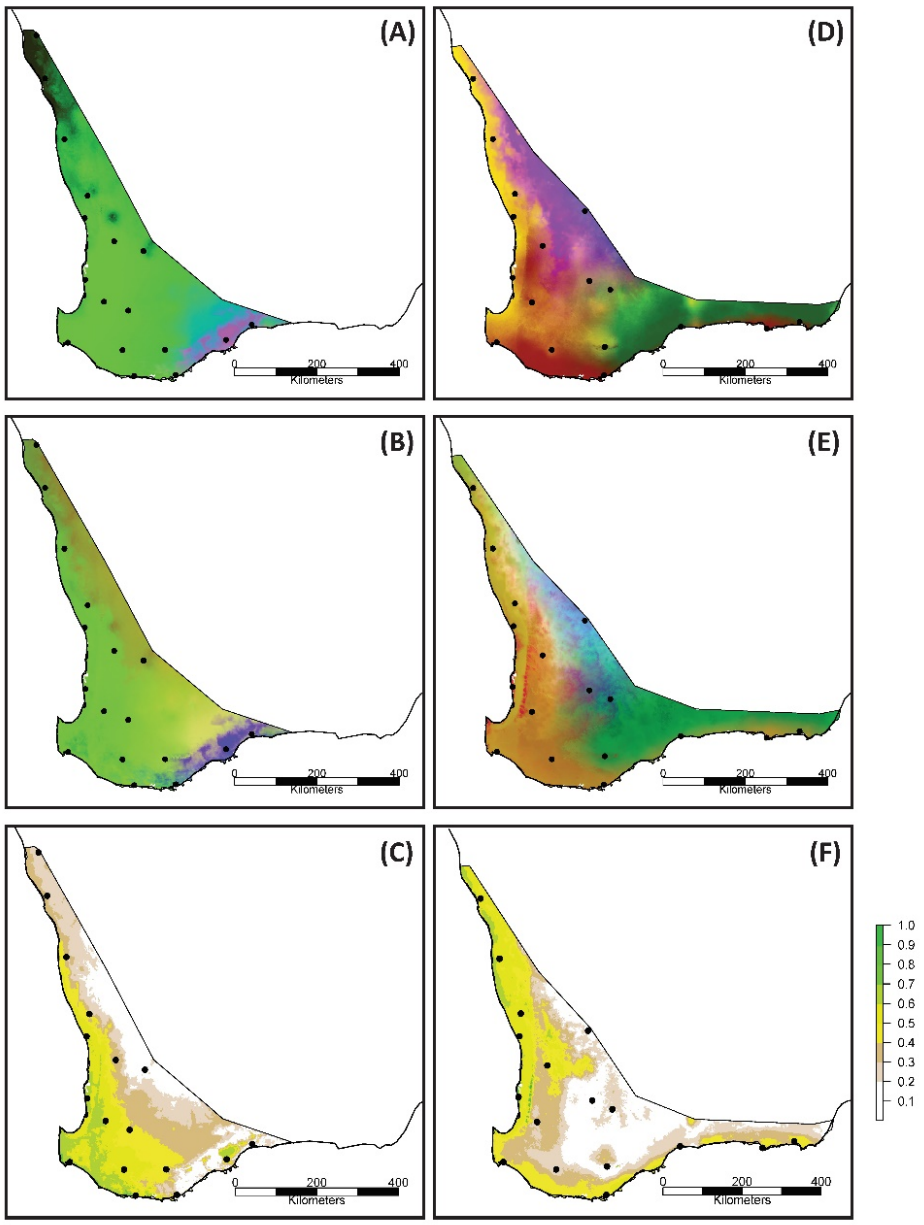


Figure 2.5 Spatial patterns of predicted genomic composition and differences in multivariate configuration (Procrustes residuals). Genomic compositions were derived using fitted generalized dissimilarity models to perform biologically–informed transformations of significant climatic variables for *Melaleuca rhaphiophylla* (A) reference and (B) candidate data sets; and *Nuytsia floribunda* (D) reference and (E) candidate data sets. 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 indicates similarity in predicted patterns of genetic composition. Differences in multivariate configuration between reference and candidate data sets were measured by Procrustes analysis for (C) *M. rhaphiophylla* and (F) *N. floribunda*. Procrustes residuals were scaled to allow direct comparisons between species.

2.6 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 *Nuytsia floribunda*, compared to the sympatric autotrophic species *Melaleuca rhaphiophylla*. Further, although 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.

2.6.1 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 the autotrophic *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.

The performance of genomic approaches vary under differential statistical frameworks (Lotterhos & Whitlock, 2015) and the limitations of each approach have been well-documented (de Villemereuil et al., 2014; Lotterhos & Whitlock, 2015; Rellstab et al., 2015; Hoban et al., 2016; Ahrens et al., 2018). Consequently, we opted to utilize 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., Andrew et al., 2018; Ahrens et al., 2019; Hopley & Byrne, 2019). Furthermore, the overall proportion of SNPs identified as putatively adaptive in this study were similar to those observed in plant (e.g., Shryock et al., 2017; Ahrens et al., 2019; Hopley & Byrne, 2019) and other landscape genomic studies (e.g., Dudaniec et al., 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., Bernatchez et al., 2016; Andrew et al., 2018). 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. Furthermore, 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 (Rellstab et al., 2015; Hoban et al., 2016). Methods using similar statistical approaches would probably 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 (Lotterhos & Whitlock, 2015; Forester et al., 2018). 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 (Whitlock & Lotterhos, 2015; Forester et al., 2018). 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, nonmodel organisms, such as those examined here, to be assessed in landscape genomics studies (Haasl & Payseur, 2016; Ahrens et al., 2018). 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. The 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 (Tiffin & Ross-Ibarra, 2014; Breed et al., 2019). 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).

2.6.2 Patterns of local climatic adaptation

Climatic variables of temperature and precipitation have previously been identified as important drivers of genetic variation in tree species (Manel et al., 2012; Poelchau & Hamrick, 2012; Gauli et al., 2015; Steane, McLean, et al., 2017; Supple et al., 2018; Ahrens et al., 2019), 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 family Myrtaceae; Supple et al., 2018), and linked to increased allelic variability in other tree species (Bradbury et al., 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 (Shryock et al., 2017; Hopley & Byrne, 2019), 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, and this variation is probably 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; Forester et al., 2018; Caye et al., 2019), whereas GDM analysis can also reveal nonlinear relationships. Nonetheless, even with the ability of GDM to detect both linear and nonlinear 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.

2.6.3 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 geographical 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 data sets by Fitzpatrick and Keller (2015) 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 utilized 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 these data. For example, regions with moderate to 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 (Hoffmann et al., 2015; Breed et al., 2019).

2.7 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., Shryock et al., 2017; Hopley & Byrne, 2019), 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*. Furthermore, 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). This study also 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).

2.8 References

Every reasonable effort has been made to acknowledge the owners of the copyright material. I would be pleased to hear from any copyright owner who has been omitted or incorrectly acknowledged.

Ahrens, C. W., Byrne, M., & Rymer, P. D. (2019). Standing genomic variation within coding and regulatory regions contributes to the adaptive capacity to climate in a foundation tree species. *Molecular Ecology, 28*, 2502-2516. https://doi.org/10.1111/mec.15092

Ahrens, C. W., Rymer, P. D., Stow, A., Bragg, J., Dillon, S., Umbers, K. D. L., & Dudaniec, R. Y. (2018). The search for loci under selection: Trends, biases and progress. *Molecular Ecology, 27*, 1342–1356. https://doi.org/10.1111/mec.14549

Aitken, S. N., Yeaman, S., Holliday, J. A., Wang, T., & Curtis-McLane, S. (2008). Adaptation, migration or extirpation: Climate change outcomes for tree populations. *Evolutionary Applications, 1*, 95-111. https://doi.org/10.1111/j.1752-4571.2007.00013.x

Allendorf, F. W., Hohenlohe, P. A., & Luikart, G. (2010). Genomics and the future of conservation genetics. *Nature Reviews Genetics, 11*, 697-709. https://doi.org/10.1038/nrg2844

Andrew, S. C., Jensen, H., Hagen, I. J., Lundregan, S., & Griffith, S. C. (2018). Signatures of genetic adaptation to extremely varied Australian environments in introduced European house sparrows. *Molecular Ecology, 27*, 4542-4555. https://doi.org/10.1111/mec.14897

Barney, C. W., Hawksworth, F. G., & Geils, B. W. (1998). Hosts of *Viscum album*. *European Journal of Forest Pathology, 28*, 187-208. https://doi.org/10.1111/j.1439-0329.1998.tb01249.x

Benestan, L. M., Ferchaud, A.-L., Hohenlohe, P. A., Garner, B. A., Naylor, G. J. P., Baums, I. B., . . . Luikart, G. (2016). Conservation genomics of natural and managed populations: building a conceptual and practical framework. *Molecular Ecology, 25*(13), 2967-2977. https://doi.org/10.1111/mec.13647

Bernatchez, S., Laporte, M., Perrier, C., Sirois, P., & Bernatchez, L. (2016). Investigating genomic and phenotypic parallelism between piscivorous and planktivorous lake trout (*Salvelinus namaycush*) ecotypes by means of RADseq and morphometrics analyses. *Molecular Ecology, 25*, 4773-4792. https://doi.org/10.1111/mec.13795

Blanquart, F., Kaltz, O., Nuismer, S. L., & Gandon, S. (2013). A practical guide to measuring local adaptation. *Ecology Letters, 16*, 1195-1205. https://doi.org/10.1111/ele.12150

Borcard, D., Legendre, P., & Drapeau, P. (1992). Partialling out the spatial component of ecological variation. *Ecology, 73*, 1045-1055. https://doi.org/10.2307/1940179

Bradbury, D., Smithson, A., & Krauss, S. L. (2013). Signatures of diversifying selection at EST-SSR loci and association with climate in natural *Eucalyptus* populations. *Molecular Ecology, 22*, 5112-5129. https://doi.org/10.1111/mec.12463

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

Breed, M. F., Harrison, P. A., Blyth, C., Byrne, M., Gaget, V., Gellie, N. J. C., . . . Mohr, J. J. (2019). The potential of genomics for restoring ecosystems and biodiversity. *Nature Reviews Genetics, 20*, 615-628. https://doi.org/10.1038/s41576-019-0152-0

Broadhurst, L. M., Lowe, A., Coates, D. J., Cunningham, S. A., McDonald, M., Vesk, P. A., & Yates, C. (2008). Seed supply for broadscale restoration: Maximizing evolutionary potential. *Evolutionary Applications, 1*, 587-597. https://doi.org/10.1111/j.1752-4571.2008.00045.x

Bromham, L., Cowman, P. F., & Lanfear, R. (2013). Parasitic plants have increased rates of molecular evolution across all three genomes. *BMC Evolutionary Biology, 13*, 126. https://doi.org/10.1186/1471-2148-13-126

Byrne, M., Coates, D. J., Forest, F., Hopper, S. D., Krauss, S. L., Sniderman, J. M. K., & Thiele, K. R. (2014). A diverse flora: Species and genetic relationships.

In H. Lambers (Ed.), *Plant life on the sandplains in southwest Australia: A global biodiversity hotspot* (pp. 81-99). Crawley, Western Australia: UWA Publishing.

Calder, D. M. (1983). Mistletoes in focus: An introduction. In M. Calder & P. Bernhardt (Eds.), *The biology of mistletoes* (pp. 1–17). Sydney: Academic Press Australia.

Calladine, A., Pate, J. S., & Dixon, K. W. (2000). Haustorial development and growth benefit to seedlings of the root hemiparasitic tree *Nuytsia floribunda* (Labill.) R.Br. in association with various hosts. *Annals of Botany, 85*, 733-740. https://doi.org/10.1006/anbo.2000.1129

Capblancq, T., Luu, K., Blum, M. G. B., & Bazin, E. (2018). Evaluation of redundancy analysis to identify signatures of local adaptation. *Molecular Ecology Resources, 18*, 1223-1233. https://doi.org/10.1111/1755-0998.12906

Cattell, R. B. (1966). The scree test for the number of factors. *Multivariate Behavioural Research, 1*, 245-276. https://doi.org/10.1207/s15327906mbr0102_10

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

Chen, H. (2018). VennDiagram: Generate high-resolution venn and euler plots. R package version 1.6.20. Retrieved from https://CRAN.R-project.org/package=VennDiagram

Davis, M. B., & Shaw, R. G. (2001). Range shifts and adaptive responses to quaternary climate change. *Science, 292*, 673-679. https://doi.org/10.1126/science.292.5517.673

De Mita, S., Thuillet, A., Gay, L., Ahmadi, N., Manel, S., Ronfort, J., & Vigouroux, Y. (2013). Detecting selection along environmental gradients: Analysis of eight methods and their effectiveness for outbreeding and selfing populations. *Molecular Ecology, 22*, 1383-1399. https://doi.org/10.1111/mec.12182

de Villemereuil, P., Frichot, É., Bazin, É., François, O., & Gaggiotti, O. E. (2014). Genome scan methods against more complex models: When and how much should we trust them? *Molecular Ecology, 23*, 2006-2019. https://doi.org/10.1111/mec.12705

Dudaniec, R. Y., Yong, C. J., Lancaster, L. T., Svensson, E. I., & Hansson, B. (2018). Signatures of local adaptation along environmental gradients in a range-expanding damselfly (*Ischnura elegans*). *Molecular Ecology, 27*, 2576-2593. https://doi.org/10.1111/mec.14709

Ehleringer, J. R., & Marshall, J. D. (1995). Water relations. In M. C. Press & J. D. Graves (Eds.), *Parasitic plants* (pp. 125-140). United Kingdom: Chapman and Hall.

ESRI. (2019). ArcGIS Desktop: Release 10.7.1. Redlands, CA: Environmental Systems Research Institute.

Ferrier, S. (2002). Mapping spatial pattern in biodiversity for regional conservation planning: Where to from here? *Systematic Biology, 51*, 331-363. https://doi.org/10.1080/10635150252899806

Ferrier, S., Manion, G., Elith, J., & Richardson, K. (2007). Using generalized dissimilarity modelling to analyse and predict patterns of beta diversity in regional biodiversity assessment. *Diversity and Distributions, 13*, 252-264. https://doi.org/10.1111/j.1472-4642.2007.00341.x

Ferrier, S., Watson, G., Pearce, J., & Drielsma, M. (2002). Extended statistical approaches to modelling spatial pattern in biodiversity in northeast New South Wales. I. Species-level modelling. *Biodiversity and Conservation, 11*, 2275-2307. https://doi.org/10.1023/A:1021302930424

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

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

Fitzpatrick, M. C., Sanders, N. J., Normand, S., Svenning, J., Ferrier, S., Gove, A. D., & Dunn, R. R. (2013). Environmental and historical imprints on beta diversity: Insights from variation in rates of species turnover along gradients. *Proceedings of the Royal Society B, 280*, 1-8. https://doi.org/10.1098/rspb.2013.1201

Flanagan, S. P., Forester, B. R., Latch, E. K., Aitken, S. N., & Hoban, S. (2018). Guidelines for planning genomic assessment and monitoring of locally adaptive variation to inform species conservation. *Evolutionary Applications, 11*, 1035-1052. https://doi.org/10.1111/eva.12569

Forester, B. R., Lasky, J. R., Wagner, H. H., & Urban, D. L. (2018). Comparing methods for detecting multilocus adaptation with multivariate genotype–environment associations. *Molecular Ecology, 27*, 2215-2233. https://doi.org/10.1111/mec.14584

François, O. (2016). Running structure-like population genetic analyses with R. Retrieved from http://membres-timc.imag.fr/Olivier.Francois/tutoRstructure.pdf

François, O., Martins, H., Caye, K., & Schoville, S. D. (2016). Controlling false discoveries in genome scans for selection. *Molecular Ecology, 25*, 454-469. https://doi.org/10.1111/mec.13513

Franks, S. J., & Hoffmann, A. A. (2012). Genetics of climate change adaptation. *Annual Review of Genetics, 46*, 185-208. https://doi.org/10.1146/annurev-genet-110711-155511

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

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

Gandon, S., & Michalakis, Y. (2002). Local adaptation, evolutionary potential and host–parasite coevolution: Interactions between migration, mutation,

population size and generation time. *Journal of Evolutionary Biology, 15*, 451-462. https://doi.org/10.1046/j.1420-9101.2002.00402.x

Gandon, S., & Van Zandt, P. A. (1998). Local adaptation and host–parasite interactions. *Trends in Ecology & Evolution, 13*, 214-216. https://doi.org/10.1016/S0169-5347(98)01358-5

Garnier-Géré, P. H., & Ades, P. K. (2001). Environmental surrogates for predicting and conserving adaptive genetic variability in tree species. *Conservation Biology, 15*, 1632-1644. https://doi.org/10.1046/j.1523-1739.2001.00180.x

Gauli, A., Vaillancourt, R. E., Bailey, T. G., Steane, D. A., & Potts, B. M. (2015). Evidence for local climate adaptation in early-life traits of Tasmanian populations of *Eucalyptus pauciflora*. *Tree Genetics & Genomes, 11*(104), 1-14. https://doi.org/10.1007/s11295-015-0930-6

Gorter, F. A., Scanlan, P. D., & Buckling, A. (2016). Adaptation to abiotic conditions drives local adaptation in bacteria and viruses coevolving in heterogeneous environments. *Biology letters, 12*, 20150879. https://doi.org/10.1098/rsbl.2015.0879

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

Greischar, M. A., & Koskella, B. (2007). A synthesis of experimental work on parasite local adaptation. *Ecology Letters, 10*, 418-434. https://doi.org/10.1111/j.1461-0248.2007.01028.x

Gruber, B., Unmack, P. J., Berry, O. F., & Georges, A. (2018). dartr: An R package to facilitate analysis of SNP data generated from reduced representation genome sequencing. *Molecular Ecology Resources, 18*, 691-699. https://doi.org/10.1111/1755-0998.12745

Haasl, R. J., & Payseur, B. A. (2016). Fifteen years of genomewide scans for selection: Trends, lessons and unaddressed genetic sources of complication. *Molecular Ecology, 25*, 5-23. https://doi.org/10.1111/mec.13339

Hijmans, R. J. (2020). raster: Geographic data analysis and modelling. R package version 3.0-12. Retrieved from https://CRAN.R-project.org/package=raster

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology, 25*, 1965-1978. https://doi.org/10.1002/joc.1276

Hoban, S., Kelley, J. L., Lotterhos, K. E., Antolin, M. F., Bradburd, G., Lowry, D. B., . . . Whitlock, M. C. (2016). Finding the genomic basis of local adaptation: Pitfalls, practical solutions, and future directions. *The American Naturalist, 188*, 379-397. https://doi.org/10.1086/688018

Hocking, P. J., & Fineran, B. A. (1983). Aspects of the nutrition of root parasitic Loranthaceae. In M. Calder & P. Bernhardt (Eds.), *The biology of mistletoes* (pp. 229-258). Australia: Academic Press.

Hoffmann, A., Griffin, P., Dillon, S., Catullo, R., Rane, R., Byrne, M., . . . Sgrò, C. (2015). A framework for incorporating evolutionary genomics into biodiversity conservation and management. *Climate Change Responses, 2*, 1-23. https://doi.org/10.1186/s40665-014-0009-x

Hopley, T., & Byrne, M. (2019). Gene flow and genetic variation explain signatures of selection across a climate gradient in two riparian species. *Genes, 10*, 579. https://doi.org/10.3390/genes10080579

Jombart, T. (2008). adegenet: A R package for the multivariate analysis of genetic markers. *Bioinformatics, 24*, 1403-1405. https://doi.org/10.1093/bioinformatics/btn129

Jombart, T., & Ahmed, I. (2011). adegenet 1.3-1: New tools for the analysis of genome-wide SNP data. *Bioinformatics, 27*, 3070-3071. https://doi.org/10.1093/bioinformatics/btr521

Kaltz, O., & Shykoff, J. A. (1998). Local adaptation in host–parasite systems. *Heredity, 81*, 361-370. https://doi.org/10.1046/j.1365-2540.1998.00435.x

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

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

Analysis in Population Genomics: Methods and Protocols (pp. 67-89). Grenoble, France: Humana Press.

Kuijt, J. (1969). *The biology of parasitic flowering plants*. Berkeley, California: University of California Press.

Larsson, J. (2019). eulerr: Area-proportional Euler and Venn diagrams with ellipses. R package version 6.0.0. Retrieved from https://cran.r-project.org/package=eulerr

Li, Y. F., Costello, J. C., Holloway, A. K., & Hahn, M. W. (2008). "Reverse ecology" and the power of population genomics. *Evolution, 62*, 2984-2994. https://doi.org/10.1111/j.1558-5646.2008.00486.x

Lotterhos, K. E., & Whitlock, M. C. (2015). The relative power of genome scans to detect local adaptation depends on sampling design and statistical method. *Molecular Ecology, 24*, 1031-1046. https://doi.org/10.1111/mec.13100

Luu, K., Bazin, E., & Blum, M. G. B. (2017). pcadapt: An R package to perform genome scans for selection based on principal component analysis. *Molecular Ecology Resources, 17*, 67-77. https://doi.org/10.1111/1755-0998.12592

Manel, S., Gugerli, F., Thuiller, W., Alvarez, N., Legendre, P., Holderegger, R., . . . Taberlet, P. (2012). Broad-scale adaptive genetic variation in alpine plants is driven by temperature and precipitation. *Molecular Ecology, 21*, 3729-3738. https://doi.org/10.1111/j.1365-294X.2012.05656.x

Manion, G., Lisk, M., Ferrier, S., Nieto-Lugilde, D., Mokany, K., & Fitzpatrick, M. C. (2018). gdm: Generalized dissimilarity modelling. R package version 1.3.11. Retrieved from https://CRAN.R-project.org/package=gdm

Nadeau, S. (2014). *Genetic population structure and adaptation to climate across the range of eastern white pine (Pinus strobus L.) and western white pine (Pinus monticola Douglas ex. D. Don).* (MSc Thesis). The University of British Columbia, Vancouver.

Norton, D. A., & Carpenter, M. A. (1998). Mistletoes as parasites: Host specificity and speciation. *Trends in Ecology & Evolution, 13*, 101-105. https://doi.org/10.1016/S0169-5347(97)01243-3

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., . . . Wagner, H. (2019). vegan: Community ecology package. Retrieved from https://CRAN.R-project.org/package=vegan

Pate, J. S. (1995). Mineral relationships of parasites and their hosts. In M. C. Press & J. D. Graves (Eds.), *Parasitic plants* (pp. 80-102). London: Chapman and Hall.

Peres-Neto, P. R., & Jackson, D. A. (2001). How well do multivariate data sets match? The advantages of a Procrustean superimposition approach over the Mantel test. *Oecologia, 129*, 169-178. https://doi.org/10.1007/s004420100720

Poelchau, M. F., & Hamrick, J. L. (2012). Differential effects of landscape-level environmental features on genetic structure in three codistributed tree species in Central America. *Molecular Ecology, 21*, 4970-4982. https://doi.org/10.1111/j.1365-294X.2012.05755.x

Press, M. C., & Phoenix, G. K. (2005). Impacts of parasitic plants on natural communities. *New Phytologist, 166*, 737-751. https://doi.org/10.1111/j.1469-8137.2005.01358.x

Prober, S. M., Byrne, M., McLean, E. H., Steane, D. A., Potts, B. M., Vaillancourt, R. E., & Stock, W. D. (2015). Climate-adjusted provenancing: A strategy for climate-resilient ecological restoration. *Frontiers in Ecology and Evolution, 3*(65), 1-5. https://doi.org/10.3389/fevo.2015.00065

R Core Team. (2019). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/

Rellstab, C., Gugerli, F., Eckert, A. J., Hancock, A. M., & Holderegger, R. (2015). A practical guide to environmental association analysis in landscape genomics. *Molecular Ecology, 24*, 4348-4370. https://doi.org/10.1111/mec.13322

Richardson, J. L., Brady, S. P., Wang, I. J., & Spear, S. F. (2016). Navigating the pitfalls and promise of landscape genetics. *Molecular Ecology, 25*, 849-863. https://doi.org/10.1111/mec.13527

Sansaloni, C., Petroli, C., Jaccoud, D., Carling, J., Detering, F., Grattapaglia, D., & Kilian, A. (2011). Diversity Arrays Technology (DArT) and next-generation sequencing combined: Genome-wide, high throughput, highly informative genotyping for molecular breeding of *Eucalyptus*. *BMC Proceedings, 5*, P54. https://doi.org/10.1186/1753-6561-5-S7-P54

Savolainen, O., Lascoux, M., & Merilä, J. (2013). Ecological genomics of local adaptation. *Nature Reviews Genetics, 14*, 807-820. https://doi.org/10.1038/nrg3522

Shryock, D. F., Havrilla, C. A., DeFalco, L. A., Esque, T. C., Custer, N. A., & Wood, T. E. (2015). Landscape genomics of *Sphaeralcea ambigua* in the Mojave Desert: A multivariate, spatially-explicit approach to guide ecological restoration. *Conservation Genetics, 16*, 1303-1317. https://doi.org/10.1007/s10592-015-0741-1

Shryock, D. F., Havrilla, C. A., DeFalco, L. A., Esque, T. C., Custer, N. A., & Wood, T. E. (2017). Landscape genetic approaches to guide native plant restoration in the Mojave Desert. *Ecological Applications, 27*, 429-445. https://doi.org/10.1002/eap.1447

Stapley, J., Reger, J., Feulner, P. G. D., Smadja, C., Galindo, J., Ekblom, R., . . . Slate, J. (2010). Adaptation genomics: The next generation. *Trends in Ecology & Evolution, 25*, 705-712. https://doi.org/10.1016/j.tree.2010.09.002

Steane, D. A., McLean, E. H., Potts, B. M., Prober, S. M., Stock, W. D., Stylianou, V. M., . . . Byrne, M. (2017). Evidence for adaptation and acclimation in a widespread eucalypt of semi-arid Australia. *Biological Journal of the Linnean Society, 121*, 484-500. https://doi.org/10.1093/biolinnean/blw051

Steane, D. A., Potts, B. M., McLean, E. H., Collins, L., Holland, B. R., Prober, S. M., . . . Byrne, M. (2017). Genomic scans across three eucalypts suggest that adaptation to aridity is a genome-wide phenomenon. *Genome Biology and Evolution, 9*, 253-265. https://doi.org/10.1093/gbe/evw290

Storey, J. D., Bass, A. J., Dabney, A., & Robinson, D. (2019). qvalue: Q-value estimation for false discovery rate control. R package version 2.18.0. Retrieved from http://github.com/jdstorey/qvalue

Storfer, A., Patton, A., & Fraik, A. K. (2018). Navigating the interface between landscape genetics and landscape genomics. *Frontiers in Genetics, 9*(68), 1-14. https://doi.org/10.3389/fgene.2018.00068

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

Tiffin, P., & Ross-Ibarra, J. (2014). Advances and limits of using population genetics to understand local adaptation. *Trends in Ecology & Evolution, 29*, 673-680. https://doi.org/10.1016/j.tree.2014.10.004

Vavrek, M. J. (2011). fossil: Palaeoecological and palaeogeographical analysis tools. Retrieved from https://palaeo-electronica.org/2011_1/238/index.html

Walters, S. J., Robinson, T. P., Byrne, M., Wardell-Johnson, G. W., & Nevill, P. (2020). Dataset for: Contrasting patterns of local adaptation along climatic gradients between a sympatric parasitic and autotrophic tree species. *DRYAD*. https://doi.org/10.5061/dryad.0p2ngf1xn

Wang, I. J., & Bradburd, G. S. (2014). Isolation by environment. *Molecular Ecology, 23*, 5649-5662. https://doi.org/10.1111/mec.12938

Watson, D. M. (2001). Mistletoe–a keystone resource in forests and woodlands worldwide. *Annual Review of Ecology and Systematics, 32*, 219-249. https://doi.org/10.1146/annurev.ecolsys.32.081501.114024

Weir, B. S., & Cockerham, C. C. (1984). Estimating *F*-statistics for the analysis of population structure. *Evolution, 38*, 1358-1370. https://doi.org/10.1111/j.1558-5646.1984.tb05657.x

Weissman, D. B., & Barton, N. H. (2012). Limits to the rate of adaptive substitution in sexual populations. *PLOS Genetics, 8*, e1002740. https://doi.org/10.1371/journal.pgen.1002740

Whitlock, M. C., & Lotterhos, K. E. (2015). Reliable detection of loci responsible for local adaptation: Inference of a null model through trimming the distribution of F_{ST}. *The American Naturalist, 186*, S24-S36. https://doi.org/10.1086/682949

Wickham, H. (2016). ggplot2: Elegant graphics for data analysis. New York: Springer-Verlag. Retrieved from https://github.com/tidyverse/ggplot2

Woodall, G. S., & Robinson, C. J. (2003). Natural diversity of *Santalum spicatum* host species in south-coast river systems and their incorporation into profitable and biodiverse revegetation. *Australian Journal of Botany, 51*, 741-753. https://doi.org/10.1071/BT02118

Yates, C. J., Robinson, T., Wardell-Johnson, G. W., Keppel, G., Hopper, S. D., Schut, A. G. T., & Byrne, M. (2019). High species diversity and turnover in granite inselberg floras highlight the need for a conservation strategy protecting many outcrops. *Ecology and Evolution, 9*, 7660-7675. https://doi.org/10.1002/ece3.5318

Yates, F. (1934). Contingency tables involving small numbers and the χ^2 Test. *Supplement to the Journal of the Royal Statistical Society, 1*, 217-235. https://doi.org/10.2307/2983604

2.9 Supplementary information

Table S2.9.1 Coordinates and climatic data for *Melaleuca rhaphiophylla* populations sampled across south-western Australia. Latitude and longitude are presented in the Geocentric Datum of Australia (GDA94) coordinate system. Mean ± *SD* are provided for seven least-correlated bioclimatic variables used in environmental association analysis and generalized dissimilarity modelling.

Code	Population	Latitude	Longitude	No.	IT (%)	TS (%)	MTWQ (°C)	MTHQ (°C)	PS (%)	PWQ (mm)	PDQ (mm)
BEE	Beekeepers	-29.858401	115.166946	16	49.8 ± 0.11	470 ± 1.92	15.9 ± 0.01	26.4 ± 0.04	83.6 ± 0.11	280 ± 0.51	30.0 ± 0.00
BRO	Brookton	-32.292704	117.181293	16	48.2 ± 0.09	530 ± 0.46	11.2 ± 0.05	23.9 ± 0.03	68.5 ± 0.05	192 ± 2.00	33.3 ± 1.00
COL	Collie	-33.392608	116.174289	16	50.7 ± 0.11	434 ± 1.88	10.8 ± 0.07	21.3 ± 0.03	76.6 ± 0.56	430 ± 12.3	52.6 ± 0.51
FI2	Fitzgerald River 2	-34.219893	119.282186	16	57.2 ± 0.06	332 ± 2.10	13.2 ± 0.01	20.4 ± 0.05	36.5 ± 0.05	191 ± 0.00	73.0 ± 0.00
FIT	Fitzgerald River	-33.889790	119.936441	16	55.1 ± 0.00	295 ± 0.00	13.5 ± 0.00	19.9 ± 0.00	35.4 ± 0.00	171 ± 0.00	68.0 ± 0.00
FRA	Frankland	-35.003319	116.944696	16	52.2 ± 0.00	285 ± 0.00	12.7 ± 0.00	19.6 ± 0.00	61.0 ± 0.00	478 ± 0.00	81.0 ± 0.00
GUL	Gull Rock	-34.984665	118.007202	16	53.5 ± 0.05	284 ± 3.37	12.4 ± 0.08	19.2 ± 0.01	53.5 ± 0.28	348 ± 0.96	75.7 ± 0.48
HOW	Howatharra	-28.539697	114.673371	16	48.6 ± 0.02	440 ± 0.65	14.6 ± 0.01	25.3 ± 0.00	86.6 ± 0.04	260 ± 0.00	22.0 ± 0.00
KAL	Kalbarri	-27.602539	114.452794	16	50.5 ± 0.10	488 ± 6.26	16.4± 0.27	27.1 ± 0.44	81.2 ± 0.64	189 ± 2.87	20.4 ± 0.50
MOO	Moore River	-31.088800	115.758129	16	50.7 ± 0.01	467 ± 0.63	13.4 ± 0.01	24.8 ± 0.00	84.1 ± 0.13	323 ± 0.50	29.0 ± 0.00
MUI	Lake Muir	-34.442654	116.647456	16	52.7 ± 0.11	356 ± 0.98	10.9 ± 0.02	19.5 ± 0.01	66.1 ± 0.10	352 ± 3.07	51.9 ± 0.34
MUN	Mundaring	-32.081164	116.433993	16	49.6 ± 0.00	487 ± 0.00	10.9 ± 0.00	22.7 ± 0.00	77.4 ± 0.00	360 ± 0.00	45.0 ± 0.00
SCO	Scott	-34.281607	115.258321	16	50.3 ± 0.64	297 ± 6.37	14.3 ± 0.11	20.7 ± 0.09	78.6 ± 0.23	544 ± 3.28	53.6 ± 0.73
STI	Stirling Range	-34.436082	117.729739	16	51.8 ± 0.00	401 ± 0.00	11.9 ± 0.00	20.5 ± 0.00	49.6 ± 0.00	186 ± 0.00	50.0 ± 0.00
TOW	Towerrining	-33.582913	116.792366	16	49.8 ± 0.02	467 ± 0.50	10.8 ± 0.00	22.0 ± 0.02	64.1 ± 0.18	233 ± 0.00	44.4 ± 0.50
YAL	Yalgorup	-32.910649	115.699491	16	45.6 ± 0.27	379 ± 2.50	14.7 ± 0.05	22.8 ± 0.06	84.8 ± 0.05	490 ± 6.21	44.4 ± 0.51
YAN	Yanchep	-31.573307	115.686179	16	49.3 ± 0.00	396 ± 0.00	14.1 ± 0.00	23.7 ± 0.00	83.0 ± 0.00	393 ± 0.00	39.0 ± 0.00
TOTAL					50.9 ± 2.65	401 ± 78.5	13.0 ± 1.75	22.3 ± 2.45	68.9 ± 16.2	319 ± 116	47.8 ± 17.9

Abbreviations: IT: isothermality, BIO3; MTHQ: mean temperature of the warmest quarter, BIO10; MTWQ: mean temperature of the wettest quarter, BIO8; PDQ: precipitation of the driest quarter, BIO17; PS: precipitation seasonality, BIO15; PWQ: precipitation of the wettest quarter, BIO16; TS: temperature seasonality, BIO4.

Table S2.9.2 Coordinates and climatic data for *Nuytsia floribunda* populations sampled across south-western Australia. Latitude and longitude are presented in the Geocentric Datum of Australia (GDA94) coordinate system. Mean ± *SD* are provided for seven least-correlated bioclimatic variables used in environmental association analysis and generalized dissimilarity modelling.

Code	Population	Latitude	Longitude	No.	IT (%)	TS (%)	MTWQ (°C)	MTHQ (°C)	PS (%)	PWQ (mm)	PDQ (mm)
ARI	Cape Arid	-33.827230	122.976605	16	54.8 ± 0.07	325 ± 2.83	12.3 ± 0.03	20.3 ± 0.04	43.2 ± 0.07	220 ± 0.81	72.8 ± 0.45
ART	Arthur River	-32.939991	117.625571	14	46.7 ± 0.19	487 ± 0.15	10.7 ± 0.05	22.4 ± 0.05	53.1 ± 0.15	162 ± 0.51	44.0 ± 0.00
BEE	Beekeepers	-29.855458	115.167284	16	49.8 ± 0.12	469 ± 1.40	15.9 ± 0.01	26.3 ± 0.03	83.5 ± 0.08	279 ± 0.48	30.0 ± 0.00
COL	Collie	-33.407033	116.162747	16	50.5 ± 0.07	434 ± 2.22	10.7 ± 0.05	21.1 ± 0.10	75.5 ± 0.54	414 ± 7.60	53.0 ± 0.00
FIT	Fitzgerald River	-33.934041	119.952421	15	55.1 ± 0.37	282 ± 2.09	13.8 ± 0.06	20.0 ± 0.08	37.9 ± 0.57	181 ± 2.06	66.8 ± 0.56
GRA	Cape le Grand	-33.969691	122.135667	16	55.0 ± 0.10	299 ± 0.54	12.7 ± 0.02	20.0 ± 0.00	56.5 ± 0.08	316 ± 0.25	68.9 ± 0.25
GUL	Gull Rock	-34.991241	117.997172	16	53.5 ± 0.26	281 ± 2.61	12.4 ± 0.07	19.2 ± 0.05	53.7 ± 0.26	349 ± 1.13	75.3 ± 0.48
HOW	Howatharra	-28.544409	114.666134	16	48.6 ± 0.03	437 ± 2.48	14.6 ± 0.03	25.3 ± 0.02	86.5 ± 0.12	260 ± 0.40	22.0 ± 0.00
MOO	Moore River	-31.049457	115.730544	16	50.6 ± 0.11	458 ± 6.17	13.4 ± 0.02	24.6 ± 0.13	84.0 ± 0.17	326 ± 1.28	30.6 ± 0.81
MUI	Lake Muir	-34.438038	116.668582	16	52.6 ± 0.05	359 ± 0.47	10.9 ± 0.01	19.6 ± 0.02	65.7 ± 0.17	339 ± 2.23	51.0 ± 0.00
MUN	Mundaring	-32.178490	116.434698	16	49.8 ± 0.00	495 ± 0.00	10.7 ± 0.00	22.6 ± 0.00	77.5 ± 0.00	369 ± 0.00	45.0 ± 0.00
SCO	Scott	-34.273679	115.267326	16	51.1 ± 0.42	305 ± 4.35	14.1 ± 0.01	20.8 ± 0.10	78.6 ± 0.37	540 ± 2.31	52.7 ± 0.48
STI	Stirling Range	-34.373122	118.017002	16	53.3 ± 0.41	338 ± 3.21	11.4 ± 0.12	18.6 ± 0.17	46.0 ± 0.12	169 ± 4.44	50.6 ± 1.93
TAR	Tarin Rock	-33.129954	118.156419	12	46.4 ± 0.13	475 ± 2.71	10.5 ± 0.02	21.9 ± 0.05	47.4 ± 0.68	146 ± 2.46	47.0 ± 1.48
YAL	Yalgorup	-32.867193	115.671697	16	45.3 ± 0.36	375 ± 2.16	14.8 ± 0.07	22.8 ± 0.01	84.8 ± 0.21	491 ± 3.33	45.0 ± 0.00
YAN	Yanchep	-31.543548	115.696037	15	49.1 ± 0.15	399 ± 1.97	13.9 ± 0.09	23.6 ± 0.04	83.1 ± 0.22	400 ± 3.42	39.4 ± 0.83
YOR	Yorkrakine Rock	-31.422851	117.512310	16	45.5 ± 0.00	548 ± 0.00	12.9 ± 0.00	25.0 ± 0.00	51.2 ± 0.00	139 ± 0.00	35.0 ± 0.00
TOTAL				264	50.5 ± 3.17	397 ± 82.0	12.7 ± 1.64	22.0 ± 2.30	65.6 ± 16.7	303 ± 117	48.8 ± 15.1

Abbreviations: IT: isothermality, BIO3; MTHQ: mean temperature of the warmest quarter, BIO10; MTWQ: mean temperature of the wettest quarter, BIO8; PDQ: precipitation of the driest quarter, BIO17; PS: precipitation seasonality, BIO15; PWQ: precipitation of the wettest quarter, BIO16; TS: temperature seasonality, BIO4.

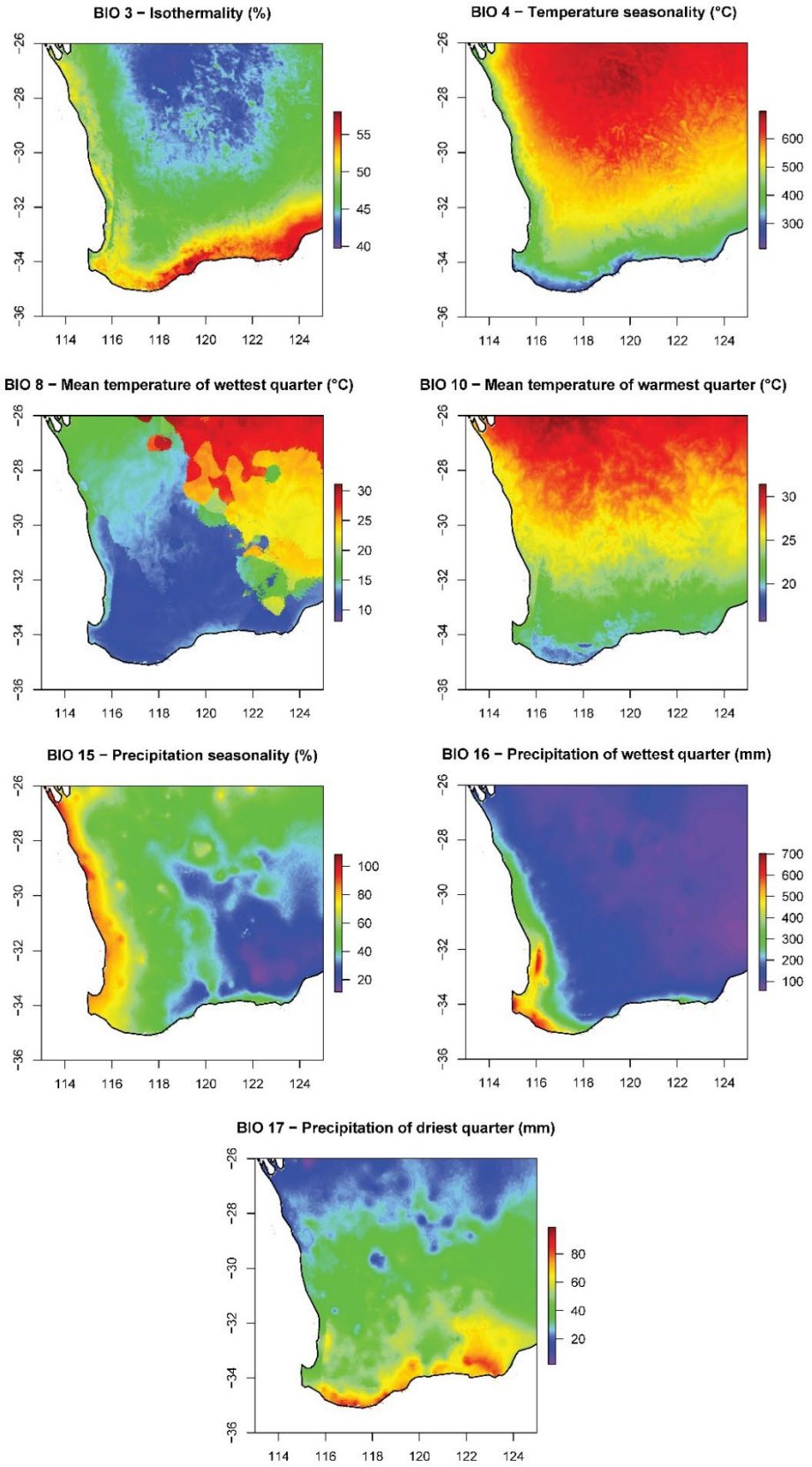


Figure S2.9.1 Climate maps of the seven uncorrelated climatic variables in south-western Australia.

CHAPTER 3

ASSOCIATION OF ADAPTIVE GENETIC VARIATION WITH CLIMATIC VARIABLES DIFFERS BETWEEN A HOST-SPECIFIC MISTLETOE AND ITS HOST

3.1 Preface

*This chapter consists of an accepted manuscript titled '**Association of adaptive genetic variation with climatic variables differs between a host-specific mistletoe and its host**' [Evolutionary Applications 2021]. The content from section 3.2 onwards is the same as the accepted manuscript with only minor changes in formatting of references and lettering in figures to ensure consistency within the thesis. Permissions to include this work in this thesis are included in Appendix I.*

The previous chapter (Chapter 2) compared adaptive genetic variation between a generalist parasite and co-occurring autotrophic species, revealing differing patterns of climate adaptation. The previous chapter also revealed adaptive genetic variation in the parasite to have greater association with temperature variables, and discussed potential explanations based on the parasitic life history (e.g., influence of temperature on higher transpiration rate). This current data chapter (Chapter 3) also presents a comparative genetics study examining adaptive genetic variation in two co-occurring species — except this time between a host-specific parasite and its host species. The study presented in this chapter makes a significant contribution to this thesis as it demonstrates that differing patterns of climate adaptation are also found between a host-specific parasite and its host, which has not been studied prior. This work builds on the findings from the previous chapter, and suggests that differences in patterns of adaptive genetic variation are not limited to a single parasitic taxa. Additionally, this chapter also found adaptive genetic variation in the host-specific parasite to be associated with temperature variables, supporting the finding in the previous chapter and indicating that temperature may be an important selective pressure for parasitic plants more broadly. This work leads to the subsequent chapter (Chapter 4) that compares patterns of neutral genetic variation and phylogeographic history between the two parasite-autotroph species pairs.

3.1.1 Acknowledgements

This work was supported by the Australian Government through an Australian Government Research Training Program Scholarship and the Australian Research Council Industrial Transformation Training Centre for Mine Site Restoration (project number ICI150100041). We thank Bronwyn Macdonald for assistance with laboratory work and Tara Hopley for assistance running statistical analyses. Data analysis was

supported with resources provided by the Pawsey Supercomputing Centre with funding from the Australian Government and the Government of Western Australia.

3.1.2 Data accessibility

The data that support the findings of this study will be made openly available in the DRYAD Digital Repository prior to publication of this manuscript.

3.1.3 Author contributions

SJW conducted the study and wrote the manuscript. All authors conceived the ideas and designed the study. Samples were collected and processed by SJW. Data analysis was performed by SJW with input from PN, TPR and MB. The manuscript was edited and approved by all authors.

Walters, S. J., T. P. Robinson, M. Byrne, G. W. Wardell-Johnson, and P. Nevill. (In press). Association of adaptive genetic variation with climatic variables differs between a host-specific mistletoe and its host. *Evolutionary Applications.*

3.2 Abstract

Parasitism is a pervasive phenomenon in nature with the relationship between species driving evolution in both the parasite and host. Due to their host-dependent lifestyle, parasites may adapt to the abiotic environment in ways that differ from their hosts or from free living relatives; yet rarely has this been assessed. Here, we test two competing hypotheses related to whether adaptive genetic variation in a host-specific mistletoe associates with the same, or different, climatic variables as its host species. We sampled 11 populations of the host-specific mistletoe *Amyema gibberula* var. *tatei* (*n* = 154) and 10 populations of its associated host *Hakea recurva* subsp. *recurva* (*n* = 160). Reduced-representation sequencing was used to obtain genome-wide markers and adaptive genetic variation detected using genome scan methods. Climate associations were identified using generalised dissimilarity modelling and these were mapped geographically to visualise the spatial patterns of genetic composition. Our results supported our hypothesis of parasites and host species responding differently to climatic variables. Temperature was relatively more important in predicting allelic turnover in the host-specific mistletoe while precipitation was more important for the host. This suggests that parasitic plants and host species may respond differently to selective pressures, potentially as a result of differing nutrient acquisition strategies. Specifically, mistletoes acquire water from hosts (rather than the abiotic environment), which may provide a buffer to precipitation as a selective pressure. Applying these methods to a comparative study in a host-parasite system has also highlighted factors that affect the study of selection pressure on nonmodel organisms, such as differing adaptation rates and lack of reference genomes.

3.3 Introduction

Parasitism is ubiquitous across the tree of life (Musselman & Press, 1995; Poulin & Morand, 2000; Poulin, 2011) with host-parasite interactions driving evolution in both partners. These interactions are typically investigated with respect to coevolution (e.g., Laine, 2008; Lopez Pascua et al., 2012), and due to their host dependant lifestyle, parasites may adapt to the abiotic environment in similar ways to their hosts (e.g., Gorter et al., 2016). This makes host-parasite systems an interesting model for studying local adaptation (Kaltz & Shykoff, 1998; Kawecki & Ebert, 2004).

Previous studies in host-parasite systems have shown that local adaptation can be influenced by heterogeneity of the abiotic environment (Thompson, 2005; Nuismer & Gandon, 2008; Wolinska & King, 2009), with evidence for the influence of temperature and nutrient levels emerging across plant-pathogens (Laine, 2007, 2008; Mboup et al., 2012) and other parasitic microorganisms (Mitchell et al., 2005; Lopez Pascua et al., 2012; Gorter et al., 2016). Despite an increasing number of local adaptation studies integrating the abiotic environment within laboratory experiments, few studies have examined the association of adaptation with climatic gradients in field-based settings, which could provide a crucial understanding of adaptation patterns in natural populations (Thompson, 2005). Furthermore, while adaptation studies across a range of taxonomic groups have recently benefitted from genome-wide markers (reviewed in Ahrens et al., 2018), these approaches have rarely been applied to host-parasite systems (but see Hartmann et al., 2019; Walters et al., 2020 for recent examples).

Mistletoes are aerial parasites that acquire water and mineral nutrients via a specialised haustorium structure, unique to parasitic plants (Kuijt, 1969; Musselman & Press, 1995). Mistletoes are important for the functioning of ecosystems worldwide (Press & Graves, 1995; Watson, 2001; Press & Phoenix, 2005), providing food resources for fauna (Press & Phoenix, 2005) and increasing nutrient cycling within plant communities (March & Watson, 2010). Mistletoe-host systems provide a useful model for local adaptation studies as the parasite is sessile, vector-dispersed and entirely reliant on a single individual host for survival and persistence (Calder, 1983; Press & Graves, 1995). Climatic factors are known to influence the distribution, survival (e.g., de Buen & Ornelas, 2002; Scalon & Wright, 2015) and genetic variation in mistletoes (e.g., Ramírez-Barahona et al., 2017). For example, a previous study found that patterns of genetic variation in a mistletoe varied along precipitation and seasonality gradients (Ramírez-Barahona et al., 2017), which are also important drivers of local adaptation in autotropic plants (Shryock et al., 2017; Steane et al., 2017). However, few studies have examined whether signatures of selection in parasitic plants mimic those of their hosts.

A parasitic lifestyle may enable mistletoes to adapt to climatic gradients in different ways from their host species. Mistletoes are susceptible to xylem cavitation when water potentials drop too low (Ehleringer & Marshall, 1995) and they must have higher

transpiration rates than hosts to maintain a positive water gradient (Ehleringer et al., 1985; Stewart & Press, 1990). Therefore, climatic factors that affect transpiration rates (i.e., temperature) may provide a stronger selection pressure for mistletoes than that experienced by their hosts. Consequently, mistletoes have their own ecological or climatic niche requirements and will only occur where suitable abiotic conditions overlap with appropriate host species (e.g., Lira-Noriega & Peterson, 2014; Ramírez-Barahona et al., 2017).

A recent study found associations between genome-wide variation and climatic variables to be different between a parasitic plant and a sympatric autotrophic species (Walters et al., 2020). However, the parasite examined (*Nuytsia floribunda*) was a generalist root parasite that can utilise different host species simultaneously, and develop haustorium with additional hosts when water is limiting (Hocking & Fineran, 1983). In contrast, host-specific mistletoes have few host species and a life-long association with a single host (Norton & Carpenter, 1998). Therefore, patterns of genome-wide variation could more closely resemble that of the host.

We sought to test these two competing hypotheses in our study of a mistletoe and its specific host species. Our null hypothesis was that adaptive genetic variation in the host-specific mistletoe would associate with the same climatic variables as the host species. Due to the semi-arid climate of the study landscape, precipitation may be relatively more important than temperature in predicting allelic turnover for both species. Our alternative hypothesis was that adaptive genetic variation in the host-specific mistletoe would associate with different climatic variables than the host species. Specifically, as mistletoes rely on higher transpiration rates to create a positive water gradient with the host (Ehleringer et al., 1985; Stewart & Press, 1990), temperature may be relatively more important than precipitation in predicting allelic turnover for the mistletoe.

Here, we applied a genotyping-by-sequencing approach to test these hypotheses by examining the patterns of genome-wide variation between a mistletoe *Amyema gibberula* var. *tatei* (Blakely) Barlow (family Loranthaceae) and its host species, *Hakea recurva* Meisn. subsp. *recurva* (family Proteaceae). We aimed to develop our understanding of the association of adaptive genetic variation along climatic gradients between parasites and their associated host species. Specifically, we examined: (a) the

association of adaptive genetic variation with climatic variables and the relative importance of temperature, precipitation and geographical distance with allelic turnover; and (b) the predicted spatial pattern of adaptive genetic variation.

3.4 Materials and methods

3.4.1 Study species and sample collection

The host-specific mistletoe *Amyema gibberula* var. *tatei* and its host *Hakea recurva* subsp. *recurva* have a widespread distribution in the mid-west and wheatbelt regions of Western Australia, spanning ~500 km north-south and ~300 km east-west (Figure 3.1). Temperature and precipitation vary across the species' distribution with a semi-arid climate in the north-east and dry Mediterranean climate in the south-west. The host occurs as a tree or shrub to 6 m in height, is pollinated by insects and has gravity/wind dispersed seed. In contrast, the mistletoe is a host-specific hemiparasitic aerial shrub that occurs only on *Hakea* species, and almost entirely on *H. recurva subsp. recurva* (Start, 2015). Like other *Amyema* species in Australia, flowers are bird-pollinated and the fleshy fruit are dispersed by the mistletoe bird, *Dicaeum hirundinaceum* (Liddy, 1983).

Sampling locations were distributed throughout the entire range of the mistletoe to capture the full geographical and climatic space in terms of precipitation and temperature that the species occupies in south-western Australia. To allow comparison between species, host populations were sampled to cover a similar geographical and climatic range. Host populations were generally found on granite outcrops, rocky ridges and rocky sandstone slopes while the distribution of mistletoe populations was dependent on the availability of host plants. Leaf tissue was collected from 160 host and 154 mistletoe plants with 10 – 16 individuals per species sequenced from 10 and 11 populations, respectively (Table S3.8.1and Table S3.8.2). Only one mistletoe was collected from each host plant and we aimed for a minimum sampling distance of 20 m between individuals to avoid sampling related individuals, with this achieved for 93% of host and 85% of mistletoe individuals. Host plants and attached mistletoes were sampled together when possible, with approximately 61% of host individuals collected with a respective mistletoe. Samples were stored on silica gel and the location of each individual sampled was recorded using a GARMIN eTrex 10 GPS.

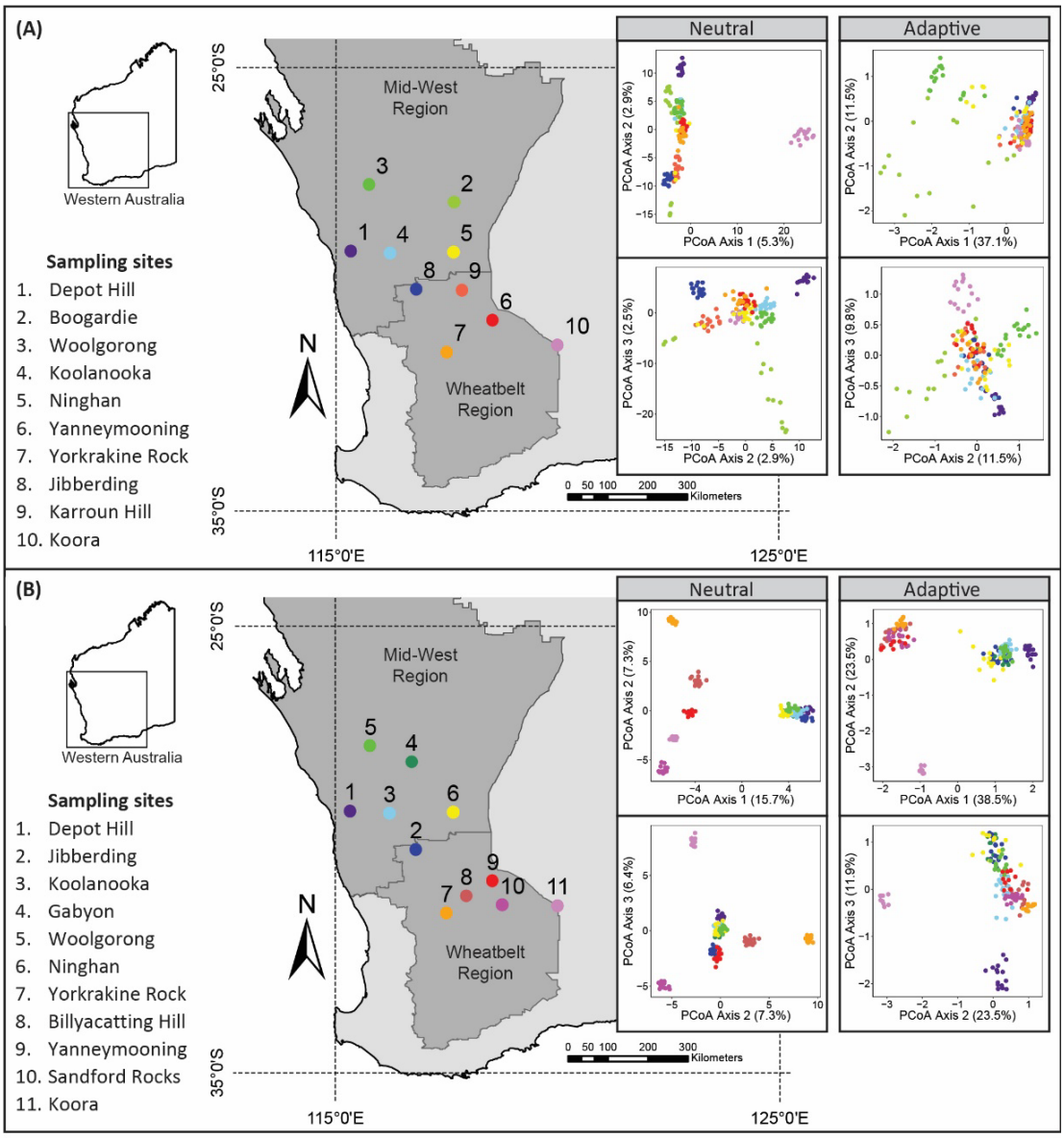


Figure 3.1 Sampling sites and principal coordinate analysis (PCoA) of neutral and adaptive genetic variation for (A) *Hakea recurva* subsp. *recurva* and (B) *Amyema gibberula* var. *tatei*. Neutral datasets (14,848 and 1,631 SNPs for each species, respectively) contained SNPs not identified as outliers, or with significant environment association, in any genome scan method. Adaptive datasets (35 and 36 SNPs for each species, respectively) contained SNPs identified by two or more genome scan methods. Maps show the geographical location of sample sites. Samples within the PCoAs are colour-coded by site and the percentage on each axis indicates how much genome-wide variation between individuals was explained by the axis.

3.4.2 Climatic data assemblage

Climatic data for fifteen variables covering annual and quarterly temperature averages, annual and quarterly precipitation totals, and seasonality, was downloaded in raster format at 1 km cell resolution from the Worldclim2.0 database (Hijmans et al., 2005; Fick & Hijmans, 2017). Point information was extracted using the coordinates of each sampled individual using the Spatial Analyst toolbox in ARCMAP version 10.7.1 (ESRI,

2019). Variables were split into temperature and precipitation groups, and we used Spearman rank correlation tests in the R STATS package (R Core Team, 2019) to assess pairwise collinearity between variables within each group. To minimise inclusion of highly correlated factors (Rellstab et al., 2015), we selected variables that had within-group pairwise correlations of $|r| < 0.8$ and that varied across the study area. This dataset comprised four temperature variables (isothermality, temperature seasonality, mean temperature of the wettest quarter and mean temperature of the driest quarter) and three precipitation variables (annual precipitation, precipitation seasonality and precipitation of the warmest quarter). Variables in the final dataset were plotted in the R package RASTER version 3.0-12 (Figure S3.8.1; Hijmans, 2020) and population-level means are provided in Table S3.8.1and Table S3.8.2.

3.4.3 SNP generation and bioinformatics

DNA for each species was extracted from ~40 mg of silica-dried leaf tissue using the CTAB method of Doyle and Doyle (1990) with the addition of 2% PVP (polyvinylpyrrolodine) and 0.2% β-mercaptoethanol to the extraction buffer. DNA was visualised on a 1% agarose gel and quantified using a QUBIT 2.0. Samples with DNA concentration above 80 ng/uL were diluted to 70 ng/uL and approximately 20 uL of purified DNA in TE buffer was sent to Diversity Arrays Technology Pty Ltd (Canberra, Australia) for individual genotyping with DArTseq technology (Sansaloni et al., 2011). DNA from a subset of individuals (4.4% for the host and 14.3% for the mistletoe) was replicated across multiple plates, but processed independently, to ensure between-plate genotyping continuity.

Briefly, DArTseq uses high-throughput reduced representation sequencing to assay millions of markers for genome-wide single nucleotide polymorphisms (SNPs; Sansaloni et al., 2011; Kilian et al., 2012). Reduced representation sequencing is advantageous in that it produces an abundance of short-read data for numerous individuals and can be performed without prior genomic knowledge of species (Manel et al., 2016). While availability of reference genomes and transcriptomes can allow loci to be linked to gene function (Tiffin & Ross-Ibarra, 2014), signals of selection can still be obtained for nonmodel species that lack prior genomic knowledge (Savolainen et al., 2013).

Genome reduction was undertaken using a combination of two methylation-sensitive restriction enzymes, *Pst*I/*Mse*l for the host and *Pst*I/*Hpa*II for the mistletoe, with the digestion and adaptor ligation process described by Kilian et al. (2012). High-density sequencing was run on the Illumina HiSeq 2500 platform and sequence alignment performed *de novo* using Diversity Array Technology's propriety analytical pipeline as prior genomic information was not available for either species (or related species). Sequences were filtered to remove those with a Phred score < 30 and the remainder were collapsed into identical sequences. SNP marker calling was performed with Diversity Array Technology's propriety DARTSOFT14 pipeline. Approximately 25% of samples were regenotyped as technical replicates to allow a measure of DArTseq reproducibility at each locus to be calculated. NCBI BLAST (Camacho et al., 2009) of bacteria and fungi databases was used to remove microbial DNA from barcoded sequences. Remaining sequences were trimmed and split into individual organism data.

Further quality control filtering was performed using the package DARTR version 1.1.11 (Gruber et al., 2018) in R (R Core Team, 2019) to ensure only high-quality data was used for downstream analysis. Specifically, we removed replicates of individuals from the SNP datasets and then filtered the dataset to retain: 1) SNPs with less than 5% missing data, 2) SNPs with DArTseq reproducibility score > 0.98, 3) SNPs with minor allele frequency greater than 5% and 4) individuals with < 20% missing data. Downstream genetic analyses typically assume loci are not closely linked (see Hoban et al., 2016). Therefore, as a final filtering step we randomly selected only one SNP per fragment to be retained in the dataset.

3.4.4 Landscape and population genetic analyses

To separate adaptive and neutral genetic variation, we used a combination of one population differentiation (PD) test and two environment association (EA) analyses to detect loci under selection using a consensus approach as recommended by De Mita et al. (2013). Specifically, we used the PD method PCADAPT (Luu et al., 2017), which uses principal component analysis to identify SNPs with excessive association to population structure, but not to specific environmental variables. In comparison, EA approaches account for neutral population structure to detect SNPs with significant associations to environmental variables (e.g., temperature and precipitation; Hoban et al., 2016), although, unlike PD tests, they can lose power under certain demographic

scenarios (Lotterhos & Whitlock, 2015; Forester et al., 2018). The two EA approaches used in this study each have a different statistical approach in correcting for population structure. Specifically, latent factor mixed models (LFMM; Frichot et al., 2013; Caye et al., 2019) use a least-squares estimation approach and BAYPASS (Gautier, 2015) uses Bayesian hierarchical modelling.

PCADAPT uses a robust Mahalanobis test statistic to identify SNPs in which z-scores do not follow the same distribution as those of the larger dataset and these are considered as outliers (Luu et al., 2017). The analysis was implemented in the R package PCADAPT version 4.1.0 (Luu et al., 2017) with the false discovery rate (FDR) set to 5%. The optimum number of principal components (PCs) was identified by running PCADAPT with $K = 10$ and interpreting the scree plot using Cattell's rule (Cattell, 1966). Secondly, PCADAPT was run with the optimum number of PCs and a MAF threshold of 0.05 to calculate the test statistic and p-values for each locus. To correct for FDR, p-values were transformed into q-values using the R package QVALUE version 2.18.0 (Storey et al., 2019) and SNPs with $q < 0.05$ were identified as outliers.

LFMM uses allele frequency data and an imputed number of latent factors to calculate an exact solution for latent factor regression models, while controlling for confounding variables (Caye et al., 2019). The analysis was implemented in the R package LFMM version 1.0 (Caye et al., 2020). We estimated the number of latent factors (K) following the package vignette, performing principal component analysis (PCA) on the dataset using the R function *prcomp* (R Core Team, 2019). Results of the PCA were plotted as screeplots (Figure S3.8.2) and interpreted using Cattell's rule (Cattell, 1966). Missing genetic data was imputed in the R package LEA version 2.8 (Frichot & François, 2015) using K latent factors, and each climatic variable was scaled to a standard deviation of one. LFMM analysis was run for each climatic variable through ridge estimates using K latent factors. For each climatic variable, z-scores were used to derive a genomic inflation factor (λ) that were used to adjust p-values based on a chi-squared (χ^2) distribution (François et al., 2016). To control for FDR, a Benjamini–Hochberg p-value correction was applied according to Frichot and François (2015) and SNPs with $q < 0.05$ were considered to have a significant SNP–environment association.

BAYPASS tests for covariance between population-level allele frequencies and environmental variables while correcting for demographic effects (Gautier, 2015). The core model in BAYPASS was run four times with default settings in addition to nval of 100,000, burnin of 50,000, npilot of 30 and pilotlenth of 5000, with results averaged over runs. The XtX statistic was calibrated using the *simulate.baypass* function according to the manual to create a pseudo-observed dataset that was run in BAYPASS using the same settings as the core model. The results were used to identify SNPs with an XtX statistic below 3% (representing balancing selection) or above 97% (representing directional selection), which were considered outliers. To identify association with environmental variables, outlier SNPs (both balancing and directional) were removed to create a neutral dataset that was run in BAYPASS with the same settings as the core model. The average of four runs was used to create a neutral covariance matrix. Finally, the auxiliary model was run in BAYPASS using the neutral covariance matrix and the seven climatic variables with the same settings as the core model. Bayes factors were obtained from the mean of four runs and were transformed into deciban units (dB) using the $10\log_{10}(BF)$ transformation. Values of 20 deciban units or more were considered as strong evidence for significant SNP–environment associations (Kass & Raftery, 1995).

We plotted the total number of significant SNPs for each method using the R package VENNDIAGRAM version 1.6.20 (Chen, 2018) and split the SNPs into neutral and adaptive datasets. Neutral SNPs were considered to be those not identified as outliers, or with significant environment association, by any genome scan method and adaptive SNPs were considered to be those identified by two or more methods (Forester et al., 2018). We estimated global and pairwise F_{ST} (Weir & Cockerham, 1984) between populations for neutral and adaptive datasets using the R package HIERFSTAT version 0.04-22 (Goudet, 2005), with the latter used as input for the spatial modelling. Additionally, we used principal coordinate analysis (PCoA) in the R package DARTR version 1.1.11 (Gruber et al., 2018) to examine differences in genetic structure for neutral and adaptive genetic datasets and plotted the first three PCoA axes using R package GGPLOT2 version 3.2.1 (Wickham, 2016).

3.4.5 Landscape genetic modelling

Generalised dissimilarity modelling (GDM; Ferrier, 2002; Ferrier et al., 2002) was used to examine and compare the association of genome-wide variation with climatic

gradients between the mistletoe and host. Specifically, we compared: (a) the association of adaptive and neutral genetic variation with climatic variables, using the method described by Fitzpatrick and Keller (2015) on applying GDMs to genome-wide markers; and (b) the relative importance of temperature, precipitation and geographical distance in predicting allelic turnover, using variation partitioning (Borcard et al., 1992). Pairwise F_{ST} matrices (scaled to between zero and one were used as the biological response variable and predictor datasets were assembled with geographical coordinates of each population along with the seven climatic variables.

GDM analysis was implemented in the R package GDM version 1.3.11 (Manion et al., 2018) to assess the relative importance of each climatic variable against allelic turnover. For each dataset, we used a backwards elimination procedure with 500 permutations and three splines to measure significance ($\alpha = 0.05$) of each climatic variable (Ferrier et al., 2007; Fitzpatrick et al., 2013). Only significant climatic variables were retained in the final GDM models. We summed the spline coefficients to quantify the relative importance of each predictor variable (Fitzpatrick et al., 2013; Yates et al., 2019).

Monotonic I-spline turnover functions were calculated for predictor variables in the final GDM models and these were mapped using GGPLOT2 to visualise the relationship between allelic turnover and climatic variables. Spline height represented the amount of explained genetic variation, when holding all other variables constant, and spline slope indicated the rate of genetic differentiation across the range of the predictor (Fitzpatrick et al., 2013; Fitzpatrick & Keller, 2015). Next, we partitioned the deviance resulting from the GDM models into geographical distance, temperature and/or precipitation variables to evaluate the contributions of each in explaining allelic turnover (Borcard et al., 1992; Yates et al., 2019). Partitioned deviance was plotted in Venn diagrams using the R package EULERR version 6.0.0 (Larsson, 2019).

To examine the predicted spatial patterns of adaptive and neutral genetic variation, we visualised the GDM models using the spatial interpolation method of Fitzpatrick and Keller (2015). Briefly, we used fitted GDMs to transform significant climatic variables into genetic importance values, then used PCA to reduce the transformed variables into three PCs, which were composited into an RGB raster image (R = PC1, G = PC2, B = PC3). Similar colours correspond to similar predicted patterns of genetic

composition. To compare mapped genetic patterns between the two datasets (neutral SNPs; adaptive SNPs) for each species, we used Procrustes analysis (Peres-Neto & Jackson, 2001) to measure and map the similarity of multivariate configuration following the approach of Fitzpatrick and Keller (2015). Procrustes residuals measure the absolute difference in patterns of predicted genetic compositions between neutral and adaptive datasets for each species. Further, to allow direct comparison between species, we scaled residuals by the largest and smallest value observed across both species following the method of Walters et al. (2020). Finally, residuals were mapped geographically to identify areas with the largest differences in genetic composition patterns between SNP datasets.

3.5 Results

3.5.1 SNP generation

DArTseq technologies produced SNP datasets that comprised 118,880 SNPs across 80,296 loci for host *Hakea recurva* subsp. *recurva* (n = 160) and 15,187 SNPs across 10,415 loci for host-specific mistletoe *Amyema gibberula* var. *tatei* (n = 154). All replicates had greater than 97% genetic similarity. Following further quality control filtering the working datasets comprised 15,422 SNPs for the host and 2,055 SNPs for the mistletoe with global missing data of 1.20% and 1.12%, respectively. All individuals were retained in the datasets for both species.

3.5.2 Landscape and population genetic analyses

PCAdapt identified 488 SNPs as outliers for the host (mean $\chi^2 = 6.32$, df = 5) and 225 SNPs as outliers for the mistletoe (mean $\chi^2 = 5.79$, df = 2). LFMM identified 59 significant SNP–environment associations for the host involving 47 SNPs. In contrast, LFMM identified 272 significant SNP–environment associations for the mistletoe involving 135 SNPs. Similarly, BayPass identified 88 SNP–environment associations involving 81 SNPs for the host, and 107 SNP–environment associations for the mistletoe involving 105 SNPs. For both EA approaches, the number of significant SNP–environment associations varied between climatic variables and no SNPs for either species were significantly associated across all variables (Table 3.1).

Overall, 574 SNPs were identified by at least one analytical method for the host (Figure 3.2A) and those same methods identified 424 SNPs for the mistletoe (Figure 3.2B). While all these SNPs showed either higher than expected differentiation

between populations or significant association with climatic variables, they may not all be directly affected by selection but could be physically linked to loci under selection. Accordingly, only SNPs identified by more than one genome scan method were included in adaptive datasets (35 and 36 SNPs for the host and mistletoe, respectively). By contrast, neutral datasets (14,848 and 1,631 SNPs for the host and mistletoe, respectively) comprised SNPs not identified as outliers, or with significant environment association, in any genome scan method. Overall, global F_{ST} was greater in the mistletoe than the host. Specifically, F_{ST} values for the neutral datasets were 0.079 and 0.330 for the host and mistletoe, respectively, while values for the adaptive datasets were 0.370 and 0.734 (host and mistletoe, respectively).

Table 3.1 Number of SNPs with significant environment association for *Hakea recurva* subsp. *recurva* (*n* = 15,422 SNPs) and *Amyema gibberula* var. *tatei* (*n* = 2,055 SNPs). Environment association analyses (LFMM and BAYPASS) were run on each species across seven climatic variables. The total number of unique SNPs identified by the two approaches is shown for each variable.

Climatic variable	***Hakea recurva* subsp. *recurva***			***Amyema gibberula* var. *tatei***		
	LFMM	**BayPass**	**Total**	**LFMM**	**BayPass**	**Total**
IT (BIO3)	3	0	3	102	1	103
TS (BIO4)	7	5	12	15	0	15
MTWQ (BIO8)	2	36	38	18	54	66
MTDQ (BIO9)	1	1	2	0	13	13
AP (BIO12)	15	20	27	48	19	60
PS (BIO15)	4	13	15	79	0	79
PWQ (BIO18)	27	13	35	10	20	29

Abbreviations: AP, annual precipitation; IT, isothermality; MTDQ, mean temperature of the driest quarter; MTWQ, mean temperature of the wettest quarter; PS, precipitation seasonality; PWQ, precipitation of the warmest quarter; TS, temperature seasonality.

PCoA identified differing genetic structure between species and datasets (Figure 3.1). For the neutral datasets, the first PCoA axis separated out the most south-eastern population of the host while the remaining populations were separated along the second and third axes, collectively explaining 10.7% of genetic variation (Figure 3.1A). By contrast, the first PCoA axis separated the northern and southern populations of mistletoe while the second and third axes distinguished between southern populations, collectively explaining 29.4% of genetic variation (Figure 3.1B). For the adaptive datasets, the first PCoA axis separated out the north-eastern populations, with host individuals separated along the second axis, and the first three axes explained 58.4% of the genetic variation (Figure 3.1A). In the mistletoe, the first PCoA axis

separated the northern and southern populations but the second axis separated out the most south-eastern population, and the first three axes explained 73.9% of genetic variation (Figure 3.1B).

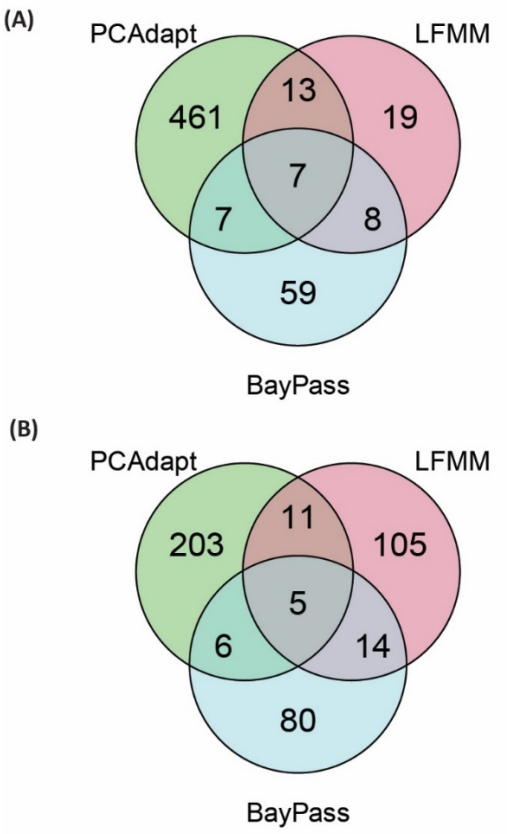


Figure 3.2 Venn diagrams of SNPs identified by three genome scan methods for (A) *Hakea recurva* subsp. *recurva* and (B) *Amyema gibberula* var. *tatei*. The diagrams show a comparison between SNPs obtained from PCADAPT, LFMM and BAYPASS methods. For the environment association methods (LFMM and BAYPASS), SNPs that were significant for multiple environmental variables were only included once in the Venn diagram.

3.5.3 Landscape genetics modelling

Following the GDM backwards elimination procedure, no predictor variables were significant ($\alpha = 0.05$) for the neutral dataset of the host although we opted to use mean temperature of the wettest quarter ($p = 0.06$) in the final GDM model (Table 3.2). Two significant variables were retained in the GDM model for the host adaptive dataset. In contrast, one significant predictor variable was retained in the GDM model for the mistletoe neutral dataset while three variables were retained in the GDM model for the adaptive dataset. Overall, predictor variables in the final GDM models explained a lower percentage of model deviance in neutral datasets for both species, compared to adaptive datasets (Table 3.2).

Table 3.2 Model fit of generalized dissimilarity modelling for *Hakea recurva* subsp. *recurva* and *Amyema gibberula* var. *tatei* datasets. Neutral datasets contained SNPs that were not identified as outliers, or with significant environment association, in any genome scan method (14,848 and 1,631 SNPs for each species, respectively). Adaptive datasets contained only SNPs identified by two or more genome scan methods (35 and 36 SNPs for each species, respectively). Models contain only significant predictor variables ($p < 0.05$), except for the *H. recurva subsp. recurva* neutral dataset ($p = 0.06$).

Model	***Hakea recurva* subsp. *recurva***		***Amyema gibberula* var. *tatei***	
	Neutral dataset	**Adaptive dataset**	**Neutral dataset**	**Adaptive dataset**
Predictor variables	MTWQ	Geo + AP	MTWQ	Geo + TS + MTWQ
Model deviance	7.79	4.56	8.36	3.58
Percentage explained	35.34	69.55	32.83	75.92
p-value	0.109	0.000	0.023	0.000

Abbreviations: AP, annual precipitation (BIO12); Geo, geographical distance; MTWQ, mean temperature of the wettest quarter (BIO8); TS, temperature seasonality (BIO4).

GDM analysis removed the variation associated with geographical distance and compared the remaining variation – the partial genetic distance – to climatic variables with patterns varying by both predictor variable and species (Figure 3.3**)**. Specifically, geographical distance showed a near linear relationship with genetic distance with the spline predicting a gradual change in allelic turnover across the range (Table 3.3; Figure 3.3A). Additionally, geographical distance had the greatest spline height for both adaptive datasets, indicating that this was the most important predictor of allelic turnover. In contrast, all three climatic variables in the final GDM models had a non-linear relationship with partial genetic variation (Figure 3.3B-D). Temperature seasonality was a significant predictor of the mistletoe adaptive dataset with the largest change in allelic turnover predicted to occur below 580% (Figure 3.3B). Mean temperature of the wettest quarter was the only predictor variable for the neutral datasets in both species and was also an important predictor for the mistletoe adaptive dataset, with the largest change in allelic turnover predicted to occur below 13°C (Figure 3.3C). Finally, annual precipitation was a significant predictor of the host adaptive dataset with the largest change in allelic turnover below 280 mm (Figure 3.3D).

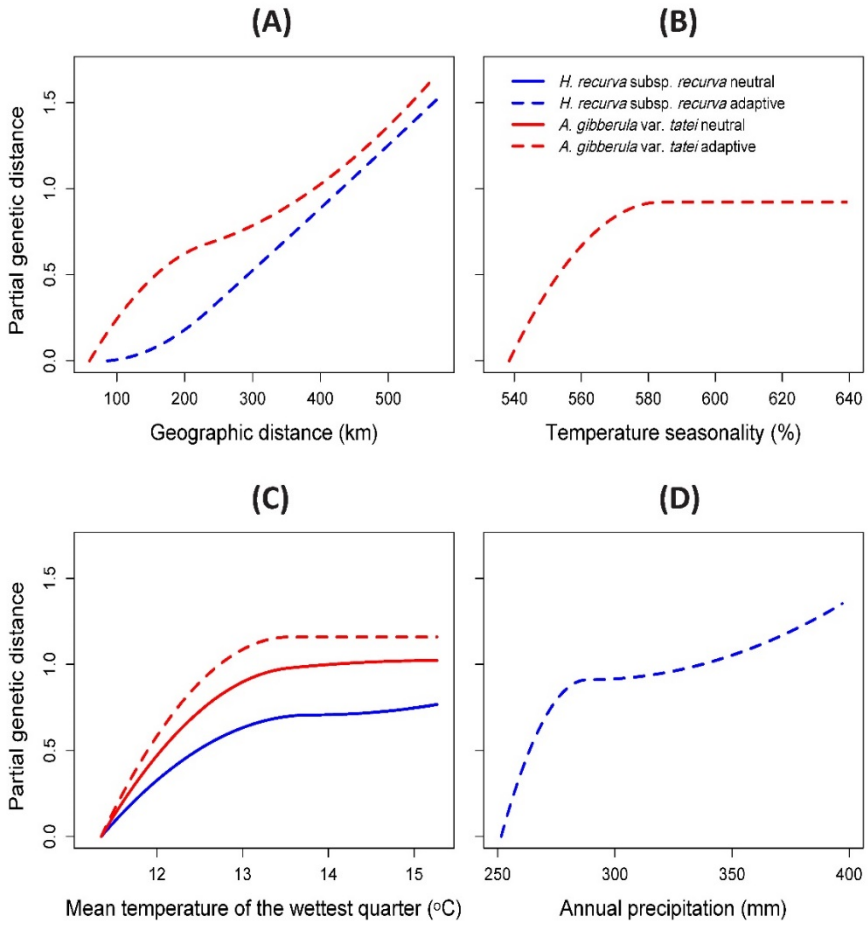


Figure 3.3 Generalised dissimilarity model-fitted I-splines showing allelic turnover across predictor variables for *Hakea recurva* subsp. *recurva* and *Amyema gibberula* var. *tatei*. Neutral datasets (14,848 and 1,631 SNPs for each species, respectively) contained SNPs not identified as outliers, or with significant environment association, in any genome scan method. Adaptive datasets (35 and 36 SNPs for each species, respectively) contained SNPs identified by two or more genome scan methods. Allelic turnover was only plotted if the dataset had a significant relationship with the predictor variables: (A) geographical distance, (B) temperature seasonality (BIO4), (C) mean temperature of the wettest quarter (BIO8) and (D) annual precipitation (BIO12). 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.

Geographical distance explained approximately 43% of the GDM model deviance of adaptive datasets for both species (Figure 3.4). Precipitation explained a similar proportion of GDM model deviance as geographical distance for the host and no variation was explained by temperature for this species (Figure 3.4A). In contrast, precipitation did not explain any of the GDM model deviance for the mistletoe while temperature explained over 65%, although a large proportion of allelic turnover was also explained by geographical distance (Figure 3.4B). Unexplained variation in GDM model deviance was similar between species.

Table 3.3 Relative importance of predictor variables in generalized dissimilarity models for *Hakea recurva* subsp. *recurva* and *Amyema gibberula* var. *tatei*. Neutral datasets contained SNPs that were not identified as outliers, or with significant environment association, in any genome scan method (14,848 and 1,631 SNPs for each species, respectively). Adaptive datasets contained only SNPs identified by two or more genome scan methods (35 and 36 SNPs for each species, respectively). Relative importance values were obtained from the summations of the three spline coefficients for each significant predictor variables. Cells with no value indicate that the variable was not a significant predictor of that model.

	Hakea recurva* subsp. *recurva		***Amyema gibberula* var. *tatei***	
Relative importance	**Neutral dataset**	**Adaptive dataset**	**Neutral dataset**	**Adaptive dataset**
Geo	-	1.54	-	1.67
IT (BIO3)	-	-	-	-
TS (BIO4)	-	-	-	0.92
MTWQ (BIO8)	0.77	-	1.02	1.16
MTDQ (BIO9)	-	-	-	-
AP (BIO12)	-	1.36	-	-
PS (BIO15)	-	-	-	-
PWQ(BIO18)	-	-	-	-

Abbreviations: AP, annual precipitation; Geo, geographical distance; IT, isothermality; MTDQ, mean temperature of the driest quarter; MTWQ, mean temperature of the wettest quarter; PS, precipitation seasonality; PWQ, precipitation of the warmest quarter; TS, temperature seasonality.

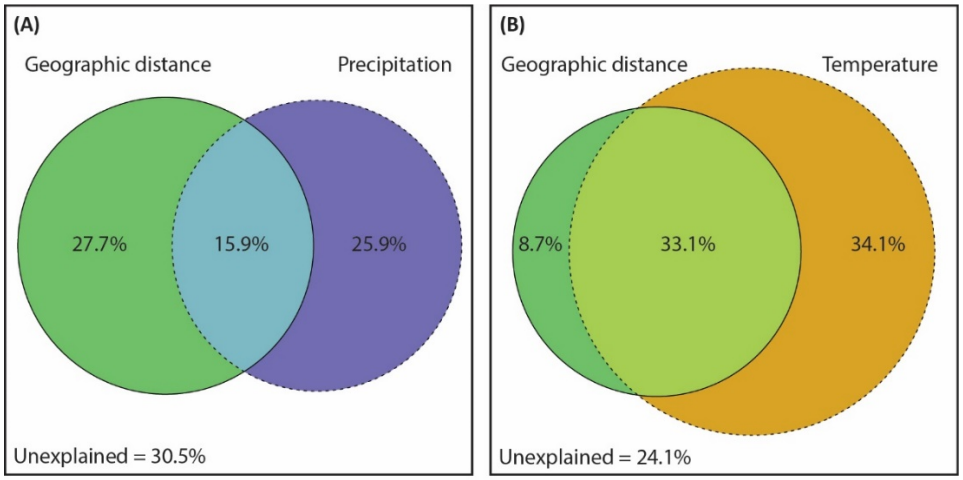


Figure 3.4 Partitioning of generalized dissimilarity model deviance by predictor variables for (A) *Hakea recurva* subsp. *recurva* and (B) *Amyema gibberula* var. *tatei*. Three sets of predictor variables were used (geographical distance, temperature and precipitation variables) for adaptive datasets (35 and 36 SNPs for each species, respectively) that contained SNPs identified by two or more genome scan methods.

Spatial patterns of predicted genetic composition were similar for neutral datasets of both species, but not for adaptive datasets (Figure 3.5). Specifically, rapid turnover in genetic composition of neutral datasets was similarly predicted in the south-eastern region for both species (Figure 3.5A, D). In contrast, the turnover of genetic composition for adaptive datasets was predicted to occur more rapidly in the eastern

region for the host (Figure 3.5B) but the western and southern regions for the mistletoe (Figure 3.5E). Procrustes residuals, which compared multivariate configuration between neutral and adaptive datasets, varied spatially across the distribution of both species. In general, residuals were higher in the southern half of the range for each species, indicating less congruence between SNP datasets (Figure 3.5C, F).

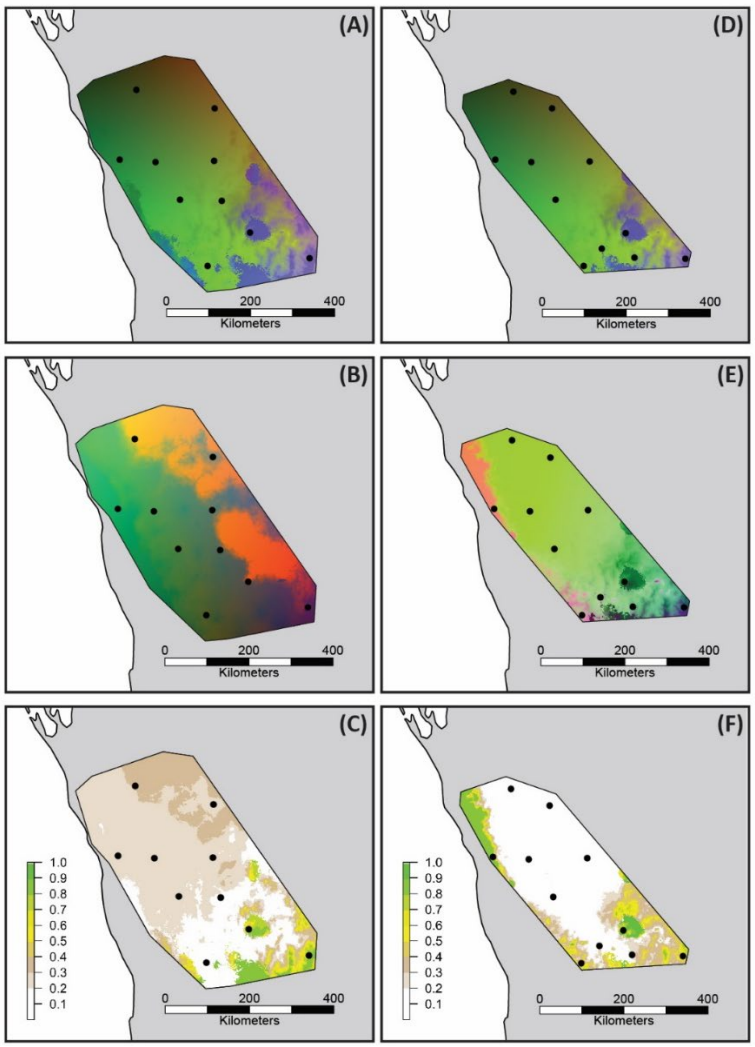


Figure 3.5 Spatial patterns of predicted genetic composition and differences in multivariate configuration (Procrustes residuals) for (A-C) *Hakea recurva* subsp. *recurva* and (D-F) *Amyema gibberula* var. *tatei*. Genetic compositions were derived using fitted generalized dissimilarity models to perform biologically-informed transformations of significant climatic variables for neutral datasets (A, D) and adaptive datasets (B, E). Neutral datasets (14,848 and 1,631 SNPs for each species, respectively) contained SNPs not identified as outliers, or with significant environment association, in any genome scan method. Adaptive datasets (35 and 36 SNPs for each species, respectively) contained SNPs identified by two or more genome scan methods. Principal component analysis was used to reduce the 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 neutral and adaptive datasets were measured by Procrustes analysis (C, F). Procrustes residuals were scaled to allow direct comparisons between species.

3.6 Discussion

Our investigation of genome-wide variation along climatic gradients in a host-specific mistletoe and its host found that adaptive genetic variation was associated with different climatic variables for each species. This supported our hypothesis that these species respond differently to climatic variables. Specifically, temperature was relatively more important in predicting allelic turnover for the mistletoe, while precipitation was more important for the host. This could reflect a parasitic life history. While genome-wide markers have been used to examine climate adaptation in numerous taxa, applying these methods to a comparative study of nonmodel organisms in a host-parasite system has presented some distinct challenges, which we discuss below.

3.6.1 Associations with climatic variables in a mistletoe-host system

Mistletoes and their hosts have been previously recorded as having similar relationships between physiological parameters and climate (Scalon & Wright, 2015). However, this is the first study to compare the associations of genome-wide variation along climatic gradients in mistletoe-host systems. Despite similar associations of neutral genetic variation between the mistletoe and its host, associations of adaptive genetic variation with climatic variables were different. Specifically, GDM analysis indicated adaptive genetic variation in the mistletoe to be associated with temperature. Seasonality and mean temperature of the wettest quarter were particularly associated with the mistletoe, as also correlated with population differentiation in another mistletoe (Ramírez-Barahona et al., 2017). One explanation for the importance of temperature is that mistletoes (and other parasitic plants) must maintain a hydrostatic gradient to draw water from hosts, which is achieved through increased transpiration rates relative to host plants (Stewart & Press, 1990; Ehleringer & Marshall, 1995). As temperature influences transpiration rates, temperature may provide a greater selective pressure for the mistletoe in comparison to the host. While these patterns of genome-wide variation are consistent with local adaptation, further validation of the role of temperature is needed. Experimental work in other host-parasite systems have found temperature to influence local adaptation (Laine, 2007, 2008); although this information is not yet available for parasitic plants.

GDM analysis showed adaptive genetic variation in the host to be associated with precipitation, which is consistent with findings of other studies on autotrophic plants (e.g., Manel et al., 2012; Shryock et al., 2017; Steane et al., 2017; Walters et al., 2020). However, precipitation was not found to associate with adaptive genetic variation in the mistletoe. This was despite recent experimental evidence suggesting that population differentiation in mistletoes can vary along gradients of water availability and precipitation seasonality (Ramírez-Barahona et al., 2017). One explanation for the difference in relative importance of precipitation in this study could be the different water acquisition strategies between the mistletoes and its hosts, which may respond differently to abiotic selective pressures. Specifically, acquisition of water from host plants (rather than the abiotic environment) may provide a buffer between the mistletoe and climatic conditions. This could provide a more uniform environment with reduced selection pressures.

Similar observations on associations of genome-wide variation with climatic variables have also been found for a root hemiparasite and sympatric autotroph (Walters et al., 2020), although association with climatic variables does not necessarily imply that adaptation is present. Another explanation for the difference between species in our study could be that mistletoes have a different ecological/climatic niche to their hosts (Lira-Noriega & Peterson, 2014). Alternatively, the variables assessed here may correlate with other environmental gradients (e.g., solar radiation, altitude; Garnier-Géré & Ades, 2001; Gauli et al., 2015) that may be true drivers of adaptation in these species. While this study provides evidence consistent with local adaptation, further experimental work is needed to explicitly confirm the effect of climate adaptation in these species.

In this study, we found adaptive genetic variation to positively correlate with geographical distance (i.e., isolation by distance), similar to that in other plant species (e.g., Shryock et al., 2017; Supple et al., 2018). Geographical distance has been previously found to influence genetic structure between populations of mistletoes (Yule et al., 2016; Nyagumbo et al., 2017) and other parasitic species (Feurtey et al., 2016), but not always for host-specific mistletoes (Jerome & Ford, 2002). For both species in our study, the most south-eastern population was notably different from other populations, which is likely due to the isolated occurrence of this population on the edge of the species' range. Over time, geographical isolation between parasitic

populations can lead to co-speciation with hosts or host-shift speciation (reviewed in de Vienne et al., 2013).

3.6.2 Factors affecting the study of selection pressure in nonmodel organisms using genome-wide markers

Genome-wide markers have been used to identify signals of selection across many taxonomic groups (reviewed in Ahrens et al., 2018); yet applying these methods to a comparative study in a host-parasite system has posed some distinct challenges. Firstly, demographic and life histories can differ widely between species; for instance, parasites generally have much shorter generation times (Huyse et al., 2005). Faster generation times leads to more frequent genome replication that collects more DNA mutations per unit of time and, therefore, adaptation can proceed more rapidly (Smith & Donoghue, 2008; Bromham et al., 2013). Mutation rates can also vary between parasites (Nieberding & Olivieri, 2007), therefore, the detection of genome-wide variation in other host-parasite systems may differ to that observed here. Population sizes were also observed to differ between our species, which would influence the genetic variability within populations (Charlesworth, 2009).

Another complicating factor for our study is that both nonmodel species lack reference genomes or transcriptomes and, consequently, we have not been able to verify the gene function of adaptive SNPs. While signals of selection can still be obtained for species that lack prior genomic knowledge (Savolainen et al., 2013), it is likely that not all SNPs identified as outliers, or those with significant environment association, were directly under selection but rather physically linked to loci under selection (i.e., genetic hitchhiking; reviewed by Barton, 2000). The exact effect of genetic hitchhiking on genome scans depends upon a number of evolutionary parameters (Lotterhos & Whitlock, 2015), the majority of which are unknown for our study species. Consequently, we sought to minimise the inclusion of false positives in our adaptive dataset by using a consensus approach of multiple genome scan methods (De Mita et al., 2013). Future work could expand upon these findings by using reference genomes to map the gene function of both SNPs identified as outliers and those with significant environment association (Tiffin & Ross-Ibarra, 2014; Bragg et al., 2015; Breed et al., 2019).

Lastly, while our study has enabled association of adaptive genetic variation with different climatic gradients, it does not provide insight into the genetic architecture of climate adaptation in these species *per se*. For instance, polygenic adaptation of many loci with small effect that result in phenotypic changes may be difficult to detect with genome scans in comparison to loci with a single, large effect (Pritchard & Di Rienzo, 2010; Le Corre & Kremer, 2012). This could influence the number of SNPs identified as adaptive as the ratios of loci with small and large effects may differ between these species, although this information was not available *a priori*. Furthermore, we also found stronger population differentiation in the mistletoe and this is known to influence the detection of loci under selection (de Villemereuil et al., 2014; Flanagan et al., 2018). Although population differentiation was accounted for in our EA analyses, combining these results with phenotypic data could further our understanding of local climatic adaptation between mistletoes and their hosts.

3.6.3 Comparison with other host-parasite systems

Our findings on the association of adaptive genetic variation with climatic variables in a mistletoe-host system were similar that of a previous study on a generalist root parasite and co-occurring autotroph (Walters et al., 2020). For both parasitic plants, there was a stronger association of adaptive genetic variation to temperature variables, in comparison to precipitation variables for host species. While this could be indicative of the parasitic life history, specifically their different water acquisition strategies, further examination is needed to confirm this pattern in other parasitic plants. Furthermore, patterns on the association of adaptive genetic variation to climatic variables may differ in other host-parasite systems that often have fewer differences in gene flow and population structure between parasites and hosts (e.g., Dybdahl & Lively, 1996; McCoy et al., 2005; Feurtey et al., 2016). Examining the association of genome-wide variation could be crucial to understanding climate adaptation, particularly as parasite evolution depends upon the physical environment (Laine, 2008). Therefore, extending this work to other parasitic plants (e.g., annual species), or other host-parasite systems, would further increase our understanding of the association of adaptive genetic variation to climatic variables in natural populations.

3.7 References

Every reasonable effort has been made to acknowledge the owners of the copyright material. I would be pleased to hear from any copyright owner who has been omitted or incorrectly acknowledged.

Ahrens, C. W., Rymer, P. D., Stow, A., Bragg, J., Dillon, S., Umbers, K. D. L., & Dudaniec, R. Y. (2018). The search for loci under selection: Trends, biases and progress. *Molecular Ecology, 27*, 1342–1356. https://doi.org/10.1111/mec.14549

Barton, N. H. (2000). Genetic hitchhiking. *Philosophical Transactions: Biological Sciences, 355*, 1553-1562. https://doi.org/10.1098/rstb.2000.0716

Borcard, D., Legendre, P., & Drapeau, P. (1992). Partialling out the spatial component of ecological variation. *Ecology, 73*, 1045-1055. https://doi.org/10.2307/1940179

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

Breed, M. F., Harrison, P. A., Blyth, C., Byrne, M., Gaget, V., Gellie, N. J. C., . . . Mohr, J. J. (2019). The potential of genomics for restoring ecosystems and biodiversity. *Nature Reviews Genetics, 20*, 615-628. https://doi.org/10.1038/s41576-019-0152-0

Bromham, L., Cowman, P. F., & Lanfear, R. (2013). Parasitic plants have increased rates of molecular evolution across all three genomes. *BMC Evolutionary Biology, 13*, 126. https://doi.org/10.1186/1471-2148-13-126

Calder, D. M. (1983). Mistletoes in focus: An introduction. In M. Calder & P. Bernhardt (Eds.), *The biology of mistletoes* (pp. 1–17). Sydney: Academic Press Australia.

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., & Madden, T. L. (2009). BLAST+: Architecture and applications. *BMC Bioinformatics, 10*, 421. https://doi.org/10.1186/1471-2105-10-421

Cattell, R. B. (1966). The scree test for the number of factors. *Multivariate Behavioural Research, 1*, 245-276. https://doi.org/10.1207/s15327906mbr0102_10

Caye, K., Jumentier, B., & François, O. (2020). *lfmm*: Latent factor mixed models: R package version 1.0. Retrieved from https://CRAN.R-project.org/package=lfmm

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

Charlesworth, B. (2009). Effective population size and patterns of molecular evolution and variation. *Nature Reviews Genetics, 10*, 195-205. https://doi.org/10.1038/nrg2526

Chen, H. (2018). VennDiagram: Generate high-resolution venn and euler plots. R package version 1.6.20. Retrieved from https://CRAN.R-project.org/package=VennDiagram

de Buen, L. L., & Ornelas, J. F. (2002). Host compatibility of the cloud forest mistletoe *Psittacanthus schiedeanus* (Loranthaceae) in Central Veracruz, Mexico. *American Journal of Botany, 89*, 95-102. https://doi.org/10.3732/ajb.89.1.95

De Mita, S., Thuillet, A., Gay, L., Ahmadi, N., Manel, S., Ronfort, J., & Vigouroux, Y. (2013). Detecting selection along environmental gradients: Analysis of eight methods and their effectiveness for outbreeding and selfing populations. *Molecular Ecology, 22*, 1383-1399. https://doi.org/10.1111/mec.12182

de Vienne, D. M., Refrégier, G., López-Villavicencio, M., Tellier, A., Hood, M. E., & Giraud, T. (2013). Cospeciation vs host-shift speciation: Methods for testing, evidence from natural associations and relation to coevolution. *New Phytologist, 198*, 347-385. https://doi.org/10.1111/nph.12150

de Villemereuil, P., Frichot, É., Bazin, É., François, O., & Gaggiotti, O. E. (2014). Genome scan methods against more complex models: When and how much

should we trust them? *Molecular Ecology, 23*, 2006-2019. https://doi.org/10.1111/mec.12705

Doyle, J. J., & Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. *Focus, 12*, 13-15.

Dybdahl, M. F., & Lively, C. M. (1996). The geography of coevolution: Comparative population structures for a snail and its trematode parasite. *Evolution, 50*, 2264-2275. https://doi.org/10.2307/2410696

Ehleringer, J. R., & Marshall, J. D. (1995). Water relations. In M. C. Press & J. D. Graves (Eds.), *Parasitic plants* (pp. 125-140). United Kingdom: Chapman and Hall.

Ehleringer, J. R., Schulze, E.-D., Ziegler, H., Lange, O. L., Farquhar, G. D., & Cowar, I. R. (1985). Xylem-tapping mistletoes: Water or nutrient parasites? *Science, 227*, 1479-1481. https://doi.org/10.1126/science.227.4693.1479

ESRI. (2019). ArcGIS Desktop: Release 10.7.1. Redlands, CA: Environmental Systems Research Institute.

Ferrier, S. (2002). Mapping spatial pattern in biodiversity for regional conservation planning: Where to from here? *Systematic Biology, 51*, 331-363. https://doi.org/10.1080/10635150252899806

Ferrier, S., Manion, G., Elith, J., & Richardson, K. (2007). Using generalized dissimilarity modelling to analyse and predict patterns of beta diversity in regional biodiversity assessment. *Diversity and Distributions, 13*, 252-264. https://doi.org/10.1111/j.1472-4642.2007.00341.x

Ferrier, S., Watson, G., Pearce, J., & Drielsma, M. (2002). Extended statistical approaches to modelling spatial pattern in biodiversity in northeast New South Wales. I. Species-level modelling. *Biodiversity and Conservation, 11*, 2275-2307. https://doi.org/10.1023/A:1021302930424

Feurtey, A., Gladieux, P., Hood, M. E., Snirc, A., Cornille, A., Rosenthal, L., & Giraud, T. (2016). Strong phylogeographic co-structure between the anther-smut fungus and its white campion host. *New Phytologist, 212*, 668-679. https://doi.org/10.1111/nph.14125

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

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

Fitzpatrick, M. C., Sanders, N. J., Normand, S., Svenning, J., Ferrier, S., Gove, A. D., & Dunn, R. R. (2013). Environmental and historical imprints on beta diversity: Insights from variation in rates of species turnover along gradients. *Proceedings of the Royal Society B, 280*, 1-8. https://doi.org/10.1098/rspb.2013.1201

Flanagan, S. P., Forester, B. R., Latch, E. K., Aitken, S. N., & Hoban, S. (2018). Guidelines for planning genomic assessment and monitoring of locally adaptive variation to inform species conservation. *Evolutionary Applications, 11*, 1035-1052. https://doi.org/10.1111/eva.12569

Forester, B. R., Lasky, J. R., Wagner, H. H., & Urban, D. L. (2018). Comparing methods for detecting multilocus adaptation with multivariate genotype–environment associations. *Molecular Ecology, 27*, 2215-2233. https://doi.org/10.1111/mec.14584

François, O., Martins, H., Caye, K., & Schoville, S. D. (2016). Controlling false discoveries in genome scans for selection. *Molecular Ecology, 25*, 454-469. https://doi.org/10.1111/mec.13513

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

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

Garnier-Géré, P. H., & Ades, P. K. (2001). Environmental surrogates for predicting and conserving adaptive genetic variability in tree species. *Conservation Biology, 15*, 1632-1644. https://doi.org/10.1046/j.1523-1739.2001.00180.x

Gauli, A., Vaillancourt, R. E., Bailey, T. G., Steane, D. A., & Potts, B. M. (2015). Evidence for local climate adaptation in early-life traits of Tasmanian populations of *Eucalyptus pauciflora*. *Tree Genetics & Genomes, 11*(104), 1-14. https://doi.org/10.1007/s11295-015-0930-6

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

Gorter, F. A., Scanlan, P. D., & Buckling, A. (2016). Adaptation to abiotic conditions drives local adaptation in bacteria and viruses coevolving in heterogeneous environments. *Biology letters, 12*, 20150879. https://doi.org/10.1098/rsbl.2015.0879

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

Gruber, B., Unmack, P. J., Berry, O. F., & Georges, A. (2018). dartr: An R package to facilitate analysis of SNP data generated from reduced representation genome sequencing. *Molecular Ecology Resources, 18*, 691-699. https://doi.org/10.1111/1755-0998.12745

Hartmann, F. E., Rodríguez de la Vega, R. C., Carpentier, F., Gladieux, P., Cornille, A., Hood, M. E., & Giraud, T. (2019). Understanding adaptation, coevolution, host specialization, and mating system in castrating anther-smut fungi by combining population and comparative genomics. *Annual Review of Phytopathology, 57*, 431-457. https://doi.org/10.1146/annurev-phyto-082718-095947

Hijmans, R. J. (2020). raster: Geographic data analysis and modelling. R package version 3.0-12. Retrieved from https://CRAN.R-project.org/package=raster

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas.

International Journal of Climatology, 25, 1965-1978. https://doi.org/10.1002/joc.1276

Hoban, S., Kelley, J. L., Lotterhos, K. E., Antolin, M. F., Bradburd, G., Lowry, D. B., . . . Whitlock, M. C. (2016). Finding the genomic basis of local adaptation: Pitfalls, practical solutions, and future directions. *The American Naturalist, 188*, 379-397. https://doi.org/10.1086/688018

Hocking, P. J., & Fineran, B. A. (1983). Aspects of the nutrition of root parasitic Loranthaceae. In M. Calder & P. Bernhardt (Eds.), *The biology of mistletoes* (pp. 229-258). Australia: Academic Press.

Huyse, T., Poulin, R., & Théron, A. (2005). Speciation in parasites: A population genetics approach. *Trends in Parasitology, 21*, 469-475. https://doi.org/10.1016/j.pt.2005.08.009

Jerome, C. A., & Ford, B. A. (2002). Comparative population structure and genetic diversity of *Arceuthobium americanum* (Viscaceae) and its *Pinus* host species: Insight into host–parasite evolution in parasitic angiosperms. *Molecular Ecology, 11*, 407-420. https://doi.org/10.1046/j.0962-1083.2002.01462.x

Kaltz, O., & Shykoff, J. A. (1998). Local adaptation in host–parasite systems. *Heredity, 81*, 361-370. https://doi.org/10.1046/j.1365-2540.1998.00435.x

Kass, R. E., & Raftery, A. E. (1995). Bayes factors. *Journal of the American Statistical Association, 90*, 773-795. https://doi.org/10.2307/2291091

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

Kilian, A., Wenzl, P., Huttner, E., Carling, J., Xia, L., Blois, H., . . . Uszynski, G. (2012). Diversity Arrays Technology: A generic genome profiling technology on open platforms. In F. Pompanon & A. Bonin (Eds.), *Data Production and Analysis in Population Genomics: Methods and Protocols* (pp. 67-89). Grenoble, France: Humana Press.

Kuijt, J. (1969). *The biology of parasitic flowering plants*. Berkeley, California: University of California Press.

Laine, A.-L. (2007). Pathogen fitness components and genotypes differ in their sensitivity to nutrient and temperature variation in a wild plant–pathogen association. *Journal of Evolutionary Biology, 20*, 2371-2378. https://doi.org/10.1111/j.1420-9101.2007.01406.x

Laine, A.-L. (2008). Temperature-mediated patterns of local adaptation in a natural plant–pathogen metapopulation. *Ecology Letters, 11*, 327-337. https://doi.org/10.1111/j.1461-0248.2007.01146.x

Larsson, J. (2019). eulerr: Area-proportional Euler and Venn diagrams with ellipses. R package version 6.0.0. Retrieved from https://cran.r-project.org/package=eulerr

Le Corre, V., & Kremer, A. (2012). The genetic differentiation at quantitative trait loci under local adaptation. *Molecular Ecology, 21*. https://doi.org/10.1111/j.1365-294X.2012.05479.x

Liddy, J. (1983). Dispersal of Australian mistletoes: The Cowiebank study. In M. Calder & P. Bernhardt (Eds.), *The biology of mistletoes* (pp. 101-116). Australia: Academic Press.

Lira-Noriega, A., & Peterson, A. T. (2014). Range-wide ecological niche comparisons of parasite, hosts and dispersers in a vector-borne plant parasite system. *Journal of Biogeography, 41*, 1664-1673. https://doi.org/10.1111/jbi.12302

Lopez Pascua, L., Gandon, S., & Buckling, A. (2012). Abiotic heterogeneity drives parasite local adaptation in coevolving bacteria and phages. *Journal of Evolutionary Biology, 25*, 187-195. https://doi.org/10.1111/j.1420-9101.2011.02416.x

Lotterhos, K. E., & Whitlock, M. C. (2015). The relative power of genome scans to detect local adaptation depends on sampling design and statistical method. *Molecular Ecology, 24*, 1031-1046. https://doi.org/10.1111/mec.13100

Luu, K., Bazin, E., & Blum, M. G. B. (2017). pcadapt: An R package to perform genome scans for selection based on principal component analysis. *Molecular Ecology Resources, 17*, 67-77. https://doi.org/10.1111/1755-0998.12592

Manel, S., Gugerli, F., Thuiller, W., Alvarez, N., Legendre, P., Holderegger, R., . . . Taberlet, P. (2012). Broad-scale adaptive genetic variation in alpine plants is driven by temperature and precipitation. *Molecular Ecology, 21*, 3729-3738. https://doi.org/10.1111/j.1365-294X.2012.05656.x

Manel, S., Perrier, C., Pratlong, M., Abi-Rached, L., Paganini, J., Pontarotti, P., & Aurelle, D. (2016). Genomic resources and their influence on the detection of the signal of positive selection in genome scans. *Molecular Ecology, 25*, 170-184. https://doi.org/10.1111/mec.13468

Manion, G., Lisk, M., Ferrier, S., Nieto-Lugilde, D., Mokany, K., & Fitzpatrick, M. C. (2018). gdm: Generalized dissimilarity modelling. R package version 1.3.11. Retrieved from https://CRAN.R-project.org/package=gdm

March, W. A., & Watson, D. M. (2010). The contribution of mistletoes to nutrient returns: Evidence for a critical role in nutrient cycling. *Austral Ecology, 35*, 713-721. https://doi.org/10.1111/j.1442-9993.2009.02056.x

Mboup, M., Bahri, B., Leconte, M., De Vallavieille-Pope, C., Kaltz, O., & Enjalbert, J. (2012). Genetic structure and local adaptation of European wheat yellow rust populations: The role of temperature-specific adaptation. *Evolutionary Applications, 5*, 341-352. https://doi.org/10.1111/j.1752-4571.2011.00228.x

McCoy, K. D., Boulinier, T., & Tirard, C. (2005). Comparative host–parasite population structures: Disentangling prospecting and dispersal in the black-legged kittiwake *Rissa tridactyla*. *Molecular Ecology, 14*, 2825-2838. https://doi.org/10.1111/j.1365-294X.2005.02631.x

Mitchell, S. E., Rogers, E. S., Little, T. J., & Read, A. F. (2005). Host-parasite and genotype-by-environment interactions: Temperature modifies potential for selection by a sterilizing pathogen. *Evolution, 59*, 70-80. https://doi.org/10.1111/j.0014-3820.2005.tb00895.x

Musselman, L. J., & Press, M. C. (1995). Introduction to parasitic plants. In M. C. Press & J. D. Graves (Eds.), *Parasitic plants*. London, UK: Chapman & Hall.

Nieberding, C. M., & Olivieri, I. (2007). Parasites: Proxies for host genealogy and ecology? *Trends in Ecology & Evolution, 22*, 156-165. https://doi.org/10.1016/j.tree.2006.11.012

Norton, D. A., & Carpenter, M. A. (1998). Mistletoes as parasites: Host specificity and speciation. *Trends in Ecology & Evolution, 13*, 101-105. https://doi.org/10.1016/S0169-5347(97)01243-3

Nuismer, S. L., & Gandon, S. (2008). Moving beyond common-garden and transplant designs: Insight into the causes of local adaptation in species interactions. *The American Naturalist, 171*, 658-668. https://doi.org/10.1086/587077

Nyagumbo, E., Ndagurwa, H. G. T., & Mushonga, K. (2017). High genetic variation and low gene flow among populations of *Viscum verrucosum* in semi-arid savanna, southwest Zimbabwe. *Ecological Genetics and Genomics, 3-5*, 41-46. https://doi.org/10.1016/j.egg.2017.08.001

Peres-Neto, P. R., & Jackson, D. A. (2001). How well do multivariate data sets match? The advantages of a Procrustean superimposition approach over the Mantel test. *Oecologia, 129*, 169-178. https://doi.org/10.1007/s004420100720

Poulin, R. (2011). The many roads to parasitism: A tale of convergence. *Adv Parasitol, 74*, 1-40. https://doi.org/10.1016/b978-0-12-385897-9.00001-x

Poulin, R., & Morand, S. (2000). The diversity of parasites. *The Quarterly Review of Biology, 75*, 277-293. https://doi.org/10.1086/393500

Press, M. C., & Graves, J. D. (1995). *Parasitic Plants*. London, UK: Chapman & Hall.

Press, M. C., & Phoenix, G. K. (2005). Impacts of parasitic plants on natural communities. *New Phytologist, 166*, 737-751. https://doi.org/10.1111/j.1469-8137.2005.01358.x

Pritchard, J. K., & Di Rienzo, A. (2010). Adaptation - Not by sweeps alone. *Nature Reviews Genetics, 11*, 665-667. https://doi.org/10.1038/nrg2880

R Core Team. (2019). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/

Ramírez-Barahona, S., González, C., González-Rodríguez, A., & Ornelas, J. F. (2017). The influence of climatic niche preferences on the population genetic

structure of a mistletoe species complex. *New Phytologist, 214*, 1751-1761. https://doi.org/10.1111/nph.14471

Rellstab, C., Gugerli, F., Eckert, A. J., Hancock, A. M., & Holderegger, R. (2015). A practical guide to environmental association analysis in landscape genomics. *Molecular Ecology, 24*, 4348-4370. https://doi.org/10.1111/mec.13322

Sansaloni, C., Petroli, C., Jaccoud, D., Carling, J., Detering, F., Grattapaglia, D., & Kilian, A. (2011). Diversity Arrays Technology (DArT) and next-generation sequencing combined: Genome-wide, high throughput, highly informative genotyping for molecular breeding of *Eucalyptus*. *BMC Proceedings, 5*, P54. https://doi.org/10.1186/1753-6561-5-S7-P54

Savolainen, O., Lascoux, M., & Merilä, J. (2013). Ecological genomics of local adaptation. *Nature Reviews Genetics, 14*, 807-820. https://doi.org/10.1038/nrg3522

Scalon, M. C., & Wright, I. J. (2015). A global analysis of water and nitrogen relationships between mistletoes and their hosts: Broad-scale tests of old and enduring hypotheses. *Functional Ecology, 29*, 1114-1124. https://doi.org/10.1111/1365-2435.12418

Shryock, D. F., Havrilla, C. A., DeFalco, L. A., Esque, T. C., Custer, N. A., & Wood, T. E. (2017). Landscape genetic approaches to guide native plant restoration in the Mojave Desert. *Ecological Applications, 27*, 429-445. https://doi.org/10.1002/eap.1447

Smith, S. A., & Donoghue, M. J. (2008). Rates of molecular evolution are linked to life history in flowering plants. *Science, 322*, 86-89. https://doi.org/10.1126/science.1163197

Start, A. N. (2015). The mistletoe flora of southern Western Australia, with a particular reference to host relationships and fire. *Australian Journal of Botany, 63*, 636-646. https://doi.org/10.1071/BT15028

Steane, D. A., McLean, E. H., Potts, B. M., Prober, S. M., Stock, W. D., Stylianou, V. M., . . . Byrne, M. (2017). Evidence for adaptation and acclimation in a widespread eucalypt of semi-arid Australia. *Biological Journal of the Linnean Society, 121*, 484-500. https://doi.org/10.1093/biolinnean/blw051

Stewart, G. R., & Press, M. C. (1990). The physiology and biochemistry of parasitic angiosperms. *Annual Review of Plant Physiology and Plant Molecular Biology, 41*, 127-151. https://doi.org/10.1146/annurev.pp.41.060190.001015

Storey, J. D., Bass, A. J., Dabney, A., & Robinson, D. (2019). qvalue: Q-value estimation for false discovery rate control. R package version 2.18.0. Retrieved from http://github.com/jdstorey/qvalue

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

Thompson, J. N. (2005). *The Geographic Mosaic of Coevolution*. Chicago, IL: University of Chicago Press.

Tiffin, P., & Ross-Ibarra, J. (2014). Advances and limits of using population genetics to understand local adaptation. *Trends in Ecology & Evolution, 29*, 673-680. https://doi.org/10.1016/j.tree.2014.10.004

Walters, S. J., Robinson, T. P., Byrne, M., Wardell-Johnson, G. W., & Nevill, P. (2020). Contrasting patterns of local adaptation along climatic gradients between a sympatric parasitic and autotrophic tree species. *Molecular Ecology, 29*, 3022-3037. https://doi.org/10.1111/mec.15537

Watson, D. M. (2001). Mistletoe–a keystone resource in forests and woodlands worldwide. *Annual Review of Ecology and Systematics, 32*, 219-249. https://doi.org/10.1146/annurev.ecolsys.32.081501.114024

Weir, B. S., & Cockerham, C. C. (1984). Estimating *F*-statistics for the analysis of population structure. *Evolution, 38*, 1358-1370. https://doi.org/10.1111/j.1558-5646.1984.tb05657.x

Wickham, H. (2016). ggplot2: Elegant graphics for data analysis. New York: Springer-Verlag. Retrieved from https://github.com/tidyverse/ggplot2

Wolinska, J., & King, K. C. (2009). Environment can alter selection in host–parasite interactions. *Trends in Parasitology, 25*, 236-244. https://doi.org/10.1016/j.pt.2009.02.004

Yates, C. J., Robinson, T., Wardell-Johnson, G. W., Keppel, G., Hopper, S. D., Schut, A. G. T., & Byrne, M. (2019). High species diversity and turnover in granite inselberg floras highlight the need for a conservation strategy protecting many outcrops. *Ecology and Evolution, 9*, 7660-7675. https://doi.org/10.1002/ece3.5318

Yule, K. M., Koop, J. A. H., Alexandre, N. M., Johnston, L. R., & Whiteman, N. K. (2016). Population structure of a vector-borne plant parasite. *Molecular Ecology, 25*, 3332-3343. https://doi.org/10.1111/mec.13693

3.8 Supplementary information

Table S3.8.1 Coordinates and climatic data for *Hakea recurva* subsp. *recurva* populations sampled across south-western Australia. Latitude and longitude are presented in the Geocentric Datum of Australia (GDA94) coordinate system. Mean ± *SD* are provided for seven least-correlated bioclimatic variables used in environmental association analysis and generalised dissimilarity modelling.

Code	Population	Latitude	Longitude	No.	IT (%)	TS (°C)	MTWQ (°C)	MTDQ (°C)	AP (mm)	PS (%)	PWQ (mm)
BOO	Boogardie	-28.039939	117.674462	16	42.8 ± 0.14	653 ± 1.24	14.2 ± 0.01	20.3 ± 0.03	266 ± 0.87	40.0 ± 0.44	68.1 ± 0.25
DEP	Depot Hill	-29.143822	115.346400	16	49.7 ± 0.00	538 ± 0.00	15.3 ± 0.00	24.6 ± 0.00	397 ± 0.00	76.2 ± 0.00	39.0 ± 0.00
JIB	Jibberding	-30.000985	116.826976	16	45.8 ± 0.01	586 ± 0.02	14.0 ± 0.00	25.2 ± 0.01	298 ± 0.34	53.0 ± 0.09	36.0 ± 0.00
KAR	Karroun Hill	-30.024129	117.856322	16	44.4 ± 0.00	613 ± 0.48	13.1 ± 0.01	22.2 ± 0.02	284 ± 0.34	46.4 ± 0.12	45.0 ± 0.00
KOO	Koolanooka	-29.193801	116.228194	16	45.0 ± 0.00	588 ± 0.00	14.3 ± 0.00	22.3 ± 0.00	320 ± 0.00	55.3 ± 0.00	46.0 ± 0.00
KOR	Koora	-31.260612	120.011578	16	45.6 ± 0.00	544 ± 0.00	11.3 ± 0.00	22.2 ± 0.00	292 ± 0.00	43.6 ± 0.00	47.0 ± 0.00
NIN	Ninghan	-29.172953	117.660768	16	43.8 ± 0.14	628 ± 1.83	13.6 ± 0.06	20.4 ± 2.81	284 ± 1.15	46.4 ± 0.62	52.5 ± 0.52
WOO	Woolgorong	-27.644765	115.761262	16	46.0 ± 0.07	615 ± 0.79	15.2 ± 0.00	23.9 ± 0.04	252 ± 1.26	52.6 ± 0.21	45.6 ± 0.50
YAN	Yanneymooning	-30.708080	118.547847	16	45.0 ± 0.03	584 ± 0.55	11.6 ± 0.02	24.3 ± 0.04	267 ± 0.89	47.1 ± 0.17	37.3 ± 0.45
YOR	Yorkrakine Rock	-31.421116	117.512880	16	45.5 ± 0.02	548 ± 0.26	12.9 ± 0.01	23.2 ± 0.02	320 ± 0.50	51.2 ± 0.17	40.0 ± 0.00
TOTAL				160	45.4 ± 1.70	590 ± 36.5	13.6 ± 1.30	22.9 ± 1.80	298 ± 39.3	51.2 ± 9.50	45.6 ± 8.90

Abbreviations: AP: annual precipitation, BIO12; IT: isothermality, BIO3; MTDQ: mean temperature of the driest quarter, BIO9; MTWQ: mean temperature of the wettest quarter, BIO8; PS: precipitation seasonality, BIO15; PWQ: precipitation of the warmest quarter, BIO18; TS: temperature seasonality, BIO4.

Table S3.8.2 Coordinates and climatic data for *Amyema gibberula* var. *tatei* populations sampled across south-western Australia. Latitude and longitude are presented in the Geocentric Datum of Australia (GDA94) coordinate system. Mean ± *SD* are provided for seven least-correlated bioclimatic variables used in environmental association analysis and generalised dissimilarity modelling.

Code	Population	Latitude	Longitude	No.	IT (%)	TS (°C)	MTWQ (°C)	MTDQ (°C)	AP (mm)	PS (%)	PWQ (mm)
BIL	Billyacatting Hill	-31.042439	117.959293	16	45.4 ± 0.01	555 ± 0.10	11.8 ± 0.00	25.3 ± 0.00	320 ± 0.25	44.5 ± 0.12	44.1 ± 0.25
DEP	Depot Hill	-29.143822	115.346400	16	49.7 ± 0.00	538 ± 0.00	15.3 ± 0.00	24.6 ± 0.00	397 ± 0.00	76.2 ± 0.00	39.0 ± 0.00
GAB	Gabyon	-28.043533	116.736323	10	44.6 ± 0.06	639 ± 0.28	14.3 ± 0.00	23.7 ± 0.00	249 ± 1.03	49.3 ± 0.14	57.0 ± 0.00
JIB	Jibberding	-30.000985	116.826976	16	45.8 ± 0.01	586 ± 0.02	14.0 ± 0.00	25.2 ± 0.01	298 ± 0.34	53.0 ± 0.09	36.0 ± 0.00
KOO	Koolanooka	-29.193801	116.228194	16	45.0 ± 0.00	588 ± 0.00	14.3 ± 0.00	22.3 ± 0.00	320 ± 0.00	55.3 ± 0.00	46.0 ± 0.00
KOR	Koora	-31.260612	120.011578	16	45.6 ± 0.00	544 ± 0.00	11.3 ± 0.00	22.2 ± 0.00	292 ± 0.00	43.6 ± 0.00	47.0 ± 0.00
NIN	Ninghan	-29.172953	117.660768	12	43.7 ± 0.11	629 ± 1.93	13.5 ± 0.07	19.7 ± 2.01	285 ± 0.90	46.3 ± 0.58	52.7 ± 0.49
SAN	Sandford Rocks	-31.242659	118.760163	16	45.4 ± 0.00	558 ± 0.00	12.3 ± 0.00	23.1 ± 0.00	335 ± 0.00	40.5 ± 0.00	53.0 ± 0.00
WOO	Woolgorong	-27.678167	115.789340	12	46.0 ± 0.09	615 ± 1.38	15.2 ± 0.11	23.8 ± 0.14	253 ± 2.84	52.6 ± 0.22	45.5 ± 0.52
YAN	Yanneymooning	-30.708080	118.547847	12	45.0 ± 0.03	584 ± 0.60	11.6 ± 0.02	24.3 ± 0.04	266 ± 0.79	47.2 ± 0.15	37.2 ± 0.39
YOR	Yorkrakine Rock	-31.421116	117.512880	12	45.5 ± 0.03	548 ± 0.30	12.9 ± 0.01	23.2 ± 0.03	320 ± 0.58	51.1 ± 0.19	40.0 ± 0.00
TOTAL					45.7 ± 1.46	577 ± 32.0	13.3 ± 1.37	23.5 ± 1.63	308 ± 40.4	51.1 ± 9.69	44.9 ± 6.36

Abbreviations: AP: annual precipitation, BIO12; IT: isothermality, BIO3; MTDQ: mean temperature of the driest quarter, BIO9; MTWQ: mean temperature of the wettest quarter, BIO8; PS: precipitation seasonality, BIO15; PWQ: precipitation of the warmest quarter, BIO18; TS: temperature seasonality, BIO4.

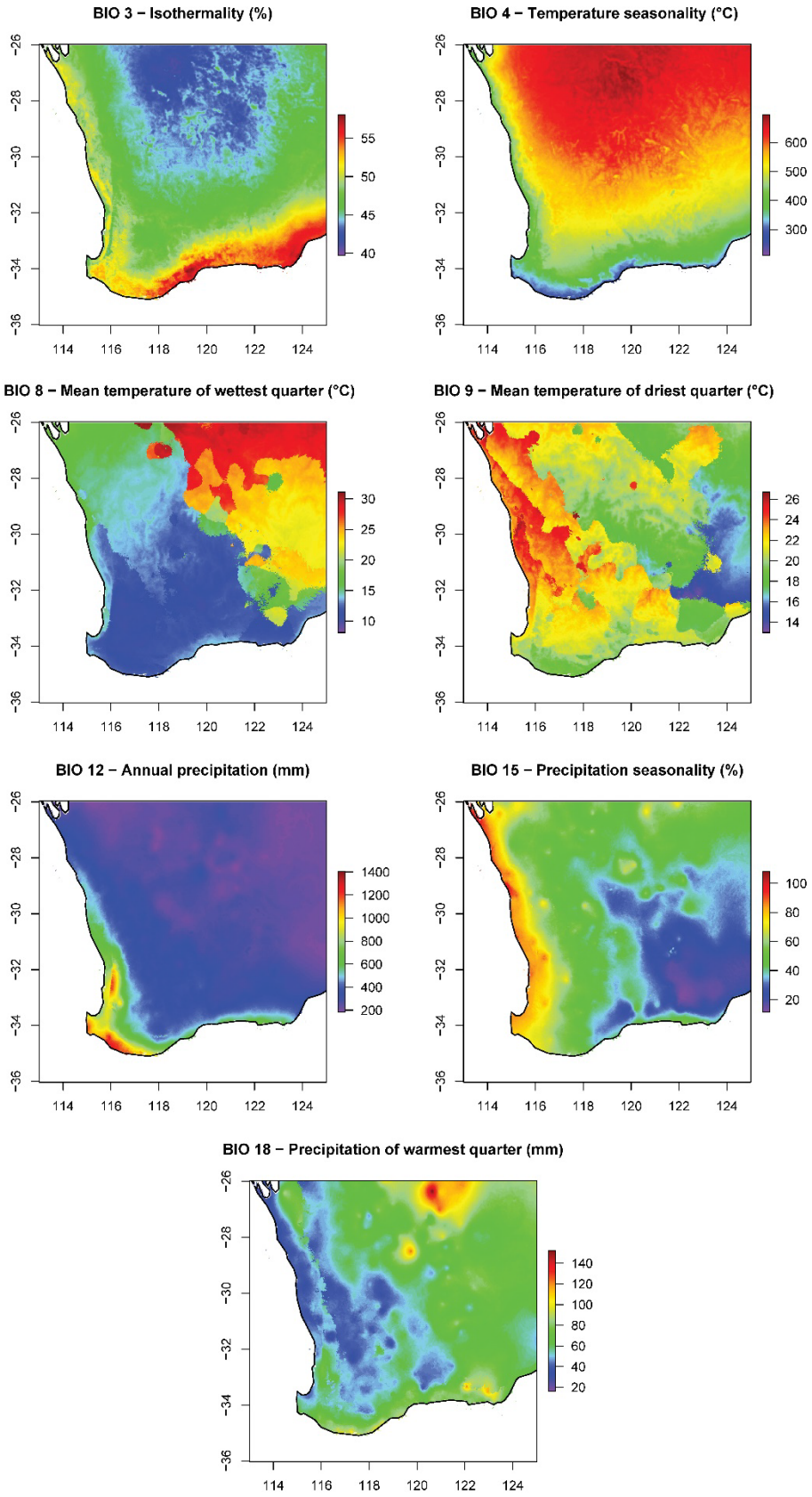


Figure S3.8.1 Climate maps of the seven uncorrelated climatic variables in south-western Australia.

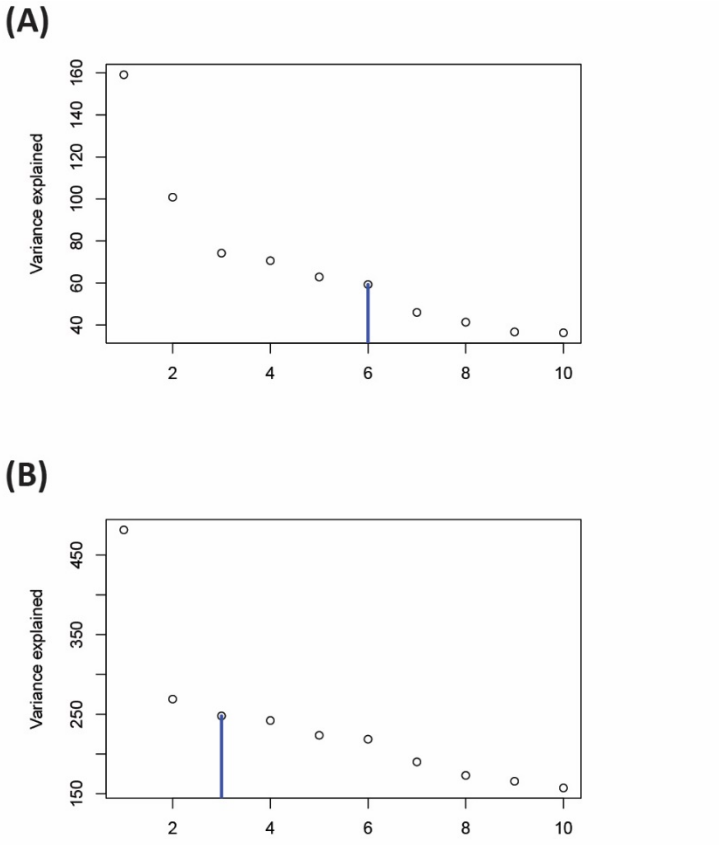


Figure S3.8.2 Screeplots for principal component analysis of (A) *Hakea recurva* subsp. *recurva* and (B) *Amyema gibberula* var. *tatei*.

CHAPTER 4

NUTRIENT ACQUISITION STRATEGY AFFECTS SPATIAL PATTERNS OF GENETIC VARIATION

4.1 Preface

*This chapter consists of a manuscript under review titled '**Nutrient acquisition strategy affects spatial patterns of genetic variation**' [Journal of Biogeography]. The content from section 4.2 onwards is the same as the submitted manuscript with only minor changes in formatting of references and lettering in figures to ensure consistency within the thesis. Permissions to include this work in this thesis are included in Appendix I.*

The introductory chapter (Chapter 1) argues the importance of examining both adaptive (examined in Chapters 2 and 3) and neutral genetic variation to understand patterns of long-term adaptation and persistence within populations and species. Although previous studies have examined neutral genetic variation within host-parasite systems, these patterns have not yet been studied using multiple parasite-autotroph plant pairs with different levels of host-specialisation. The current data chapter (Chapter 4) presents a comparative phylogeography study examining patterns of neutral genetic variation within two parasite-autotroph species pairs, and between two parasites with different levels of host-specialisation. Genome-wide markers were combined with chloroplast sequence data to assess patterns of contemporary and historical genetic diversity, differentiation and structure across the landscape. This chapter makes a significant contribution to this thesis as it demonstrates that both generalist and host-specialised parasitic plants have dissimilar patterns of neutral genetic variation and phylogeographic history compared to their co-occurring autotrophic hosts. This increases our knowledge of genetic diversity patterns in parasitic plants compared to other co-occurring species, and in host-parasite systems more broadly. This work leads into the final data chapter (Chapter 5) that combines the information on both adaptive and neutral genetic variation to delineate genetic provenances under multiple future climate scenarios.

4.1.1 Acknowledgements

This work was supported by the Australia Government through an Australian Government Research Training Program Scholarship and the Australian Research Council Industrial Transformation Training Centre for Mine Site Restoration (project number ICI150100041). We thank Bronwyn Macdonald for assistance with laboratory work and Tony Start for guidance on mistletoe identification.

4.1.2 Data accessibility

Data generated for this study will be made publicly accessible on the DRYAD repository and chloroplast sequences will be archived on Genbank prior to publication of this manuscript.

4.1.3 Author contributions

SJW conducted the study and wrote the manuscript. The research and study design was conceived by SJW, PN, MB, and GW. Samples were collected and processed by SJW. Data analysis was performed by SJW with input from PN, JRPW and MB. The manuscript was edited and approved by all authors.

Walters, S. J., M. Byrne, J. R. P. Worth, G. W. Wardell-Johnson, and P. Nevill. [Under review] Nutrient acquisition strategy affects spatial patterns of genetic variation. *Journal of Biogeography.*

4.2 Abstract

Parasitism is ubiquitous across the tree of life, yet comparative phylogeographic studies of multiple host-parasite pairs are rare. Here, we investigated whether different levels of host-specialisation in parasitic plants result in contrasting genetic diversity and differentiation compared with their host species. Genotyping-by-sequencing and chloroplast sequence data were used to compare phylogeographic patterns in two parasite-autotroph species pairs with overlapping ranges: generalist parasite *Nuytsia floribunda* and sympatric autotroph *Melaleuca rhaphiophylla*, and the host-specific mistletoe *Amyema gibberula* var. *tatei* and its host *Hakea recurva* subsp. *recurva*. We evaluated genetic diversity, population differentiation and phylogeographic patterns within each species pair and between the two parasites. We found lower nuclear genetic diversity and higher population differentiation in both parasitic species compared to the co-occurring autotrophs, and genetic diversity was lowest in the host-specific mistletoe. Haplotype diversity, genetic differentiation and phylogeographic structure were similar within the two pairs of co-occurring species, and all four species showed signals of historical persistence across the landscape. However, fine scale phylogeographic patterns and historical gene flow differed within species pairs and between the two parasites. Lower diversity and higher structure in the parasites compared to hosts at the nuclear level suggests that different nutrient acquisition strategies can affect genetic structure. Stronger signals in the host-specific species compared to the generalist parasite was expected from greater dependence on fewer host species for establishment and survival. While fine scale patterns of phylogeographic variation suggest idiosyncratic responses of species in this ancient landscape, our findings are also indicative of widespread persistence of hosts and parasites. This supports the idea that host-parasite relationships have been maintained for long periods across their ranges and have been similarly affected by genetic drift.

4.3 Introduction

The distribution of genetic variation across the landscape is fundamentally driven by microevolutionary processes that are influenced by a species' life history (Loveless & Hamrick, 1984). Comparative phylogeographic studies can identify associations between genetic variation and life history traits, providing vital information for understanding biogeographic influences on species, and informing conservation and

management (Hoffmann et al., 2015; Broadhurst et al., 2017). For plants, studies comparing genetic diversity and structure between species have focussed on divergent characteristics such as growth form, range size and disjunction, mating system, pollination syndrome and seed dispersal mechanisms (Loveless & Hamrick, 1984; Hamrick & Godt, 1996; Duminil et al., 2007; Broadhurst et al., 2017), but the influence of parasitism has been overlooked. This is despite the presence of parasitism across numerous plant families (Press & Phoenix, 2005) and the importance of parasitic plants within ecosystems globally (Watson, 2001; Press & Phoenix, 2005).

Parasitic plants acquire nutrients from host species via a specialised haustoria and are reliant on hosts for long-term survival (Kuijt, 1969; Calder, 1983). The spatial structure of host populations can play an important role in determining the distribution and genetic structure of parasitic populations (Nadler, 1995; Barrett et al., 2008). Moreover, shared geological and climatic conditions potentially result in parasites and host species with a native, long term association having common evolutionary histories (Thompson, 2005). To date, comparative studies have examined genetic diversity and differentiation on single host-parasite systems both for naturally occurring parasitic plants (e.g., Jerome & Ford, 2002) and other organisms (e.g., Dybdahl & Lively, 1996; McCoy et al., 2005; Feurtey et al., 2016; Hartmann et al., 2020). However, no study has compared multiple host-parasite systems with different levels of host-specificity. Here, we focus on comparing genetic diversity and differentiation of two naturally occurring parasitic plants – one a generalist and the other a host-specific species – with their respective co-occurring autotrophic host species.

In general, parasitic species are expected to have higher population differentiation and lower gene flow between populations than non-parasites (Price, 1980). For instance, Jerome and Ford (2002) observed lower gene flow in a naturally occurring parasitic angiosperm compared to its host species, potentially a result of the parasites' requirement for both a suitable biotic and abiotic environment. However, this is not universally observed in parasitic plants (e.g., Mutikainen & Koskela, 2002) and could be a result of different levels of host-specialisation. Host generalists are more likely to have higher levels of genetic variation, lower population differentiation and less genetic structure than host-specific species (Huyse et al., 2005; Barrett et al., 2008). Specifically, the reliance of host-specific species on fewer hosts may increase the risk

of localised extinction and subsequent loss of genetic diversity, in comparison to generalists that have numerous hosts (Kuijt, 1969; Okubamichael et al., 2016).

Long-term associations between naturally occurring parasitic plants and autotrophic hosts may lead to the sympatric species experiencing the same major geological and historical events, which can result in similar phylogeographic patterns between species (Avise, 2000, 2009). Due to their closer relationship with fewer host species, highly host-specific parasites are more likely to have common phylogeographic patterns with hosts (Nieberding & Olivieri, 2007). Nevertheless, generalist species can still demonstrate phylogeographic patterns concordant with non-parasitic taxa, given the effects of common history on species occupying a common landscape (e.g., Byrne, 2007). Further, as highly host-specific species are less likely to experience demographic stability (Barrett et al., 2008), patterns of phylogeographic diversity and divergence are likely to differ between parasitic species with different levels of host-specialisation.

Here, we aimed to investigate the influence of parasitism and host dynamics on contemporary and historical patterns of genetic diversity, differentiation and phylogeography in two host-parasite systems with a native, long term association and overlapping ranges, across south-western Australia. Firstly, we compared the generalist root parasite *Nuytsia floribunda* (Labill.) R.Br. ex G.Don (Loranthaceae) with the sympatric autotroph *Melaleuca rhaphiophylla* Schauer (Myrtaceae), one of numerous host species (Calladine et al., 2000). This host species was selected as it had a similar distributional area to *N. floribunda*, both species are insect-pollinated with wind/gravity dispersed seed, and they can occur together within the landscape. Secondly, we compared the host-specific mistletoe *Amyema gibberula* var. *tatei* (Blakely) Barlow (Loranthaceae) with its co-occurring host species *Hakea recurva* Meisn. subsp. *recurva* (Proteaceae; Start, 2015). Specifically, we used genotyping-by-sequencing and the same analytical approach to quantify and compare the nuclear genetic diversity, differentiation and genetic structure for each parasite-autotroph pair, and then compared results between the two parasitic species with different host-specialisations. Secondly, we used chloroplast sequence data to quantify and compare the historical phylogeographic patterns for each parasite-autotroph pair, and then compared results between the two parasitic species.

Consistent with the prediction for parasites (Price, 1980), we expected both parasitic plants would have lower nuclear genetic diversity, higher differentiation and lower gene flow than co-occurring autotrophs. However, due to the association between host-specialisation and genetic diversity (Barrett et al., 2008), we predicted that the generalist parasite would have higher nuclear genetic diversity, lower differentiation and have less genetic structure than the host-specific mistletoe. Further, we hypothesized that historical phylogeographic patterns would be similar within the two pairs of co-occurring species, although common patterns are more likely in host-specific species (Nieberding & Olivieri, 2007). Finally, due to the association between host-specialisation and demographic stability (Barrett et al., 2008), we expected lower haplotype diversity and higher differentiation in the host-specific mistletoe.

4.4 Materials and methods

4.4.1 Study species

Two pairs of co-occurring parasitic and autotrophic plant species were examined in this study. The first species pair comprised the generalist root hemiparasite *Nuytsia floribunda* and the sympatric autotrophic species *Melaleuca rhaphiophylla*. The second species pair comprised the obligatory mistletoe *Amyema gibberula* var. *tatei* (hereafter *Amyema gibberula*) and its host species *Hakea recurva* subsp. *recurva* (hereafter *Hakea recurva*). All four species have a widespread distribution across south-western Australia (Figure 4.1). *Nuytsia floribunda* occurs on sandplains, sandy swamps and at the base of rocky outcrops with host plants present nearby. *Melaleuca rhaphiophylla* occurs along sandy waterways and swamps. The occurrence of *A. gibberula* depends on the availability of the host plant. *Hakea recurva* occurs on granite outcrops, rocky ridges and rocky sandplain slopes. All species except *A. gibberula* occur as trees or shrubs to 6 - 10 m in height, are insect pollinated and have wind and/or gravity dispersed seed (Lamont, 1985; Flora of Australia, 1999; Brophy et al., 2013). *Amyema* species are bird-pollinated aerial shrubs with bird-dispersed seed (Calder, 1983). Distribution data was obtained from Florabase records of specimens lodged at the Western Australian Herbarium (https://florabase.dpaw.wa.gov.au) and sampling sites for each species were selected using random sampling to ensure sites were independent (> 50 km separation).

4.4.2 Sample collection and DNA extraction

Leaf tissue was collected for genetic analysis from 265 *N. floribunda*, 272 *M. rhaphiophylla*, 154 *A. gibberula* and 160 *H. recurva* plants. A total of 17 sites were sampled for *N. floribunda* and *M. rhaphiophylla*, 11 sites for *A. gibberula* and 10 sites for *H. recurva*. The number of individuals varied from 10–16 per site (Table S4.9.1, Table S4.9.2, Table S4.9.3 and Table S4.9.4 in Supplementary Information). Where possible, a minimum distance of 20 m between individuals was used for the autotrophic species and the mistletoe to avoid sampling related individuals. As *N. floribunda* is clonal (Pate, 1995), a distance of 50 m (or 30 m in sites with few individuals) was applied to avoid sampling the same, or related, individuals. The collection of *N. floribunda* individuals was not limited to those with a *M. rhaphiophylla* host, with individuals likely to have numerous haustoria connections with a range of host plants. Furthermore, a few sites of *N. floribunda* were also outside the distributional range of *M. rhaphiophylla* but were sampled to capture the full geographical and climatic range of the species. For *A. gibberula*, only one mistletoe was collected from each *H. recurva* host and, where possible, host plants were sampled together with mistletoes. Approximately 61% of *H. recurva* individuals were collected with a respective mistletoe. Tissue samples were stored on silica gel and the location of each individual recorded using a GARMIN eTrex 10 GPS.

Several methods were used for DNA extractions. Genomic DNA from ~10 mg *N. floribunda* and ~8 mg *M. rhaphiophylla* leaf tissue was extracted at Diversity Arrays Technology Pty Ltd (Canberra, Australia) for nuclear analysis using an in-house extraction protocol. DNA for *N. floribunda* chloroplast analysis was extracted using the Invisorb DNA Plant HTS 96 Kit (Stratec Molecular, Germany). DNA for chloroplast analysis in *M. rhaphiophylla* and for nuclear and chloroplast analysis in *A. gibberula* and *H. recurva* were extracted from ~40 mg leaf tissue using the CTAB method of Doyle and Doyle (1990) with the addition of 2% PVP (polyvinylpyrrolodine) and 0.2% β-mercaptoethanol to the extraction buffer. Extracted DNA was quantified using a QUBIT 2.0 Fluorometer (Life Technologies) and visualised on a 1% agarose gel. DNA concentration above 80 ng/uL were diluted to 70 ng/uL for nuclear genotyping and a 1:20 dilution was used for chloroplast sequencing.

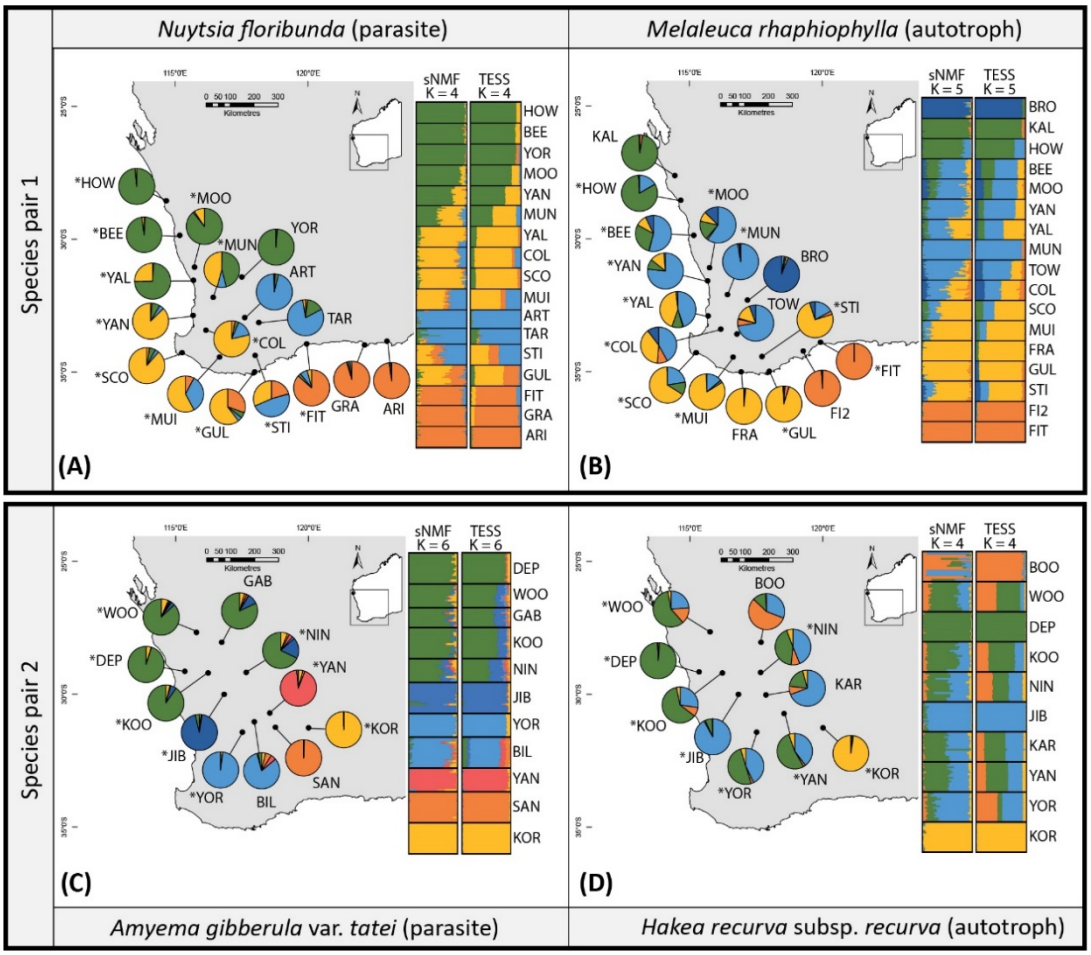


Figure 4.1 Population genetic structure for two pairs of sympatric parasitic and autotrophic plant species in south-western Australia. Generalist parasite (A) *Nuytsia floribunda* (*n* = 264) was paired with sympatric autotroph (B) *Melaleuca rhaphiophylla* (*n* = 272), and host-specific (C) *Amyema gibberula* var. *tatei* (*n* = 154) paired with host (D) *Hakea recurva* subsp. *recurva* (*n* = 160). Bar charts show results from sNMF and TESS analysis of individual membership proportions. Asterisks indicate sites sampled for both species within each species pair.

4.4.3 Nuclear DNA genotyping

Nuclear DNA was genotyped at Diversity Arrays Technology Pty Ltd using high-density reduced-representation sequencing to detect single nucleotide polymorphisms (SNPs; Sansaloni et al., 2011; Kilian et al., 2012). At least 3.5% of samples from each species were replicated across plates and processed independently to check for repeatability of genotyping between plates. Double digest complexity reduction was performed using a combination of methylation-sensitive restriction enzymes (*Pst*I/*Hpa*II for *M. rhaphiophylla* and *A. gibberula*, and *Pst*I/*Mse*I for *N. floribunda* and *H. recurva*) and uniquely barcoded adaptors, with digestion and adaptor ligation described by Kilian et al. (2012). High density sequencing was performed on the Illumina HiSeq 2500 platform with sequences for these nonmodel species aligned *de*

novo using Diversity Arrays Technology's propriety analytical pipeline. Poor-quality sequences (Phred score < 30) were removed and identical sequences collapsed prior to SNP marker calling in the propriety DARTSOFT14 pipeline. Approximately 25% of samples per species were re-genotyped to calculate reproducibility at each locus (RepAvg). NCBI BLAST (Camacho et al., 2009) was used to identify and remove microbial DNA from barcoded sequences, which were trimmed and split into individual data.

Further quality control filtering was performed in the R package DARTR version 1.1.11 (Gruber et al., 2018) to ensure only high-quality SNPs were used for nuclear analysis. First, similarity between replicates of individuals were examined and replicated samples subsequently removed from the dataset. We then filtered datasets for each species to retain: (a) loci with call rates > 95%; (b) RepAvg score > 0.98; (c) minor allele frequency > 5%; (d) individuals with < 20% missing data; (e) only one loci on the same SNP fragment. Finally, to produce a neutral dataset for analysis, we removed all loci previously identified as putatively adaptive for all four species (Walters et al., 2020, 2021); although previous studies have found similar levels of nuclear genetic diversity and differentiation when using all SNPs and only neutral SNPs (e.g., Van Wyngaarden et al., 2017).

4.4.4 Single nucleotide polymorphism data analysis

Prior to nuclear genetic diversity analysis, SNP datasets were subsampled to correct for different numbers of SNPs obtained for each species. Datasets were randomly subsampled to 1,500 SNPs using the *seploc* function in the R package ADEGENET version 2.1.2 (Jombart, 2008; Jombart & Ahmed, 2011). Genetic diversity parameters of observed heterozygosity (H_O), within-population gene diversity (H_S), overall gene diversity (H_T) and inbreeding coefficient (F_{IS}) were estimated for the subsampled datasets in the R package HIERFSTAT version 0.04-22 (Goudet, 2005). Further, to correct for uneven sample sizes, estimates of allelic richness (A_R) were calculated using rarefaction to the smallest sample size (i.e., ten) in ADZE (Szpiech et al., 2008). Population-level diversity parameters were compared: (a) within parasite-autotroph species pairs; and (b) between the two parasitic species, using Mann-Whitney-Wilcoxon independent samples test ($\alpha = 0.05$) performed in the R package CAR version 3.0-2 (Fox & Weisberg, 2019).

Population differentiation (pairwise F_{ST}) for nuclear data was calculated in the R package HIERFSTAT (Goudet, 2005) using the full filtered datasets according to the F_{ST} method by Weir and Cockerham (1984), which is appropriate for genetic markers with few allelic states (Meirmans & Hedrick, 2011). The resulting distance matrices were used to build unrooted neighbour-joining trees for each species using the *bionj* algorithm (Gascuel, 1997) in the R package APE version 5.3 (Paradis & Schliep, 2018), which is highly informative at displaying population structure (Kalinowski, 2011). We also performed an analysis of molecular variance (AMOVA), implemented in the R package PEGAS version 0.12 (Paradis, 2010) with 1,000 permutations, to partition genetic variation within and among populations for each species.

Where paired species occurred at the same site, we used Mantel tests implemented in the R package VEGAN version 2.5-6 (Oksanen et al., 2019) with Pearson's correlation coefficient and 9,999 permutations to test for correlations between pairwise genetic distance matrices of paired species measured as $F_{ST}/(1-F_{ST})$. We also used Mantel tests to test for correlation between genetic data and environmental distance across the sampled area. Population-level genetic distances were again measured as $F_{ST}/(1-F_{ST})$ with geographical distance calculated in the R package FOSSIL version 0.3.7 (Vavrek, 2011) and converted to the natural logarithm for analysis.

To further investigate population structure we used two clustering methods; sparse non-negative matrix factorization (sNMF; Frichot et al., 2014) and TESS3 ancestry estimation (Caye et al., 2016), both of which use least-square estimates of ancestry coefficients. Both were implemented using R packages, sNMF in LEA version 2.4 (Frichot & François, 2015) and TESS3 in TESS3R version 1.1 (Caye et al., 2016). We tested values of ancestral population groups (K) between one and 12 with ten replicates run for each species. For sNMF we used the default alpha regularization parameter with 5% masked genotypes. Average cross entropy values (sNMF) and cross-validation scores (TESS3) were plotted with the value of K selected as the point of inflection, or where the decrease in cross entropy values became minimal (François, 2016).

4.4.5 Chloroplast DNA sequencing

For each species, a subset of eight individuals per site were selected for chloroplast DNA sequencing. Twelve chloroplast regions previously shown to have variability in

Australian flora (Byrne & Hankinson, 2012) were screened on a subsample of individuals for each species to examine variability of the chloroplast regions. Subsamples comprised eight individuals across four sampling sites (three sites from the range edge and one from the range centre) selected to maximise identification of regions showing variation. We tested seven intergenic spacer regions (ndhF-rpl32, rpl32-trnL, trnV-ndhC, trnQ-rps16, trnS-trnG52S, psbD-trnT, psbA-trnH), one intron (trnG-trnG52G) and five D-loop regions (rpl16-F71R1516, petB-sak23Fsak24R, petD-sak17Fsak18R, atpF-sak21Fsak22R, nhdA-sak26Fsak28R).

DNA amplification was carried out in 25 μL volumes with 5 – 10 ng of template DNA, 0.5 U of Taq DNA polymerase (Invitrogen), 5 μL of 5 × PCR buffer (Invitrogen), F and R primers (2.5 μM for intergenic and intron primers; 12.5 μM for D-loop primers), and $MgCl_2$ (1.5 mM for trnG intron, trnS-trnG52G spacer and all D-loop regions; 2.5 mM for remaining intergenic spacers except 3.0 mM trnV-ndhC). Amplifications were performed with 35 cycles in a Mastercycler ep thermal cycler (EppendorfAG, Hamburg, Germany), following the PCR cycling conditions described by Shaw et al. (2007). D loop primers and the trnG intron were annealed at 50°C and all intergenic spaces were annealed at 52°C except psbA-trnH that was annealed at 55°C. Raw PCR products were run on gel electrophoresis and regions that successfully amplified were sequenced using forward and reverse primers at the Australian Genome Research Limited Facility (Perth, Australia).

Three regions for each species were selected for further analysis to maximise the amount of variation while retaining adequate sequence quality (regions listed in Table S4.9.5). Remaining individuals selected for chloroplast DNA sequencing were amplified for the selected regions and sequenced using the protocols described above. Sequences were assessed for read quality, edited and aligned by region using MAFFT version 7.450 (Katoh et al., 2002; Katoh & Standley, 2013) in GENEIOUS PRIME version 2020.0.5 (Biomatters, Ltd., New Zealand). Aligned sequences were then trimmed to equal lengths for each region and the three regions concatenated for each individual.

4.4.6 Chloroplast DNA phylogeographical analysis

Diversity statistics for chloroplast DNA were calculated in DNASP version 6 (Rozas et al., 2017) including the number of haplotypes (h), number of private haplotypes (h_p), haplotype diversity (H_D), nucleotide diversity (π) and neutrality tests of historical

population stability (Tajima's *D*, Fu's F_S statistic, and Ramos-Osnins and Roazas R_2 test). Significance of neutrality values were assessed using a null distribution of 10,000 coalescent-based simulations. For diversity analysis, indels were coded as multistate characters according to the modified complex indel coding method of Simmons and Ochoterena (2000) implemented in SEQSTATE version 1.4.1 (Müller, 2005). Haplotype richness (H_R) was calculated for each species by rarefaction to the minimum sample size (i.e., 80) in ADZE (Szpiech et al., 2008). Estimates of ordered (N_{ST}) and unordered (G_{ST}) population differentiation, and the presence of phylogeographic structure within species (i.e., when $N_{ST} > G_{ST}$), were calculated using 1,000 random permutations in SPADS version 1.0 (Dellicour & Mardulyn, 2014) and evaluated for structure according to Pons and Petit (1996). These diversity statistics were also calculated for the simple sequence repeat (cpSSR) regions only, which can have faster rates of evolution than other markers.

Haplotype relationships were visualised using the median joining maximum parsimony network approach (Bandelt et al., 1999) implemented in NETWORK version 10.1.0.0 (Fluxus Technology Ltd, 2020). Indels were reduced to one character in length, all characters had equal weighting and ε was set to 0. The post-processing MP option (Polzin & Daneshmand, 2003) was applied to identify the shortest network trees and haplotypes were mapped across the species range. This process was conducted firstly for all chloroplast markers and then repeated for the base pair substitutions only as they have a slower rate of evolution and may reflect historical structuring more accurately than other markers. To further assess phylogeographic structure, we used spatial analysis of molecular variance (SAMOVA) (Dupanloup et al., 2002), which uses a simulated annealing procedure to identify groups of populations that are maximally differentiated but geographically homogenous. SAMOVA analysis was implemented in SPADS version 1.0 (Dellicour & Mardulyn, 2014) to calculate the proportion of genetic variance between groups of populations (φ_{CT}) for the number of groups between two and the total number of sampling sites minus one with 10,000 iterations and 10 repetitions.

4.5 Results

4.5.1 Single nucleotide polymorphism data analysis

Genotyping-by-sequencing produced: (a) 36,881 nuclear SNPs for the generalist parasite and 52,450 SNPs for the sympatric autotroph; and (b) 15,187 SNPs for the host-specific mistletoe and 118,880 SNPs for its host. Replicates for all species had greater than 97% genetic similarity except for one *Nuytsia floribunda* and one *Melaleuca rhaphiophylla* replicate (88.5 and 82.4% similarity, respectively). For the specified order of filtering, the parameter that resulted in the greatest reduction in SNPs for all species was call rate, which reduced the number of SNPs by 47-75%. Together, the call rate, RepAvg and MAF filters removed > 80% of SNPs, and all individuals were retained except one *N. floribunda* individual. The working datasets produced after filtering consisted of: (a) 6,670 SNPs for the generalist parasite (n = 264) and 5,209 SNPs for the sympatric autotroph (n = 272); and (b) 1,631 SNPs for the host-specific mistletoe (n = 154) and 14,848 SNPs for its host (n = 160).

Nuclear genetic diversity parameters of subsampled datasets varied within the two pairs of co-occurring species (Table 4.1). Allelic richness, observed heterozygosity and within population diversity were all significantly lower in the generalist parasite compared to the sympatric autotroph, while inbreeding coefficient was significantly higher ($p < 0.05$). For each species, sampling sites on the northern and eastern edges of the range had the lowest genetic diversity: these were Yorkrakine Rock and Cape Arid for the generalist parasite (Table S4.9.1); and Fitzgerald River and Fitzgerald River 2 for the autotroph (Table S4.9.2). Allelic richness, observed heterozygosity and within population diversity were all significantly lower in the host-specific parasite compared to its host ($p < 0.05$) but no difference was detected between inbreeding coefficients. Genetic diversity was lowest in the most south-eastern site (Koora) for both species (Table S4.9.3 and Table S4.9.4). Low genetic diversity was also observed in other southern sampling sites for the mistletoe, but not the host. Overall, all nuclear genetic diversity parameters except the inbreeding coefficient were significantly higher in the generalist parasite compared to the host-specific mistletoe ($p < 0.05$; Table 4.1).

Table 4.1 Nuclear diversity statistics and significance test results for two pairs of sympatric parasitic and autotrophic plant species in south-western Australia. (A) Generalist parasite *Nuytsia floribunda* (n = 264) and sympatric autotroph *Melaleuca rhaphiophylla* (n = 272). (B) Host-specific mistletoe *Amyema gibberula* var. *tatei* (n = 154) and host species *Hakea recurva* subsp. *recurva* (n = 160). Datasets for all species were randomly subsampled to 1,500 SNPs and estimates of allelic richness (A_R) were rarefied to the smallest sample size.

	Species	A_R	H_O	H_S	H_t	F_{IS}
(A)	*Nuytsia floribunda*	1.63 ± 0.01	0.229 ± 0.005	0.248 ± 0.005	0.301	0.046 ± 0.008
	Melaleuca rhaphiophylla	1.72 ± 0.01	0.355 ± 0.007	0.286 ± 0.005	0.317	-0.244 ± 0.009
	Pairwise comparison	***	***	***	-	***
(B)	*Amyema gibberula* var. *tatei*	1.49 ± 0.01	0.160 ± 0.005	0.193 ± 0.005	0.288	0.087 ± 0.010
	Hakea recurva subsp. *recurva*	1.70 ± 0.01	0.236 ± 0.005	0.268 ± 0.005	0.290	0.098 ± 0.008
	Pairwise comparison	***	***	**	-	NS
Pairwise comparison between parasites		*	**	*	-	NS

Parameters: A_R = allelic richness, H_O = observed heterozygosity, H_S = within population gene diversity, H_T = overall gene diversity, and F_{IS} = fixation index. All means are presented with ± 1 *SE*. Mann-Whitney-Wilcoxon independent samples test (α = 0.05) were used for pairwise significance testing within each species pair and between the two parasitic species (*** = $p < 0.001$; ** = $0.001 \leq p < 0.01$; * = $0.01 \leq p$ 0.05; *NS* = not significant).

Nuclear population differentiation was stronger in the generalist parasite (global F_{ST} = 0.185, pairwise = 0.035 - 0.420; Figure S4.9.1A) than the sympatric autotroph (global F_{ST} = 0.102; pairwise = 0.022 - 0.290; Figure S4.9.1B). Similarly, the host-specific mistletoe had stronger nuclear population differentiation (global F_{ST} = 0.359; pairwise = 0.088 - 0.638; Figure S4.9.1C) than its host (global F_{ST} = 0.087; pairwise = 0.022 - 0.211; Figure S4.9.1D). Overall, the host-specific mistletoe was the most strongly differentiated species, with a global F_{ST} twice that of the generalist parasite. Additionally, AMOVA revealed significant ($p < 0.05$) partitioning of genetic variation by populations for all species with similar proportions for the generalist parasite (29.7%) and co-occurring autotroph (23.5%), but much greater differentiation in the host-specific mistletoe (49.1%) compared to its host (14.7%).

Mantel tests showed significant association between pairwise genetic distance matrices of the 12 paired sampling sites for the generalist parasite-autotroph pair and 8 paired sampling sites for mistletoe-host pair ($p < 0.05$; Figure S4.9.2). However, significant isolation by distance relationships were also detected in all four species ($p < 0.05$), which could explain the association between genetic distance matrices. Specifically, geographical distance explained: (a) 58.1% of the variation in genetic distance for the generalist parasite and 38.4% for the co-occurring autotroph; and (b) 26.0% for the host-specific mistletoe and 49.4% for its host.

Structure analysis indicated the presence of four groups for the generalist parasite and five groups for the sympatric autotroph (Figure 4.1A, B). Neighbour-joining trees for both species showed the northern and southern sites to be most diverged for both species while sampling sites in the centre of the species' range were more closely related (Figure 4.2A, B), reflecting a pattern of isolation by distance. While the divergence of populations in the generalist parasite was gradual across the species range, the two eastern-most sites for the autotroph (Fitzgerald River and Fitzgerald River 2) had stronger divergence compared to other populations. In contrast, structure analysis indicated the presence of six groups for the host-specific mistletoe and four groups for its host (Figure 4.1C, D). Neighbour-joining trees showed mistletoe populations in the southern half of the species' range to have greater divergence than northern populations (Figure 4.2C). For the host, neighbour-joining trees showed the south-eastern population (Koora) to be the most diverged with remaining populations more closely related (Figure 4.2D). Mixing of inferred clusters within populations was also lower for the parasites, particularly for the host-specific species, compared to autotrophic host species.

4.5.2 Chloroplast DNA phylogeographical analysis

Chloroplast regions assessed for each species varied in length (see Table S4.9.5) with a total of: (a) 2,143 bp for the generalist parasite and 2,357 bp for the sympatric autotroph; and (b) 2,161 bp for the host-specific mistletoe and 2,755 bp for its host. Number of haplotypes and rarefied haplotype richness varied between the two species pairs with similar values for co-occurring species (Table 4.2). The generalist parasite had more variable sites but a similar number of haplotypes overall and per sampling site (Table S4.9.1) as the co-occurring autotroph (Table S4.9.2). In contrast, the host-specific mistletoe had fewer variable sites, fewer haplotypes overall and per sampling site (Table S4.9.3), than its co-occurring host (Table S4.9.4). Overall, the generalist parasite had proportionally more variable sites, more haplotypes and higher rarefied haplotype richness than the host-specific mistletoe when considering all chloroplast markers. However, when considering only the cpSSR regions, both parasitic species had the same number of variable sites and haplotypes (Table S4.9.6).

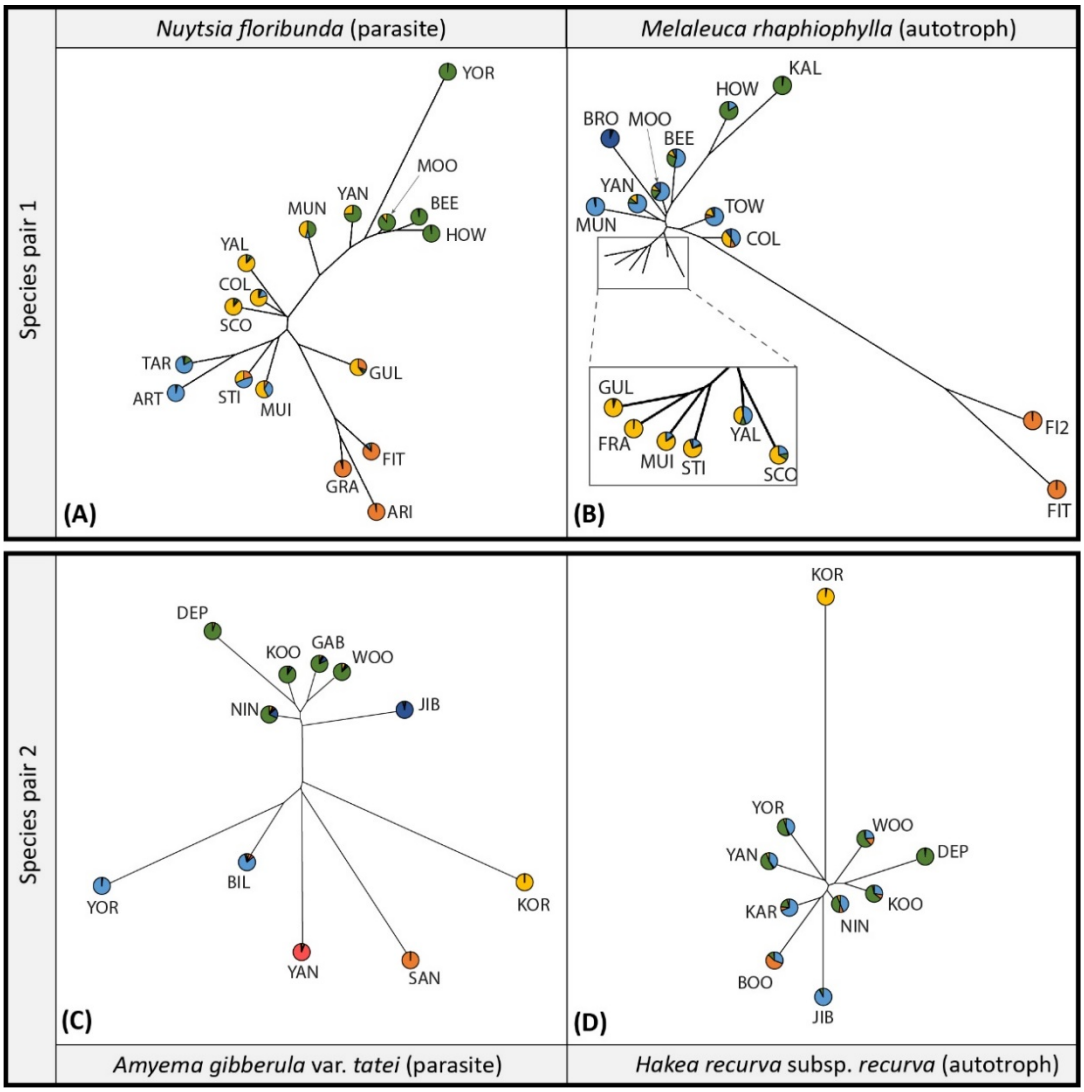


Figure 4.2 Unrooted neighbour-joining trees for two pairs of parasitic and autotrophic plant species. Generalist parasite (A) *Nuytsia floribunda* (6,670 SNPs) was paired with sympatric autotroph (B) *Melaleuca rhaphiophylla* (5,209 SNPs), and host-specific (C) *Amyema gibberula* var. *tatei* (1,631 SNPs) paired with host (D) *Hakea recurva* subsp. *recurva* (14,848 SNPs). Pairwise genetic distances were based on Weir and Cockerham's (1984) F_{ST} values and branch lengths are scaled to genetic distance between populations. The terminus of each branch represents a sampling site and pie charts are identical to those from Figure 4.1.

Mean haplotype and nucleotide diversity was greater in the autotroph compared to the generalist parasite (Table 4.2). Four sites of the generalist parasite contained only one haplotype with the greatest number of haplotypes being three at Cape le Grand and Yanchep (Table S4.9.1). All three haplotypes were private to Yanchep. Ten sites for the autotroph contained only one haplotype, four of which were private. The greatest number of haplotypes was at Yalgorup, with all three haplotypes unique to the site (Table S4.9.2). In contrast, mean haplotype and nucleotide diversity were similar between the host-specific mistletoe and host (Table 4.2). Six sites of the host-specific mistletoe contained only one haplotype while the other five sites contained two

haplotypes. Six haplotypes were unique to a single mistletoe site (Table S4.9.3). One site of the host contained only one haplotype. The greatest number of haplotypes was five in Koolanooka, two of which were private (Table S4.9.4). Overall, levels of mean haplotype and nucleotide diversity were marginally higher in the generalist parasite compared to the host-specific mistletoe. Tests of neutrality were not significant for any four species although Ramos-Osnins and Raza's R_2 statistic was universally lower in the mistletoe-host pair. Estimates of overall chloroplast population differentiation (G_{ST} and N_{ST}) were significant for all four study species ($p < 0.05$).

Table 4.2 Chloroplast haplotype diversity parameters, genetic differentiation parameters and neutrality tests for two pairs of sympatric parasitic and autotrophic plant species in south-western Australia. (A) Generalist parasite *Nuytsia floribunda* (*n* = 136) and sympatric autotroph *Melaleuca rhaphiophylla* (*n* = 136). (B) Host-specific mistletoe *Amyema gibberula* var. *tatei* (*n* = 88) and host species *Hakea recurva* subsp. *recurva* (*n* = 80).

	Species	*S*	*h*	H_R	H_D	π	D	F_S	R_2	G_{ST}	N_{ST}	N_{ST} -G_{ST}
(A)	*Nuytsia floribunda*	112	16	14.3	0.783 ± 0.028	0.00101 ± 0.00006	-0.663 (p = 0.288)	-4.515 (p = 0.062)	0.127 (p = 0.863)	0.589 (p = 0.0)	0.705 (p = 0.0)	0.116 (p = 0.003)
	Melaleuca rhaphiophylla	31	17	15.9	0.923 ± 0.007	0.00230 ± 0.00006	1.158 (p = 0.903)	0.713 (p = 0.651)	0.142 (p = 0.963)	0.799 (p = 0.0)	0.890 (p = 0.0)	0.091 (p = 0.005)
(B)	*Amyema gibberula* var. *tatei*	12	9	8.8	0.773 ± 0.035	0.00053 ± 0.00004	-0.907 (p = 0.207)	-2.550 (p = 0.124)	0.074 (p = 0.311)	0.807 (p = 0.0)	0.834 (p = 0.0)	0.026 (p = 0.161)
	Hakea recurva subsp. *recurva*	73	11	11	0.736 ± 0.044	0.00056 ± 0.00006	-1.042 (p = 0.153)	-3.231 (p = 0.078)	0.067 (p = 0.216)	0.230 (p = 0.0)	0.179 (p = 0.0)	-0.052 (p = 0.811)

Parameters: S = total number of variable sites, h = total number of haplotypes, H_R = haplotype richness rarefied to the lowest sample size, H_D = haplotype diversity, π = nucleotide diversity, G_{ST} = unordered genetic differentiation, N_{ST} = ordered genetic differentiation, D = Tajima's D, F_S = Fu's F_S statistic, and R_2 = Ramos– Osnins and Raza's R_2 statistic. Means are presented with ± 1 *SD* and p-values are given in parentheses.

Significant phylogeographical structure ($N_{ST} > G_{ST}$) was detected in the generalist-autotroph species pair, but not for either species in the mistletoe-host pair (Table 4.2). Visual interpretation of the haplotype network and maps indicated more complex patterns in the generalist-autotroph species pair (Figure 4.3A, B) and simpler star-like networks for mistletoe-host pair (Figure 4.3C, D). Geographical structuring was observed for the generalist parasite with two dominant haplotypes: HAP3 that was found in 54 individuals across 9 sampling sites, and HAP1 that was present in 31 individuals across 6 sites (Table S4.9.7). These haplotypes had ancestral positions within the network, and one was dominant in the northern sites and the other in the southern sites, although they co-occurred at one location (Stirling Range; Figure 4.4A). This differed from the sympatric autotroph where geographical structuring was present, but there was no obvious common ancestral haplotype (Table S4.9.7). More divergent haplotypes were observed on the southern extreme of the species range (five mutations from HAP13) and at the Yalgorup site on the western coast that was comprised solely of private haplotypes (three mutations from HAP10; Figure 4.4B). In contrast, networks for the mistletoe-host pair indicated a common ancestral haplotype (HAP1 in both species; Figure 4.4C, D). For the mistletoe, this dominant haplotype was found in 37 individuals across 6 sites with three sampling sites comprising solely of HAP1 (Table S4.9.7; Figure 4.4C). For the host, the dominant HAP1 haplotype was found in 38 individuals across 8 sites with only one site in the southern extreme of the species' range comprising solely of HAP1 (Table S4.9.7; Figure 4.4D). Sampling sites in the mistletoe generally had single haplotypes compared to multiple haplotypes in all but one of the sites of the autotroph. Despite these differences within species pairs, simpler patterns of haplotype distribution were observed when considering only base pair substitutions, particularly for the parasitic species (Figure S4.9.3**).** Lastly, SAMOVA analysis identified: (a) 12 groups with the highest genetic differentiation for the generalist parasite ($\varphi_{CT} = 0.758$) and 15 groups for the sympatric autotroph ($\varphi_{CT} = 0.905$); and (b) eight groups for the host-specific mistletoe ($\varphi_{CT} = 0.862$) and four groups for its host ($\varphi_{CT} = 0.261$).

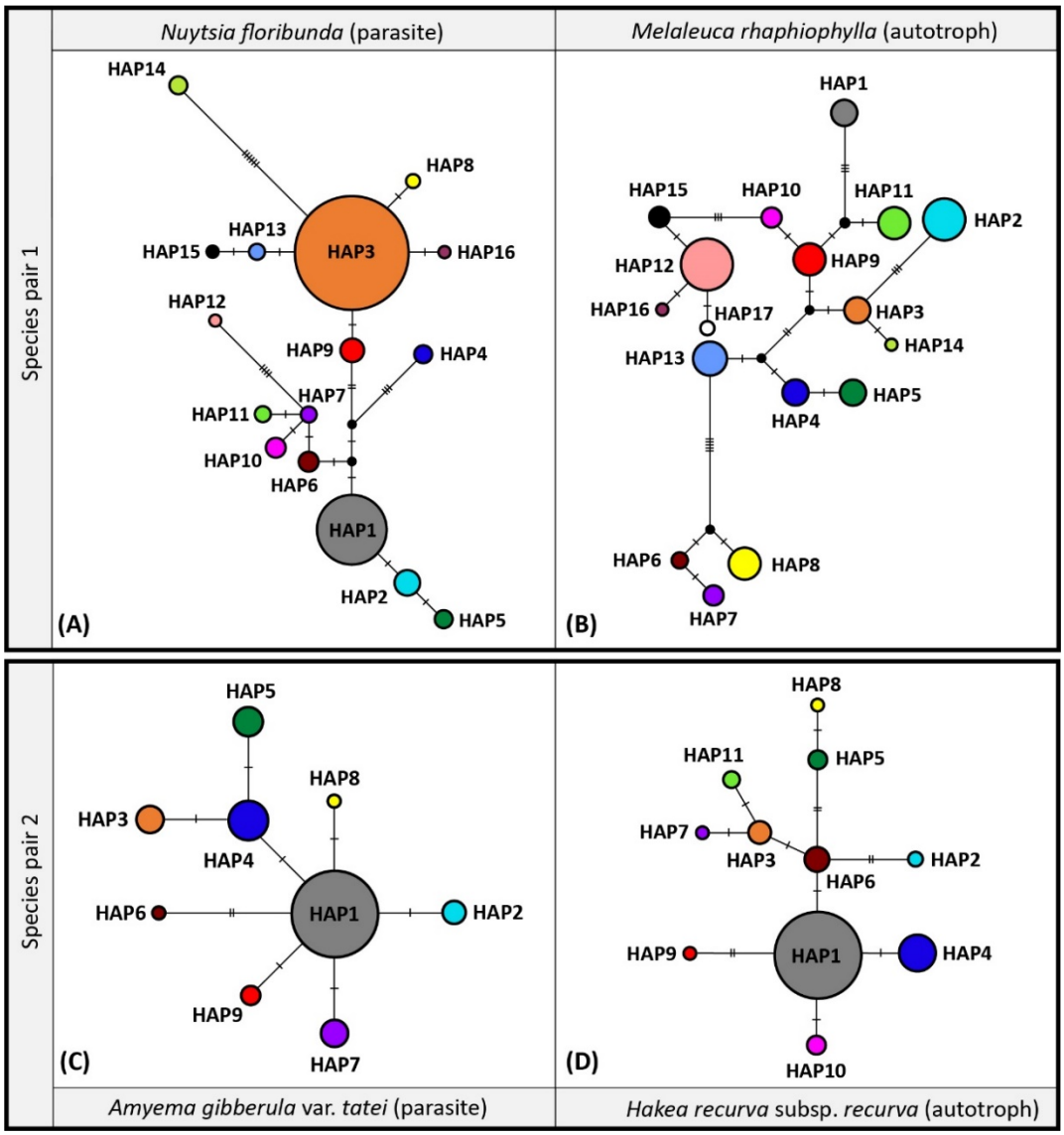


Figure 4.3 Median-joining network of evolutionary relationships between chloroplast DNA haplotypes. Relationships were compared between two pairs of sympatric parasitic and autotrophic plant species in south-western Australia. Generalist parasite (A) *Nuytsia floribunda* (n = 136) was paired with sympatric autotroph (B) *Melaleuca rhaphiophylla* (n = 136), and host-specific (C) *Amyema gibberula* var. *tatei* (n = 88) paired with host (D) *Hakea recurva* subsp. *recurva* (n = 80). Circle colours correspond to the geographical map in Figure 4.4 and circle size proportional to the number of individuals of that haplotype. Branch lengths are approximately proportional to the number of mutations with the cross bar indicating the exact number of mutations. Small black circles indicate unobserved hypothetical haplotypes.

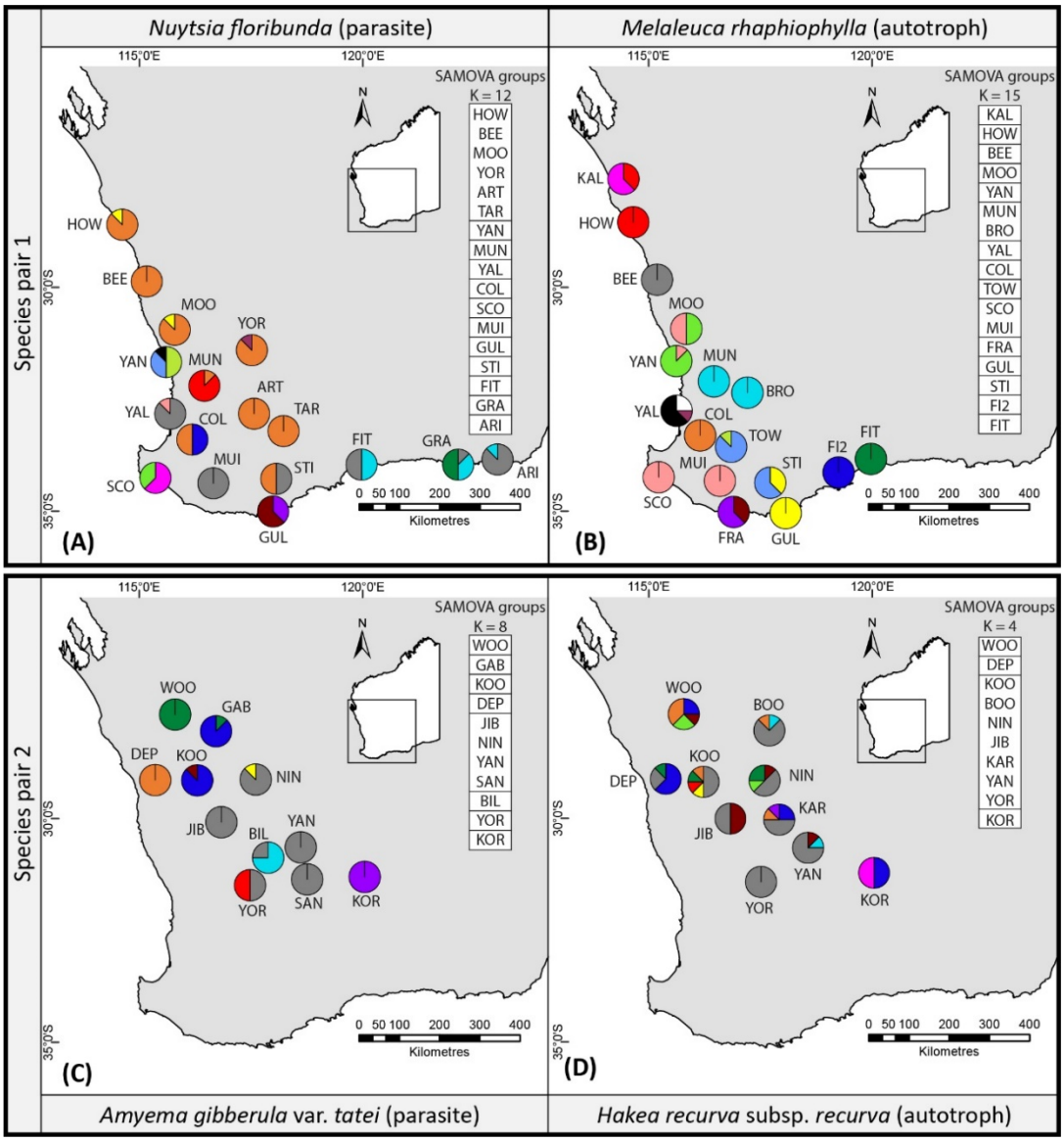


Figure 4.4 Geographical distribution of chloroplast DNA haplotypes for two pairs of sympatric parasitic and autotrophic plant species in south-western Australia. Generalist parasite (A) *Nuytsia floribunda* (*n* = 136) was paired with sympatric autotroph (B) *Melaleuca rhaphiophylla* (*n* = 136), and host-specific (C) *Amyema gibberula* var. *tatei* (*n* = 88) paired with host (D) *Hakea recurva* subsp. *recurva* (*n* = 80). Sampling sites are represented by coloured circles and reflect the geographical distribution in south-western Australia. SAMOVA analysis identified groups of populations with the highest genetic differentiation. Each box represents one group and sites within the same box are in the same SAMOVA group.

4.6 Discussion

This is the first study, to our knowledge, to investigate phylogeographic patterns in two parasite-autotroph plant pairs, despite the occurrence of parasitism across numerous plant families. This provides vital information for understanding biogeographic influences on host-parasite systems. Our analysis supports the expectation that parasitic plants would have lower levels of nuclear diversity and

higher differentiation than co-occurring autotrophs. This was observed in both parasites, although signals were stronger in the host-specific species, and likely reflects small population size and bottleneck effects of parasitic plants due to their dependence on host species for population establishment and maintenance. Broad level patterns of haplotype diversity, genetic differentiation and phylogeographical structure were similar with signals of historical persistence found in all four species, indicating widespread persistence of the host and parasites across their ranges. However, fine scale phylogeographic patterns differed between co-occurring species and between the two parasites indicating some idiosyncratic responses within species.

4.6.1 Contemporary genetic diversity and differentiation

As predicted, the two parasitic species had lower contemporary genetic diversity and higher genetic differentiation than the co-occurring autotrophs. Unlike autotrophic species, parasitic plants can have both biotic requirements for establishment (Fineran & Hocking, 1983; Norton & Carpenter, 1998) and specific climatic niches (e.g., Lira-Noriega & Peterson, 2014; Ramírez-Barahona et al., 2017). This may result in more limited establishment of parasite individuals compared to autotrophic species, which could ultimately reduce population size and levels of genetic diversity. While the generalist parasite had the same pattern as the co-occurring autotroph of low diversity and high differentiation in peripheral sampling sites (Cape Arid, Yorkrakine Rock), consistent with isolation by distance and the central-marginal hypothesis (Eckert et al., 2008), this was not the case for the host-specific species. Although the site with the lowest genetic diversity and highest differentiation in the host-specific species was identical to that in the host (Koora), the majority of the southern mistletoe sampling sites had low genetic diversity and high differentiation (Sandford Rocks, Yanneymooning, Yorkrakine Rock) compared to the northern sites. This comparative pattern differed to that observed for the generalist parasite species pair and could be explained by differences in parasitic life history.

Specifically, host-specialisation may influence genetic diversity levels as host-specific parasites are more constrained by availability of hosts than generalists, and populations may develop from few founding individuals, resulting in lower genetic heterozygosity (Barlow, 1983). Additionally, the host-specific species in this study had more fragmented and isolated populations compared to the other study species. This would make the species more easily affected by random genetic drift and be more likely to

have lower genetic diversity compared to more abundant species (Hamrick et al., 1992). Other explanations for lower nuclear genetic diversity in the host-specific mistletoe could include smaller effective population sizes (Leimu et al., 2006) and increased susceptibility to demographic stochasticity through localised extinction and/or recolonization events compared to generalists (Barlow, 1983; Barrett et al., 2008). Such events would lower nuclear genetic diversity within populations (either through genetic drift or founder effects) and increase genetic differentiation between populations, as we observed in this study.

Despite the expectation that contemporary gene flow would be lower in parasitic species, we found comparatively lower gene flow only in the host-specific species. Low gene flow has been found in studies of mistletoe species in other landscapes (e.g., Jerome & Ford, 2002; Nyagumbo et al., 2017). As genetic structure is influenced by host-specificity (Huyse et al., 2005), it is possible that the differences in genetic structure patterns between species pairs is a result of different levels of parasite host-specialisation. Another explanation is that other life history factors may be influencing contemporary gene flow in parasitic plants. For instance, unlike the generalist that has the same pollination syndrome as the two autotrophic species, the host-specific mistletoe is predominantly bird-pollinated (Calder, 1983). Previous work in the same landscape found lower-than-expected gene flow associated with bird pollinated species that was attributed to territoriality amongst bird species (Nistelberger et al., 2015; Millar et al., 2017). Furthermore, geographic distance explained comparatively less genetic variation in the host-specific mistletoe, which may further suggest that other factors (i.e., dependence on a specific host species, reliance on avian dispersers, climatic requirements and/or other landscape features) may have a greater effect on genetic patterns within this species. To distinguish the influence of parasitism from other life history factors, future work could focus on comparing contemporary gene flow between a generalist and host-specific parasite with the same pollination syndrome.

Patterns of contemporary genetic diversity and differentiation in the autotrophic plant species were comparable to other plant species across these landscapes (e.g., Millar et al., 2016; Millar et al., 2017; Binks et al., 2018). The low genetic diversity, higher inbreeding level and strong differentiation in the eastern population (Fitzgerald River and Fitzgerald River 2) for *M. rhaphiophylla* indicated a restriction of gene flow from

other populations. A similar observation has been made for another widespread species, *Calothamnus quadfridus*, across the same landscape (Binks et al., 2018). In this study, greater differentiation of populations on the species' margins, compared to populations in the centre of the range, indicates a pattern of isolation by distance, and reflects the expectation of the central-marginal hypothesis (Eckert et al., 2008). A similar pattern was also found in *H. recurva* with the highest differentiation, and lowest genetic diversity, in the population on the south-east margin of the species' range (Koora), suggesting isolation of this population within the landscape. This peripheral population occurs on a low-lying granite outcrop that can allow populations to persist within more hostile landscapes (Hopper et al., 1997) and which have been previously found to harbour populations of other species in these landscapes (e.g., Nistelberger et al., 2014; Tapper et al., 2014). Despite geographic distance explaining a greater proportion of genetic variation in the autotrophic species (and the generalist parasite), there were still a large proportion of unexplained variance that could be attributed to other factors. For example, reliance on insect pollinators, climatic factors (i.e., wind direction/strength), and/or topographic features (i.e., direction of water movement across the landscape). While the focus of this study was to compare between autotrophic and parasitic species, future work could use landscape genetic analysis to further examine landscape and climatic effects on gene flow within these species.

4.6.2 Historical diversity, divergence and persistence

Haplotype diversity and genetic differentiation levels in the two autotrophic species also reflect patterns observed in other plant species within mesic and semi-arid regions of south-western Australia (e.g., Byrne et al., 2002; Nistelberger et al., 2014; Dalmaris et al., 2015; Millar et al., 2016, 2017), providing a framework for interpretation of patterns in their parasites. As expected, broad scale phylogeographic patterns were similar in all species with high haplotype diversity across the species and low diversity within sampling sites, consistent with signals of localised persistence observed in many species across this landscape (Byrne, 2007, 2008; Byrne et al., 2014). Compared to the autotroph, the generalist parasite had lower haplotype diversity, genetic differentiation and a greater admixture of haplotypes, indicating comparatively greater connectivity between sites. However, haplotype diversity and genetic differentiation levels were within the range of that observed in other species within this region (Byrne

et al., 2003; Nistelberger et al., 2014; Dalmaris et al., 2015; Llorens et al., 2017) and neutrality tests indicated historical persistence in the generalist parasite populations. The differing patterns could be explained by the wide host range of the generalist parasite that may limit the establishment of congruent phylogeographic patterns with a single host species. In contrast, while the host-specific mistletoe had similar overall haplotype diversity to the host, and neutrality tests for both species indicated historical persistence, genetic differentiation and population-level haplotype diversity levels were not congruent between the species. The mistletoe showed patterns more similar to the generalist parasite and its autotroph where sampling sites generally had a single haplotype and common haplotypes occurring across sites, although the common haplotypes in *A. gibberula* occurred in central sampling sites rather than also across sites at the edge of the range. One explanation for the differing patterns between parasites and their host species could be that parasites are more likely to undergo host-shift speciation rather than co-speciation (de Vienne et al., 2013). Therefore, congruent genetic patterns with a single host species would be rare, even for parasites with a narrow host range. The lack of similarity in the patterns within the host specific parasite and its host may also reflect the effect of fluctuating population dynamics in its dependence on the host even though the high haplotype diversity in the host indicates long term population persistence.

Despite the prediction of lower haplotype diversity in the host-specific species, diversity at the species-level was similar between the two parasites. However, population differentiation was lower in the generalist species, which could be explained by variation in parasitic life histories. Highly host-specific species have lower demographic stability and, therefore, are more likely to experience localised population extinction/recolonization than parasites with a wide host range (Barlow, 1983; Barrett et al., 2008). This may result in loss of local populations over time, which would increase differentiation between remaining populations. A second explanation could be faster generation times in mistletoe species that would increase genetic differentiation more rapidly than the longer-lived generalist species (Huyse et al., 2005). This could also explain the higher–than–expected haplotype diversity and that a greater proportion of haplotypes were derived via mutations at cpSSRs. Notwithstanding the influence of a parasitic life history, these differences in differentiation could also be attributed to other life history traits that effect historical

gene flow and connectivity between populations (Hamrick & Godt, 1996). For instance, unexpectedly low gene flow was been previously found in other fleshy fruited bird-dispersed shrub species that was attributed to behavioural traits and limited migratory behaviour in seed dispersers (Worth et al., 2010).

Congruent patterns of fine scale phylogeographic structure were not observed in either species pair, contrary to that observed in other host-parasite systems (e.g., Feurtey et al., 2016; Hartmann et al., 2020), nor between the autotrophs. This is not unexpected in this ancient landscape where persistence of plant populations across their range is a common response to the climatic cycles of the Pleistocene (Byrne, 2008), as this landscape is old, climatically stable and has remained unglaciated since the Paleozoic Era (Hopper & Gioia, 2004). *Melaleuca rhaphiophylla* had no common haplotypes and predominantly shallow divergence and most sites had one haplotype. The greatest divergence was observed in three sites on the southern margin of the species' range and in the diverse site at Yalgorup, both in areas suggested to have been stable habitat since the early Pleistocene (Byrne, 2008). High genetic differentiation in the species supports the expectation for lower gene flow in taxa with gravity-dispersed seed (Duminil et al., 2007) with neutrality tests and divergence patterns indicating historical persistence of populations in localised refugia. In contrast, *H. recurva* had a common haplotype, but sites also had high diversity and some shared haplotypes, particularly in the central and northern regions of the species' range. Neutrality tests and multiple haplotypes within the same site both indicate historical persistence within this species, with some populations in areas that have been previously identified as historical mesic refugia within the semi-arid landscape (e.g., Koolanooka; Millar et al., 2017). These differences may be species related or may reflect different patterns of phylogeographic structure between the mesic and semi-arid study landscapes. While historical climatic oscillations resulted in fluctuations across both mesic and arid zones (reviewed in Byrne, 2007, 2008), the mesic zone likely retained conditions more conducive to population persistence and connectivity (Byrne et al., 2008). However, the levels of diversity within sites were higher in *H. recurva* that occurs in the semi-arid area compared to *M. rhaphiophylla* that occurs in the more mesic region. This is the opposite of patterns generally found in species where sites in the semi-arid region often show lower diversity than those in the more mesic areas. For example, Tapper et al.

(2014) found higher haplotype diversity in populations in the mesic regions in the granite rock endemic *Stypandra glauca* than in the semi-arid region.

Overall, these results indicate that although phylogeographic history in parasitic plants may be influenced by a complex interaction of life history traits (e.g., parasitism, seed dispersal mechanism), the signals of widespread persistence suggest that host-parasite relationships have been sustained across these landscapes over long periods. Comparing phylogeographic patterns between co-occurring parasitic and autotrophic species provides vital information for understanding biogeographic influences on host-parasite systems. Furthermore, comparing patterns between the two parasites provides information related to their host specificity. However, these patterns could also reflect differences between the mesic and semi-arid study landscapes. Accordingly, further work is needed to disentangle the influence of life history traits on genetic and phylogeographic variation in parasitic plants within the same landscape.

4.7 Conclusion

Parasitism is ubiquitous across the tree of life (Musselman & Press, 1995; Poulin & Morand, 2000; Poulin, 2011), and parasitic plants are functionally important within many ecosystems (Watson, 2001; Press & Phoenix, 2005). This is the first study to compare contemporary and historical patterns of genetic diversity, differentiation and phylogeographic patterns between two pairs of co-occurring parasitic and autotrophic plant species. As such, it provides vital information for understanding biogeographic influences on host-parasite systems. Here, our analysis supports the expectation of lower nuclear genetic diversity and higher population differentiation in parasites compared to sympatric autotrophs, suggesting that different nutrient acquisition strategies can affect genetic structure. Stronger signals in the host-specific species may be the result of host-specialisation influencing demographic structure and gene flow within species (Barrett et al., 2008). Fine scale phylogeographic patterns differed in all four species, suggesting idiosyncratic responses of species in this ancient landscape. Additionally, our findings were also indicative of widespread persistence in all four species, which is a common response for plant species within these landscapes (Byrne, 2008). This also supports the idea that host-parasite relationships have been maintained for a long time across their ranges and have been similarly affected by genetic drift.

4.8 References

Every reasonable effort has been made to acknowledge the owners of the copyright material. I would be pleased to hear from any copyright owner who has been omitted or incorrectly acknowledged.

Avise, J. C. (2000). *Phylogeography: The history and formation of species*. Cambridge, MA: Harvard University Press.

Avise, J. C. (2009). Phylogeography: Retrospect and prospect. *Journal of Biogeography, 36*, 3-15. https://doi.org/10.1111/j.1365-2699.2008.02032.x

Bandelt, H.-J., Forster, P., & Röhl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution, 16*, 37-48. https://doi.org/10.1093/oxfordjournals.molbev.a026036

Barlow, B. A. (1983). Biogeography of Loranthaceae and Viscaceae. In M. Calder & P. Bernhardt (Eds.), *The biology of mistletoes* (pp. 19-46). Sydney: Academic Press Australia.

Barrett, L. G., Thrall, P. H., Burdon, J. J., & Linde, C. C. (2008). Life history determines genetic structure and evolutionary potential of host–parasite interactions. *Trends in Ecology & Evolution, 23*, 678-685. https://doi.org/10.1016/j.tree.2008.06.017

Binks, R. M., Gibson, N., Ottewell, K. M., Macdonald, B., & Byrne, M. (2018). Predicting contemporary range-wide genomic variation using climatic, phylogeographic and morphological knowledge in an ancient, unglaciated landscape. *Journal of Biogeography, 46*, 503-514. https://www.doi.org/10.1111/jbi.13522

Broadhurst, L., Breed, M., Lowe, A., Bragg, J., Catullo, R., Coates, D., . . . Byrne, M. (2017). Genetic diversity and structure of the Australian flora. *Diversity and Distributions, 23*, 41-52. https://doi.org/10.1111/ddi.12505

Brophy, J. J., Craven, L. A., & Doran, J. C. (2013). *Melaleucas. Their botany, essential oils and uses*. Canberra: ACIAR Monograph No. 156. Australian Centre for International Agricultural Research (ACIAR).

Byrne, M. (2007). Phylogeography provides an evolutionary context for the conservation of a diverse and ancient flora. *Australia Journal of Botany, 55*, 316-325. https://www.doi.org/10.1071/BT06072

Byrne, M. (2008). Evidence for multiple refugia at different time scales during Pleistocene climatic oscillations in southern Australia inferred from phylogeography. *Quaternary Science Reviews, 27*, 2576-2585. https://doi.org/10.1016/j.quascirev.2008.08.032

Byrne, M., Coates, D. J., Forest, F., Hopper, S. D., Krauss, S. L., Sniderman, J. M. K., & Thiele, K. R. (2014). A diverse flora: Species and genetic relationships. In H. Lambers (Ed.), *Plant life on the sandplains in southwest Australia: A global biodiversity hotspot* (pp. 81-99). Crawley, Western Australia: UWA Publishing.

Byrne, M., & Hankinson, M. (2012). Testing the variability of chloroplast sequences for plant phylogeography. *Australian Journal of Botany, 60*, 569-574. https://doi.org/10.1071/BT12146

Byrne, M., Macdonald, B., & Brand, J. (2003). Phylogeography and divergence in the chloroplast genome of Western Australian Sandalwood (*Santalum spicatum*). *Heredity, 91*, 389-395. https://doi.org/10.1038/sj.hdy.6800346

Byrne, M., Macdonald, B., & Coates, D. (2002). Phylogeographical patterns in chloroplast DNA variation within the *Acacia acuminata* (Leguminosae: Mimosoideae) complex in Western Australia. *Journal of Evolutionary Biology, 15*, 576-587. https://www.doi.org/10.1046/j.1420-9101.2002.00429.x

Byrne, M., Yeates, D. K., Joseph, L., Kearney, M., Bowler, J., A. J. Williams, M. A. J., . . . Wyrwoll, K.-H. (2008). Birth of a biome: Insights into the assembly and maintenance of the Australian arid zone biota. *Molecular Ecology, 17*, 4398-4417. https://www.doi.org/10.1111/j.1365-294X.2008.03899.x

Calder, D. M. (1983). Mistletoes in focus: An introduction. In M. Calder & P. Bernhardt (Eds.), *The biology of mistletoes* (pp. 1–17). Sydney: Academic Press Australia.

Calladine, A., Pate, J. S., & Dixon, K. W. (2000). Haustorial development and growth benefit to seedlings of the root hemiparasitic tree *Nuytsia floribunda* (Labill.) R.Br. in association with various hosts. *Annals of Botany, 85*, 733-740. https://doi.org/10.1006/anbo.2000.1129

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., & Madden, T. L. (2009). BLAST+: Architecture and applications. *BMC Bioinformatics, 10*, 421. https://doi.org/10.1186/1471-2105-10-421

Caye, K., Deist, T. M., Martins, H., Michel, O., & François, O. (2016). TESS3: Fast inference of spatial population structure and genome scans for selection. *Molecular Ecology Resources, 16*, 540-548. https://www.doi.org/10.1111/1755-0998.12471

Dalmaris, E., Ramalho, C. E., Poot, P., Veneklaas, E. J., & Byrne, M. (2015). A climate change context for the decline of a foundation tree species in south-western Australia: Insights from phylogeography and species distribution modelling. *Annals of Botany, 116*, 941-952. https://www.doi.org/10.1093/aob/mcv044

de Vienne, D. M., Refrégier, G., López-Villavicencio, M., Tellier, A., Hood, M. E., & Giraud, T. (2013). Cospeciation vs host-shift speciation: Methods for testing, evidence from natural associations and relation to coevolution. *New Phytologist, 198*, 347-385. https://doi.org/10.1111/nph.12150

Dellicour, S., & Mardulyn, P. (2014). spads 1.0: A toolbox to perform spatial analyses on DNA sequence data sets. *Molecular Ecology Resources, 14*, 647-651. https://www.doi.org/10.1111/1755-0998.12200

Doyle, J. J., & Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. *Focus, 12*, 13-15.

Duminil, J., Fineschi, S., Hampe, A., Jordano, P., Salvini, D., Vendramin, G. G., & Petit, R. J. (2007). Can population genetic structure be predicted from life-history traits? *The American Naturalist, 169*, 662-672. https://doi.org/10.1086/513490

Dupanloup, I., Schneider, S., & Excoffier, L. (2002). A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology, 11*, 2571-2581. https://www.doi.org/10.1046/j.1365-294X.2002.01650.x

Dybdahl, M. F., & Lively, C. M. (1996). The geography of coevolution: Comparative population structures for a snail and its trematode parasite. *Evolution, 50*, 2264-2275. https://doi.org/10.2307/2410696

Eckert, C. G., Samis, K. E., & Lougheed, S. C. (2008). Genetic variation across species' geographical ranges: The central–marginal hypothesis and beyond. *Molecular Ecology, 17*, 1170–1188. https://www.doi.org/10.1111/j.1365-294X.2007.03659.x

Feurtey, A., Gladieux, P., Hood, M. E., Snirc, A., Cornille, A., Rosenthal, L., & Giraud, T. (2016). Strong phylogeographic co-structure between the anther-smut fungus and its white campion host. *New Phytologist, 212*, 668-679. https://doi.org/10.1111/nph.14125

Fineran, B. A., & Hocking, P. J. (1983). Features of parasitism, morphology and haustorial anatomy in Loranthaceous root parasites. In M. Calder & P. Bernhardt (Eds.), *The biology of mistletoes* (pp. 205-228). Sydney: Adademic Press Australia.

Flora of Australia. (1999). *Flora of Australia Volume 17B, Proteaceae 3, Hakea to Dryandra*. Melbourne: ABRS/CSIRO Australia.

Fluxus Technology Ltd. (2020). Network v10.1.0.0. Retrieved from https://www.fluxus-engineering.com/index.htm

Fox, J., & Weisberg, S. (2019). An R Companion to applied regression. Thousand Oaks, CA: Sage. Retrieved from https://socialsciences.mcmaster.ca/jfox/Books/Companion/

François, O. (2016). Running structure-like population genetic analyses with R. Retrieved from http://membres-timc.imag.fr/Olivier.Francois/tutoRstructure.pdf

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

Frichot, E., Mathieu, F., Trouillon, T., Bouchard, G., & François, O. (2014). Fast and efficient estimation of individual ancestry coefficients. *Genetics, 196*, 973-983. https://www.doi.org/10.1534/genetics.113.160572

Gascuel, O. (1997). BIONJ: An improved version of the NJ algorithm based on a simple model of sequence data. *14*, 685–695. https://www.doi.org/10.1093/oxfordjournals.molbev.a025808

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

Gruber, B., Unmack, P. J., Berry, O. F., & Georges, A. (2018). dartr: An R package to facilitate analysis of SNP data generated from reduced representation genome sequencing. *Molecular Ecology Resources, 18*, 691-699. https://doi.org/10.1111/1755-0998.12745

Hamrick, J. L., & Godt, M. J. W. (1996). Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions of the Royal Society B, 351*, 1291-1298. https://doi.org/10.1098/rstb.1996.0112

Hamrick, J. L., Godt, M. J. W., & Sherman-Broyles, S. L. (1992). Factors influencing levels of genetic diversity in woody plant species. *New Forests, 6*, 95-124. https://doi.org/10.1007/BF00120641

Hartmann, F. E., Snirc, A., Cornille, A., Godé, C., Touzet, P., Van Rossum, F., . . . Giraud, T. (2020). Congruent population genetic structures and divergence histories in anther-smut fungi and their host plants *Silene italica* and the *Silene nutans* species complex. *Molecular Ecology, 29*, 1154-1172. https://www.doi.org/10.1111/mec.15387

Hoffmann, A., Griffin, P., Dillon, S., Catullo, R., Rane, R., Byrne, M., . . . Sgrò, C. (2015). A framework for incorporating evolutionary genomics into biodiversity conservation and management. *Climate Change Responses, 2*, 1-23. https://doi.org/10.1186/s40665-014-0009-x

Hopper, S. D., Brown, A. P., & Marchant, N. G. (1997). Plants of Western Australian granite outcrops. *Journal of the Royal Society of Western*

Australia, 80, 141-158. Retrieved from https://www.rswa.org.au/publications/Journal/80(3)/80(3)hopper.pdf

Hopper, S. D., & Gioia, P. (2004). The southwest Australian floristic region: Evolution and conservation of a global hotspot of biodiversity. *Annual Review of Ecology, Evolution and Systematics, 35*, 623-650. https://doi.org/10.1146/annurev.ecolsys.35.112202.130201

Huyse, T., Poulin, R., & Théron, A. (2005). Speciation in parasites: A population genetics approach. *Trends in Parasitology, 21*, 469-475. https://doi.org/10.1016/j.pt.2005.08.009

Jerome, C. A., & Ford, B. A. (2002). Comparative population structure and genetic diversity of *Arceuthobium americanum* (Viscaceae) and its *Pinus* host species: Insight into host–parasite evolution in parasitic angiosperms. *Molecular Ecology, 11*, 407-420. https://doi.org/10.1046/j.0962-1083.2002.01462.x

Jombart, T. (2008). adegenet: A R package for the multivariate analysis of genetic markers. *Bioinformatics, 24*, 1403-1405. https://doi.org/10.1093/bioinformatics/btn129

Jombart, T., & Ahmed, I. (2011). adegenet 1.3-1: New tools for the analysis of genome-wide SNP data. *Bioinformatics, 27*, 3070-3071. https://doi.org/10.1093/bioinformatics/btr521

Kalinowski, S. T. (2011). The computer program STRUCTURE does not reliably identify the main genetic clusters within species: Simulations and implications for human population structure. *Heredity, 106*, 625-632. http://www.doi.org/10.1038/hdy.2010.95

Katoh, K., Misawa, K., Kuma, K., & Miyata, T. (2002). MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research, 30*, 3059-3066. https://www.doi.org/10.1093/nar/gkf436

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution, 30*, 772-780. https://www.doi.org/10.1093/molbev/mst010

Kilian, A., Wenzl, P., Huttner, E., Carling, J., Xia, L., Blois, H., . . . Uszynski, G. (2012). Diversity Arrays Technology: A generic genome profiling technology on open platforms. In F. Pompanon & A. Bonin (Eds.), *Data Production and Analysis in Population Genomics: Methods and Protocols* (pp. 67-89). Grenoble, France: Humana Press.

Kuijt, J. (1969). *The biology of parasitic flowering plants*. Berkeley, California: University of California Press.

Lamont, B. B. (1985). Dispersal of the winged fruits of *Nuytsia floribunda* (Loranthaceae). *Australian Journal of Ecology, 10*, 187-193. https://doi.org/10.1111/j.1442-9993.1985.tb00880.x

Leimu, R., Mutikainen, P., Koricheva, J., & Fischer, M. (2006). How general are positive relationships between plant population size, fitness and genetic variation? *Journal of Ecology, 94*, 942-952. https://doi.org/10.1111/j.1365-2745.2006.01150.x

Lira-Noriega, A., & Peterson, A. T. (2014). Range-wide ecological niche comparisons of parasite, hosts and dispersers in a vector-borne plant parasite system. *Journal of Biogeography, 41*, 1664-1673. https://doi.org/10.1111/jbi.12302

Llorens, T. M., Tapper, S. L., Coates, D. J., McArthur, S., Hankinson, M., & Byrne, M. (2017). Does population distribution matter? Influence of a patchy versus continuous distribution on genetic patterns in a wind-pollinated shrub. *Journal of Biogeography, 44*, 361-374. https://doi.org/10.1111/jbi.12843

Loveless, M. D., & Hamrick, J. L. (1984). Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics, 15*, 65-95. https://doi.org/10.1146/annurev.es.15.110184.000433

McCoy, K. D., Boulinier, T., & Tirard, C. (2005). Comparative host–parasite population structures: Disentangling prospecting and dispersal in the black-legged kittiwake *Rissa tridactyla*. *Molecular Ecology, 14*, 2825-2838. https://doi.org/10.1111/j.1365-294X.2005.02631.x

Meirmans, P. G., & Hedrick, P. W. (2011). Assessing population structure: F_{ST} and related measures. *Molecular Ecology Resources, 11*, 5-18. https://www.doi.org/10.1111/j.1755-0998.2010.02927.x

Millar, M. A., Byrne, M., Coates, D. J., & Roberts, J. D. (2016). Contrasting diversity and demographic signals in sympatric narrow-range endemic shrubs of the south-west Western Australian semi-arid zone. *Biological Journal of the Linnean Society, 118*, 315-329. https://doi.org/10.1111/bij.12736

Millar, M. A., Byrne, M., Coates, D. J., & Roberts, J. D. (2017). Comparative analysis indicates historical persistence and contrasting contemporary structure in sympatric woody perennials of semi-arid south-west Western Australia. *Biological Journal of the Linnean Society, 120*, 771-787. https://doi.org/10.1093/biolinnean/blw005

Müller, K. (2005). SeqState: Primer design and sequence statistics for phylogenetic DNA datasets. *Applied Bioinformatics, 4*, 65-69. https://www.doi.org/10.2165/00822942-200504010-00008

Musselman, L. J., & Press, M. C. (1995). Introduction to parasitic plants. In M. C. Press & J. D. Graves (Eds.), *Parasitic plants*. London, UK: Chapman & Hall.

Mutikainen, P., & Koskela, T. (2002). Population structure of a parasitic plant and its perennial host. *Heredity, 89*, 318-324. https://www.doi.org/10.1038/sj.hdy.6800142

Nadler, S. A. (1995). Microevolution and the genetic structure of parasite populations. *The Journal of Parasitology, 81*, 395-403. https://doi.org/10.2307/3283821

Nieberding, C. M., & Olivieri, I. (2007). Parasites: Proxies for host genealogy and ecology? *Trends in Ecology & Evolution, 22*, 156-165. https://doi.org/10.1016/j.tree.2006.11.012

Nistelberger, H., Gibson, N., Macdonald, B., Tapper, S.-L., & Byrne, M. (2014). Phylogeographic evidence for two mesic refugia in a biodiversity hotspot. *Heredity, 113*, 454-463. https://www.doi.org/10.1038/hdy.2014.46

Nistelberger, H. M., Byrne, M., Coates, D., & Roberts, J. D. (2015). Genetic drift drives evolution in the bird-pollinated, terrestrial island endemic *Grevillea*

georgeana (Proteaceae). *Botanical Journal of the Linnean Society, 178*, 155-168. https://www.doi.org/10.1111/boj.12270

Norton, D. A., & Carpenter, M. A. (1998). Mistletoes as parasites: Host specificity and speciation. *Trends in Ecology & Evolution, 13*, 101-105. https://doi.org/10.1016/S0169-5347(97)01243-3

Nyagumbo, E., Ndagurwa, H. G. T., & Mushonga, K. (2017). High genetic variation and low gene flow among populations of *Viscum verrucosum* in semi-arid savanna, southwest Zimbabwe. *Ecological Genetics and Genomics, 3-5*, 41-46. https://doi.org/10.1016/j.egg.2017.08.001

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., . . . Wagner, H. (2019). vegan: Community ecology package. Retrieved from https://CRAN.R-project.org/package=vegan

Okubamichael, D. Y., Griffiths, M. E., & Ward, D. (2016). Host specificity in parasitic plants—perspectives from mistletoes. *AoB PLANTS, 8*(plw069). https://doi.org/10.1093/aobpla/plw069

Paradis, E. (2010). pegas: An R package for population genetics with an integrated–modular approach. *Bioinformatics, 26*, 419-420. https://www.doi.org/10.1093/bioinformatics/btp696

Paradis, E., & Schliep, K. (2018). ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics, 35*, 526-528. https://doi.org/10.1093/bioinformatics/bty633

Pate, J. S. (1995). Mineral relationships of parasites and their hosts. In M. C. Press & J. D. Graves (Eds.), *Parasitic plants* (pp. 80-102). London: Chapman and Hall.

Polzin, T., & Daneshmand, S. V. (2003). On Steiner trees and minimum spanning trees in hypergraphs. *Operations Research Letters, 31*, 12-20. https://doi.org/10.1016/S0167-6377(02)00185-2

Pons, O., & Petit, R. J. (1996). Measuring and testing genetic differentiation with ordered versus unordered alleles. *Genetics, 144*, 1237-1245. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1207615/

Poulin, R. (2011). The many roads to parasitism: A tale of convergence. *Adv Parasitol, 74*, 1-40. https://doi.org/10.1016/b978-0-12-385897-9.00001-x

Poulin, R., & Morand, S. (2000). The diversity of parasites. *The Quarterly Review of Biology, 75*, 277-293. https://doi.org/10.1086/393500

Press, M. C., & Phoenix, G. K. (2005). Impacts of parasitic plants on natural communities. *New Phytologist, 166*, 737-751. https://doi.org/10.1111/j.1469-8137.2005.01358.x

Price, P. W. (1980). *Evolutionary biology of parasites*. Princeton, NJ: Princeton University Press.

Ramírez-Barahona, S., González, C., González-Rodríguez, A., & Ornelas, J. F. (2017). The influence of climatic niche preferences on the population genetic structure of a mistletoe species complex. *New Phytologist, 214*, 1751-1761. https://doi.org/10.1111/nph.14471

Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J. C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S. E., & Sánchez-Gracia, A. (2017). DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular Biology and Evolution, 34*, 3299-3302. https://www.doi.org/10.1093/molbev/msx248

Sansaloni, C., Petroli, C., Jaccoud, D., Carling, J., Detering, F., Grattapaglia, D., & Kilian, A. (2011). Diversity Arrays Technology (DArT) and next-generation sequencing combined: Genome-wide, high throughput, highly informative genotyping for molecular breeding of *Eucalyptus*. *BMC Proceedings, 5*, P54. https://doi.org/10.1186/1753-6561-5-S7-P54

Shaw, J., Lickey, E. B., Schilling, E. E., & Small, R. L. (2007). Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The tortoise and the hare III. *American Journal of Botany, 94*, 275-288. https://www.doi.org/10.3732/ajb.94.3.275

Simmons, M. P., & Ochoterena, H. (2000). Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology, 49*, 369-381. https://www.doi.org/10.1093/sysbio/49.2.369

Start, A. N. (2015). The mistletoe flora of southern Western Australia, with a particular reference to host relationships and fire. *Australian Journal of Botany, 63*, 636-646. https://doi.org/10.1071/BT15028

Szpiech, Z. A., Jakobsson, M., & Rosenberg, N. A. (2008). ADZE: A rarefaction approach for counting alleles private to combinations of populations. *Bioinformatics, 24*, 2498-2504. https://www.doi.org/10.1093/bioinformatics/btn478

Tapper, S.-L., Byrne, M., Yates, C. J., Keppel, G., Hopper, S. D., Van Niel, K., . . . Wardell-Johnson, G. W. (2014). Prolonged isolation and persistence of a common endemic on granite outcrops in both mesic and semi-arid environments in south-western Australia. *Journal of Biogeography, 41*, 2032-2044. https://www.doi.org/10.1111/jbi.12343

Thompson, J. N. (2005). *The Geographic Mosaic of Coevolution*. Chicago, IL: University of Chicago Press.

Van Wyngaarden, M., Snelgrove, P. V. R., DiBacco, C., Hamilton, L. C., Rodríguez-Ezpeleta, N., Jeffery, N. W., . . . Bradbury, I. R. (2017). Identifying patterns of dispersal, connectivity and selection in the sea scallop, *Placopecten magellanicus*, using RADseq-derived SNPs. *Evolutionary Applications, 10*, 102-117. https://www.doi.org/10.1111/eva.12432

Vavrek, M. J. (2011). fossil: Palaeoecological and palaeogeographical analysis tools. Retrieved from https://palaeo-electronica.org/2011_1/238/index.html

Walters, S. J., Robinson, T. P., Byrne, M., Wardell-Johnson, G. W., & Nevill, P. (2020). Contrasting patterns of local adaptation along climatic gradients between a sympatric parasitic and autotrophic tree species. *Molecular Ecology, 29*, 3022-3037. https://doi.org/10.1111/mec.15537

Walters, S. J., Robinson, T. P., Byrne, M., Wardell-Johnson, G. W., & Nevill, P. (In press). Association of adaptive genetic variation with climatic variables differs between a host-specific mistletoe and its host. *Evolutionary Applications*.

Watson, D. M. (2001). Mistletoe–a keystone resource in forests and woodlands worldwide. *Annual Review of Ecology and Systematics, 32*, 219-249. https://doi.org/10.1146/annurev.ecolsys.32.081501.114024

Weir, B. S., & Cockerham, C. C. (1984). Estimating *F*-statistics for the analysis of population structure. *Evolution, 38*, 1358-1370. https://doi.org/10.1111/j.1558-5646.1984.tb05657.x

Worth, J. R. P., Jordan, G. J., Marthick, J. R., McKinnon, G. E., & Vaillancourt, R. E. (2010). Chloroplast evidence for geographic stasis of the Australian bird-dispersed shrub *Tasmannia lanceolata* (Winteraceae). *Molecular Ecology, 19*, 2949-2963. https://www.doi.org/10.1111/j.1365-294X.2010.04725.x

4.9 Supplementary information

Table S4.9.1 Site-level chloroplast and nuclear diversity statistics for *Nuytsia floribunda* sampled at 17 sites each across south-western Australia. The dataset was randomly subsampled to 1,500 SNPs and estimates of allelic richness (A_R) were rarefied to a sample size of ten. Latitude and longitude are presented in the Geocentric Datum of Australia (GDA94) coordinate system.

					Nuclear DNA						Chloroplast DNA				
Sampling site	**Code**	**Latitude**	**Longitude**	**No.**	A_R	H_O	H_S	F_{IS}	h	hp	H_D	π	D	F_S	R_2
Cape Arid	ARI	-33.827230	122.976605	16	1.54±0.01	0.183±0.005	0.217±0.005	0.110±0.009	2	-	0.250±0.180	0.00012±0.00009	-1.055 (p>0.10)	-0.182	0.331
Arthur River	ART	-32.939991	117.625571	14	1.60±0.01	0.226±0.006	0.232±0.005	0.031±0.008	1	-	0.00	0.00	NA	NA	NA
Beekeepers	BEE	-29.855458	115.167284	16	1.59±0.01	0.206±0.005	0.227±0.005	0.079±0.008	1	-	0.00	0.00	NA	NA	NA
Collie	COL	-33.407033	116.162747	16	1.75±0.01	0.274±0.005	0.290±0.004	0.004±0.007	2	1	0.571±0.094	0.00112±0.00019	2.101 (p<0.05)	3.933	0.286
Fitzgerald River	FIT	-33.934041	119.952421	15	1.61±0.01	0.184±0.005	0.244±0.005	0.185±0.009	2	-	0.571±0.094	0.00028±0.00005	1.444 (p>0.10)	0.966	0.286
Cape le Grand	GRA	-33.969691	122.135667	16	1.59±0.01	0.219±0.005	0.236±0.005	0.025±0.008	3	1	0.679±0.122	0.00040±0.00010	0.242 (p>0.10)	-0.146	0.219
Gull Rock	GUL	-34.991241	117.997172	16	1.70±0.01	0.239±0.005	0.273±0.005	0.123±0.009	2	2	0.536±0.123	0.00026±0.00006	1.167 (p>0.10)	0.866	0.268
Howatharra	HOW	-28.544409	114.666134	16	1.58±0.01	0.220±0.005	0.223±0.005	-0.037±0.007	2	-	0.250±0.180	0.00012±0.00009	-1.055 (p>0.10)	-0.182	0.331
Moore River	MOO	-31.049457	115.730544	16	1.64±0.01	0.236±0.005	0.248±0.005	0.025±0.008	2	-	0.250±0.180	0.00012±0.00009	-1.055 (p>0.10)	-0.182	0.331
Lake Muir	MUI	-34.438038	116.668582	16	1.69±0.01	0.257±0.005	0.269±0.005	0.009±0.008	1	-	0.00	0.00	NA	NA	NA
Mundaring	MUN	-32.178490	116.434698	16	1.68±0.01	0.264±0.005	0.266±0.005	-0.033±0.007	2	1	0.250±0.180	0.00012±0.00009	-1.055 (p>0.10)	-0.182	0.331
Scott	SCO	-34.273679	115.267326	16	1.71±0.01	0.264±0.005	0.276±0.005	0.010±0.008	2	2	0.536±0.123	0.00026±0.00006	1.167 (p>0.10)	0.866	0.268
Stirling Range	STI	-34.373122	118.017002	16	1.71±0.01	0.259±0.005	0.276±0.005	0.031±0.007	2	-	0.571±0.094	0.00056±0.00009	1.794 (0.10>p>0.05)	2.216	0.286
Tarin Rock	TAR	-33.129954	118.156419	12	1.62±0.01	0.227±0.005	0.246±0.005	0.075±0.009	1	-	0.00	0.00	NA	NA	NA
Yalgorup	YAL	-32.867193	115.671697	16	1.68±0.01	0.254±0.005	0.264±0.005	-0.005±0.007	2	1	0.250±0.180	0.00049±0.00035	-1.535 (0.10>p>0.05)	1.946	0.331
Yanchep	YAN	-31.543548	115.696037	15	1.67±0.01	0.241±0.005	0.258±0.005	0.031±0.008	3	3	0.679±0.122	0.00124±0.00021	1.428 (p>0.10)	2.119	0.238
Yorkrakine Rock	YOR	-31.422851	117.512310	16	1.43±0.01	0.143±0.005	0.168±0.005	0.119±0.008	2	1	0.250±0.180	0.00012±0.00009	-1.055 (p>0.10)	-0.182	0.331
Total	-	-	-	264	1.63±0.01	0.229±0.005	0.248±0.005	0.046±0.008	16	12	0.783±0.028	0.00101±0.00006	-0.663 (p=0.288)	-4.515 (p=0.062)	0.127 (p=0.863)

Parameters: nuclear diversity: No. = sample size, A_R = allelic richness, H_O = observed heterozygosity, H_S = within population gene diversity, F_{IS} = fixation index. Chloroplast diversity: S = total number of variable sites, h = total number of haplotypes, h_p = number of private haplotypes, H_D = haplotype diversity, π = nucleotide diversity, D = Tajima's D, F_S = Fu's F_S statistic, and R_2 = Ramos– Osnins and Raza's R_2 statistic. Mean ± *SE* (A_R, H_O, H_S, F_{IS}), mean ± *SD* (H_D, π) and *p*-values (D) presented.

Table S4.9.2 Site-level chloroplast and nuclear diversity statistics for *Melaleuca rhaphiophylla* sampled at 17 sites each across south-western Australia. The dataset was randomly subsampled to 1,500 SNPs and estimates of allelic richness (A_R) were rarefied to a sample size of ten. Latitude and longitude are presented in the Geocentric Datum of Australia (GDA94) coordinate system.

					Nuclear DNA							Chloroplast DNA			
Sampling site	**Code**	**Latitude**	**Longitude**	**No.**	A_R	H_O	H_S	F_{IS}	h	h_p	H_D	π	D	F_S	R_2
Beekeepers	BEE	-29.858401	115.166946	16	1.76±0.01	0.387±0.007	0.301±0.004	-0.307±0.007	1	1	0.000	0.000	NA	NA	NA
Brookton	BRO	-32.292704	117.181293	16	1.73±0.01	0.391±0.008	0.296±0.005	-0.346±0.008	1	-	0.000	0.000	NA	NA	NA
Collie	COL	-33.392608	116.174289	16	1.77±0.01	0.388±0.007	0.306±0.004	-0.316±0.008	1	1	0.000	0.000	NA	NA	NA
Fitzgerald River 2	FI2	-34.219893	119.282186	16	1.51±0.01	0.157±0.005	0.204±0.005	0.208±0.009	1	1	0.000	0.000	NA	NA	NA
Fitzgerald River	FIT	-33.889790	119.936441	16	1.45±0.01	0.149±0.005	0.188±0.005	0.191±0.009	1	1	0.000	0.000	NA	NA	NA
Frankland	FRA	-35.003319	116.944696	16	1.76±0.01	0.391±0.007	0.302±0.005	-0.321±0.007	2	2	0.536±0.123	0.00023±0.00005	1.167 (p>0.10)	0.866	0.268
Gull Rock	GUL	-34.984665	118.007202	16	1.75±0.01	0.384±0.007	0.297±0.005	-0.315±0.008	1	-	0.000	0.000	NA	NA	NA
Howatharra	HOW	-28.539697	114.673371	16	1.71±0.01	0.365±0.007	0.285±0.005	-0.299±0.008	1	-	0.000	0.000	NA	NA	NA
Kalbarri	KAL	-27.602539	114.452794	16	1.67±0.01	0.326±0.007	0.266±0.005	-0.192±0.008	2	1	0.536±0.123	0.00046±0.00011	1.449 (p>0.10)	2.083	0.268
Moore River	MOO	-31.088800	115.758129	16	1.79±0.01	0.391±0.006	0.310±0.004	-0.290±0.007	2	-	0.571±0.094	0.00122±0.00020	2.184 (**p<0.05**)	4.619	0.286
Lake Muir	MUI	-34.442654	116.647456	16	1.76±0.01	0.388±0.007	0.301±0.004	-0.307±0.007	1	-	0.000	0.000	NA	NA	NA
Mundaring	MUN	-32.081164	116.433993	16	1.74±0.01	0.387±0.007	0.295±0.005	-0.327±0.007	1	-	0.000	0.000	NA	NA	NA
Scott	SCO	-34.281607	115.258321	16	1.74±0.01	0.364±0.007	0.290±0.005	-0.277±0.007	1	-	0.000	0.000	NA	NA	NA
Stirling Range	STI	-34.436082	117.729739	16	1.77±0.01	0.395±0.007	0.306±0.004	-0.318±0.008	2	-	0.536±0.123	0.00137±0.00031	1.813 (0.10>p>0.05)	5.011	0.268
Towerrining	TOW	-33.582913	116.792366	16	1.78±0.01	0.397±0.007	0.307±0.004	-0.333±0.007	2	1	0.250±0.180	0.00043±0.00031	-1.535 (0.10>p>0.05)	1.946	0.331
Yalgorup	YAL	-32.910649	115.699491	16	1.77±0.01	0.383±0.007	0.302±0.004	-0.287±0.007	3	3	0.607±0.164	0.00029±0.00009	-0.448 (p>0.10)	-0.478	0.197
Yanchep	YAN	-31.573307	115.686179	16	1.77±0.01	0.393±0.007	0.305±0.004	-0.309±0.007	2	-	0.250±0.180	0.00053±0.00038	-1.595 (0.10>p>0.05)	2.407	0.331
Total	-	-	-	272	1.72±0.01	0.355±0.007	0.286±0.005	-0.244±0.009	17	10	0.923±0.007	0.00230±0.00006	1.158 (p=0.903)	0.713 (p=0.651)	0.142 (p=0.963)

Parameters: nuclear diversity: No. = sample size, A_R = allelic richness, H_O = observed heterozygosity, H_S = within population gene diversity, F_{IS} = fixation index. Chloroplast diversity: S = total number of variable sites, h = total number of haplotypes, h_p = number of private haplotypes, H_D = haplotype diversity, π = nucleotide diversity, D = Tajima's D, F_S = Fu's F_S statistic, and R_2 = Ramos– Osnins and Raza's R_2 statistic. Mean ± *SE* (A_R, H_O, H_S, F_{IS}), mean ± *SD* (H_D, π) and p-values (D) presented.

Table S4.9.3 Site-level chloroplast and nuclear diversity statistics for *Amyema gibberula* var. *tatei* sampled at 11 sites each across south-western Australia. The dataset was randomly subsampled to 1,500 SNPs and estimates of allelic richness (A_R) were rarefied to a sample size of ten. Latitude and longitude are presented in the Geocentric Datum of Australia (GDA94) coordinate system.

					Nuclear DNA							Chloroplast DNA			
Sampling site	**Code**	**Latitude**	**Longitude**	**No.**	A_R	H_O	H_S	F_{IS}	h	h_p	H_D	π	D	F_S	R_2
Billyacatting	BIL	-31.042439	117.959293	16	1.52±0.01	0.185±0.005	0.206±0.005	0.055±0.007	2	1	0.429±0.169	0.00020±0.00008	0.334 (p>0.10)	0.536	0.214
Depot Hill	DEP	-29.143822	115.346400	16	1.50±0.01	0.170±0.005	0.192±0.005	-0.004±0.010	1	1	0.00	0.00	NA	NA	NA
Gabyon	GAB	-28.043533	116.736323	10	1.64±0.01	0.215±0.005	0.255±0.005	0.154±0.011	2	-	0.250±0.180	0.00012±0.00008	-1.055 (p>0.10)	-0.182	0.331
Jibberding	JIB	-30.000985	116.826976	16	1.58±0.01	0.204±0.005	0.234±0.005	-0.051±0.009	1	-	0.00	0.00	NA	NA	NA
Koolanooka	KOO	-29.193801	116.228194	16	1.68±0.01	0.229±0.005	0.268±0.005	0.094±0.010	2	1	0.250±0.180	0.00035±0.00025	-1.448 (p>0.10)	1.415	0.331
Koora	KOR	-31.260612	120.011578	16	1.15±0.01	0.041±0.003	0.059±0.004	0.133±0.010	1	1	0.00	0.00	NA	NA	NA
Ninghan	NIN	-29.172953	117.660768	12	1.68±0.01	0.234±0.005	0.270±0.005	0.029±0.012	2	1	0.250±0.180	0.00012±0.00008	-1.055 (p>0.10)	-0.182	0.331
Sandford Rocks	SAN	-31.242659	118.760163	16	1.30±0.01	0.115±0.005	0.120±0.005	-0.049±0.006	1	-	0.00	0.00	NA	NA	NA
Woolgorong	WOO	-27.678167	115.789340	12	1.63±0.01	0.197±0.005	0.253±0.005	0.136±0.010	1	-	0.00	0.00	NA	NA	NA
Yanneymooning	YAN	-30.708080	118.547847	12	1.38±0.01	0.084±0.004	0.151±0.005	0.353±0.013	1	-	0.00	0.00	NA	NA	NA
Yorkrakine Rock	YOR	-31.421116	117.512880	12	1.29±0.01	0.091±0.004	0.114±0.005	0.103±0.007	2	1	0.571±0.094	0.00026±0.00004	1.444 (p>0.10)	0.966	0.286
Total	-	-	-	154	1.49±0.01	0.160±0.005	0.193±0.005	0.087±0.010	9	6	0.773±0.035	0.00053±0.00004	-0.907 (p=0.207)	-2.550 (p=0.124)	0.074 (p=0.311)

Parameters: nuclear diversity: No. = sample size, A_R = allelic richness, H_O = observed heterozygosity, H_S = within population gene diversity, F_{IS} = fixation index. Chloroplast diversity: S = total number of variable sites, h = total number of haplotypes, h_p = number of private haplotypes, H_D = haplotype diversity, π = nucleotide diversity, D = Tajima's D, F_S = Fu's F_S statistic, and R_2 = Ramos– Osnins and Raza's R_2 statistic. Mean ± *SE* (A_R, H_O, H_S, F_{IS}), mean ± *SD* (H_D, π) and p-values (D) presented.

Table S4.9.4 Site-level chloroplast and nuclear diversity statistics for *Hakea recurva* subsp. *recurva* sampled at 10 sites each across south-western Australia. The dataset was randomly subsampled to 1,500 SNPs and estimates of allelic richness (A_R) were rarefied to a sample size of ten. Latitude and longitude are presented in the Geocentric Datum of Australia (GDA94) coordinate system.

				Nuclear DNA							Chloroplast DNA				
Sampling site	**Code**	**Latitude**	**Longitude**	**No.**	A_R	H_O	H_S	F_{IS}	h	h_p	H_D	π	D	F_S	R_2
Boogardie	BOO	-28.039939	117.674462	16	1.70±0.01	0.235±0.005	0.267±0.005	0.109±0.008	3	-	0.464±0.200	0.00044±0.00020	-1.030 (p>0.10)	0.506	0.182
Depot Hill	DEP	-29.143822	115.346400	16	1.66±0.01	0.237±0.005	0.257±0.005	0.065±0.008	3	-	0.607±0.164	0.00048±0.00022	-0.727 (p>0.10)	0.671	0.257
Jibberding	JIB	-30.000985	116.826976	16	1.69±0.01	0.235±0.005	0.265±0.005	0.092±0.008	2	-	0.571±0.094	0.00021±0.00004	1.444 (p>0.10)	0.966	0.286
Karroun Hill	KAR	-30.024129	117.856322	16	1.75±0.01	0.239±0.004	0.282±0.004	0.142±0.008	4	1	0.750±0.139	0.00057±0.00017	-0.020 (p>0.10)	-0.375	0.181
Koolanooka	KOO	-29.193801	116.228194	16	1.73±0.01	0.244±0.005	0.274±0.004	0.085±0.007	5	2	0.786±0.151	0.00080±0.00021	-0.345 (p>0.10)	-1.001	0.141
Koora	KOR	-31.260612	120.011578	16	1.52±0.01	0.195±0.005	0.211±0.005	0.059±0.008	2	1	0.571±0.094	0.00042±0.00007	1.794 (0.10>p>0.05)	2.216	0.286
Ninghan	NIN	-29.172953	117.660768	16	1.76±0.01	0.246±0.004	0.289±0.004	0.113±0.008	4	-	0.750±0.139	0.00072±0.00017	0.000 (p>0.10)	0.081	0.195
Woolgorong	WOO	-27.644765	115.761262	16	1.71±0.01	0.235±0.005	0.273±0.005	0.123±0.008	4	-	0.821±0.101	0.00068±0.00015	0.788 (p>0.10)	-0.034	0.228
Yanneymooning	YAN	-30.708080	118.547847	16	1.72±0.01	0.239±0.004	0.276±0.004	0.118±0.007	3	-	0.464±0.200	0.00034±0.00018	-0.812 (p>0.10)	0.071	0.232
Yorkrakine Rock	YOR	-31.421116	117.512880	16	1.73±0.01	0.257±0.005	0.282±0.004	0.073±0.007	1	-	0.000	0.000	NA	NA	NA
Total	-	-	-	160	1.70±0.01	0.236±0.005	0.268±0.005	0.098±0.008	11	4	0.736±0.044	0.00056±0.00006	-1.042 (p=0.153)	-3.231 (p=0.078)	0.067 (p=0.216)

Parameters: nuclear diversity: No. = sample size, A_R = allelic richness, H_O = observed heterozygosity, H_S = within population gene diversity, F_{IS} = fixation index. Chloroplast diversity: S = total number of variable sites, h = total number of haplotypes, h_p = number of private haplotypes, H_D = haplotype diversity, π = nucleotide diversity, D = Tajima's D, F_S = Fu's F_S statistic, and R_2 = Ramos– Osnins and Raza's R_2 statistic. Mean ± *SE* (A_R, H_O, H_S, F_{IS}), mean ± *SD* (H_D, π) and p-values (D) presented.

Table S4.9.5 Variation observed in three non-coding chloroplast regions for two pairs of sympatric parasitic and autotrophic plant species in south-western Australia: (A) Generalist parasite *Nuytsia floribunda* and autotroph *Melaleuca rhaphiophylla*; and (B) host-specific mistletoe *Amyema gibberula* var. *tatei* and autotroph *Hakea recurva* subsp. *recurva*.

Species	Region	Aligned length	Indels		Base substitutions				
			No.	No. of bases	No. of transitions	No. of transversions	Total	Total mutations	Variability (%)
Nuytsia floribunda	petB	742	2	1,1	1	0	1	3	0.4
	petD	743	1	2	0	0	0	1	0.3
	rpl32F-trnL	658	16	1,1,1,13,16,2,6,3,11,2,5,15,1,19,4,6	0	1	1	17	16.3
Melaleuca rhaphiophylla	petD	737	1	1	3	1	4	5	0.7
	ndhA	875	4	1,1,1,1	0	6	6	10	1.1
	trnG	745	2	11,1	2	2	4	4	2.1
Amyema gibberula var. *tatei*	atpF	663	2	1,1	2	0	2	4	0.6
	petB	655	1	1	2	0	2	3	0.5
	rpl16	843	1	4	1	0	1	2	0.6
Hakea recurva subsp. *recurva*	ndhA	872	0	0	2	2	4	4	0.5
	trnQ-rps16	1,187	1	6	0	1	1	2	0.6
	trnV-ndhC	696	5	23,5,9,6,18	0	1	1	6	8.9

Table S4.9.6 Chloroplast haplotype diversity parameters, genetic differentiation parameters and neutrality tests for chloroplast simple sequence repeat (cpSSR) regions only. Two pairs of sympatric parasitic and autotrophic plant species in south-western Australia: (A) Generalist parasite *Nuytsia floribunda* (*n* = 136) and sympatric autotroph *Melaleuca rhaphiophylla* (*n* = 136); and (B) Host-specific mistletoe *Amyema gibberula* var. *tatei* (*n* = 88) and host species *Hakea recurva* subsp. *recurva* (*n* = 80).

Pair	Species	*S*	*h*	H_R	H_D	π	D	F_S	R_2	G_{ST}	N_{ST}	N_{ST} -G_{ST}
(A)	*Nuytsia floribunda*	3	4	3.6	0.306±0.047	0.00015±0.00002	-0.748 (*p*=0.243)	-1.386 (*p*=0.266)	0.169 (*p*=0.856)	0.589 (*p*=0.0)	0.705 (*p*=0.0)	0.116 (*p*=0.001)
	Melaleuca rhaphiophylla	5	12	11.4	0.883±0.009	0.00074±0.00002	1.244 (*p*=0.908)	-2.377 (*p*=0.191)	0.288 (*p*=1.00)	0.799 (*p*=0.0)	0.890 (*p*=0.0)	0.091 (*p*=0.001)
(B)	*Amyema gibberula* var. *tatei*	3	4	4	0.543±0.054	0.00032±0.00004	0.313 (*p*=0.659)	0.342 (*p*=0.602)	0.174 (*p*=0.912)	0.807 (*p*=0.0)	0.834 (*p*=0.0)	0.026 (*p*=0.158)
	Hakea recurva subsp. *recurva*	0	1	1	0.00±0.00	0.00±0.00	NA	NA	NA	0.230 (*p*=0.0)	0.175 (*p*=0.0)	-0.055 (*p*=0.860)

Parameters: *S* = total number of variable sites, *h* = total number of haplotypes, H_R = haplotype richness rarefied to the lowest sample size, H_D = haplotype diversity, π = nucleotide diversity, G_{ST} = unordered genetic differentiation, N_{ST} = ordered genetic differentiation, D = Tajima's D, F_S = Fu's F_S statistic, and R_2 = Ramos– Osnins and Raza's R_2 statistic. Means are presented with ± 1 *SD* and *p*-values are given in parentheses.

Table S4.9.7 Chloroplast haplotypes based on three non-coding DNA regions for two pairs of sympatric parasitic and autotrophic plant species in south-western Australia: (A) Generalist parasite *Nuytsia floribunda* and autotroph *Melaleuca rhaphiophylla*; and (B) host-specific mistletoe *Amyema gibberula* var. *tatei* and autotroph *Hakea recurva* subsp. *recurva.*

Haplotype	No. individuals	Sampling site
	Nuytsia floribunda	
1	31	ARI(7), FIT(4), GRA(1), MUI(8), STI(4), YAL(7)
2	8	ARI(1), FIT(4), GRA(3)
3	54	ART(8), BEE(8), COL(4), HOW(7), MOO(7), MUN(1), STI(4), TAR(8), YOR(7)
4	4	COL(4)
5	4	GRA(4)
6	5	GUL(5)
7	3	GUL(3)
8	2	HOW(1), MOO(1)
9	7	MUN(7)
10	5	SCO(5)
11	3	SCO(3)
12	1	YAL(1)
13	3	YAN(3)
14	4	YAN(4)
15	1	YAN(1)
16	1	YOR(1)
	Melaleuca rhaphiophylla	
1	8	BEE(8)
2	16	BRO(8), MUN(8)
3	8	COL(8)
4	8	FI2(8)
5	8	FIT(8)
6	3	FRA(3)
7	5	FRA(5)
8	11	GUL(8), STI(3)
9	11	HOW(8), KAL(3)
10	5	KAL(5)
11	11	MOO(4), YAN(7)
12	21	MOO(4), MUI(8), SCO(8), YAN(1)
13	12	STI(5), TOW(7)
14	1	TOW(1)
15	5	YAL(5)
16	1	YAL(1)
17	2	YAL(2)
	Amyema gibberula* var. *tatei	
1	37	BIL(2), JIB(8), NIN(7), SAN(8), YAN(8), YOR(4)

2	6	BIL(6)
3	8	DEP(8)
4	14	GAB(7), KOO(7)
5	9	GAB(1), WOO(8)
6	1	KOO(1)
7	8	KOR(8)
8	1	NIN(1)
9	4	YOR(4)
Hakea recurva* subsp. *recurva		
1	38	BOO(6), DEP(2), JIB(4), KAR(4), KOO(4), NIN(4), YAN(6), YOR(8)
2	2	BOO(1), YAN(1)
3	6	BOO(1), KAR(1), KOO(1), WOO(3)
4	13	DEP(5), KAR(2), KOR(4), WOO(2)
5	4	DEP(1), KOO(1), NIN(2)
6	7	JIB(4), NIN(1), WOO(1), YAN(1)
7	1	KAR(1)
8	1	KOO(1)
9	1	KOO(1)
10	4	KOR(4)
11	3	NIN(1), WOO(2)

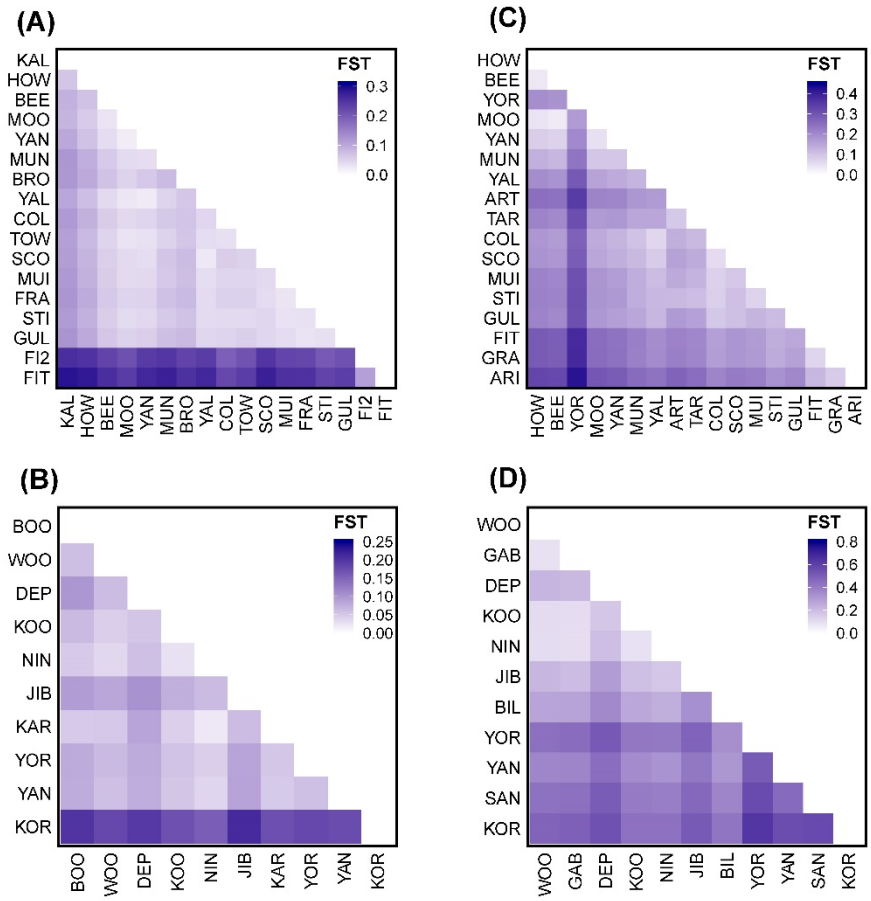


Figure S4.9.1 Pairwise F_{ST} values among sampling sites for two pairs of sympatric parasitic and autotrophic plant species in south-western Australia. Values are presented visually as a heatmap matrix for (A) generalist parasite *Nuytsia floribunda* (6,670 neutral SNPs), (B) sympatric autotroph *Melaleuca rhaphiophylla* (5,209 neutral SNPs), (C) host-specific mistletoe *Amyema gibberula* var. *tatei* (1,931 neutral SNPs) and (D) co-occurring primary host *Hakea recurva* subsp. *recurva* (14,848 neutral SNPs).

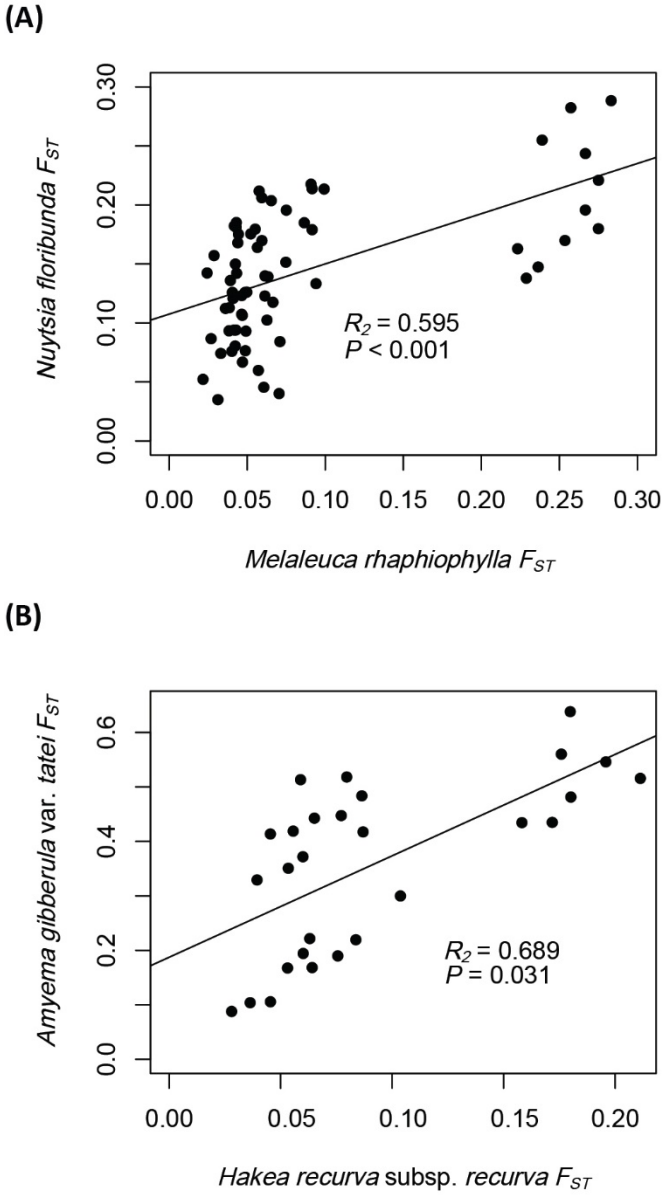


Figure S4.9.2 Pairwise genetic distance (F_{ST}; Weir and Cockerham 1984) between sampling sites for two pairs of sympatric parasitic and autotroph plant species. (A) Pairwise F_{ST} for 12 sites where both generalist parasite *Nuytsia floribunda* and autotroph *Melaleuca rhaphiophylla* were collected; and (B) pairwise F_{ST} for 8 sites where both host-specific mistletoe *Amyema gibberula* var. *tatei* and host *Hakea recurva* subsp. *recurva* were collected. Mantel tests of the correlation between the paired genetic distances were statistically significant after 9,999 permutations.

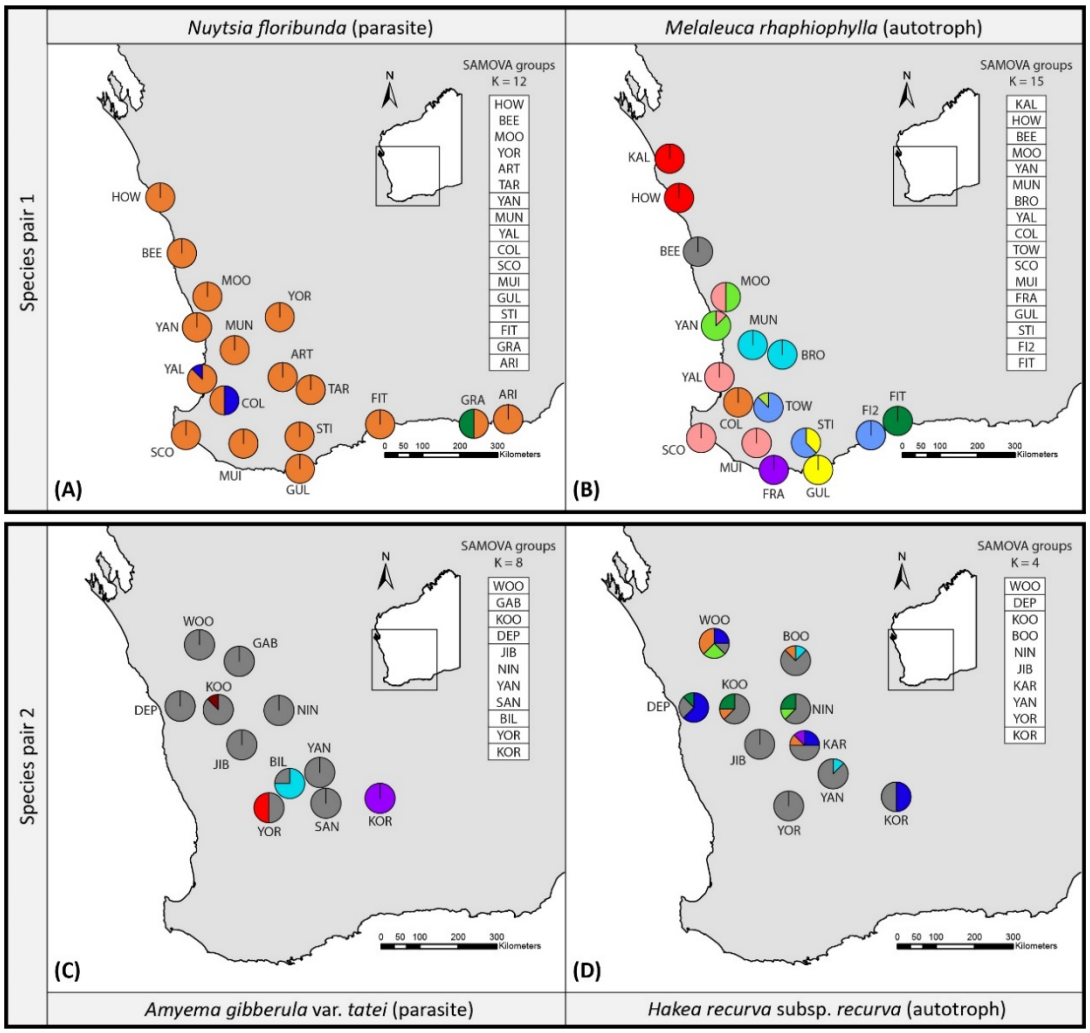
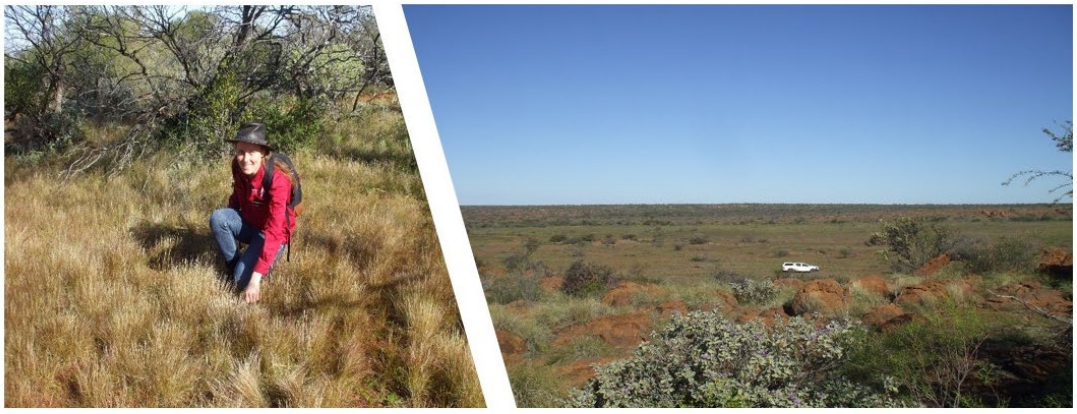


Figure S4.9.3 Geographical distribution of chloroplast DNA haplotypes for single nucleotide polymorphism (SNP) regions only. Haplotype maps were compared between two pairs of sympatric parasitic and autotrophic plant species in south-western Australia. Generalist parasite (A) *Nuytsia floribunda* (*n* = 136) was paired with sympatric autotroph (B) *Melaleuca rhaphiophylla* (*n* = 136), and host-specific (C) *Amyema gibberula* var. *tatei* (*n* = 88) paired with host (D) *Hakea recurva* subsp. *recurva* (*n* = 80). Sampling sites are represented by coloured circles and reflect the geographical distribution in south-western Australia.

CHAPTER 5

SEED SOURCING IN THE GENOMICS ERA: MULTISPECIES PROVENANCE DELINEATION FOR CURRENT AND FUTURE CLIMATES



5.1 Preface

*This chapter consists of a manuscript in preparation titled '**Seed sourcing in the genomics era: Multispecies provenance delineation for current and future climates**'. Permissions to include this work in this thesis are included in Appendix I.*

The introductory chapter (Chapter 1) argues the importance of using information on both adaptive and neutral genetic variation when designing conservation and restoration programs; yet this approach has only been applied to a limited number of plant species. The previous three data chapters (Chapters 2, 3 and 4) provide information on the genetic variation between two pairs of co-occurring plant species and this final data chapter (Chapter 5) synthesises this information for use in conservation and restoration planning. Specifically, the genetic data is combined with a spatial modelling approach to delineate provenances under current and future climate scenarios. The study presented in this chapter makes a significant contribution to this thesis as it synthesises the information on adaptive and neutral genetic variation to provide practical information on the geographical extent of provenances under current and future climate scenarios. This work revealed differing provenance patterns in co-occurring parasitic and autotrophic plant species, indicating that seed sourcing approaches may differ between species with different life history traits. The work in this chapter also identified a similar direction of change in provenances under future climate scenarios between these co-occurring species, but that responses varied between habitats. Lastly, this chapter presents a landscape genomics approach to delineate provenances for co-occurring species under multiple climate scenarios.

5.1.1 Acknowledgements

This work was supported by the Australian Government through an Australian Government Research Training Program Scholarship and the Australian Research Council Industrial Transformation Training Centre for Mine Site Restoration (project number ICI150100041).

5.1.2 Data accessibility

No new data was generated as part of this work.

5.1.3 Author contributions

Author Contributions: SJW conducted the study and wrote the manuscript. All authors conceived the ideas and designed the study. Data analysis was performed by SJW with input from PN, TPR and MB. The manuscript was edited and approved by all authors.

Walters, S. J., T. P. Robinson, M. Byrne, G. W. Wardell-Johnson, and P. Nevill. Seed sourcing in the genomics era: Multispecies provenance delineation for current and future climates. *In preparation*.

5.2 Abstract

Genomic data is increasingly used to delineate provenances, which should ideally incorporate information on both neutral (e.g., gene flow and genetic drift) and adaptive evolutionary processes (e.g., natural selection). Recently, provenance delineation in plant species has begun to incorporate adaptive variation, but this has only been completed on a small number of plant species with a limited range of life history traits. Rarely is provenance delineation applied to species across different habitats within a single study, and in the context of future climate scenarios. Here, we present a multispecies case study using neutral and adaptive genetic data to delineate provenances under current and future climate scenarios (SSP2 and SSP3) of two species co-occurring in a mesic environment and two species co-occurring in a semi-arid environment. We found that the spatial distribution of provenances and the amount of genomic change required to track the predicted climatic conditions over time differed within the pairs of co-occurring species. Additionally, future climate scenarios had differing effects on provenance patterns between the two habitats with the greatest changes in geographical extent observed in the mesic pair. This suggests that provenance guidelines can be both species and habitat dependent. We discuss how these results can be utilised to design seed sourcing strategies for successful restoration, and also how these methods could be broadly applied to delineate provenances in other species and areas to facilitate evidence-based restoration strategies.

5.3 Introduction

Ecological restoration is the process of restoring a degraded ecosystem to reinstate species' diversity, composition and long-term ecological functioning comparable to that of a reference ecosystem (Clewell et al., 2000; McDonald et al., 2016; Gann et al., 2019). The importance of restoring natural ecosystems that have been degraded is becoming increasingly recognised globally (Hobbs & Harris, 2001; Suding, 2011). For example, the Bonn Challenge is a global restoration effort launched in 2011 that aims to restore 350 million hectares of degraded land by 2030 (IUCN, 2011), and the United Nations General Assembly recently declared 2021-2030 as the Decade on Ecosystem Restoration (United Nations, 2019). Achieving ambitious global restoration targets will inherently require the collection of increasingly large quantities of biodiverse seed

(Merritt & Dixon, 2011; Broadhurst et al., 2016; Nevill et al., 2018). Sourcing an adequate quantity of genetically appropriate seed, from either wild populations or seed production areas (Broadhurst et al., 2015; Nevill et al., 2016), will be paramount to the success of global restoration efforts (Vander Mijnsbrugge et al., 2010; Broadhurst et al., 2015; Breed et al., 2019).

The delineation of provenances – the geographical location of a plant population or seed source (Broadhurst et al., 2008; Breed et al., 2019) – is particularly important for successful restoration, as the use of appropriate seed sources can improve plant establishment, avoid inbreeding, outbreeding depression and genetic swamping (Hufford & Mazer, 2003), and prevent the introduction of maladapted genotypes (McKay et al., 2005). Traditionally, plant provenances were determined by geographical distance between populations (e.g., Mortlock, 2000; but see Breed et al., 2018) as local genotypes were presumed to have evolved to suit local environmental conditions. However, locally collected seed does not guarantee superior fitness for a variety of reasons (e.g., Bischoff et al., 2010), and geographical distance may be a poor predictor of demographic history and adaptation of populations to environmental conditions (Breed et al., 2019).

Ideally, seed collection strategies should be designed to maximise genetic diversity of restored vegetation to promote resilience and ensure that the restored area has the ability to adapt to changing conditions in the future (Broadhurst et al., 2008; Bischoff et al., 2010). This requires that the delineation of provenances reflect both neutral and adaptive evolutionary processes (Funk et al., 2012). Previous work on delineating provenances within plant species using genetic data have focussed predominantly on identifying neutral evolutionary processes such as genetic drift and gene flow (e.g., Krauss & He, 2006; Rossetto & Hogbin, 2013; Rossetto et al., 2020), although work has recently begun to incorporate adaptive processes using genomic data (e.g., Shryock et al., 2017; Carvalho et al., 2021; Fremout et al., 2021).

Landscape genomic approaches are particularly good at identifying adaptive genetic variation (Balkenhol et al., 2017), which is important for maintaining evolutionary potential within restored populations (Proft et al., 2018) and potentially facilitating population adaptation to changing climates (Sgrò et al., 2011; Hoffmann et al., 2015). Associating adaptive variation with current and future climate scenarios provides a

promising approach to guide seed sourcing under changing climates (Williams et al., 2014; Breed et al., 2019). Specifically, this approach can be used to forecast the potential provenances that best match future climatic conditions, which can be incorporated into provenance and seed sourcing decision-making (e.g., Prober et al., 2015). This predictive approach has been previously used in single-species studies (e.g., Supple et al., 2018; Ahrens et al., 2020; Ingvarsson & Bernhardsson, 2020), but has not yet been applied to co-occurring species or multiple future climate scenarios.

Despite growing recognition of the potential advantages on applying genomics approaches to the development of seed sourcing guidelines (Mijangos et al., 2015; Breed et al., 2019), provenance delineation in co-occurring species with different life history traits is rarely undertaken. To-date, multispecies studies have largely focussed on provenance delineation using predominantly neutral genetic variation (e.g., Rossetto et al., 2019; Rossetto et al., 2020), which can vary with life history traits (Duminil et al., 2007; Broadhurst et al., 2017). Consequently, co-occurring species with disparate life histories may have different provenances (e.g., Krauss & Koch, 2004), even for sympatric species within the same genus (e.g., Rossetto et al., 2020). Furthermore, while delineation of provenances using adaptive genomic data has been recently conducted in a small number of species globally (e.g., Shryock et al., 2017; Supple et al., 2018; Rossetto et al., 2019; Carvalho et al., 2021), these studies have only covered species with a limited number of life history traits (e.g., life form, pollination and seed dispersal mechanism), and within a single habitat. Therefore, additional work is needed to better understand the geographical extent of provenances in multiple species with diverse life histories and across different habitats within a single study.

Here, we present a case study using genomic data to delineate provenances under current and future climate scenarios in multiple pairs of co-occurring species across diverse habitats. Specifically, we use a landscape genomics approach to model adaptive genetic diversity under current climatic conditions in two species co-occurring in a mesic environment and two species co-occurring in a semi-arid environment, and project patterns onto two future environmental landscapes. Then, we determine the genomic similarity of seed sourcing locations to hypothetical restoration sites, both under current and future climate scenarios. The aims of this study were to: (a) compare patterns of genetic differentiation between different habitats as a precursor

to modelling of adaptive variation; (b) extrapolate models of adaptive genetic diversity to multiple future climate scenarios; (c) compare the effect of current and future climate scenarios on provenance patterns within species pairs and between different habitats; and (d) compare the genomic change required to track between current and future climates. The implications of changing climates on restoration seed sourcing is discussed.

5.4 Materials and methods

5.4.1 Study species

As a case study, we selected four plant species that vary in life history traits and are widespread across south-western Australia (Figure 5.1). The four species were arranged in pairs across two different habitats to gain an understanding of the spatial distribution of provenances in co-occurring species across different geographical landscapes. The first species pair comprised *Melaleuca rhaphiophylla* Schauer (Myrtaceae) and *Nuytsia floribunda* (Labill.) R.Br ex G.Don (Loranthaceae), which were distributed across a mesic landscape in the southwest of Australia and have both been previously used in restoration projects (e.g., van Etten et al., 2011; Brundrett et al., 2018). The second species pair consisted of *Hakea recurva* Meisn. subsp. *recurva* (Proteaceae; hereafter *Hakea recurva*), which is currently used in mining restoration programs (e.g. Commander et al., 2017), and *Amyema gibberula* var. *tatei* (Blakely) Barlow (Loranthaceae; hereafter *Amyema gibberula*); both species were distributed across a semi-arid landscape. *Nuytsia floribunda* and *A. gibberula* are hemiparasites that can photosynthesize carbon but rely on host species for water and mineral nutrients (Musselman & Press, 1995). In contrast, *M. rhaphiophylla* and *H. recurva* are autotrophic and rely solely on the abiotic environment for resource acquisition. All species except *A. gibberula* are insect pollinated with wind and/or gravity dispersed seed (Lamont, 1985; Flora of Australia, 1999; Brophy et al., 2013). *Amyema* species are bird-pollinated with bird-dispersed seed (Calder, 1983) and are often fire sensitive (Start, 2015).

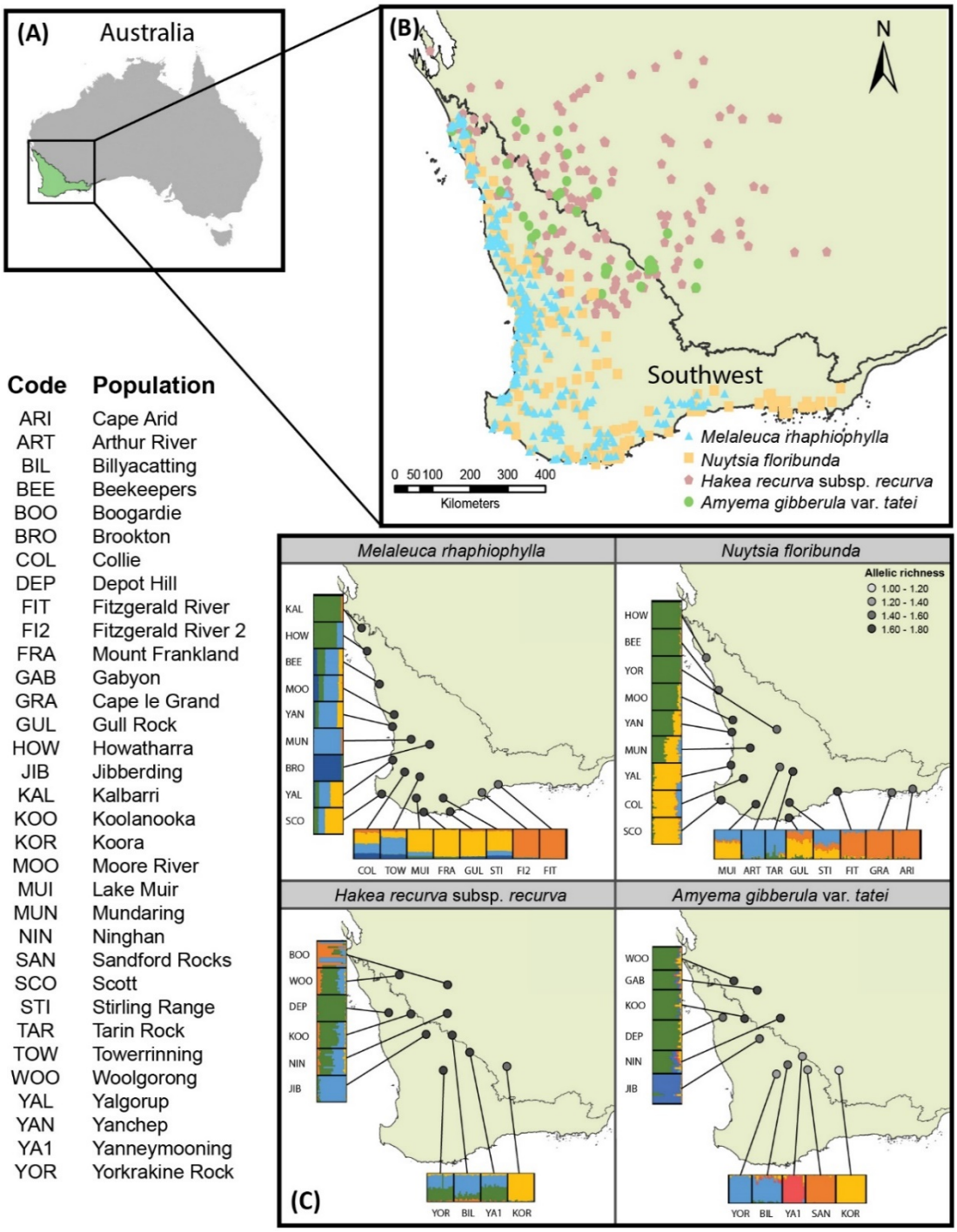


Figure 5.1 Map of the study region in south-western Australia, and the spatial distribution of neutral genetic structure for two species co-occurring in a mesic habitat (*Melaleuca rhaphiophylla* and *Nuytsia floribunda*) and two species co-occurring in a semi-arid habitat (*Hakea recurva* subsp. *recurva* and *Amyema gibberula* var. *tatei*). (A) Location of the study region within the Australian continent. (B) Species distributions across south-western Australia (distribution data obtained from Florabase: https://florabase.dpaw.wa.gov.au/). (C) The spatial distribution of neutral genetic variation. Bar plots show ancestral genetic clusters detected by sparse non-negative matrix factorization and dots are coloured by population-level values of allelic richness (A_R).

5.4.2 Genetic data

For all four study species, we used publicly available genomic data archived on the DRYAD database (Walters et al., 2020b; Walters, Robinson, et al., 2021b). Collection of samples, genetic sequencing methods and genome scans for adaptive loci in this data have been described previously (Walters et al., 2020a; Walters, Robinson, et al., 2021a), but we summarise the methods here to provide context for this study.

Briefly, samples were collected across the geographical and climatic range of the species using a random sampling approach. Samples were obtained from 17 populations of *M. rhaphiophylla* and *N. floribunda*, 10 populations of *H. recurva* and 11 populations of *A. gibberula* (Figure 5.1). To avoid sampling related plants, a minimum sampling distance of 20 m between individuals was applied for all species, where possible. Leaf tissue was collected from each sampled plant and was stored on silica gel with the location recorded using a GARMIN eTrex 10 GPS device.

The genetic data for all species were characterised by reduced-representation sequencing with species *M. rhaphiophylla* and *N. floribunda* published by Walters et al. (2020a), and *H. recurva* and *A. gibberula* published by Walters, Robinson, et al. (2021a). Here, we provide a summary of those methods to provide context for this study. Briefly, nuclear DNA was genotyped at Diversity Arrays Technology Pty Ltd using a double digest complexity reduction sequencing method (DArTseq) to detect thousands of genome-wide single nucleotide polymorphisms (SNPs; Sansaloni et al., 2011; Kilian et al., 2012). Complexity reduction was performed using a combination of two restriction enzymes per species (*Pst*I/*Hpa*II for *M. rhaphiophylla* and *A. gibberula*, and *Pst*I/*Mse*I for *N. floribunda* and *H. recurva*), and high density sequencing was performed on the Illumina HiSeq 2500 platform (further information on DArTseq digestion and ligation can be found in Kilian et al. (2012)). All species were aligned *de novo* as prior genomic information (e.g., reference genomes) were not available. Marking calling was performed in the propriety DARTSOFT14 pipeline with ~25% of individuals within a species regenotyped to calculate a reproducibility measure (RepAvg), and microbial DNA was removed from barcoded sequences using NCBI BLAST (Camacho et al., 2009). Raw SNP datasets were filtered to retain: (a) loci with call rates > 95%; (b) RepAvg > 0.98; (c) minor allele frequency > 5%; (d) individuals with call rates > 80%; and (e) one SNP per fragment. This methodology

produced 5,531 SNPs for *M. rhaphiophylla* (*n* = 272), 6,727 SNPs for *N. floribunda* (*n* = 264), 15,422 SNPs for *H. recurva* (*n* = 160) and 2,055 SNPs for *A. gibberula* (*n* = 154; Walters et al., 2020a; Walters, Robinson, et al., 2021a).

Genetic data for all species was also split into neutral and adaptive datasets in these previous studies (Walters et al., 2020a; Walters, Robinson, et al., 2021a), and here we utilise both datasets in our delineation of provenances. Briefly, these studies used genome scan methods (both outlier tests and environment association analyses) to separate neutral and adaptive genetic diversity. Neutral data were downloaded as SNP datasets and adaptive data were downloaded as pairwise F_{ST} matrices from publicly available data within the DRYAD Digital Repository (Walters et al., 2020b; Walters, Robinson, et al., 2021b). The neutral SNP datasets comprised 5,209 SNPs for *M. rhaphiophylla*, 6,670 SNPs for *N. floribunda*, 14,848 SNPs for *H. recurva* and 1,631 SNPs for *A. gibberula*. F_{ST} matrices for the adaptive datasets were calculated from 98 SNPs for *M. rhaphiophylla*, 33 SNPs for *N. floribunda*, 35 SNPs for *H. recurva* and 36 SNPs for *A. gibberula*.

5.4.3 Distribution data and climatic variables

Distribution data for all four species were downloaded as point data from the Atlas of Living Australia (http://www.ala.org.au). Datasets were filtered in ARCMAP version 10.7.1 (ESRI, 2019) to remove: (a) any record not lodged with a herbarium; (b) specimens growing outside the usual range (i.e., in botanic gardens); and (c) records of specimens that could not be located during leaf tissue collection. The distribution data for all species was visualised in ARCMAP.

Eight climatic variables that have been previously identified as significant predictors of adaptive genomic variation these species were used for this study (Walters et al., 2020a; Walters, Robinson, et al., 2021a). These were: isothermality (BIO3), temperature seasonality (BIO4), mean temperature of the wettest quarter (BIO8), mean temperature of the warmest quarter (BIO10), annual precipitation (BIO12), precipitation seasonality (BIO15), precipitation of the wettest quarter (BIO16), precipitation of the driest quarter (BIO17). Climatic variables were downloaded in raster format at 2.5 arc-minute resolution from the Worldclim2.1 database (Hijmans et al., 2005; Fick & Hijmans, 2017).

For future predictions, we used the same climatic variables predicted for 2080-2100 based on the GCM MIROC6 model for two shared socio-economic pathways (SSPs): SSP2-45 (low-medium emission scenario) and SSP3-70 (medium-high emissions scenario; O'Neill et al., 2017). Briefly, the SSPs have been defined based on information from a suite of elements considered to be important determinants of climate change mitigation and/or adaptation such as technology, policies and institutions, and human development (but see O'Neill et al., 2017 for further detail). SSP2 represents an emissions scenario with medium challenges to mitigation (e.g. moderate levels of environmental awareness, development of some low-carbon technology, and intermediate international cooperation) and adaptation (e.g. some engineered infrastructure, moderate inequality and policy partially aimed toward sustainability) (O'Neill et al., 2017; Riahi et al., 2017). In contrast, SSP3 represents high challenges to emission mitigation (e.g. high energy demand with a fossil-fuel dominated energy supply, slow change to technology, and a lack of cooperation internationally) and adaptation (e.g. slow development of technology, increased inequality, and barriers to trade) (O'Neill et al., 2017; Riahi et al., 2017).

5.4.4 Patterns of genetic differentiation across habitats

Firstly, to investigate the patterns of genetic differentiation across the different habitats, we used a genetic clustering approach, sparse non-negative matrix factorization (sNMF; Frichot et al., 2014). This approach was implemented in the R package LEA version 2.4 (Frichot & François, 2015) using the previously published neutral SNP datasets. We tested values from one to 12 ancestral populations (K) for each species with ten replicates run for each value of K. The most likely K was selected as the value with minimal decrease in cross entropy values (François, 2016). Population differentiation (F_{ST}) was calculated in the R package HIERFSTAT version 0.04-22 (Goudet, 2005) according to the F_{ST} method by Weir and Cockerham (1984), and pairwise F_{ST} values were plotted in the R package GGPLOT2 version 3.2.1 (Wickham, 2016). To assess genetic diversity, we estimated global and population-level allelic richness (A_R), observed heterozygosity (H_O) and the inbreeding coefficient (F_{IS}) in the R package HIERFSTAT (Goudet, 2005). Population-level genetic diversity parameters were mapped for each species in ARCMAP version 10.7.1 (ESRI, 2019).

5.4.5 Modelling adaptive genetic diversity under current and future climatic conditions

To model adaptive genetic variation against climate, we used generalised dissimilarity modelling (GDM; Ferrier, 2002; Ferrier et al., 2002), as applied to genomic data using the approach of Fitzpatrick and Keller (2015). For all the study species, we used GDM models that have been previously modelled for current climatic data (Walters et al., 2020a; Walters, Robinson, et al., 2021a), and here we extend these models to project patterns of adaptive genetic diversity to the two future climate scenarios (SSP2 and SSP3).

For all species, the GDM models developed previously had 70-76% of genetic differentiation explained by the predictor variables, with model deviances of 13.08 for *M. rhaphiophylla* and 9.83 for *N. floribunda* (Walters et al., 2020a), and 4.56 for *H. recurva* and 3.58 for *A. gibberula* (Walters, Robinson, et al., 2021a). Using these previously developed models, and the downloaded pairwise F_{ST} matrices of adaptive genetic diversity, we implemented GDM analysis using the R package GDM version 1.3.11 (Manion et al., 2018). Predictor variables comprised geographical coordinates and significant climatic variables for each species (Table 5.1). The GDM models were projected onto the current environmental landscape, using the spatial interpolation method of Fitzpatrick and Keller (2015). Firstly, we used the *gdm.transform* function to transform the environmental layers based on the GDM model and performed a principal components analysis (PCA) using the *prcomp* function (R Core Team, 2019). This transformed variables into three principal components (PCs) that were predicted across space for the distributional area of each species.

To project the GDM model onto two future climatic landscapes (SSP2 and SSP3), we used the *predict* function in the GDM R package (Manion et al., 2018) to predict the distribution of adaptive genetic variation across habitats in the future. We also used the time = TRUE option to calculate the amount of genomic change required to track the predicted environmental conditions over time (i.e., the genomic vulnerability; Bay et al., 2018).

Table 5.1 Climatic variables used in generalised dissimilarity models for two pairs of sympatric plant species in south-western Australia: *Melaleuca rhaphiophylla* and *Nuytsia floribunda* co-occur across a mesic habitat; and *Hakea recurva* subsp. *recurva* and *Amyema gibberula* var. *tatei* co-occur across a semi-arid habitat.

Species	Geo	IT (BIO3)	TS (BIO4)	MTWQ (BIO8)	MTHQ (BIO10)	AP (BIO12)	PS (BIO15)	PWQ (BIO16)	PDQ (BIO17)
Melaleuca rhaphiophylla		x	x				x		x
Nuytsia floribunda	x	x	x	x	x			x	
Hakea recurva subsp. *recurva*	x					x			
Amyema gibberula var. *tatei*	x		x	x					

Abbreviations: AP, annual precipitation; Geo, geographical distance; IT, isothermality; MTHQ, mean temperature of the warmest quarter; MTWQ, mean temperature of the wettest quarter; PDQ, precipitation of the driest quarter; PS, precipitation seasonality; PWQ, precipitation of the wettest quarter; TS, temperature seasonality.

5.4.6 Visualising provenances under current and future climate scenarios

Finally, to investigate provenances under current climatic conditions, and compare to the change under future climate scenarios, we selected four hypothetical restoration sites across the two study habitats (Figure 5.2). The hypothetical study sites selected for the mesic species were situated on the Swan Coastal Plain (31.75° S, 115.94° E) and Mallee subregion (33.57° S and 119.04° E). The hypothetical study sites for the semi-arid species were located in the Yalgoo (29.19° S, 116.76° E) and Coolgardie regions (30.84° S, 119.54° E). All of these sites correspond to currently active mines that are undergoing progressive rehabilitation. For each hypothetical restoration site, we extracted the GDM values from the transformed current climatic data for each species. We then calculated the genetic similarity of potential seed sources to the restoration site, both under current climatic conditions and the two future climate scenarios. The resulting genetic similarity datasets for each species were visualised across the landscape under each of the three climate scenarios using R (R Core Team, 2019).

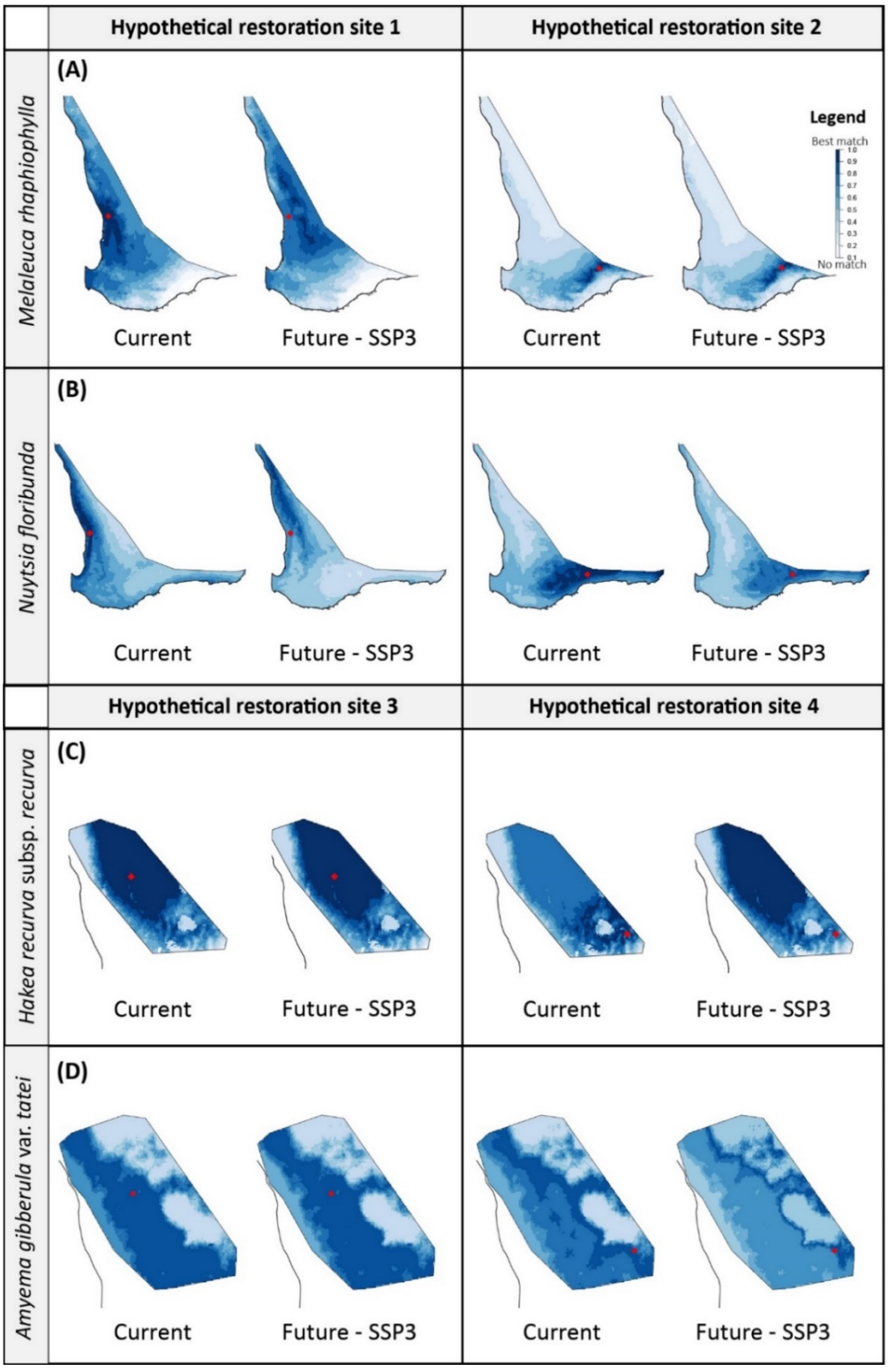


Figure 5.2 Predicted genomic similarity of hypothetical restoration sites (red diamonds) to potential seed sourcing locations for two pairs of sympatric plant species across south-western Australia. Two species, (A) *Melaleuca rhaphiophylla* and (B) *Nuytsia floribunda*, co-occur across a mesic habitat and two species, (C) *Hakea recurva* subsp. *recurva* and (D) *Amyema gibberula* var. *tatei,* co-occur across a semi-arid habitat. Potential seed sourcing locations were projected for current climate conditions under a medium-high emissions scenario for 2080-2100 (SSP3). Areas that best match climatic conditions at the hypothetical restoration site are shown in dark blue.

5.5 Results

5.5.1 Patterns of genetic differentiation across habitats

For both habitats, genetic clustering showed a pattern of isolation by distance within species, and that the most differentiated populations commonly had the lowest allelic richness (Figure 5.1C). Admixture levels within individuals varied by population and species, although populations with the lowest admixture generally also had stronger population differentiation and lower genetic diversity. Population differentiation (pairwise F_{ST}) varied between species (Figure S5.8.3), with a global F_{ST} of 0.103 for *M. rhaphiophylla*, 0.185 for *N. floribunda*, 0.087 for *H. recurva* and 0.352 for *A. gibberula.* Genetic diversity parameters differed between all species (Table 5.2). Population-level parameters of allelic richness (A_R, Figure 5.1C) observed heterozygosity (H_O, Figure S5.8.1) and inbreeding coefficient (F_{IS}, Figure S5.8.2) also varied between populations in all species.

Table 5.2 Measures of neutral genetic diversity for two pairs of sympatric plant species in south-western Australia: *Melaleuca rhaphiophylla* and *Nuytsia floribunda* co-occur across a mesic habitat, and *Hakea recurva* subsp. *recurva* and *Amyema gibberula* var. *tatei* co-occur across a semi-arid habitat. A_R = allelic richness, H_O = observed heterozygosity, F_{IS} = inbreeding co-efficient. Values are presented as mean ± *SE*.

Species	A_R	H_O	F_{IS}
Melaleuca rhaphiophylla	1.683 ± 0.005	0.355 ± 0.004	-0.241 ± 0.005
Nuytsia floribunda	1.659 ± 0.005	0.228 ± 0.002	0.075 ± 0.004
Hakea recurva subsp. *recurva*	1.775 ± 0.003	0.233 ± 0.001	0.120 ± 0.002
Amyema gibberula var. *tatei*	1.487 ± 0.011	0.160 ± 0.005	0.168 ± 0.010

5.5.2 Visualising provenances under current and future climate scenarios

Comparing the effect of multiple climate scenarios indicated that provenance patterns differ between habitats and species, but also within habitats (Figure 5.2). For *M. rhaphiophylla*, areas that best matched current climatic conditions occurred in the immediate area at both hypothetical restoration sites (Figure 5.2A). A similar result was found for *N. floribunda*, although the areas with a similar genetic composition entended further along the coast (Figure 5.2B). Under the low-medium emissions scenario (SSP2), the areas that best matched the predicted future climate for both species shifted further inland, but only for one of the two hypothetical restoration sites (Figure S5.8.4). For the second hypothetical restoration site, the geographical area that

best matched the future climate was largely the same as that for current conditions. A similar pattern was observed under the medium-high emissions scenario (SSP3; Figure 5.2A; B), although the seed sourcing areas that best matched the climatic conditions at the hypothetical restoration sites reduced under future climate scenarios.

Similarly, for the semi-arid pair, areas that best matched current climatic conditions at the hypothetical restoration site occur across majority of the range of the species at one restoration site, but not the other (Figure 5.2C; D). Under the low-medium emissions scenario (SSP2), there were few changes in the distribution of areas that best matched the predicted future climate of the hypothetical restoration site in the centre of the species range (Figure S5.8.4C; D), and there was little additional change under the medium-high emissions scenario (SSP3). However, at the second hypothetical restoration site the area that best matched the projected future climate varied from that of the current climate (Figure 5.2C; D).

5.5.3 Tracking between current and future climates

Patterns of temporal variation (i.e., difference between the GDM models projected onto current climatic conditions and the GDM models projected onto future climate scenarios) differed between species within the same habitats. For *M. rhaphiophylla*, the model predicted that greater genetic change would be required in the south-eastern area of the species range under the low-medium emission future climate scenario (SSP2), but in the central area under the medium-high emissions future climate scenario (SSP3; Figure 5.3A). In contrast, for *N. floribunda*, the model predicted that greater genetic change would be required in the central area of the species range under both future climate scenarios (Figure 5.3B). For *H. recurva*, the model predicted areas to require greater genetic change to be scattered throughout the species range for both climate scenarios, although marginally stronger under the medium-high emissions scenario (SSP3; Figure 5.3C). Lastly, for *A. gibberula*, the model predicted that greater genetic change would be required in the south-eastern and south-western areas of the species range under both future climate scenarios (Figure 5.3D), with genetic change relatively higher than the other study species.

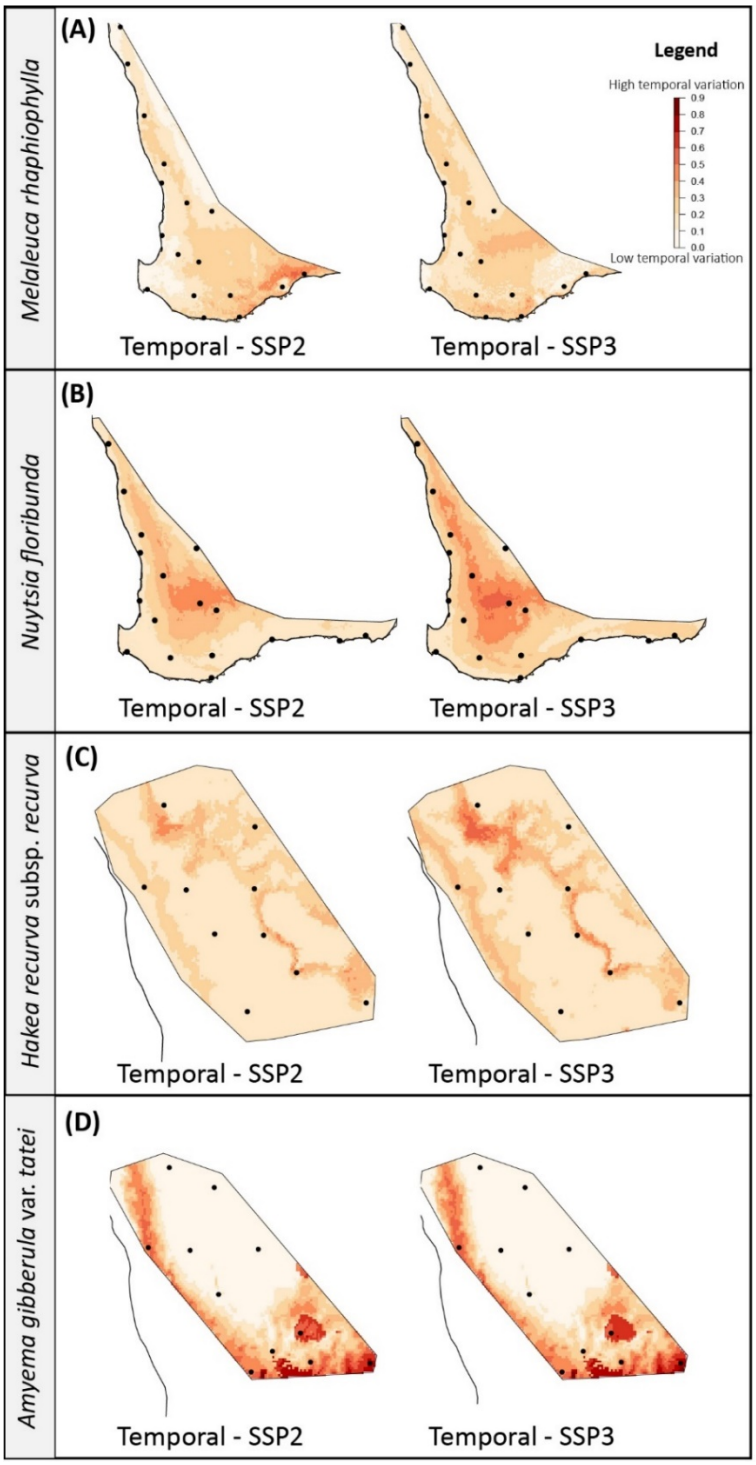


Figure 5.3 Predicted temporal variation in the distribution of adaptive genetic variation for two pairs of sympatric plant species across south-western Australia. Two species, (A) *Melaleuca rhaphiophylla* and (B) *Nuytsia floribunda*, co-occur across a mesic habitat and two species, (C) *Hakea recurva* subsp. *recurva* and (D) *Amyema gibberula* var. *tatei*, co-occur across a semi-arid habitat. The temporal variation was derived from the differences between the GDM projected onto current climatic conditions and the GDM projected onto future climate scenarios for 2080-2100: a low-medium emission scenario, SPP2; and a medium-high emissions scenario, SSP3. Areas with higher temporal variation (depicted in dark red) require greater genetic change to track climate between current and future conditions. Black points are sampling sites.

5.6 Discussion

Sourcing genetically appropriate seed is vital for restoration success (Vander Mijnsbrugge et al., 2010; Broadhurst et al., 2015); this can be aided through delineation of seed provenances using genomic approaches (Vander Mijnsbrugge et al., 2010; Breed et al., 2019). Our work combined spatial modelling with data on adaptive genetic variation to provide insight into provenance patterns for different species and habitats under multiple climate scenarios. We found that the geographical extent of provenances differed within the pairs of co-occurring species, and between the different habitats. Identifying provenances that best matched future climate scenarios also indicated different responses to changing climate between species from mesic and semi-arid and habitats, as well as between populations within a single species.

5.6.1 Provenances vary by species and habitat

The importance of understanding connectivity between populations and maintaining adequate genetic diversity in restored populations is well-established (Broadhurst et al., 2008; Bischoff et al., 2010; Weeks et al., 2011; Mijangos et al., 2015). In this study, we found a pattern of isolation by distance in all four species, as opposed to distinct molecular lineages observed in other widespread plant species in south-western Australia such as *Eucalyptus salubris* (Steane et al., 2015). This is important for provenancing as it indicates that genetic differences between populations increase with geographical distance, likely a result of genetic drift. Therefore, provenancing approaches need to consider the levels of genetic diversity within, and between, populations to avoid negative impacts to long-term viability of restored populations (Hufford & Mazer, 2004; McKay et al. 2005). For instance, avoiding the collection of seed from potentially inbred populations with low genetic diversity (e.g., Fitzgerald River and Fitzgerald River 2 for *Melaleuca rhaphiophylla*, Yorkrakine Rock for *Nuytsia floribunda*, Koora for *Hakea recurva,* Koora and Yanneymooning for *Amyema gibberula*),which may otherwise result in poor restoration outcomes (e.g. inbreeding depression; Hufford & Mazer, 2003). Understanding the patterns of differentiation between populations is also crucial when designing provenance approaches to avoid mixing germplasm from highly divergent populations that may lead to outbreeding depression (Edmands, 2007; Allendorf et al., 2010). There was no

significant differentiation in the species studied here that would be necessary to consider in provenance approaches.

Linking adaptive genetic diversity to climatic variables is a second layer of information that provides crucial information on the evolutionary potential of plant populations (Proft et al., 2018) and can inform provenance decisions for restoration (Breed et al., 2019). Here, we found that plant species within the same habitat had different patterns of genetic composition and similarity to hypothetical restoration sites. While, this reflects earlier work that has found different climatic drivers of adaptation within these species (Walters et al., 2020a; Walters, Robinson, et al., 2021a), it also suggests that different provenance approaches could be needed for species, even within the same habitat. Contrasting patterns of provenances and/or seed transfer zones have been previously observed in co-occurring plant species (e.g., Shryock et al., 2017; Rossetto et al., 2020; Carvalho et al., 2021), and even between closely related species with similar functional characteristics (e.g., Rossetto et al., 2020).

In this study, use of the same approach in the delineation of provenances for multiple species in different habitats has provided a number of insights that would not be observed from a single species, or habitat, alone. Firstly, provenance patterns will vary between species within the same landscape, although similarities may exist. For instance, for all four species examined here the most differentiated populations occurred on the edges of the species' range, although the relative location differed between species. Secondly, the scale of provenances differed between habitats with the semi-arid species having larger areas that best matched the current climate of the hypothetical restoration sites. The differences between habitats could be explained by more rapid turnover in climatic gradients across the mesic south-west landscape, where changes in species distributions as a result of climate change have already been detected (e.g., Yates et al., 2010; Booth, 2017). The implication for provenancing is that species occurring in habitats with sharp climatic gradients are likely to have greater differences in genetic composition compared to species occurring in areas with more gradual gradients (e.g. semi-arid areas in this study) and, therefore, provenancing approaches (e.g. admixture provenancing; Breed et al., 2013) may need to be developed for specific habitats.

5.6.2 Modelling changes between current and future climates

Correlating adaptive genetic variation with both current and future climate scenarios can provide important information for seed sourcing under changing climates (Fitzpatrick & Keller, 2015; Breed et al., 2019). In this study, model predictions revealed that the effect of the two future climate scenarios were broadly similar within the pairs of co-occurring species, and with similarities observed between habitats. Furthermore, these results also showed that patterns of genetic composition can vary between populations within a single species. The implication for designing provenancing approaches is that a single approach may not be suitable for all populations throughout the species' range. For example, a climate-adjusted provenancing approach (Prober et al., 2015) that collects seed from populations in the direction of future climatic conditions may be applicable when areas that best match current with future climate scenarios move further inland and/or northward (e.g. *N. floribunda*). However, this same approach would not similarly be suitable for populations where the areas that best match between current and future climate conditions are similar (e.g. *H. recurva*). For the latter, a provenancing approach that collects seed from geographically distant populations could be disadvantageous in bringing in genetically different material into the restored population that is not necessarily better adapted to future climate. In this case, local provenancing or composite provenancing (Broadhurst et al., 2008) approaches may result in germplasm being used that is better matched to both current and future climate scenarios.

In this study, we found that the magnitude of genetic change required to track between current and future climatic conditions varied between the species. Areas with greater magnitude indicate populations that are less likely to adapt to future climates (Bay et al., 2018). While previous single species studies have assessed temporal variation in genetic composition for plant species within other landscapes (e.g., Bay et al., 2018; Supple et al., 2018; Ahrens et al., 2020; Ingvarsson & Bernhardsson, 2020), no study has yet taken a comparative approach for multiple species with the same habitat. The advantage of a comparative approach is that it may identify areas common to numerous species predicted to require more intense natural selection to track climate change. This information could be used to inform conservation and restoration practices within these habitats such as identifying areas where assisted migration may need to be prioritised to ensure long-term species persistence (Prober et al., 2015). Notably, for

three of the four study species the higher emission scenario (SSP3) resulted in greater change in genetic composition compared to low-medium emissions scenario (SSP2). However, for *M. rhaphiophylla*, the areas of greatest change under the SSP2 emissions scenario were fewer than SSP3. This could be due to the closer association of the species with creek lines and watercourses that may make the species less susceptible to changes in climatic conditions (i.e. reduced annual rainfall).

5.6.3 Application of GDMs for provenance delineation

Generalised dissimilarity modelling provides a powerful approach to generating spatial predictions of adaptive variation and genomic vulnerability under climate change scenarios that can be incorporated into provenancing decisions for climate change (Fitzpatrick & Keller, 2015; Proft et al., 2018). To date, predictive provenancing studies using GDMs have predominately utilised future climate models based on representative concentration pathway predictions (RCPs; e.g., Supple et al., 2018; Ahrens et al., 2020; Ingvarsson & Bernhardsson, 2020). This is the first study to utilise the newer shared socioeconomic pathways (SSPs; described in O'Neill et al., 2017). While the RCP models were based on projected concentrations of greenhouse gases, the SSP scenarios consider whether or not reductions in emissions will be achieved based on different air pollution policy futures (Riahi et al., 2017). Utilising SSP scenarios for provenance delineation could be advantageous as they span a wider range of emissions, and therefore, provenance patterns may not be limited to a specific emissions estimate that may change over time.

One complicating factor for this GDM modelling was that sampling intensity varied across these habitats, which could reduce statistical power in predictive models (Supple et al., 2018). Sampling additional populations in future studies may increase the accuracy of this modelling approach. Another complicating factor was that analyses of individual genes were beyond the scope of this study due to low resolution sequencing data (compared to whole genome sequencing) and the lack of prior genomic information. Provenancing guidelines developed using these GDM approaches would also benefit from the complementary establishment of common garden field trials using propagules from areas of different genetic compositions to validate the findings against phenotypic differences.

One limitation in projecting genetic similarity to both current and future climate scenarios was the availability of climatic data at similar resolutions. As in other spatial modelling applications (e.g., Franklin et al., 2013), finer scaled climate projections generally produce more detailed spatial predictions. Although a very fine scale resolution was available for the current climatic data used in this study (approximately 1km^2; Fick & Hijmans, 2017), it was not similarly available for the future climate scenarios. Therefore, we opted to use the coarser 2.5 arc-minute spatial resolution for all datasets, resulting in less detailed predictions for the current climate data, and thus resulting in less detailed predictions of provenance. This could affect seed sourcing strategies as a single value of genetic similarity relates to a 21km^2 area that may contain a number of localised microclimates. Future work could further refine these provenance predictions for current as higher resolution spatial datasets become available.

5.6.4 Implication for restoration seed sourcing

Provenance delineation is a crucial step in the sourcing of seed for restoration (Merritt & Dixon, 2011), but is rarely applied to species of different habitats within a single study, or to multiple future climate scenarios. The landscape genomics approaches utilised in this study have great potential to increase our understanding of the relationship between environmental and genomic variation, potentially improving restoration outcomes. While similar modelling approaches have been used elsewhere (e.g. Shryock et al., 2017; Carvalho et al., 2021), the effect of future climate is seldom examined. The approach to provenance delineation developed here could be used in other regions to design contemporary and future seed sourcing strategies by identifying areas of genetic similarity to restoration sites (i.e. composite provenancing; Broadhurst et al., 2008), and the direction of genetic change required under future climates (e.g. climate-adjusted provenancing; Prober et al., 2015).

Ideally, seed collection strategies should promote resilience in restored vegetation and ensure that the restoration has the ability to adapt to changing conditions in the future (Broadhurst et al., 2008; Bischoff et al., 2010). For the species included in this study, incorporating adaptability to future climates into selection of germplasm used at hypothetical restoration sites could involve collecting seed from the different areas identified by GDM modelling that have similar future climatic conditions at the restoration sites (i.e., admixture strategy; Breed et al., 2013; Prober et al., 2015).

Finally, information on provenances harbouring adaptive variation relevant to future climate scenarios could be used in the designing of seed production areas to ensure long-term native seed supply for large-scale restoration activities (Broadhurst et al., 2015; Nevill et al., 2016).

While it is not currently feasible to conduct provenance delineation for every species, especially in biodiverse regions such as south-western Australia (Hopper & Gioia, 2004), the cost of genetic sequencing technology is decreasing. It is now possible to select a representative sample of species to conduct genetic sequencing and provenance delineation on a landscape-scale (e.g., Restore and Renew; Rossetto & Hogbin, 2013; Rossetto et al., 2019). For such a program, we advocate for the selection of a representative multispecies sample with a broad range of functional diversity (i.e. life history traits) and across different habitats, as these may both influence genetic patterns, and the examination of provenances under multiple climate scenarios. This would provide a greater understanding of the spatial distribution of adaptive variation and the predicted response of multiple species to future climate change.

5.7 References

Every reasonable effort has been made to acknowledge the owners of the copyright material. I would be pleased to hear from any copyright owner who has been omitted or incorrectly acknowledged.

Ahrens, C. W., James, E. A., Miller, A. D., Scott, F., Aitken, N. C., Jones, A. W., . . . Rymer, P. D. (2020). Spatial, climate and ploidy factors drive genomic diversity and resilience in the widespread grass *Themeda triandra*. *Molecular Ecology, 29*, 3872-3888. https://doi.org/10.1111/mec.15614

Allendorf, F. W., Hohenlohe, P. A., & Luikart, G. (2010). Genomics and the future of conservation genetics. *Nature Reviews Genetics, 11*, 697-709. https://doi.org/10.1038/nrg2844

Balkenhol, N., Dudaniec, R. Y., Krutovsky, K. V., Johnson, J. S., Cairns, D. M., Segelbacher, G., . . . Joost, S. (2017). Landscape genomics: Understanding relationships between environmental heterogeneity and genomic characteristics of populations. In O. P. Rajora (Ed.), *Population genomics concepts, approaches and applications* (pp. 261-322). Cham: Springer. https://doi.org/10.1007/978-3-030-04589-0

Bay, R. A., Harrigan, R. J., Underwood, V. L., Gibbs, H. L., Smith, T. B., & Ruegg, K. (2018). Genomic signals of selection predict climate-driven population declines in a migratory bird. *Science, 359*, 83-86. https://doi.org/10.1126/science.aan4380

Bischoff, A., Steinger, T., & Müller-Schärer, H. (2010). The importance of plant provenance and genotypic diversity of seed material used for ecological restoration. *Restoration Ecology, 18*, 338-348. https://doi.org/10.1111/j.1526-100X.2008.00454.x

Booth, T. H. (2017). Impacts of climate change on eucalypt distributions in Australia: An examination of a recent study. *Australian Forestry, 80*, 208-215. https://doi.org/10.1080/00049158.2017.1365402

Breed, M. F., Harrison, P. A., Bischoff, A., Durruty, P., Gellie, N. J. C., Gonzales, E. K., . . . Bucharova, A. (2018). Priority actions to improve provenance

decision-making. *BioScience, 68*, 510-516. https://doi.org/10.1093/biosci/biy050

Breed, M. F., Harrison, P. A., Blyth, C., Byrne, M., Gaget, V., Gellie, N. J. C., . . . Mohr, J. J. (2019). The potential of genomics for restoring ecosystems and biodiversity. *Nature Reviews Genetics, 20*, 615-628. https://doi.org/10.1038/s41576-019-0152-0

Breed, M. F., Stead, M. G., Ottewell, K. M., Gardner, M. G., & Lowe, A. J. (2013). Which provenance and where? Seed sourcing strategies for revegetation in a changing environment. *Conservation Genetics, 14*, 1-10. https://doi.org/10.1007/s10592-012-0425-z

Broadhurst, L., Breed, M., Lowe, A., Bragg, J., Catullo, R., Coates, D., . . . Byrne, M. (2017). Genetic diversity and structure of the Australian flora. *Diversity and Distributions, 23*, 41-52. https://doi.org/10.1111/ddi.12505

Broadhurst, L., Driver, M., Guja, L., North, T., Vanzella, B., Fifield, G., . . . Bush, D. (2015). Seeding the future – the issues of supply and demand in restoration in Australia. *Ecological Management & Restoration, 16*, 29-32. https://doi.org/10.1111/emr.12148

Broadhurst, L. M., Jones, T. A., Smith, F. S., North, T., & Guja, L. (2016). Maximizing seed resources for restoration in an uncertain future. *BioScience, 66*, 73-79. https://doi.org/10.1093/biosci/biv155

Broadhurst, L. M., Lowe, A., Coates, D. J., Cunningham, S. A., McDonald, M., Vesk, P. A., & Yates, C. (2008). Seed supply for broadscale restoration: Maximizing evolutionary potential. *Evolutionary Applications, 1*, 587-597. https://doi.org/10.1111/j.1752-4571.2008.00045.x

Brophy, J. J., Craven, L. A., & Doran, J. C. (2013). *Melaleucas. Their botany, essential oils and uses*. Canberra: ACIAR Monograph No. 156. Australian Centre for International Agricultural Research (ACIAR).

Brundrett, M., Longman, V., Wisolith, A., Jackson, K., Collins, M., & Clarke, K. (2018). *Banksia woodland restoration project: Annual report 6: January – December 2017*. Department of Biodiversity, Conservation and Attractions, Perth.

Calder, D. M. (1983). Mistletoes in focus: An introduction. In M. Calder & P. Bernhardt (Eds.), *The biology of mistletoes* (pp. 1–17). Sydney: Academic Press Australia.

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., & Madden, T. L. (2009). BLAST+: Architecture and applications. *BMC Bioinformatics, 10*, 421. https://doi.org/10.1186/1471-2105-10-421

Carvalho, C. S., Forester, B. R., Mitre, S. K., Alves, R., Imperatriz-Fonseca, V. L., Ramos, S. J., . . . Jaffé, R. (2021). Combining genotype, phenotype, and environmental data to delineate site-adjusted provenance strategies for ecological restoration. *Molecular Ecology Resources, 21*, 44-58. https://doi.org/10.1111/1755-0998.13191

Clewell, A., Rieger, J., & Munro, J. (2000). *Guidelines for developing and managing ecological restoration projects*. Retrieved from Tuscon, Arizona: www.ser.org

Commander, L., Golos, P., Elliot, C., Merino-Martin, L., Stevens, J., & Miller, B. (2017) Sinosteel Midwest Corporation: Practitioner restoration manual. Botanic Gardens and Parks Authority for Sinosteel Midwest Corporation.

Duminil, J., Fineschi, S., Hampe, A., Jordano, P., Salvini, D., Vendramin, G. G., & Petit, R. J. (2007). Can population genetic structure be predicted from life-history traits? *The American Naturalist, 169*, 662-672. https://doi.org/10.1086/513490

Edmands S. (2007) Between a rock and a hard place: Evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Molecular Ecology, 16*:463-475. https://doi.org/10.1111/j.1365-294X.2006.03148.x

ESRI. (2019). ArcGIS Desktop: Release 10.7.1. Redlands, CA: Environmental Systems Research Institute.

Ferrier, S. (2002). Mapping spatial pattern in biodiversity for regional conservation planning: Where to from here? *Systematic Biology, 51*, 331-363. https://doi.org/10.1080/10635150252899806

Ferrier, S., Watson, G., Pearce, J., & Drielsma, M. (2002). Extended statistical approaches to modelling spatial pattern in biodiversity in northeast New

South Wales. I. Species-level modelling. *Biodiversity and Conservation, 11*, 2275-2307. https://doi.org/10.1023/A:1021302930424

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

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

Flora of Australia. (1999). *Flora of Australia Volume 17B, Proteaceae 3, Hakea to Dryandra*. Melbourne: ABRS/CSIRO Australia.

François, O. (2016). Running structure-like population genetic analyses with R. Retrieved from http://membres-timc.imag.fr/Olivier.Francois/tutoRstructure.pdf

Franklin, J., Davis, F. W., Ikegami, M., Syphard, A. D., Flint, L. E., Flint, A. L., & Hannah, L. (2013). Modeling plant species distributions under future climates: How fine scale do climate projections need to be? *Global Change Biology, 19*, 473-483. https://doi.org/10.1111/gcb.12051

Fremout T., Thomas E., Bocanegra-González K.T., Aguirre-Morales C.A., Morillo-Paz A.T., Atkinson R., Kettle C., González-M R., Alcázar-Caicedo C., González M.A., Gil-Tobón C., Gutiérrez J.P., Moscoso-Higuita L.G., López-Lavalle L.A.B., De Carvalho D., and Muys B. (2021). Dynamic seed zones to guide climate-smart seed sourcing for tropical dry forest restoration in Colombia. *Forest Ecology and Management, 490*, 119127. https://doi.org/10.1016/j.foreco.2021.119127

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

Frichot, E., Mathieu, F., Trouillon, T., Bouchard, G., & François, O. (2014). Fast and efficient estimation of individual ancestry coefficients. *Genetics, 196*, 973-983. https://www.doi.org/10.1534/genetics.113.160572

Funk, W. C., McKay, J. K., Hohenlohe, P. A., & Allendorf, F. W. (2012). Harnessing genomics for delineating conservation units. *Trends in Ecology & Evolution, 27*(9), 489-496. https://doi.org/10.1016/j.tree.2012.05.012

Gann, G. D., McDonald, T., Walder, B., Aronson, J., Nelson, C. R., Jonson, J., . . . Dixon, K. W. (2019). International principles and standards for the practice of ecological restoration. Second edition. *Restoration Ecology, 27*(S1), S1–S46. https://doi.org/10.1111/rec.13035

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

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology, 25*, 1965-1978. https://doi.org/10.1002/joc.1276

Hobbs, R. J., & Harris, J. A. (2001). Restoration ecology: Repairing the earth's ecosystems in the new millennium. *Restoration Ecology, 9*, 239-246. https://doi.org/10.1046/j.1526-100x.2001.009002239.x

Hoffmann, A., Griffin, P., Dillon, S., Catullo, R., Rane, R., Byrne, M., . . . Sgrò, C. (2015). A framework for incorporating evolutionary genomics into biodiversity conservation and management. *Climate Change Responses, 2*, 1-23. https://doi.org/10.1186/s40665-014-0009-x

Hopper, S. D., & Gioia, P. (2004). The southwest Australian floristic region: Evolution and conservation of a global hotspot of biodiversity. *Annual Review of Ecology, Evolution and Systematics, 35*, 623-650. https://doi.org/10.1146/annurev.ecolsys.35.112202.130201

Hufford, K. M., & Mazer, S. J. (2003). Plant ecotypes: Genetic differentiation in the age of ecological restoration. *Trends in Ecology & Evolution, 18*, 147-155. http://doi.org/10.1016/S0169-5347(03)00002-8

Ingvarsson, P. K., & Bernhardsson, C. (2020). Genome-wide signatures of environmental adaptation in European aspen (*Populus tremula*) under current

and future climate conditions. *Evolutionary Applications, 13*, 132-142. https://doi.org/10.1111/eva.12792

IUCN. (2011). Bonn Challenge. Retrieved from http://www.bonnchallenge.org/content/challenge

Kilian, A., Wenzl, P., Huttner, E., Carling, J., Xia, L., Blois, H., . . . Uszynski, G. (2012). Diversity Arrays Technology: A generic genome profiling technology on open platforms. In F. Pompanon & A. Bonin (Eds.), *Data Production and Analysis in Population Genomics: Methods and Protocols* (pp. 67-89). Grenoble, France: Humana Press.

Krauss, S., & Koch, J. (2004). Rapid genetic delineation of provenance for plant community restoration. *The Journal of Applied Ecology, 41*, 1162-1173. https://doi.org/10.1111/j.0021-8901.2004.00961.x

Krauss, S. L., & He, T. (2006). Rapid genetic identification of local provenance seed collection zones for ecological restoration and biodiversity conservation. *Journal for Nature Conservation, 14*, 190-199. https://doi.org/10.1016/j.jnc.2006.05.002

Lamont, B. B. (1985). Dispersal of the winged fruits of *Nuytsia floribunda* (Loranthaceae). *Australian Journal of Ecology, 10*, 187-193. https://doi.org/10.1111/j.1442-9993.1985.tb00880.x

Manion, G., Lisk, M., Ferrier, S., Nieto-Lugilde, D., Mokany, K., & Fitzpatrick, M. C. (2018). gdm: Generalized dissimilarity modelling. R package version 1.3.11. Retrieved from https://CRAN.R-project.org/package=gdm

McDonald, T., Jonson, J., & Dixon, K. (2016). National standards for the practice of ecological restoration in Australia. *Restoration Ecology, 24*(S1), S4-S32. Retrieved from http://www.seraustralasia.com/standards/contents.html

McKay, J. K., Christian, C. E., Harrison, S., & Rice, K. J. (2005). "How local is local?"—A review of practical and conceptual issues in the genetics of restoration. *Restoration Ecology, 13*, 432-440. https://doi.org/10.1111/j.1526-100X.2005.00058.x

Merritt, D. J., & Dixon, K. W. (2011). Restoration seed banks — A matter of scale. *Science, 332*, 424-425. https://doi.org/10.1126/science.1203083

Mijangos, J. L., Pacioni, C., Spencer, P. B. S., & Craig, M. D. (2015). Contribution of genetics to ecological restoration. *Molecular Ecology, 24*, 22-37. https://doi.org/10.1111/mec.12995

Mortlock, W. (2000). Local seed for revegetation: Where will all that seed come from? *Ecological Management & Restoration, 1*, 93-101. https://doi.org/10.1046/j.1442-8903.2000.00029.x

Musselman, L. J., & Press, M. C. (1995). Introduction to parasitic plants. In M. C. Press & J. D. Graves (Eds.), *Parasitic plants*. London, UK: Chapman & Hall.

Nevill, P. G., Cross, A. T., & Dixon, K. W. (2018). Ethical seed sourcing is a key issue in meeting global restoration targets. *Current Biology, 28*, R1378-R1379. https://doi.org/10.1016/j.cub.2018.11.015

Nevill, P. G., Tomlinson, S., Elliott, C. P., Espeland, E. K., Dixon, K. W., & Merritt, D. J. (2016). Seed production areas for the global restoration challenge. *Ecology and Evolution, 6*, 7490-7497. https://doi.org/10.1002/ece3.2455

O'Neill, B. C., Kriegler, E., Ebi, K. L., Kemp-Benedict, E., Riahi, K., Rothman, D. S., . . . Solecki, W. (2017). The roads ahead: Narratives for shared socioeconomic pathways describing world futures in the 21st century. *Global Environmental Change, 42*, 169-180. https://doi.org/10.1016/j.gloenvcha.2015.01.004

Prober, S. M., Byrne, M., McLean, E. H., Steane, D. A., Potts, B. M., Vaillancourt, R. E., & Stock, W. D. (2015). Climate-adjusted provenancing: A strategy for climate-resilient ecological restoration. *Frontiers in Ecology and Evolution, 3*(65), 1-5. https://doi.org/10.3389/fevo.2015.00065

Proft, K. M., Jones, M. E., Johnson, C. N., & Burridge, C. P. (2018). Making the connection: Expanding the role of restoration genetics in restoring and evaluating connectivity. *Restoration Ecology, 26*, 411-418. http://doi.org/10.1111/rec.12692

R Core Team. (2019). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/

Riahi, K., van Vuuren, D. P., Kriegler, E., Edmonds, J., O'Neill, B. C., Fujimori, S., . . . Tavoni, M. (2017). The shared socioeconomic pathways and their energy, land use, and greenhouse gas emissions implications: An overview. *Global Environmental Change, 42*, 153-168. https://doi.org/10.1016/j.gloenvcha.2016.05.009

Rossetto, M., Bragg, J., Kilian, A., McPherson, H., van der Merwe, M., & Wilson, P. D. (2019). Restore and Renew: A genomics-era framework for species provenance delimitation. *Restoration Ecology, 27*, 538-548. https://doi.org/10.1111/rec.12898

Rossetto, M., & Hogbin, T. (2013). Restore and renew NSW: Practical provenance and fitness information for restoration practitioners. *Australasian Plant Conservation: Journal of the Australian Network for Plant Conservation, 22*(2), 22.

Rossetto, M., Wilson, P. D., Bragg, J., Cohen, J., Fahey, M., Yap, J. L. S., & van der Merwe, M. (2020). Perceptions of similarity can mislead provenancing strategies—An example from five co-distributed *Acacia* species. *Diversity, 12*(8), 306. http://doi.org/10.3390/d12080306

Sansaloni, C., Petroli, C., Jaccoud, D., Carling, J., Detering, F., Grattapaglia, D., & Kilian, A. (2011). Diversity Arrays Technology (DArT) and next-generation sequencing combined: Genome-wide, high throughput, highly informative genotyping for molecular breeding of *Eucalyptus*. *BMC Proceedings, 5*, P54. https://doi.org/10.1186/1753-6561-5-S7-P54

Sgrò, C. M., Lowe, A. J., & Hoffmann, A. A. (2011). Building evolutionary resilience for conserving biodiversity under climate change. *Evolutionary Applications, 4*, 326-337. https://doi.org/10.1111/j.1752-4571.2010.00157.x

Shryock, D. F., Havrilla, C. A., DeFalco, L. A., Esque, T. C., Custer, N. A., & Wood, T. E. (2017). Landscape genetic approaches to guide native plant restoration in the Mojave Desert. *Ecological Applications, 27*, 429-445. https://doi.org/10.1002/eap.1447

Start, A. N. (2015). The mistletoe flora of southern Western Australia, with a particular reference to host relationships and fire. *Australian Journal of Botany, 63*, 636-646. https://doi.org/10.1071/BT15028

Suding, K. N. (2011). Toward an era of restoration in ecology: Successes, failures, and opportunities ahead. *Annual Review of Ecology, Evolution, and Systematics, 42*, 465-487. https://doi.org/10.1146/annurev-ecolsys-102710-145115

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

United Nations. (2019). *United Nations Decade on Ecosystem Restoration (2021–2030). Seventy-third session. A/RES/73/284*. Retrieved from https://undocs.org/A/RES/73/284

van Etten, E. J. B., McCullough, C. D., & Lund, M. A. (2011). Sand mining restoration on the Swan Coastal Plain using topsoil – Learning from monitoring of previous rehabilitation attempts. In the *Eighth International Heavy Minerals Conference 2011, 5 – 6 October 2011* (pp. 323-338). Perth, Western Australia: The Australasian Institute of Mining and Metallurgy.

Vander Mijnsbrugge, K., Bischoff, A., & Smith, B. (2010). A question of origin: Where and how to collect seed for ecological restoration. *Basic and Applied Ecology, 11*, 300-311. https://doi.org/10.1016/j.baae.2009.09.002

Walters, S. J., Robinson, T. P., Byrne, M., Wardell-Johnson, G. W., & Nevill, P. (2020a). Contrasting patterns of local adaptation along climatic gradients between a sympatric parasitic and autotrophic tree species. *Molecular Ecology, 29*, 3022-3037. https://doi.org/10.1111/mec.15537

Walters, S. J., Robinson, T. P., Byrne, M., Wardell-Johnson, G. W., & Nevill, P. (2020b). Dataset for: Contrasting patterns of local adaptation along climatic gradients between a sympatric parasitic and autotrophic tree species. *DRYAD*. https://doi.org/10.5061/dryad.0p2ngf1xn

Walters, S. J., Robinson, T. P., Byrne, M., Wardell-Johnson, G. W., & Nevill, P. (2021a). Association of adaptive genetic variation with climatic variables differs between a host-specific mistletoe and its host. *Evolutionary Applications, In press*.

Walters, S. J., Robinson, T. P., Byrne, M., Wardell-Johnson, G. W., & Nevill, P. (2021b). Dataset for: Association of adaptive genetic variation with climatic variables differs between a host-specific mistletoe and its host. *DRYAD*.

Weeks, A. R., Sgro, C. M., Young, A. G., Frankham, R., Mitchell, N. J., Miller, K. A., . . . Hoffmann, A. A. (2011). Assessing the benefits and risks of translocations in changing environments: A genetic perspective. *Evolutionary Applications, 4*, 709-725. https://doi.org/10.1111/j.1752-4571.2011.00192.x

Weir, B. S., & Cockerham, C. C. (1984). Estimating *F*-statistics for the analysis of population structure. *Evolution, 38*, 1358-1370. https://doi.org/10.1111/j.1558-5646.1984.tb05657.x

Wickham, H. (2016). ggplot2: Elegant graphics for data analysis. New York: Springer-Verlag. Retrieved from https://github.com/tidyverse/ggplot2

Williams, A. V., Nevill, P. G., & Krauss, S. L. (2014). Next generation restoration genetics: Applications and opportunities. *Trends in Plant Science, 19*, 529-537. https://doi.org/10.1016/j.tplants.2014.03.011

Yates, C. J., McNeill, A., Elith, J., & Midgley, G. F. (2010). Assessing the impacts of climate change and land transformation on *Banksia* in the south west Australian floristic region. *Diversity and Distributions, 16*, 187-201. https://doi.org/10.1111/j.1472-4642.2009.00623.x

5.8 Supplementary information

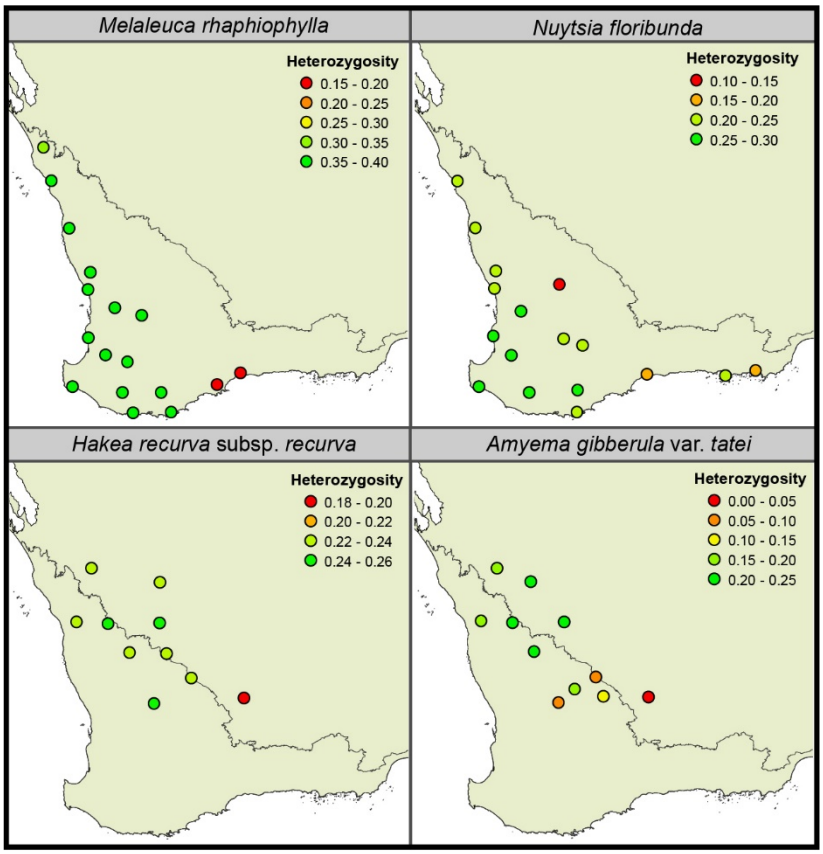


Figure S5.8.1 The spatial distribution of the observed heterozygosity (H_O) across the range of two pairs of sympatric plant species across south-western Australia. Two species (*Melaleuca rhaphiophylla* and *Nuytsia floribunda*) co-occur across a mesic habitat and two species (*Hakea recurva* subsp. *recurva* and *Amyema gibberula* var. *tatei*) co-occur across a semi-arid habitat.

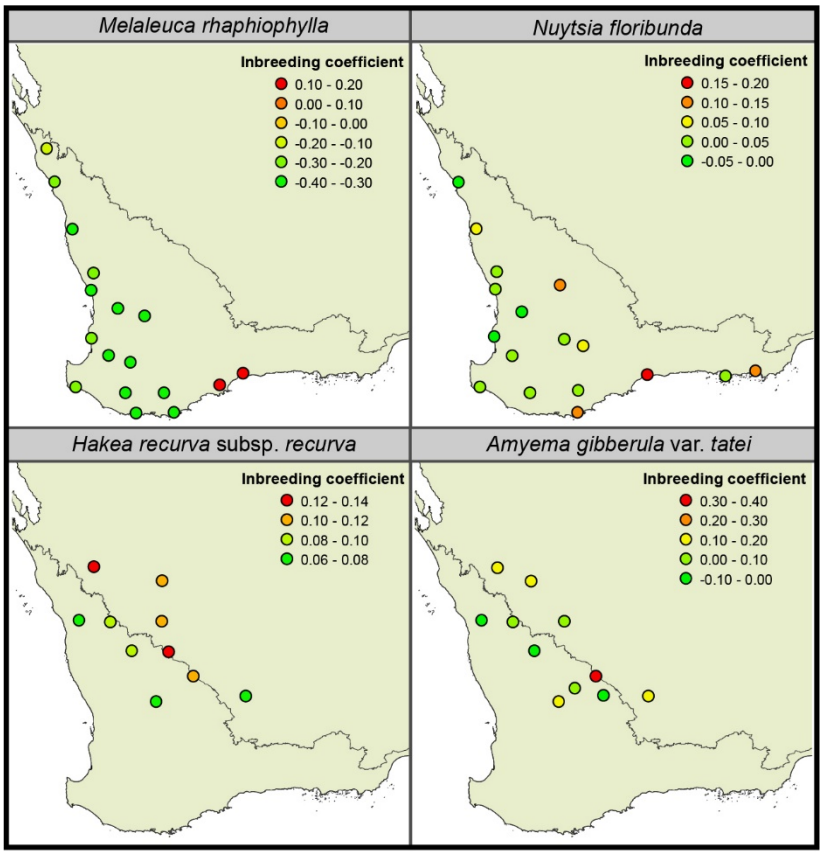


Figure S5.8.2 The spatial distribution of the inbreeding coefficient (F_{IS}) across the range of two pairs of sympatric plant species across south-western Australia. Two species (*Melaleuca rhaphiophylla* and *Nuytsia floribunda*) co-occur across a mesic habitat and two species (*Hakea recurva* subsp. *recurva* and *Amyema gibberula* var. *tatei*) co-occur across a semi-arid habitat.

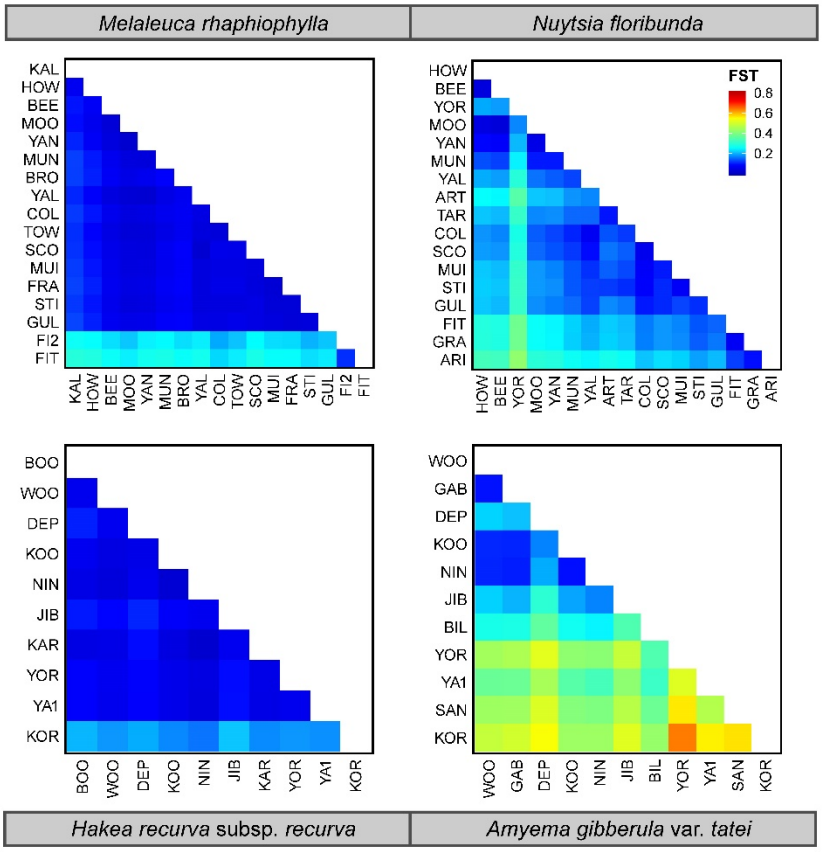


Figure S5.8.3 Heatmap matrix of pairwise F_{ST} values among populations for two pairs of sympatric plant species across south-western Australia. Two species (*Melaleuca rhaphiophylla* and *Nuytsia floribunda*) co-occur across a mesic habitat and two species (*Hakea recurva* subsp. *recurva* and *Amyema gibberula* var. *tatei*) co-occur across a semi-arid habitat.

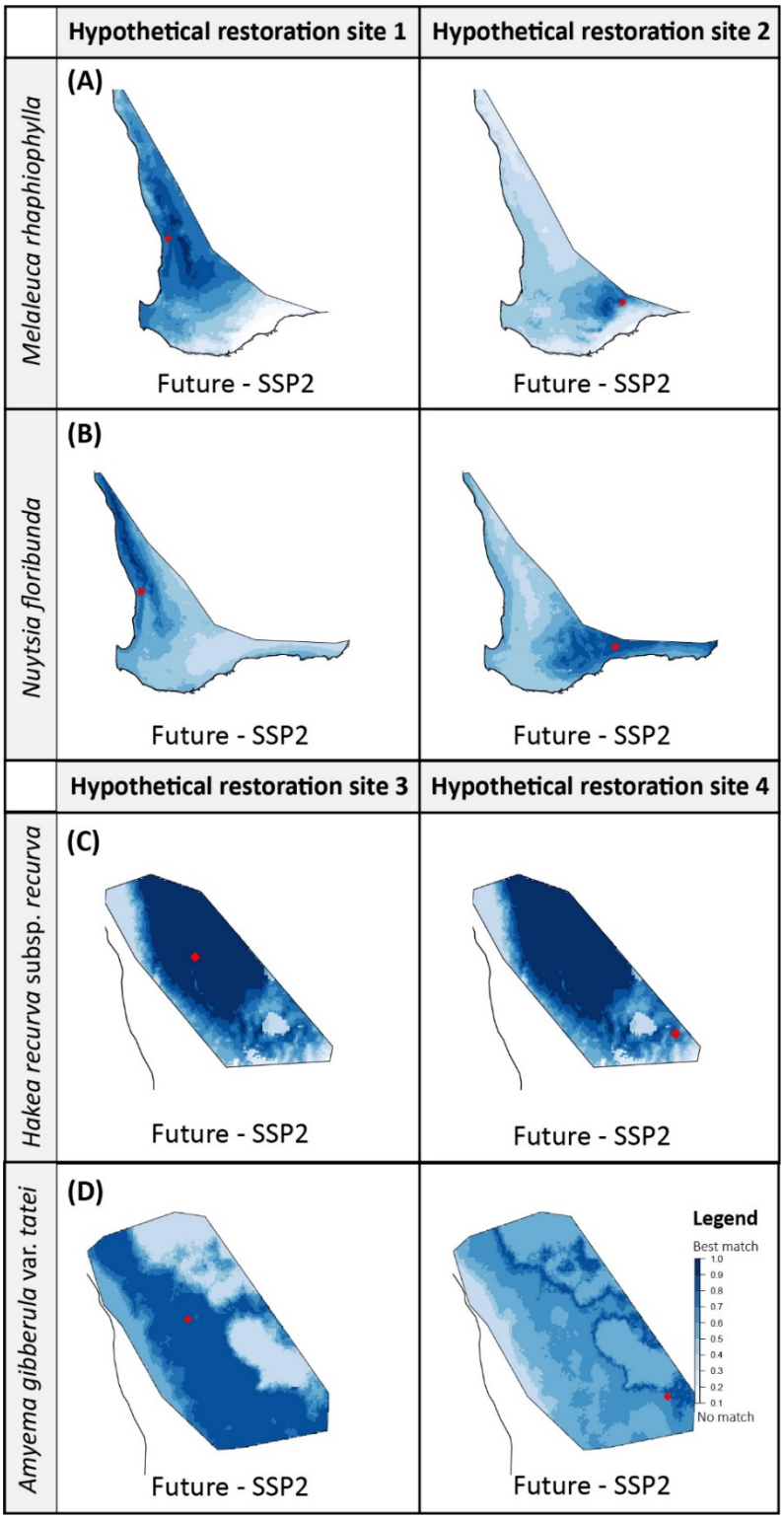


Figure S5.8.4 Predicted genomic similarity of hypothetical restoration sites (red diamonds) to potential seed sourcing locations for two pairs of sympatric plant species across south-western Australia. Two species, (A) *Melaleuca rhaphiophylla* and (B) *Nuytsia floribunda*, co-occur across a mesic habitat and two species, (C) *Hakea recurva* subsp. recurva and (D) *Amyema gibberula* var. *tatei*, co-occur across a semi-arid habitat. Potential seed sourcing locations were projected for current climate conditions under a low-medium emission scenario for 2080-2100 (SPP2). Areas that best match climatic conditions at the hypothetical restoration site are shown in dark blue.

CHAPTER 6

GENERAL DISCUSSION

6.1 Summary of findings

This thesis represents the first study of both adaptive and phylogeographic genetic variation in co-occurring plant species with different nutrient acquisition strategies. In this final discussion chapter I begin by compiling and discussing the main findings from the previous chapters (Figure 6.1). The limitations and significance of this thesis are then discussed in context of this research. Finally, I identify and discuss the future research directions that have arisen from the work within this thesis. The main findings from the thesis are summarised below:

#1 Comparative approaches are useful to examine patterns of adaptive genetic variation in multiple co-occurring species. Comparative studies using genome-wide markers can provide insight into patterns of adaptive variation across the landscape (Bragg et al., 2015; Balkenhol et al., 2017); although studies typically only examine two plant species (e.g., Shryock et al., 2017; Hopley & Byrne, 2019) or numerous species with the same life history traits (e.g., Steane et al., 2017). In this thesis, I use a comparative genomics approach to compare patterns of adaptive genetic variation (Chapters 2 and 3) for two pairs of co-occurring species with different parasitic life histories. Using this approach, I found similar patterns of climate adaptation in both parasitic species, which differed to that of co-occurring autotrophic hosts. This indicates that my findings on genetic patterns for parasitic plants were not limited to a single taxa, and suggests that these patterns could be representative of parasitic plants more broadly. These findings demonstrate the value of comparative studies in understanding the association of genetic patterns with life history traits; this approach will be further enhanced through future application of whole genome sequencing (Gagnaire, 2020).

#2 Adaptive genetic variation associates with different climatic variables in parasitic and autotrophic plant species. Adaptive genetic variation can indicate adaptation of populations to local environmental conditions (Savolainen et al., 2013). In this thesis, I used a landscape genetic approach to assess the association of adaptive genetic variation with climatic variables between two pairs of parasitic and autotrophic plant species (Chapters 2 and 3). I found signals of adaptation to climate for both parasites with greater association of adaptive genetic variation to temperature variables, compared to autotrophic species. Spatial patterns of adaptive genetic

variation also differed between the co-occurring species, suggesting that plant species with different nutrient acquisition strategies may respond differently to climatic selective pressures. Temperature has been previously correlated with genetic variation in a mistletoe species (Ramírez-Barahona et al., 2017), and may provide a greater selective pressure to parasitic plants as they must maintain higher transpiration rates (which are influenced by temperature) to create a water gradient with host plants (Ehleringer et al., 1985; Stewart & Press, 1990). Therefore, temperature may be a more important driver of adaptive genetic variation in parasitic plants. Other climatic variables (i.e. solar radiation) and landscape factors (i.e. barriers to seed dispersal by birds) that were not assessed within this study could also be incorporated into future studies. Nonetheless, my data in these two chapters increases our knowledge of adaptation to climate in parasitic species within a field-based setting as studies to date have largely focussed on the abiotic environment within laboratory settings (e.g., Laine, 2008; Lopez Pascua et al., 2012; Gorter et al., 2016), and have rarely examined parasitic plants. More broadly, these findings add to the growing body of literature on climate adaptation in plant species (reviewed in Balkenhol et al., 2017 and in Ahrens et al., 2018).

#3 Applying comparative genomics to host-parasite systems poses some distinct challenges. In Chapters 2 and 3, I used a comparative approach between two pairs of parasitic and autotrophic plant species. While I found differences in the patterns of climate adaptation between the co-occurring species, other differences were identified between parasitic and autotrophic species that may influence the detection of adaptation within these species. Firstly, I found the parasitic species to have stronger population structuring than host species (Chapter 4), which can make it more difficult to detect loci under selection (de Villemereuil et al., 2014; Flanagan et al., 2018). Secondly, generation times likely differ between the species, particularly for the host-species mistletoe. This could allow adaptation to proceed more rapidly in this species compared to its host (Smith & Donoghue, 2008; Bromham et al., 2013). Thirdly, prior genomic information was not available for any of these nonmodel organisms so I could not further explore the link between adaptive genetic variation and possible gene function within these specific species (Tiffin & Ross-Ibarra, 2014; Bragg et al., 2015; Breed et al., 2019); although future studies could use BLAST to annotate candidate SNPs against sequences found in other species. However, I have been able to compare

patterns of climate adaptation between these co-occurring species, offering insight into patterns of climate adaptation within host-parasitic systems across the landscape (Savolainen et al., 2013; Bragg et al., 2015); although further work through phenotypic studies is needed to confirm the exact mechanism of adaptation within these species. Similar comparative studies have been previously undertaken in co-occurring plants (e.g., Yeaman et al., 2016) and insect species (e.g., Yadav et al., 2021), yet this is the first study to explicitly examine host-parasite systems.

#4 Patterns of nuclear diversity and genetic structure differed between sympatric parasitic and autotrophic plant species. Understanding the variation in patterns of gene flow and connectivity between species with different life history characteristics is crucial for conservation and management planning (Bragg et al., 2015; Broadhurst et al., 2017). In Chapter 4, I compared the nuclear genetic diversity and population structuring between two pairs of co-occurring parasitic and autotrophic plant species. I found the parasites to have lower levels of genetic diversity and higher population differentiation than respective autotrophs, as expected for parasitic species (Price, 1980). Additionally, I found that genetic structure in the parasitic plants did not mirror that of sympatric autotrophs, particularly for the host-specific mistletoe. This suggests that sympatric species with different nutrient acquisition strategies have different patterns of genetic diversity and structure, corroborating earlier findings in parasitic plants (e.g., Jerome & Ford, 2002) and other host-parasitic systems (e.g., Jossart et al., 2017).

#5 Patterns of genetic variation differed between generalist and host-specific species. The level of host-specialisation can influence the neutral genetic diversity (Barrett et al., 2008) and phylogeographic patterns in parasitic species (Nieberding & Olivieri, 2007). In Chapter 4, I compared the genetic diversity and phylogeographic history between a generalist and a host-specific parasite. I found the generalist parasite had higher nuclear genetic diversity and lower population structuring than the host-specific species. Through analysis of chloroplast diversity, I also found historical diversity and divergence to differ between the generalist and host-specific parasites. This suggests that genetic diversity patterns vary between parasites with different levels of host-specialisation. These findings may be due to the reliance of host-specific species on a limited number of hosts, which may increase the risk of localised

extinction events and loss of genetic diversity (Kuijt, 1969; Okubamichael et al., 2016).

#6 Phylogeographic patterns differed between parasitic and autotrophic plant species. Co-occurring species that experience the same geological and historical events may have similar phylogeographic histories (Avise, 2000, 2009), which influence contemporary genetic diversity and structure. In Chapter 4, I used a phylogeographic approach to compare patterns of historical diversity, divergence and persistence in two pairs of sympatric parasitic and autotrophic plant species. I found that all four species had genetic signals of persistence within the landscape but that specific phylogeographic patterns differed between the co-occurring species and mesic vs. semi-arid habitats. This suggests that, despite experiencing a similar geological history and climatic oscillations of the Pleistocene, co-occurring plant species do not necessarily have comparable phylogeographic histories. For this study, these differences could be due to the disparate parasitic life histories in the co-occurring species, which also have adaptive genetic variation correlated with different climatic variables, or a result of other landscape features (e.g., landscape position). Overall, the findings in this chapter contribute to the understanding of evolutionary history of parasitic plant species across the landscape (Funk et al., 2012).

#7 Provenances patterns differ in co-occurring species and between different habitats. Understanding the distribution of genetic provenances is important to guide seed collection strategies for restoration (Vander Mijnsbrugge et al., 2010). In Chapter 5, I combined the genetic data with a spatial modelling approach to delineate areas that best matched the predicted genetic composition of hypothetical restoration sites. I found the distribution of provenances to differ within the two pairs of co-occurring species with different nutrient acquisition strategies, as it does between species with other life history traits (e.g., Shryock et al., 2017; Rossetto et al., 2019). Additionally, the geographical extent of provenances also differed between species from mesic and arid habitats. Specifically, species occurring across steeper climatic gradients in the mesic landscape had a reduced geographical extent of provenances, with greater magnitude of genetic change required to track between current and future climates. Together, this suggests that seed sourcing approaches may differ by both species and habitat.

#8 Species with disparate life history traits can have a similar direction of change in genetic provenances under future climate scenarios. Ideally, seed sourcing strategies should aim to collect genetically diverse seed to maximise the adaptive potential of restored vegetation to current and future climatic conditions (Broadhurst et al., 2008; Breed et al., 2019). In Chapter 5, I projected the spatial modelling to delineate areas that best matched the predicted genetic composition required under current and future climate scenarios. I found the area that best matched future climate scenarios shifted for each species and that the direction of change was similar within the two pairs of co-occurring species. This finding suggests that climate change will likely have a similar effect on seed sourcing in species with disparate life histories. Additionally, I found that future climate scenarios had differing effects on provenance patterns between the two habitats with the greatest changes in geographical extent observed in the mesic pair. . This suggests that seed sourcing approaches for future climates could vary across different habitats.

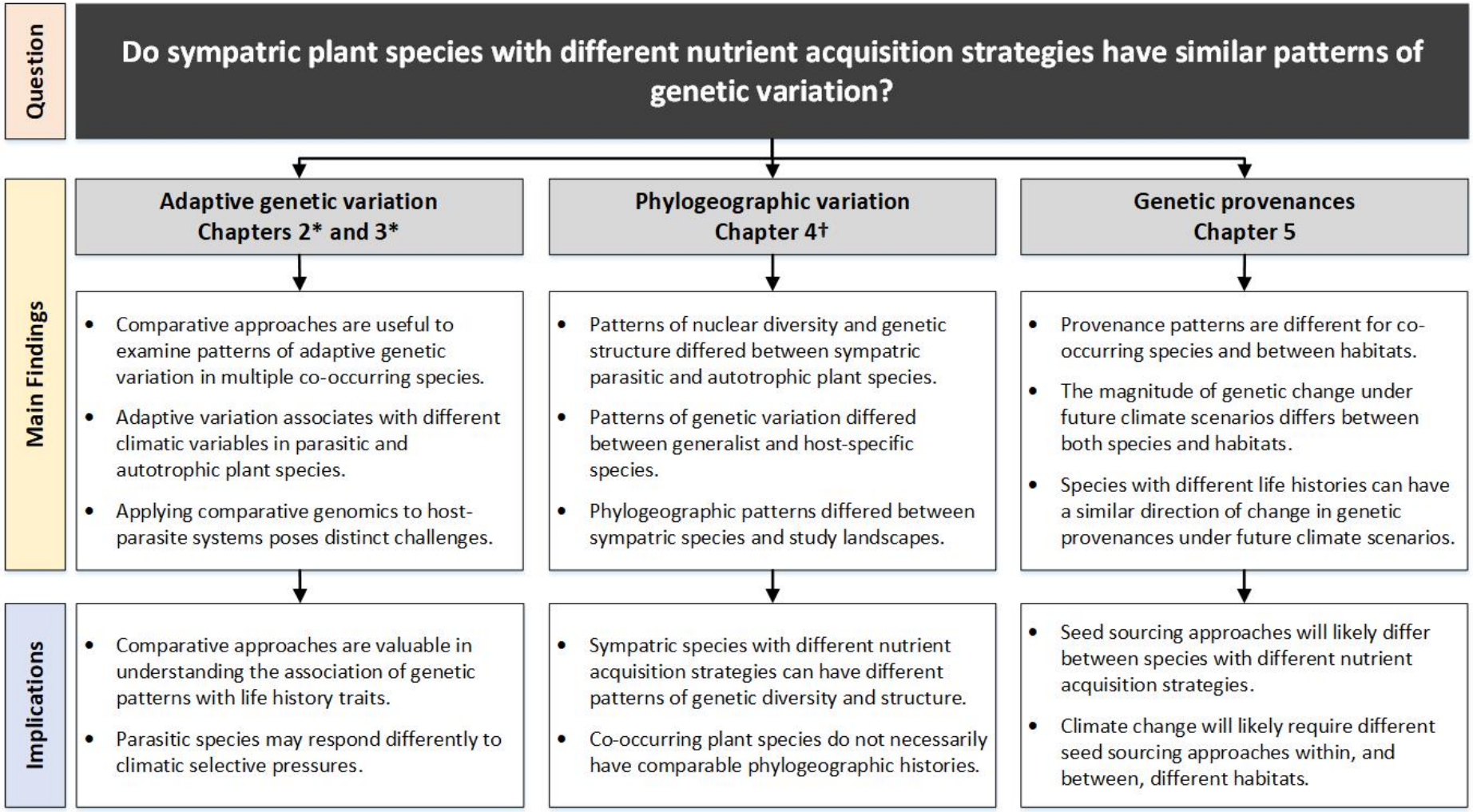


Figure 6.1 Conceptual framework of the main findings and implications of the research in this thesis. *Paper published or accepted. †Manuscript under review.

6.2 Limitations of this work

The research within this thesis demonstrates the potential of applying multidisciplinary approaches to understanding patterns of genetic variation in co-occurring species; yet there remains several limitations to this work. Firstly, while advancements in genome sequencing technology have enabled nonmodel organisms to be studied using landscape genomics (Haasl & Payseur, 2016; Ahrens et al., 2018), adaptive genetic variation cannot be linked to the mechanism of adaptation without phenotypic studies. Specifically, controlled experiments (i.e. common garden studies, reciprocal transplant experiments; Kawecki & Ebert, 2004; Blanquart et al., 2013) of genotyped individuals could be used to link gene frequencies to fitness and confirm drivers of adaptation in these species. Nonetheless, in the absence of phenotypic studies, patterns of adaptive variation can still be compared between species to provide insight to climate adaptation across the landscape (Savolainen et al., 2013). In this thesis, I compared patterns of adaptive genetic variation between two pairs of co-occurring nonmodel species and, although I was not able to infer the underlying mechanisms of adaptation in these species, I was still able to compare patterns of climate adaptation across the landscape.

A second limitation to landscape genetic studies is that loci identified by genome scans may not necessary be directly under selection but instead linked to loci under selection (i.e., genetic hitch-hiking; Barton, 2000). The chance of neutral loci being incorrectly identified as adaptive (and vice versa) depends upon numerous evolutionary parameters within species that influence the accurate detection of adaptive loci (Lotterhos & Whitlock, 2015). In this thesis, I used a consensus of multiple genome scan methods when identifying adaptive genetic variation to minimise the inclusion of false positives (De Mita et al., 2013). However, I also found stronger genetic structure in the parasitic species when compared to sympatric autotrophic species. Although neutral genetic structure was accounted for in the environment-association analyses (Lotterhos & Whitlock, 2015; Forester et al., 2018), stronger population structure may have made it more difficult to detect loci under selection (de Villemereuil et al., 2014; Flanagan et al., 2018).

A third limitation of this thesis is that multicollinearity among climatic variables may conceal true drivers of climate adaptation. Specifically, adaptive genetic variation may be associated with a climatic variable that does not itself drive adaptation, but is

correlated with an environmental variable that does (Hoban et al., 2016). In this thesis, I selected the climatic variables to minimise multicollinearity and these variables explained a large proportion of the allelic turnover in statistical modelling for all four species. However, environmental variables that were not examined in this work could also drive adaptation within species (e.g., solar radiation, altitude; Garnier-Géré & Ades, 2001; Gauli et al., 2015). Confirming the exact climatic drivers of adaptation in these species will be aided by phenotypic studies (discussed further in section 6.4).

A fourth limitation of this work is that the two parasite-autotroph species pairs were distributed across two different geographical landscapes and sampling intensity varied between these areas. In this thesis, I paired the parasitic plants with autotrophs co-occurring within the same geographical landscape, such that paired species would likely have experienced similar geological and historical events. The intention of this was to provide comparative information on sympatric species in multiple landscapes. However, sampling intensity varied between the landscapes predominantly due to the generalist parasite being more ubiquitous across the landscape than the host-specific species. The host-specific species was more patchily distributed with fewer populations available for sampling, which may reduce statistical power of spatial modelling in some geographical areas (Supple et al., 2018). Sampling additional populations across the landscape would have increased the accuracy of statistical models and provided finer detailed patterns of genetic variation across the landscape.

Finally, the spatial analysis of genetic provenances was limited by the availability of climatic data at the same resolution for all climatic scenarios. Very fine scale resolution climatic layers were available for current climatic data (30 arc-seconds; Fick & Hijmans, 2017) but this resolution was not available for the future climate scenarios, which had a lowest resolution of 2.5 arc-minutes. In this thesis, I used the very fine scale resolution climatic data in Chapters 2 and 3 to associate adaptive genetic variation with climatic variables. However, as the future climate data did not have the same resolution, I opted to use the courser 2.5 arc-minute spatial resolution for all climatic datasets in the final data chapter (Chapter 5) to ensure consistency between the different climatic scenarios. As finer scaled climate projections generally produce more detailed spatial predictions (e.g., Franklin et al., 2013), this resulted in comparatively less detailed spatial predictions for the genetic provenances compared to the earlier chapters.

6.3 Significance of thesis

The research presented within this thesis has made a significant and original contribution to the study of genetic variation through novel applications of analytical methods, application of genetic approaches to new study systems, and the use of a multidisciplinary approach to investigate genome-wide patterns of genetic variation in two pairs of co-occurring plant species. Firstly, this research presents a novel application of Procrustes Analysis (Peres-Neto & Jackson, 2001) in comparing and visualising multivariate configuration. Procrustes Analysis has previously been used within landscape genetics modelling to compare two genetic datasets (Fitzpatrick & Keller, 2015) but the research within this thesis (Chapters 2 and 3) presents the first application of this analytical techniques within a comparative species study (discussed further in Chapter 2; Walters et al., 2020). The research within this thesis is also the first to assess temporal variation of genetic composition between current and future climates in a comparative species study (Chapter 5). This approach has only been applied prior in single species studies (e.g., Supple et al., 2018; Ahrens et al., 2020; Ingvarsson & Bernhardsson, 2020). This thesis also used the most recent future climate predictions – the shared socioeconomic pathways (O'Neill et al., 2017) – which have not yet been applied to provenance delineation using genome-wide data.

Secondly, this thesis presents the first work to apply genomic and spatial approaches to study climate adaptation within multiple host-parasite systems (Chapters 2 and 3). Previously, studies on host-parasite systems have focussed on assessing only neutral genetic variation (e.g., Jerome & Ford, 2002; Feurtey et al., 2016) or on assessing adaptation to the abiotic environment within laboratory settings (e.g., Laine, 2008; Lopez Pascua et al., 2012; Gorter et al., 2016). No research had combined genetic and spatial approaches to assess adaptation to climate in field based studies. Examining adaptive genetic variation will be important to increasing our understanding of climate adaptation ecologically important species (e.g., parasitic plants; Press & Graves, 1995; Watson, 2001; Press & Phoenix, 2005).

Third, this thesis provides species-specific information on adaptive genetic variation (Chapters 2 and 3), neutral genetic variation (Chapter 4), and genetic provenances (Chapter 5) for two species co-occurring across a mesic habitat and two species co-occurring across a semi-arid habitat. Information on species-specific genetic patterns

can be used at a local level for species conservation and in designing seed collection strategies for restoration (Broadhurst et al., 2008; Funk et al., 2012; Hoffmann et al., 2015; Flanagan et al., 2018).

Finally, this thesis applies a multidisciplinary approach to investigate genome-wide patterns of genetic variation within multiple co-occurring plant species with different life history traits, which has rarely been completed prior. Most comparative studies on climate adaptation using genome-wide markers generally only consider two plant species (e.g., Shryock et al., 2017; Hopley & Byrne, 2019) or multiple species with similar life histories (e.g., Steane et al., 2017). Similarly, phylogeographic studies commonly compare patterns between two co-occurring species (e.g., Amico & Nickrent, 2009; Binks et al., 2015; Millar et al., 2016, 2017) but rarely between multiple pairs of sympatric species. This thesis uses a combination of genetic techniques and spatial modelling approaches to detect climate adaptation (Chapters 2 and 3) and phylogeographic patterns (Chapter 4) in two pairs of co-occurring plant species with different life history traits. This enabled patterns of climate adaptation, genetic diversity and phylogeographic history to be compared both within and between the two species pairs. Furthermore, while a multidisciplinary approach to delineating genetic provenances using genome-wide markers and spatial modelling has been utilised previously (e.g., Shryock et al., 2017; Supple et al., 2018; Rossetto et al., 2019; Rossetto et al., 2020; Carvalho et al., 2021), rarely do these studies compare genetic provenances across multiple climate scenarios or using the shared socioeconomic pathways (SSPs; O'Neill et al., 2017). In this thesis, I combined the genetic data with a spatial modelling in a multidisciplinary approach to assess genetic provenances for both species pairs under current climate and two future SSP scenarios (Chapter 5). This thesis can be used to guide future work using multidisciplinary approaches to inform seed scouring strategies for future climates.

6.4 Future directions

6.4.1 New aims and questions arising from the thesis

The application of genome-wide markers to this multidisciplinary, comparative study, has highlighted some additional aims and questions for future research. Although not exhaustive, the list below represents topics of future enquiry that I consider to be some of the logical extensions to the work within this thesis.

Future direction #1. The association of genome-wide markers with climatic variables provides evidence consistent with local adaptation but this does not confirm the exact drivers of adaptation. For instance, the association of temperature variables with adaptive genetic variation could reflect a parasitic life history. However, as previously discussed in the limitations section, it could also arise from multi-collinearity between variables (Hoban et al., 2016). Phenotype studies such as common garden studies and reciprocal transplant experiments (Kawecki & Ebert, 2004; Blanquart et al., 2013) could be used in future work to complement molecular genetic research and confirm the drivers of adaptation in co-occurring species.

Future direction #2. A lack of research on adaptive genetic variation in parasitic species means that it is not yet possible to surmise whether the findings in this thesis will be replicated in other host-parasite systems. Whilst the patterns of neutral genetic variation in this thesis corroborated findings from other studies on parasitic plants (e.g., Jerome & Ford, 2002), patterns of genetic structure contrasted to those found in other host-parasite systems (e.g., Feurtey et al., 2016; Hartmann et al., 2020). In the future, this work could be expanded to analyse climate adaptation within other host-parasite systems, particularly where parasitic species have population structures that mirror that of the host.

Future direction #3. Comparing species across two landscapes provides information that is not limited to a single study landscape but this can make applying a consistent sampling strategy more difficult. In this thesis, one species was more patchily distributed across the landscape than the others, which meant that sampling intensity varied between landscapes. This can have implications for spatial modelling (Supple et al., 2018). Future studies should focus on comparing species pairs that have similar distribution patterns across the same, or respective, landscapes.

6.4.2 Recommendations on methods for future studies

Over the duration of this thesis, the field of landscape genomics, its application to understanding climate adaptation and delineation of genetic provenances, have progressed rapidly. Here, I propose some future directions in these techniques that underpin potential additional work in these evolving fields.

Future direction #4. References genomes and transcriptomes are currently unavailable for many species – including those within this thesis. Expanding the

database of reference genomes to cover nonmodel species would enable future studies to identify potential underlying mechanisms of adaptation (Tiffin & Ross-Ibarra, 2014; Bragg et al., 2015; Breed et al., 2019), that could then be verified through phenotypic studies. Additionally, future studies could use BLAST to annotate candidate SNPs against sequences found in other species, which could discover conserved gene functions that exist across taxa.

Future direction #5. Variation in sampling intensity in species across the landscape can affect the power of predictive models (Supple et al., 2018). In this thesis, sampling intensity varied between species pairs and some geographical areas that were predicted to be genetically distinct only had only population sampled within the area. Future work in landscape genetics could increase the number of populations sampled and reduce the individuals sampled per population to increase the power of statistical models (De Mita et al., 2013). Additionally, populations could be paired across the landscape to further verify the association between adaptive genetic variation and climatic variables (Storfer et al., 2018).

Future direction #6. Finer scaled climate projections produce more detailed spatial predictions (Franklin et al., 2013). While the current bioclimatic data was available in the highest 1km^2 resolution, predictions for future climate scenarios were only available to 21km^2 resolution. Here, I have used the coarser spatial resolution for genetic provenance delineation to ensure consistency between current and future datasets, although this resulted in comparatively less detailed spatial predictions. Future work could further refine the predictions of genetic provenances as higher resolution datasets for future climate scenarios become available.

6.5 Thesis conclusion

Understanding spatial patterns of adaptive and neutral genetic variation in plant species is important for the delineation of conservation units and provenances for seed sourcing. To date, comparative studies have provided useful information on the effect of numerous life history traits across plant taxa, but few have assessed species with different parasitic life histories. The overarching question of this thesis was “Do plant species with different nutrient acquisition strategies have similar patterns of genetic variation?”. This thesis demonstrates that co-occurring parasitic and autotrophic plant species have different patterns of adaptive and neutral genetic variation. It shows

adaptive genetic variation to be associated with different climatic variables in the parasitic species, suggesting that parasitic plants may respond differently to climatic selective pressures. This thesis also revealed contrasting patterns of neutral genetic variation between sympatric species with different parasitic life histories, and between parasites with different levels of hosts-specialisation. Finally, the landscape genomics approach found different provenance patterns between two pairs of sympatric parasitic and autotrophic plant species under multiple climate scenarios, but also between the mesic and arid habitats. This indicates that seed sourcing approaches will be both species and habitat dependent, and approaches to provenance will be influenced by differential response to climate change. With further development and application to a broader range of taxa with different life histories, comparative landscape genomics studies will continue to increase our understanding of the spatial patterns of genetic variation in plant species.

6.6 References

Every reasonable effort has been made to acknowledge the owners of the copyright material. I would be pleased to hear from any copyright owner who has been omitted or incorrectly acknowledged.

Ahrens, C. W., James, E. A., Miller, A. D., Scott, F., Aitken, N. C., Jones, A. W., . . . Rymer, P. D. (2020). Spatial, climate and ploidy factors drive genomic diversity and resilience in the widespread grass *Themeda triandra*. *Molecular Ecology, 29*, 3872-3888. https://doi.org/10.1111/mec.15614

Ahrens, C. W., Rymer, P. D., Stow, A., Bragg, J., Dillon, S., Umbers, K. D. L., & Dudaniec, R. Y. (2018). The search for loci under selection: Trends, biases and progress. *Molecular Ecology, 27*, 1342–1356. https://doi.org/10.1111/mec.14549

Amico, G. C., & Nickrent, D. L. (2009). Population structure and phylogeography of the mistletoes *Tristerix corymbosus* and *T. aphyllus* (Loranthaceae) using chloroplast DNA sequence variation. *American Journal of Botany, 96*, 1571-1580. https://doi.org/10.3732/ajb.0800302

Avise, J. C. (2000). *Phylogeography: The history and formation of species*. Cambridge, MA: Harvard University Press.

Avise, J. C. (2009). Phylogeography: Retrospect and prospect. *Journal of Biogeography, 36*, 3-15. https://doi.org/10.1111/j.1365-2699.2008.02032.x

Balkenhol, N., Dudaniec, R. Y., Krutovsky, K. V., Johnson, J. S., Cairns, D. M., Segelbacher, G., . . . Joost, S. (2017). Landscape genomics: Understanding relationships between environmental heterogeneity and genomic characteristics of populations. In O. P. Rajora (Ed.), *Population genomics concepts, approaches and applications* (pp. 261-322). Cham: Springer. https://doi.org/10.1007/978-3-030-04589-0

Barrett, L. G., Thrall, P. H., Burdon, J. J., & Linde, C. C. (2008). Life history determines genetic structure and evolutionary potential of host–parasite interactions. *Trends in Ecology & Evolution, 23*, 678-685. https://doi.org/10.1016/j.tree.2008.06.017

Barton, N. H. (2000). Genetic hitchhiking. *Philosophical Transactions: Biological Sciences, 355*, 1553-1562. https://doi.org/10.1098/rstb.2000.0716

Binks, R. M., Millar, M. A., & Byrne, M. (2015). Not all rare species are the same: contrasting patterns of genetic diversity and population structure in two narrow-range endemic sedges. *Biological Journal of the Linnean Society, 114*(4), 873-886. 10.1111/bij.12465

Blanquart, F., Kaltz, O., Nuismer, S. L., & Gandon, S. (2013). A practical guide to measuring local adaptation. *Ecology Letters, 16*, 1195-1205. https://doi.org/10.1111/ele.12150

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

Breed, M. F., Harrison, P. A., Blyth, C., Byrne, M., Gaget, V., Gellie, N. J. C., . . . Mohr, J. J. (2019). The potential of genomics for restoring ecosystems and biodiversity. *Nature Reviews Genetics, 20*, 615-628. https://doi.org/10.1038/s41576-019-0152-0

Broadhurst, L., Breed, M., Lowe, A., Bragg, J., Catullo, R., Coates, D., . . . Byrne, M. (2017). Genetic diversity and structure of the Australian flora. *Diversity and Distributions, 23*, 41-52. https://doi.org/10.1111/ddi.12505

Broadhurst, L. M., Lowe, A., Coates, D. J., Cunningham, S. A., McDonald, M., Vesk, P. A., & Yates, C. (2008). Seed supply for broadscale restoration: Maximizing evolutionary potential. *Evolutionary Applications, 1*, 587-597. https://doi.org/10.1111/j.1752-4571.2008.00045.x

Bromham, L., Cowman, P. F., & Lanfear, R. (2013). Parasitic plants have increased rates of molecular evolution across all three genomes. *BMC Evolutionary Biology, 13*, 126. https://doi.org/10.1186/1471-2148-13-126

Carvalho, C. S., Forester, B. R., Mitre, S. K., Alves, R., Imperatriz-Fonseca, V. L., Ramos, S. J., . . . Jaffé, R. (2021). Combining genotype, phenotype, and environmental data to delineate site-adjusted provenance strategies for ecological restoration. *Molecular Ecology Resources, 21*, 44-58. https://doi.org/10.1111/1755-0998.13191

De Mita, S., Thuillet, A., Gay, L., Ahmadi, N., Manel, S., Ronfort, J., & Vigouroux, Y. (2013). Detecting selection along environmental gradients: Analysis of eight methods and their effectiveness for outbreeding and selfing populations. *Molecular Ecology, 22*, 1383-1399. https://doi.org/10.1111/mec.12182

de Villemereuil, P., Frichot, É., Bazin, É., François, O., & Gaggiotti, O. E. (2014). Genome scan methods against more complex models: When and how much should we trust them? *Molecular Ecology, 23*, 2006-2019. https://doi.org/10.1111/mec.12705

Ehleringer, J. R., Schulze, E.-D., Ziegler, H., Lange, O. L., Farquhar, G. D., & Cowar, I. R. (1985). Xylem-tapping mistletoes: Water or nutrient parasites? *Science, 227*, 1479-1481. https://doi.org/10.1126/science.227.4693.1479

Feurtey, A., Gladieux, P., Hood, M. E., Snirc, A., Cornille, A., Rosenthal, L., & Giraud, T. (2016). Strong phylogeographic co-structure between the anther-smut fungus and its white campion host. *New Phytologist, 212*, 668-679. https://doi.org/10.1111/nph.14125

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

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

Flanagan, S. P., Forester, B. R., Latch, E. K., Aitken, S. N., & Hoban, S. (2018). Guidelines for planning genomic assessment and monitoring of locally adaptive variation to inform species conservation. *Evolutionary Applications, 11*, 1035-1052. https://doi.org/10.1111/eva.12569

Forester, B. R., Lasky, J. R., Wagner, H. H., & Urban, D. L. (2018). Comparing methods for detecting multilocus adaptation with multivariate genotype–environment associations. *Molecular Ecology, 27*, 2215-2233. https://doi.org/10.1111/mec.14584

Franklin, J., Davis, F. W., Ikegami, M., Syphard, A. D., Flint, L. E., Flint, A. L., & Hannah, L. (2013). Modeling plant species distributions under future climates: How fine scale do climate projections need to be? *Global Change Biology, 19*, 473-483. https://doi.org/10.1111/gcb.12051

Funk, W. C., McKay, J. K., Hohenlohe, P. A., & Allendorf, F. W. (2012). Harnessing genomics for delineating conservation units. *Trends in Ecology & Evolution, 27*(9), 489-496. https://doi.org/10.1016/j.tree.2012.05.012

Gagnaire, P. (2020). Comparative genomics approach to evolutionary process connectivity. *Evolutionary Applications, 13,* 1320-1334. https://doi.org/10.1111/eva.12978

Garnier-Géré, P. H., & Ades, P. K. (2001). Environmental surrogates for predicting and conserving adaptive genetic variability in tree species. *Conservation Biology, 15*, 1632-1644. https://doi.org/10.1046/j.1523-1739.2001.00180.x

Gauli, A., Vaillancourt, R. E., Bailey, T. G., Steane, D. A., & Potts, B. M. (2015). Evidence for local climate adaptation in early-life traits of Tasmanian populations of *Eucalyptus pauciflora*. *Tree Genetics & Genomes, 11*(104), 1-14. https://doi.org/10.1007/s11295-015-0930-6

Gorter, F. A., Scanlan, P. D., & Buckling, A. (2016). Adaptation to abiotic conditions drives local adaptation in bacteria and viruses coevolving in heterogeneous environments. *Biology letters, 12*, 20150879. https://doi.org/10.1098/rsbl.2015.0879

Haasl, R. J., & Payseur, B. A. (2016). Fifteen years of genomewide scans for selection: Trends, lessons and unaddressed genetic sources of complication. *Molecular Ecology, 25*, 5-23. https://doi.org/10.1111/mec.13339

Hartmann, F. E., Snirc, A., Cornille, A., Godé, C., Touzet, P., Van Rossum, F., . . . Giraud, T. (2020). Congruent population genetic structures and divergence histories in anther-smut fungi and their host plants *Silene italica* and the *Silene nutans* species complex. *Molecular Ecology, 29*, 1154-1172. https://www.doi.org/10.1111/mec.15387

Hoban, S., Kelley, J. L., Lotterhos, K. E., Antolin, M. F., Bradburd, G., Lowry, D. B., . . . Whitlock, M. C. (2016). Finding the genomic basis of local

adaptation: Pitfalls, practical solutions, and future directions. *The American Naturalist, 188*, 379-397. https://doi.org/10.1086/688018

Hoffmann, A., Griffin, P., Dillon, S., Catullo, R., Rane, R., Byrne, M., . . . Sgrò, C. (2015). A framework for incorporating evolutionary genomics into biodiversity conservation and management. *Climate Change Responses, 2*, 1-23. https://doi.org/10.1186/s40665-014-0009-x

Hopley, T., & Byrne, M. (2019). Gene flow and genetic variation explain signatures of selection across a climate gradient in two riparian species. *Genes, 10*, 579. https://doi.org/10.3390/genes10080579

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

Jerome, C. A., & Ford, B. A. (2002). Comparative population structure and genetic diversity of *Arceuthobium americanum* (Viscaceae) and its *Pinus* host species: Insight into host–parasite evolution in parasitic angiosperms. *Molecular Ecology, 11*, 407-420. https://doi.org/10.1046/j.0962-1083.2002.01462.x

Jossart, Q., De Ridder, C., Lessios, H. A., Bauwens, M., Motreuil, S., Rigaud, T., . . . David, B. (2017). Highly contrasted population genetic structures in a host–parasite pair in the Caribbean Sea. *Ecology and Evolution, 7*, 9267-9280. https://doi.org/10.1002/ece3.3413

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

Kuijt, J. (1969). *The biology of parasitic flowering plants*. Berkeley, California: University of California Press.

Laine, A.-L. (2008). Temperature-mediated patterns of local adaptation in a natural plant–pathogen metapopulation. *Ecology Letters, 11*, 327-337. https://doi.org/10.1111/j.1461-0248.2007.01146.x

Lopez Pascua, L., Gandon, S., & Buckling, A. (2012). Abiotic heterogeneity drives parasite local adaptation in coevolving bacteria and phages. *Journal of*

Evolutionary Biology, 25, 187-195. https://doi.org/10.1111/j.1420-9101.2011.02416.x

Lotterhos, K. E., & Whitlock, M. C. (2015). The relative power of genome scans to detect local adaptation depends on sampling design and statistical method. *Molecular Ecology, 24*, 1031-1046. https://doi.org/10.1111/mec.13100

Millar, M. A., Byrne, M., Coates, D. J., & Roberts, J. D. (2016). Contrasting diversity and demographic signals in sympatric narrow-range endemic shrubs of the south-west Western Australian semi-arid zone. *Biological Journal of the Linnean Society, 118*, 315-329. https://doi.org/10.1111/bij.12736

Millar, M. A., Byrne, M., Coates, D. J., & Roberts, J. D. (2017). Comparative analysis indicates historical persistence and contrasting contemporary structure in sympatric woody perennials of semi-arid south-west Western Australia. *Biological Journal of the Linnean Society, 120*, 771-787. https://doi.org/10.1093/biolinnean/blw005

Nieberding, C. M., & Olivieri, I. (2007). Parasites: Proxies for host genealogy and ecology? *Trends in Ecology & Evolution, 22*, 156-165. https://doi.org/10.1016/j.tree.2006.11.012

O'Neill, B. C., Kriegler, E., Ebi, K. L., Kemp-Benedict, E., Riahi, K., Rothman, D. S., . . . Solecki, W. (2017). The roads ahead: Narratives for shared socioeconomic pathways describing world futures in the 21st century. *Global Environmental Change, 42*, 169-180. https://doi.org/10.1016/j.gloenvcha.2015.01.004

Okubamichael, D. Y., Griffiths, M. E., & Ward, D. (2016). Host specificity in parasitic plants—perspectives from mistletoes. *AoB PLANTS, 8*(plw069). https://doi.org/10.1093/aobpla/plw069

Peres-Neto, P. R., & Jackson, D. A. (2001). How well do multivariate data sets match? The advantages of a Procrustean superimposition approach over the Mantel test. *Oecologia, 129*, 169-178. https://doi.org/10.1007/s004420100720

Press, M. C., & Graves, J. D. (1995). *Parasitic Plants*. London, UK: Chapman & Hall.

Press, M. C., & Phoenix, G. K. (2005). Impacts of parasitic plants on natural communities. *New Phytologist, 166*, 737-751. https://doi.org/10.1111/j.1469-8137.2005.01358.x

Price, P. W. (1980). *Evolutionary biology of parasites*. Princeton, NJ: Princeton University Press.

Ramírez-Barahona, S., González, C., González-Rodríguez, A., & Ornelas, J. F. (2017). The influence of climatic niche preferences on the population genetic structure of a mistletoe species complex. *New Phytologist, 214*, 1751-1761. https://doi.org/10.1111/nph.14471

Rossetto, M., Bragg, J., Kilian, A., McPherson, H., van der Merwe, M., & Wilson, P. D. (2019). Restore and Renew: A genomics-era framework for species provenance delimitation. *Restoration Ecology, 27*, 538-548. https://doi.org/10.1111/rec.12898

Rossetto, M., Wilson, P. D., Bragg, J., Cohen, J., Fahey, M., Yap, J. L. S., & van der Merwe, M. (2020). Perceptions of similarity can mislead provenancing strategies—An example from five co-distributed *Acacia* species. *Diversity, 12*(8), 306. http://doi.org/10.3390/d12080306

Savolainen, O., Lascoux, M., & Merilä, J. (2013). Ecological genomics of local adaptation. *Nature Reviews Genetics, 14*, 807-820. https://doi.org/10.1038/nrg3522

Shryock, D. F., Havrilla, C. A., DeFalco, L. A., Esque, T. C., Custer, N. A., & Wood, T. E. (2017). Landscape genetic approaches to guide native plant restoration in the Mojave Desert. *Ecological Applications, 27*, 429-445. https://doi.org/10.1002/eap.1447

Smith, S. A., & Donoghue, M. J. (2008). Rates of molecular evolution are linked to life history in flowering plants. *Science, 322*, 86-89. https://doi.org/10.1126/science.1163197

Steane, D. A., Potts, B. M., McLean, E. H., Collins, L., Holland, B. R., Prober, S. M., . . . Byrne, M. (2017). Genomic scans across three eucalypts suggest that adaptation to aridity is a genome-wide phenomenon. *Genome Biology and Evolution, 9*, 253-265. https://doi.org/10.1093/gbe/evw290

Stewart, G. R., & Press, M. C. (1990). The physiology and biochemistry of parasitic angiosperms. *Annual Review of Plant Physiology and Plant Molecular Biology, 41*, 127-151. https://doi.org/10.1146/annurev.pp.41.060190.001015

Storfer, A., Patton, A., & Fraik, A. K. (2018). Navigating the interface between landscape genetics and landscape genomics. *Frontiers in Genetics, 9*(68), 1-14. https://doi.org/10.3389/fgene.2018.00068

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

Tiffin, P., & Ross-Ibarra, J. (2014). Advances and limits of using population genetics to understand local adaptation. *Trends in Ecology & Evolution, 29*, 673-680. https://doi.org/10.1016/j.tree.2014.10.004

Vander Mijnsbrugge, K., Bischoff, A., & Smith, B. (2010). A question of origin: Where and how to collect seed for ecological restoration. *Basic and Applied Ecology, 11*, 300-311. https://doi.org/10.1016/j.baae.2009.09.002

Walters, S. J., Robinson, T. P., Byrne, M., Wardell-Johnson, G. W., & Nevill, P. (2020). Contrasting patterns of local adaptation along climatic gradients between a sympatric parasitic and autotrophic tree species. *Molecular Ecology, 29*, 3022-3037. https://doi.org/10.1111/mec.15537

Watson, D. M. (2001). Mistletoe–a keystone resource in forests and woodlands worldwide. *Annual Review of Ecology and Systematics, 32*, 219-249. https://doi.org/10.1146/annurev.ecolsys.32.081501.114024

Yadav, S., Stow, A. J., & Dudaniec, R. Y. (2021) Microgeographical adaptation corresponds to elevational distributions of congeneric montane grasshoppers. *Molecular Ecology, 30*, 481-498. https://doi.org/10.1111/mec.15739

Yeaman, S., Hodgins, K. A., Lotterhos, K. E., Suren, H., Nadeau, S., Degner, J. C., . . . Aitken, S. N. (2016). Convergent local adaptation to climate in distantly related conifers. *Science, 353*, 1431-1433. https://doi.org/10.1126/science.aaf7812

APPENDIX I. COPYRIGHT STATEMENTS

Chapter 2: Published in the journal Molecular Ecology. As an author, permission is automatically granted to reproduce this copyright material if it constitutes less than half of the total material in such publication.

To Whom It My Concern, I, Sheree Jaye Walters, contributed to the conceptualisation and design of the study, collected and processed the samples, analysed the data, and wrote and edited the resulting manuscript:

Walters, S. J., T. P. Robinson, M. Byrne, G. W. Wardell-Johnson, and P. Nevill. (2020). Contrasting patterns of local adaptation along climatic gradients between a sympatric parasitic and autotrophic tree species. *Molecular Ecology 29:* 3022-3037. https://www.doi.org/10.1111/mec.15537.

_____

I, as a co-author, endorse that this level of contribution by the candidate indicated above is appropriate.

Dr Todd Robinson

_____

Dr Margaret Byrne

_____

Dr Grant Wardell-Johnson

_____

Dr Paul Nevill

_____

Chapter 3: Accepted at Evolutionary Applications. As an author, permission is automatically granted to reproduce this copyright material if it constitutes less than half of the total material in such publication.

To Whom It My Concern, I, Sheree Jaye Walters, contributed to the conceptualisation and design of the study, collected and processed the samples, analysed the data, and wrote and edited the resulting manuscript:

Walters, S. J., T. P. Robinson, M. Byrne, G. W. Wardell-Johnson, and P. Nevill. (In press). Association of adaptive genetic variation with climatic variables differs between a host-specific mistletoe and its host. *Evolutionary Applications.*

_____

I, as a co-author, endorse that this level of contribution by the candidate indicated above is appropriate.

Dr Todd Robinson

_____

Dr Margaret Byrne

_____

Dr Grant Wardell-Johnson

_____

Dr Paul Nevill

_____

Chapter 4: Under review at Evolutionary Applications. As an author, permission is automatically granted to reproduce this copyright material if it constitutes less than half of the total material in such publication.

To Whom It My Concern, I, Sheree Jaye Walters, contributed to the conceptualisation and design of the study, collected and processed the samples, analysed the data, and wrote and edited the resulting manuscript:

Walters, S. J., M. Byrne, J. R. P. Worth, G. W. Wardell-Johnson, and P. Nevill. [Under review] Comparative phylogeography of parasitic and autotrophic plants reveals the complex effects of life history traits on the distribution of genetic variation. *Journal of Biogeography.*

_____

I, as a co-author, endorse that this level of contribution by the candidate indicated above is appropriate.

Dr Margaret Byrne

_____

Dr James Worth

_____

Dr Grant Wardell-Johnson

_____

Dr Paul Nevill

_____

Chapter 5: In preparation for publication.

To Whom It My Concern, I, Sheree Jaye Walters, contributed to the conceptualisation and design of the study, collected and processed the samples, analysed the data, and wrote and edited the resulting manuscript:

Walters, S. J., T. P. Robinson, M. Byrne, G. W. Wardell-Johnson, and P. Nevill. Seed sourcing in the genomics era: Multispecies provenance delineation for current and future climates. *[In preparation]*.

_____

I, as a co-author, endorse that this level of contribution by the candidate indicated above is appropriate.

Dr Todd Robinson

_____

Dr Margaret Byrne

_____

Dr Grant Wardell-Johnson

_____

Dr Paul Nevill

_____

Walters, S. J., T. P. Robinson, M. Byrne, G. W. Wardell-Johnson, and P. Nevill. (2020). Contrasting patterns of local adaptation along climatic gradients between a sympatric parasitic and autotrophic tree species. Molecular Ecology 29: 3022-3037.

https://www.doi.org/10.1111/mec.15537.