Genetic variation and resilience to climate change in Mediterranean-type trees

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THESIS DECLARATION

I, João Carlos Melanda Filipe, declare that:

This thesis is my account of my research and has been substantially accomplished during enrolment in this degree, except where other sources are fully acknowledged. All co-authors, where stated and certified by my Principal Supervisor or Executive Author, have agreed that the works presented in this thesis represent substantial contributions from myself. The thesis contains as its main content, work that has not previously been submitted for a degree at any other university. In the future, no part of this thesis will be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of Murdoch University and where applicable, any partner institution responsible for the joint-award of this degree. This thesis does not contain any material previously published or written by another person, except where due reference has been made in the text and, where relevant, in the Authorship Declaration that follows. This thesis does not violate or infringe any copyright, trademark, patent, or other rights whatsoever of any person.

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This thesis contains published work and/or work prepared for publication, some of which has been co-authored.

This research project was conducted on unceded Whadjuk land, from the Noongar people, First Nations of Australia. I acknowledge them as the traditional custodians of this land, pay my respects to elders, past, present, and emerging, and recognise that the violence of colonialism committed is ongoing and pervasive. All settlers must work to understand and mitigate it.

This PhD candidature was partially carried out during the COVID-19 pandemic of 2020-2021. Most laboratory work, data analysis, and writing were conducted during Western Australian lockdown periods and/or while COVID-19 related restrictions were imposed.

Signature:

Date: 19 of November, 2021

ABSTRACT

Climate models predict that temperature means will continuously increase globally, and that heatwaves and drought periods will become more frequent and intense, particularly in Mediterranean-type climates. The Southwest Western Australia (SWWA) Biodiversity Hotspot has extensive forest environments that have been subject to heatwaves and drought-induced forest mortality in recent years, impacting forest carbon sequestration and local ecological structure. Although, species may persist through enhanced physiological tolerance, phenotypic plasticity and/or genetic adaptation. Genetic variation is critical for ecological adaptive capacity - the potential and ability to adjust to, and persist through, external factors - and consequently, the evolutionary potential of the species. Evolution to a specific environment through natural selection results in patterns of local adaptation (when a local population experiences higher fitness compared to non-local counterparts). Local adaptation can be identified by either genome wide surveys that link genetic variants to climate variables or measuring plant traits indicative of plant performance and survival through reciprocal transplants in common environments.

Exploring genetic adaptation patterns associated with physiological tolerance to climate can guide forest management approaches to enhance forests' resilience to climate change, such as assisted gene migration.

The genetic survey (Chapter 2) sampled natural jarrah populations and obtained 13,534 independent single nucleotide polymorphic (SNP) markers across the genome. Three genotype-association analyses were used to identify putatively adapted SNPs associated with independent climate variables. While overall levels of population differentiation were low (F_{ST}=0.04), environmental association analyses found a total of 2,336 unique SNPs associated with temperature and precipitation variables, with 1,440 SNPs annotated to genic regions. Considerable allelic turnover was identified for SNPs associated with temperature seasonality and mean precipitation of the warmest quarter, suggesting that both temperature and precipitation are important factors in adaptation. SNPs with similar gene functions, had analogous allelic turnover along climate gradients, while SNPs among temperature and

precipitation variables had uncorrelated patterns of adaptation. These contrasting patterns provide evidence that there may be standing genomic variation adapted to current climate gradients, providing the basis for adaptive management strategies to bolster forest resilience in the future.

The second experimental chapter (Chapter 3) explored seed germination response to temperature in jarrah and marri populations from wide-ranging climate origins, to estimate the thermal optima and constraints. Seeds from across the entire geographic distribution were collected from independent populations of each species. Patterns of germination observed differences between species on a thermal gradient plate (5-40°C) and provided a temperature range for explicit germination tests. Germination tests were carried out at five constant temperatures between 9 and 33°C. We discuss how the germination niche (1) differs between species, (2) varies among populations, and (3) relates to climatic origin. Temperature response of germination differed among species, specifically the optimum temperature for germination (jarrah – 23.4°C; marri - 31°C). Temperature response of germination also differed among populations within species and was related to the climate-origin only for marri.

Lastly, for the third experimental chapter (Chapter 4), a reciprocal transplant common garden experiment was used to investigate variation in marri`s functional traits using hyperspectral data. Hyperspectral remote sensing has the potential to assess plant functional status rapidly and non-destructively across climatic gradients to support conservation and management strategies, such as assisted migration, for forests under climate change. This study explored the variability of functional traits in marri to estimate patterns of local adaptation. Trees from natural populations spanning marri's geographic distribution were grown in two common garden plantation sites with different climate settings. High-resolution field-based spectral measurements were collected from leaves of adult plants at both sites in two seasons (summer and autumn). Partial least squares regression analyses of full reflectance spectra highlighted differences among populations, sites, and seasons in spectral regions associated with photosynthetic pigments and water content, among other spectral traits, related to leaf condition and stress responses. Variation in these traits was further explored with analyses of

spectral indices tailored to pigment and water absorptions. Analyses of spectral indices variation identified significant differences between populations, suggesting there is heritable variation in climatic tolerances, but stronger effects of season and site.

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I, Professor Giles Hardy, certify that the student's statements regarding their contribution to each of the works listed above are correct. As all co-authors' signatures could not be obtained, I hereby authorise inclusion of the co-authored work in the thesis. Principal supervisor signature: Date: 19/11/2021

Chapter 1.

General Introduction

The following general introduction is a brief state of the art of key questions and terminology on climate change and forest dynamics, ecology, and adaptation. More focus in detailed issues and methodology topics related to the main body of this project are given individually for each of the research chapters (2, 3 and 4).

1.1. Climate change: a global threat

Recently described as a new geologic era, the Anthropocene portrays the profound environmental impacts from human actions. Although a proper definition of the Anthropocene is still debated (Zalasiewicz, 2015), it is widely recognised today as a new geological period characterized by a boom of human activities and their environmental consequences, which includes anthropogenic climate change (Subramanian, 2019). Approximately 25% of animal and plant species are being directly impacted by human activities, with an estimated one million species facing extinction, many within decades (IUCN/SSC Red List, 2019; IPBES, 2019). Human-induced events, such as habitat fragmentation, pollution, and climate change, are major triggers of what has been addressed as the Earth's sixth mass extinction (Ceballos et al., 2017; Torres-Romero et al., 2020; McCallum, 2021).

Within this context, the last report of the Intergovernmental Panel on Climate Change (IPCC, 2021) estimates that global temperature warming can reach from 1.5°C to 5 °C, with a projected average of 3 °C, at the end of the century. The modelled climate shift pace (CMIP6 – IPCC, 2021) for the next 65 years is up to 3000 times faster than those observed since the last glacial maximum (Sandel et al., 2017). Whether organisms will have the ability to follow the spatial changes of their appropriate native habitats (where conditions are ideal for survival) under climate change has been a major research question during the last decades (Pecl et al., 2017). As a direct outcome from climate change, species distribution models (Parmesan & Yohe, 2003), have been confirming that terrestrial species have migrated poleward, in both hemispheres, at a median rate of 16.9km per decade since the early 19th century (the beginning of the industrial era) (Chen et al., 2011; Pecl et al., 2017).

Nearly 50,000 of the ~390,000 described vascular plant species (approximately 20% of the total land plant species), are predicted to become extinct during the next few decades (Brummitt et al., 2015; Cronk, 2016; Humphreys et al., 2019, Knapp et al., 2020). Extensive tree mortality and dieback events have been progressively detected throughout forests and woodlands globally due to intense periods of warming and soil water deficit in recent decades

(Allen et al., 2010, Matusick et al., 2018; Goulden and Bales, 2019; Powers et al., 2020). Such events are occurring throughout vegetated continents worldwide (Meir & Woodard, 2010; Lewis et al., 2011). Native range contractions, associated with local extinction events, and biodiversity losses, have been associated with climate change impacts and predicted to continue (Choat et al.,2018; Enquist et al., 2019; Brodribb et al, 2020).

1.2. Climate change impacts on forest ecosystems

Climate change is now considered a key pressure on forest ecosystem function and, in some extreme cases, survival (Bellard et al., 2013; Pacifici et al., 2015; Urban, 2015; Brondizio et al., 2019). Forest biomes are extensive ecosystems that makeup approximately 30% of the Earth's land surfaces (Bonan, 2008) and deliver countless benefits to both humans and natural ecosystems through carbon sequestration, habitat and food sources for fauna, hydrological systems regulation, economic and industry services. As climate change intensifies, considerable adverse effects on forest composition and dynamics, including extensive range collapses and biodiversity losses (Reyer et al., 2015; IPCC, 2021), structural changes (Cavin et al., 2013) and altered hydrological patterns (Vose et al., 2016) are predicted to occur at both local and global scales. Additionally, there is growing evidence that heat-induced tree mortality can cause abrupt changes in forest biomass stocks (Chaste et al., 2019; Breshears et al., 2021), which consequently alters carbon budgets (Breshears and Allen, 2002), enhancing a cyclic warming of the earth atmosphere (IPCC, 2021). The climate change induced biodiversity loss is expected to heavily impact ecosystem's structure and, hence, impact forest's composition, functioning and services (Johnson et al., 2017; Roe, 2019; Schmeller et al., 2020). This is particularly alarming in biodiversity hotspots (Le Roux et al., 2019), where ecosystems can soon become unsustainable under conditions of severely reduced rainfall and increased temperatures (Trew & Maclean, 2021). Severely impacted areas include the Amazon rainforest, where the predicted trees' species extinction may reach 58% by 2050 (Gomes et al., 2019); and South-west Western Australia, where the warming and drying trends (BOM & CSIRO, 2020), and extreme events such as heatwaves, are having major impacts on plant communities' structure and function (Malcolm et al., 2006, Bader et al., 2014; Challis et al.,

2016), with observed impacts for several keystone tree species, including widespread canopy collapses (Matusick et al., 2013; 2018).

Reported events such as tree canopy collapses consistently suggest a limited capacity of trees to acclimate, efficiently and rapidly, to increased drought periods and higher temperatures (Teskey et al., 2015; Konôpková et al., 2018). Because plants are the foundation for most terrestrial ecosystems, documenting plants' vulnerability and resilience is an urgent need. Predicting the impacts of drought and warming temperatures on biomes and plant lineages remains a challenging task for research, as most predictions rely on species distribution models and climatic niche data lack the species genetic adaptation and physiological tolerance patterns (Fitzpatrick et al., 2008; McDowell et al., 2008; Yates et al., 2010; Urban, 2015).

1.3. Plants adaptation to climate

1.3.1. Genetic basis of adaptation

While some variation in biotic and abiotic factors exists in natural systems (Staudinger et al., 2013), rapid and extreme shifts are challenging for most organisms (Levin and Poe 2017, Carlo et al., 2018). If new climatic scenarios are no longer suitable for species to maintain their normal phenology and physiology, species must either respond to new conditions through genetic or plastic adaptation mechanisms, shift their geographical range or, in worst case scenarios, go extinct (Guisan & Zimmermann 2000; Bellard et al., 2012; Soto-Correa et al., 2012). In other words, the likelihood of extirpation can be reduced through ecological (e.g., local acclimation; migration by seed dispersal into climatically appropriate ranges) and evolutionary (e.g., genetic adaptation, phenotypic plasticity) strategies (Anderson et al., 2011; Christmas et al., 2016). Hence, quantifying species physiological thresholds, as well as assessing genetic adaptation signatures, is key to understanding how plants will cope with extreme climatic-induced events, such as drought and heatwaves, in the future (Allen et al., 2010; Razgour et al., 2019)

Species migration may be restricted by natural geographic barriers, such as water masses and mountain ranges, and in modern landscapes, habitat fragmentation and urbanisation also

represent major challenges for gene flow (Parmesan & Yohe, 2003; Aitken et al., 2008; Zhu et al., 2012). Therefore, ability to persist through environmental stressors over generations in the same native range will greatly depend on evolutionary strategies such as plastic responses and/or genetic variation (Sexton et al., 2011; Kremer et al., 2012). This variation (standing or acquired from mutations or gene migrations) is critical for genetic adaptive capacity and, consequently, the evolutionary potential of the species (Reed & Frankham, 2003; Reed, 2011). Natural selection acts upon standing genetic variation which drives the evolution of local adaptation patterns across environmental gradients. (Williams, 1966). Thereby, maintenance of standing genetic variation is a key factor for adaptation to changing conditions in native habitats (Matuszewski et al., 2015; Guzella et al., 2018, Chhatre et al., 2019).

Unfortunately, rapid environmental shifts can hinder local adaptation, resulting in a mismatch of adapted genotypes and novel climate settings (Aitken & Whitlock, 2013; Browne et al., 2019). Most forest trees are long-living organisms, and adaptation may not occur fast enough to keep up with climate change rates (Davis & Shaw, 2001), a phenomenon known as adaptational lag. With long life cycles and extensive gene flow (pollen and seed dispersal), trees tend to show more extensive evolutionary scales (*i.e.,* time scales of evolution by natural selection from one generation to the next), and complex patterns of adaptation. Local adaptation is unlikely unless selection forces (e.g, climate gradients, altitude, herbivores presence) are very strong. Despite extensive gene flow, that may counteract selection processes, some tree species still display local adaptation patterns, with local populations showing higher fitness, compared to non-local counterparts (Savolainen et al., 2007, 2013; Lind et al., 2017). Genetic studies identifying local adaptation in trees, particularly those including functional traits simultaneously, are limited, and therefore the prevalence of local adaptation patterns in tree species is still largely unknown (Aspinwall et al., 2015).

Alternatively, phenotypic plasticity, i.e., the ability of a genotype to express distinct phenotypes if exposed to different environments, may also provide an efficient, or least fast, response to climate change in trees (Nicotra et al., 2010). In the context of rapid global climate change, plastic responses through tolerance traits related to temperature and precipitation extremes can enable some genotypes to persist under increasing adverse conditions.

However, natural environments are often very heterogenous (spatially and temporally), and populations are frequently exposed to numerous environmental factors; therefore, species might have to simultaneously adjust to numerous pressures such as reduced precipitation and increasing temperatures (Valladares et al., 2007), which again may prove to be challenging in the face of rapid climate shifts. The ability to adjust physiological and phenological traits to changing environments through phenotypic plasticity has been extensively recorded in tree species, such as eucalypts (Prober et al., 2016), and shown to be heritable by subsequent generations (Matesanz et al., 2010; Nicotra et al., 2010).

1.3.2. Physiological tolerance and functional traits

Plant functional traits are physiological, morphological, and other traits associated with survival and reproductive success, and are indicative of the fitness (performance and survival in specific environments) of individuals (Violle et al., 2007, Nicotra et al., 2010). Evolution to a specific environment through natural selection results in patterns of local genetic adaptation and phenotypic trait variation, allowing local populations to experience higher phenotypic fitness compared to non-local counterparts (Kawecki & Ebert, 2004). In other words, natural selection operates through differential fitness. The expression of functional traits is influenced by the individual's genotype (G), the environment (E) conditions, and their interactions (GxE). Inter- and intraspecific variation in functional traits can be found across different environments, such as along climate gradients, which might be indicative of local adaptation (Reich, 2014). Some functional traits specifically are highly responsive to climate stressors in plants, e.g., decreased leaf water potential being associated with drought (Nolan et al., 2017; Scoffoni et al., 2017); hindered growth, biomass production (French et al., 2017, 2019) and lower chlorophyll content associated with heatwaves (Notarnicola et al., 2021); and insufficient leaf gas exchange rates linked to both drought and heatwave conditions (Duan et al., 2017). Climatic factors such as temperature and precipitation of origin are well known to be associated with interspecific variation for several functional traits in plant species (e.g., hydraulic capacity (Li et al., 2018) morphological (Cochrane et al., 2016) and thermoregulation (Perez & Feeley, 2020)). Studies with eucalypt species (*Eucalyptus* spp. and *Corymbia* spp.) have found intraspecific variation in their capacity to respond to climatic shifts through

functional traits, such as morphological and growth traits (Costa e Silva et al., 2006; Jordan et al., 2020), photosynthetic and respiratory capacities (Aspinwall et al., 2017), and hydraulic properties (Blackman et al., 2017). Such studies have shown that tree populations can display variation in functional traits, potentially through distinct genomic signatures dependent on the climate of origin, suggesting local adaptation to climate. Estimating traits variation and their adaptability, and identifying potential agents of selection in trees, can deliver evidence on how the species will respond to future climate scenarios across its distribution.

Under hot or dry conditions, water shortage can negatively influence several plant processes, including functional traits related to growth, metabolism and photosynthesis (Lawlor & Cornic, 2002). Increased water use efficiency through the evolution of functional traits may reduce water loss, increasing plant fitness (e.g., stomatal closure during dry periods, reduced leaf surface area to decrease transpiration rates) (Nicotra and Davidson, 2010). Leaf water content and evapotranspiration rates (combination of water evaporation and transpiration) are then considered primary indicators of the plant water stress status (Egilla et al., 2005; Cui et al., 2018). Drought-stressed trees can also reveal variation in photosynthetic pigments (e.g., chlorophyll) content (Ustin et al., 1998; Zarco-Tejada et al., 2002), red discoloration produced by accumulation of secondary metabolites such as anthocyanins and carotenoids (Barry et al., 2008) or generalized loss of photosynthetic tissues due to defoliation or necrosis. Chlorophyll content, a major photosynthetic pigment, is widely regarded as a generic indicator of plant stress (Gitelson et al., 2003, Sampson et al., 2003). High-temperature stress can influence the biochemical processes involved in photosynthesis changing pigment concentrations, reducing overall plant growth' (Teskey et al., 2015) and, in worst cases, cause tissue death (Vacca et al., 2004). Although plant death is rarely caused by heat stress alone, temperature is similarly a major trigger for plant stress. Particularly when high temperatures are coupled with water debt, which can increase plant mortality rates can increase substantially (Teskey et al., 2015). The role of warmer temperatures, especially extreme heatwave events, has been identified as a global main driver for tree mortality across diverse ecosystems (Matusick et al., 2013; Williams et al., 2013; Allen et al., 2015).

1.4. Description of study species

The research focuses on *Corymbia calophylla* (R.Br.) K.D. Hill & L.A.S. Johnson (marri) and *Eucalyptus marginata* Donn ex. Sm. (jarrah). From the Myrtaceae family, both species are endemic to the South-west of Western Australia (SWWA). They are two of the most common tree species in the region and are often found to share the same habitat, making them co-occurring and co-dominant foundation trees. SWWA has a Mediterranean-type climate (MTC) and is a global biodiversity hotspot (Klausmeyer & Shaw 2009; Mittermeier et al. 2011). Ecosystems with MTC are considered particularly vulnerable to the impacts from climate change due to their high plant diversity and endemism. Intense declines in health and growth of dominant tree species have been associated with climatic changes in Mediterranean regions globally, including SWWA (e.g., Barbeta et al., 2011; Carnicer et al., 2011; Sarris et al., 2011; Sanchez-Salguero et al., 2012, Matusick, 2013, 2018). From a conservation and land management perspective, it is necessary to investigate the potential drivers of observed declines across the entire geographic and climatic range of a species.

1.4.1. *Corymbia calophylla* (Marri)

Corymbia calophylla, previously classified as *Eucalyptus calophylla*, is an economically important and ecologically a keystone species in the forests and woodlands of the SWWA. This bloodwood, commonly known as marri, is a large tree growing up to 40 meters tall in the wild in appropriate conditions, with very large buds and fruits (Boland et al., 2006). Marri is widely distributed from north of Geraldton (28°S) to Cape Riche (34°S), inland beyond Narrogin (33°S 117°E) and on the Swan Coastal Plain, reflecting its tolerance of a wide range of environments, with annual rainfall between 360mm to 1150mm, and the average annual temperatures varies from 14°C to 21°C. Marri seeds are a major food source for nationally listed threatened birds (Baudin's black cockatoo - *Zanda baudinii* and Carnaby's black cockatoo - *Calyptorhynchus latirostris*). In addition, marri was one of the tannin-rich *Eucalyptus* kinos (total tannin content of 44%) that were a valuable source of antibacterial compounds for early European settlers and played an important role in traditional medicines of Australian Aboriginal people (Martius et al., 2012). Consequently, a decline in its overall health will have significant long-term detrimental impacts on ecosystem function, particularly affecting carbon sequestration,

habitat and food sources for fauna, and hydrological systems regulation (CCWA, 2013; Bradshaw, 2015). Past physiological studies suggest that marri can adapt to repeated drought events and fragmented soils (the breakdown and crumbling of soil aggregates resulting from extreme drought; Abbott et al., 1989). The physiological ability that allows stomata to close earlier during drought periods makes this trait crucial for the species survival and adaptation to drought (Szota et al., 2011). Marri is one of the most fire tolerant species in Western Australia, possibly related to its bark thickness and large diameter (Grant et al., 1997).

1.4.2. *Eucalyptus marginata* (Jarrah)

Eucalyptus marginata, commonly known as jarrah, is also one of the most common endemic trees of SWWA. The jarrah forest extends for about 64 000 km^2 (Gardner, 1987), being unusual for a single eucalypt species to cover such a large area. Its ecosystem is home to a diverse flora and fauna with important conversation values (Gardner, 1987). *E. marginata* occurs, generally, where the rainfall isohyet exceeds 600 mm. Like marri, jarrah is usually a large tree able to grow up to 40 meters tall in the wild in appropriate conditions. However, it can take the form of mallee in places at the edges of its distribution, such as Mount Lesueur and in the Stirling Ranges (Brooker, 2012; Barret, 2016). It is a stringybark with rough, greyish-brown, vertically grooved, fibrous bark which sheds in long flat strips, and the stalked flower buds are arranged in umbels of between 4 and 8, each bud, with flowers 1–2 cm in diameter and bloom in spring and early summer (Wrigley, 2012). Jarrah is a vital component in its ecosystem, offering abundant habitats for animal life – especially birds and bees – while it is alive, and in the hollows that form as the heartwood decays. When it falls, it is very common to provide shelter to ground-dwellers such as the chuditch (*Dasyurus geoffroii*), a carnivorous marsupial (Wrigley, 2012). As a foundation tree and similarly to marri, jarrah is a vital component in the ecosystem with a significant role in regulating local hydrological systems and carbon storage (CCWA, 2013; Bradshaw, 2015). Since the middle of the 19th century, jarrah has been a major component of timber production from SWWA forests (CCWA, 2013; Davison, 2015), although harvesting has been reduced in recent years. Studies have found that water availability was a limiting factor for the establishment of seedlings in a greenhouse trial (Stoneman et al., 1994), while warm and wet conditions were confirmed as beneficial for early stages of jarrah growth

at rehabilitation sites (McChesney et al., 1995; Standish et al., 2015). Once established, mature jarrah trees are fairly tolerant to summer drought (Koch & Samsa 2007; Standish et al., 2015), but vulnerable to waterlogging and dieback, through root-rot caused by the oomycete *Phytophthora cinnamomi* (Davison, 2018).

1.5. South-west Western Australia forest decline

All over Australia, species of the most common eucalypt genera (*Eucalyptus*, *Corymbia* and *Angophora*) have shown tree health decline and population size reduction since the mid-20th century (Jurskis, 2005). The SWWA has experienced intense and continued drought over the last three decades (Bates et al., 2008; BOM& CSIRO, 2020). This stress factor is one of the main explanations for forest declines and, more recently (following the summer of 2010 – 2011), sudden stand mortality in the jarrah-marri forest (Brouwers et al., 2013; Evans et al., 2013; Matusick et al., 2013). The deterioration in marri trees has occurred since the 1970s, with factors such as drought and fungal pathogens considered the key causes of this decline (Kimber, 1981). Szota et al., (2011) reported that *C. calophylla* (marri) shows the capacity to endure prolonged periods of drought. However, evidence suggests that heatwaves and extreme drought events have caused widespread mortality of marri trees throughout its distribution (Matusick et al., 2013). More recently, the canker disease triggered by *Quambalaria coyrecup*, combined with climate change, were considered the main responsible factors for this decline (Paap et al., 2016; Paap et al., 2017). Continued longitudinal surveys of marri showing symptoms of canker in SWWA reported that the decline in marri health across its natural habitat is indeed the result of a combination of factors, including climate change and other anthropogenic disturbances, and dieback associated with *Phytophthora* spp., (Croeser et al., 2018). A recent investigation into structural resistance and resilience to climate change-type drought of the jarrah-marri forest, Matusick et al., (2018) found that extreme climate can cause structural shifts in a resprouting Mediterranean-type forest, providing evidence for a shift to an alternate state, particularly with repeated disturbance in the jarrah forest in SWWA. The consequences of the health decline of marri and jarrah are significant in many of its distribution areas, including economic and social, but mainly ecological effects. These are mostly related to the loss of wildlife habitats and food resources

for threatened cockatoo species and the decline of pollen production, essential to the health of native insect pollinators (Yates et al., 2005).

1.6. Project overview and aims

The general aim of this PhD project was to assess patterns of standing genetic adaptation, phenotypic plasticity of plant traits, and patterns of local adaptation to the environment associated with climate, across endemic foundation trees sourced from climate-origins with variable rainfall and temperature. Jarrah and marri are ideal tree species for studies of genetic adaptation and phenotypic plasticity of traits, especially for a comparative approach of endemic species since they share the same native distribution.

The genetic diversity and structure of marri have been evaluated in a recent study of twenty-seven populations using microsatellite markers (Sampson et al., 2018) that showed high diversity and little genetic structure with isolation by distance across the distribution. The same study also investigated the historical diversity using analysis of chloroplast DNA. It showed evidence of the origins of the distribution in the northern part of the distribution, with subsequent expansion southwards during the Pleistocene. An investigation of adaptive capacity in marri found evidence for adaptation to climate, primarily temperature (Ahrens et al., 2019a). Three provenance trials of marri have previously been established at three different sites in Western Australia: Mundijong (approximately 30km from South of Perth), Margaret River (270 km south of Perth), and Mt Barker (392km SSE of Perth). These trials contain marri trees from 18 locations throughout its distribution and, on average, ten families per provenance. Surveys and trials have been conducted at these sites to estimate the growth rates, overall health score, and physiological characteristics such as leaf water transport capacity and leaf hydraulic vulnerability. Preliminary results indicated that growth and *Quambalaria* leaf blight disease resistance traits expressed differential responses among provenances, and the differences were consistent between experimental sites. Moreover, these traits were correlated with the climate of origin (Ahrens et al., 2019b).

The genetic diversity of jarrah has been briefly investigated in a study of populations across its range, including two outlier populations. Like marri, jarrah showed high diversity and little

genetic structure, including the outliers (Wheeler et al., 2003). Investigation of historical diversity showed strong differentiation between populations within the main distribution (Wheeler et al., 2006). An investigation of growth in a provenance trial (O'Brien et al., 2010) showed that populations from different origins across the species' distribution are differently locally adapted.

Vulnerable populations at risk of climate change may benefit from assisted genetic flow (Aitken & Whitlock, 2013). For plants, assisted flow usually involves selecting seeds from a suitable provenance (i.e., climate-adjusted provenancing) for use in revegetation and restoration (Prober et al., 2015, Hoffmann et al., 2019). The Forest Management Plan 2014–2023 (CCWA, 2014) for SWWA forests has provision for the implementation of assisted gene migration (AGM) in management strategies for response to climate change. Potential risks of AGM include loss of current lineages, local adaptation disruptions and, for species with low gene flow rates, outbreeding depression (Aitken and Whitlock 2013). Therefore, before implementing AGM, it is crucial to investigate the species genetic structure, and patterns of adaptive and plastic variation related to climate conditions. Identifying the genetic basis of local adaptation and selective factors is still challenging, particularly for species with limited genomic resources, while the complex molecular mechanisms involved are still being unravelled (Capblancq et al., 2020; Mayol et al., 2020).

Considering the long life of trees, and the rapid on-going climate shifts, the environment is also likely to have altered such that a particular site no longer experiences the same conditions under which the trees originally evolved. Such temporal variation in environment may be particularly important for trees, not only with respect to past adaptation but also in the context of climate change, requiring more adaptive sourcing strategies (Breed et al., 2013). Genetic surveys, common garden experiments and provenance trials were used to identify adaptive and plastic processes acting on physiological tolerance and functional traits.

Chapter 2 investigated standing genetic variability of adult jarrah trees associated with climate gradients. Twenty-eight populations were sampled across the geographic distribution of jarrah to generate a genome-wide single nucleotide polymorphic (SNP) dataset. Environmental association analysis was used to identify putatively adapted SNPs associated with independent climate variables. Annotation and gene ontology inferred on the gene functions associated with adaptive SNPs, and landscape genomic analysis identified allelic turnover for SNPs associated with temperature and precipitation gradients

Chapter 3 explored patterns of local adaptation on germination sensitivity from both jarrah and marri to temperature. Seed germination response to temperature treatments was used to estimate the thermal optima and constraints among populations from wide ranging climate-origins. Seeds from across the entire geographic distribution were collected from 12 independent populations of each species. It is discussed how the germination niche (1) differs between species, (2) varies among populations, and (3) relates to climatic origin.

Chapter 4 explored variability and phenotypic plasticity of functional traits in marri. High-resolution field-based spectral measurements were collected from leaves of juvenile plants at two of the common garden plantations (Mundijong and Margaret River) in two seasons (summer and winter). Analyses of full reflectance spectra was used to assess differences among populations, plantations, and seasons in spectral regions associated with photosynthetic pigments and water content. Variation in these functional traits was further explored with analyses of vegetation indices (VIs) tailored to pigment and water absorptions.

Chapter 2.

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

Chapter 2 of this thesis, 'Signatures of natural selection in a foundation tree along Mediterranean climatic gradients', is published as a journal article in Molecular Ecology. For consistency, all references and figures formatting in this thesis were kept according to the rules of Molecular Ecology.

Abstract

Temperature and precipitation regimes are rapidly changing, resulting in forest dieback and extinction events, particularly in Mediterranean-type climates (MTC). Forest management that enhances forests' resilience is urgently required, however adaptation to climates in heterogeneous landscapes with multiple selection pressures is complex. For widespread trees in MTC we hypothesized that: patterns of local adaptation are associated with climate; precipitation is a stronger factor of adaptation than temperature; functionally related genes show similar signatures of adaptation; and adaptive variants are independently sorting across the landscape. We sampled 28 populations across the geographic distribution of *Eucalyptus marginata* (jarrah), in South-west Western Australia, and obtained 13,534 independent single nucleotide polymorphic (SNP) markers across the genome. Three genotype-association analyses that employ different ways of correcting population structure were used to identify putatively adapted SNPs associated with independent climate variables. While overall levels of population differentiation were low (F_{ST} = 0.04), environmental association analyses found a total of 2,336 unique SNPs associated with temperature and precipitation variables, with 1,440 SNPs annotated to genic regions. Considerable allelic turnover was identified for SNPs associated with temperature seasonality and mean precipitation of the warmest quarter, suggesting that both temperature and precipitation are important factors in adaptation. SNPs with similar gene functions, had analogous allelic turnover along climate gradients, while SNPs among temperature and precipitation variables had uncorrelated patterns of adaptation. These contrasting patterns provide evidence that there may be standing genomic variation adapted to current climate gradients, providing the basis for adaptive management strategies to bolster forest resilience in the future.

2.1. Introduction

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

Mediterranean-type climates (MTC) are defined by reliable precipitation and temperature regimes, with predictable summer periods of low rainfall and hot temperatures, and winter periods of high rainfall and moderate temperatures. Ecological studies in the Mediterranean basin consistently identify more frequent drought periods, together with warmer temperatures, as main drivers for declines in oaks (*Quercus* spp.) (Corcobado et al., 2014; Gentilesca et al., 2017) and pines (*Pinus* spp.) (Camarero et al., 2018). In the South-west Western Australia biodiversity hotspot, a region with MTC, the 2010-11 extreme drought and heatwave conditions resulted in large scale forest collapses in eucalypts (Matusick et al., 2013). While some variation in climatic factors exists in natural systems (Staudinger et al., 2013), the rapid and extreme shifts associated with anthropogenic climate change are challenging for most organisms to persist (Levin & Poe 2017; Carlo et al., 2018).

If new climatic scenarios are no longer suitable for species to maintain their normal ecology and physiology, they either shift their geographical range or, in worst case scenarios, go extinct (Aitken et al., 2008; Bellard et al., 2012). Although, species may persist through enhanced physiological tolerance, phenotypic plasticity and/or genetic adaptation (Anderson et al., 2011; Christmas et al., 2016). Maintenance of standing genetic variation (allelic variation at a locus held within existing population) is a key factor for adaptation to changing conditions

in native habitats (Guzella et al., 2018, Chhatre et al., 2019) and for persistence through environmental stressors over generations (Sexton et al., 2011; Kremer et al., 2012). Genetic variation is critical for ecological adaptive capacity - the potential and ability to adjust to, and persist through, external factors - and consequently, the evolutionary potential of the species (Reed et al., 2011). Evolution to a specific environment through natural selection results in patterns of local adaptation, when a local population experiences higher fitness compared to non-local counterparts (Kawecki & Ebert, 2004).

Recent improvements in DNA sequencing and statistical methodology have made it possible to investigate genetic divergence and the effects of environmental factors on the process of local genetic adaptation (Honjo & Kudoh, 2019; Gougherty et al., 2020). Environmental association analyses (EAA) have been gaining traction in the last decade (Ahrens et al., 2018), allowing identification of possible candidate genes involved in environmental adaptation from tens of thousands of genome-wide single-nucleotide polymorphisms (SNPs) sourced from populations across environmental gradients. For example, EAAs have been used to explore adaptive genetic variation on diverse and widespread woody plant genera, like *Quercus* (Martins et al., 2018; Gugger et al., 2021), *Populus* (Ingvarsson & Bernhardsson, 2020; Gougherty et al., 2021) and *Corymbia* (Ahrens et al., 2019a). These studies have identified functional genes involved in adaptation to climatic factors that can be interpreted as divergent selection linked to population-specific environmental variables (i.e., local adaptation to climate). However, different climate factors identify different sets of adaptive candidates, and few studies have focused on how sets of putatively adaptive SNPs sort across the landscape. If adaptive SNPs independently sort across the landscape (here, we define independent assortment as contrasting distributions of adaptation across the landscape), then understanding these species' adaptative patterns to climate may prove to be difficult.

Identifying the genetic basis of local adaptation and selective environmental factors is still challenging. Genetic patterns that confer climate adaptations are mostly polygenic (Savolainen et al., 2013) and complex to investigate (Lind et al., 2018), particularly for species with limited genomic resources (Mayol et al., 2019; Capblancq et al., 2020). Non-synonymous mutations in coding genes result in amino acid changes, which can yield changes in gene

functions (Kryazhimskiy & Plotkin, 2008). However, mutations in gene regions are not the only ways genetic variation can impact adaptation. For example, mutations in cis-regulatory regions can often result in quicker adaptive processes (Wittkopp & Kalay, 2012). Beneficial mutations can be under selection among populations spread across that environment. Groups of genes found to be significantly associated with environment can be categorised into broader functional groups using gene ontology (GO) enrichment analysis (The Gene Ontology Consortium, 2019). GO terms have been used to predict polygenic adaptive biological processes and molecular functions associated with putatively adaptive SNPs in tree species (Jordan et al., 2017; Collevatti et al., 2019). However, few studies investigate how genes of similar function develop patterns of adaptation across complex landscapes. If genes with related functions are found to be adaptive to the same climate variable, this might be indicative of additive genetic variation controlling adaptation to the environment.

This study investigated the putative patterns of local adaptation associated with climate gradients across complex landscapes. To test hypotheses associated with signals of adaptation, we focused on *Eucalyptus marginata* Donn ex. Sm. (jarrah) because of its high genetic diversity and low population differentiation (Wheeler et al., 2003) and its ecological importance in the biodiverse hotspot of South-west Western Australia (SWWA). This region has prolonged periods of extensive drying, with an estimated reduction of 20% in rainfall, from the 1970s to the present (Water Corporation, 2020), documented impacts of drought and heatwave events (Matusick et al., 2013), and the future (2030) climate is projected to show increased frequency and intensity of extremes (BOM & CSIRO, 2020). Furthermore, jarrah provenance trials have demonstrated genetic variation in functional traits associated with precipitation factors (O'Brien et al., 2007; Koch & Samsa, 2007), indicating potential local adaptation to drought stress. Ecological studies have also confirmed that water availability is critical for jarrah seedling survival and persistence (Stoneman et al., 1994; McChesney et al., 1995; Standish et al., 2015). Considering these studies on jarrah, we hypothesize that (1) populations show strong genetic patterns of local adaptation to climate; (2) precipitation is a stronger determinant of genetic adaptation compared to temperature; (3) functionally related genes show similar signatures of adaptation to climate; and (4) adaptive variants are independently sorting across the landscape. Lastly, we use this information to map the

biological turnover of loci across the landscape to facilitate informed strategies for forest management that incorporate current patterns of genetic variation. We discuss how active management strategies, such as assisted gene migration (Hoffman et al., 2015; Prober et al., 2015; Aitken & Bemmels, 2016) may incorporate the results from this study and be employed to build adaptive capacity to climate change.

2.2. Methods

2.2.1. Sample collection and DNA extraction

Leaf samples from a total of 280 individual mature trees from 28 natural jarrah populations across the geographic range of the species (Figure 2.1), including one outlier population (JIL; located outside jarrah's natural range), were collected during 2019 (Table 2.1). Geographic coordinates were recorded for all sampled individuals from each population using a handheld GPS device (Magellan eXplorist 310) (Table 2.1 shows a central point for each population). The sampling, which covered a total area of approximately 80,000 km2, included independent (> 50 km separation) and replicate (across similar climate of origin) populations over both temperature and precipitation gradients to ensure adequate partitioning of the adaptive and neutral genetic variation. For each population, mature leaves were collected from ten trees at least 100m apart from each other. Leaves were immediately stored in silica gel until freeze-dried using FreeZone 6 Liter Benchtop Freeze Dryer (Labconco Corporation, USA), and kept in silica gel at room temperature until DNA extraction could be performed. For each sample, genomic DNA was extracted from 40mg of freeze-dried leaf material. Each leaf sample was independently ground into fine powder and a modified CTAB-DNA extraction protocol was employed (Doyle & Doyle, 1990), with 0.1M sodium sulphite (Byrne et al., 2001) and 2% w/v polyvinylpyrrolidone (MW 40,000) added to the extraction buffer. Quality of extracted DNA was estimated using gel electrophoresis and quantified using the Qubit dsDNA BR assay kit on a Qubit fluorometer (Invitrogen, Carlsbad, CA).

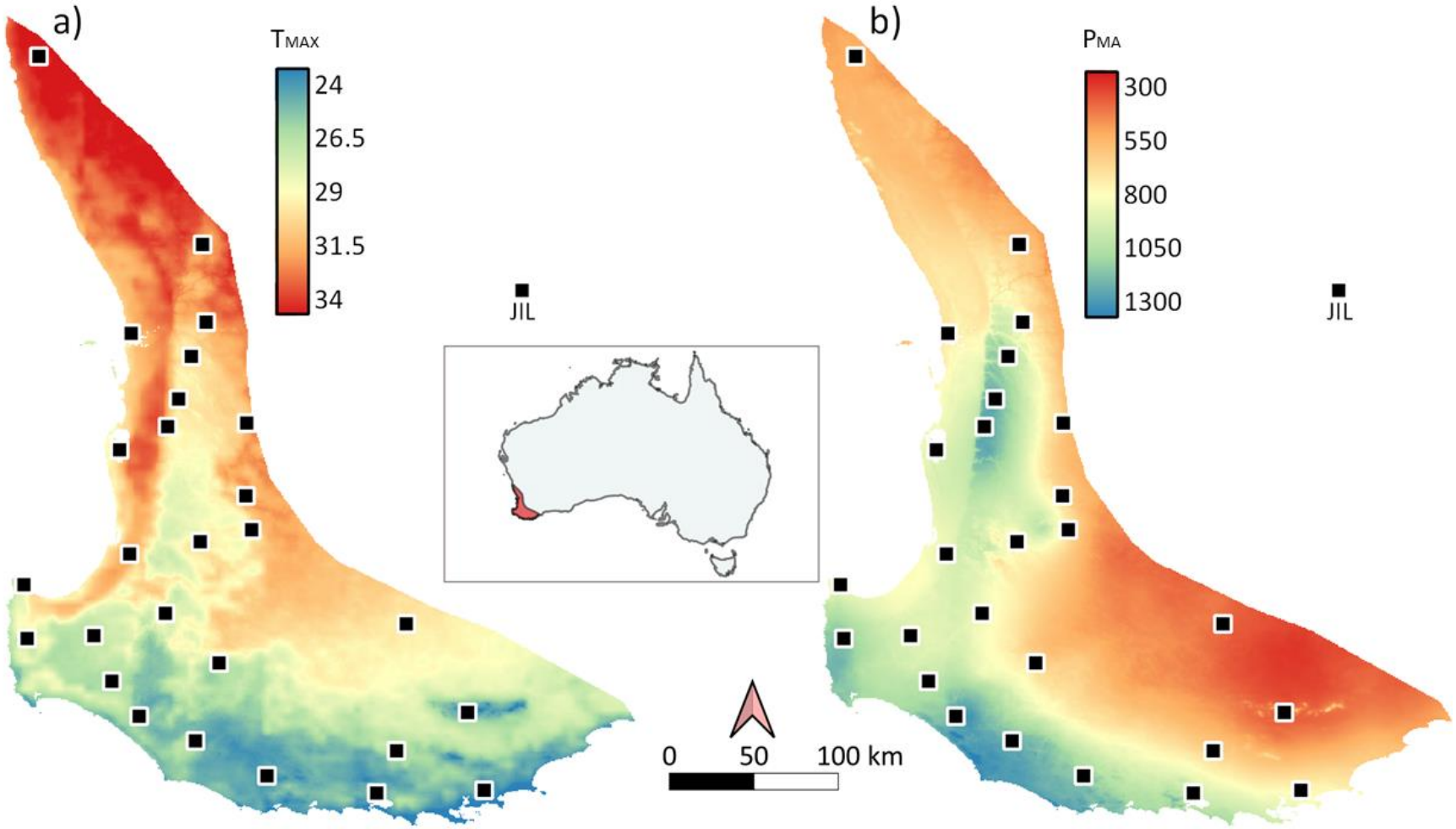


Figure 2.1. Sampling locations of jarrah in South-west Western Australia (black squares). Two climate gradients are shown for the species distribution area: a) maximum temperature of the warmest month, (°C; TMAX) and b) mean annual precipitation (mm; PMA). Bio-climatic layers from worldclim.org (Fick & Hijmans, 2017). Insert shows distribution of jarrah in Australia.

Table 2.1. Locations and climatic variables for the 28 sampled populations of jarrah in SWWA. Lat = latitude; Long = longitude; T_{SEAS} = temperature seasonality; T_{MAX} = mean maximum temperature of the warmest month; T_{MIN} = mean minimum temperature of the coldest month; P_{MA} = mean annual precipitation; and P_{WQ} = mean precipitation of the warmest quarter. Temperature (T) and precipitation (P) variables are expressed in degrees Celsius (°C) and millimetres (mm) respectively.

Population	Code	Latitude	Longitude	T_{SEAS}	T_{MAX}	T_{MIN}	P_{MA}	P_{WQ}
Mt Lesueur	LES	-30.1644	115.1991	41.1	32.2	8.2	578	35
Julimar	JUL	-31.3491	116.2470	49.0	33.1	6.1	635	44
Jilakin Rock*	JIL	-31.6647	118.3261	52.8	33.2	5.0	326	46
Chidlow	CHI	-31.8622	116.2266	47.4	32.3	6.1	876	54
Perry Lakes	PER	-31.9436	115.7838	37.6	30.4	9.4	765	38
Dale	DAL	-32.1017	116.1900	45.9	31.5	6.2	1053	58
Serpentine	SER	-32.3451	116.072	43.9	30.6	6.4	1151	57
Lupton	LUP	-32.5292	116.5003	48.3	31.4	4.3	705	45
Whittaker	WHI	-32.5499	116.0100	43.1	29.9	5.8	1190	62
Peel	PEE	-32.6920	115.7103	37.5	30.4	8.3	888	42
Saddleback	SAD	-32.9967	116.535	46.1	30.8	4.3	681	44
Godfrey	GOD	-33.2142	116.5712	45.0	30.2	4.1	661	45
Yourdaming	YOU	-33.3035	116.2407	43.9	30.4	4.1	851	46
Eaton	EAT	-33.3177	115.7482	39.2	30.5	6.7	853	47
Meelup	MEE	-33.5939	115.088	30.1	27.4	9.1	839	43
Grimwade	GRI	-33.7612	115.9988	40.2	29.6	5.3	881	53
Katanning	KAT	-33.8294	117.5731	41.9	29.2	5.2	457	50
Bramley	BRA	-33.9035	115.0871	28.8	26.1	8.8	1072	54
Mowen	MOW	-33.9133	115.5434	34.7	27.8	6.9	965	54
Nannup	NAN	-33.9852	115.7778	36.1	28.3	6.6	928	56
Kingston	KIN	-34.0825	116.3374	38.8	28	5.1	785	61
Milylannup	MIL	-34.1928	115.6654	32.3	26.6	7.4	1027	64
Stirling Range	STI	-34.3850	117.9927	35.4	26.9	5.8	493	67
Carey	CAR	-34.4257	115.8223	30.6	26	7.6	1112	72
Boorara	BOO	-34.6126	116.2060	31.4	25.9	6.9	1126	79
Plantagenet	PLA	-34.6402	117.4987	33.7	26.7	6.5	738	79
Beadmore	BEA	-34.8171	116.4834	31.3	25.8	7.0	1088	83
Denmark	DEN	-34.9535	117.3805	30.3	25.8	7.6	976	88

*Outlier population

2.2.2. Genotyping by DArTseq platform

Sequencing of the 280 jarrah individuals was undertaken using DArTSeq^{TM} technology at Diversity Arrays Technology Pty Ltd (Canberra, Australia). This technology uses a double digestion complexity reduction method for next generation sequencing (Kilian et al., 2012). The reduction of the genome is accomplished by using a combination of PstI and HpaII enzymes in digestion/ligation reactions with different adapters corresponding to two different restriction-enzyme overhangs. The PstI-compatible adapter is designed to include flowcell attachment sequence, sequencing primer sequence and varying length barcode region. Diversity Arrays Technology's proprietary bioinformatics pipeline was used to demultiplex and align the raw fastq files. Identical sequences were then collapsed into "fastqcall files". These files were used in the secondary pipeline for DArT P/L's proprietary SNP calling algorithm (DArTsoft14). Minimum read depth for each individual was set to six and average read depth was 30.93 across all SNPs, guaranteeing call quality for all SNPs and individuals. For the SNP calling algorithm, only nucleotide substitutions were considered a SNP. Only one random SNP was retained on each 75 base pair (bp) sequence to avoid linkage disequilibrium bias. All SNPs were mapped to the *Eucalyptus grandis* genome to obtain chromosome number and bp position to support the linkage disequilibrium analysis. The full data set was then filtered in R (R Core Development Team, 2020) using custom scripts. We applied a minor allele frequency filter of 2% (i.e., an allele frequency of 0.02), which equates to a minor allele count of at least 11 calls, minimising inclusion of sequencing errors. Missing data was set to 6% across individuals (SNPs were kept if they were called in at least 263 individuals). These thresholds were chosen because this translates to, on average, the presence of genetic information from nine individuals per population, which is adequate for EAA type of method and identifying SNPs under selection (Ahrens et al., 2021a). Linkage disequilibrium (LD) was calculated within each of the chromosomes using the function LD.Measures in LDcorSV (Mangin et al., 2012). To guarantee adequate independence between SNPs and prevent potential linkage bias, the dataset was filtered by the within chromosome pairwise LD R^2 coefficient (only one of the SNPs was randomly retained for analysis if the R^2 is > 0.5.

2.2.3. Environmental variables

Temperature and precipitation variables have been commonly assessed as predictors for environmental adaptation in eucalypts for phenotypic and genotypic variants (Correia et al., 2018; Aspinwall et al., 2019; Pritzkow et al., 2020). Climatic data for all populations was downloaded from the 19 variables in the WorldClim v2 database (Fick & Hijmans, 2017) at a spatial resolution of 30 arcsec. Climate data for each population was extracted using the R package *raster* (Hijmans, 2021) from the geo-located GPS coordinates of the sampled populations. Principal component analysis (PCA) of environmental variables was performed with R package *ade4* (Chessel et al., 2004) and a Pearson's correlation coefficient matrix was calculated between all 19 climate variables using the *cor* function. For later environmental association analysis, we tested a total of five climate variables. Two of these represent extreme temperature and precipitation variables and we predicted they would drive patterns of adaptation in MTC regions: maximum temperature of the warmest month (T_{MAX}) and precipitation of the warmest quarter (P_{WQ}). Three other temperature and precipitation variables were selected as independent climatic factors, based on PCA and Pearson's correlation coefficients (If the $r > 0.7$ of magnitude between two variables, these were considered strongly correlated and thereafter not included in further analysis; Table S2.2, tabs 16-17) and are known to be important for local adaptation in eucalypts (Queirós et al., 2020; Rocha et al., 2020): minimum temperature of the coldest month (T_{MIN}), mean annual precipitation (P_{MA}), and temperature seasonality (T_{SEAS}).

2.2.4. Environmental Association Analysis

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

manual (Frichot & François, 2015) and choosing the *k*-value with the lowest cross entropy score for the LFMM analysis.

To elucidate the association between climate and genetic variation, three approaches were applied: a redundancy analysis (RDA), latent factor mixed models (LFMM) and BAYPASS. The three distinct approaches apply different statistical frameworks to identify population structure, which should reflect on the performance for each method (Lotterhos & Whitlock, 2015), but simultaneously allow a greater confidence in the identified associations (Ahrens et al., 2018). The detailed description for each method, including advantages and limitations, are broadly documented elsewhere (Lotterhos & Whitlock, 2015; Rellstab et al., 2015; Hoban et al., 2016; Ahrens et al., 2018). Therefore, we apply a multi-approach methodology as suggested by De Mita et al., (2013), providing a powerful detection of potential adaptive loci, regardless of limitations from each method (Rellstab et al., 2015).

Briefly, RDA is a multivariate method that assumes linear relationships from explanatory variables on response variables, thus allowing the estimation of genetic variance related to each distinct environmental factor simultaneously (Forester et al., 2018). RDA and LFMM require complete data sets, therefore we imputed missing data as the most common allele in the locus from the optimal ancestral cluster (*k*) as defined in the SNMF output. The explanatory variables (i.e., climate) were then constrained by the dependent variables (i.e., individuals), using the rda function in the *VEGAN* package 2.5-1 in R (Oksanen et al., 2018). The anova.cca function was used to test for RDA significance using 999 permutations (randomised environmental variables). We did not explicitly control for population structure because RDA without explicit population structure inputs improves the output (Forester et al., 2018).

The LFMM was also applied to test for climate associations (Frichot et al., 2013), which applies a univariate regression model to assess genotype-environment associations while using the optimal k-value estimated in SNMF to control for ancestral population structure. This method is described as highly efficient to identify polygenic associations, even across diverse demographic sampling (De Mita et al., 2013; Lotterhos & Whitlock, 2015). The LFMM analyses were independently performed for each of the climate variables, consisting of 30,000 iterations each (15,000 discarded as initial burn-in). Median z-scores were combined from a

total of 5 runs for each variable and recalibrated by manually adjusting the genomic inflation factor, λ, and then dividing the scores by λ. Adjusted *p*-values are computed by flattening the histogram (false discoveries were controlled with the Benjamin-Hochberg algorithm using q = 0.01), which ideally should display a peak close to zero. We used λ = 0.45 (this optimally flattened the histogram after testing other λ values as recommended in LFMM manual) in the adjustment function to flatten the histogram and followed the steps and R script available from the LFMM manual. To account for multiple comparisons, we applied a false discovery rate (FDR) threshold of 0.05 to all runs.

Lastly, a hierarchical clustering model implemented in BAYPASS (Gautier, 2015) was applied, based on the model from BayEnv (Coop et al., 2010). A population covariance matrix (Ω) was generated by running the core model. Each run had 100,000 iterations (50,000 discarded as initial burn-in), repeated five times and averaged. The covariance matrix was then used in the AUX covariate mode (100,000 iterations; 50,000 as burn-in), repeated five times and averaged for final results. Significant SNPs were identified if they had a Bayes Factor (BF) > 3 (Kass & Raftery, 1995). Like LFMM, BAYPASS is based on a mixed linear model to account for potentially confounding allele frequency variances due to population structure. However, the difference between the two approaches may provide a means of identifying any influence of population structure (Forester et al., 2018; Ahrens et al., 2021a).

2.2.5. Annotation and gene ontology analysis

To investigate the potential role of adaptive SNPs, identified by the three EAAs, in coding regions of genes, genomic annotation was run using the *blastn* function (Altschul et al., 1997) from BLAST (https://blast.ncbi.nlm.nih.gov/). The 75 bp sequences associated with each putatively adaptive SNP were annotated using the *Eucalyptus grandis* genome (Myburg et al., 2014) and gene annotation considered significant if their blast output values met two related thresholds: an *E*-value < 1 × 10−8 and a blast-score of at least 60.0. Chromosome number and location of SNPs were recorded, as well as annotated gene functions. Annotated genes were used in a comparative analysis with the co-occurring tree marri (*Corymbia calophylla*) from Ahrens et al., (2019a) to identify shared adaptive genes between the two species.

The putatively adaptive SNPs in genes were also used to predict broader biological functions using GO enrichment analysis through the web interface PlantRegMap (Tian et al., 2020). GO terms are organized within three categories: molecular function, cellular component and biological process. We explored the biological process aspect from the GO analysis, which refers to a category of broad processes accomplished by multiple genes or gene products. For each climate variable, Fisher's exact test was used to test for significantly over-represented GO terms, with a threshold of *p*-value < 0.01. Significant GO terms are defined by a set of genes associated with a specific climate variable. We use this output to explore how functionally related candidate SNPs associated with each climate variable are linked to GO terms, which might be additive for environmental responses (e.g., abiotic stress response) (Kulbaba et al., 2019). We only developed a subset of significant GO terms for landscape scale patterns to illustrate our point of variation among similarly functioning genes. We chose GO terms with the highest number of SNPs and/or with biological processes directly related to the climate variable (specifically response and/or tolerance to heat, cold and drought) for further landscape genomic analysis.

2.2.6. Landscape genomics

We used generalized dissimilarity modelling (GDM) to visualise the relationship between allele frequency of putatively adaptive SNPs and climate (Fitzpatrick & Keller, 2015). GDM is a statistical method that predicts spatial patterns of allelic turnover across geographic regions due to climate by generating an I-spline turnover plot for each tested predictor and uses percent deviance explained as a measure of model fit (Ferrier et al., 2007). Specifically, the GDM spline plots show the association between predicted ecological distances and genetic dissimilarities (F_{ST} matrix) while partitioning out variance explained by geographic distance; the y-axis on the spline plots is therefore labelled as partial genetic distance, as it describes a portion of genetic distance, and the height of each spline indicates the magnitude of genomic turnover of a SNP along the climate gradient. GDM analyses was run using the *gdm* package v 1.3.7 in R (Manion et al., 2018), considering an input genotypic matrix (pairwise F_{ST} for single putatively adaptive SNPs or SNP groups from GO terms) and a pairwise climate matrix that includes geographic coordinates. The *HierFSTAT* package in R was used to create population pairwise F_{ST} matrices with the putatively adaptive SNPs associated with each climate variable.

GDM was independently applied to all the putatively adaptive SNPs identified by the EAA as significant. For each climate variable, SNP GDM models with the highest value of deviance explained was selected for plotting and mapping of predicted allelic turnover to test the hypothesis of adaptive variants being independently sorted across the landscape.

Following the GDM transformation of the climate variables for each SNP, PCA was applied on the GDM transformed environmental layer using R. The PCA was predicted across geographic space using the predict function in the *raster* package and visualised using the plot function, revealing a 0-1 allelic turnover map. To test the hypothesis of additive variation, we ran GDM analyses on groups of SNPs related to specific GO terms for each of the five climate variables and visualised how the allelic turnover within the GO term was related to that climate. By running GDM analysis on groups of SNPs, we can quantify an 'additive score' of deviance explained for each set of SNPs to compare the importance of GO terms. To test the independent assortment hypothesis among SNPs, we directly compared maps derived from the GDM models that explained the most deviance for each of the five bioclimatic factors using a Spearman's correlation coefficient test in the *spatialEco* package (Evans, 2017).

2.3. Results

2.3.1. Sequencing and population structure

A total of 78,198 SNPs were generated by DArTseq technologies and filtered down to 13,534 independent SNPs, with 8,824 SNPs mapped to the 11 *Eucalyptus grandis* chromosomes. The number of SNPs per chromosome varied from 599 to 1,083, with a mean of 802 SNPs per chromosome. Of the remaining SNPs, 477 fell on unspecified scaffolds and 4,233 on regions that could not be aligned to the *E. grandis* genome (unknown location). Population differentiation was low (global F_{ST} = 0.04), and similar to that identified in a previous RFLP analysis of variation (F_{ST} = 0.034; Wheeler et al., 2003), with population pairwise F_{ST} values ranging from 0.011 to 0.18 (Table S2.2, tab 18). The cross-entropy analysis estimated that the optimal number of clusters (*k*-value with the lowest entropy score) was 6 (Figure S2.1). SNMF analysis with six clusters revealed substantial admixture in populations. Five of the clusters could be geographically described (Figure 2.2, S2.2), one cluster was primarily located in the southern area, one in the central area and two in the northern area, where one cluster was

dominant in populations along the coast. A fifth cluster occurred in the outlier population (JIL; blue colour), and the sixth cluster was present in four individuals (two individuals from BRA and BOO). The LES population, which is the northmost population, displays mixed affinity, being similar to both southern (green) and northern (yellow and red) populations. This is consistent with the northern areas harbouring ancestral variation in other co-occurring species (e.g., marri; Sampson et al., 2018), or could indicate possible historic human influence through Aboriginal movement of plants (Lullfitz et al., 2020a, 2020b).

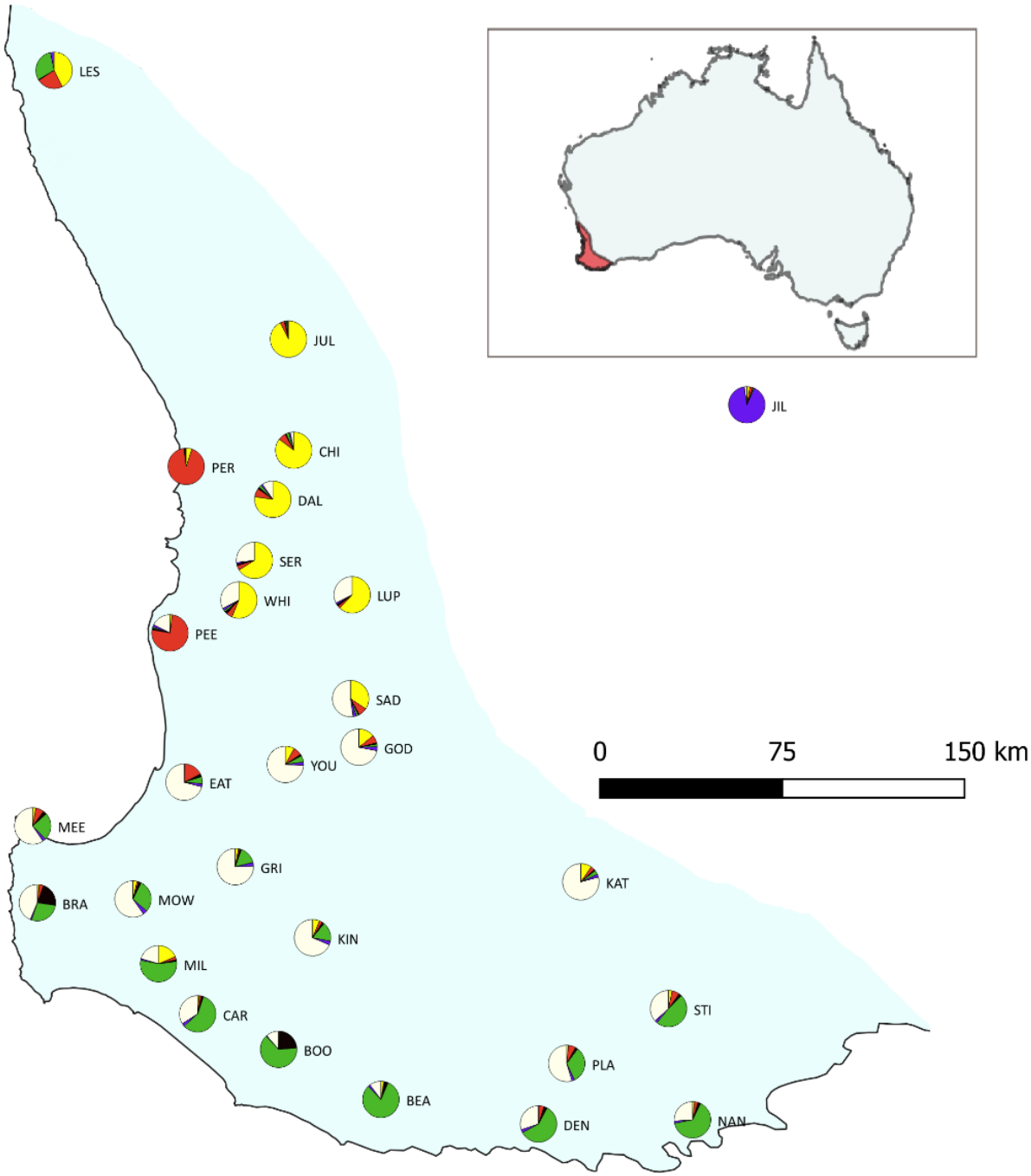


Figure 2.2. Distribution of sampled jarrah displaying population membership proportion for *k* = 6 genetic clusters, depicted as pie charts. Refer to Table 2.1 for more details on each population.

2.3.2. Environmental association analysis

The full set of 13,534 independent SNPs were considered for environmental association analysis. All three EAA approaches found putatively adaptive SNPs for each of the five climate variables (Table S2.2, tabs 1-5). The RDA approach identified fewer putatively adaptive SNPs than BAYPASS and LFMM that identified similar numbers (Figure 2.3; Table S2.1). The proportion of overlapped SNPs is different for each variable (Figure 2.3). Overall, 2,336 unique SNPs were flagged to be associated with at least one of the tested climate variables across the three EAA approaches. RDA analysis (Figure 2.3) identified between 16 (T_{SEAS}) and 57 (T_{MAX}) SNPs significantly associated with each of the climate variables, for a total of 168 SNPs. All five climate variables were shown to be significantly associated with variation in the RDA (T_{SEAS}: $F = 3.98$, $p = 0.001$; T_{MAX}: $F = 2.26$, $p = 0.001$; T_{MIN}: $F = 2.11$, $p = 0.001$; P_{MA}: $F = 1.80$ $p = 0.001$; P_{WQ}: $F = 1.49$, $p = 0.001$). LFMM identified between 263 (P_{MA}) and 411 (T_{MAX}) SNPs with significant correlations, with a total of 1,753 putatively adaptive SNPs. BAYPASS identified between 284 (T_{MIN}) and 888 (T_{MAX}) SNPs with significant correlations, with a total of 2,327 putatively adaptive SNPs. Putatively adaptive SNPs found for all environmental variables from each EAA method were used in further analyses.

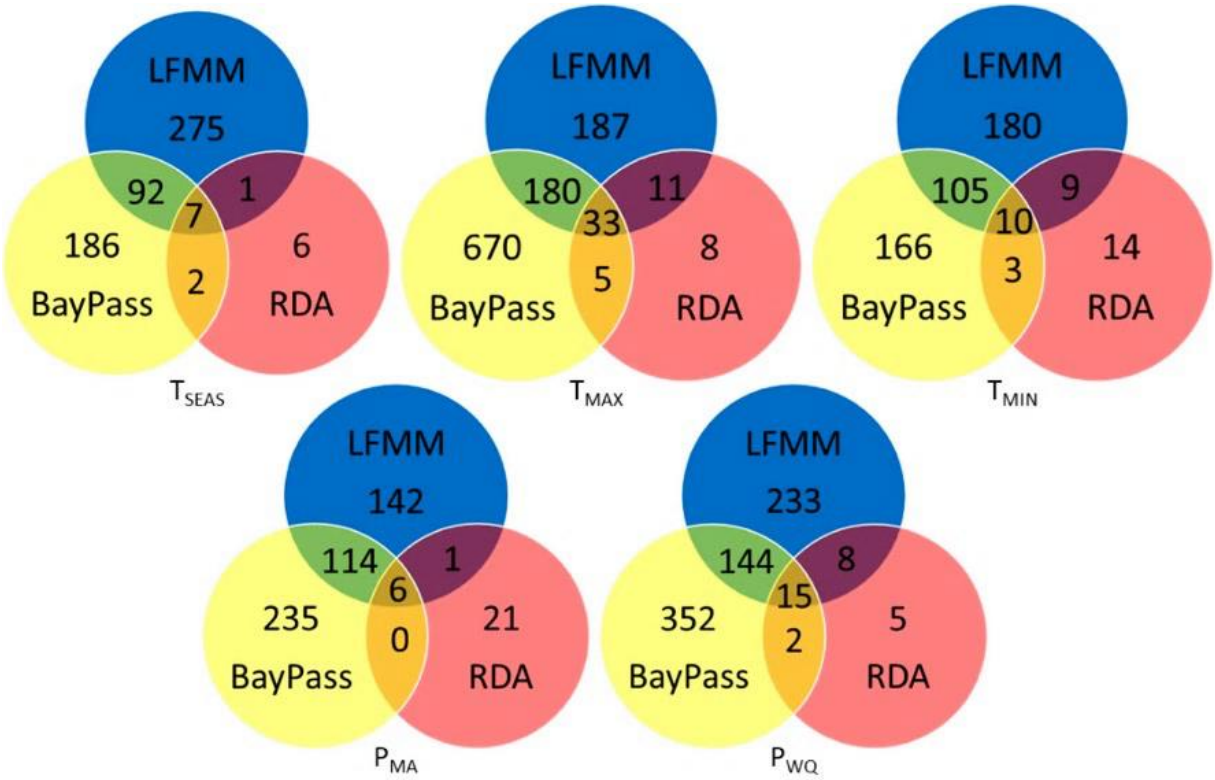


Figure 2.3. Summary of environmental association analysis in jarrah. Venn diagrams show the intersections between the three approaches of environmental association analyses (RDA, red; LFMM, blue; BAYPASS, yellow) considering adaptive SNPs associated with each of the climate variables: T_{SEAS} = temperature seasonality; T_{MAX} = mean maximum temperature of the warmest month; T_{MIN} = mean minimum temperature of the coldest month; P_{MA} = mean annual precipitation; P_{WQ} = mean precipitation of the warmest quarter.

2.3.3. Annotation and gene ontology

Of the 2,336 unique putatively adaptive SNPs associated with the climate variables, 1,440 SNPs were linked to functionally annotated genes, which represents 10.6% of the total independent SNPs tested for EAA (13,534). Full annotation results for SNPs associated with each variable are given in Supporting Information (Table S2.2, tabs 6–10). T_{MAX} delivered the highest amount of linked functionally annotated genes (474), followed by P_{WQ} (312), T_{SEAS} (237), P_{MA} (214) and T_{MIN} (203). Annotated SNPs (Table 2.2) included, for example, JAR00198, associated with both T_{SEAS} and T_{MIN}, located in a trans-cinnamate 4-monooxygenase (TCMO) gene; two SNPS associated with T_{MAX}, JAR00038 and JAR00207 were found on transcription repressor MYB6 and transcription factor MYB44 genes respectively; P_{MA}, JAR02395, located in a peroxidase 72 gene.

Gene ontology enrichment analysis explored how groups of annotated SNPs relate to similar functions (Table S2.2, tabs 11-15). Enriched GO terms in the biological process category are highlighted for each of the five bioclimatic variables (Table 2.3). A GO term associated with response to light stimulus (GO:0009416) was found with the SNPs related to T_{SEAS}.

Genes associated with this GO term are linked to cellular response processes (in terms of components movement, enzyme production, and secretion and protein expression) from abiotic stimulus, specifically electromagnetic radiation and light. A GO term related to karrikin stimulus was found associated with T_{MIN} (GO:0080167). As for P_{MA} and P_{WQ}, GO terms with high counts of SNPs were found for each variable (GO:0044763 and GO:1901566, respectively) as well as a term related to UV response (GO:0009411) associated with PMA.

Table 2.2. Gene annotation showing the top five SNPs (Blast score = 125) for jarrah, with NCBI blast e-value score, relative ranks based on levels of significance for each EAA and chromosome number (chr) from *Eucalyptus grandis* genomic mapping (un = unknown) for each environmental variable. T_{SEAS} = temperature seasonality; T_{MAX} = mean maximum temperature of the warmest month; T_{MIN} = mean minimum temperature of the coldest month; P_{MA} = mean annual precipitation; and P_{WQ} = mean precipitation of the warmest quarter). SNPs that were also found associated with GO terms (Table 2.3) are in bold.

Climate	SNP	RDA (*p*-value)	LFMM (*p*-value)	BAYPASS (BF)	chr	Blast e-val	Gene annotation from the *Eucalyptus grandis* genome
T_{SEAS}	JAR00166	-	0.00034	4.708	un	1.0E-28	Mitochondrion
	JAR00198	-	0.00064	6.788	10	1.0E-28	Trans-cinnamate 4-monooxygenase
	JAR00273	-	3.11E-05	21.663	11	1.0E-28	Mitochondrion
	JAR00499	-	0.00071	-	8	1.0E-28	Probable LRR receptor-like serine/threonine-protein
	JAR00662	-	4.78E-06	-	6	1.0E-28	UPF0496 protein
T_{MAX}	JAR00038	-	-	3.270	6	8.0E-29	Transcription repressor MYB6
	JAR00207	-	-	3.054	6	8.0E-29	Transcription factor MYB44
	JAR00209	-	-	9.466	11	8.0E-29	AT-hook motif nuclear-localized protein 16
	JAR00214	-	-	11.262	6	8.0E-29	Protein indeterminate-domain 1
	JAR00262	-	-	6.154	4	8.0E-29	Uncharacterized
T_{MIN}	JAR00013	-	-	9.801	10	8.0E-29	Mitochondrion
	JAR00166	-	-	18.303	un	1E-28	Mitochondrion
	JAR00198	-	0.00026	6.788	10	1E-28	Trans-cinnamate 4-monooxygenase
	JAR00273	-	1.32E-06	-	11	1E-28	Mitochondrion
	JAR00620	0.242	-	-	11	8.0E-29	Uncharacterized
P_{MA}	JAR00027	-	0.00014	10.316	7	1.0E-28	Mitochondrion
	JAR00500	-	-	6.788	4	1.0E-28	Putative yippee-like protein Os10g0369500
	JAR01426	-	0.0004	-	11	1.0E-28	Tyrosine decarboxylase 1
	JAR01512	-	0.0001	-	5	1.0E-28	Uncharacterized
	JAR02395	-	0.00092	-	9	1.0E-28	Peroxidase 72
P_{WQ}	JAR00214	0.454	0.00053	-	6	8E-29	Protein indeterminate-domain 1
	JAR00273	-	-	11.889	11	8E-29	10 kDa chaperonin
	JAR00499	-	0.00021	-	8	1E-28	Probable LRR receptor-like serine/ threonine-protein
	JAR00690	-	0.00031	6.266	1	8E-29	Zinc finger protein ZAT5
	JAR01091	-	5.34E-07	4.388	7	1E-28	LOB domain-containing protein 1-like

Table 2.3. Overrepresented gene ontology (GO) terms for SNPs identified in jarrah for each environmental variable. The GDM % deviance explained is expressed as additive score for groups of SNPs. T_{SEAS} = temperature seasonality; T_{MAX} = mean maximum temperature of the warmest month; T_{MIN} = mean minimum temperature of the coldest month; P_{MA} = mean annual precipitation; and P_{WQ} = mean precipitation of the warmest quarter by count of SNPs and/or relevant biological function

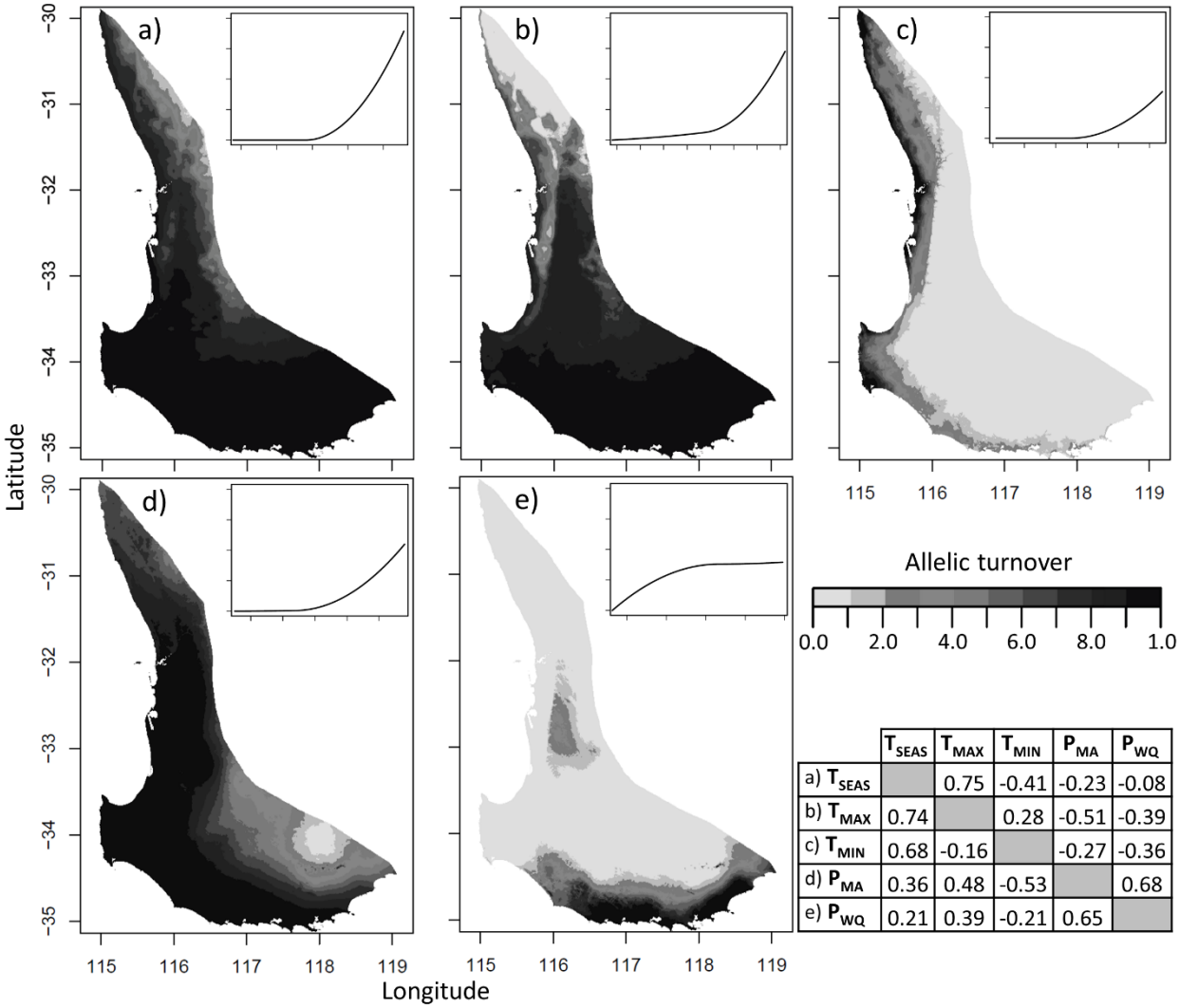
Climate	GO id	GO term	GDM % deviance explained	*p*-value	SNPs	Count
T_{SEAS}	GO:0009314	Response to light stimulus	3.66	0.00261	JAR02551, JAR04603, JAR00284, JAR02659, JAR06621, JAR04257, JAR07363, JAR00198, JAR01133, JAR07395	10
T_{MAX}	GO:0000271	Polysaccharide biosynthetic process	4.61	0.0067	JAR05227, JAR06489, JAR06314, JAR08046, JAR12549, JAR11847, JAR08134, JAR12439	8
	GO:0010104	Regulation of ethylene-activated signalling pathway	2.80	0.0097	JAR09402, JAR12137	2
T_{MIN}	GO:0071840	Cellular component organization or biogenesis	2.80	0.00859	JAR05151, JAR02381, JAR00166, JAR02528, JAR04603, JAR03088, JAR05858, JAR01284, JAR06869, JAR05668, JAR04700, JAR07286	12
	GO:0080167	Response to karrikin	14.27	0.00391	JAR06869, JAR00198, JAR03623	3
P_{MA}	GO:0044763	Single-organism cellular process	3.01	0.0061	JAR07368, JAR04995, JAR05607, JAR07695, JAR05954, JAR12984, JAR11156, JAR00284, JAR11454, JAR08984, JAR13223, JAR06091, JAR07363, JAR08184, JAR12280	15
	GO:0009411	Response to UV	4.62	0.0059	JAR00284, JAR07363	2
P_{WQ}	GO:1901566	Organonitrogen compound biosynthetic process	21.22	0.0064	JAR12369, JAR00189, JAR00543, JAR11122, JAR05879, JAR12666, JAR02347, JAR11253, JAR11737, JAR06747, JAR07829, JAR06097, JAR10308, JAR12789, JAR12316, JAR00476, JAR13196, JAR11797, JAR11414, JAR11170, JAR10452	21

2.3.4. Landscape Modelling

The GDM analysis was applied independently to all putatively adaptive SNPs associated with the five climatic variables (Figure S2.4), and the models that explained the highest deviance for each variable were selected to display spatial patterns of allelic turnover (Figure 2.4): T_{SEAS} – JAR00269 (39.2%); $_{TMAX}$ – JAR11943 (25.5%); T_{MIN} – JAR01172 (16.8%); P_{MA} – JAR10596 (21.9%) and P_{WQ} – JAR06621 (36.9%). The GDM model for the SNP associated with T_{SEAS} (JAR00269) explained more deviance than any other putatively adaptive SNP across the five climate variables, followed by the model for P_{WQ} (JAR06621). There was rapid turnover noticeable for the three temperature variables from the coastal to eastern populations in the north of the range, and more gradual turnover from the northern populations to the southern populations (Figure 2.4a, b, c). But even among the three temperature variables, there were major differences in adaptive patterns. For instance, while T_{SEAS} and T_{MAX} displayed a similar rapid turnover from the coastal to eastern populations in the north of the range, and fairly gradual turnover from the northern populations to the southern populations, T_{MIN} followed the same trend in the northern region, but a rapid turnover is present between the coastal and inland populations in the south region. In contrast, the precipitation variables showed rapid turnover in the southern or central parts of the distribution, and more gradual turnover in the northern distribution (Figure 2.4d, e). In southern areas, P_{WQ} showed a rapid turnover between coastal and inland southern populations, while P_{MA} showed a more gradual pattern in this region. Correlation coefficients between allelic turnover maps showed clear differences between some of the adaptive landscapes (Figure 2.4; bottom right table, below the diagonal), as r2 values ranged between the absolute values of 0.21 to 0.74, and often different than correlation between their respective climate variables (above the diagonal). These differences are indicative of contrasting spatial patterns.

The groups of SNPs associated with selected GO terms (Table 2.3) were also used in a combined GDM analysis to measure allelic turnover across climatic gradients and interpreted as an additive pattern of adaptation (Figure 2.5). The patterns of allelic turnover varied by climatic variable: overall, GDM showed small to moderate response, in terms of deviance explained. The GDM model explained more deviance for the group of SNPs linked to the GO term associated with P_{WQ} (n = 21; 21.22%, Figure 2.5e) than any other climate variable

association using GO terms, followed by GO terms associated with T_{MIN} (n = 3; 14.27%, Figure 2.5c). Models for T_{SEAS}, T_{MAX} and P_{MA} explained a similar deviance for allelic turnover composition (< 5% for each group of SNPs).



	T_{SEAS}	T_{MAX}	T_{MIN}	P_{MA}	P_{WQ}
a) T_{SEAS}		0.75	-0.41	-0.23	-0.08
b) T_{MAX}	0.74		0.28	-0.51	-0.39
c) T_{MIN}	0.68	-0.16		-0.27	-0.36
d) P_{MA}	0.36	0.48	-0.53		0.68
e) P_{WQ}	0.21	0.39	-0.21	0.65	

Figure 2.4. Predicted spatial variation of allelic turnover based on the outputs from the GDM models that explained the most deviance for each climate variable (between climate and SNP) for jarrah. a) T_{SEAS} – JAR00269; b) T_{MAX} – JAR11943; c) T_{MIN} – JAR01172; d) P_{MA} – JAR10596 and e) P_{WQ} – JAR06621. Insets are spline plots of partial genetic distance (y-axis) by climatic distance (x-axis) for the individual SNP (dimensions of the plot are the same as in Figure 2.4). Bottom right table shows the pairwise Spearman's correlation coefficient (r^2) between the two allelic turnover maps (below diagonal) and between the climate variables (above the diagonal).

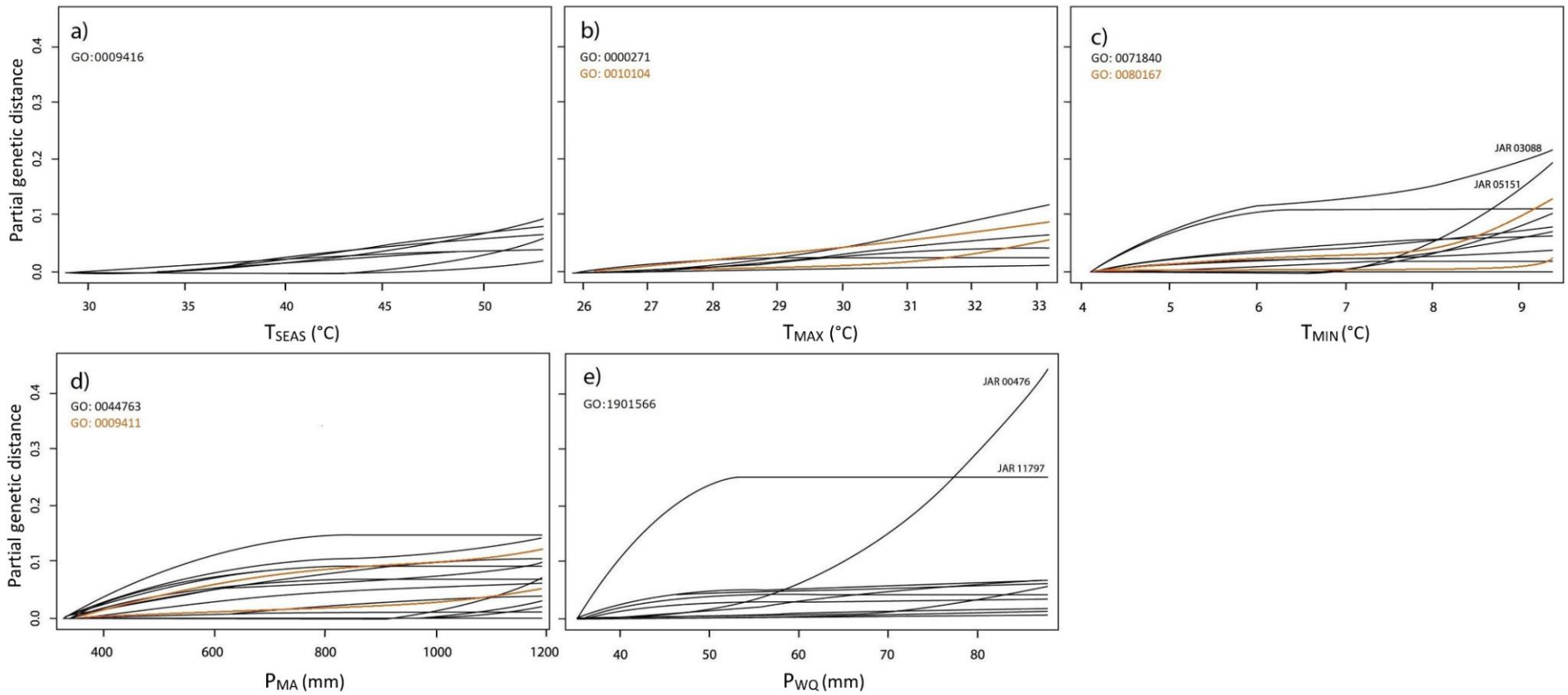


Figure 2.5. Geographic generalized dissimilarity modelling (GDM) in jarrah showing SNPs allelic turnover for gene functions (GO terms) across each environmental variable. a) T_{SEAS} = temperature seasonality; b) T_{MAX} = mean maximum temperature of the warmest month; c) T_{MIN} = mean minimum temperature of the coldest month; d) P_{MA} = mean annual precipitation; and e) P_{WQ} = mean precipitation of the warmest quarter. GO terms with different SNP sets in the same plot are represented with different colours (black or orange).

2.4. Discussion

This study identified putative patterns of climate adaptation in jarrah, with several strong associations between candidate SNPs and climatic gradients. The results provide support for our hypothesis of strong patterns of local adaptation to climate across the distribution of jarrah, although, contrary to our second hypothesis, we found adaptation to both temperature and precipitation variables rather than primarily with precipitation. As expected, annotation highlighted functional genes associated with biological processes, some of which relate to abiotic stress factors and provide good candidates for adaptations. Furthermore, the landscape genomics modelling assessed the magnitude of allelic turnover for putatively adaptive SNPs and highlighted temperature seasonality, mean maximum temperature of the warmest month and precipitation of the warmest quarter as explaining significantly more variation than other climate drivers. These patterns indicate that adaptive variants are independently sorting across the landscape, which is consistent with our fourth hypothesis. We discuss the mechanisms for adaptation to climate across complex landscapes for forest trees, including a direct comparison with a co-dominant co-occurring foundation species, before providing the scientific basis for implementation of management and conservation strategies to promote the resilience of foundation tree species. These associations, indicating potential local adaptation, were found despite high levels of gene flow among populations across the distribution, a common characteristic among eucalypt species (Jones et al., 2002; Supple et al., 2018; Murray et al., 2019; Ahrens et al., 2019a). Low differentiation among populations indicates that application of EAA in jarrah is appropriate to identify alleles putatively under selection. As expected, the three EAA approaches identified different sets of putatively adaptive SNPs for each climate variable. One limitation of EEAs is the identification of SNPs that are found to be under selection but are in fact not (false positives). While false positives are an inherent limitation in EAA studies, EAAs can consistently identify adaptive SNPs, even if the selection coefficient is small (Ahrens et al., 2021a). We focus our interpretation on SNPs that are within gene space to lessen the impact of false positives, despite that candidate SNPs identified outside of gene space could be true positives. For instance, SNPs could be in promoter regions, which are known to have high proportion of adaptive variants (Wittkopp & Kalay, 2012), SNPs could form a large haplotype block with

genes that are under selection (Todesco et al., 2020), or SNPs could be in linkage disequilibrium with adaptive SNPs. Future work should focus on improving the genomic resources of the species to elucidate these complex issues that are beyond the scope of this work.

2.4.1. Adaptation to temperature and precipitation

GDM models on all putatively adaptive SNPs found the highest deviance explained for a SNP associated with T_{SEAS} (39.2%), closely followed by P_{WQ} (36.9%), with overall results showing low to moderate deviance across the five climate variables. Furthermore, P_{WQ} was linked to GO:1901566, with the highest number of putatively adaptive SNPs (21) and also showed the highest deviance explained by the GDM analysis (Figure 2.5e). Overall, both temperature and precipitation variables are linked to adaptive genetic variants; although, GO and GDM analysis highlighted the specific precipitation variable (P_{WQ}) as a stronger driver of adaptation.

The annotations of putatively adaptive genes were made based on the reference genome of *Eucalyptus grandis*, a distant relative, so we provide a pertinent but cautious preliminary interpretation of functional results until a full jarrah reference genome becomes available. Gene functions associated with the temperature and precipitation variables show biological functions associated with response and adaptation to these abiotic factors. For example, the KCS gene family (JAR02659), that was associated with T_{SEAS}, has been linked to cold and light responses (Joubès et al., 2008) in *Arabidopsis*, being involved in the biosynthesis of waxes that cover the leaves surface. Two SNPs, also associated with T_{SEAS}, (JAR13256 and JAR08936) are linked to the ABC transporter gene families, which have been shown to be associated with heat response and abiotic stress tolerance during seed germination (Zhang et al., 2012; Hwang et al., 2016). Control of seed germination during periods of thermal stress could be a crucial mechanism for selecting phenotypes that are more adapted to Mediterranean type of climates with hot and dry summers. In that sense, we can envision how genes that control seed germination response could be selected for or against in such climates (Rix et al., 2015). A SNP associated with P_{WQ} (JAR13490) was found in the chromatin-remodelling factor PKL gene that has been consistently linked to multiple plant development processes, particularly

to the abscisic acid (ABA) pathway regulation (Perruc et al., 2007). ABA is a phytohormone that is well known for controlling stomatal closure (Rajab et al., 2019, Maheshwari et al., 2020), thus being crucial for efficient drought response (Yu et al., 2019, Zhang et al., 2020). Drought is a known selective force in the region, and the ability to control stomates would also allow for the reduction of transpiration during dry periods, a physiological ability that has been shown to be crucial in the co-occurring marri (Challis et al., 2020). These are just a sample of the many compelling gene functions associated with either temperature or precipitation found across the five tested climatic variables, identifying these variables as potential drivers of local adaptation.

2.4.2. Functionally related genes have similar adaptive patterns

In our GO enrichment analysis, we focused on biological processes related to abiotic stress responses such as drought, cold and heat. Generally, we found biological GO terms with gene overrepresentations, consistent with expectations under our third hypothesis: functionally related genes have similar patterns of correlation with climate. For instance, a GO term related to karrikin stimulus was found associated with T_{MIN} (GO:0080167). Karrikins are a group of phytohormones that control several aspects of plant germination and growth (Nelson et al., 2012). A study with *Arabidopsis* showed that karrikin signalling can inhibit seed germination under heat stress (Wang et al., 2018), possibly to avoid germination under conditions unfavourable to seedling establishment.

Many plants' functional traits are polygenic, involving complex interactions controlled by multiple genes, so it is expected that patterns of climate adaptation are also the result of combined effects from several alleles of small-effect (Wadgymar et al., 2017). Indeed, climatic variables are expected to not be the main driver for variation in some putatively adaptive SNPs, as the genes associated can be pleiotropic and may be under selection from other biotic or abiotic factors. For example, although precipitation and temperature are consistently highlighted as key factors influencing plant distribution and ecology, soil properties greatly affect these settings, as water availability depends on the interaction between climatic variables and soil characteristics (Piedallu et al., 2013). The identification and understanding

of adaptive genetic variations might then be improved by including other relevant abiotic factors such as soil characteristics. Nevertheless, by hierarchically categorising gene functions through GO enrichment, we were able to find adaptive patterns across the distribution, highlighting likely polygenic adaptations to climate variables in this species.

2.4.3. Adaptive variants are independently sorted

Across the species geographic distribution, climatic heterogeneity explains significant genomic variation. Two distinct climate variables in particular, T_{SEAS} and P_{WQ}, showed strong associations with genomic variants. The patterns of genomic turnover associated with the studied climatic variables are aligned with the climatic gradients of the region (Figure 2.4). These associations are indicative of the multidimensional patterns of adaptation resulting in uncorrelated intraspecific selection among SNPs (White & Butlin, 2021). Here, we define dimensionality as the interaction between uncorrelated climate variables to independently describe each habitat. Our dimensionality is driven by climate, and the independent sorting of putatively adaptive SNPs is indicative of this complex pattern. It has been modelled that local adaptation increases with dimensionality (MacPherson et al., 2015), and it likely leads to dimensionality of phenotypic traits (Kirkpatrick and Meyer 2004; McGuigan et al., 2005). Indeed, there is evidence of intraspecific variation among growth traits (e.g., height and diameter) that are locally adapted in jarrah (O'Brien et al., 2007, 2010; Koch & Samsa, 2007).

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

2.4.4. Landscape adaptations of forests

Comparative analysis can provide broader patterns for forest management, where concurrent genetic and spatial patterns of local adaptation within co-occurring tree species provides strong evidence for environmental fitness and evolution (Bragg et al., 2015). Our analysis here identified SNPs associated with temperature and precipitation in jarrah; while a similar study on a co-occurring species, marri, found SNPs associated with temperature to explain more deviance than precipitation (Ahrens et al., 2019a), thereby suggesting that temperature is a stronger driver of local adaptation for marri. It is interesting that there were similarities in functional genes associated with several adaptive variants between jarrah and marri (e.g., ABC transporters and CBL gene families). Comparison across both species identified a set of 26 genes that were also found to be associated with at least one of the five variables analysed (Table S2.3). Most of these shared genes are associated with either T_{MAX} (16) or P_{WQ} (12) in jarrah; while for marri, the majority of the shared genes are associated with T_{MAX} (24), which is consistent with adaptation to both temperature and precipitation in jarrah and with temperature in marri. While this comparison shows that some functional genes share adaptive patterns, there were more genes that were different, indicating that the same adaptive management plan may not be effective for both species.

2.4.5. Management perspectives

Our analysis of standing genetic variation across the distribution of jarrah found potential links between putatively adaptive SNPs and climate factors, which may provide a source of adaptation to future climate conditions. The evidence that genetic variants are involved with climate adaptation occurred as either associations with specific annotated gene functions or biological processes associated with climate factors. Our analysis here, and that of the co-dominant species marri (Ahrens et al., 2019a), are also consistent with results from recent genomic studies on other eucalypt species in other regions of Australia (Steane et al., 2017; Jordan et al., 2017, 2020), providing evidence of adaptation to climate in natural populations and stressing the role of temperature (particularly T_{SEAS} and T_{MAX}) and precipitation (P_{WQ}) variables. The presence of climate adaptation provides a basis for implementation of assisted

gene migration for forest management strategies (Aitken & Bemmels, 2016) and climate adjusted provenance (i.e., sourcing of seed from populations in the direction of climate change for use in restoration sites to enhance adaptation to future climate) in restoration practices (Prober et al., 2015). As a foundation tree, jarrah is a vital component in the ecosystem and has a significant role in regulating local hydrological systems and carbon storage (CCWA, 2013; Bradshaw, 2015). Additionally, it offers abundant habitats for a wide variety of groups, from vascular flora and lichens to terrestrial vertebrates and birds (Whitford & Williams, 2002; Whitford et al., 2015), as well as unique food sources for fauna, especially birds (Wrigley, 2012; Lee et al., 2013). The Forest Management Plan 2014–2023 (CCWA, 2013) for SWWA forests has provision for implementation of assisted gene migration in management strategies for response to climate change. Our findings of standing variation harbouring putative adaptations to climate associated with temperature and precipitation factors provides an evidence base for design and implementation of such strategies. In addition, phenotypic approaches on other eucalypt species have also highlighted the role of local climate in the development of adaptive traits (Costa e Silva et al., 2019; Ahrens et al., 2019b, Ahrens et al., 2021b). Expanding this work to a phenotypic approach in jarrah (such as O'Brien et al., 2007) for identifying patterns of plasticity and adaptation associated with climate would contribute to further understanding the association of genomic and phenotypic diversity across environmental gradients. While it appears that genetic variants associated with similarly functioning genes are adapting to the environment in similar ways, we also found that putative adaptations among climate variables are sorted through the landscape in contrasting ways. This makes implementation of assisted gene migration strategies more complex and multidimensional. In fact, our findings support recommendations for sourcing germplasm from multiple sources to bolster the adaptability in adaptively depauperate populations and provide a basis for more active selection of functionally related genes, potentially increasing the diversity and adaptability through new combinations of genetic variation.

2.5. Supplementary materials

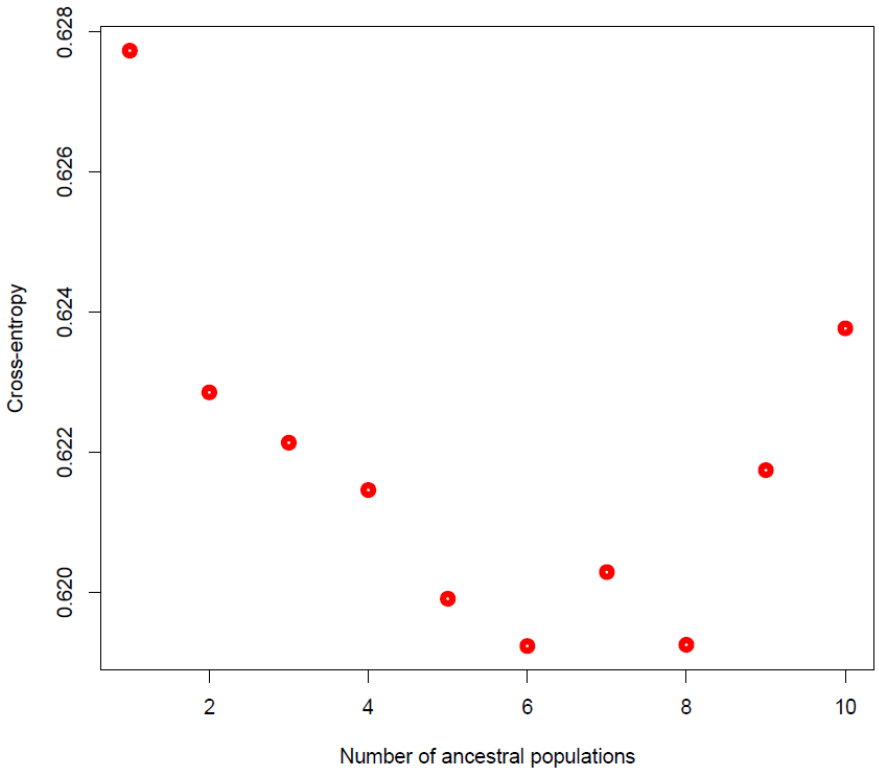


Figure S2.1. Cross entropy scores for all *K*s in the SNMF analysis of *Eucalyptus marginata*. This shows that a *k*=6 optimally describes the data.

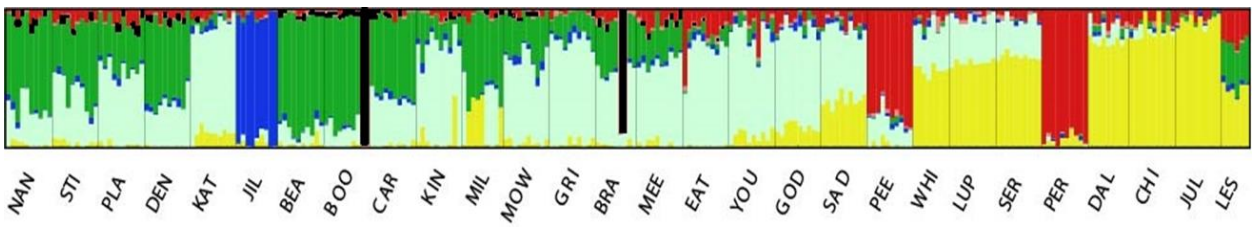


Figure S2.2. Individual assignment of ancestral genetic clusters in *Eucalyptus marginata* using SNMF (*k*=6) in the *LEA* package. Populations are labelled below the figure, arranged from southeast (left) to northwest (right) and denoted by the vertical black lines.

Table S2.1. Number of adaptive SNPs identified as having significant correlations with climate variables by RDA, LFMM and BAYPASS from a total data set of 13,534 SNPs in *Eucalyptus marginata*. Total unique adaptive SNPs found for each variable by the 3 EAAs together are shown in last column. T_{SEAS} = temperature seasonality; T_{MAX} = mean maximum temperature of the warmest month; T_{MIN} = mean minimum temperature of the coldest month; P_{MA} = mean annual precipitation; and P_{WQ} = mean precipitation of the warmest quarter.

Climate	RDA	LFMM	BAYPASS	Total unique adaptive SNPs
T_{SEAS}	16	375	287	569
T_{MAX}	57	411	888	1094
T_{MIN}	36	304	284	487
P_{MA}	28	263	355	519
P_{WQ}	31	400	513	759

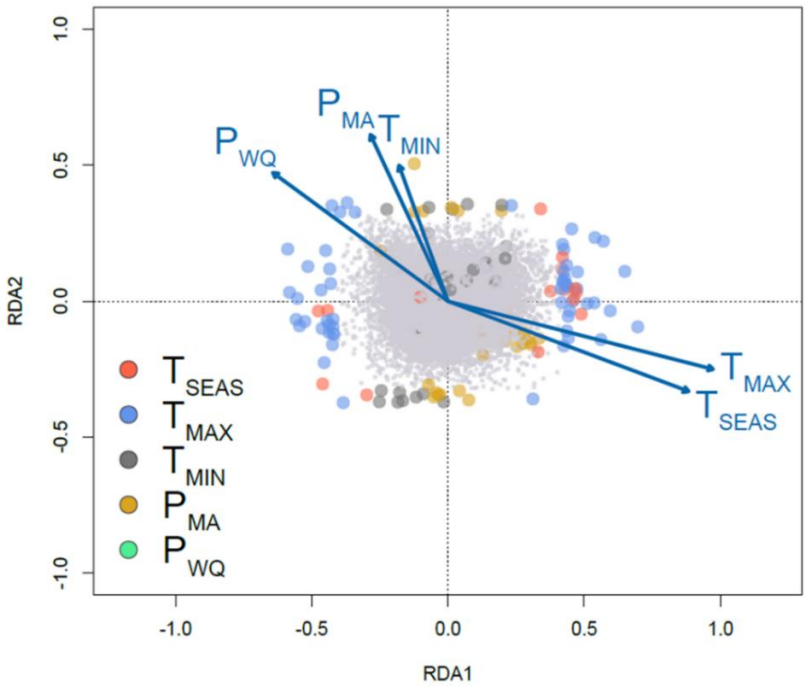


Figure S2.3. Identification of SNPs under putative selection using redundancy analysis (RDA) in *Eucalyptus marginata*. All points are SNPs: grey points are SNPs not associated with the five climate variables; colours indicate significant correlation with a climate variable. T_{SEAS} = temperature seasonality; T_{MAX} = mean maximum temperature of the warmest month; T_{MIN} = mean minimum temperature of the coldest month; P_{MA} = mean annual precipitation; and P_{WQ} = mean precipitation of the warmest quarter.

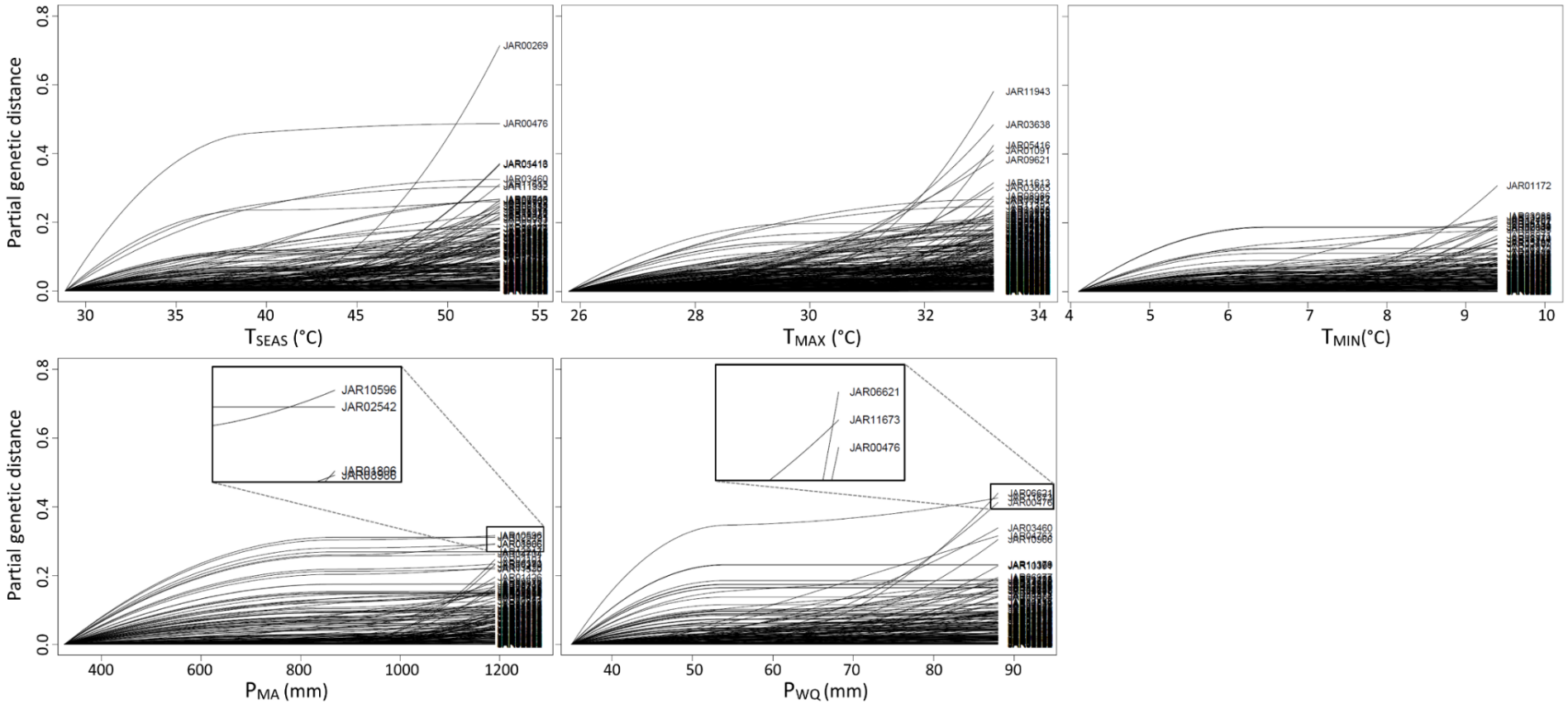


Figure S2.4. Geographic generalized dissimilarity modelling (GDM) in *Eucalyptus marginata* showing allelic turnover from all adaptive SNPs across each environmental variable. T_{SEAS} = temperature seasonality; T_{MAX} = mean maximum temperature of the warmest month; T_{MIN} = mean minimum temperature of the coldest month; P_{MA} = mean annual precipitation; and P_{WQ} = mean precipitation of the warmest quarter.

Table S2.3. SNPs identified in jarrah shared with those identified in a with previous study on *Corymbia calophylla* (Ahrens et al., 2019a).

			Jarrah					Marri	
SNP	**Shared locus**	**Gene annotation from the *Eucalyptus grandis* genome**	T_{SEAS}	T_{MAX}	T_{MIN}	P_{MA}	P_{WQ}	T_{MAX}	P_{MA}
JAR00785	LOC104441236	Coatomer subunit epsilon-1		X			X	X	
JAR00931	LOC104426747	Rapid alkalinization factor					X	X	
JAR01122	LOC104432985	Protein CHROMATIN REMODELING 19				X			
JAR02908	LOC104414468	Long-chain-alcohol oxidase FAO1				X		X	
JAR03482	LOC104450728	Serine/threonine-protein kinase-like protein CCR4		X			X	X	
JAR04628	LOC104449331	Alpha-amylase-like	X	X				X	X
JAR06045	LOC104435040	Glucan endo-1,3-beta-glucosidase 12	X	X			X		X
JAR06122	LOC104429802	Trans-resveratrol di-O-methyltransferase		X				X	
JAR06639	LOC104455075	Zinc finger protein ZAT1		X		X	X	X	
JAR06977	LOC104419420	Zibosome biogenesis protein WDR12 homolog	X	X			X	X	
JAR07135	LOC104441479	E3 ubiquitin-protein ligase UPL5			X		X	X	
JAR07318	LOC104417750	CTD small phosphatase-like protein 2		X				X	
JAR07672	LOC104415414	Uncharacterized		X			X	X	
JAR07772	LOC104435662	Uncharacterized		X			X	X	
JAR08036	LOC104446079	Heavy metal-associated isoprenylated plant protein	X	X				X	
JAR08342	LOC104431117	Probable glucan endo-1,3-beta-glucosidase At4g16260	X	X				X	
JAR08645	LOC104449205	Uncharacterized		X			X	X	
JAR08706	LOC104442342	Beta-amyrin synthase					X	X	
JAR11002	LOC104451511	Uncharacterized			X			X	
JAR11272	LOC104441755	Probable xyloglucan galactosyltransferase GT20	X	X				X	
JAR11414	LOC104422748	Peptidyl-prolyl cis-trans isomerase Pin1		X			X	X	
JAR12096	LOC104446870	Glutamate receptor 2.9	X	X				X	
JAR12217	LOC104414728	GEM-like protein 5				X		X	X
JAR12339	LOC104457401	Wall-associated receptor kinase-like 8				X		X	
JAR12574	LOC104428148	Oligopeptide transporter 3			X			X	
JAR13352	LOC104418537	Uncharacterized protein At4g06744				X		X	

Chapter 3.

Germination temperature sensitivity of two Mediterranean-type tree species as a vulnerability predictor to global warming

Abstract

Climate change is shifting temperatures from historic patterns putting at risk the sustainability of forest ecosystems. South-west Western Australia (SWWA) has extensive forest environments that have been subject to heatwaves-induced forest mortality in recent years. These rapid changes may impact community composition, population dynamics and the geographic range of species. Thus, there is a need to understand how ecosystems might regenerate under climate change by assessing native tree species' thermal limits and capacity to adapt to future temperature increases. Seed germination is crucial for population survival from seeding species and is directly regulated by local environmental conditions such as temperature. Seeds can germinate over a range of temperatures, with thresholds beyond and below germination is unlikely to occur, and within which there is an optimum germination temperature. Rapid changes in temperature associated with global warming may hinder germination timing and success, leaving plant populations vulnerable to decline and extinction. This study assessed seed germination response to temperature in *Eucalyptus marginata* (jarrah) and *Corymbia calophylla* (marri), endemic foundation species in the SWWA, to estimate the thermal optima and constraints among populations from wide-ranging climate origins. Seeds from across the entire geographic distribution were collected from 12 populations where both species co-occur. Patterns of germination were investigated using a thermal gradient plate (5-40°C). Germination trials were conducted at five constant temperatures between 9 and 33°C to test how the germination niche (1) differs between species, (2) varies among populations, and (3) relates to climatic origin. Temperature response of germination differed among species, with jarrah having a lower optimal temperature and thermal limit than marri (Topt = 15.3^{o}C and 21.2^{o}C; ED50 = 23.4^{o}C and 31°C, respectively). The thermal limit for germination differed among populations within both species, however only marri showed evidence for adaptation to thermal origins. The reduced thermal limit and reduced adaptive capacity quantified in jarrah indicates this foundation tree has a reduced recruitment window in northern forest populations currently experiencing warm temperatures. These results imply climate change may have a greater impact on jarrah compared to marri. The latter has the capacity for germination over a greater temperature range. As such the forest composition will alter in the future. This study provides critical information to assist in developing forest adaptive management strategies.

3.1. Introduction

Climate conditions are important selective factors promoting the divergent evolution of populations within species (Aitken & Bemmels, 2016; Martins et al., 2018). Rapid anthropogenic climate change is driving populations to become progressively uncoupled from the climate they were originally adapted (Cheaib et al., 2012; Cobb et al., 2017). Vulnerability to climate for a given species will greatly depend on life-history characteristics, genetic variation, and phenotypic plasticity (Aitken et al., 2008), but also their overall geographic distribution and local ecological niche (Thuiller et al., 2005; Broennimann et al., 2006). Warming effects may also affect species, or population within species, very differently. In species like trees, with slow migration rates and the long time required for evolutionary change (Savolainen et al., 2007; Oddou-Muratorio & Davi, 2014), the dissociation between population climatic adaptations and contemporary climate is expected to be accentuated in the near future. This adaptation 'lag' might cause reductions in population fitness, species distribution, and even local extinctions (Ooi, 2012; Kopp & Matuszewski, 2013; Wiens et al., 2016).

Mediterranean-type climate (MTC) regions of the world, defined with predictable summer periods of low rainfall and hot temperatures, and winter periods of high rainfall and moderate temperatures, have been flagged as potentially highly vulnerable to climate change (Cowling et al., 2004). Ecological studies in these regions consistently associate declines in native trees (e.g., reduced seedling establishment, inhibited growth and canopy collapses) to warming temperature and intense drought (Corcobado et al., 2014; Gentilesca et al., 2017, Camarero et al., 2018). Under projected climate change scenarios (IPCC 2021), there are modelled predictions for major declines in the distribution and diversity of the plant communities in areas such as the South-west Western Australia (SWWA) region (Fitzpatrick et al., 2008, Yates et al., 2010), a recognised biodiversity hotspot. Broad scale tree canopy collapses have already been recorded in SWWA because of heatwaves and drought events (Matusick et al., 2013, 2018; Ruthrof et al., 2018). Forest communities in this region will likely require more intensive management in the foreseeable future. Thus, understanding of the patterns of adaptive genetic variation related to climate is crucial to predict how species and populations will be affected by climate change, and to develop effective management plans and conservation strategies (Keenan, 2015; Aitken & Bemmels, 2016).

Climate adaptation management strategies include assisted gene flow (Aitken & Whitlock, 2013) through the controlled migration of seed material. Direct seeding approaches with locally collected seeds in forest tree species often report little success (Ceccon et al., 2016), with low germination rates (Merritt & Dixon, 2011). For example, in woodland restoration areas in SWWA, field surveys after seed sowing of seven *Eucalyptus* species reported a germination success of <1% (Hallett et al., 2014). Overall seeding success is strongly influenced by environmental conditions (abiotic factors such as temperature and rainfall conditions (Walck et al., 2011; Rosbakh & Poschlod, 2015), and species-specific evolutionary traits (e.g., seed morphology, dormancy, and local adaptation patterns; Donohue et al., 2010; Meineri et al., 2013; Fernandez-Pascual et al., 2020). Populations from widespread species may be locally adapted to their specific climate niches (Anderson & Wadgymar 2020; Vesela et al., 2020). Consequently, they may demonstrate an advantage through seed germination at sites where climate is shifting towards conditions similar to their home site. Seed sourcing for assisted gene migration can be improved by assessing current geographic patterns of adaptive capacity to climate (Hoffman et al., 2015), providing information to implement seed sourcing from populations in the direction of climate change to enhance adaptation to future climate (Prober et al., 2015).

A foremost question related to contemporary seed biology research is how climate variables might disturb the overall fitness and phenological traits such as germination (Cochrane et al., 2020). Temperature is a major driver in the control of seed germination (Probert, 2000), which rarely occurs outside the range of temperatures for normal physiological functions, and for which species are evolutionarily adapted. Seeds of many plant species generally germinate over a range of temperatures, and within this range, there will be an optimal temperature (or temperatures), below and above which germination might be delayed or deeply suppressed (Mott & Groves, 1981).

The main objectives of this study were to identify inter- and intraspecific variation for the thermal thresholds, including optimal temperature, for germination in two SWWA eucalypt foundation tree species, *Eucalyptus marginata* (jarrah) and *Corymbia calophylla* (marri). Most Western Australian eucalypt species are thought to exhibit highly variable germination responses to environmental factors (Bell et al., 1993; Bell et al., 1995). In the Mediterranean-

type climate of SWWA, species are considered to have low-temperature optima for germination that coincides with a period of highest moisture availability. However, resprouting species appear to be less sensitive to temperature conditions for germination than obligate seeders in the region (Bell et al., 1995). Variability in seedling densities has been observed between the co-dominant, overstorey species jarrah and marri, as part of restoration sites monitoring in the Northern Jarrah Forest, in SWWA (Koch, 2007; Norman & Koch, 2009). Maintaining viable natural populations and achieving restoration targets under forestry, mining and ecological restoration requires understanding the causes of variability in seedling establishment.

Species within the same habitat, and populations from contrasting climate-origins, may have different germination requirements. This was explored in this study through sampling populations from wide-ranging climate-origins for both jarrah and marri testing for intraspecific variation in germination temperature response. This study explicitly quantified how the germination niche (a) differs between species, (b) varies among populations, and (c) relates to climatic temperature of origin. Specifically, the hypotheses were 1) the germination niche will differ between species, where marri will have higher thermal limits than jarrah which has limited recruitment in the northern (warm) forests (Koch, 2007; Norman & Koch, 2009), and 2) germination responses will vary between populations where maximum temperatures will be positively correlated with germination thermal limits (following genetic adaptation; Ahrens et al, 2019a). Assessing the optimal temperature niche for germination can inform on the vulnerability from species, and populations within species, to potential climate change (Cochrane et al., 2014; Cochrane, 2016), and guide seed sourcing for restoration practices (Prober et al., 2015; Hoffman et al., 2015).

3.2. Methods

3.2.1. Study species

Eucalyptus marginata Donn ex. Sm. (jarrah) and *Corymbia calophylla* (R. Br.) K.D. Hill & L.A.S. Johnson (marri) are co-occurring endemic foundation tree species in the forests and woodlands of SWWA. They are found extensively across climate gradients, occurring at sites with mean maximum temperatures of the warmest month (T_{MAX}) varying from 24°C to 34°C and mean annual rainfall ranging (P_{MA}) from 350mm to 1500mm (Figure 3.1). Jarrah and marri trees are primarily pollinated by animals, including birds, mammals and insects (Vesk et al., 2010). Both species are not obligate seeders, which means they can regenerate through resprouting even after severe heatwave or fire-induced damage (Matusick et al., 2013).

Jarrah flower buds start to occur around the beginning of December (early summer) and flowering happens from spring to summer (between September and the following December). Jarrah recruitment in nature occurs when seeds are released from mature and dried capsules in the upper canopy (Cremer, 1965; Christensen, 1971), usually between the third December and March (summer to autumn). Individual adult trees live through years of heavy seed production, usually once every four to seven years (Abbott & Loneragan, 1986; Florence, 2004). The full jarrah reproductive cycle typically takes 24–27 months (Johnstone & Kirby, 1992). Jarrah seeds have been previously described as viable if between 4 mm (Cargill et al., 2019) and 4.5 mm (Abbott & Loneragan, 1986). The morphology of jarrah seeds and chaff is similar, but normally separated by size (chaff fragments are usually smaller than four mm) and colour (seeds are black, chaff is usually dark-red; Abbott & Loneragan, 1986; Cargill et al., 2019). Jarrah's germination in nature is usually influenced by the soil water potential, temperature and surface characteristics, atmospheric humidity, allelopathic inhibition, light, and pathogens (Battaglia & Reid, 1993; Cargill et al., 2019). Jarrah seed germination in nature has been reported as highly variable, between 1.6% (Gill et al., 1992) and 90% (Abbott & Loneragan, 1986); while emergence from seed in restoration sites has also been reported as very variable, from 2 to 38% (McChesney et al., 1995). Marri flowers initiate in August (late winter), flowering starts in early January (mid-summer), capsules are developed between March and December, and seed are released during the following January (summer;

Johnstone & Kirkby, 1992). Marri seed is usually 12 - 13mm long and easy to distinguish from the smaller chaff (Boland et al., 1980; Johnstone & Kirby, 1992).

3.2.2 Seed collection

Seeds from 120 individual mature trees from 12 natural populations were collected for each species across their co-occurring geographic range (Figure 3.1; Table 3.1) during autumn-winter of 2019 (from March to August). Geographic coordinates were recorded for all sampled individuals from each population using a handheld GPS device (Magellan eXplorist 310) (Table 3.1 shows a central point for each population). The sampling, which covered an approximate area of 80,000 km^2, included independent (>50 km separation) and replicate (across similar climate of origin) populations over both temperature and precipitation gradients. Mature fruits from each population were collected from ten trees at least 100m apart and kept in silica gel until placed at room temperature. Seed release occurs when the capsules dry and valves open. The seeds were extracted by shaking them in closed sieves (Figure 3.2), separating most chaff from seeds. After extraction, seeds were stored at 3°C until use. Seeds found to be damaged or predated by insect larvae were discarded. Whole seeds that were plump with no signs of discolouration were assumed to be viable.

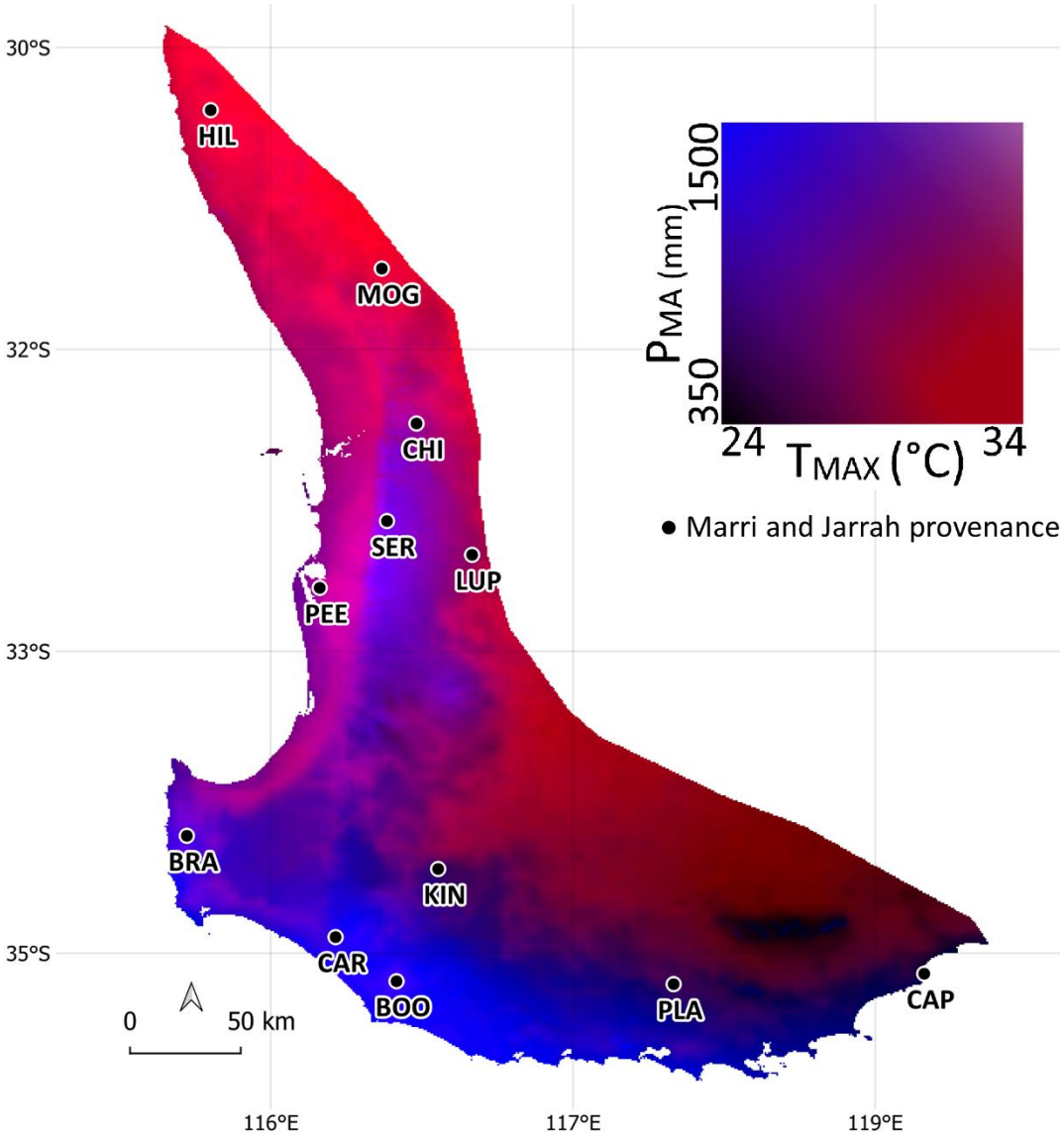


Figure 3.1. Sampled wild populations from marri and jarrah in South-west Western Australia (black dots). Two climate gradients are shown overlapped with colour blending for the species distribution area: maximum temperature of the warmest month (°C; T_{MAX} in the red display) and mean annual precipitation (mm; P_{MA} in the blue display). Bio-climatic layers from worldclim.org (Fick & Hijmans, 2017).

Table 3.1. The study's locations and climate-origin of each sampled wild marri and jarrah population along with four climate gradients for the species distribution area: minimum temperature of coldest month (°C; T_{MIN}) mean annual temperature (°C; T_{MA}), maximum temperature of the warmest month (°C; T_{MAX}) and mean annual precipitation (mm; P_{MA}). Bio-climatic layers from worldclim.org (Fick & Hijmans, 2017).

Population	Code	Latitude	Longitude	Climate of origin	T_{MIN} (°C)	T_{MA} (°C)	T_{MAX} (°C)	P_{MA} (mm)
Hill River	HIL	-30.164	115.199	Warm and dry	8.4	19.6	33.9	558
Mogumber	MOG	-31.098	116.050	Warm and dry	6.1	18.7	33.5	554
Lupton	LUP	-32.529	116.500	Warm and dry	4.3	15.7	30.1	688
Chidlow	CHI	-31.862	116.226	Warm and wet	6.1	16.8	30.7	883
Serpentine	SER	-32.345	116.072	Warm and wet	6.4	16.3	29.7	1161
Peel Inlet	PEE	-32.692	115.710	Warm and wet	8.3	18.1	32.0	894
Bramley	BRA	-33.903	115.087	Cool and wet	8.8	16.6	27.6	1109
Carey	CAR	-34.425	115.822	Cool and wet	7. 6	15.7	26.1	1164
Boorara	BOO	-34.612	116.206	Cool and wet	6.9	15.5	26.9	1194
Kingston	KIN	-34.082	116.337	Cool and dry	5.1	14.5	26.4	724
Cape Riche	CAP	-34.601	118.742	Cool and dry	6.3	16.1	25.7	585
Plantagenet	PLA	-34.653	117.499	Cool and dry	6.5	14.9	26.5	660

Figure 3.2. Marri (a) and jarrah (b) fruits and seeds, with closed brass sieve used for seed extraction and separation from the chaff. Seeds sown in 35mm plastic Petri dishes on 0.75% w/v water agar for marri (c) and jarrah (d).

3.2.3. Experiment 1: Temperature gradient plate

The germination response for jarrah and marri was first examined simultaneously over a gradient of alternating and constant temperatures using a bi-directional temperature gradient plate (TGP; Model GRD1, Grant Instruments, Cambridge, UK; temperature range: +5 to 45°C). This first experiment was a single TGP run as a screening process to investigate temperature values where germination is optimal and two axes of the thermal niche for germination – minimum and maximum thermal limits. The TGP allows the description of fuller spectrums of responses to temperature, by qualifying thresholds more broadly than step by step temperature treatments delivered by incubators. Two populations were used for this trial, representing contrasting home-site temperature and rainfall regimes: a cool temperature, high rainfall southern population, Boorara (BOO), and a warm, dry northern population, Hill River (HIL). Seeds were sown in 35mm plastic Petri dishes on 0.75% w/v water agar. Seed numbers per dish were 10, for jarrah, and 5, for marri. The reason for this difference is the considerable variation in seed size between the two species (Figure 3.2). Each species-population was exposed to 49 temperature combinations, between 5 and 40°C, with a 12-h photoperiod (all cells were exposed to a daily 12h photoperiod). This method simultaneously delivers 196 (the total numbers of individual cells) different temperature combinations (constant and alternating). i.e., 5 to 40°C day-time temperatures combined with 5 to 40°C night-time temperatures. The moisture level was constant and equal for all plates (plates were sealed with parafilm to minimise moisture loss by evaporation). Temperature conditions cannot be replicated during an alternating bi-directional run on the TGP, so each temperature condition was represented by only one dish. Seed germination was recorded three times weekly, and germinated seeds showing a radicle at least double the length of the seed were removed. At the end of the experiment, non-germinated seeds with a hard, white endosperm were considered as viable; empty (i.e., non-viable) seeds were not considered in the original count after a cut-test. The germination test run ended when no new germinants were recorded for five consecutive days.

3.2.4. Experiment 2: Cabinet incubators

The second experiment was conducted using germination cabinet incubators (TRI-145-1-SD; Thermoline Australia Pty Ltd.; NSW, Australia) after assessing minimum and maximum limits for the thermal niche germination with the TGP. The 12 sampled populations from each species were tested under five constant temperatures: 9, 15, 21, 27 and 33°C, with a 12h day/12h night photoperiod. Seeds were sown in 100mm plastic Petri dishes on 0.75% w/v water agar, each dish containing the same number of seeds, ten, for both species, to avoid confounding effects in the data analyses. A total of three independent runs were performed. In each run, populations were represented by three dishes for each temperature treatment (30 seeds). A total of 90 seeds from each species-population was tested for each constant temperature after the three runs, for a species total of 1,080 seeds (12 x 90). The moisture level was constant and equal for all sowed plates (plates were sealed with parafilm to minimise moisture loss by evaporation). Seed germination was recorded three times per week, with germinated seeds showing a radicle at least double the length of the seed removed. At the end of the experiment, non-germinated seeds were checked for viability with a cut-test and empty seeds were removed from the original count. Each run ended when no new germinants were recorded for five consecutive days.

3.2.5. Data analysis and visualisation

Experiment 1 data was visualised through contour plots showing percentage germination on the bi-directional TGP. Contour plots were generated in R v4.1.1 (R Core Development Team, 2020) using the function '*levelplot'* from package *lattice* v0.20-45 (Sarkar, 2008). Individual plots for each species-population tested were created: jarrah-HIL, jarrah-BOO, marri-HIL and marri-BOO. Maximum percentage germination (Gmax) and time to 50% germination (T50) for each species were calculated from the experiment 2 data. Both Gmax and T50 were calculated in R with the function '*summaryBy'* from *doBy* v 4.6.11 package (Højsgaard et al., 2009). The count data per day were transformed into time-to-event data (Onofri et al., 2014), and a three-parameter log-logistic function was applied (*LL.3*), setting the argument "type" to "*event*", which returned the following parameters: proportion of seeds that germinated

during the experiment out of the total number of seeds present at the beginning of the experiment, and the optimal temperature for germination (Td estimate) along with the upper and lower temperature for germination (Tmin and Tmax, respectively). The LL.3 model failed at the population level but was successfully run for each species separately. To define the response curves for each species-populations, and identify the optimal mean temperature threshold conditions for germination, the semi-parametric method of the dose-response model (drm) was applied, using the function *drm* of the '*drc*' package (Ritz et al., 2015), which takes into account the characteristic features of germination curves: the repeated observations of the seeds over time, the observation intervals, and the lack of interdependence between proportions and variance homogeneity (Ritz et al., 2013). The *drm* function was fitted with a continuous curve following the decline in germination after Topt to calculate the temperature value to achieve 85, 50 and 15% of germinated seeds (ED85, ED50 and ED15, respectively), along with 95% confidence intervals. The drm model was applied on all individual populations separately, and groups of populations by climate-origin within species.

To explore how germination temperature has adapted to climate-origin, germination metrics for each marri and jarrah population were related to the climate at the seed source location. Current climate (1970-2000) temperature and precipitation layers for sampled populations were extracted from WorldClim v2 database (Fick & Hijmans, 2017) with a spatial resolution of approximately 1 km^2, including annual mean temperature (T_{MA}), maximum temperature of the warmest month (T_{MAX}), annual precipitation (P_{MA}), and precipitation of warmest quarter (P_{WQ}). Three (T_{MAX}, P_{MA}, and P_{WQ}) of these represent variables which were shown to drive patterns of genetic adaptation in jarrah (Chapter 2); similarly, T_{MAX} and P_{MA} were also shown to be associated with genomic variation in marri (Ahrens et al., 2019a). Additionally, annual mean temperature and P_{MA} are known to be important for local adaptation in other eucalypt species (Queirós et al., 2020; Rocha et al., 2020). A linear model with ED50 as the response variable was used to test for the significance of each climate predictor (T_{MA}, T_{MAX}, P_{MA} and P_{WQ}) separately. Significant relationships ($p < 0.05$) were visualised using *ggplot2* in R (Wickham, 2009). Monthly climate variables to define annual germination windows under current and future climate (2061 – 2080) were also extracted from WorldClim v2: monthly

total precipitation (P_{MM}) and monthly average maximum temperature (T_{MMAX}). Future climate data were modelled from GCM projections of BCC-CSM2-MR (Wu et al., 2019).

3.3. Results

3.3.1. Experiment 1: Temperature gradient plate

The TGP revealed high levels of seed viability (non-viable seeds <0.5% for jarrah, <1% for marri) with germination completed in 5 weeks. There was great variation in the temperature dimensions for germination and its timing across the two species. The contour plots illustrate the species-specific nature of seed responses to temperature (Figure 3.5). The time to first germination in jarrah was 7 days, while for marri, the first germination was recorded after 2 days; marri recorded no new germinants from day 22 onwards, while jarrah germinated until day 30.

Jarrah showed an overall very low and constrained temperature window for germination. The total mean proportion of germination was 19%; the maximum proportion of germination was moderate and similar between populations: cool-wet jarrah-BOO at 66%, and warm-dry jarrah-HIL reached 53%. Although, distinct response patterns are observable between populations across the plate (Figure 3.5). Jarrah-BOO displayed an almost symmetrical shape of greater germination (25 to 66%) around the diagonal of constant temperatures in the bottom-left corner, where temperatures were lower (closer to 5°C), with very low (< 20%) or absent germination as it got further from the centre of that shape and across the rest of the plate. While for jarrah-HIL this shape slightly moved towards the top direction of the plate, where higher temperatures occurred during the night period, but with several pockets of moderate-low germination (25 - 35%) distributed randomly across the plate.

Contrastingly, marri displayed several temperature combinations where plate cells reached 100% germination, for both populations (Figure 3.5). The total mean germination proportion for marri was 64%. The warm-dry marri-HIL showed two main areas of high germination (> 80%), with no consistent pattern. However, most of these areas were inside the lower half of the plate, where the higher temperatures occurred during the diurnal phase, which might

indicate light requirement for germination. The response pattern for cool-wet marri-BOO appears to be more defined, with again two main areas for high germination, although these were roughly centred with the diagonal line of constant temperatures, and high proportions of germination occurred above and below the line. Differently from warm-dry HIL, marri-BOO showed low germination (< 25%) close to the top edge, and a pocket of 0% at the bottom right corner of the plate, where the temperature was close to 40°C.

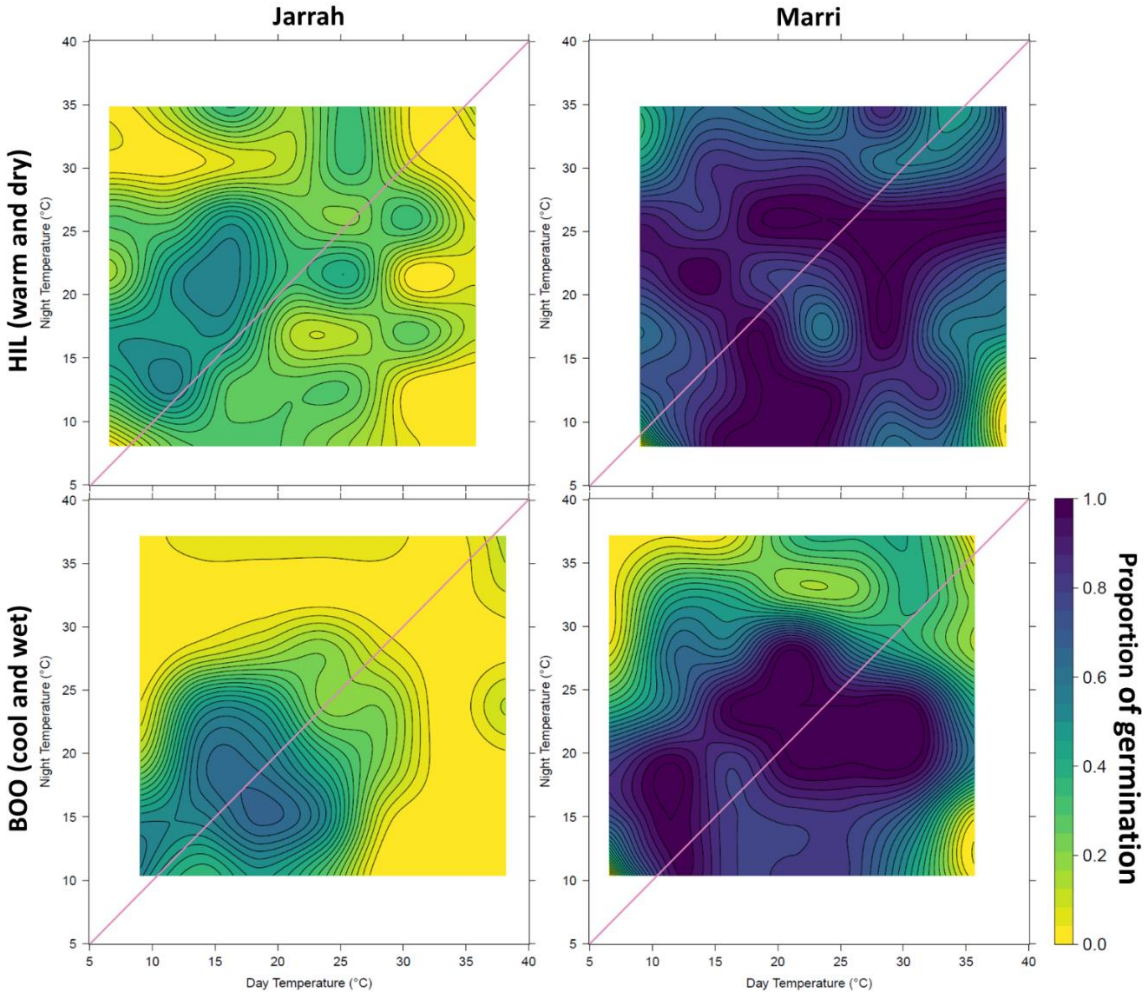


Figure 3.5. Bi-directional temperature gradient plate contour plots for seeds from jarrah and marri populations of HIL (Hill River, warm and dry) and Boorara (BOO, cool and wet). The colour gradation from dark purple (100%) to bright yellow (0%) represents decreasing percentage germination. Isopleths connect points of equal percentage germination. Constant temperatures are presented on the diagonal line from the top-right corner (maximum temperature 40°C) to the bottom-left corner of the plots (lowest temperature 5°C). All points above and below the diagonal line represent alternating temperature regimes, with the greatest amplitude at each graph's top-left and bottom-right corners. The diagonal line from the bottom left to top right corner of each plot also signifies the divide between diurnal cycles that have light during the warmer day regime (bottom right section) and dark during the warmer day regime (top left section).

3.3.2. Experiment 1: Cabinet incubators

The overall results from the TGP trial suggest that a temperature range from 9 to 33°C would be adequate to explore germination temperature response from these species for the cabinet incubators trials. The three independent runs for the cabinets lasted 5 weeks each. After each run, cut-tests found a negligible proportion of non-viable seeds (< 1% for both species), which were removed from the original count. The mean time (averaged from three runs) for first germination in jarrah was 6.5 days, and for marri was 7 days. Mean maximum germination percentage (Gmax) and mean time to 50% germination in days (T50) were calculated for marri and jarrah (n = 1,080 individuals for each species) exposed to five constant temperatures for 35 days in cabinet incubators: 9, 15, 21, 27 and 33°C (Figure 3.6). For the lowest temperature treatment (9°C), jarrah showed a high Gmax (78%), while marri recorded a lower mean value (60%); when the temperature was increased to 15°C, both species show a similar high response, around 80% (marri – 82%; jarrah – 79%). Although, at 21°C, the species were again separated; jarrah drastically decreased from 21 to 27°C, shifting from 64% to 18% Gmax, until a record low was registered at 33°C, with 14% Gmax. Marri was not indifferent to the increase in temperature, but with a smother drop and scoring mean Gmax consistently higher than jarrah, with the jump from 21 to 33°C resulting in a drop from 70% to 30% Gmax. The T50 response was, to some extent, paralleled with Gmax: at the lowest temperature (9°C), jarrah required less time to achieve 50% germination (T50 = 15.5 days), while marri only achieved 50% germination after 18.5 days; similarly to Gmax, at 15°C both species showed a similar response for T50, at around 11.5 days, and from 21°C onwards the species were separated, with jarrah scoring higher T50; interestingly, at the highest temperature (33°C), the response was again shifted and marri recorded a higher T50.

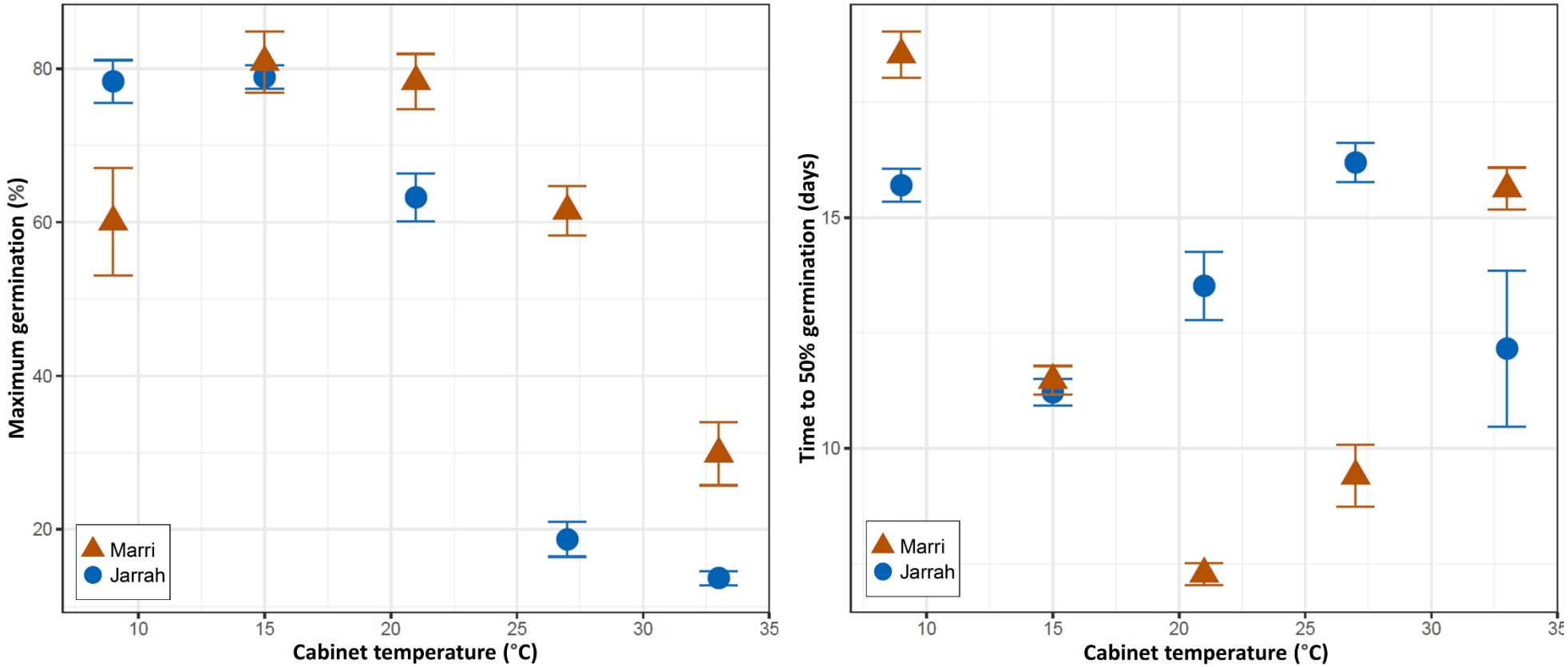


Figure 3.6. Mean maximum germination percentage (Gmax; left panel) and mean time to 50% germination in days (T50; right panel), with SE bars, for marri and jarrah exposed to constant temperatures for 35 days: 9, 15, 21, 27 and 33°C.

3.3.3. Dose response modelling

Jarrah registered an overall 48% maximum seed germination, while marri had 61% seed germination. The time-to-event modelling approach combines the cabinet germination data (Gmax and T50) to estimate the overall germination success and critical temperature parameters for each species, such as Topt, here expressed as Td estimates from the time-to-event model. The Topt for marri was higher (Td = 21.2°C) than jarrah (Td = 15.3°C). The minimum and maximum temperature threshold estimates were not reliable as the entire temperature response was not captured for each species. To investigate the germination response curve across the temperature range 9 – 33°C a log-logistic dose-response model was applied on population groups by climate-origin (warm-dry, warm-wet, cool-dry and cool-wet), and on individual populations within species. For groups of climate-origins, the ED85, ED50 and ED15 parameters were calculated (Table 3.2), which relate to the temperature dose necessary for an outcome of 85, 50 and 15% germination proportion, respectively. All the ED parameters for grouped populations of marri scored higher temperature predictions comparatively to the jarrah counterparts. The highest ED50 was recorded for marri warm-dry climate (33°C), followed by the warm-wet (32.1°C), both higher than the estimated ED50 for the cool climates (29.4°C); for jarrah, ED50 shows less variation between climate groups (<1 °C), ranging from 23.4°C (cool-dry) to 24.4°C (warm-dry). Noteworthy, both warm climate groups had a >8.5°C ED50 magnitude difference with respective counterparts between species.

Table 3.2. Dose response model ED85, ED50 and ED15 estimates for climate-origin population groups within marri and jarrah.

	DRM prediction	**85% germination**	**50% germination**	**15% germination**
Species	**Climate of origin**	**ED85 estimate (SE)**	**ED50 estimate (SE)**	**ED15 estimate (SE)**
Marri	Warm and dry	31.6 (1.7)	33.0 (0.3)	34.4 (1.8)
	Warm and wet	28.3 (1.5)	32.1 (0.7)	36.0 (1.2)
	Cool and wet	24.5 (0.7)	29.4 (0.5)	34.3 (0.7)
	Cool and dry	25.4 (0.8)	29.4 (0.5)	33.4 (0.8)
Jarrah	Warm and dry	19.9 (0.7)	24.4 (0.5)	29.9 (0.9)
	Warm and wet	20.2 (0.6)	23.5 (0.4)	27.3 (0.7)
	Cool and wet	19.9 (0.6)	23.7 (0.3)	28.4 (0.7)
	Cool and dry	20.5 (0.4)	23.4 (0.3)	26.8 (0.5)

Individual response curves from both species were plotted for visualisation of population responses between and within species (Figure 3.7). For most warm populations, jarrah recorded a higher proportion of germination than marri for the lower tier of temperatures (9 and 15°C), with a swap between species occurring around 21°C, with germination depressed onwards for both species and marri keeping a higher germination proportion for the higher temperatures. The exceptions for this pattern are HIL (warm-dry) and PEE (warm-wet), for which marri scores higher proportion of germination across the whole range of temperature dose. The same three warm populations for both species (MOG, SER and LUP) recorded an extremely low germination response (< 5%) for the highest temperature treatment (33°C). On the cool climate populations (Figure 3.7), the variation between jarrah and marri was smaller, with the dose response curves following a very similar shape for most of the counterparts, with very close gaps between them, or even overlapping (PLA, 9 – 15°C). Jarrah cold-climate populations showed a higher germination response than marri at lower temperatures (9 and 15°C) for two populations only (BRA and KIN), while marri cold populations consistently recorded higher germination proportion than jarrah for higher temperatures (> 21°C), similarly to the patterns found across warm populations.

The temperature threshold for germination was estimated as ED50, which provides a meaningful comparison among populations and species for the temperature upper limit of germination. Jarrah scored lower ED50 compared to marri in all co-occurring populations. While the jarrah population with the warmest climate-origin (HIL, T_{MAX} = 33.9°C) recorded the highest ED50 for jarrah (27.7 ± 1.1°C), the two most temperature-sensitive jarrah populations also belong to the warm climate-origin (MOG, T_{MAX} = 33.5°C, ED50 = 21.3 ± 0.3°C; and CHI, T_{MAX} = 30.7°C, ED50 = 21.9 ± 0.5°C). Marri showed a much clearer signature of climate-origin with two cool-climate marri populations showing the lowest ED50, PLA and BRA (27.1 ± 0.8°C and 27.8 ± 0.8°C, respectively) and, similar to jarrah, the warmest population HIL recorded the highest ED50 (35.9 ± 1.4°C).

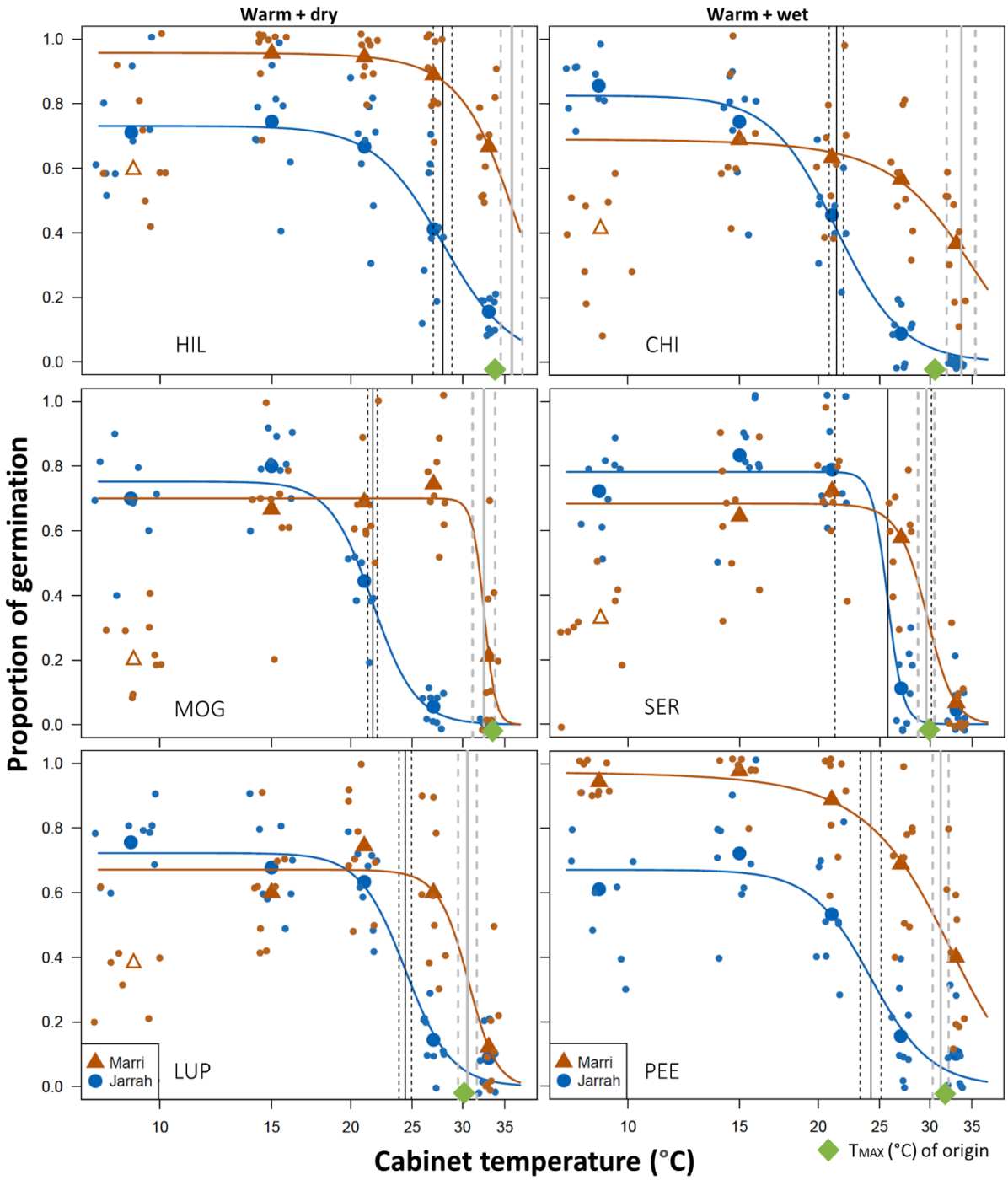


Figure 3.7. Germination response curves for marri and jarrah (warm-origin populations) exposed to constant temperatures for 35 days: 9, 15, 21, 27 and 33°C. Each small data point is the proportion of germination from individual dishes, bigger data points are the population mean under each temperature dose. Vertical lines denote ED50 (± SE in dashed lines) for each marri (grey) and jarrah (black) population. Green diamond marks the mean maximum temperature of the warmest month (T_{MAX}, °C) of origin foreach population. Empty triangles denote marri populations for which mean proportion of germination at 9°C was dropped to improve the model fit. (See Table 3.2 for population details).

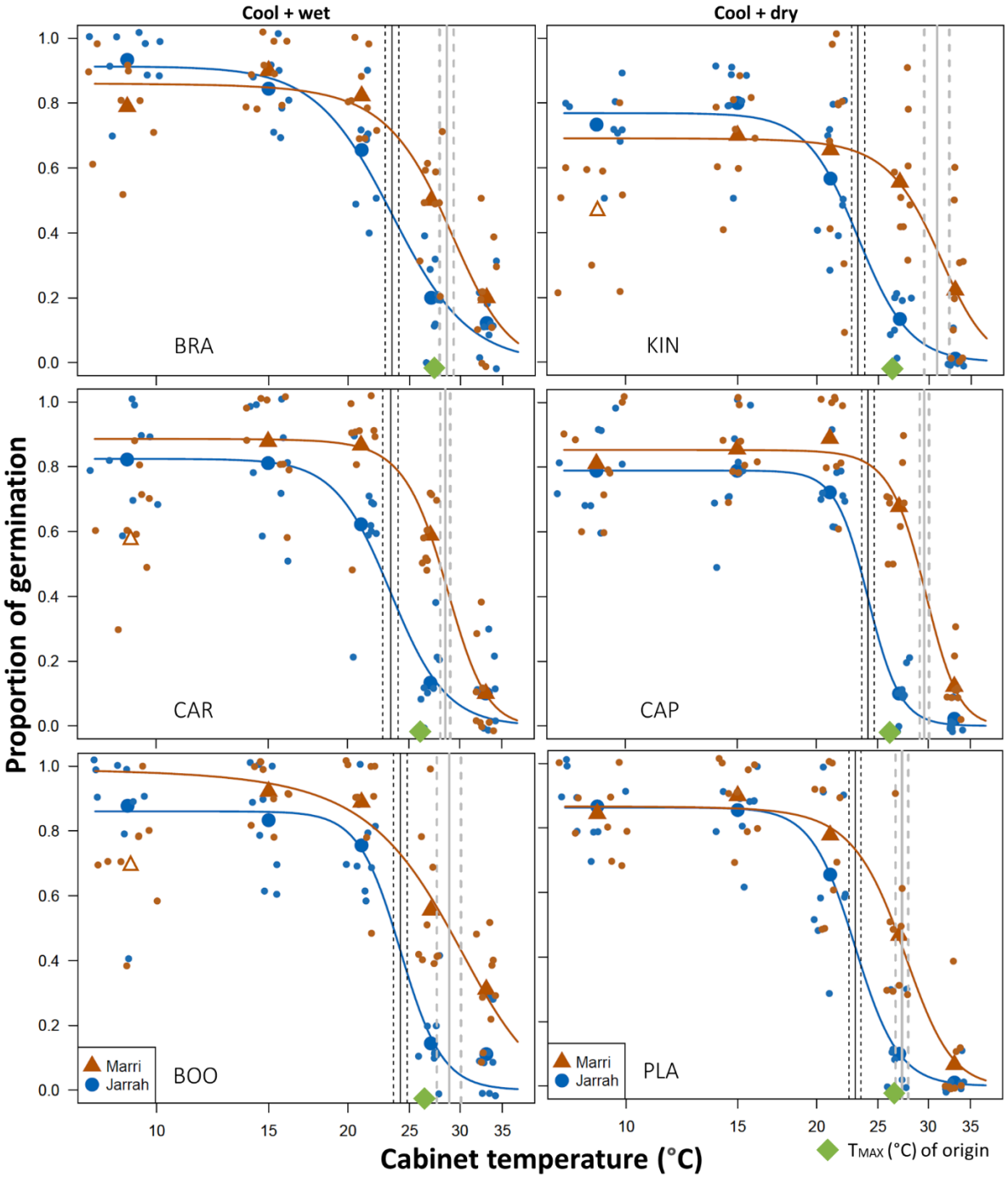


Figure 3.7 (continued). Germination response curves for marri and jarrah (cool-origin populations) exposed to constant temperatures for 35 days: 9, 15, 21, 27 and 33°C. Each small data point is the proportion of germination from individual dishes, bigger data points are the population mean under each temperature dose. Vertical lines denote ED50 (± SE in dashed lines) for each marri (grey) and jarrah (black) population. Green diamond marks the mean maximum temperature of the warmest month (T_{MAX}, °C) of origin foreach population. Empty triangles denote marri populations for which mean proportion of germination at 9°C was dropped to improve the model fit. (See Table 3.2 for population details).

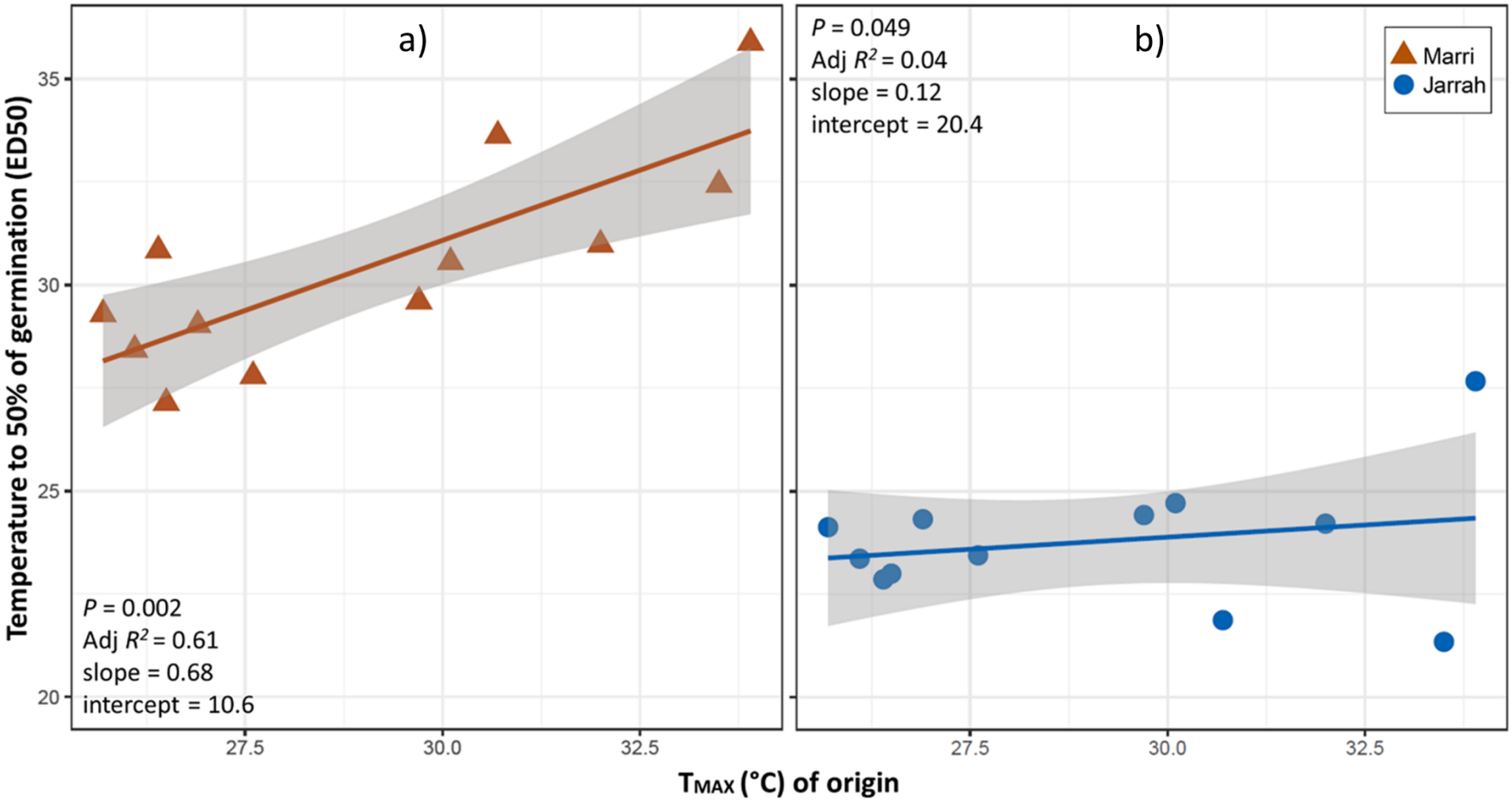


Figure 3.8. Correlation scatter plots of the temperature required to reach 50% germination (ED50) against mean maximum temperature of the warmest month (T_{MAX}, °C) of origin for each marri (a) and jarrah (b) population.

The thermal threshold of germination (ED50) was significantly predicted by T_{MAX} of origin for marri and for jarrah populations ($p = 0.002$, $R^2 = 0.61$; $p = 0.049$, $R^2 = 0.04$, respectively) (Figure 3.8). Marri showed a strong positive correlation where ED50 increased 0.68°C per degree Celsius in T_{MAX}, indicative of local adaptation. Jarrah populations showed a weak relationship with a relatively flat slope (0.12) between the T_{MAX} and ED50. T_{MAX} for sampled marri populations ranged from 25.7°C (CAP) to 33.9°C (HIL). Warm-origin marri populations displayed overlapping ED50 estimates, or slightly lower (<0.3°C magnitude), to respective T_{MAX}; noticeable exceptions are HIL (T_{MAX} = 33.9°C) and CHI (T_{MAX} =30.7°C), with higher ED50 of 35.8°C and 33.6°C, respectively. Cool origin marri populations showed higher ED50 than respective T_{MAX}, with the largest magnitude found for KIN (+4.5°C). All jarrah populations display ED50 values lower than respective T_{MAX} of origin, however the difference magnitude was greater for warm origin (>5°C) compared to cool origin populations (<4°C). Other climate metrics (T_{MA}, P_{MA} and P_{WQ}) were not significant predictors of ED50 among populations ($P > 0.05$).

3.4. Discussion

Climate change is leading to forest dieback events and threatens the recruitment of natural forests. Investigating the temperature constraints for germination can provide information on the vulnerability of species, and populations within species, to potential climate warming and guide seed sourcing for restoration and forest management practices. This study developed a multi-species comparative framework, testing key hypotheses to inform the fundamental understanding of the germination response of tree species to temperature in MTC forests. A major finding was that temperature response of germination differed significantly among species, where jarrah had a lower Topt and ED50 than marri. The thermal limit from germination also differed among populations within both species, however only marri showed strong correlation with temperature of origin. The vulnerability and adaptive capacity to climate change is discussed in the context of the temperature variability and window of time for germination.

3.4.1. Germination range

Both study species germinated over a wide range of temperatures (c. 10 to 30°C). Although the TGP results allow for only limited quantification, it is still noteworthy that marri showed requirements for fluctuating temperatures for optimal germination in the TGP (c. 21/26°C), while germination of jarrah seeds was enhanced with more constant cool temperatures (c. 15/18°C). Minimum germination temperatures were not well defined especially for jarrah which had high levels of germination at 9°C (Gmax = 78%), although the rate of germination was reduced (T50 = 15.5 days). Eucalypts from temperate climates such as MTC commonly germinate over wide temperature ranges, while extensive seed dormancy is more often observed for species from arid zones. For example, for species from temperate regions such as *Corymbia maculata* and *E. resinifira*, the optimum temperate niche was reported as 13 to 28°C (Grant, 1997); while in *E. salmonophloia,* an endemic from central arid zones of Western Australia, the optimum germination temperature niche is reportedly very narrow (Yates et al., 1996), with highest germination rates and maximum germination between 20 to 25°C, and at either side of the optimum range, germination was greatly depressed. Marri germination scores declined at 9°C in both percentage and rate (Gmax = 60%; T50 X days). Maximum germination achieved for both marri and jarrah were very similar (marri – 82%; jarrah – 79%) and occurred at the same temperature treatment (15°C), under which the time to achieve 50% germination was also the most similar between species (T50 = 11.5 days). The upper edge of germination (ED85 temperature to achieve 85% germination) were higher and more variable in marri (24.5°C to 31.6°C) than the jarrah (19.9°C to 20.5°C). Jarrah showed a much lower and narrow germination niche (<9°C to 20°C) than marri (9°C to 27°C). A rise in germination with increasing temperatures, as already demonstrated in cooler climate regions (Mondoni et al., 2012; Fernández-Pascual et al., 2020), may be expected until threshold temperatures are exceeded and when germination would constrain recruitment.

3.4.2. Current conditions for germination

Germination in marri and jarrah was cued by cooler temperatures. For plants with transient seed banks, regular and reliable germination cues are vital in maintaining populations and communities (Bewley & Halmer, 2006; Moreira et al., 2010; Mackenzie et al., 2016). These cues include, but are not limited to, specific levels of light, moisture, and temperature. Seeds

will not germinate if placed in a set of unfavourable water conditions, or when the temperature is too high or too low. In Mediterranean-type climates, germination is generally delayed during hot and dry periods, when seedlings would be vulnerable to desiccation. Germination is instead cued by conditions that are most suitable. for seeding establishment, such as the winter rainfall period starting in late autumn and continuing through to early spring where soil moisture levels would be optimal (Doussi & Thanos, 2002; Cristaudo et al., 2019). The present study supports this finding with high levels of germination found for both species in cool (15°C) conditions.

Optimum temperature response of germination differed among species. Temperature is critical to define the time and place for a seed to germinate (Gresta et al., 2010; Baskin & Baskin, 2014), and seasonal temperature variations are key determinants of seedling establishment (Humphries et al., 2018; Yi et al., 2019). Changes in the mean temperatures, and the frequency and intensity of temperature extremes (e.g., heatwaves) can have a powerful impact on the achievement of plants' life-cycle events. Jarrah showed a lower Topt, with higher levels of germination at 9°C compared to marri (78% to 60%, respectively). Optimizing germination during cool temperature conditions is a key risk-avoidance strategy against environmental stressors, as it increases the chance of seedling survival under longer periods of favourable conditions such as lower evaporation rates in the soil (Duncan et al., 2019). In a previous study of six *Eucalyptus* species from dry and wet sclerophyll forests, Rawal et al., (2015) showed that under constant and adequate moisture conditions, all species expressed the highest germination success under cool and mild temperatures. A similar result was reported for *E. blakelyi* (sub-humid with hot summers and no dry season climate origin), which expressed optimal germination success at milder temperatures from 15 to 25°C (Li et al., 2003). Early germination may improve plant fitness but does not ensure effective establishment (Verdú & Traveset, 2005), and only one species (marri) in the present study was predicted to maintain germination rates. Species that experience the same seasonal temperatures may still have different recruitment rates due to the sensitivity of seeds to available soil moisture (Cochrane et al., 2014), and *Eucalyptus* species germination are differently affected by water availability (Emery & Collette, 2021). Measuring responses to thermal extremes can be easily underestimated, as seeds under natural conditions experience multiple global change drivers simultaneously, a factor which may erode their flexibility

(Valladares et al., 2007). Although marri showed stronger thermal tolerance, rising temperatures will also be accompanied by substantial declines in winter and spring (and annual) rainfall (Hope et al., 2015). Moisture is a significant niche determinant, with heat stress tolerance during seedling germination and emergence complexly linked to water availability. Changing environmental conditions will set limits on germination and seedling establishment but little is known of how these will impact on endemic species of the region. Future work with co-occurring species should include a moisture variable to thoroughly address the full extent of the species adaptation to the temperature-moisture interaction.

Marri is currently limited by available rainfall during summer, while jarrah is constrained by both rainfall and temperature during the warm season (Table S3.1). The window of germination can be determined when soil moisture and temperatures are optimal. Both jarrah and marri do occur over the same latitudinal gradient in SWWA (from 30°S to 36°S) where maximum temperature of the warmest month (T_{MAX}) and minimum temperature of the coldest month (T_{MIN}) vary considerably (T_{MAX} from 24 to 34°C; T_{MIN} from 3 to 10°C). Germination in summer would be prevented by lack of rainfall, and high temperatures in the northern (warm) forests. Soil moisture in winter would be adequate for germination, however minimum temperatures may inhibit germination (Jaganathan et al., 2015). As such, the optimal conditions for germination may be late Autumn, with early rainfall, if not early spring when temperatures are likewise optimal. This window of germination varies by species and populations across the range (Table S3.1). Marri's current germination window across its range seems to be mostly limited by decreased rainfall (P_{MM} < 30mm; Abbott, 1984) during summer (from December to March). Overall, northern warm climate-origin marri populations have a shorter annual germination window (6-7 months) compared to southern cool-origin populations (8-9 months), which follows the expected north-south decreased rainfall gradient in SWWA, even within the same season. Similarly, jarrah's current germination window excludes the summer season, although it is also limited by current monthly maximum temperatures as well, even for months when rainfall would be appropriate. While cool-origin jarrah populations have a similar germination window of eight months, the window of time for germination is highly variable among jarrah populations from warm-origins (4 to 8 months).

3.4.3. Germination predictions under global warming

The ED50 estimates showed that marri has a much higher Topt threshold (31°C) than jarrah (23.4°C). Climate change is shifting the conditions for natural populations, and it is uncertain if they will have the ability to migrate fast enough to keep up with the current rate of climate change and be able to persist in future scenarios (Jump & Peñuelas, 2005; Corlett & Westcott, 2013). The persistence of plant populations relies mainly on *in situ* responses to environmental stressors, such as warming temperatures, which may represent physiological constraints on the species recruitment. In such context, the range of the germination niche, particularly the range of the germination temperature window, might be affected. Recruitment constraints may provide a measure of susceptibility to climate change: species that only germinate over a narrow temperature window are potentially most at risk. Temperatures outside that window might reduce opportunity for germination and seedling establishment.

Interspecific variation for germination thresholds among co-occurring species is not uncommon. Cochrane (2017) modelled observed germination data for 26 SWWA eucalypt species (including jarrah) to predict optimum germination responses (mean time to germination, germination timing and success) under current (1950–2000 averages) and future (2070) climate scenarios. Considerable interspecific variability was found for current germination thresholds. While several species showed extensive physiological tolerance for high germination temperatures, other species showed a narrow and restricted germination temperature niche than might result in germination decline under forecast conditions. Indeed, Cochrane's (2017) study found that jarrah had wide physiological tolerance for high germination temperatures and the capacity to germinate outside current and forecasted future autumn–winter wet season temperatures. It also predicted that jarrah germination levels would likely decline in the warmer summer months but increase over winter in the future. However, this work had some limitations. Firstly, the projected models were built on TGP observations, which offers limited quantitative data. While the present study also found a broad germination range for both species on TGP investigations, jarrah was found to have reduced tolerance to high temperatures when quantified in cabinets. Secondly, Cochrane's (2017) study only included a single jarrah population (similar climate-origin of the present

study's PEE). Since the present study's framework incorporated populations from contrasting climate-origin provenances, it is possible to profile population-specific temperature threshold predictions from distinct climate-origins across the species range based on ED50 estimates (Table S3.1).

Under predicted scenarios, by 2070 there will be a 1–2°C increase in summer temperatures, a 0.5–2.0°C increase in winter temperature and a 5–20% decrease in annual rainfall across SWWA (IPCC, 2021). A 2°C rise in temperature has already been shown to impact on seed responses including seed production, seed mass, seedling emergence and establishment and soil seed bank dynamics (Williams et al., 2007; Hovenden et al., 2008; Hedhly et al., 2009; Ooi et al., 2009). Changes in recruitment can lead to population decline and alter vegetation community patterns (species composition and abundance), paving the way for other ecological issues such as biological invasions (Parolo & Rossi 2008). Levels of recruitment may be affected by total germination and/or the rate of germination. Some species may increase these parameters as temperatures rise, while in others they may be reduced (Williams et al., 2007; Milbau et al., 2009). For example, in the northern MOG population the current temperature window of germination in an average season would be only 4 months in jarrah, compared to 7 months in marri. In contrast to jarrah, marri appears to have higher thermal limit for germination and more variation among populations, indicating it is less vulnerable to a warming climate. This endemic species from SWWA has demonstrated a similar variable response to warming temperatures as other woody (Cochrane, 2016, 2017) and non-woody (Dwyer & Erickson 2016) species from the region. Despite observed declines in germination under the highest temperatures across all sampled populations, marri is not expected to suffer major germination depression due to predicted future changes in temperature in SWWA. The germination predicted for 2061-2080 was the same in 6/12 populations and the modest contraction in the time for germination (-1 month) was driven by reduced rainfall rather than thermal limits of germination.

3.4.3. Adaptive capacity from co-occurring species and conservation implications

Marri's populations showed strong thermal tolerance with signatures of local adaptation in germination temperature response. Various tree species show adaptable. germination tactics to cope with environmental oscillations (Nicotra et al., 2010). This adaptability may preserve

co-existence in vegetation communities under current climates (Turcotte & Levine 2016). Similarly, flexible germination responses may allow populations to establish and persist as global temperatures rise (Clauss & Venable 2000), provided seeds can recognise and respond to germination cues and establish when optimal conditions arise (Donohue et al., 2010). The estimated ED50 for marri's populations showed high positive correlation (slope = 0.6) with a temperature variable (T_{MAX}) of origin, which is indicative of local adaptation to a temperature gradient. Since this species has high levels of gene flow and connectivity across its range (Sampson et al., 2018), marri might be well equipped to persist under intensifying warming for the next few decades.

Jarrah's temperature niche did not relate to climate of origin. Contrastingly to marri, the co-occurring jarrah showed a weak correlation between population's ED50 and a current temperature gradient. Additionally, jarrah's current germination window is predicted to decline further across its entire range with future warming (Table S3.1). Nevertheless, the furthest north population of Hill River (HIL) showed enhanced thermal limits to germination and potential adaptive capacity for the species to persist with climate warming. With recent observations of jarrah dieback caused by disease (notably *Phytophthora cinnamomi*) and impacts of extreme events, such heatwaves (Matusick, 2016, 2018), HIL may be a valuable source of thermal adaptive capacity for other regions of the northern jarrah forest. Given this population is geographically and genetically isolated (Chapter 2) natural dispersal may be limited and active management strategies such as assisted gene migration may be needed (Aitken & Bemmels, 2015).

Ex situ conservation is an essential complement to *in situ* conservation approaches (Guerrant et al., 2004) and allows genetic resources for current or future use to be captured and managed, especially when natural populations are small and/or threatened. For example, Coates et al., (2015) compared *ex situ* seed collections and three extant geographically disjunct populations of the rare SWWA endemic species *Banksia browniii* and found that a substantial proportion of gene diversity and allelic richness had been lost within few decades due to the introduced pathogen *Phytophthora cinnamomi*. Programs for *ex situ* conservation of forest species include living collections, conservation stands and seed banks. Seed banks are invaluable worldwide resources for *ex situ* genetic diversity conservation and increasingly

utilized as part of the management to protect threatened flora (Walters, 2015). Seeds for several SWWA woody species, including eucalypts, have been stored in seed banks for nearly 30 years without significant loss of viability (Crawford et al., 2007). The jarrah population of HIL showed a noteworthy thermal adaptive capacity that might be of potential conservation interest. The *ex situ* preservation of this population's germplasm might be of crucial relevance for the species conservation management under future climate change. While marri populations may have enhanced capacity to persist in the wild without active conservation management, thermal adaptation in germination (among other functional and physiological traits; Ahrens, 2019b, 2021) provides a valuable genetic resource for restoration and forestry to establish diversity and resilient forests for the future.

The window of opportunity for germination may narrow with a warming and drying climate, particularly for jarrah (Table S3.1), with later germination increasing the risk of exposure to unfavourable conditions. These shifts in germination timing will be in response to both temperature and moisture conditions. Delayed germination is an example of a diversified bet-hedging strategy (Venable 2007; Childs et al., 2010), and trade-offs between germination traits (e.g., faster but lower germination or slower but higher germination) may limit the ability of co-occurring species to adapt to a changing climate. Single-species frameworks are useful tools for predicting the future direction of a select species; nevertheless, exploring fitness strategies in a set of co-occurring species simultaneously provides unique opportunities to identify processes and patterns driving community assembly (Pearson et al., 2018), as well as the chance for species coexistence (Gremer & Venable, 2014; Huebner et al., 2018).

3.4.4. Conclusions

Jarrah has a lower and narrower germination temperature niche than marri, which is particularly concerning for the diversity and functional resilience of the northern (warm-origin) forest. This foundation tree species shows limited capacity to persist in the north range under increasing temperatures and will likely require earlier active management to counter the effects of climate change and avoid extinction locally. Marri displays greater thermal limit and adaptive capacity for the future temperature settings, and as such may not require active management in the near future.

Studies such as this one provides critical fundamental information regarding the species adaptive capacity to the pressure of warming with increasing pulse events leading to dieback events and disrupting natural recruitment processes. These unique insights support conservation and restoration efforts by determining the species that are vulnerable at the germination stage and may require targeted on-ground actions such as supplementary seeding with local material to enhance establishment rates, or assisted gene migration to promote adaptation of thermal limits (Aitken & Bemmels., 2015). The Forest Management Plan 2014–2023 for SWWA forests has provision for implementation of assisted gene migration in management strategies for response to climate change. Seed sourcing for assisted gene migration should consider the respective populations physiological tolerance and adaptive capacity to climate stressors (such as heatwaves and droughts). The present findings of variation in seed performance, harbouring potential adaptations to climate associated with temperature factors, provides an evidence base for design and implementation of such strategies.

Chapter 4.

Remotely sensed functional traits can indicate an adaptive capacity to climate change of trees from a Mediterranean-type ecosystem

Abstract

Forests of South-west Western Australia (SWWA) have experienced drought-induced mortality in recent years. Climate models predict that heatwaves and extreme droughts will become more frequent and intense. However, dominant tree species may have adaptive capacity to respond to climate change through genetically (adaptation) and environmentally (plasticity) determined variation in physiological tolerance to heat and drought extremes. Spectroscopy is a promising tool for plant phenotyping, producing quantitative estimates of functional traits, including stress response indicative of plant vulnerability to climate change. This study explored the variability of functional traits in *Corymbia calophylla* (marri), a foundation species endemic to SWWA, to estimate patterns of genetic adaptation and phenotypic plasticity. Trees from natural populations spanning marri's geographic distribution were grown in two common garden plantation sites with different climate settings (cool-wet and warm-dry). High-resolution field-based spectral measurements were collected from leaves of adult plants at both sites in two seasons (summer and autumn). Partial least squares regression analyses of full reflectance spectra highlighted differences among populations, sites, and seasons in spectral regions associated with photosynthetic pigments and water content, among other spectral traits, related to leaf condition and stress responses. Trait variation was further explored with analyses of spectral indices tailored to pigment and water absorptions. Significant differences in spectral indices between populations were identified, suggesting there is genetic variation in climatic tolerances. However, the plastic effects of site and season were much stronger than population differentiation. Marri cool-origin populations showed the capacity to optimize traits related to photosynthesis under warmer and drier site conditions. Hyperspectral remote sensing has the potential to assess plant functional status rapidly and non-destructively across climatic gradients to support conservation and tree breeding for sustainable forests under climate change.

4.1. Introduction

Contemporary climate change is a major pressure on ecosystem function and structure (Levin & Poe, 2017; Brondizio et al., 2019). There are ongoing rapid shifts in precipitation and temperature patterns globally, with some areas becoming wetter and others drier, while global surface temperature is predicted to rise by 1–4°C on average by the end of the current century. Additionally, extreme events such as heatwaves, floods and prolonged droughts have increased over recent decades in numerous regions of the world (IPCC, 2021). Regions with Mediterranean-type climates (MTC) are particularly affected by climate change. MTC are defined by reliable precipitation and temperature regimes, with predictable summer periods of low rainfall and hot temperatures, and winter periods of high rainfall and moderate temperatures. Climate changes in regions with MTC have already been observed (Rubel and Kottek, 2010), and models (CMIP6 – IPCC, 2021) predict continued drying and warming trends for these areas at alarming rates. Ecological studies in the Mediterranean basin consistently identify more frequent drought periods, together with warmer temperatures, as primary drivers for declines in oaks (*Quercus* spp.) (Corcobado et al., 2014; Gentilesca et al., 2017) and pines (*Pinus* spp.) (Camarero et al., 2018). In areas of Western Australia with MTC, the 2010-11 extreme drought and heatwave conditions resulted in large-scale forest collapses in eucalypts (*Eucalyptus* spp. and *Corymbia* spp.) (Matusick et al., 2013).

Despite rapid climate change, plant species may persist through environmental (e.g., phenotypic plasticity), ecological (e.g., migration by seed dispersal into climatically appropriate ranges) and evolutionary (e.g., genetic adaptation) strategies (Anderson et al., 2011; Christmas et al., 2016). Evolution to a specific set of environmental conditions through natural selection results in patterns of local genetic adaptation and phenotypic traits variation among populations in different environments, allowing local populations to experience higher fitness compared to non-local counterparts (Kawecki & Ebert, 2004). Standing genetic variation (allelic variation at a locus held within an existing population) is a critical factor for adaptation to environmental conditions in native habitats (Hermisson & Pennings, 2017; Exposito-Alonso et al., 2018) and may be critical for populations to persist through climatic stressors over generations in their native ranges (Hoffman & Sgrò, 2011; Sofi et al., 2021).

Variation in phenotypic expression can be found across different environments, such as along climate gradients, and indicate local genetic adaptation (Reich, 2014). By linking phenotypic traits to specific functions (e.g., defence, photosynthesis, transpiration), fitness proxies for reproductive, growth and survival performance of the species can be developed (Laughlin & Messier, 2015). Functional traits include morphological (e.g., height, leaf area and stem diameter), physiological (e.g., water potential), biochemical (e.g., nutrients and pigments concentration), and phenological (e.g., flowering time and seed dormancy) features affecting individual performance in response to environmental factors such as temperature and precipitation (Violle et al., 2007). Some plant functional traits are highly responsive to climate stressors. For example: decreased leaf water potential associated with drought (Nolan et al., 2017; Scoffoni & Sack, 2017); hindered growth, biomass production (French et al., 2017, 2019), and lower chlorophyll content associated with heatwaves (Notarnicola et al., 2021); and decreased leaf gas exchange associated with both drought and heatwave conditions (Duan et al., 2017).

Drought-stressed trees can reveal variation in water content and photosynthetic pigments (e.g., chlorophyll) (Zarco-Tejada et al., 2002; Li-Ping et al., 2006), red discoloration produced by the accumulation of other pigments such as anthocyanins and carotenoids (Barry et al., 2008) or generalized loss of photosynthetic tissues due to defoliation or necrosis. Leaf water content is a primary indicator of the plant water stress status (Egilla et al., 2005; Cui et al., 2018). Likewise, chlorophyll content is regarded as a generic indicator of plant health (Gitelson et al., 2003, Sampson et al., 2003), and methods to quantify chlorophyll content in eucalypt trees with remote sensing have been developed (Datt, 1999; Coops et al., 2003). Specific wavelength regions of the electromagnetic spectra are highly sensitive to moisture content, pigment concentration, and general vegetation health. For example, leaf pigments, such as chlorophyll, absorb light mainly the visible wavelength (Curran et al., 1991, Gamon & Surfus 1999). The absorption features of photosynthetic pigments and water have been used to develop spectral indices (SIs) as proxies for leaf functional traits, and investigate climate stress responses (Dash et al., 2017; Hawryło et al., 2018; Cogato et al., 2020).

Climatic factors such as temperature and precipitation of origin are well known to be associated with functional traits such as hydraulic capacity (Li et al., 2018), morphological (Cochrane et al., 2016) and thermoregulation (Perez & Feeley, 2020). Nevertheless, previous studies with eucalypt species (*Eucalyptus* spp. and *Corymbia* spp.) have found intraspecific variation in their capacity to respond to climatic shifts through functional traits, such as morphological and growth traits (Costa e Silva et al., 2006; Jordan et al., 2020), photosynthetic and respiratory capacities (Aspinwall et al., 2017), and hydraulic properties (Blackman et al., 2017). Such studies have shown that tree populations can display variation in functional traits, potentially through distinct genomic signatures dependent on the climate of origin, suggesting local adaptation to climate. Estimating trait variation and their adaptability and potential selection agents in trees is urgently needed to understand how the species will respond to future climate scenarios and inform predictions.

Identifying intraspecific patterns of adaptation through functional traits is challenging, particularly for species with high levels of plasticity (Shipley et al., 2016), which can mask the effects of local adaptation driven by standing genetic variation (Chevin et al., 2010). For example, measurements in wild tree populations consistently deliver intraspecific patterns of correlation between leaf morphological traits and the populations' climate of origin (Steane et al., 2017). However, this variation could indicate the presence of either adaptive genetic variation or phenotypic plasticity, i.e., the ability of a genotype to express distinct phenotypes if exposed to different environments. To minimise the effect of phenotypic plasticity and more directly assess patterns of genetic variability, common garden experiments can be performed (Mátyás, 1994). These typically use populations from different landscapes and climate origins grown in a common environment to investigate the genetic differences among source populations (Blanquart et al., 2013). A single common garden experiment cannot determine if the genetic variation between populations is adaptive to their climate of origin. To investigate the potential presence of local genetic adaptation patterns, common gardens can be replicated using reciprocal transplants, with the same set of populations across environmental gradients, facilitating the study of genotype-by-environment effects while accounting for confounding effects of the corresponding home environment (De Villemereuil et al., 2016). In the context of reciprocal transplant experiments, genetic variation is assessed as differential traits expression between populations (genotype effect; G) under a common

environment; phenotypic plasticity as traits' differences within populations under changes in the environment (environment effect; E); and local adaptation as genotype-by-environment interactions (GxE), where the variation in traits under environmental change is differentially expressed among populations.

This study investigated the patterns of local adaptation through intraspecific variation of functional traits associated with climate gradients in *Corymbia calophylla* (family Myrtaceae; R. Br. K.D. Hill and L.A.S. Johnson; marri). Marri is a foundation tree with ecological importance in the biodiversity hotspot of South-west Western Australia (SWWA). This region has a MTC and has previously experienced a prolonged period of extensive drying with an estimated reduction of 20% in rainfall from the 1970s to the present (Water Corporation, 2020), particularly in autumn and early winter. The future (2030) climate for SWWA is projected to show mean annual warming around 0.5 to 1.1 °C above the climate of 1986–2005, and increased incidences of meteorological drought (BOM & CSIRO, 2020). Investigations into the resistance and resilience to climate change of SWWA forests found that long-term declines in precipitation combined with warmer temperatures can impact canopy health and regeneration capacities in marri (Matusick et al., 2016), and will likely contribute to continued drought-induced tree mortality. Recent evidence suggests that extreme heatwaves and drought events have indeed caused the recent widespread mortality of marri trees throughout their distribution (Matusick et al., 2018), and that continuous canopy collapses can impact forest landscape structure and distribution (Andrew et al., 2016). These novel climate scenarios are affecting the native forests of SWWA, which will likely require more intensive management, such as assisted gene migration seed-sourcing through a climate-adjusted provenancing approach (Prober et al., 2015; Aitken & Bemmels, 2016). Although climate adaptation management strategies can be implemented in forest management and woodlands restoration programs (Prober et al., 2016) and seed sourcing for climate adaptation has already been incorporated into the Western Australia Forest Management Plan 2014–2023 (CCWA, 2013), more research is required before specific recommendations can be employed.

The climatic association of traits variation in marri has been assessed in previous studies. Aspinwall et al., (2017), showed enhanced photosynthetic capacity in the cool-wet

populations compared to the warm-dry provenances, and Challis et al., (2020) detected higher drought tolerance from warm-dry provenances (evaluating time to hydraulic failure through measurements of stem hydraulic conductance) relative to cool-wet counterparts. Such provenance trials in marri have demonstrated variation in functional traits associated with climates of origin, indicating potential local adaptation to climate gradients. However, these greenhouse studies have limitations, such as extrapolating the results to trees growing in native natural environments. Additionally, conventional methods for plant traits measurements like the ones used above are time-consuming and, in most cases, unable to provide real-time estimates of the measured trait.

Remote sensing methods using high-resolution spectroscopy can produce real-time quantitative estimates of biophysical (e.g., canopy cover, biomass) and biochemical properties (e.g., leaf water and chlorophyll content) in a quick, affordable, and non-destructive way (Ustin & Jacquemoud, 2020). These estimates are developed by exploring the variations in leaf optical properties (i.e., measurements of absorption and reflectance), influenced by the physical and chemical status at the leaf and canopy scales (Zhang et al., 2021). Ahrens et al., (2019b) applied this methodology to estimate the variability of functional traits across 12 provenances with different climates of origin growing in a common garden in a natural setting within the native distribution of marri. While this study confirmed trait variation between provenances associated with natural climate gradients, it only accounted for a single environment (i.e., a single experimental common garden). Therefore, the extent to which the intraspecific trait variation in marri results from local adaptation to climate is poorly demonstrated. Additionally, this study was conducted during the autumn season (wet and cool), while plants are unlikely to be stressed by climate conditions. Thus, its ability to assess climate tolerances relevant to climate change to warmer, drier conditions may have been limited.

A multi-environment approach was taken to address key knowledge gaps in understanding seasonal and site plasticity in which 15 natural populations from across the geographic distribution of marri were grown in two common garden plantation sites with different climate settings (cool-wet and warm-dry) and different seasons (autumn and summer). Leaf traits were estimated using high-resolution field-based spectral measurements collected

from hundreds of adult plants exposed to natural water availability and temperature levels under field conditions across the sites and seasons (cool-wet autumn to summer; summer cool-wet to warm-dry). The hypotheses tested were: 1) populations will differ in their functional traits (G), indicative of genetic variation; 2) functional traits will differ among sites and seasons (E), indicating plastic responses; and 3) functional traits expressed by different populations will vary differentially across sites and seasons (GxE), indicative of local adaptation. Understanding organisms' genetic variation and adaptive capacity to cope with climate can contribute to developing conservation strategies in affected landscapes (Hoffman et al., 2015; Thurman et al., 2020). Further, these results can contribute to deliver a scientific basis for adopting assisted migration and establishing conservation and management strategies.

4.2. Methods

A reciprocal transplant methodology in common gardens was applied to investigate potential local adaptation patterns and phenotypic plasticity in marri (section 4.2.1). Fifteen populations (i.e., different genotypes) from across marri's native distribution were grown in common natural environments within the species native range (section 4.2.2). Plant functional traits were assessed through remotely sensed hyperspectral data, which is highly sensitive to plant pigments and water content, common indicators of plant stress status (section 4.2.3). Two levels of statistical analysis of the spectral data were performed; each tested the three hypotheses and assessed the effects of genotype, environment, and genotype-by-environment interactions on plant traits. The first was a multivariate exploratory analysis to evaluate the overall effects of the experimental design across the full reflectance spectra and identify the spectral regions most sensitive to environment or population of origin. These results were also used to guide the selection of SIs possessing known relationships with functional traits to evaluate in the second, targeted univariate analysis.

4.2.1. Study species

Corymbia calophylla, commonly known as marri, is a foundation tree species in the forests and woodlands of SWWA. It can be found extensively across climate gradients, occurring at sites with mean annual rainfall ranging (P_{MA}) from 350mm to 1500mm, and mean maximum

temperatures of the warmest month (T_{MAX}) varying from 24°C to 34°C (Figure 4.1). There is good connectivity among populations throughout the core distribution of the marri forests and woodlands with low levels of genetic structure facilitated by insect and bird pollination (O'Brien & Krauss, 2010; Sampson et al., 2018). Marri shows some capacity to endure seasonal periods of drought through physiological traits of stomatal control (e.g., early stomatal closing during drought periods) and osmotic adjustments (e.g., leaves showing lower osmotic potential at zero turgor during warmer periods) (Szota et al., 2011). Trees can resprout from lignotubers and epicormic buds following intense drought or fire, releasing seed from large woody fruits to germinate readily given optimal temperature and water conditions in winter and spring (Matusick et al., 2016).

4.2.2. Experimental design

Two common garden plantation sites were established in 2014 at Margaret River (MR) and Mundijong (MU), Western Australia (Figure 4.1). Both sites are located within marri's main native distribution. They have different temperature and annual precipitation regimes: MR is in the southern cool-wet region (T_{MAX} = 26.5°C; P_{MA} = 1,070mm), while MU is in the northern warm-dry region (T_{MAX} = 31.8°C; P_{MA} = 813mm). Since their establishment, the sites have been permanently fenced to decrease interference from large herbivores and are managed as plantations. Further details, including seed collection, for the plantations design, can be found in Ahrens et al., (2019b).

Two separate analyses were developed, sampling trait responses to environmental variation across sites and seasons (Figure 4.1; Table 4.1). The sampling covered 15 populations, each represented by 8-10 families, and 2 to 10 individuals were sampled for each family (depending on availability within each site). The first analysis (site) sampled 13 populations from both plantation sites during the same season (summer); Margaret River was sampled during three days in mid-January 2019, and Mundijong during three days in mid-January 2020. The second analysis (season) sampled 12 populations from MR across two different seasons (summer and autumn). Margaret River summer was sampled in January 2019 and Margaret River autumn in March 2017. Both analyses cover populations from four contrasting climate combinations across the natural distribution: warm and dry (HIL, MOG, LUP, TOO; see Table 4.1 for

definitions of the population codes), warm and wet (CHI, SER, WHI, PEE), cool and dry (KIN, CAP, PLA) and cool and wet (LEN, BRA, CAR, BOO).

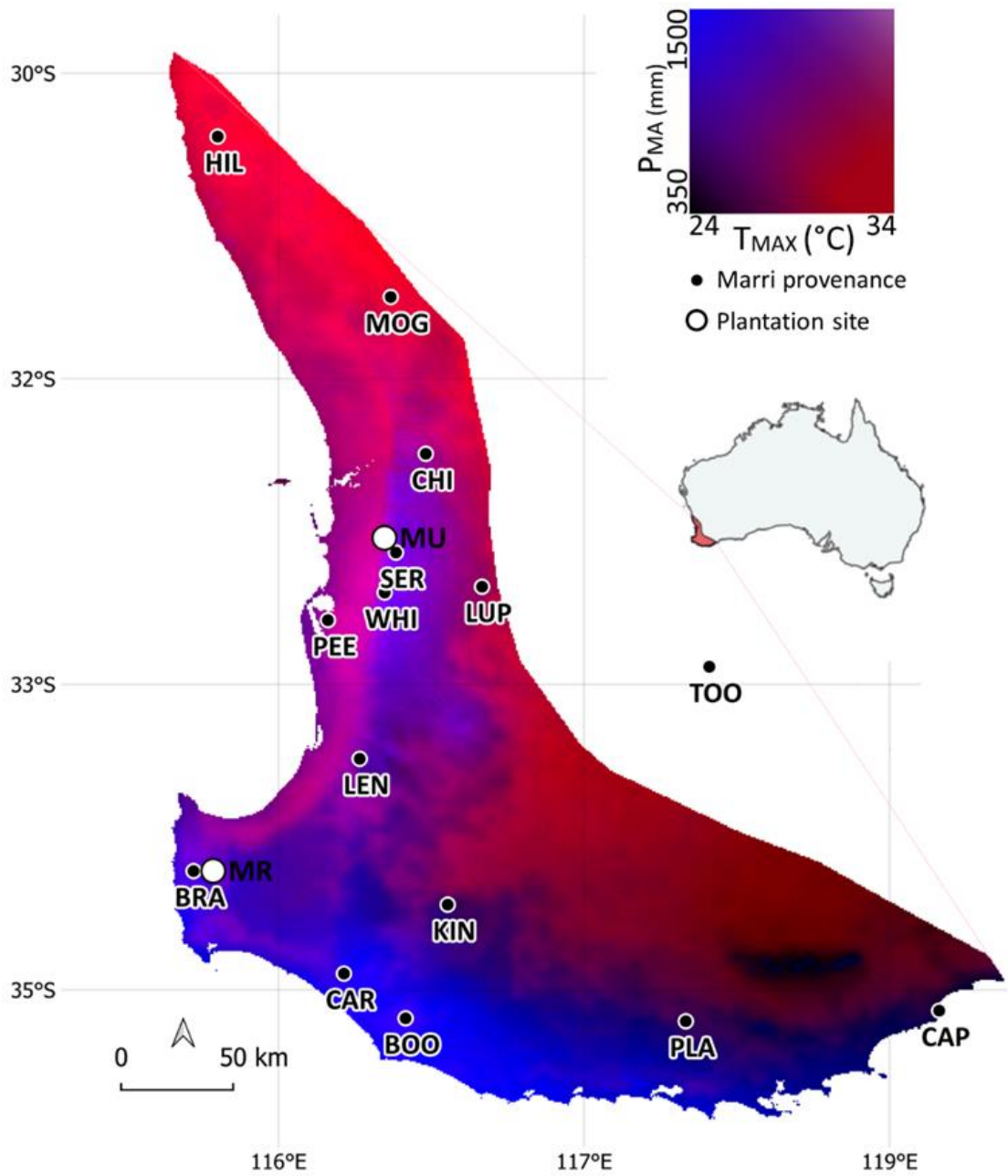


Figure 4.1. Sampled wild populations of marri in South-west Western Australia (black dots) and common garden sites (white dots). Two climate gradients are shown overlapped with colour blending for the species distribution area: maximum temperature of the warmest month (°C; T_{MAX} in the red display) and mean annual precipitation (mm; P_{MA} in the blue display). Bio-climatic layers from worldclim.org (Fick & Hijmans, 2017). The insert shows the main distribution of marri in Australia.

Table 4.1 - Locations and climate-origin of each marri wild population within the study, along with the total number of sampled families and individuals for each site and season.

						Mundijong site (MU)		Margaret River site (MR)			
						Summer		Summer		Autumn	
Population	Code	Latitude	Longitude	T_{MAX} (°C)	P_{MA} (mm)	Families	Individuals	Families	Individuals	Families	Individuals
Warm, dry climate											
Hill River	HIL	-30.311	115.201	31.9	558	8	21	9	23	9	79
Mogumber	MOG	-31.098	116.050	33.5	579	10	29	10	23	10	34
Lupton	LUP	-32.520	116.499	31.1	635	-	-	10	22	10	35
Lake Toolibin*	TOO	-32.937	117.632	31.2	358	10	29	10	24	-	-
Warm, wet climate											
Chidlow	CHI	-31.868	116.223	32.7	900	10	27	10	27	10	34
Serpentine	SER	-32.352	116.076	30.7	1,173	10	29	10	24	10	93
Whittaker	WHI	-32.549	116.021	30.5	1,189	10	27	10	21	-	-
Peel Inlet	PEE	-32.684	115.742	30.4	894	-	-	8	20	8	28
Cool, dry climate											
Kingston	KIN	-34.081	116.330	27.4	824	9	27	10	24	10	31
Cape Riche	CAP	-34.601	118.742	25.7	585	8	15	8	17	8	74
Plantagenet	PLA	-34.653	117.499	26.5	760	10	30	10	24	10	30
Cool, wet climate											
Lennard	LEN	-33.365	115.898	28.8	928	10	30	10	23	-	-
Bramley	BRA	-33.916	115.083	26.1	1,072	9	27	9	18	9	34
Carey	CAR	-34.419	115.821	25.9	1,106	10	29	10	25	10	33
Boorara	BOO	-34.638	116.123	25.6	1,160	10	27	10	24	10	99

*Outlier population

4.2.3. Spectral measurements and analyses

4.2.3.1. Spectral data collection

Leaf reflectance data were collected using an ASD field spectroradiometer (FieldSpec 4, Malvern Panalytical Ltd) with a spectral range of 350 – 2,500 nm. The sensor has a 1.4 nm sampling interval with a spectral resolution (full bandwidth at half maximum) of 3 nm for the visible and near-infrared regions (350 – 1,000 nm); and 1.1 nm sampling interval with a spectral resolution between 10 nm and 12 nm for the shortwave infrared regions (1,001 – 2,500 nm). Measurements were taken with the aid of a leaf clip attached to a plant probe with its own light source over a 4-hr period (9 a.m. to noon) each day of sampling. The leaf clip stabilizes the leaf in the plant probe to maintain the light beam at an angle of 45°. Young but fully expanded leaves were collected from the plant's middle canopy. Three measurements were taken from three different spots for each leaf, avoiding the leaf's midrib and edges. These three measurements were then averaged into a single value. The raw measurements were converted to estimates of percent reflectance based on contemporaneous measurements from a 99% Spectralon calibration panel (Labsphere, Inc.). A reference measurement of the calibration panel was taken before the first measurement each day and every 30 min onwards.

4.2.3.2. PLSR analyses of full spectra

Full reflectance spectra (350 – 2,500nm) are illustrated in Figure 4.2 for all sampled marri individuals across each unique environmental setting. Redundancy and collinearity of variables are common features of hyperspectral data. Additionally, the number of response variables in these analyses is usually much higher than the number of samples, which hinders building a robust statistical model. In such context, multivariate techniques, such as partial least squares regression (PLSR; Wold et al., 2001) analyses, are an attractive analytical choice. This multivariate method generalizes and combines features from standard principal component analysis (PCA) and multiple regression analysis (Abdi, 2010). While PCA determines the orthogonal axes that best explain the variance structure of a single multivariate dataset, PLSR calculates new axes as latent variables (i.e., components) for both the predictor (*X*) and response (*Y*) sets of variables that are most explanatory of each other

(Haenlein & Kaplan, 2004). The regression coefficients for each component are calculated through a leave-one-out cross-validation approach. While the optimum number of components used for the regression was determined by comparing the coefficients of multiple determination (R^2) of predictions obtained from models using different numbers of components (Esbensen et al., 2002). Correlation loadings between an input variable (in either the predictor or the response set) and the respective components were produced by the PLSR modelling to indicate the relationship between predictors and response. The generated axes for each component were organized by decreasing strength of the relationship they describe between the predictor (*X*-loadings) and the response (*Y*-loadings) variables.

The PLSR models were used to associate differences across the full reflectance spectra (i.e., the response variables) to the predictor variables of the reciprocal transplant experimental design: genotype (G; populations), environment (E; site or season), and their interaction (GxE). The advantage of applying this method in the current study is that PLSR reduces large amounts of collinear spectral variables to fewer, non-correlated components, while relating these to the predictor variables (Heckmann et al., 2017; Meacham-Hensold et al., 2019). Two PLSR models were built for each analysis: *PLSR-site* and *PLSR-season.* Analysis of site *(PLSR-site)* was developed to estimate the influence of the environmental variable *site* (E) on the wavelength response. While analysis of season (*PLSR-season*) was used to infer on the influence of *season* (E) on traits, while also accounting for the influence of *population* (G) and the interaction between *population* and each of the environmental variables (GxE). The open-source partial least squares package *'pls'* (Mevik et al., 2018) created the PLSR models in R v4.1.1 (R Core Development Team, 2020). The optimum number of components was selected by screening R^2 (Esbensen et al., 2002) values for key wavelength regions spanning broad spectral regions, representative of targeted leaf traits: green (551nm), yellow (615nm), red (676nm), red-edge (725nm), near-infrared (NIR; 870nm and 979nm), and short-wave infrared (SWIR; 1653nm and 2223nm) and identifying the number of components at which the amount of explained variation in these wavelength regions plateaued. Once selected, the corresponding number of components from the PLSR model was used to predict significant leaf spectral variation associated with each predictor variable (Asner et al., 2011; Serbin et al., 2012). Highlighted X-loadings relate to the correlation strength of each predictor (site and

populations) on spectra variation and were grouped based on the loadings' magnitude values (strongly related: > 3; moderately related: 1 - 3; not related: < 1).

4.2.3.3. Spectral indices

The PLSR exploratory analyses can predict variations in specific wavelength regions (i.e., red, red-edge, green) across the full untransformed reflectance spectra associated with the independent variables of interest (population and site or season). To better determine whether the variations found by the PLSR modelling are genotype (G; population), environment (E; site/season), or GxE dependent, univariate statistical analyses were developed with SIs to investigate significant effects. A set of SIs was selected (Table 4.2) based on wavelength regions with strong loadings identified by PLSR models, and the functional traits they were sensitive to. Specifically, the aim was to explore SI indicative of leaf water and pigments content, since these traits are highly indicative of vegetation health status (Sampson et al., 2003; Cui et al., 2018).

Green, yellow and red regions (500 – 750nm) of leaf and canopy reflectance spectra are influenced by pigments such as chlorophyll, anthocyanins and carotenoids, and SI developed with these spectral regions are generally well correlated with defoliation in eucalypts using spectrometry measurements (e.g., le Maire et al., 2012, 2013; Barry et al., 2008; Duarte et al., 2020; dos Santos et al., 2020). The overtone for water absorption is one of the most prominent signals for water presence in the near-infrared (NIR) wavelength. This region has been used to predict water status in the remote sensing of plants (Peñuelas et al., 1993; Sims & Gamon, 2003). Spectral reflectance at 900nm and 970nm has since been included in several SIs to detect water status that has been shown to have high correlations with water content in foliage. Five SI were calculated ($mNDVI_{705}$, RGI, WBI, NDRE and PRI; Table 4.2) and further analysed as independent datasets under analyses of site and season. Pearson correlation analysis was applied to measure the linear correlation coefficient (r) between these variables and detect redundancy among SI's. If the $r > 0.7$ of magnitude between two variables, these were considered strongly correlated (Ratner, 2009).

4.2.3.4. Data analyses

To test for the effect of genotype (G), environment (E) and their interactions (G×E), linear mixed-effects regression models (LMM) were applied on each SI separately, fitted by restricted maximum likelihood. The LMM were developed using the '*lme'* function in *the nlme* package (Pinheiro et al., 2020) in R v4.1.1 (R Core Development Team, 2020). The effects of genotype (G; population), across the three unique environmental settings: Mundijong (MU) – summer, Margaret River (MR) – summer and Margaret River (MR) – autumn, were first determined. The fixed effect for the LMM built was '*population'*. Separate LMM models were built for each environment set to test the effect of population (G). To determine the effects of environment (E; site/season) and the GxE interactions, two separate analyses of site and season were applied, where the fixed effects were *'population',* '*site*' or '*season*', and the interactions '*population:site'* or '*population:season'*, respectively. For all LMM, *'family'* was included as a random effect. Model assumptions were checked by visually inspecting residuals for normality and homoscedasticity assumptions (Zuur & Ieno, 2016). The Shapiro-Wilk test additionally tested the normality of residuals. When necessary, *Box-Cox* transformations (Box & Cox, 1964) were applied to obtain an approximately normal distribution. A variance function (using *weights = varIdent()*) was included in the model build to account for the heterogeneity of variances between groups and meet the homoscedasticity assumption (Pinheiro & Bates, 2009), where the terms were *'population',* '*site*' or '*season*'. Fitted LMMs were subjected to type III analyses of variance (ANOVA) with Satterthwaite's method to create a summary of the *F*- and *p* statistics with the *car* package (Fox & Weisberg, 2019). Effects with $p < 0.05$ were considered statistically significant. Least-square means for each LMM were computed using the '*lsmeans'* function from the *lsmeans* package in R and compared through the Tukey *post-hoc* test to investigate significant differences among populations within sites and seasons when the models' main effects were found significant**.**

Table 4.2. Selected spectral indices from PLSR analysis. Rxxx is the % reflectance at xxx nm.

Spectral index	Formula	Measured biophysical response	Reference
mNDVI$_{705}$ (modified normalized difference vegetation)	$\frac{R_{750} - R_{705}}{R_{750} + R_{705} - 2xR_{445}}$	Leaf chlorophyll content	Sims & Gamon, 2002
RGI (red-green ratio)	$\frac{R_{600} : R_{700}}{R_{500} : R_{600}}$	Leaf anthocyanins content	Gamon & Surfus, 1999
WBI (water band)	$\frac{R_{970}}{R_{900}}$	Leaf water content	Peñuelas et al., 1993
NDRE (normalised difference red edge)	$R_{790} - \frac{R_{720}}{R_{790}} + R_{720}$	Leaf chlorophyll content	Barnes et al., 2000
PRI (photochemical reflectance)	$\frac{R_{531} - R_{570}}{R_{531} + R_{570}}$	Xanthophyll response to light / photosynthetic efficiency.	Gamon et al., 1997

4.3. Results

4.3.1. PLSR models of full spectra

In general, all sampled spectra (Figure 4.2) displayed a similar shape, with typical features of leaf reflectance spectra such as the photosynthetic pigments reflectance red-edge around 700 – 750nm, and the broad decline in reflectance across the whole SWIR range caused by liquid water absorption. For both developed PLSR models, *PLSR-site* and *PLSR-season*, there was a noticeable variation of R^2 values across the select key wavelength regions (Figure S4.1). While the amount of variation explained plateaued early for most wavelength regions, at around 4-5 components, extra components were needed to capture variation in the NIR (*PLSR-site*; Figure S4.1a) and red (*PLSR-season;* Figure S4.1b) wavelengths. Thus, ten components were used for the final models, as the optimal number of components to maximize explanatory capacity across the full spectra. The amount of variation explained by the PLSR models R^2 differed between the models (Figure 4.3): mean *PLSR-site* R^2 = 0.072 (SD = 0.03; range: 0.02 – 0.16) and mean *PLSR-season* R^2 = 0.130 (SD = 0.05; range: 0.03 – 0.26), with the environmental variable *'season'* having a greater explanatory capacity of spectral variation than *'site'* (*PLSR-site*). Across the full spectra, there was a notable variation of explanatory capacity within each model. For example, the explanatory capacity for *PLSR-site* tended to be the highest around the visible region (500 – 700nm) and lowest in the red-edge and NIR plateau (700 – 1,250nm). While *PLSR-season* displayed the greatest explanatory capacity for the red-edge spectral region (700 –750nm), with R^2 = 0.260, and was relatively higher than the typical R^2 for *PLSR-site* across other key spectral regions, including the visible (especially green band, 500 – 550nm), NIR plateau, and SWIR regions, compared to *PLSR-site*. Generally, lower R^2 values for *PLSR-season* were coincident with the edges of spectral features, while higher values were found in wavelength regions with higher reflectance (Figure 4.3).

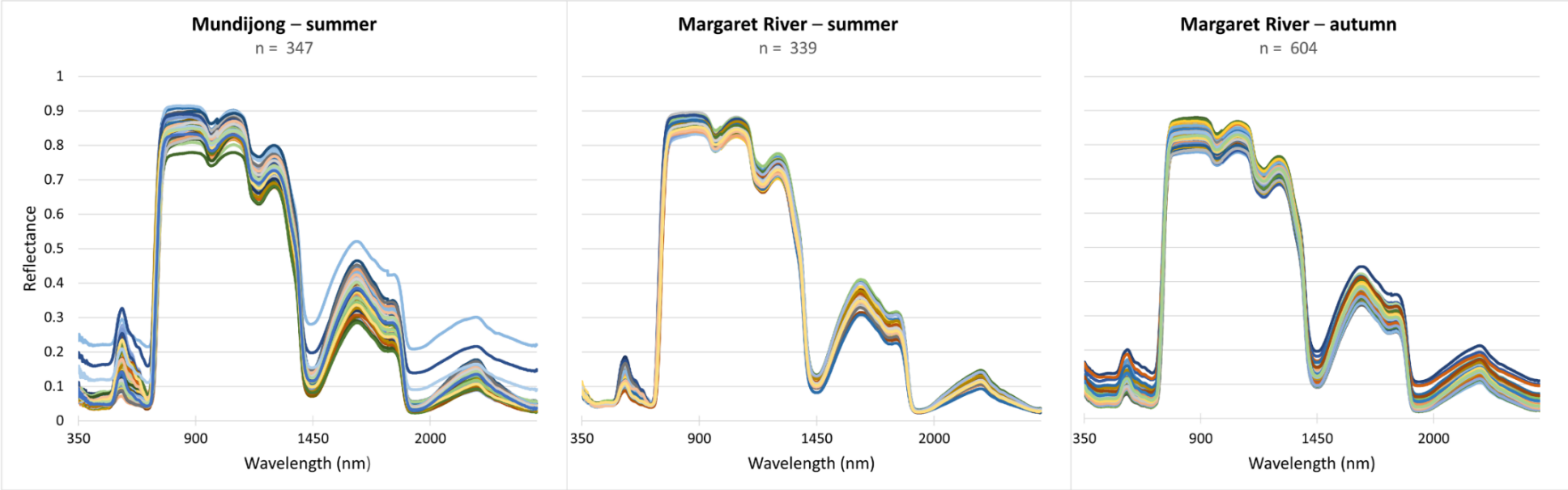


Figure 4.2. Leaf reflectance (350 – 2,500nm) for sampled marri individuals (n = number of individuals measured) from each measured environment: Mundijong – summer, Margaret River – summer and Margaret River – autumn.

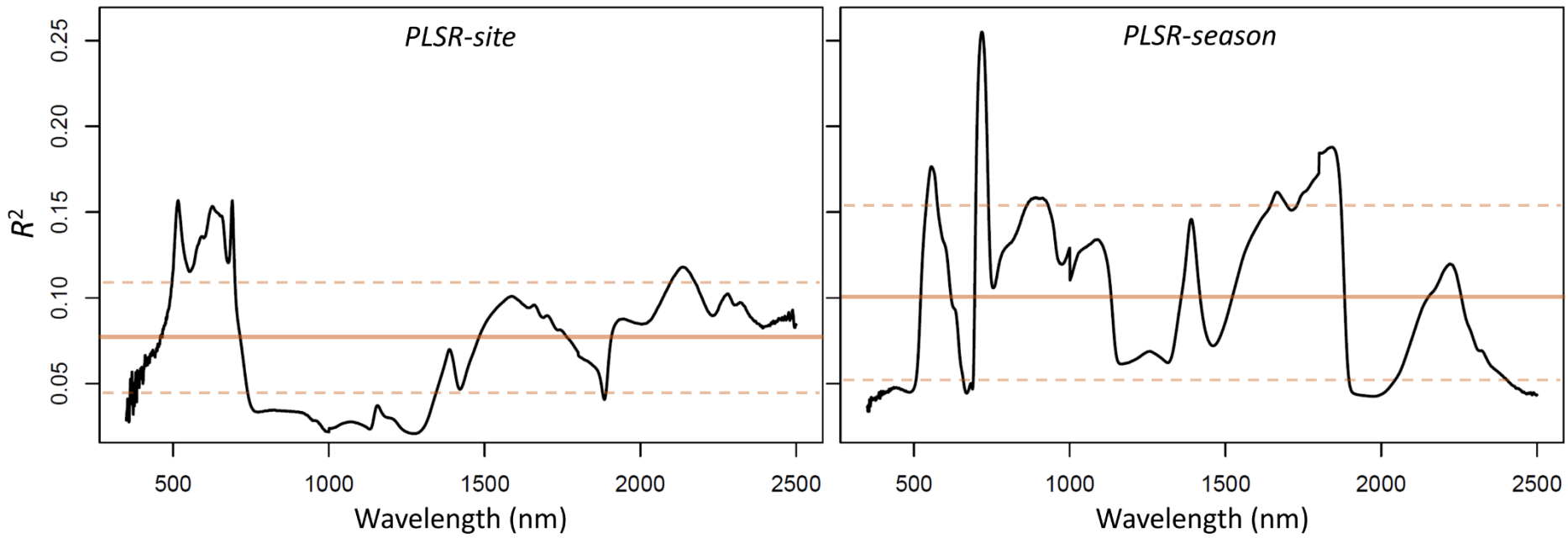


Figure 4.3. R^2 for *PLSR-site* and *PLSR-season* models built with ten components across the full reflectance spectra (350 – 2,500nm). Orange line marks the mean R^2 value for each model across all of the reflectance wavelength regions (standard deviation denoted in dashed lines): *PLSR-site* mean R^2 = 0.073 (SD = 0.03; range: 0.02 – 0.16); *PLSR-season* mean R^2 = 0.120 (SD = 0.05; range: 0.03 – 0.26).

The PLSR loadings (Figures 4.4a and 4.4b) for a given component showed variations in the reflectance spectra related to the predictive variables (population, site and season). The *X*-loadings relate to the predictor variables, thus highlighting contrasts between the environments (MR vs MU, for site comparison; summer vs autumn, for season comparison), and between specific populations. *Y*-loadings relate to the response variable (i.e., spectral reflectance) and display variation in specific wavelength regions associated with that component and, by extension, the predictor variables with *X*-loadings for that same component. The PLSR components are ordered by decreasing strength of the association they refer to between the predictor and the response variables. To aid in interpretations, populations were grouped based on the magnitude of the *X*-loadings values for each component (strongly related: >3; moderately related: 1-3; not related: <1). Although both predictive models (*PLSR-site* and *PLSR-season*) were built with ten components, results are provided here for the loadings for the first four components only. After that, the outputs did not identify new spectral responses and/or notable contrasts between populations. It is noteworthy that both PLSR models across several components found strong *Y*-loadings associated with spectral variation that is very subtle (e.g., shifts of the red-edge position) due to averaging effects within and across populations, and challenging to describe based on visual inspection of the reflectance plots.

In both models, *PLSR-site* and *PLSR-season*, the first component identified the environment (E) effect as the predictor variable explaining the most variation across the spectra, as seen from the strong *X*-loading for site (Figure 4.4a) and season (Figure 4.4b) terms. Patterns of spectral variation associated with each environment (E) effect are identified by the *Y*-loadings for component 1. The *'site'* effect (Figure 4.4a) was related to variation in the visible reflectance near the photosynthetic pigments reflectance features of the green-yellow wavelength regions (500 – 650nm), the red-edge region (700 – 750 nm) and in the SWIR, which is affected by strong water absorption features (1,500 – 1,700nm and 2,200 – 2,300nm). Observable in the reflectance spectral panel from component 1 (Figure 4.4a), MU reflectance (blue line) was higher in the visible and SWIR regions, when compared to the MR reflectance curve (black line). The red-edge region showed likewise strong *Y*-loadings, although the differences between sites were not visually discernible. As for the *'season'* effect (Figure 4.4b), the *Y*-loadings identified variations throughout most of the spectrum, with

some similarities with the 'site' effect (e.g., green-yellow wavelength regions and particularly strongest loadings for the red-edge). Notably, summer reflectance (black line) was higher in the visible and NIR regions, but lower in the SWIR, contrasting with the autumn's reflectance (blue line) for these spectral regions.

Figure 4.4a. *PLSR-site* model loadings built with ten components (first four components are shown). Barplots (left side) display the *X*-loadings; on the right side, thin top plots display the *Y*-loadings (horizontal line denotes *Y*-loading = 0) for each component and respective reflectance spectral panels (spectral variation related to predictors, based on strong *Y*-loadings, is grey highlighted). Coloured-highlighted *X*-loadings relate to the correlation strength of each predictor (site and populations) on spectral variation (blue – strong positive; black – strong negative; green – moderate positive; red – moderate negative; grey – weak correlation); predictors with similar correlation strength are grouped by colour for display on the spectral panels. PLSR loadings for the interaction effect (*'population:site')* are not displayed since no noteworthy correlations over the main effects were found. (See Table 4.1 for population details). →

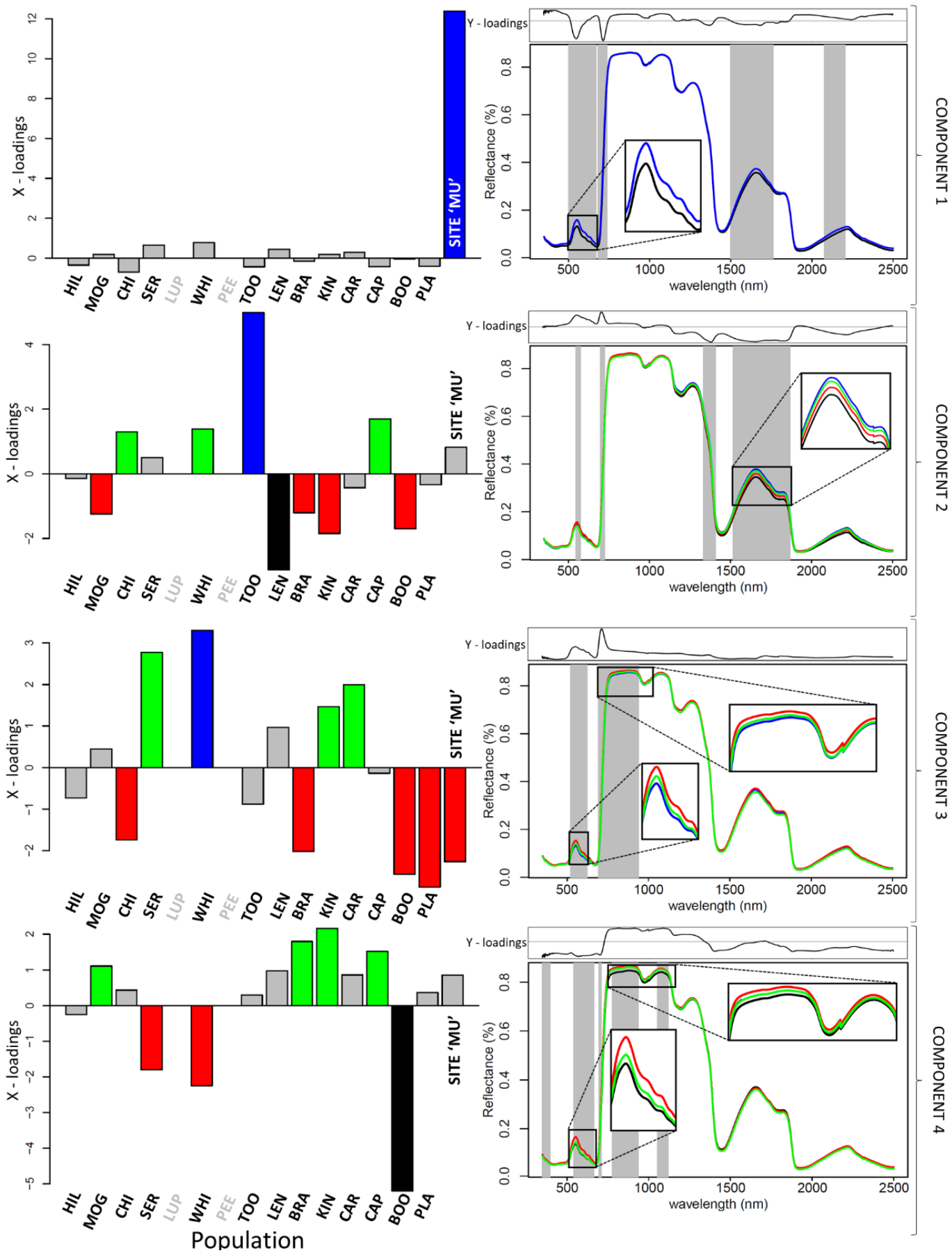
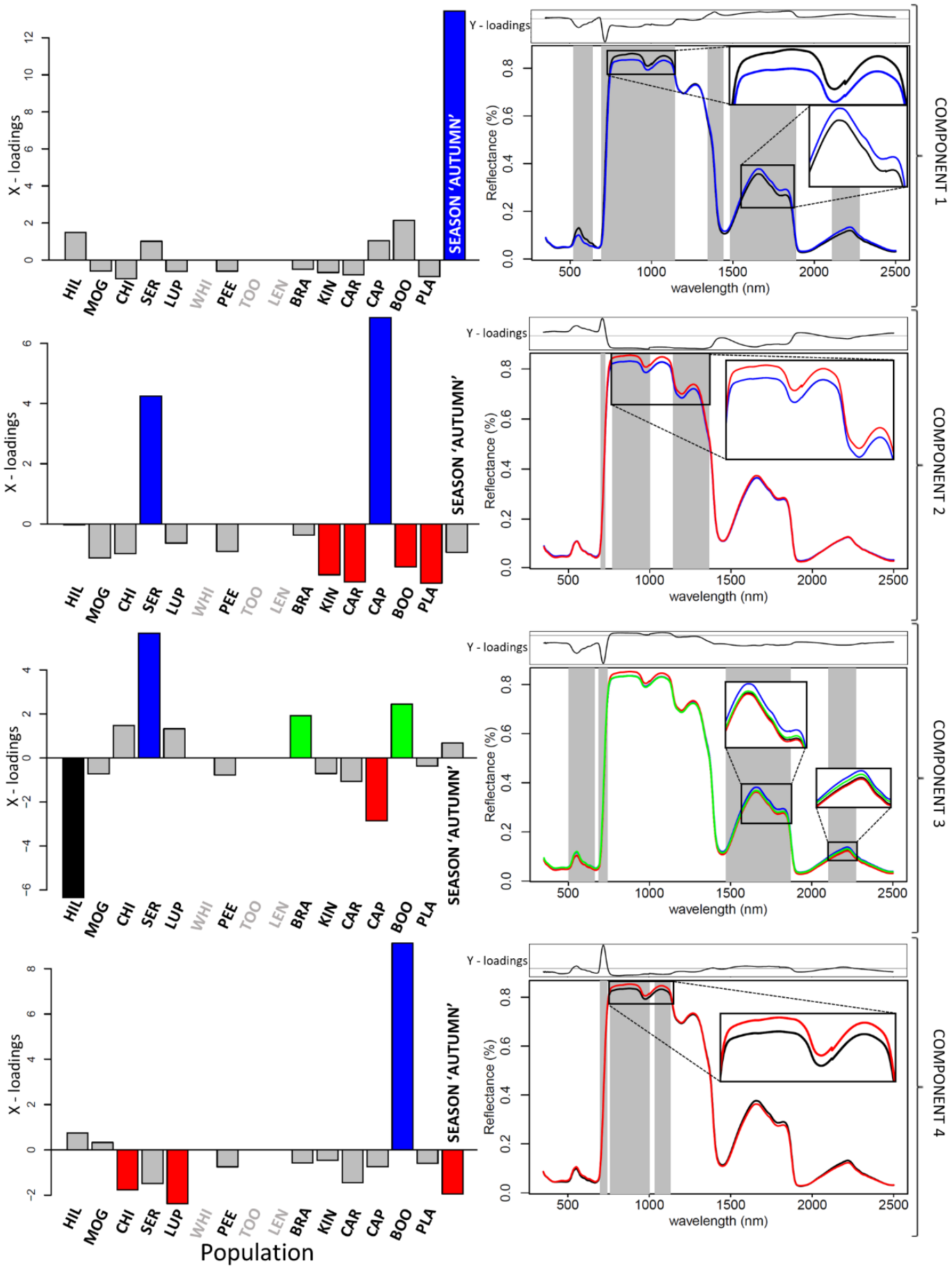

COMPONENT 1
COMPONENT 2
COMPONENT 3
COMPONENT 4
Y - loadings
Reflectance (%)
wavelength (nm)
X - loadings
SITE 'MU'
HIL
MOG
CHI
SER
LUP
WHI
PEE
TOO
LEN
BRA
KIN
CAR
CAP
BOO
PLA
Population

Figure 4.4b. *PLSR-season* model loadings built with ten components (first four components are shown). Barplots (left side) display the *X*-loadings; on the right side, thin top plots display the *Y*-loadings (horizontal line denotes *Y*-loading = 0) for each component and respective reflectance spectral panels (spectral variation related to predictors, based on strong *Y*-loadings, is grey highlighted). Coloured-highlighted *X*-loadings relate to the correlation strength of each predictor (site and populations) on spectral variation (blue – strong positive; black – strong negative; green – moderate positive; red – moderate negative; grey – weak correlation); predictors with similar correlation strength are grouped by colour for display on the spectral panels. PLSR loadings for the interaction effect (‘*population:season’)* are not displayed since no noteworthy correlations over the main effects were found. (See Table 4.1 for population details). →


COMPONENT 1
COMPONENT 2
COMPONENT 3
COMPONENT 4
Y - loadings
Reflectance (%)
wavelength (nm)
X - loadings
SEASON 'AUTUMN'
HIL
MOG
CHI
SER
LUP
WHI
PEE
TOO
LEN
BRA
KIN
CAR
CAP
BOO
PLA
Population

Regarding the genotype (G) influence, which was evaluated by the *'population'* effect, the remaining components identified different aspects of this main effect on the analysis of site. *PLSR-site* (Figure 4.4a) related the second component to the TOO and LEN populations with strong positive and negative (respectively) *X*-loadings, and a tier of moderate predictor loadings for CHI, WHI and CAP (positive correlation), and MOG, BRA, KIN and BOO (negative correlation). The response *Y*-loadings highlighted spectral variation related to these populations across the green wavelength regions (550-565nm), red-edge (700-750nm), and the SWIR: around 1,400 – 1,450nm, and 1,500 – 1,900nm. Populations with positive *X*-loadings for this component showed higher reflectance across the highlighted green and SWIR regions, contrasting with populations with negative *X*-loadings that displayed lower reflectance values for these same regions. Populations with moderate negative *X*-loadings showed a subtle shift of the red-edge towards shorter wavelengths.

The second component from *PLSR-season* (Figure 4.4b) similarly identified spectral variation related to the *'population'* effect, although with different correlation *X*-loadings for each population: strong positive loadings were for SER and CAP, while moderate negative loadings were found for KIN, CAR, BOO and PLA. The main spectral variation identified by the *Y*-loadings and related to the genotype effect on the second component from *PLSR-season* was found in the red-edge and two NIR regions: 750 – 1,000nm and 1,150 – 1,350nm. Populations with negative *X*-loadings show higher reflectance across the NIR region, contrasting with populations with positive *X*-loadings that displayed lower reflectance values for this same region. Variations in the red-edge regions were not visually discernible here.

From the *PLSR-site,* the third component identified WHI as having a strong positive correlation with the related spectral variation, followed by moderate positive correlation from SER, KIN and CAR, and moderate negative correlation from CHI, BRA, BOO and PLA. The strongest *Y*-loadings were found for the red-edge (700 – 750 nm), followed by moderate loadings associated with the visible green-yellow wavelength regions (500 – 650nm) and NIR plateau (750 – 950nm). Populations negatively correlated with variation from these components showed higher reflectance for the highlighted spectral regions and a subtle shift of the red-edge position towards shorter wavelengths. Finally, the fourth component from *PLSR-site* identified BOO with a strong negative; SER and WHI and moderate negative; and MOG, BRA,

KIN and CAP with moderate positive correlations with spectral variation in the visible wavelength regions, the red-edge, and two wavelength regions from the NIR region (750 – 900nm and 1,050 – 1,100nm). Interestingly, BOO (strong negative correlation) showed the lowest reflectance for the highlighted spectral regions (visible and NIR plateau), while moderate negative correlations identified for SER and WHI drove the highest reflectance for these same regions, as well as a shift of the red-edge towards shorter wavelengths.

The third component from *PLSR-season* related SER and HIL (strong positive and negative correlation, respectively) to highlighted spectral variation in the red-edge (700 – 750), visible (500 – 600nm), and the SWIR regions (1,500 – 1,700nm and 2,200 – 2,300nm). Populations positively correlated with variation from these components showed higher reflectance for the highlighted spectral regions and a subtle shift of the red-edge position towards shorter wavelengths. Its fourth component identified a very strong positive *X*-loading from the BOO population, associated with spectral variation with strong *Y*-loadings in the red-edge (700 – 750nm), followed by the NIR region: 750 – 1,000nm and 1,050 – 1,200nm. Similarly to component 2, populations with negative *X*-loadings from component 3 also show higher reflectance across the NIR region, contrasting with the population with strong positive *X*-loadings (BOO) that display lower reflectance values for this same region.

The GxE effect could be identified by including the interaction terms *population:site* and *population:season* in the *PLSR-site* and *PLSR-season* models, respectively. Although the inclusion of the interaction term in both models improved the returned R^2 values (PLSR models without interaction term: *PLSR-site* average R^2 =0.072; PLSR-season average R^2 = 0.101), the PLSR loadings for both models did not identify strong associations between predictor and response variables for interaction terms. Therefore, only the main effects (population, and site or season) are displayed in Figures 4.4a,b and further discussed.

4.3.2 Spectral indices variation

Five SIs were calculated for all sampled marri individuals across different sites and seasons (Table 4.3). Pearson's correlation analysis (Table S4.1) found that $mNDVI_{705}$ and NDRE were strongly correlated with each other ($r > 0.7$) in both analyses of site and season. The population (G) effect was not significant for any measured index in the summer at either site (Table 4.4). However, population was significant for the analyses of autumn measurements from the MR site for the following indices: $mNDVI_{705}$ ($p < 0.001$), WBI ($p < 0.001$), NDRE ($p < 0.001$), and PRI ($p = 0.04$). Mean (± 1SE) SIs values and population pairwise *P* values (Tukey *post-hoc*) to inform significant differences between populations within MR – autumn are given in Figure S4.2 and Table S4.2, respectively. The population that recorded the most intraspecific differences for $mNDVI_{705}$ was the cool-dry CAP, being different from six other populations, followed by the population furthest away from the MR plantation site, HIL, with three significant differences. These two provenances (CAP and HIL) recorded the lowest values for $mNDVI_{705}$ (Figure S4.2), among populations growing in the cool-wet site during autumn. The home population from the MR site, BRA (cool-wet), recorded a significantly lower WBI from the same climate origin BOO-population, and from HIL, which comes from the contrasting warm-dry climate origin of marri. The northernmost population (HIL) also recorded the lowest value for PRI and was significantly different from two populations from contrasting climate-origins (PLA and BOO, both from cool-origin)

Table 4.3. Mean (± 1SE) spectral indices values for marri across Mundijong (MU) – summer, Margaret River (MR) – summer and Margaret River (MR) – autumn. Spectral indices abbreviations: $mNDVI_{705}$ – modified normalized difference vegetation index; RGI – red-green index; WBI – water band index; NDRE – normalized difference red-edge; PRI – photochemical reflectance index.

	Site and season		
Spectral index	**MU – summer**	**MR – summer**	**MR – autumn**
$mNDVI_{705}$	0.607 ± 0.0074	0.657 ± 0.0062	0.753 ± 0.0024
RGI	0.683 ± 0.0044	0.667 ± 0.0041	0.704 ± 0.0025
WBI	0.938 ± 0.0007	0.942 ± 0.0005	0.950 ± 0.0002
NDRE	0.252 ± 0.0047	0.286 ± 0.0042	0.349 ± 0.0021
PRI	-0.010 ± 0.0020	-0.007 ± 0.0016	0.026 ± 0.0005

The environment (E) effect was significant under both analyses of site and season for all measured SI (Table 4.4; Figure 4.4), except PRI (p = 0.104) in the analysis of site. When comparing sites, $mNDVI_{705}$, WBI and NDRE had significantly higher values at MR than MU site ($p < 0.001$), while an opposite trend was found for RGI, which had a lower value at the MR site compared to MU ($p < 0.007$). Comparing seasons, all measured SIs mean values were significantly higher during autumn than the summer season. The pattern of variation between populations was overwhelmingly consistent, with all populations showing higher mean values for the measured SIs during summer compared to autumn (Figure 4.6). A single exception was the BOO (cool - wet) population, which displayed an opposite direction for RGI, with a lower mean value for this SI during autumn than summer. When measured across the two seasons, such patterns indicate an overall strong plastic response from all populations.

There were significant GxE interactions for all SIs under analyses of site and season (Table 4.4; Figure 4.6). The interaction responses in comparing sites were more variable and contrasting within each SI. For $mNDVI_{705}$, most populations showed lower mean values in MU than MR, with few contrasting exceptions: BRA (cool - wet), CAP and PLA (cool - dry). For RGI, responses were mixed but generally contrasting between sites; noteworthy, some populations from the cool climates (PLA, CAR, CAP and BRA) displayed the strongest differences among sites, with considerably higher values in the MU site. Interestingly, KIN (cool - dry) and BOO (cool – wet) followed an opposite direction. The responses for WBI showed a pattern, to some extent, like the comparison of season for the same SI, with most populations showing a similar trend of reduced values from one environment to the other, which might indicate a plastic response from most populations for WBI. A single exception was WHI (warm - wet) that followed an opposite direction; LEN and CAR (cool - wet) display almost unnoticeable differences between sites. For NDRE, three populations from the cool climates (PLA, CAP and BRA) seem to follow an opposite response direction from the other populations, with higher values at MU than MR; Similarly, for PRI, some cool-climate populations (BRA, LEN, CAP and PLA) also displayed an opposite direction to the other populations, with higher values at MU than MR, but joined by WHI (warm - wet) in this pattern.

Table 4.4. Linear mixed-effects model *F* and *p* values for spectral indices testing for significance of genotype (G; population) across Mundijong (MU) – summer (n=347), Margaret River (MR) – summer (n=339) and Margaret River (MR) – autumn (n=604); environment (E; site/season) and GxE under the analysis of site (MU – summer x MR – summer) and of season (MR – summer x MR – autumn). Significant values ($p < 0.05$) are shown in bold. Spectral indices abbreviations: $mNDVI_{705}$ - modified normalized difference vegetation index; RGI - red-green index; WBI – water band index; NDRE – normalized difference red-edge; PRI – photochemical reflectance index.

	MU – summer		**MR – summer**		**MR – autumn**		**Analysis of Site**				**Analysis of season**			
	G (pop)		G (pop)		G (pop)		E (site)		GxE		E (season)		GxE	
Spectral index	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
$mNDVI_{705}$	1.052	0.408	0.896	0.547	**4.981**	**<0.001**	32.475	**<0.001**	3.596	**<0.001**	284.720	**<0.001**	14.898	**<0.001**
RGI	1.006	0.449	1.534	0.119	1.859	0.051	7.455	**0.007**	2.482	**<0.001**	82.627	**<0.001**	5.487	**<0.001**
WBI	0.754	0.696	0.983	0.467	**3.898**	**<0.001**	22.884	**<0.001**	2.387	**<0.001**	220.030	**<0.001**	11.500	**<0.001**
NDRE	1.048	0.412	1.251	0.264	**5.488**	**<0.001**	29.617	**<0.001**	4.037	**<0.001**	214.860	**<0.001**	12.458	**<0.001**
PRI	0.853	0.596	0.659	0.774	**2.711**	**0.004**	2.653	0.104	1.672	**0.029**	497.810	**<0.001**	22.990	**<0.001**

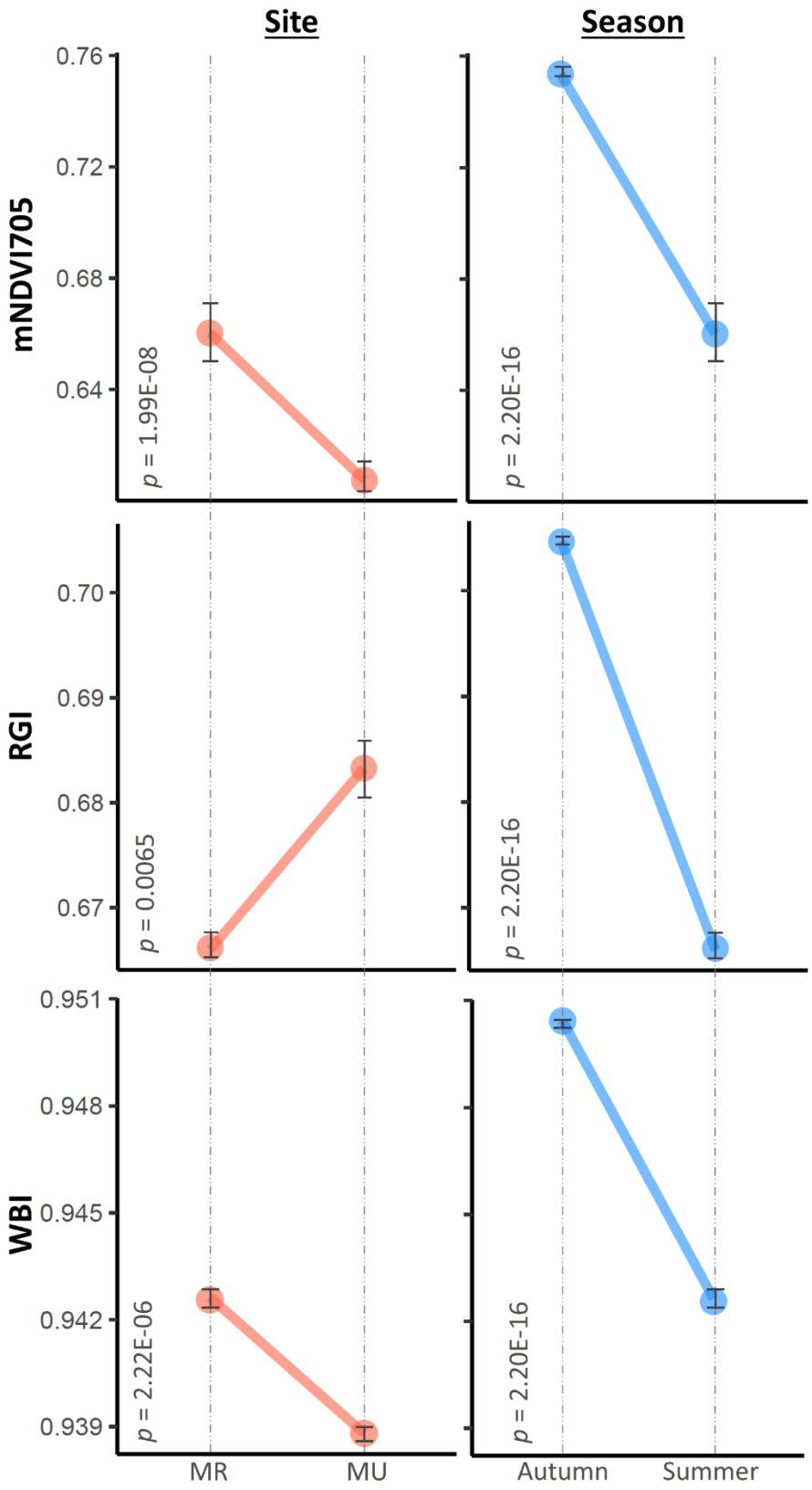


Figure 4.5. Environment effect on measured spectral indices, with SE of the mean, under the analyses of site (left) season (right). Spectral indices abbreviations: $mNDVI_{705}$ - modified normalized difference vegetation index; RGI - red-green index; WBI – water band index.

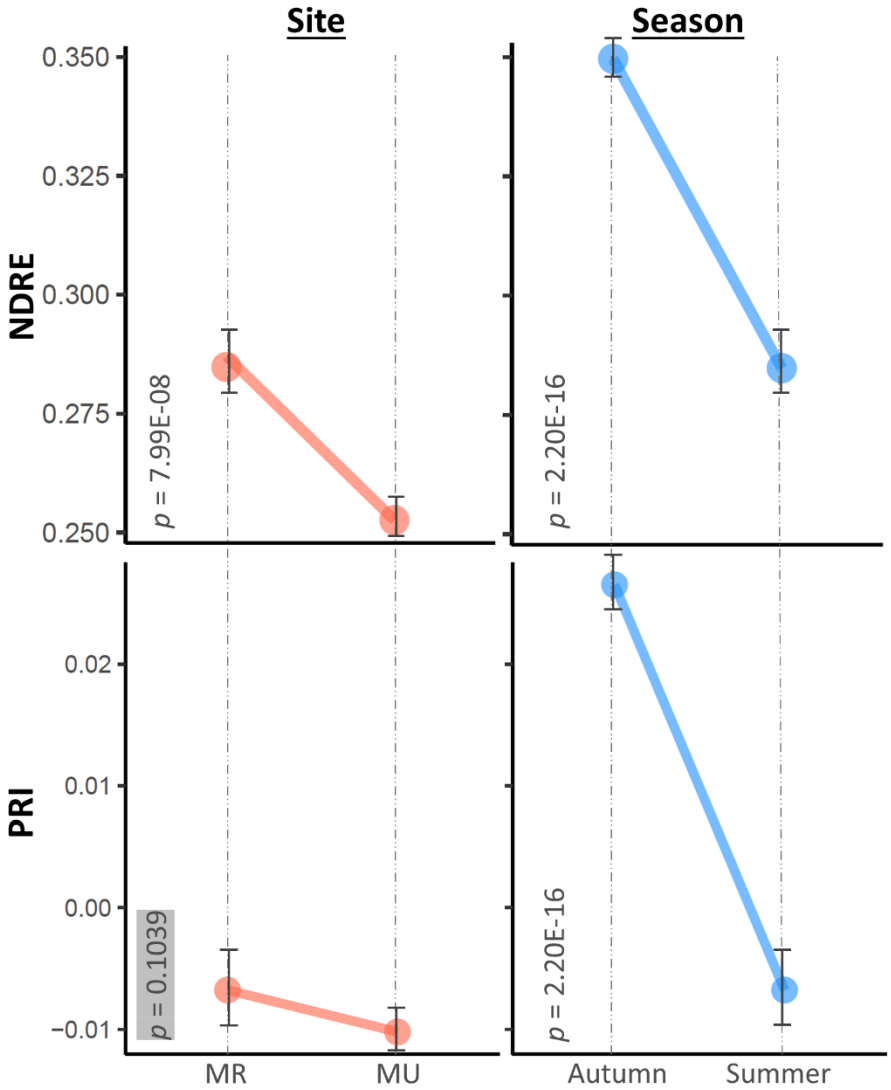


Figure 4.5 (continued). Environment effect on measured spectral indices, with SE of the mean, under the analyses of site (left) and season (right). Spectral indices abbreviations: NDRE – normalized difference red-edge; PRI – photochemical reflectance index. Non-significant difference ($p > 0.05$) is highlighted in grey.

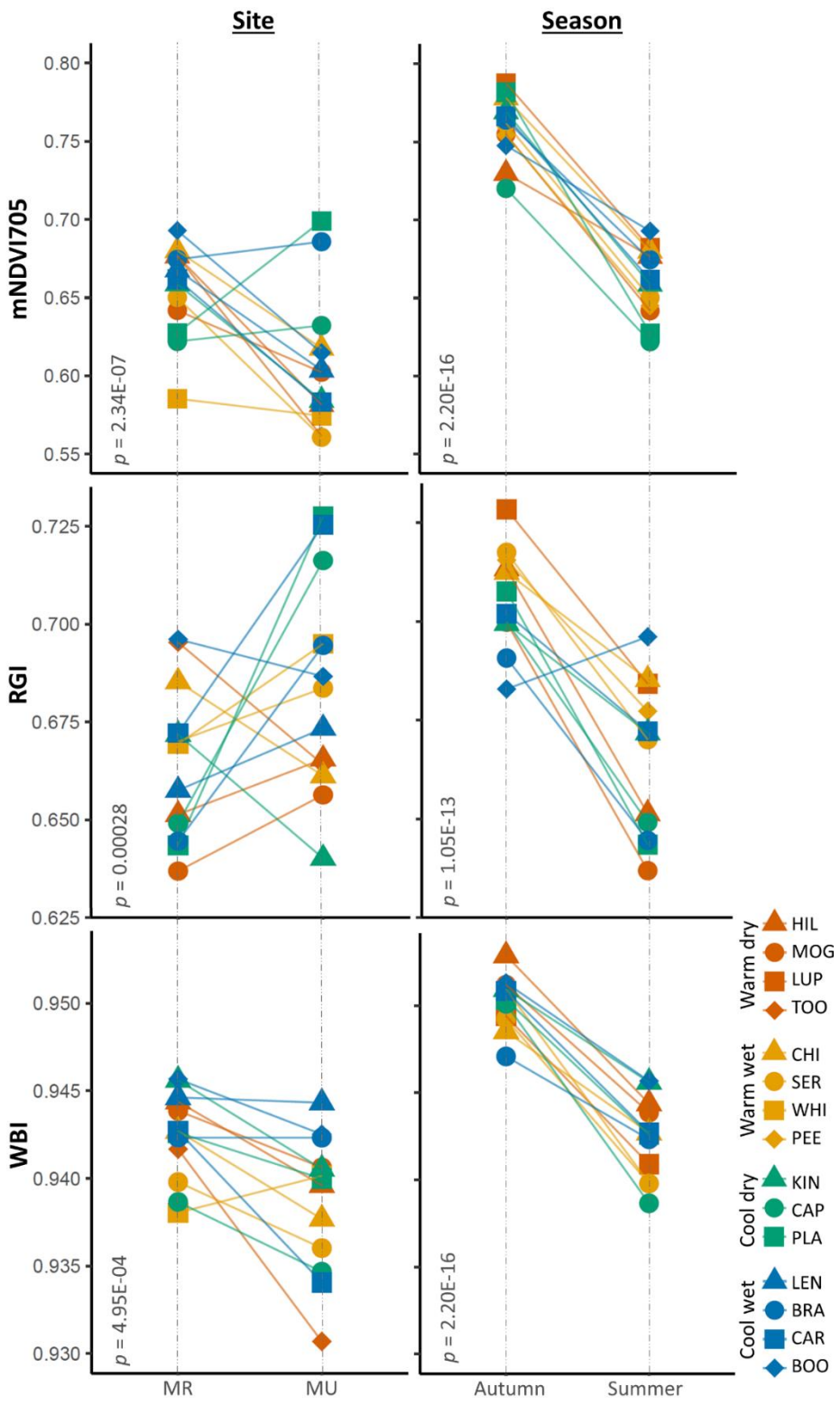


Figure 4.6. GxE interaction effect on measured spectral indices under the analysis of site (left) and analysis of season (right). Spectral indices abbreviations: $mNDVI_{705}$ - modified normalized difference vegetation index; RGI - red-green index; WBI – water band index. (See Table 4.1 for population details)

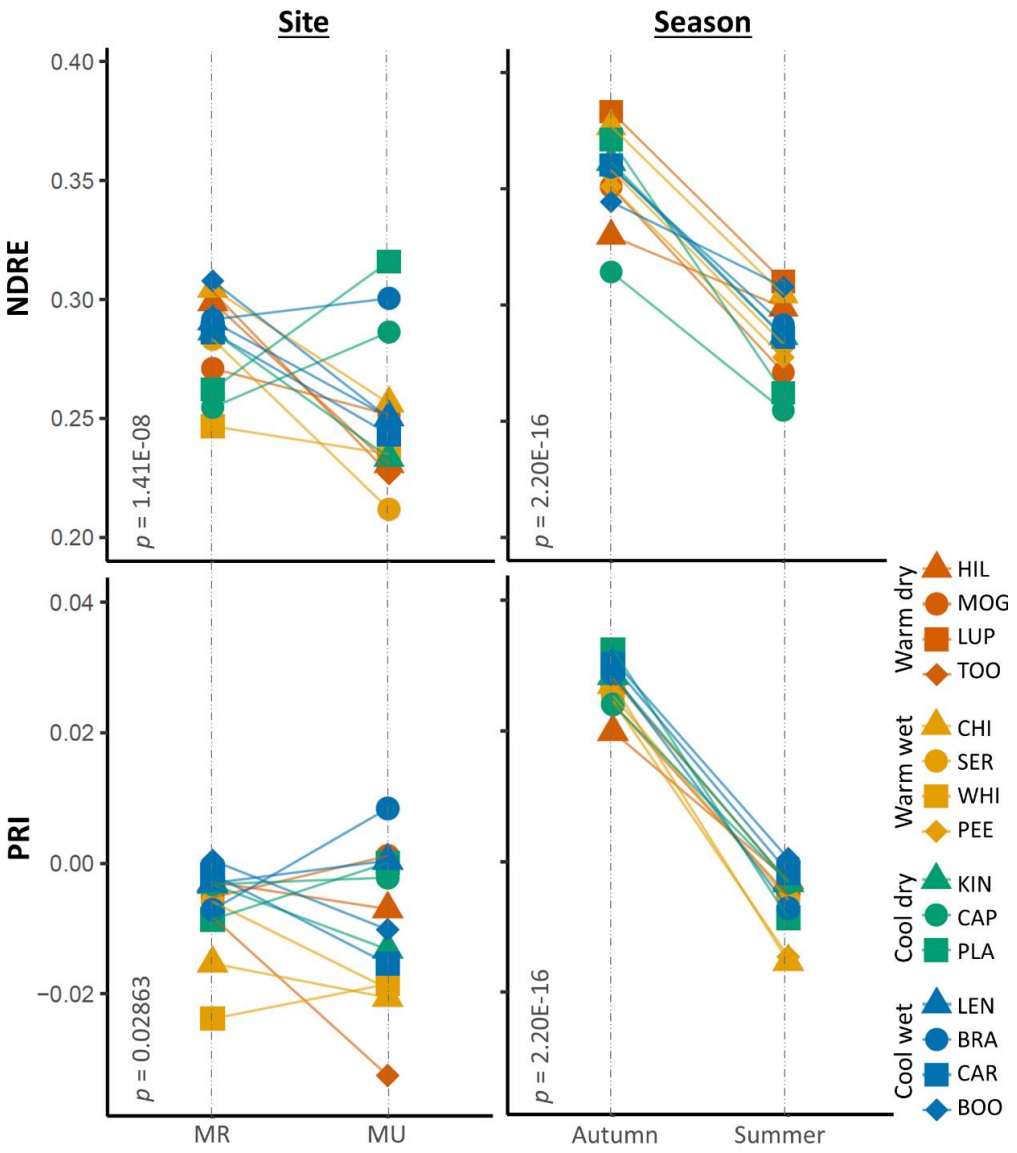


Figure 4.6 (continued). GxE interaction effect on measured spectral indices under the analysis of site (left) and analysis of season (right). Spectral indices abbreviations: NDRE – normalized difference red-edge; PRI – photochemical reflectance index. (See Table 4.1 for population details).

4.4. Discussion

The present study quantified functional traits variation using spectral analyses in experimental sites with reciprocally planted populations from diverse climate origins. A multi-analyses approach using both full spectra and spectral subsets was applied to quantify genetic and plastic effects, along with evidence for local adaptation. While there was support for genetic differences and local adaptation among populations, seasonal plasticity had the largest effect, followed by plasticity among sites. The implications of the findings are discussed.

4.4.1 Genetic variation from populations

There was intraspecific variation in particular spectral features mainly related to water balance and photosynthetic pigments for the sampled populations of marri. This outcome is consistent with past physiological studies, where marri individuals from different populations have provenance-specific background differences in water management (Blackman et al., 2017; Challis et al., 2020) and photosynthesis capacity (Aspinwall et al., 2017). The genotype (G) effect was strongly demonstrated by the *'population'* effect on the full leaf reflectance spectra, which was evaluated through multivariate exploratory analyses (PLSR) on site and season. This full-spectrum based modelling approach identified specific regions across the spectrum that are most sensitive to different genotypes. The correlations between variation in spectral features with different provenances of origin may be the first step to elucidate the influence of genotype on traits expression. Highlighted variations occurred in wavelength regions from the visible, red-edge, NIR and SWIR regions, with different populations of origin driving those variances, although identified differences among populations differed between sites and seasons.

The reflectance spectra revealed that pigment content varied among populations within sites and seasons. Light absorption by a variety of leaf pigments, including photosynthetic pigments (i.e., chlorophyll and carotenoids) dominates the reflectance spectrum in the visible region (400 – 700 nm) (Croft & Chen, 2018). During photosynthesis, chlorophyll pigments *a* and *b* absorb in a wide variety of visible wavelength regions, including the violet-blue (400 – 500 nm) and red (600 –700 nm); while there is lower absorption over the green wavelength

regions (500 – 600 nm) (Croft et al., 2017), thus the typical green appearance of healthy vegetation. Additionally, changes in the xanthophyll cycle, a biochemical process known to play a photoprotective role during photosynthesis under intense light stress (Taiz et al., 2006), were shown to alter the reflectance around 530nm (Gamon et al., 1992). Generally, depressed content for leaf pigments, particularly chlorophyll, is strongly associated with higher reflectance for the visible wavelength regions (Ustin & Jacquemoud, 2020), and a shift of the red-edge position towards shorter wavelengths (Sims & Gamon, 2002). Comparisons of site and season displayed strong genotype predictor loadings linked to spectral variations in the red-edge (700 – 750nm), followed by moderate correlations highlighted for the visible green-yellow wavelength regions (500 – 650nm). Although, the mixed climate-origin genotypes overall identified as predictors for variation in these wavelength regions indicate that marri populations might be adapted in different ways regarding photosynthetic capacity, consistent with a previous study (Aspinwall et al., 2017). Several physiological studies have shown other plant genera and populations from distinct climate-origins displaying distinct and/or divergent adaptive responses in acclimation of photosynthesis (Atkin et al., 2006, Ishikawa et al., 2007., Zhang et al., 2015). Spatial heterogeneity of environmental stressors, such as spatially diverse climate warming, might hinder convergent physiological acclimation in marri, since the biochemical mechanisms of photosynthesis are highly sensitive to temperature fluctuations (Weih & Karlsson, 2001; Campbell et al., 2007).

Reflectance variation across the NIR might indicate variation in leaf nitrogen (N) content between population. There seems to be a tendency for cool-origin populations (KIN, CAR, BOO and PLA) to have higher reflectance across the NIR in the comparison of seasons, which might be associated with higher N content. (Gamon et al., 1995; Ollinger et al., 2008; Lepine et al., 2016). This could indicate an enhanced capacity for photosynthesis across seasons, since leaf N is a major component of chlorophyll (Allison et al., 1997). Leaf N is also a significant component of Rubisco (ribulose-1, 5-bisphosphate carboxylase/oxygenase), a major enzyme involved in the first step of carbon fixation during photosynthesis (Lambers et al., 2008). There is evidence that the portioning of leaf N to photosynthetic enzymes such as Rubisco is driven by climate conditions (Luo et al., 2021), which can be an acclimation strategy of photosynthesis to alternating temperature and light conditions (Yin et al., 2018). Population variation in foliar N can be associated with a greater allocation to photosynthetic

enzymes in trees, maintaining increased photosynthetic capacity. Several NIR wavelength regions are associated with leaf nitrogen (N), including 760 – 900nm (Wang et al., 1993), 780nm (Stone et al., 1996) and 1,100nm (Inoue et al., 1998), across different plant genera. More recently, plant N stress was shown to be detectable with subtle changes in the red-edge wavelength regions of 700 – 720nm (Barnes et al., 2000), and Oliveira et al., (2017) identified red-edge wavelength regions as strong predictors of N concentration for two *Eucalyptus* species. The mechanism for the correlation between leaf N and photosynthetic capacity is poorly understood, and several studies found variable results within and among species (Ellsworth and Reich, 1993; Grassi et al., 2005; Kattge et al., 2009) and canopy positions (Meir et al., 2002). Alternatively, Croft et al., (2017) found a stronger correlation of photosynthetic parameters to leaf chlorophyll content, than between leaf N content, indicating that leaf chlorophyll could be a better proxy for leaf photosynthetic capacity than leaf N content.

Spectral variation indicated that population of origin influences water management traits. The comparison of site showed the tendency for warm populations (TOO, CHI and WHI) to display higher reflectance across the SWIR (1,500 – 1,900nm), thus indicating lower leaf water content. In contrast, a group of cool-origin populations (LEN, BRA, KIN and BOO) was identified with higher water content, expressed by lower reflectance in the SWIR. In fresh leaves, water exhibits strong absorption in wavelength regions located at 1,450 and 1,900nm from the SWIR region (Peñuelas & Filella, 1998). Indeed, several studies have accurately reported a strong correlation between the spectral reflectance between 1,650 and 1,850nm and the leaf water content (Tian et al., 2010), including MTC vegetation (Chakroun et al., 2015). The warm-dry population TOO was identified by the second component from comparing sites with the lowest leaf water content, in contrast to LEN (cool-dry), which displayed the highest water content. Comparison of seasons similarly identified a genotype effect in the SWIR features, although with some contrasting directions, as HIL (warm-dry) displayed the highest water content and SER (warm-wet) displayed the lowest.

Some subtle variations in spectral wavelength regions were highlighted by the PLSR loadings but were almost imperceptible to identify in plots of average reflectance spectra. However, these changes are still relevant to investigating the physiological responses of plants under biotic stress. Variation in red-edge wavelength regions, for example, was consistently

identified across most of the explored components from both PLSR comparisons of site and season as correlated with the genotype predictors. Although, because those variations were at such a small and subtle scale, it was challenging to identify in which exact direction the genotypes and populations were driving those variations to. For example, higher leaf chlorophyll content is associated with the red-edge position shifting to slightly longer wavelengths (Gates et al., 1965). For hyperspectral measurements, it is difficult to visually identify these subtle shifts in the location and variation of specific regions, such as the red-edge reflectance, driven by a variety of morphological, physiological or phenological leaf features that manifest the life history of the plant organism (e.g., pigments, leaf water content, leaf internal structure), and affect the patterns of scattering, absorption and reflectance of electromagnetic energy (Ollinger, 2011; Asner et al., 2011). The more specific narrow-band SIs can be deployed to reduce the effect of leaf structure, and other confounding factors (Chen et al., 2010; Li et al., 2014) while also diminishing the overfitting effect of full-spectrum analysis (Anderegg et al., 2020).

Climate of origin was related to variation in functional traits. The intraspecific spectral variation was further elucidated through univariate analysis on targeted SIs with known relationships with functional traits across the three unique environments: MU – summer, MR – summer and MR – autumn. However, a significant population effect was not observed in summer, and population differences were found only in the mildest site-season environment setting (MR – autumn). These SI results give weak support for the first hypothesis of populations showing genetic variation across different environments. Nonetheless, pairwise comparisons between populations within MR – autumn found significant differences between some populations across four measured SIs ($mNDVI_{705}$, WBI, NDRE and PRI). Both $mNDVI_{705}$ (Sims & Gamon, 2002) and NDRE (Barnes et al., 2000) indicate leaf chlorophyll content. Indeed, they were highly correlated with each other; thus, further analysis focused on $mNDVI_{705}$ as the SI indicative of chlorophyll content. The water band index (WBI; Peñuelas, 1993) is a SI developed to estimate leaf water content (Sims & Gamon, 2003). Both WBI and $mNDVI_{705}$ are widely used to assess plant drought and heat responses in MTC biomes (Claudio et al., 2006; Pu et al., 2003, Bussotti et al., 2015; Arevalo-Ramirez et al., 2020). Variation in these indices among populations found in a reciprocal transplant and common garden

experiment could indicate genetic differentiation in the photosynthetic and water retention/transpiration capacities.

Several populations seem to be driving differences for functional traits associated with leaf photosynthetic pigment and water content, (HIL, BRA, BOO, and CAP). Similarly, PLSR analyses also identified a group of populations with strong correlations with variation in spectral regions associated with photosynthetic pigments and water content (HIL, SER, BOO and CAP). Most of these populations represent the coastal north-south edges of marri distribution (see Figure 4.1), with very contrasting climate of origin: HIL – warm and dry, SET – warm and wet, CAP – cool and dry, BOO – cool and wet (Table 4.1 for climate details). It can be hypothesised that hot-dry summers, wide intra-annual temperature fluctuations and cold-wet winters might be selective factors driving different photosynthetic and water storage capacities. Noteworthy, the sample size for MR – autumn (n = 604) was much greater compared to either MR – summer (n = 339) or MU – summer (347), which might hamper a robust comparison between environments and hamper biological interpretations. Additionally, there was not a significant population effect during summer, thus the few differences found across MR – autumn were likely the result of phenotypic plasticity. Autumn was the mildest of the two measured seasons, and it is also possible that potential intraspecific differences might be masked under the stressful hot and dry summer conditions.

4.4.2 Environmental plasticity

Plasticity is a major driver for traits differentiation for marri growing in different sites and seasons. Both data analyses, PLSR and LMM on SIs, identified the '*environment*' effect as the strongest. The multivariate analysis of the full spectrum found that environment is a major predictor of spectral variation, based on strong *X*-loadings for the site and season terms. The comparison of sites was made under the hot-dry season of summer, and the MU site is in the hot-dry region of marri distribution, where summer conditions are more intense. Noteworthy in the comparison of sites is the higher reflectance across the visible region (500 – 700nm), and a subtle shift of the red-edge towards shorter wavelengths, recorded for plants growing in MU. This variation is potentially indicative of depressed content of photosynthetic pigments (i.e., chlorophyll pigments *a* and *b*), and consequently reduced photosynthetic capacity (Croft et al., 2017), being driven by the hot-dry site on growing marri plants. There

were also differences between sites across the strong water absorption features from the SWIR region (1,500 and 1,700nm), indicating that trees growing in MR had higher water content compared to trees from MU (Peñuelas & Filella, 1998). The comparison of season similarly found strong plasticity for the same spectral features, with plants growing during summer displaying reduced photosynthetic capacity. The season comparison also found strong variation across the NIR plateau, as well as across the strong water absorption features from the SWIR region (1,450 and 1,900nm), indicating that trees growing during summer had a lower water content compared to trees during autumn (Peñuelas & Filella, 1998). In the univariate analyses of SIs across both sites and seasons, the environment (E) effect was significant for all SIs, except PRI under site analysis. This consistent pattern, of strong environment effect across comparison of sites and seasons, strongly supports the second hypothesis that functional traits differ between sites and seasons. As expected, it finds the spatial and temporal environment conditions as major drivers for differential phenotypic expression.

Season effect was a stronger driver for traits variation than site effect, with mean values amplitude of SIs between seasons consistently larger than between sites for all measured traits. Under decreased rainfall conditions, typical of MTC summer seasons, deep-root water reserves are unlikely to be refilled (Milly, 1996), hampering trees' capacity to access water during prolonged drought conditions (Hahm et al., 2019). Seasonal plasticity in functional traits associated with heat and drought responses is common in MTC and semi-arid biomes (Zunzunegui et al., 2011; Dominguez et al., 2012; Bloomfeld et al., 2018; Puglielli & Varone, 2018). These include the adjustment of leaf physiological and morphological traits in response to short-term mild drought stress in eucalypts (Myers & Neales, 1984; Merchant et al., 2010; Pritzkow et al., 2020). The ability to adjust drought tolerance traits may enable plants to better cope with seasonally adverse conditions (Basu et al., 2016), such as the dry summer periods in MTC regions.

The measured traits (except RGI) exhibited similar tendencies across comparisons of site and season, with higher mean values recorded for the cool-wet site of MR compared to the warm-dry MU, and for the cool-wet season of autumn compared to warm-dry summer. There is some consistency with the PLSR analyses of site and season, where overall the warmer and

drier environments (MU and summer) drove variation in spectral reflectance associated with lower pigment and water leaf content. SIs indicative of photosynthesis capacity and efficiency ($mNDVI_{705}$ and PRI, respectively) and water management (WBI) responded similarly across both comparisons. The photochemical reflectance index, PRI, is a spectral physiological index that, due to its sensitivity to radiance-use capacity and the xanthophyll cycle, is an indirect measure of photosynthetic efficiency (Gamon et al., 1997; Garbulsky, et al., 2011). This index was also associated with water-use efficiency in a traits' correlation study in marri (Ahrens et al., 2019b). A typical response to drought stress is stomatal control to regulate water loss through transpiration (Anderegg et al., 2018), a process that can also interfere with photosynthesis efficiency (Way et al., 2004; Flexas et al., 2004). Marri has an excellent capacity for leaf osmotic adjustment leading to earlier stomatal closure during drought periods (Szota et al., 2011). The paralleled responses from different SIs, indicative of distinct functional traits, suggest that marri's capacity to perform photosynthesis and manage water content might be associated. Indeed, high efficiency in water management has been associated with high photosynthetic capacity in other eucalypt species (Whitehead & Beadle, 2004). Nevertheless, both site and season seem to be driving differences in the traits' responses, with warm and dry conditions driving distinct photosynthetic and water management capacity.

An exception for the paralleled responses pattern was observed for RGI under the comparisons of site, where trees from MU had a significantly higher mean value for this trait than MR. The red-green index, RGI, has been well correlated with anthocyanins/chlorophyll ratio (Gamon & Surfus, 1999), and consequently with the percentage of leaf discoloration in eucalypts (Coops et al., 2004). Anthocyanins are flavonoid red pigments known for their antioxidative function when plants are exposed to environmental stress (Hatier et al., 2009), particularly relevant to shield the photosynthetic mechanisms under extreme UV radiation and heat (Gould et al., 2018; Landi et al., 2021), hence the green to brown colour changes that typically occur after dry and warmer periods. A considerable RGI difference between seasons was observed, although with lower mean value during summer. The RGI direction found for the comparison of season, which is indicative of higher anthocyanins content during the cool-wet autumn (Gamon & Surfus, 1999) might be unexpected. The reason might be linked to the sampling time, given that the autumn season was sampled shortly after the end

of summer, when leaves might generate the highest content of anthocyanins shortly after the warm-dry period (Renner & Zohner, 2019). If marri leaves showed a higher concentration of this photoprotective pigment in early autumn, this could indicate that during the summer period the trees might have been able to maintain photosynthetic efficiency under exposure to increased UV radiation and heat (Steyn et al., 2002).

4.4.3 Local adaptation

There was evidence for local adaptation of functional traits in comparing sites. Multivariate PLSR modelling did not find strong associations between spectral reflectance response and the predictor interaction of population with environment. However, significant GxE interactions were found for all SIs under LMM analysis of site and season, potentially indicative of local adaptation. This supports the third hypothesis that functional traits expressed by different populations will vary differentially across sites and seasons.

The traits' responses were overwhelmingly plastic and followed the same direction across the comparison of seasons. A single exception for the seasonal trend was found for one population, BOO (cool-wet), on the measured RGI. The lower anthocyanins/chlorophyll ratio found for this population during early autumn might indicate depression, or at least a delay, in the production of the photoprotective pigment after the warm-dry summer season. Although growing in the same climate-origin region as MR (cool-wet), the BOO home site is located further south (Figure 4.1), at a cooler (T_{MAX} = 25.6°C) and lower altitude (75m) area compared to the plantation site of MR (T_{MAX} = 26.6°C; elevation = 130m). Anthocyanins' content is shown to increase significantly within the same species across short natural gradients of altitude and latitude (Spinardi et al., 2019), including at a MTC biome vegetation (Del Valle et al., 2015). Potentially this is an adaptive strategy for photoprotection at locations exposed to intense UV radiation (Lee & Gould, 2002). Thus, it can be hypothesised that this marri population might be locally adapted to a cooler and lower altitude region for this functional trait.

Cool-origin populations might be locally adapted towards the production of chlorophyll. Most populations followed a similar plastic response in chlorophyll content, with higher values at the cool-wet site than the warm-dry site. Previous physiological studies on marri (Aspinwall

et al., 2017), and other temperate eucalypt species (Crous et al., 2013), suggest that these trees commonly experience reduced rates of photosynthesis at warmer temperatures. However, a group of populations (BRA, CAP and PLA) from the cool-origin region exhibited a contrasting response, with greater $mNDVI_{705}$ at the warm experimental site (MU) compared to all other populations. One population in particular, PLA, showed a high slope of $mNDVI_{705}$ increase from the cool to the warm site. This red-edge index is a chlorophyll content indicator (Sims & Gamon, 2002), which in turn can indicate photosynthetic capacity (Blackburn & Ferwerda, 2008; Croft et al., 2017). These results potentially reflect population-wide acclimation in chlorophyll production under different climate gradients, but some cool-origin populations show contrasting responses. Provenances from cool-origin regions experience lower temperature and light exposure conditions in their southern native range. Increased leaf photosynthetic pigments content might be an adaptive response to efficiently absorb more light energy (Zhang et al., 2016) under unfavourable conditions.

Photosynthetic efficiency differed between populations and might be climate-origin dependent. Similar to chlorophyll production, a group of cool-climate populations (BRA, LEN, CAP and PLA) showed higher photosynthetic efficiency at the warm-dry site, expressed by the PRI (Garbulsky et al., 2011). The differential expression of PRI across sites is indicative of populations having different photosynthetic inhibition under diverse light conditions (Grace et al., 2007). Cool-origin populations also expressed increased capacity to maintain photosynthetic efficiency while growing at a warmer site. The increased anthocyanins/chlorophyll displayed by BRA, CAP, CAR and PLA (cool-origin provenances) from the cooler to the warmer site is expressed by the RGI. The biosynthesis of anthocyanins is also a tolerance mechanism to cold stress (Li et al., 2016; Sicilia et al., 2020) by preventing protein aggregation, which can cause freezing damage to membranes and tissues (Schulz et al., 2016). For these reasons, marri populations from cool-origins might have an innate increased capacity to produce these red leaf pigments under environmental stress. This adaptation might confer an advantage while growing in warmer and higher latitude sites, since anthocyanins also maintain the photosynthetic mechanisms under strong UV radiation and heat (Costa et al., 2015). Alternatively, it can also be hypothesised that trees from warm-climate origins showed less photosynthetic stress under warmer conditions, thus the reduced production of the photoprotective pigment. The results from the present study, and those

from previous investigations (Aspinwall et al., 2017; Ahrens et al., 2019b) indicate that some cool-origin marri populations might be well adapted regarding photosynthetic capacity and efficiency while growing in a warmer site.

Water management capacity between populations growing in different sites was mostly plastic, although some genotypes showed a local adaptive response. Most populations expressed an expected and similar trend of lower water content at the warm-dry site MU, compared to warm wet MR, measured by the WBI (Peñuelas, 1993). Interestingly, a warm-wet provenance (WHI) showed higher water content at the warm-dry site. Leaf water content is a major determinant for plant growth, development and survival (Carl & VanLoocke, 2015). The water balance in plants is dependent on a cyclic and complex mechanism, influenced by environmental factors, such as climate, and the efficiency of physiological processes such as evapotranspiration and photosynthesis (McElrone et al., 2013). Conversely, stable water content is crucial for efficient photosynthesis (Lawlor et al., 2002), making water loss unavoidable at the leaf surface level for the intake of CO_2 (Heinen et al., 2009). The response from WHI across the comparison of sites is intriguing. Higher water content in a warmer and drier site might indicate an exceptional adaptive capacity to retain water through physiological leaf traits (i.e., evapotranspiration and stomatal conductance) during adverse conditions (Zavaleta et al., 2003).

4.4.4. Implications for tree breeding and conservation management

Marri showed high plasticity and limited genetic contribution for functional traits variation. The findings agree with previous greenhouse-scale common garden studies with marri provenances. For example, Ahrens et al., (2021c) also showed high levels of physiological plasticity from eight marri populations in response to variable heatwave severity through the enhanced capacity for thermal tolerance, compared to a few genotypic effects. However, the short-term physiological plasticity measured in a greenhouse study has limited applicability, since the results are extrapolated to trees growing in native natural environments. Despite the numerous advantages of controlled environments for stress experiments (Telfer et al., 2018), it has been shown that plants respond differently to stress under field and controlled conditions (Sprenger et al., 2016). Thus, expanded approaches to accurately quantify and validate plant stress-responses are needed in the field (Hartman et al., 2014) such as the

outcomes from the present study. However, in the present study, the time scales of stressors, and the stress response traits evaluated, should also be acknowledged as a limitation. For the most part, the natural climate variation (associated with site and seasonal variation) has been applied over a long time (since plantation sites establishment in 2014) – either by a gradual change between seasons or, for the site effect, the individual's whole life-history (c. 3 to 5 years) is related to the same site. This time frame allows the plants to acclimate to the conditions (Valladares et al., 2000; Niinemets & Valladares, 2004), and therefore, to exhibit a plastic response once measured. Thus, there could be more explicit drivers for trait variation that were not assessed here, such as heatwaves and prolonged intense droughts.

Phenotypic plasticity and genetic adaptation of important heat and drought tolerance plant traits may facilitate the resistance and resilience in marri trees against future warming and drought events. Genetic diversity and plastic responses within a species enable breeding populations to better adapt, or acclimate, to changing environments and selection pressures (Stoutjesdijk, 2013). Breeding programs normally pursue access to genetic resources from the natural wild populations, or from *ex situ* supplies created to store selected genetic material, such as seed banks or tissue cultures. Typically, several objectives are pursued simultaneously for a breeding program for any species or population (Stoutjesdijk, 2013), including the adaptive capacity to environmental stresses. Highly plastic genotypes for functional traits may confer mechanisms for adjustment in changing environments (Arnold et al., 2019). Marri populations showed high plasticity for functional traits associated with heat and drought tolerance, for example, in adjusting photosynthetic pigments and water content under distinct climate settings. Once the populations or individuals with the best traits have been identified, a breeding program may commence to select and genetically improve the material, for example, through cross-pollination to introduce desirable characteristics.

Populations at risk of climate change with minimal physiological tolerance and/or phenotypic plasticity may benefit from introducing genetic material from preadapted populations, known as assisted gene migration (AGM; Aitken & Whitlock, 2013). As temperatures rise, populations are moving pole-ward and leading-edge populations may benefit from introducing genetic material from trailing edge populations (Davis & Shaw, 2001). Risks of AGM include outbreeding depression (reduced fitness in offspring due to hybridisation with other

populations) (Aitken & Whitlock, 2013); however, this risk is negligible when there is not taxonomic uncertainty, recent geneflow and not disparate environmental differences (Frankham et al., 2017). Importantly, marri has high levels of gene flow among populations, therefore is not at risk of outbreeding depression from AGM (Sampson et al., 2018; Ahrens et al., 2019a), which might also explain the limited genetic effects observed in this study. Marri showed high plasticity for functional traits associated with heat and drought tolerance, in the present study and previous research (Aspinwall et al., 2017; Blackman et al., 2017; Ahrens et al., 2019b; Challis et al., 2020), which is indicative of some capacity to cope with climate change. Nevertheless, several factors, including widespread distribution and high gene flow associated with climatic variables, make it a potential candidate for AGM if this practice is deemed necessary in the future.

4.4.5. Conclusions

Intraspecific adaptation into climate in Mediterranean-type forest trees can be assessed using multi-spatial and temporal spectroscopy data. This study showed marri trees can adjust functional traits through genetic adaptation, but mostly phenotypic plasticity. Overall, some marri cool-origin populations showed adaptive capacity with optimized photosynthetic properties and efficiency under warmer and drier site conditions. The capacity for endemic foundation trees to adjust plant traits associated with environmental stressors through genetic adaptation and phenotypic plasticity may enhance tolerance to drying and warming environments under climate change.

The use of leaf reflectance to discriminate plant traits is not a novelty (Asner et al., 2011; Ball et al., 2015), but assessing genetic differences in plants growing in natural common gardens is less common. Other research exploring the genetic contribution to leaf spectral variation (e.g., O'Reilly-Wapstra et al., 2013; Cavender-Bares et al., 2016; Čepl et al., 2018), and the present study illustrate the potential to use hyperspectral sensors to obtain discriminative chemical and physiological characteristics by species genotypes. A multi-analyses approach used both full spectra and spectral subsets related to targeted plant traits to detect intraspecific genetic diversity. Multivariate PLSR methods can be applied to overcome collinearity and redundancy challenges on the leaf hyperspectral signature acquired under natural conditions at different sites and seasons. Moreover, this method successfully

detected the spectral regions most associated with genotype variation, optimizing the streamlined univariate analysis of traits that detected evidence for local adaptation.

Remote-sensed spectroscopy delivers rapid, cost-effective, standardized and easily replicated data to generate extensive information regarding adaptive strategies than traditional measurements of individual traits (Danusevicius et al., 2014; Czyż et al., 2020), allowing to better describe the variability of functional traits across distinct spatial and temporal scales Similarly to other remote-sense studies on species genetic diversity, the outcomes suggest that a multi-analyses approach with hyperspectral data is a robust tool to trace genetic variation across environmental gradients directly and efficiently.

4.5. Supplementary material

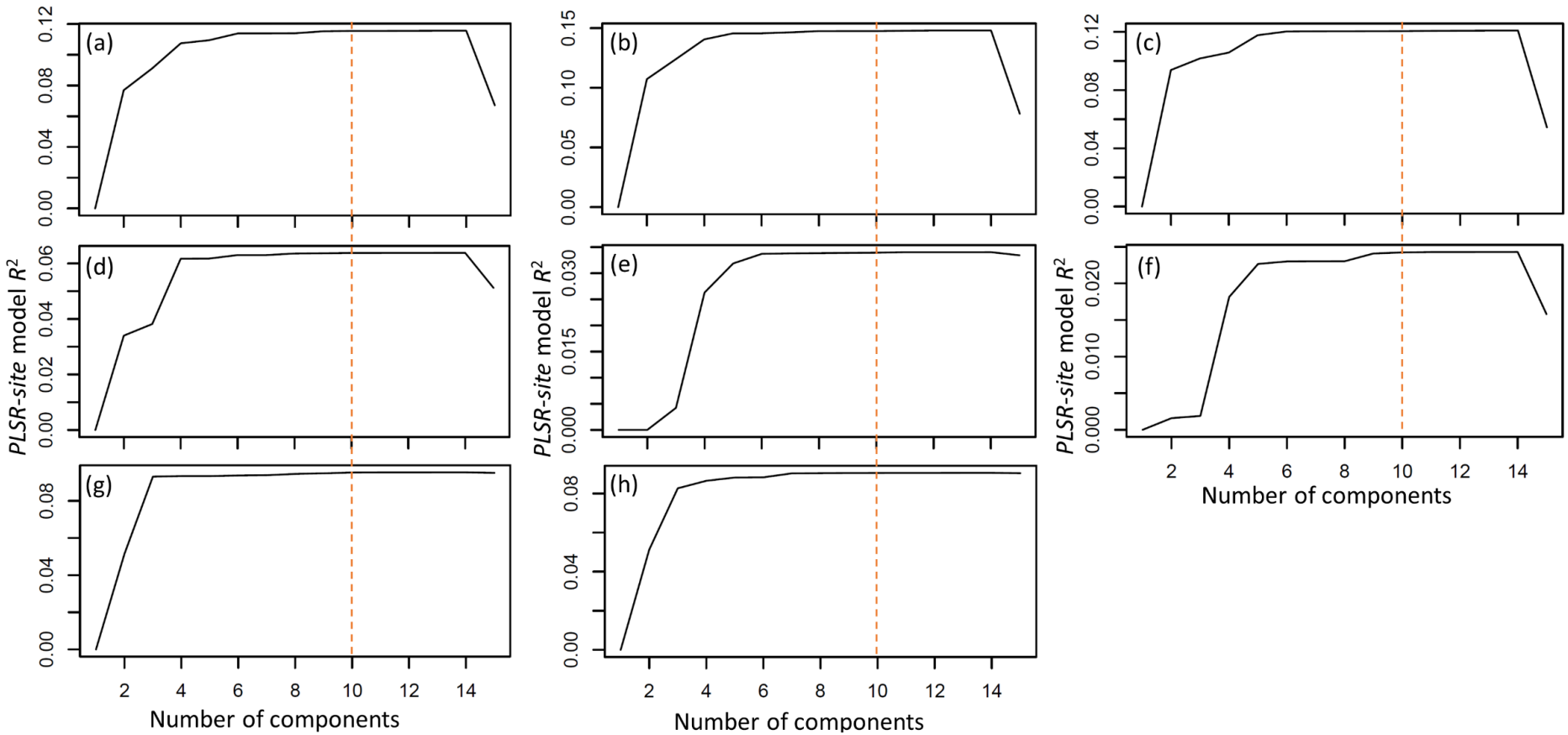


Figure S1a. PLSR model components diagnostics based on the coefficients of multiple determination (R^2) for the *PLSR-site* builds of key wavelength regions: a) green (551nm), b) yellow (615nm), c) red (676nm), d) red-edge (725nm), e) NIR1 (870nm), f) NIR2 (979nm), g) SWIR1 (1,653nm) and h) SWIR2 (2,223nm). The dashed orange line denotes the optimal number of components to maximize explanatory capacity across the full spectra.

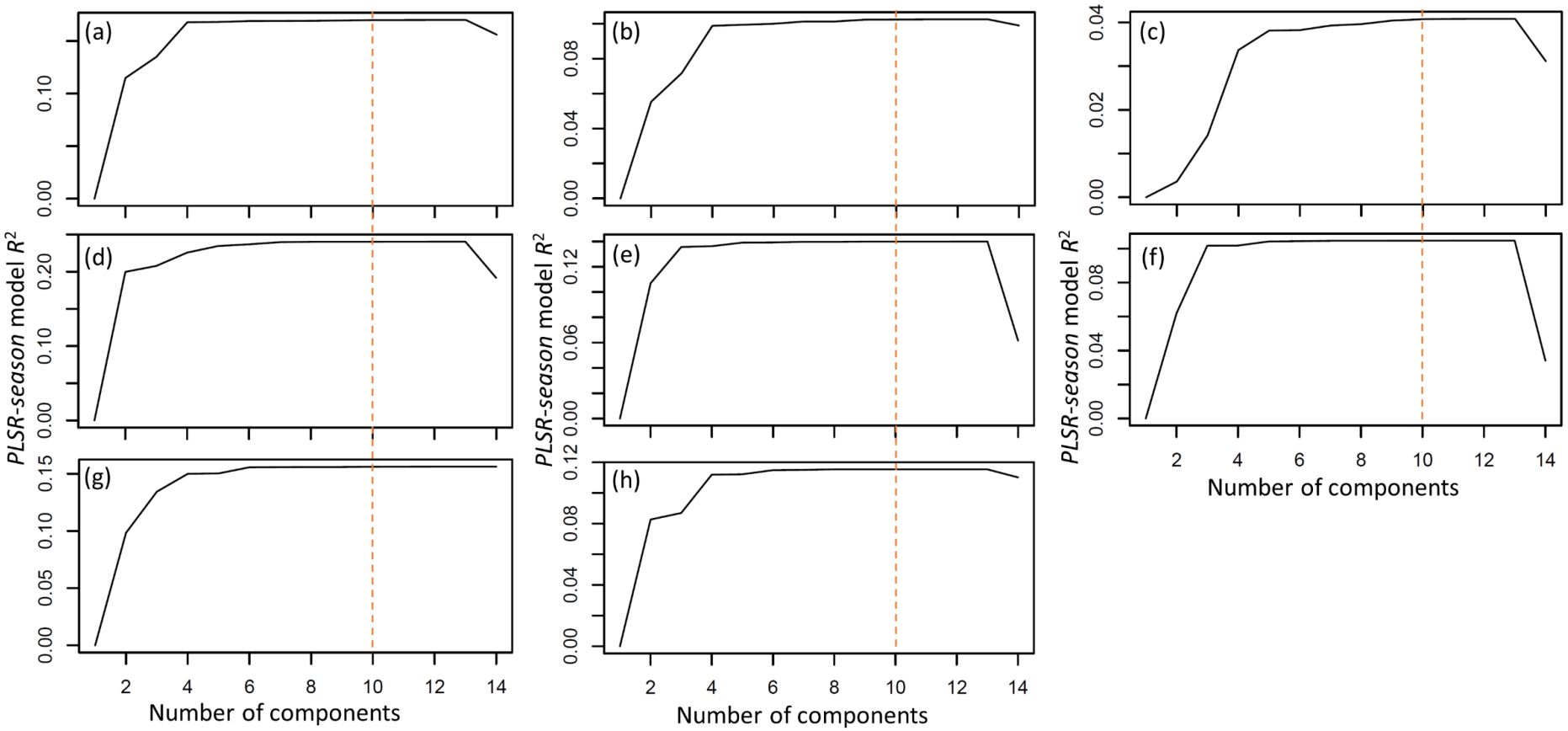


Figure S1b. PLSR model components diagnostics based on the coefficients of multiple determination (R^2) for the *PLSR-season* model builds of key wavelength regions: a) green (551nm), b) yellow (615nm), c) red (676nm), d) red-edge (725nm), e) NIR1 (870nm), f) NIR2 (979nm), g) SWIR1 (1,653nm) and h) SWIR2 (2,223nm). The dashed orange line denotes the optimal number of components to maximize explanatory capacity across the full spectra.

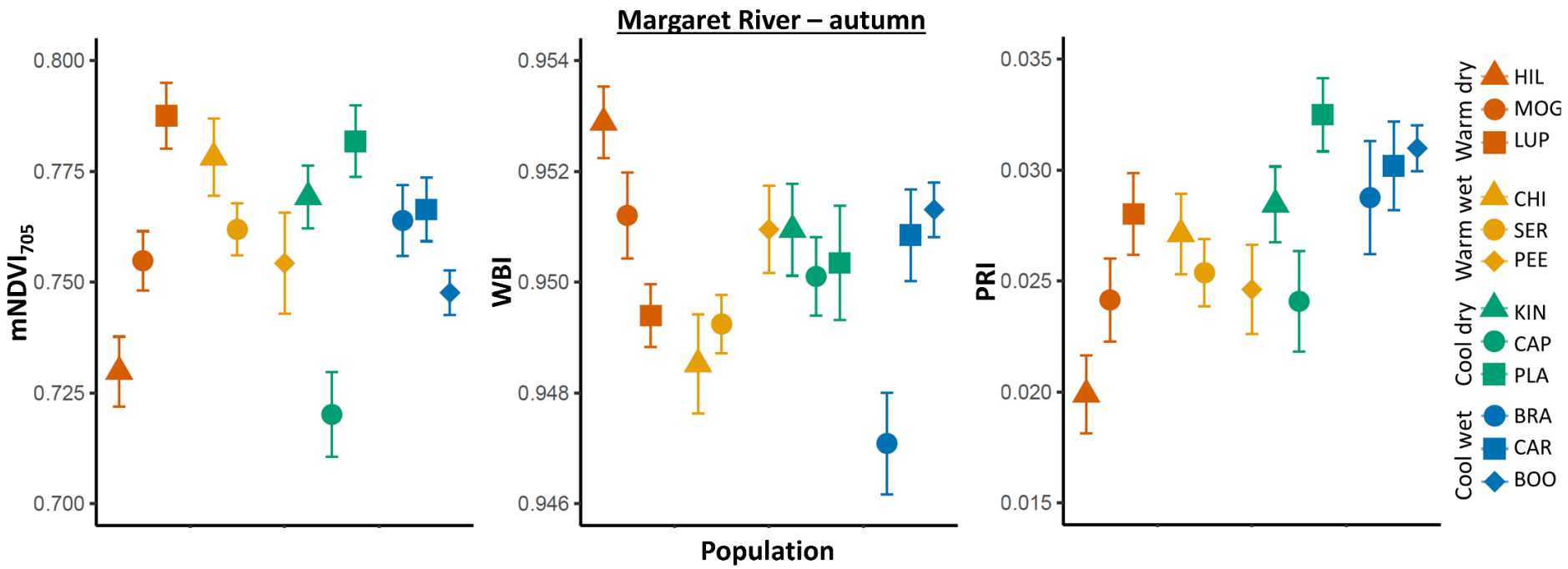


Figure S4.2. Mean (± 1SE) spectral indices values for marri across Margaret River (MR) – autumn. Spectral indices abbreviations: $mNDVI_{705}$ – modified normalized difference vegetation index; RGI – red-green index; WBI – water band index; NDRE – normalized difference red-edge; PRI – photochemical reflectance index. See Table 4.1 for populations details.

Table S4.1. Pearson's correlation coefficients (statistical significance of $p < 0.01$) of spectral indices derived from marri leaf spectral data of analysis of site (upper triangular part) and analysis of season (lower triangular part). Strongly correlated pairs ($r > 0.7$) are shown in bold. Spectral indices abbreviations: $mNDVI_{705}$ – modified normalized difference vegetation index; RGI – red-green index; WBI – water band index; NDRE – normalized difference red-edge; PRI – photochemical reflectance index.

	$mNDVI_{705}$	**RGI**	**WBI**	**NDRE**	**PRI**
$mNDVI_{705}$	-	0.31	0.44	**0.92**	0.49
RGI	0.41	-	0.06	0.34	0.04
WBI	0.18	0.22	-	0.40	0.35
NDRE	**0.87**	0.38	0.15	-	0.45
PRI	0.47	0.10	0.09	0.44	-

Table S4.2. Population pairwise (Tukey *post-hoc*) *p* values to inform significant differences between populations within MR – autumn for measured traits. Home population (BRA) is shown first. Spectral indices abbreviations: $mNDVI_{705}$ - modified normalized difference vegetation index; WBI – water band index; NDRE – normalized difference red-edge; PRI – photochemical reflectance index. Significant *p* values are shown in bold.

MR – autumn		**$mNDVI_{705}$**	**WBI**	**NDRE**	**PRI**
Population pair		Pairwise *p* value	Pairwise *p* value	Pairwise *p* value	Pairwise *p* value
BRA	CAR	1	0.1384	1	1
BRA	BOO	0.9775	**0.0071**	0.9792	1
BRA	KIN	1	0.1249	1	1
BRA	PEE	1	0.1543	1	0.9262
BRA	SER	1	0.6699	1	0.9438
BRA	LUP	0.8343	0.794	0.6406	1
BRA	PLA	0.9813	0.3551	0.9987	0.9998
BRA	CHI	0.9918	0.9919	0.8915	0.9996
BRA	MOG	1	0.0612	1	0.8677
BRA	CAP	0.0929	0.2447	**0.0464**	0.8769
BRA	HIL	0.377	**<.0001**	0.5035	0.1574
HIL	MOG	0.8178	0.9393	0.913	0.9978
HIL	LUP	**0.0011**	0.0824	**0.0006**	0.4888

HIL	CHI	**0.0132**	**0.0076**	**0.004**	0.7069
HIL	SER	0.1599	**0.0056**	0.2004	0.8176
HIL	PEE	0.8493	0.9021	0.8729	0.9975
HIL	KIN	0.1651	0.8736	0.3411	0.5316
HIL	CAP	0.9995	0.1138	0.9719	0.9594
HIL	PLA	**0.011**	0.5608	0.0626	**0.0192**
HIL	CAR	0.2372	0.8064	0.3562	0.0831
HIL	BOO	0.9407	0.7799	0.9827	**0.0095**
MOG	LUP	0.4298	0.9595	0.2281	0.9918
MOG	CHI	0.84	0.6313	0.4973	0.9993
MOG	SER	1	0.8019	0.9997	1
MOG	PEE	1	1	1	1
MOG	KIN	0.9977	1	0.9993	0.9932
MOG	CAP	0.3739	0.9967	0.2413	1
MOG	PLA	0.7805	1	0.9236	0.4092
MOG	CAR	0.9996	1	0.9996	0.7435
MOG	BOO	1	1	1	0.44
LUP	CHI	1	0.9999	1	1
LUP	SER	0.6169	1	0.4684	0.9995
LUP	PEE	0.5498	0.9917	0.4172	0.997
LUP	KIN	0.9735	0.9897	0.816	1
LUP	CAP	**0.0001**	1	**<.0001**	0.9955
LUP	PLA	1	0.9999	0.9948	0.9793
LUP	CAR	0.9263	0.9937	0.7694	0.9997
LUP	BOO	**0.0462**	0.8251	**0.0171**	0.9966
CHI	SER	0.9625	0.9999	0.8048	1
CHI	PEE	0.9009	0.8192	0.7084	0.9998
CHI	KIN	0.9999	0.791	0.9677	1
CHI	CAP	**0.0014**	0.9665	**0.0001**	0.9998
CHI	PLA	1	0.9679	1	0.9239
CHI	CAR	0.9987	0.8231	0.9526	0.9962

CHI	BOO	0.2669	0.3003	0.0752	0.9713
SER	PEE	1	0.9442	1	1
SER	KIN	1	0.9283	1	0.9996
SER	CAP	**0.0235**	0.9983	**0.0069**	1
SER	PLA	0.9315	0.998	0.9975	0.4906
SER	CAR	1	0.9471	1	0.8464
SER	BOO	0.922	0.3536	0.877	0.4799
PEE	KIN	0.9991	1	1	0.9975
PEE	CAP	0.439	0.9998	0.2243	1
PEE	PLA	0.8544	1	0.9785	0.537
PEE	CAR	0.9999	1	1	0.838
PEE	BOO	1	1	0.9999	0.6054
KIN	CAP	**0.0299**	0.9998	**0.0229**	0.9965
KIN	PLA	0.9994	1	0.9999	0.9824
KIN	CAR	1	1	1	0.9998
KIN	BOO	0.8449	1	0.9224	0.9974
CAP	PLA	**0.0012**	1	**0.002**	0.3825
CAP	CAR	**0.0475**	0.9999	**0.0238**	0.7423
CAP	BOO	0.4978	0.9502	0.3148	0.371
PLA	CAR	0.9959	1	0.9998	1
PLA	BOO	0.2243	0.9994	0.4669	1
CAR	BOO	0.9218	1	0.9343	1

Chapter 5.

General Discussion

5.1. Synthesis of aims and predictions

Assessing the ability of trees to cope with climate change is necessary for the management of forest health, productivity, and biodiversity. Mainstream species distribution models rely greatly on occurrence data, and not often consider adaptation due to selection within local populations, or plasticity due to acclimation of individuals to local environment over their lifetime (Hoffmann & Sgrò, 2011). Forecasting future responses, such as shifts in ranges, could be improved by assessing species biological characteristics that are currently missing, such as genetic adaptive profiles and the physiological tolerance thresholds. To address these fundamental and framework gaps, this project applied a comparative multi-species vulnerability assessment with wild provenances to summarize relevant information to determine a species, and populations within species, potential vulnerability to climate change.

Jarrah and marri are ideal tree species for studies of genetic adaptation and phenotypic plasticity of traits, especially for a comparative approach of endemic species since they share the same native distribution. The genetic diversity and structure of marri have been evaluated in a recent study of twenty-seven populations using microsatellite markers (Sampson et al., 2018) that showed high diversity and little genetic structure with isolation by distance across the distribution. An investigation of adaptive capacity in marri found evidence for adaptation to climate, primarily temperature (Ahrens et al., 2019a). Three provenance trials of marri have previously been established at three different sites in Western Australia. These trials contain marri trees from 18 locations throughout its distribution. Surveys and trials have been conducted at these sites to estimate the growth rates, overall health score, and physiological characteristics such as leaf water transport capacity and leaf hydraulic vulnerability.

Overall, the present study explored the mechanisms facilitating genetic adaptation and physiological tolerance to warming temperatures and drought required for population persistence in a rapidly changing climate.

5.2. Summary of key findings

5.2.1. Adaptive genetic variation associated with current climate gradients

The genome-wide association study (Chapter 2) identified putative patterns of climate adaptation in jarrah, with strong associations between candidate genetic variants and climatic predictors. The results indicate local adaptation to climate across the distribution of jarrah, with both temperature and precipitation variables driving those patterns. Annotation showed functional genes associated with biological processes, some of which relate to abiotic stress factors and provide good candidates for adaptations. Furthermore, the landscape genomics modelling assessed the magnitude of allelic turnover for putatively adaptive single nucleotide polymorphic (SNP) markers. It highlighted temperature seasonality, mean maximum temperature of the warmest month, and precipitation of the warmest quarter as explaining significantly more variation than other climate drivers. Overall, both temperature and precipitation variables were linked to adaptive genetic variants; although, gene ontology (GO) and genomic landscape analysis highlighted precipitation of the warmest quarter as a stronger driver of adaptation. Biological processes related to abiotic stress responses such as drought, cold, and heat were explored in the GO enrichment analysis. Generally, biological GO terms with gene overrepresentations were found, indicative that functionally related genes have similar patterns of correlation with climate. Across jarrah's geographic distribution, climatic heterogeneity explained significant genomic variation. The patterns of genomic turnover associated with the studied climatic variables are aligned with the temperature and precipitation gradients of the region. These associations indicate the multidimensional patterns of adaptation resulting in uncorrelated intraspecific selection among genetic variants.

5.2.2. Seed performance under warming conditions

Seed germination in marri and jarrah (Chapter 3) was cued by cooler temperatures, with high levels of germination found for both species in cooler (15°C) conditions. Both species germinated over a wide range of temperatures (c. 10 to 30°C), although jarrah showed a much lower optimum temperature for germination (Topt = 15.3°C) and narrower germination niche (<9°C to 20°C) compared to marri (Topt = 21.2°C and niche 9°C to 27°C). Marri's annual

germination window is currently limited by available rainfall during summer, while jarrah is constrained by both rainfall and temperature during the warm season. Overall, northern warm climate-origin marri populations have a shorter annual germination window (6-7 months) than southern cool-origin populations (8-9 months). Similarly, jarrah's current germination window excludes the summer season, although it is limited by current monthly maximum temperatures as well, even for the months when rainfall would be appropriate. While cool-origin jarrah populations have a similar germination window of eight months, the time window for germination is highly variable among jarrah populations from warm-origins (4 to 8 months).

Marri's populations showed strong thermal tolerance with signatures of local adaptation in germination temperature response. The estimated thermal threshold for marri's populations showed a high positive correlation with a temperature variable of origin, indicative of local adaptation to a climate gradient. Jarrah's temperature niche did not relate to the climate of origin. Contrastingly to marri, the co-occurring jarrah showed a weak correlation between the population's ED50 (temperature to achieve 50% germination) and a current temperature gradient. Additionally, jarrah's germination window is predicted to decline further with future warming across its range. Nevertheless, the northern population of Hill River (HIL) showed enhanced thermal limits to germination and potential adaptive capacity for the species to persist with climate warming.

5.2.3. Variability of plant functional traits

Environmental plasticity is a major driver for traits differentiation for marri growing in different sites and seasons (Chapter 4). The season effect was a stronger driver for variation in functional traits indicative of photosynthetic capacity and water management than the site effect. The multivariate analysis of the full spectrum found that environment is a major predictor of spectral variation. The variation found is potentially indicative of depressed content of photosynthetic pigments, and consequently reduced photosynthetic capacity being driven by the hot-dry site (Mundijong) on marri plants. There were also differences between sites across the strong water absorption features, indicating that trees growing in the cooler and wetter site (Margaret River) had higher water content than trees from the warm-dry site's conditions. The comparison of season similarly found strong plasticity for the

same spectral features, with plants growing during summer displaying reduced photosynthetic capacity and lower water content than trees during autumn.

Significant GxE interactions were found for all measured traits under the analysis of site and season, potentially indicative of local adaptation. Cool-origin populations showed some adaptive capacity towards the production of chlorophyll. Although, most populations followed a similar plastic response in chlorophyll content, with higher values at the cool-wet site than the warm-dry site. Water management capacity between populations growing in different sites was mostly plastic, although some genotypes showed a local adaptive response.

5.3. Species and populations are differently adapted to climate

Eucalypts have poor dispersal capacities, and it is expected that, rather than moving with a changing climate, the standing adaptive capacity of eucalypts will determine survival in their current location (Booth et al., 2015). Additionally, the jarrah-marri distribution is bound by an ocean barrier to the west and south and desert land to the east. Therefore, their range has strong geographic constraints to the capacity to migrate poleward (in this case, southward) to track the pole-ward shift in climate that has been observed or predicted in other species (Colwell et al., 2008; Lenoir et al., 2008; Lenoir & Svenning, 2015). Hence, marri's and jarrah's capacity to tolerate climate change will greatly depend on genetic adaptation and/or phenotypic plasticity to changing temperatures and rainfall regimes. It has been shown in previous investigations into the genetic variability of three widespread eucalypts (*Eucalyptus salubris*, *E. tricarpa* and *E. loxophleba*) (Byrne et al., 2013) that these species are likely to have some capacity to respond plastically to a changing climate.

The study of standing genetic variation across the distribution of jarrah in Chapter 2 found potential links between putatively adaptive SNPs and climate factors, which may provide a source of adaptation to future climate conditions. The evidence that genetic variants associated with climate adaptation was highlighted as either associations with specific annotated gene functions, or biological processes associated with climate factors. The analysis here, and that of the co-dominant species marri, are also consistent with results from recent genomic studies on other eucalypt species in other regions of Australia (Steane et al., 2017; Jordan et al., 2017, 2020), providing evidence of adaptation to climate in natural populations and stressing the role of temperature and precipitation variables. Comparative analysis can provide broader patterns for forest management, where concurrent genetic and spatial patterns of local adaptation within co-occurring tree species provides strong evidence for environmental fitness and evolution (Bragg et al., 2015). The analysis here (Chapter 2) identified SNPs associated with both temperature and precipitation in jarrah; while a similar study on a co-occurring species, marri, found SNPs associated with temperature to explain more deviance than precipitation (Ahrens et al., 2019a), thereby suggesting that temperature is a stronger driver of local adaptation for marri. While this comparison shows that some

functional genes share adaptive patterns, there were more genes that were different, indicating that the same adaptive management plan may not be effective for both species.

This work also contributes important and unique insights into inter and intraspecific variation of germination temperature response under warming temperatures for jarrah and marri. Plant populations' persistence relies mainly on *in situ* responses to environmental stressors, which may represent physiological constraints on the species recruitment. In such context, the range of the germination niche, particularly the range of the germination temperature window, might be affected. Recruitment constraints may provide a measure of susceptibility to climate change: species that only germinate over a narrow temperature window are potentially most at risk. Temperatures outside that window might drive very little, or no opportunity for germination. Jarrah was found to have reduced tolerance to high temperatures, and since the present study's framework incorporated populations from contrasting climate-origin provenances, it is possible to profile population-specific temperature threshold predictions from distinct climate-origins across the species range. For example, in the northern jarrah population of Mogumber (MOG), the current temperature window of germination in an average season would be only 4 months in jarrah, compared to 7 months in marri. In contrast to jarrah, marri appears to have a higher thermal limit for germination and more variation among populations, indicating it is less vulnerable to a warming climate.

Jarrah has demonstrated a similar variable response to warming temperatures as other woody (Cochrane, 2016, 2017) and non-woody (Dwyer & Erickson, 2016) species from the SWWA region. Despite observed declines in germination under the highest temperatures across all sampled populations, marri is not expected to suffer major germination depression due to predicted future changes in temperature in SWWA. The germination predicted for 2061-2080 was the same in 6/12 populations, and the modest contraction in the time for germination (-1 month) was driven by reduced rainfall rather than thermal limits of germination. Since this species has high levels of gene flow and connectivity across its range (Sampson et al., 2018), marri might be well equipped to persist under intensifying warming for the next few decades.

The findings from Chapter 4 which investigated functional traits variation across contrasting sites and seasons in marri populations indicate that this species has some capacity to respond to warmer and drier environments through heritable genetic adaptation, but mostly phenotypic plasticity. Similarly, several growth, leaf, and structural traits and resistance to climate stressors and leaf blight pathogens were found to be heritable in marri trees growing at the common garden sites (Ahrens et al., 2019b, 2020). Therefore, such plant traits may be under selection pressure from the environment. Also, in Chapter 4, some marri cool-origin populations showed adaptive capacity with optimized photosynthetic properties and efficiency under warmer and drier site conditions. This greater performance in cool-origin marri populations under water deficit and warmer conditions relative to other climate region populations is surprising considering that cool region trees growing in their home environment generally lack exposure to high temperature selection pressures common to populations from warm climate regions. Importantly, marri overall responded to variable environmental settings with high phenotypic plasticity responses, which might allow the species to persist under climate change. Seasonal plasticity in functional traits include the adjustment of leaf physiological and morphological traits in response to short-term mild drought stress in eucalypts (Myers & Neales, 1984; Merchant et al., 2010; Pritzkow et al., 2020). The ability to adjust drought tolerance traits may enable plants to better cope with seasonally adverse conditions (Basu et al., 2016), such as the dry summer periods in MTC regions. Under decreased rainfall conditions, typical of Mediterranean-type climate (MTC) summer seasons, deep-root water reserves are unlikely to be refilled (Milly, 1996), hampering trees' capacity to access water during prolonged drought conditions (Hahm et al., 2019).

5.4. Study implications for forest management

The genome-wide associations in jarrah between genetic variants and climatic factors were found despite high levels of gene flow among populations across the distribution, a common characteristic among eucalypt species (Supple et al., 2018; Murray et al., 2019; Ahrens et al., 2019a). In addition, phenotypic approaches on other eucalypt species have highlighted the role of local climate in selecting for adaptive traits (Costa e Silva et al., 2019; Ahrens et al., 2019b, Ahrens et al., 2021b). Comparative analysis can provide broader patterns for forest management, where concurrent genetic and spatial patterns of local adaptation within co-

occurring tree species provide strong evidence for environmental fitness and evolution (Bragg et al., 2015). The results are comparable with the similar approach by Ahrens et al., (2019a) on marri. The study on marri found SNPs associated with temperature to explain more deviance than precipitation (Ahrens et al., 2019a), suggesting that temperature is a stronger driver of local adaptation. Interestingly, there were similarities in functional genes associated with several adaptive variants between jarrah and marri (e.g., ABC transporters and CBL gene families). Comparison across both species identified a set of 26 genes associated with at least one of the five variables analysed. Most of these shared genes are associated with either temperature or precipitation in jarrah. While for marri, most of the shared genes are associated with temperature, which indicates adaptation driven by temperature and precipitation in jarrah, but mostly by temperature in marri. While this comparison shows that some functional genes share adaptive patterns, more genes were different, indicating that the same adaptive management plan may not be effective for both species.

Tree species and their populations are commonly genetically adapted to the historical local climate conditions where they occur (Kramer et al., 2015; Alberto et al., 2013). Considering local genetic adaptation and differentiation of populations, conservation management approaches are frequently ruled by seed zones or seed transfer boundaries to diminish the risk of maladaptation, based on the supposition that local seed sources are best adapted (O'Neill and Aitken, 2004; Bradley St Clair et al., 2007; Frank et al., 2017). Most forestry authorities in Australia have traditionally divided their forested land jurisdictions into edaphically, climatically, and ecologically similar zones, and only seed sources that originate from these regions may be planted within the same region (CCWA, 2013). However, static geographic seed zone systems may no longer be a practical long-term management approach under climate change, due to the underlying assumption that local seed sources should be optimally adapted to their respective origin provenance (Benito-Garzó & Fernández-Manjarrés 2015; Messier et al., 2019). Broadhurst et al., (2008) suggested that sourcing local seeds only might result in reduced quality restoration practices, especially in the context of wider geographical scales with heterogenous patterns of rapid climate change. For example, average rainfall in the SWWA region has already declined by 15-20% in the last 30 years (Matusick et al., 2013; Brouwers et al., 2013), which can cause a mismatch between the genetic adaptations of local populations and new climate conditions (Browne et al., 2019;

Etterson et al., 2020). This mismatch has already caused major impacts to forest health and productivity in Western Australia (Matusick et al., 2016, 2018). Moreover, the germination results from this thesis indicated that jarrah's temperature niche did not relate to the climate of origin, which might indicate an adaptational lag between physiological tolerance to temperature and current warming rates experienced in the species range. Thus, part of the management approaches can be to increase the seed source's genetic diversity to enhance the adaptive potential in climate change scenarios and reduce the risk of maladaptation (Carvalho et al., 2020; Hoffmann et al., 2021). In the context of forestry, this can be implemented by assisted gene migration (AGM) as part of reforestation activities in managed forests (McLane & Aitken, 2012; Aitken & Bemmels, 2016) through climate adjusted provenance (i.e., sourcing of seed from populations in the direction of climate change for use in restoration sites to enhance adaptation to future climate) in restoration practices (Prober et al., 2015; Pedlar et al., 2021). This approach might increase the likelihood that climate conditions to which local populations are adapted match current and future growing environments, and thereby maintain forest health and productivity for the coming decades. Populations at risk of climate change with minimal physiological tolerance and/or phenotypic plasticity may benefit from introducing genetic material from preadapted populations (AGM; Aitken & Whitlock, 2013). Assisted migration prescriptions within and outside of current species ranges are already being implemented, for example, with coniferous trees in the northern hemisphere, by moving some species and their populations (e.g., western larch - *Larix occidentalis,* in western Canada) to more northern locations or higher elevation bands across seed zone boundaries (NRCAN, 2020), to compensate for observed and projected climate change (O'Neill, 2017)

As foundation trees, jarrah and marri are vital components in the ecosystem with a significant role in regulating local hydrological systems and carbon storage (CCWA, 2013; Bradshaw, 2015). Additionally, they offer abundant habitats for a wide variety of groups, from vascular flora and lichens to terrestrial vertebrates and birds (Whitford & Williams, 2002; Whitford et al., 2015), as well as unique food sources for fauna, especially birds (Wrigley, 2012; Lee et al., 2013). The Forest Management Plan 2014–2023 (CCWA, 2013) for SWWA forests has provision for AGM in management strategies of ecologically important tree species for responding to climate change. The present study (Chapter 2) found standing variation to

harbour putative adaptations to climate associated with temperature and precipitation factors in jarrah, which may be the source of genetic material for assisted migration. Moreover, the furthest north jarrah population of Hill River (HIL) showed enhanced thermal limits to germination and potential adaptive capacity for the species to persist with climate warming (Chapter 3). With recent observations of jarrah dieback caused by disease (notably *Phytophthora cinnamomi*) and impacts of extreme events, such as heatwaves (Matusick, 2016, 2018), HIL may be a valuable source of thermal adaptive capacity for other regions of the northern jarrah forest. Given that this population is geographically and genetically isolated (Chapter 2), natural dispersal may be limited and active management strategies such as AGM may be needed (Aitken & Bemmels, 2015).

Australia's current forestry management heavily relies on *in situ* conservation as the main mechanism for the conservation of forest genetic resources, but also has *ex situ* programs for a range of forest trees and other plant species (Lott & Read, 2021). *Ex situ* conservation is an essential complement to *in situ* conservation approaches (Guerrant et al., 2004) and allows genetic resources for current or future use to be captured and managed, especially when natural populations are small and/or threatened. For example, Coates et al., (2015) compared *ex situ* seed collections and three extant geographically disjunct populations of the rare SWWA endemic species *Banksia browniii*. They found that a substantial proportion of gene diversity and allelic richness had been lost within few decades due to the introduced pathogen *Phytophthora cinnamomi*. Programs for *ex situ* conservation of forest species include living collections, conservation stands and seed banks. Seed banks are invaluable worldwide resources for *ex situ* genetic diversity conservation and are increasingly utilized as a part of the management to protect threatened flora (Walters, 2015). Seeds for several SWWA woody species, including eucalypts, have been stored in seed banks for nearly 30 years without significant loss of viability (Crawford et al., 2007). The jarrah population of HIL showed a noteworthy thermal adaptive capacity that might be of potential conservation interest. The *ex situ* preservation of this population's germplasm might be crucial for jarrah's conservation management under future climate change. While marri populations may have enhanced capacity to persist in the wild without active conservation management, thermal adaptation in germination (among other functional and physiological traits; Ahrens, 2019b, 2021)

provides a valuable genetic resource for restoration and forestry to establish diversity and resilient forests for the future.

Based on the present study which investigated adaptive and plastic responses across marri populations, AGM may be a good management option for this species, if necessary, in the future, but at present, may not be required. Nevertheless, recent studies have shown evidence of dieback in marri in response to heatwave and drought events (Poot & Veneklaas 2013; Matusick et al., 2018), suggesting that it is vulnerable to climate change-associated events. The absence of standing genetic diversity in conservation management techniques such as AGM has become increasingly acknowledged and debated (Cook & Sgrò 2018; Holderegger et al., 2019; Sandström et al., 2019). Although, before considering AGM as an option for targeted endemic flora species, the benefits of this practice should outweigh the risks (Byrne et al. 2011). For example, seeds from non-local sources may also negatively influence the gene pools of local populations due to introgression of non-local genes, which may lead to outbreeding depression (Edmands, 2007; Byrne et al., 2011; Frankham et al., 2011). Marri is not at risk of outbreeding depression (Ahrens et al. 2019a; reduced fitness in offspring as a result of hybridisation with other populations), but there may be other risks including maladaptation of introduced populations to other climate or environmental variables in the targeted region (Aitken and Whitlock 2013). For example, introducing more drought tolerant individuals from a warm-dry marri population into the cool-wet population region may reduce the high resistance of local cool-wet populations to the leaf blight pathogen common to this region, in turn, reducing fitness (Ahrens et al. 2019b).

Marri's populations showed strong thermal tolerance (Chapter 3) with signatures of local adaptation in germination temperature response. Various tree species show adaptable germination tactics to cope with environmental oscillations (Nicotra et al., 2010). This adaptability may preserve co-existence in vegetation communities under current climates (Turcotte & Levine 2016). Similarly, flexible germination responses may allow populations to establish and persist as global temperatures rise (Clauss & Venable, 2000), provided seeds can recognise and respond to germination cues and establish when optimal conditions arise (Donohue et al., 2010). Marri showed high plasticity and limited genetic contribution for functional traits variation (Chapter 4). The findings agree with previous greenhouse-scale

common garden studies with marri provenances. For example, Ahrens et al., (2021c) also showed high levels of physiological plasticity from eight marri populations in response to variable heatwave severity through the enhanced capacity for thermal tolerance, compared to a few genotypic effects. However, the short-term physiological plasticity measured in a greenhouse study has limited applicability, since the results are extrapolated to trees growing in native natural environments.

Phenotypic plasticity and genetic adaptation of important heat and drought tolerance plant traits may facilitate the resistance and resilience in marri trees against future warming and drought events. Genetic diversity and plastic responses within a species enable breeding populations to better adapt, or acclimate, to changing environments and selection pressures (Stoutjesdijk, 2013). Breeding programs normally pursue access to genetic resources from the natural wild populations, or from *ex situ* supplies created to store selected genetic material, such as seed banks or tissue cultures. Typically, several objectives are pursued simultaneously for a breeding program for any species or population (Stoutjesdijk, 2013), including the adaptive capacity to environmental stresses. Highly plastic genotypes for functional traits may confer mechanisms for adjustment in changing environments (Arnold et al., 2019). Marri populations showed high plasticity for functional traits associated with heat and drought tolerance, for example, in adjusting photosynthetic pigments and water content under distinct climate settings. Once the populations or individuals with the best traits have been identified, a breeding program may commence to select and genetically improve the material, for example, through cross-pollination to introduce desirable characteristics.

Marri showed high plasticity for functional traits associated with heat and drought tolerance, in the present study and previous research (Aspinwall et al., 2017; Blackman et al., 2017; Ahrens et al., 2019a, b; Challis et al., 2020), which is indicative of some capacity to cope with climate change. Since this species has high levels of gene flow and connectivity across its range (Sampson et al., 2018), marri might be well equipped to persist under intensifying warming for the next few decades. Continued and additional field monitoring studies should be employed to assess whether it is a high-risk species under future climate change.

5.5. Current limitations and future work

Climatic variables are expected not to be the only drivers for variation in some putatively adaptive SNPs. The associated genes can be pleiotropic and may be under selection from other biotic or abiotic factors. For example, although precipitation and temperature are consistently highlighted as key factors influencing plant distribution and ecology, soil properties greatly affect these settings, as water availability depends on the interaction between climatic variables and soil characteristics (Piedallu et al., 2013). The identification and understanding of adaptive genetic variations might be improved by including other relevant abiotic factors such as soil characteristics in future environmental association analysis. Nevertheless, by hierarchically categorising gene functions through GO enrichment, the present study found adaptive patterns across the distribution, highlighting likely polygenic adaptations to climate variables in this species.

Expanding the genotypic assessment work to phenotypic approaches in jarrah for identifying patterns of plasticity and adaptation associated with climate would contribute to further understanding the association of genomic and phenotypic diversity across environmental gradients. For instance, jarrah provenance trials have demonstrated genetic variation in functional traits associated with precipitation factors (O'Brien et al., 2007; Koch & Samsa, 2007). While it appears that genetic variants associated with similarly functioning genes are adapting to the environment in similar ways (Chapter 2), this work also found that putative adaptations among climate variables are sorted through the landscape in contrasting ways. In fact, these findings could support recommendations for sourcing germplasm from multiple sources to bolster the adaptability in adaptively depauperate populations and provide a basis for more active selection of functionally related genes, potentially increasing the diversity and adaptability through new combinations of genetic variation. Future work should focus on improving the genomic resources (e.g., genome sequencing) of the study species to elucidate on the complex issues driving local adaptation. Furthermore, genotype diversity in the seedbank might be higher than in mature trees from the same stand (Mandák et al., 2012; Schulz et al., 2018). A suggested improvement to the current genome-wide association study would be to include seedbanks, as well as mature trees from the same stands, to understand the full extent of local adaptation patterns.

Optimum temperature response of germination differed among species (Chapter 3), with jarrah showing high levels of germination at around 9°C. Optimizing germination during cool temperature conditions is a key risk-avoidance strategy against environmental stressors, as it increases the chance of seedling survival under longer periods of favourable conditions such as lower evaporation rates in the soil (Duncan et al., 2019). Early germination may improve plant fitness but does not ensure effective establishment (Verdú & Traveset, 2005), and only one species in the present study was predicted to maintain germination rates. Species that experience the same seasonal temperatures may still have different recruitment rates due to the sensitivity of seeds to available soil moisture (Cochrane et al., 2014), and *Eucalyptus* species germination are differently affected by water availability (Emery & Collette, 2021). Future germination work with co-occurring species should include a moisture variable (i.e., water availability) to thoroughly address the full extent of the species adaptation to the temperature-moisture interaction.

The field-based spectral measurements (Chapter 4) effectively showed high plasticity and limited genetic contribution for functional traits variation in marri. However, in the present study, the time scales of stressors, and the stress response traits evaluated, should also be acknowledged as a limitation. For the most part, the natural climate variation (associated with site and seasonal variation) has been applied over a long time (since plantation sites establishment in 2014) – either by a gradual change between seasons or, for the site effect, the individual's whole life-history (c. 3 to 5 years) is related to the same site. This time frame allows the plants to acclimate to the conditions (Valladares et al., 2007; Niinemets, 2015), and therefore, to exhibit a plastic response once measured. Thus, there could be more explicit drivers for trait variation that were not assessed here, such as heatwaves and prolonged intense droughts. The current framework showed that the variation in reflectance indices might be partially genetically driven. Knowledge about genetically driven pigment- or spectral reflectance- based stress markers may help to recognize genotypes resistant to abiotic stress. Moreover, the spectral signals, such as single wavelength reflectance or spectral indices, may be used as indirect markers for specific foliar parameters correlated to production or adaptive traits. This could have further implications for the use of hyperspectral data in vegetation observation and analysis.

5.6. Conclusions

This work delivered a scientific basis for the adoption of AGM in southwest forests, through a detailed understanding of adaptation to current environments, and discussed that information to bolster jarrah and marri populations for future environments. The genome-wide association study uncovered multidimensional patterns of adaptation in jarrah, meaning that patterns of adaptation occur in opposing directions. These results highlight many interesting consequences. First, complex interactions, such as pleiotropy, are important for adaptation, but often overlooked in other genotype-environment association studies. Second, these complex interactions hinder the management of tree populations, e.g., by choosing to promote variation in one gene family, we may be limiting variation in others.

Jarrah has a lower and narrower germination temperature niche than marri, which is particularly concerning for the diversity and functional resilience of northern forests. This foundation tree species shows a limited capacity to persist in its northern range under increasing temperatures and will likely require earlier active management to counter the effects of climate change and avoid extinction locally. Marri displays greater thermal limit and adaptive capacity for the future temperature settings, and as such, may not require active management in the near future. These unique insights support conservation and restoration efforts by determining the species that are vulnerable at the germination stage and may require targeted on-ground actions such as supplementary seeding with local material to enhance establishment rates, or AGM to promote the adaptation of thermal limits (Aitken & Bemmels, 2015).

Intraspecific trait adaptation to the climate in Mediterranean-type forest trees was assessed using multi-spatial and temporal spectroscopy data. This study showed marri trees can adjust functional traits through genetic adaptation, but mostly phenotypic plasticity. Overall, some marri cool-origin populations showed adaptive capacity with optimized photosynthetic properties and efficiency under warmer and drier site conditions. The capacity for endemic foundation trees to adjust plant traits associated with environmental stressors through genetic adaptation and phenotypic plasticity may enhance tolerance to drying and warming environments under climate change. However, repeated exposure to more severe and

frequent extreme climate events (e.g., heatwaves) might reduce thermal resistance (Ahrens et al., 2021c), leaving forest trees more vulnerable as the climate warms.

The Forest Management Plan 2014–2023 for SWWA forests has provision for implementation of AGM in management strategies for responding to climate change. Seed sourcing for AGM should consider the respective populations physiological tolerance and adaptive capacity to climate stressors (such as heatwaves and droughts). The present findings of variation in genetic adaptive patterns, seed performance, and functional traits, harbouring potential adaptations to climate associated with climate factors, provides an evidence base for the design and implementation of such strategies and should be taken in consideration for the development of forest management plans.

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