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An investigation into the impact of climate change on reproduction and recruitment in a model species with physical dormancy: a 'space-for-time' approach

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AN INVESTIGATION INTO THE IMPACT OF CLIMATE CHANGE ON REPRODUCTION AND RECRUITMENT IN A MODEL SPECIES WITH PHYSICAL DORMANCY:

A 'SPACE-FOR-TIME' APPROACH

A thesis submitted in fulfilment of the requirements for the award of the degree

DOCTOR OF PHILOSOPHY

From

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By

Alice Rose Hudson BSc (Hons) MSc

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CERTIFICATION

I, Alice Rose Hudson, declare that this thesis, submitted in partial fulfilment of the requirements for the award Doctor of Philosophy, in the School of Biological Sciences, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. This document has not been submitted for qualifications at any other academic institution.

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Alice Rose Hudson

14th December 2016

STATEMENT OF CONTRIBUTION

The literature review chapter (Chapter 2) and the five data chapters (i.e. Chapters 3, 4, 5, 6 & 7) presented in this thesis have been prepared as manuscripts in collaboration with my supervisors David Ayre and Mark Ooi. These chapters have been written as the following journal articles:

- **Chapter 2** – Hudson, A.R., Ayre, D.J. and Ooi, M.K.J. (2015) Physical dormancy in a changing climate. *Seed Science Research.* **25**: 66-81.
- **Chapter 3** – Hudson, A.R., Ayre, D.J. and Ooi, M.K.J. (resubmission requested; *In 2nd review*) Spatial and temporal variation in physical dormancy characteristics: causes and consequences. *Annals of Botany.*
- **Chapter 4** – Hudson, A.R., Ayre, D.J. and Ooi, M.K.J. (resubmission requested; *In prep.*) Inter-population variation in the response of flowering phenology, seed production and dormancy characteristics to experimental warming in *Acacia suaveolens. Journal of Ecology.*
- **Chapter 5** – Hudson, A.R., Ayre, D.J. and Ooi, M.K.J. (*In prep.*) Variation in the emergence, survival and growth at early life-history stages of *Acacia suaveolens* along an altitudinal gradient. *PLOS One.*
- **Chapter 6** – Hudson, A.R., Ooi, M.K.J. and Ayre, D.J. (*In prep.*) Microsatellite primers for the Australian native *Acacia suaveolens* (Fabaceae). *Applications in Plant Science.*
- **Chapter 7** – Hudson, A.R. Ooi, M.K.J. and Ayre, D.J. (*In prep.*) A genetic test of the assumptions underlying the ecological 'space-for-time' approach to climate change studies. *Conservation Genetics.*

As the primary supervisor, I, Professor David Ayre, declare that the greater part of the work in each article listed is attributed to the candidate, Alice Rose Hudson. In each of the above manuscripts, Alice led the study design and was primarily responsible for data collection, analysis and interpretation. The first draft of each manuscript was written by the candidate, who was then responsible for responding to comments made by her co-authors. The co-authors, Professor David Ayre and Doctor Mark Ooi, were responsible for assisting with study design, interpreting data and editing of the manuscripts where necessary. Alice has been solely responsible for submitting relevant manuscripts for publication to appropriate journals, and she has been in charge of responding to reviewers' comments, with assistance from her co-authors.

_____	_____
Alice Rose Hudson	Professor David Ayre
PhD Candidate	Principal Supervisor
14th December 2016	14th December 2016

LIST OF PUBLICATIONS AND PRESENTATIONS

Publications

Hudson, A., Ayre, D.J. and Ooi, M.K.J. (2015) Physical dormancy in a changing climate. *Seed Science Research.* **25**: 66-81.

Conference Presentations

Hudson, A.R., Ayre, D.J. and Ooi, M.K.J. (2015) Can warmer winters modify reproduction and seed dormancy in a fire adapted species? Ecological Society of Australia conference, Adelaide, Australia.

Hudson, A.R., Ooi, M.K.J. and Ayre, D.J. (2014) Importance of genetic diversity and phenotypic variation as determinations of germination: A case study of *Acacia suaveolens.* Postgraduate student conference, University of Wollongong, Australia.

Hudson, A.R., Ayre, D.J. and Ooi, M.K.J. (2014) Does year to year variation in seed production lead to a more resilient seed bank? Ecological Society of Australia conference, Alice Springs, Australia.

Hudson, A.R., Ayre, D.J. and Ooi, M.K.J. (2013) Physical dormancy, a help or hindrance in climate change adaptation? A case study of *Acacia suaveolens*. EcoTas13 – 5th joint conference of the Ecological Society of Australia and the New Zealand Ecological Society, Auckland, New Zealand.

Hudson, A.R., Ayre, D.J. and Ooi, M.K.J. (2013) Physical dormancy, a help or hindrance in climate change adaptation? Postgraduate student conference, University of Wollongong, Australia.

Conference Posters

Hudson, A.R., Ayre, D.J. and Ooi, M.K.J. (2015) Can warmer winters modify reproduction and seed dormancy in a fire adapted species? British Ecological Society conference, Edinburgh, UK.

STATEMENT OF STYLE

This thesis has been prepared in journal article compilation style format. With the exception of the Chapter 1 (General Introduction) and Chapter 8 (General Discussion), each chapter has been written with the aim of publication in an ecological or genetic journal. As a result of this there is some overlap between the chapters, in particular in relation to the description of the habitat type and species.

ABSTRACT

Predicting species response to future climatic change is a key focus of current plant ecology literature. In fire-prone ecosystems detailed case studies addressing how climatic warming will alter reproduction and recruitment dynamics in obligate seeding species are lacking, despite the importance of this plant group. I aim to address this knowledge gap, focusing on *Acacia suaveolens*, an obligate seeding species with physical seed dormancy (PY). In obligate seeders, fire kills the parental generation and triggers dormancy release in seeds from a seed bank. Population persistence therefore depends on successful recruitment post-fire. I used a 'space-for-time' (SFT) approach, where relationships between traits and climatic changes over geographic gradients (one altitudinal and one latitudinal) are identified and used to predict responses to future climates. By using four different experimental approaches, I also tested the assumptions of the SFT method.

Firstly, I asked if temperature and rainfall conditions at seed source sites can explain variation observed in PY characteristics. I collected seeds from 15 populations across the SFT gradients over three successive years and exposed them to one of five fire-related temperature treatments, recording germination and viability. I found a significant negative relationship between source site mean summer temperature and germination after the 80°C temperature treatment ($R^2 = 0.4$), suggesting that a higher temperature or longer heating duration is needed to break dormancy in seeds from warmer areas. Winter temperatures experienced by parental plants during seed development showed a significant negative relationship with the time to germination after the 80°C heat treatment ($R^2 = 0.27$) (possibly linked with seed weight change). However, the impact of this will depend upon competition in the post fire environment. No relationships between PY characteristics and rainfall were detected.

Secondly, I asked if the temperature experienced by parent plants during reproduction influences reproductive phenology and PY characteristics. I placed plants (grown from seeds collected across the SFT gradient) in either current or future temperature conditions during their reproductive period, recording flowering and fruiting variables. I exposed the F_1 seeds to one of five fire-related temperature treatments, recording germination and viability. In the warmer parental environment plants flowered on average 33 days later than plants in the cooler parental environment, but pods ripened on average 12 days earlier. This significantly reduced the timespan of the reproductive process, the length of which negatively correlated with seed abortion rates (R^2 = 0.24). F_1 seeds from the warmer parental environment were significantly lighter, with reduced germination after the 80°C temperature treatment than seed produced under a cooler parental environment.

Thirdly, I asked to what extent climatic and ecological variables constrain early seedling growth. I transplanted seeds and seedlings from nine altitudinal populations into nine recently burnt sites along the same gradient. I recorded early growth, development and survival. Low seedling survival at high altitude transplant sites (5-7%) was mainly due to the effects of drought (75% of seedling deaths), whilst herbivory accounted for 51% of deaths at low altitude sites. Temperature and rainfall variables were important in explaining seedling growth rates and emergence from seed, however the most important factor varied with the trait investigated.

Finally, I asked what the pre-existing genetic diversity and apparent connectivity (gene flow) among populations was. Using neutral microsatellite markers, I found significantly greater heterozygote deficiencies (F_{IS} = 0.59 ± 0.05 SE) and genetic differentiation of stands along the latitude gradient (pF_{ST} = 0.44 ± 0.02) than along the altitude gradient (F_{IS} = 0.44 ± 0.04;

pF_{ST} = 0.13 ± 0.01). Gene flow estimates were low at less than one migrant per generation. These results imply limited gene flow among stands along both gradients, limiting the chance for the transfer of climate adapted alleles.

Overall, I predict that *A. suaveolens* will be able to buffer against the impacts of climatic warming on recruitment dynamics in the short term due to wide inter- and intra-population variation in PY characteristics. Over the longer term, limited gene flow and potential shortening of the reproductive period may reduce regeneration success. The results presented within this thesis, combined with existing studies on the species, form a robust case study for predicting climate change impacts on obligate seeding species with PY in fire-prone ecosystems. I highlight the importance of parental effects as a mechanism by which climate change can modify recruitment dynamics. Assessment of the SFT assumptions, showed significant differences in gene flow and mating systems among populations to be of greatest concern.

ACKNOWLEDGEMENTS

The past four years have been amazing, challenging and rewarding. Looking back now, I can see how much I have grown both as a researcher and personally. Many people have made this PhD experience what it was, and to whom I owe great thanks.

First and foremost, I would like to thank my supervisors, David Ayre and Mark Ooi, for all their support, encouragement, and guidance over the past four years. Without them this thesis would not have been possible. They have given me confidence in my own ability as a scientist and researcher.

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adventures, but who sadly did not live to see the completion of this thesis, this is written in dedication to you. To all my family, I could not have done this without you.

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Chapter 1: GENERAL INTRODUCTION

A key goal of current plant ecology centres on predicting the response of species to future climatic change (Parmesan & Hanley, 2015). It has long been acknowledged that climatic pressures exert strong selective forces on plants (e.g. Etterson & Shaw, 2001; Jump *et al.*, 2006; Amano *et al.,* 2014), and that future climatic change is likely to do the same (Parmesan & Hanley, 2015). Whether or not a species is able to persist as the climate changes is dependent upon a multitude of environmental, ecological and genetic factors (e.g. Thomas *et al.*, 2004; Davis *et al*., 2005; Jump & Peñuelas, 2005; Hof *et al.,* 2011; Ågren & Schemske, 2012), and their relative influence, which changes over time, geographic space, and throughout the life cycle of species (Lusk *et al.,* 2008; Jackson *et al*., 2009). Investigating the response of all species, to each of these factors, across their entire life cycle and geographic distribution, is impossible. One alternative is to develop in depth 'model species' case studies, selecting key species within communities, based upon functional types or important traits (Broadhurst *et al.,* 2008; Dawson *et al.,* 2011).

The sandstone vegetation of south-eastern Australia, particularly the Sydney sandstone area, was identified by Crisp *et al.* (2001) as being one of only eight centres of high plant species richness and endemism within Australia. Within this region, the dry sclerophyllous heath / woodland is classified as one of south-east Australia's last remaining wilderness habitats extending along the east coast of New South Wales and up into the Blue Mountains National Park (Keith, 2004). However, as with many regions close to the urban interface, the impacts of climate change and habitat fragmentation continue to threaten these wilderness areas (Keith, 2004). Fire is a key driver of the population dynamics of most species within this vegetation type, with plant adaptations to fire broadly classified into two groups: obligate

seeding and resprouting (Whelan, 1995). For obligate seeding species, fire kills the parental generation, with post-fire germination from a seed bank (aerial or soil) ensuring population regeneration. Within dry sclerophyllous woodland habitat, obligate seeding species comprise an important, yet understudied component, of the perennial shrub layer of the ecosystem (Bieger *et al.*, 2014). Obligate seeding Fabaceae species are particularly prevalent (Specht, 1970; Auld, 1996; Ooi *et al.,* 2012 and references therein). These Fabaceae species have predominantly physically dormant seeds, and develop a long-lived soil seed bank during the inter-fire years. In this case, physical dormancy is broken by heat from the fire, enabling germination and the replacement of the lost parental generation. For these species, as the adult plants are generally killed by fire, the germination of seeds from the seed bank, and subsequent survival of seedlings, is crucial for population persistence. Within the plant life cycle, the seedling stage is often considered particularly vulnerable to the impacts of climatic change due to the strong influence of climatic factors on controlling seed dormancy and germination (Walck *et al.,* 2011, although see Bertrand *et al.,* 2011). Consequently, in fire-prone habitats the effects of climatic change may be particularly prevalent on early life-history stages in obligate seeding species (Keith & Myerscough, 2016).

The influence of climate on early life-history stages begins prior to seed formation and extends beyond seed germination (De Frenne *et al.,* 2012). For example, Argel & Humphreys (1983a & b) and Hoyle *et al.* (2008), amongst others, have provided evidence that the environmental conditions experienced by a maternal plant during the reproductive phase can influence the seed dormancy characteristics of the resulting seed thereby influencing seed germination and potentially recruitment dynamics (Hoyle *et al*., 2008). Moreover, when and where a seedling germinates determines the season in which the seedling will grow, in turn influencing the chances of survival and governing the phenotype the seedling will display

(Donohue *et al.,* 2005; Donohue, 2009; Donohue *et al.*, 2010). Hence, accounting for the influence of climate change on all these stages, from flowering of the maternal plant through to early seedling growth, is required to ensure an accurate representation of how a species may respond (Post *et al.,* 2008; Del Cacho *et al.,* 2013).

Understanding a species response to projected climatic changes is also governed by the distributional range of a species, and how populations differ within that range (Valladares *et al.,* 2014; Cochrane *et al*., 2015a). Adaptation of populations to local environmental conditions results in different trait means and levels of plasticity (Valladares *et al.,* 2014). Consequently, the potential of a population to respond and / or cope with the environmental challenges to which they are exposed will vary depending upon population size, environmental heterogeneity, gene flow and genetic variation, all of which influence the capacity for local adaptation (Savolainen *et al.,* 2007; Valladares *et al.*, 2014). Therefore, studying the response of a single population within a species range neglects a large amount of potential variation present throughout the species range, without which, accurately predicting that species future is not possible (Jackson *et al.,* 2009; Bell *et al.,* 2014).

Overall, in order to predict a species response to future climate change, it will be essential to focus on investigating key life-history stages (relevant to the habitat which they are from) and to quantify the level of intra- and inter-population phenotypic variation in traits associated with the identified life-history stages, and the potential for gene flow across the species range. Consequently, in this thesis I aim to assess the level of phenotypic variability existing in seed dormancy, seed germination and seedling survival in an obligate seeding species within the dry sclerophyllous woodland of south-eastern Australia. I will then investigate the influence that future climatic change may have. By addressing the following broad questions I aim to

tease apart the factors underlying the expression of phenotypic variation in traits associated with the seed and seedling life-history stages in *Acacia suaveolens*:

- Do physical dormancy traits vary with environmental conditions along altitudinal and latitudinal clines in south-eastern Australia?
- What influence does the temperature experienced by parental plants during the reproductive period have on seed production, dormancy and germination in *A. suaveolens*?
- To what extent does altitudinal adaptation influence the chances of seedling survival in *A. suaveolens?*
- How is neutral genetic variation partitioned within and among populations of *A. suaveolens* along an altitudinal and a latitudinal gradient in south-eastern Australia?
- What are the inferred levels of gene flow among populations of *A. suaveolens*?

This thesis is centred on the study species *Acacia suaveolens* (Sm.) (Willd.) (Fig. 1.1A), a commonly occurring, obligate seeding species with physically dormant seed (Morrison, 1986a; Auld, 1986a). The species has a broad, predominantly coastal, distribution, extending from Tasmania in the south, along the east coast of Australia, to the mid-coast of Queensland (Fig 1.1B) (Morrison, 1986a). The species has been well studied in respect to adult plant traits and seed dispersal factors on a localised scale within the Sydney region (e.g. Auld, 1986a, b & c). This pre-existing knowledge of adult stages and seed dispersal means the species provides an excellent opportunity to develop a detailed case study of an obligate seeding species with PY within dry sclerophyllous woodland.

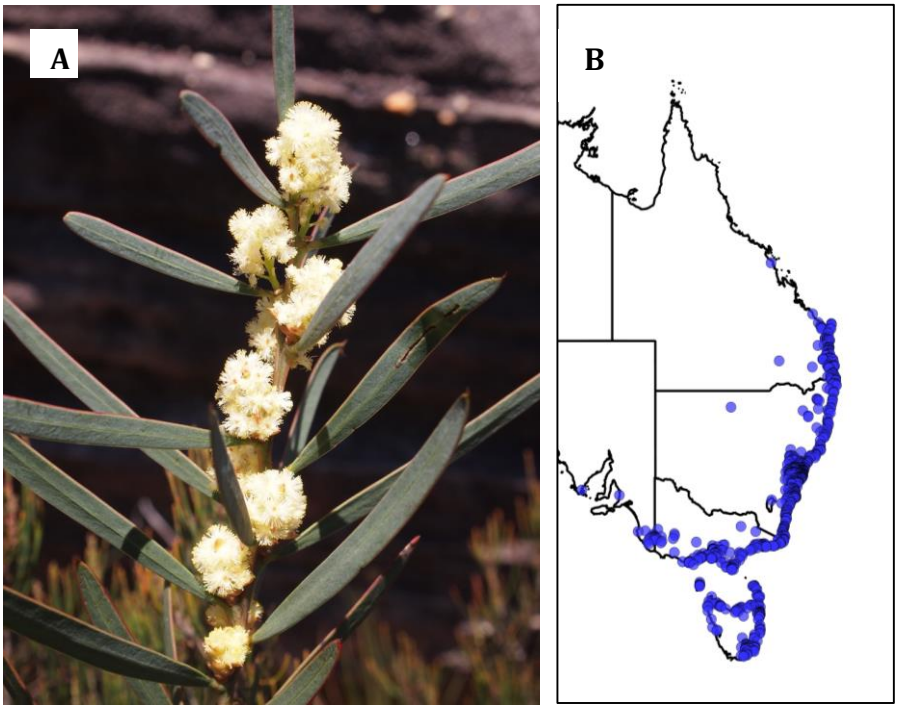

Figure 1.1. A) Photograph of the study species *Acacia suaveolens* in flower, B) distribution map of *Acacia suaveolens* in eastern Australia and Tasmania (source AVH, 2016).

In order to predict possible changes to the physical dormancy and germination traits of *A. suaveolens* under future climate change, I used a 'space-for-time' approach (Dunne *et al.*, 2004; Fukami & Wardle, 2005; Ooi *et al*., 2012), over geographic gradients. This allowed me to study the pre-existing variation in *A. suaveolens* seed dormancy and germination traits over a temperature range equal to, and greater than, that projected under climate change whilst maintaining a constant geology and broad habitat type. I established study sites along two gradients, one altitudinal and one latitudinal, each covering at least a 5°C change in average temperature (Fig. 1.2). I selected three to four positions per altitude / latitude gradient with three replicate populations per gradient position (Fig. 1.2). This design covers varying degrees

of population isolation. The established sites are used throughout the following chapters all together or as subsets (Appendix 1).

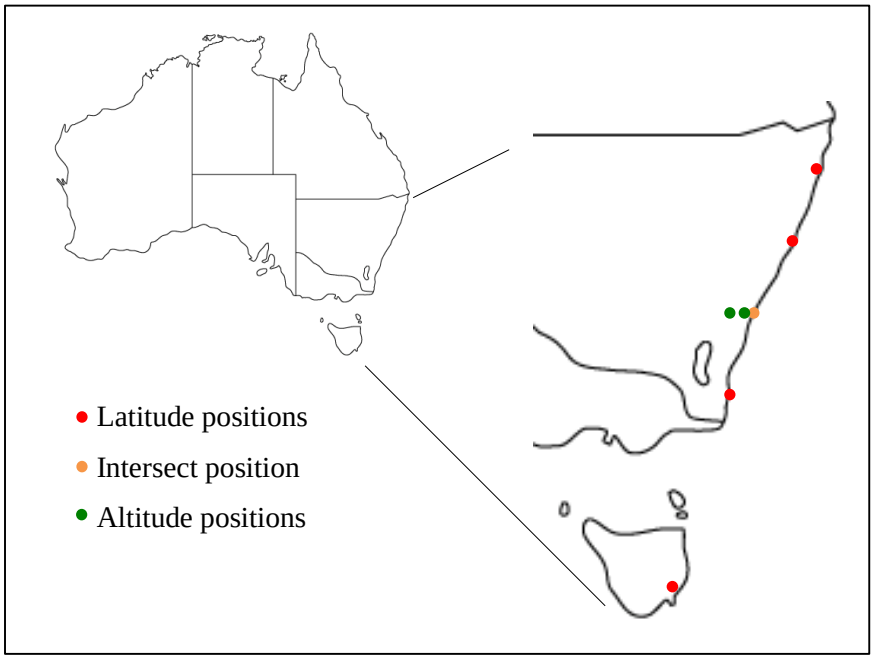

Figure 1.2. Schematic map showing the span of the study gradients. Points show the approximate locations of the altitudinal and latitudinal positions where the study populations were located (2-3 per position).

This thesis is comprised of eight chapters, including this general introduction (Chapter 1), a literature review, five data chapters and a general discussion. The literature review (Chapter 2) seeks to investigate the current state of understanding of the physical dormancy trait. It is particularly focused on phenotypic variation in characteristics associated with physical dormancy and the environmental controls involved, seeking to identify key gaps in the current literature.

In Chapter 3, I aim to characterise the level of pre-existing variation in the traits associated with physical dormancy in *A. suaveolens,* particularly focusing on the fire-related

temperatures needed to break dormancy. By characterising this variation in populations along the two climatic gradients, and over three successive years of seed production, I aim to investigate the role of environmental variables, namely the temperature and rainfall parental plants experience during the reproductive phase, on the fire-related temperatures needed to break physical dormancy.

In Chapter 4, I aim to develop this further by manipulating the temperature of the parental environment during the reproductive phase. Specifically, this will explore the role, if any, of temperature in controlling seed production and the fire-related temperatures needed to break physical dormancy.

Chapter 5 focuses on seedling germination and survival post dormancy break. By conducting transplant experiments along the altitudinal gradient I investigate the degree of altitudinal adaptation that *A. suaveolens* seedlings exhibit, and the influence of temperature and rainfall on seedling survival once dormancy has been broken.

Whilst Chapters 3 to 5 focus on the potential impact that climate change may have on the reproductive strategy of *A. suaveolens*, Chapters 6 and 7 focus on assessing the potential for gene flow among populations along the study gradients. First, I describe the development of ten microsatellite primers for *A. suaveolens* (Chapter 6) and then use these to characterise the neutral genetic diversity, breeding system and the potential for gene flow within and among populations along the study gradients (Chapter 6). I estimate the potential for the transfer of climate-adapted traits to move along the climatic gradients.

In combination these five data chapters, address the influence of climate change on the reproduction of *A. suaveolens* from flowering and seed production (Chapters 3 and 4) through dormancy break (Chapters 3 and 4) and onto early seedling survival (Chapter 5), whilst

accounting for the underlying genetic structure of the species (Chapter 7). This will then allow me to predict possible changes to reproduction and regeneration in the species under climate change projections for the south-east coast of Australia (Chapter 8).

Chapter 2: PHYSICAL DORMANCY IN A CHANGING CLIMATE

A modified version of this chapter has been published in *Seed Science Research:*

Hudson, A.R., Ayre, D.J. and Ooi, M.K.J. (2015) Physical dormancy in a changing climate. *Seed Science Research.* **25**: 66-81.

2.1. Introduction

Climate change projections indicate that many environmental factors, including mean air temperatures, average rainfall and events such as drought, fire and heat waves, will change in the future (Alexander & Arblaster, 2009; Royer *et al.*, 2011; IPCC, 2014). In order to cope with these changes, species survival will be dependent upon their ability to migrate to track the changing climate, pre-existing phenotypic plasticity and / or their ability to adapt to the new conditions they are experiencing (e.g. Thomas *et al.*, 2004; Davis *et al.*, 2005; Jump & Peñulas, 2005; Hof *et al.*, 2011). It is unlikely that many plant species will be capable of dispersal at a rate equivalent to that of projected climate changes (Honnay *et al.*, 2002; Van der Veken *et al.*, 2007; Morin *et al.*, 2008) and, as such, they will have to either maintain traits that enable them to persist, or adapt to the new conditions (Parmesan, 2006; Skelly *et al.*, 2007). Understanding the variation in important traits, the mechanisms underpinning this variation, and how this variation affects the demographic responses and persistence of plant populations to projected climate change is therefore one of the key challenges faced by researchers today.

Climate exerts a strong influence on seed dormancy and germination (Walck *et al.*, 2011). These early life-history stages are subject to strong selection pressure, as dormancy break and germination at the wrong time, or under sub-optimal conditions, can cause a sharp reduction

in fitness. Consequently, these stages are likely to be sensitive to climatic changes (Nicotra *et al.*, 2010; Walck *et al.*, 2011; McLaughlin & Zavaleta, 2012). Dormancy can be classified into four main types, namely: 1) physiological; 2) physical; 3) morphological, and 4) morphophysiological (Baskin & Baskin, 2014). To date, much of the research on the controls and variation in dormancy has been conducted on species with physiological dormancy mechanisms and on agricultural species. This has shown that environmental factors have a key role in determining the prevalence of dormancy and associated traits (e.g. Fenner, 1991; Wulff, 1995; Probert, 2000; Hoyle *et al.*, 2008; Kochanek *et al.*, 2011; Rix *et al.*, 2012; Baskin & Baskin, 2014). However, while physical dormancy (PY, also known as hardseededness) has been relatively well studied in agricultural settings, much remains unknown about how and why PY varies inter- and intra-specifically in natural ecosystems.

Physical dormancy is the result of an impermeable seed coat (testa) or endocarp which prevents water from reaching the embryo, as required for germination (Baskin *et al.*, 2000; Baskin & Baskin, 2014). As PY is believed, in many cases, to have evolved to enable plants in temporally stochastic and harsh environments to survive (Baskin *et al.*, 2000, although see Paulsen *et al.*, 2013), it may be hypothesised that plants with PY would prosper under the more variable and warmer climate that is projected for the future (IPCC, 2014). However, a number of questions need to be considered before such a conclusion can be drawn. Therefore, this review seeks to ask, firstly, what traits of PY vary, and to what extent do they vary inter- and intra- specifically? Secondly, if inter- and intra-specific variation in the PY traits exists, is this variation genetic or environmental in origin, and to what degree is it heritable? Without understanding the heritability of these traits, predicting the long-term PY responses will not be possible. While variation may buffer the population in the short term, establishing what causes this variation is crucial to understanding the long-term advantage of the traits under a

changing climate. Should environmental factors trigger changes in PY, then it may be expected that the population and community dynamics would also be affected.

Under a changing, and potentially more variable, climate (IPCC, 2014), understanding how PY traits may change is of particular interest for a number of reasons. Firstly, physical dormancy is a polyphyletic trait, resulting from convergent evolution, thus occurring throughout a variety of life histories and taxonomic lineages (see Baskin *et al.*, 2000). It is also prevalent within a number of ecologically important ecosystems worldwide, particularly in Mediterranean-type climates and tropical deciduous woodland, where approximately 27% and 37% of plants species display PY, respectively (Baskin & Baskin, 2014). Many of these ecosystems are highly diverse in terms of plant life, particularly Mediterranean-type ecosystems (Cowling *et al.*, 1996), and also contain high numbers of threatened species. Secondly, PY can allow long-term seed banks to develop (Norman *et al.*, 2002; Van Assche & Vandelook, 2006; Ooi, 2012; Ooi *et al.*, 2012; Baskin & Baskin, 2014), and any alteration of PY traits by projected environmental changes may affect the functioning of these long-term seed banks. And, thirdly, relatively little is known about the degree of variability in PY within wild species. While agricultural studies provide useful information for understanding PY, these species have undergone extensive breeding and selection processes, altering PY characteristics. Consequently, it is possible that the way they respond to climatic selection pressures during early life-history stages may differ to the response displayed by wild species.

2.2. The physical dormancy traits

Physical dormancy is usually considered a heritable trait, controlled by the testa or endocarp, which are derived from integuments of the ovule and the inner epidermal layer of the ovary wall, respectively (Evenari *et al.*, 1966; Pérez-García & Escuardo, 1997; Li *et al.*, 1999a). To

date, numerous natural mechanisms of PY breakdown have been described, particularly relating to various combinations of temperature and moisture changes, including high temperatures from summer insolation or fire (e.g. Auld & O'Connell, 1991), temperature fluctuations (e.g. Vázquez-Yanes & Orozco-Segovia, 1982) and wet heat (e.g. Van Klinken & Flack, 2005). The loss of dormancy is linked with structural changes to a specialised region of the testa known as the 'water-gap' (Box 1). While the breakdown of PY is primarily an all-or-nothing event, with an irreversible change to the water-gap region occurring, there are a number of different traits of PY that can be measured. These include: 1) initial percentage of seeds dormant at dispersal (initial PY); 2) the conditions required to break PY; 3) the time taken for the loss of PY to occur under a described suite of conditions; and 4) changes to the conditions required to break PY over time. These four traits all act together to determine when a seed will lose its dormancy. How these traits vary, and the underlying causes of the variation in each trait, will therefore alter the environment into which a seedling develops, thus influencing its chance of survival (Donohue *et al.*, 2010). Consequently, how the traits vary, what causes the variation and if any variation interacts with environmental conditions to change the timing of PY break are key research questions.

Box 1: The 'water-Gap'

A characteristic of many species with physical dormancy is the presence of a 'water-gap complex' (Baskin *et al.*, 2000; Gama-Arachchige *et al.*, 2010, 2011, 2013; Baskin & Baskin, 2014). This is defined as '*a morpho-anatomically complex structure and is composed of 1) an opening formed after PY break; 2) specialised structures that occlude the gap; and 3) associated specialized tissues'* (Gama-Arachchige *et al.*, 2013 pp. 82). For example, within the Papilionoideae and Mimosoideae of the Fabaceae the water-gap complex is characterized by the presence of a strophiole (/lens/lid). The strophiole forms part of the palisade / impermeable layer of the seed coat, but during dormancy breaking the strophiole becomes separated from the

palisade layer allowing water into the seed and imbibition to occur (Baskin *et al.*, 2000; Baskin & Baskin, 2014). In contrast, within the Malvaceae, a chalazal cap (or plug) blocks a gap within the palisade in the water-gap complex. Upon dormancy breaking, the chalazal cap separates from the impermeable layer allowing water entry (Baskin & Baskin, 2014). Consequently, the water-gap complex is often referred to as the 'weak point' of the seed coat. Although many types of water-gap complex have been proposed, Gama-Arachchige *et al.* (2013) have revised the classification creating a three class system based on the anatomy of the area.

2.3. Variation in the physical dormancy traits

In many cases, the breaking of physical dormancy is in response to an environmental stimulus, and it has been suggested that thresholds may exist, which environmental conditions must cross in order for the breaking of physical dormancy to occur (Ooi *et al.*, 2012). Considerable variation in the conditions required to break PY has been recorded between species (e.g. Jeffrey *et al.*, 1988; Auld & O'Connell, 1991; Herranz *et al.*, 1998; Ooi *et al.*, 2009), with some authors suggesting this contributes to maintaining community diversity and coexistence, by providing differentiation within the regeneration niches (Trabaud & Oustric, 1989; Moreno & Oechel, 1991; Herranz *et al.*, 1998; Ooi *et al.*, 2014). However, PY may also vary intra-specifically, in that different populations or individuals within a population may also have different requirements for breaking dormancy, providing a form of bet-hedging. In seed ecology, bet-hedging is commonly associated with the spreading of germination over time in desert annuals (Venable, 2007; Ooi *et al.*, 2009; Baskin & Baskin, 2014), but it can also be applied to ensuring that only a proportion of a population's seed bank will germinate in response to any given dormancy-breaking event in many habitat types. For example, in

fire-prone regions, heat shock from fire is usually required to break PY. However, fires are variable, ranging from low to high intensity and differing in duration, producing a range of potential temperatures that could be experienced within the soil (Whelan, 1995; Penman & Towerton, 2008). Bet-hedging within a population, manifested here as variation in the temperature required to break dormancy, would therefore increase the chance that dormancy in a proportion of seeds within the seed bank would be broken during any given fire event, thus potentially increasing overall population fitness by ensuring some germination irrespective of the fire conditions experienced. At the inter-population scale, with site-to-site environmental variation (e.g. over a climatic gradient), conditions required for breaking PY may also vary due to the influence of environmental factors on dormancy onset and formation (Ooi *et al.*, 2012).

Given the many factors which interact to cause the loss of PY (e.g. temperature, humidity, storage duration), variation within and between populations may result in different populations having different responses to climate warming and the associated extreme weather events. Consequently, it is important to understand, and if possible quantify, this variation, through investigating the variation that exists within and between populations in their dormancy-breaking requirements. Genetically determined inter-population variation might reflect the effects of both genetic drift and localised adaption, while variation within populations may reflect the effects of disruptive selection or perhaps the input of variation from diverse populations. Additionally, potential environmental links to this variation will enable predictions of the changes to PY and seed banks resulting from projected climatic changes. Quantifying this variation between species will have wider implications through influences on community composition, as well as on the ability of habitats to regenerate in zones where PY occurs.

To investigate the level of variation currently known to exist in PY at the inter-specific and inter-population level, we searched for papers using ISI Web of Science and Google Scholar, using multiple variations of the search terms 'physical dormancy', 'hardseededness', 'populations' and 'environmental variation'. We found a total of 10 papers, covering 25 species, with *Trifolium subterraneum* and *T. cherli* appearing multiple times (for *T. subterraneum*, multiple cultivars each collected from multiple sites, were included (Norman *et al.*, 2006)). Table 2.1 highlights the variation in the initial PY trait between populations as well as the methodologies used. Comparing variation in PY traits across populations or species from different studies is inherently difficult, given the variety of dormancy-breaking techniques used (Table 2.1). But due to the variety of ecosystems in which PY occurs, a standard treatment for testing PY on all species would be not feasible. For example, an 80°C heat treatment may be required to break dormancy of *Acacia* species from fire-prone environments (Auld & O'Connell, 1991), yet this would kill a *Trifolium* embryo. Consequently, we suggest standardised habitat-specific comparisons, and have grouped the studies as such. Even grouped by habitat type, simply the duration over which germination checks were conducted varied, from 28-90 days in studies from fire-prone environments and 14-28 days in agricultural studies. It is not clear how different the results would be if the shorter studies were run for the longer periods.

Across all the species studied, the average variance in initial dormancy (%) between populations of species was 19.9, with a range of 0 to 111.7. When categorised by habitat type, there were no major differences (Table 2.1). This would seem to suggest that there is little inter-population variation in the percentage of seeds with PY at the time of release (or soon after); indeed, all coefficients of variation were less than 12%. In agricultural systems (five of the ten papers studied) this is not surprising, given that agricultural selection processes are

often focused on attaining 100% soft or 100% hard seed, depending the crop species (D'hondt *et al.*, 2010). Similarly, for those species from fire prone habitats (five of the ten papers), this may be expected, given that PY in such a habitat is believed to be an adaptation enabling populations to survive fire, with little fitness advantage gained by germination occurring outside of post-fire conditions (Whelan, 1995; Ooi *et al.*, 2012, 2014). However, as only three of the eighteen families with PY are represented in Table 2.1 (Fabaceae, Cistaceae and Anacardiaceae), and only two habitat types, given a broader sample size these patterns may not hold. Finally, while all of these studies compared different populations, the geographic distance between populations may have influenced the results. Populations closer together are more likely to have increased levels of gene flow and therefore could be expected to be genetically more similar, which may act to homogenize PY.

Table 2.1. Summary of the inter-population variation in initial dormancy of PY species from the literature where multiple populations had been assessed. This includes the dormancy breaking methodologies used (- data unavailable; FP = fire-prone habitat; A = agricultural habitat).

Reference	Species	Habitat type	Populations studied	Collection method	Seed storage period	Replication	Control conditions	Observation period	Variance in % dormant in control	CV of % dormant
Auld & O'Connell, 1991	*Acacia suaveolens*	FP	2	Bulk	0-1 years	1 x 30 seeds	'Ambient room temperature'	28 d	20.48	4.68
	A. ulicifolia	FP	2	Bulk	-				9.68	3.18
	A. terminalis	FP	2	Bulk	-				53.05	8.46
	A. myrtifolia	FP	2	Bulk	-				0.25	0.52
Moreira & Pausas, 2012	*Fumana thymifolia*	FP	2	Bulk	-	4 x 50 seeds	20°C darkness	90 d	24.50	5.18
	Cistus salviifolius	FP	6	Bulk	-				12.67	3.80
	C. albidus	FP	4	Bulk	-				4.67	2.30
	C. creticus	FP	3	Bulk	-				25.33	5.30
	Ulex parviflorus	FP	5	Bulk	-				88.70	11.05
Norman *et al.*, 1998	*Trifolium glanduliferum*	A	2		1 month	4 x 200 seeds	15°C	28 d	0.50	0.72
	T. purpureum	A	2		1 month				0.50	0.83
	T. cherleri	A	2		1 month				4.50	2.25
Norman *et al.*, 2002	*T. subterraneum*	A	5	Bulk	-	4 x 100 seeds	15°C	14 da	111.70	11.77
	T. cherleri	A	3	Bulk	-				28.00	5.51
	T. hirtum	A	5	Bulk	-				30.00	5.83
	T. tomentosum	A	5	Bulk	-				1.30	1.17
	T.lappaceum	A	2	Bulk	-				0.00	0.00
	T.spumosum	A	6	Bulk	-				1.47	1.27
	T. glomeratum	A	5	Bulk	-				1.20	1.11
	T. angustifolium	A	2	Bulk	-				8.00	2.89
	T. nigrescens	A	2	Bulk	-				0.00	0.00
Norman *et al.*, 2006	*T. subterraneum* cv. Dalkeith	A	3	Bulk	-	1 x 200 seeds	15°C	14 d	12.33	4.16

	T. subterraneum cv. Geraldton	A	3	Bulk	-				26.33	6.06
	T. subterraneum cv. Dwalganup	A	3	Bulk	-				0.33	0.69
	T. subterraneum cv. Nungarin	A	3	Bulk	-				56.33	9.92
	T. subterraneum cv. Urana	A	3	Bulk	-				16.33	4.97
	T. subterraneum cv. Izmir	A	3	Bulk	-				48.00	8.77
Li *et al.*, 1999b	*Rhus aromatica*	-	3	Bulk	-	4 x 50 seeds	25/15°C in light	28 d	32.00	6.02
	R. glabra	-	6	Bulk	-				10.92	3.42
Burrows *et al.*, 2009	*A. melanoylon*	FP	3	Bulk	-	2 x 100 seeds	25°C	40 d	4.33	2.27
Bolin, 2009	*R. copallinum*	FP	4	Bulk	-	3 x 50 seeds	25°C 12/12hr light dark	28 d	0.56	0.76
Majd *et al.*, 2013	*Prospis farcata*	A	2	Bulk	1 month	1 x 25 seeds	25°C	15 d	0.00	0.00
Nichols *et al.*, 2009*	*T. subterraneum* (year 3)	A	2	Bulk	-	-	Submerged in 15° water	2 d for control; 112 d incubation	4.50	3.63
	T. subterraneum (year 16)	A	2	Bulk	-				41.41	10.95
Average by habitat		A FP							18.70 22.20	

*Nichols *et al.* (2009) assessed the same two populations three years after artificial establishment and 16 years after

Compared to variation in initial PY between populations, it may be expected that large variation in the conditions required to break PY would exist within populations, as well as between species within a habitat. Such variation between individuals and between species within a given location would act to reduce competition among seedlings, by staggering germination when suitable conditions arrive, as well as by ensuring that a portion of the seed bank germinates. As with comparing initial dormancy variation, comparing intra- and inter-specific variation in PY-breaking conditions across multiple papers, even grouped by habitat type, is difficult, due to wide variation in techniques. However, a number of individual papers have compared dormancy-breaking conditions for multiple species subjected to the same treatments. Focusing on fire-prone habitats, this can be manifested as different optimal heating temperatures required to break PY. Intra-specifically, Auld & O'Connell (1991) subjected seeds of two populations each of *Acacia suaveolens, A. ulicifolia, A. myrtifolia,* and *A. terminalis* to different dormancy-breaking temperatures and different durations of the temperature treatments that may occur during natural fires. The populations were classed as 'geographically distinct'. For all four species, the interaction between site, temperature treatment and treatment duration was significant ($P < 0.005$). Similar studies, including those by Moreira & Pausas (2012), Ooi *et al.,* (2012), Bolin (2009) and Farrell & Ashton (1978), have also shown similar intra-specific variation amongst populations of fire-prone habitats from bulk wild-collected seed. But Li *et al.* (1999b) found no variation in PY response between populations to different storage treatments in two of three *Rhus* (Anacardiaceae) species studied. Future studies should focus, where possible, on using seed collected from individual maternal plants within populations, to help quantify the variation in PY traits intra-specifically.

2.4. Genetic versus environmental control of physical dormancy

To predict future changes to plant population dynamics and their persistence, it is necessary to understand both the basis of variation in key traits and the extent to which such variation can evolve. Physical dormancy expresses a large degree of phenotypic variation and is considered an inherited, quantitative trait (e.g. Evenari *et al.*, 1966; Hill *et al.*, 1986a & b; De Souza & Marcos-Filho, 2001). Furthermore, genetic effects on PY are often assumed to only reflect the maternal genotype due to the importance of the seed coat (or endocarp) in PY, both of which are derived from parts of the maternal ovule (Roach & Wulff, 1987; Fenner, 1991; Li *et al.*, 1999a; Donohue, 2009; Baskin & Baskin, 2014). However, Ramsay (1997) has shown that hardseededness can also be passed through the paternal line in *Vicia faba*. Such conflicting assumptions raise the hypothesis that PY may be affected by changing climatic conditions through impacts on both the maternal and paternal plants and their respective genotypes.

Phenotypic variation in a trait can be the result of multiple factors, including genetic and environmental influences (Falconer, 1989). Put simply, the phenotypic variation (V_P) displayed in a trait can be expressed as:

$$V_P = V_G + V_E + V_{GE}$$

Whereby, V_G is the genetic component, V_E is the environmental component and V_{GE} is the interaction term between genetic and environmental portions (Falconer, 1989). Isolating these factors to understand their relative contributions to trait variation will enable predictions of trait responses to selection (Frankham *et al.*, 2010). However, in considering such responses it is important to recognise that selection not only shapes additive genetic variation, but also the extent of phenotypic plasticity. In the short term, if PY traits (e.g. initial dormancy, the

conditions required to break PY, time to loss of PY) show high phenotypic plasticity then the expression of PY may show an immediate response to climate change that may be important for long-lived species. However, in the longer term, if the climatic conditions become more stochastic as predicted, selection may favour individuals or species with a high degree of phenotypic plasticity for PY traits, due to their ability to alter these traits in line with climate. Consequently, it is not only genetic control that must be considered when assessing PY characteristics, but also the genetic variation within any given population. High levels of population genetic variation for PY may provide a buffer against climate change by providing a wide base upon which selection pressures can act (Lacerda *et al.*, 2004).

Genetic sources of variation can be further divided into additive, dominant and interacting partitions, the relative proportions of which influence how heritable a trait is and consequently the potential a trait has to respond to selection (Frankham *et al.*, 2010). The greater the additive genetic contribution within a population to a phenotype under a given set of environmental conditions, the higher the heritability of that trait, and therefore the greater the selection effect for a given intensity of selection (Falconer, 1989; Frankham *et al.*, 2010). However, the intensity of selection (i.e. the proportion of a population with the trait value selected for) will also alter the degree of evolutionary change. The higher the heritability value, the greater the potential of the trait to respond to the selection pressure. Consequently, without estimating the degree of heritability for traits, it is not possible to predict trait response to selection (i.e. in this case, climate change effects).

The additive genetic contribution to a trait can be estimated through narrow-sense heritability (h_2). Table 2.2 contains studies on hardseeded species where an estimate of heritability was made on initial PY. Of the papers measuring h_2, the mean estimate is 88.19% with a range of

64-98.9%. Despite wide variation in the results, there is a general trend that PY characteristics have a high heritability, suggesting the potential for evolutionary change to occur given a selection pressure. However, as heritability estimates are only valid under the set of environmental conditions used, we classified studies based on the environment in which the parental plant was grown, with those conducted under glass / vinyl house expected to have a more stable growing environment compared to field-based studies. Glass / vinyl house studies had an average h_2 of 92.6%, compared with 84.9% for studies conducted in the field, suggesting that under more variable environmental conditions (as expected for wild species) genetic components of phenotypic variance may be lower and thus a reduced evolutionary response to selection would be possible. However, the small sample size here, and the fact that all these studies are based on highly selected cultivars / accessions / lines of the species, must be considered.

Table 2.2: Species for which broad sense (H_2) and narrow sense (h_2) heritability studies have been conducted for initial PY from the literature, including the dormancy breaking methodologies used.

Reference	Family	Species	Parent growth environment	Conditions	H_2 (%)	h_2 (%)
Furbeck *et al.*, 1993	Malvaceae	*Gossypium hirsutum*	Field	7 d at 18.3°C		64.00
Nair *et al.*, 2004	Fabaceae	*Trifolium michelianum* cv. Paradana	Field	12 d at 22°C		90.50
Nair *et al.*, 2004	Fabaceae	*Trifolium michelianum*	Field	12 d at 22°C		96.10
Slattery, 1986	Fabaceae	*Trifolium subterraneum*	Glass house	Unknown	19.00	
Humphrey *et al.*, 2005	Fabaceae	*Vigna radiata*	Glass house	72 hours at 25°C		84.00
Humphrey *et al.*, 2005	Fabaceae	*Vigna radiata*	Field	72 hours at 25°C		89.00
James *et al.*, 1999	Fabaceae	*Vigna radiata* ssp. *subulata* x ssp. *radiata* cv. Berken	Glass house	3 d at unknown	99.00	
James *et al.*, 1999	Fabaceae	*Vigna radiata* ssp. *subulata* x ssp. *radiata* cv. Celera	Glass house	3 d at unknown	74.00	
Sriphadet *et al.*, 2007	Fabaceae	*Vigna radiata*	Glass house	3 d at 23-25°C		98.90
Sriphadet *et al.*, 2007	Fabaceae	*Vigna radiata*	Glass house	7 d at 23-25°C		94.80
Isemura *et al.*, 2012	Fabaceae	*Vigna radiata*	Vinyl house	1 d at 15°C	99.60	
Isemura *et al.*, 2010	Fabaceae	*Vigna umbelata*	Vinyl house	1 d at 15°C	97.20	
Veasey & Martins, 1990	Fabaceae	*Desmodium barbatum*	Field	21 d at 40°C	37.00*	
Veasey & Martins, 1990	Fabaceae	*Desmodium tormentosum*	Field	21 d at 40°C	40.00*	
Veasey & Martins, 1990	Fabaceae	*Desmodium tormentosum*	Field	21 d at 40°C	95.00*	
Veasey & Martins, 1990	Fabaceae	*Desmodium incanum*	Field	21 d at 40°C	89.00*	
Veasey & Martins, 1990	Fabaceae	*Desmodium discolour*	Field	21 d at 40°C	84.00*	
Mean					77.76	88.19

* Calculated as coefficient of determination, regarded as an upper limit of broad sense heritability (not included in estimation of mean)

Estimates of additive gene action on genetic variation may allow estimates of heritability to be calculated, but selection (and therefore the potential for evolutionary change) will also be influenced by the presence of dominant gene interactions. Studies which look more specifically at estimating additive / dominant genetic control based either on general (gca) / specific combining ability (sca) estimates (additive / dominant gene action, respectively) or the actions of identified quantitative trait loci (QTLs) have found differing results for different PY traits. For example, both Morley (1958) and Furbeck *et al.* (1993) concluded that the genetic control of initial PY is mostly additive (based on gca / sca estimates), a result matched by Kaga *et al.* (2008). However, Kaga *et al.* (2008) also found that dominance effects were higher for QTLs linked to the loss of PY in the field than for initial PY, suggesting different controls for different traits, meaning that each trait would respond differently to selection pressures exerted by climate change. Where studies have attempted to identify QTLs or possible gene numbers involved in PY (Donnelly *et al.,* 1972; Lee, 1975; Kilen & Hartwig 1978; Keim *et al.*, 1990; Ramsay, 1997; Sakamoto *et al.,* 2004; Humphrey *et al.*, 2005; Li *et al.,* 2012) all conclude that there is likely to be at least one major gene controlling PY, with minor genes interacting to confer dominance. Most studies agree that it is likely to be only a few (usually two or three) major genes that are involved with the development of an impermeable seed coat (the dominant form). Given the low number of major genes potentially involved in PY, it may be a reasonable assumption that they are either linked with the formation of the water-gap complex (Isemura *et al.,* 2012), or simply to whether a species has physically dormant seeds. Whether this follows on to the control of the different characteristics of PY beyond initial percentage hardseededness requires further investigation.

A number of caveats need to be considered when assessing heritability of PY from the literature. Arguably the key point is that the majority of these studies are based on agricultural

species, which have been subjected to intense selective breeding, often against PY. Even when recombinant inbred lines (RILs - *'a collection of strains used to map quantitative trait loci'* (Pollard, 2012)) generated from 'wild' types or accessions are crossed with cultivated accessions, there is likely to have been genetic changes altering heritability, thus limiting the inferences that can be made about PY for wild species. The agricultural focus also neglects to account for site-to-site genetic variation that may exist, for example, in how phenotypically plastic a trait is. In natural environments under a changing climate, this is a major gap, which needs to be addressed. Secondly, the methods used throughout the literature vary widely, both across the genetic methods used, and the methods to test for PY. For example, in Table 2.2, of the four studies that used *Vigna radiate,* only two used similar test conditions, limiting the cross-study comparisons.

2.5. Maternal effects

Maternal effects include important genetic and environmental influences on PY. Roach & Wulff (1987) argue that these can be understood as "*cytoplasmic genetic, endosperm nuclear and maternal contributions to the phenotype*", whilst Donohue (2009, pp. 1061), highlights five sources of influence:

> "*i) the maternal genetic effects caused by the maternal inheritance of plastids, ii) the effects of endosperm, which is triploid, with two-thirds of its genotype of maternal origin, iii) the effects of the seed coat which is maternal tissue, iv) the effects of maternal provisioning during seed development, with nutrient resources, hormones, proteins & transcripts, all capable of being provisioned to seeds by the maternal parent, and v) the maternal determination of the post progeny environment via dispersal or phenology*".

However, as with both classification systems, the factors are not independent. Because of the importance of the seed coat in PY control, and the fact that the seed coat is of maternal origin, it is commonly assumed that PY is under maternal control. To this end, a number of studies (e.g. Donnelly *et al.*, 1972; D'hondt *et al.*, 2010) do not control pollination in genetics studies, assuming no paternal effect. As previously mentioned, there is some evidence that PY can be passed through the paternal line in certain species (e.g. Ramsay, 1997), and paternal effects on seed size in general are well known (e.g. House *et al.*, 2010), all suggesting that further research into the paternal role in PY is required.

In seed ecology, distinguishing between maternal effects and environmental effects is important but difficult (Roach & Wulff, 1987). Given that a seed is attached to the maternal plant until dispersal, factors that affect the maternal plant will also affect the seed; although if these effects occur through the maternal plant (e.g. via seed provisioning), or via the direct influence of the environment on the seed, is hard to split. Roach & Wulff (1987) therefore advocated that environmental effects during the period of embryo formation to seed dispersal should be considered as maternal effects. Under such criteria, the aspects of PY variation attributed to purely environmental effects can only occur post-dispersal and relate to seed storage conditions, whilst phenotypic variation resulting from environmental variation during seed fill should be attributed to maternal environmental effects. For certain environmental effects and species this may be so, but for factors such as humidity, it may not be as clear cut.

2.6. Environmental effects through the maternal plant

Given that climate is expected to change rapidly, understanding how much of an influence environmental maternal effects have on PY variation is crucial for predicting future response patterns. Rainfall, temperature and humidity are three main factors projected to change.

However, whilst much of the existing literature considers these factors separately, interactions between them are likely to have a strong impact within natural ecosystems.

2.6.1. Rainfall

Rainfall has a strong influence on seed weight (Baskin & Baskin, 2014), but has also been shown to influence seed dormancy in PY species. Overall, the current literature seems to suggest that lower rainfall during seed development increases initial PY (Hill *et al.*, 1986b; Smith *et al.*, 1998; Norman *et al.*, 2006; Gresta *et al.*, 2007), although this is not always so (Norman *et al.*, 2002; Michael *et al.*, 2006).

Norman *et al.* (2006) studied 20 *T. subterraneum* genotypes produced at three sites differing in annual rainfall. They found that seeds from the driest site had the highest level of initial PY, while seeds produced at the wettest site had lower initial levels of PY but maintained the dormancy for a longer period of time. Similar results were found when maternal plants of *Glycine max* were subjected to water stress; they produced seeds with a higher initial PY percentage (Hill *et al.,* 1986b). In contrast, in a long-term study by Nichols *et al.* (2009) on *T. subterraneum,* the same seed mix was planted at two sites differing in mean annual rainfall (and mean annual maximum temperature). After 16 years, seeds produced at the wetter site showed higher initial PY (63.3%) compared to those from the drier site (54.2%). After 16 weeks of storage, seed from the drier site was 41.3% dormant in contrast to only 6.7% of seed from the wetter site. Although it could be suggested that under increasing drought conditions plants with PY may produce a higher percentage of initially dormant seeds, it is important to note that within natural ecosystems seed lots are often 90-100% dormant at seed dispersal (Auld & O'Connell, 1991; Ooi *et al.,* 2014). Consequently, the impact of rainfall on the ability of seeds to maintain PY is more important than initial dormancy in many natural ecosystems.

In a study by Michael *et al.* (2006), *Malva parviflora* seeds produced at wet sites differed in their dormancy-breaking response after storage treatments under natural conditions compared to those produced at a dry site. Seeds from the wetter sites showed higher dormancy maintenance (>84% dormant) compared to those from drier sites (45-55%) after one year of storage, but after two years of storage differences in dormancy were less apparent, with less than 11% left dormant compared to less than 7% for drier sites. Despite the different time frames involved, the results of Michael *et al.* (2006) contradict those of Nichols *et al.* (2009), but both show that rainfall is likely to affect the ability of seeds to maintain PY. In ecosystems where seeds are required to remain dormant for extended periods of time, such as arid or fire-prone environments, changes to rainfall patterns could have important implications for seed bank maintenance. Thus, the relationship between rainfall and the ability of seeds to maintain PY in the seed bank needs further investigation.

2.6.2. Temperature

There are few studies that have experimentally tested the effects of temperature experienced by the maternal plant during seed production on physical dormancy; however, those that do, suggest that dormancy may be altered as a result. Argel & Humphreys (1983a) studied seeds of *Stylosanthes hamata* cv. Verano (Fabaceae) grown on plants subjected to temperature treatments of either 21°C, 24°C or 27°C during the flowering phase. Plants grown at 21°C produced seed lots with initial PY of less than 20%, compared to greater than 90% initial PY for those grown at 27°C. At 24°C the results depended upon pod position, but plants produced over 50% dormant seeds. Similarly, Piano *et al.* (1996) collected seeds of 374 lines of *T. subterraneum* from 61 sites around Sardinia, Italy, and found that initial hardseededness showed a strong positive correlation with April and October temperatures (r = 0.29 and 0.25,

respectively). The range of temperatures used by Argel & Humphreys (1983a) are within those projected under the most extreme climate change scenarios for many Mediterranean regions (IPCC, 2014). Based on this and the similar pattern shown by Piano *et al.* (1996), there is some suggestion that species with PY may produce a higher proportion of dormant seeds under a future warmer climate. However, the very limited amount of data available highlights the need for considerably more research to be focused on these effects. As with rainfall effects, more focus on non-agricultural species, and on other PY traits, is required to enable more robust projections of responses under future climates.

2.6.3. Humidity

The role of humidity in triggering the onset of PY during seed development is one of the best-investigated aspects of PY. The link between the onset of PY and low humidity has been displayed in both agricultural and natural settings, with numerous studies showing the need for humidity to drop below a threshold value for PY to occur (Quinlivan & Nicol, 1971; Argel & Humphreys, 1983a; Bolingue *et al.*, 2010). Tozer & Ooi (2014) found that dormancy was induced in Australian *Acacia saligna* seeds once relative air humidity dropped below approximately 20%, usually prior to seed pod dehiscence. However, the effect of humidity-induced PY can occur pre- or post-dispersal (e.g. Bolingue *et al.*, 2010; Tozer & Ooi, 2014), suggesting that it may be less of a maternal environmental effect, and more closely linked with the direct influence of microclimate on moisture content of the seed. However, due to the presence of a distinct threshold in a number of species for the formation of PY, it is possible that this is maternally, or at least genetically, determined. Despite this, D'hondt *et al.* (2010) studied nine genotypes of *Trifolium repens* grown under 46.5-78.3%, 61.1-94.3% and 89-99.9% relative humidity (RH). The different treatments produced different levels of initial PY

(high humidity, low PY; and low humidity, high PY), but there was no interaction between clone and humidity. Most of the PY variation was attributed to phenotypic plasticity. As most Mediterranean areas are projected to face an overall drying trend (IPCC, 2014), this would produce a reduction in RH. Consequently, based on the range of plasticity and the humidities shown in D'hondt *et al.* (2010), it may be expected that the level of initial PY would therefore increase. However, as with temperature and rainfall, predictions of the impacts that this will have on other PY traits are limited due to a lack of studies.

2.7. Environmental effects post seed dispersal

According to Donohue *et al.* (2010), environmental effects post dispersal can be considered a form of maternal effect, given the role of the maternal plant in determining the post-dispersal environment. Here, we consider them independent of maternal effects, due, in part, to the significant influence that environmental effects can have on the seed bank of PY species. This is not to say that maternal effects are not present post dispersal, particularly as maternal preconditioning of response patterns may occur. Given the importance of environmental effects on the seed bank, most of the literature focus is on the impact of environmental conditions on the maintenance of PY, and changes to the conditions required to break PY with time, rather than initial PY. In some ecosystems, such as fire-prone environments, these effects can continue for several years after the death of the parent plant, distinctly separating maternal environmental conditions from post-dispersal conditions. In the context of climate change, changes to temperature, rainfall and humidity will affect the ability of seeds to maintain PY, altering seed bank accumulation, persistence, bet-hedging capacity and germination timing (Walck *et al.*, 2011; Ooi, 2012). In addition, these climatic changes will exert a strong selection pressure on populations, through their control over dormancy loss.

2.7.1. Rainfall / soil moisture

In certain species, water availability during seed storage has been shown to influence dormancy loss. *Ipomoea lacunosa* seeds initially stored at 30°C in wet sand subsequently germinated to greater than 95% at a 25/15°C temperature regime. If seeds were initially stored in dry sand, germination did not occur (Jayasuriya *et al.*, 2008a). In this case, rainfall would be required for the effects of heat to cause seeds to break dormancy. In contrast, *Cuscuta australis* seeds required dry storage in order to break dormancy (Jayasuriya *et al.*, 2008b). Once in the seed bank, soil moisture levels rather than rainfall *per se* will be important in PY loss; however, as a construct of laboratory incubator experiments, it is more common for studies to measure the effects of storage humidity on the ability of seeds to maintain PY. For example, Bolingue *et al.* (2010) found that time to 50% imbibition was five days for *Medicago trunculata* seeds stored at 57% RH, compared with 58 days for seeds stored at 5.5% RH. Moreover, the temperature at which seeds develop can interact with RH to affect PY. *Stylosanthes hamata* plants were grown at 21°C, 24°C and 27°C during fruiting, then collected seeds were stored for 120 days under RH's of 77%, 32%, 15% or 6%. At 77% RH the seed lot developed under 21°C was 100% non-dormant, while the seeds developed at 27°C were only 24% non-dormant (Argel & Humphreys, 1983b), highlighting the strong effect of storage RH on PY maintenance. Ecologically, however, RH will reflect differing soil moisture conditions experienced in the soil seed bank, which may cause different patterns of PY maintenance than variation in relative air humidity and must be taken into account when assessing such studies.

The study of Argel & Humphreys (1983b) provides evidence of preconditioning by the maternal plant, in this case during fruiting, revealing that this alters the seeds' response to

storage humidity. Consequently, whilst each climatic factor has been considered independently in this review, and a distinction has been made between post- and pre-dispersal maternal effects, interactions between these factors will undoubtedly influence the response of PY traits, namely the time to loss of PY and the conditions required to break PY.

In addition to altering dormancy-breaking requirements, water availability is crucial for ensuring germination once seeds have lost dormancy. Projections for future changes to rainfall patterns are highly variable between regions but, in general, there is consensus that more extreme rainfall events, combined with longer drought intervals, will occur (Heisler-White *et al.*, 2009). Kimball *et al.* (2010) showed that delayed arrival of winter rainfall in the south-western US over a 25-year period altered community composition, with physically dormant *Erodium cicutarium* one of the species benefiting from this change, possibly due to PY loss being related to summer conditions rather than winter (Meisert, 2002). However, while some species may benefit, an increase in sporadic rainfall may increase the prevalence of 'false start' rain events. These occur when rainfall post dormancy break provides enough moisture for seeds to imbibe, but insufficient moisture for germination to continue. Once PY seeds have lost dormancy, wet-dry cycling can reduce the viability of a seed lot, reducing the seed bank prior to the occurrence of sufficient rainfall for the germination process to complete (Ooi *et al.*, pers. comm.; although see Van Assche & Vandelook, 2010).

2.7.2. Temperature

Temperature is the most widely cited mechanism of PY loss (Baskin & Baskin, 2014) and, as such, it may be predicted that temperature changes in the future will have large impacts upon seed bank maintenance. Loss of physical dormancy is often directly related to temperature, with an increasing proportion of any seed lot losing dormancy as temperature increases

(Martin *et al.*, 1975; Auld & O'Connell, 1991; Van Assche & Vandelook, 2006; Ooi *et al.*, 2012). The temperature experienced by the seeds once they are on the soil surface or in the seed bank, and the dormancy-breaking conditions that the seeds require, combine to ensure that germination occurs during the best period for recruitment. For example, PY species that recruit into gaps have dormancy-breaking requirements related to diurnal temperature fluctuations reflecting those that occur once a gap appears (e.g. Vázquez-Yanes & Orozco-Segovia, 1982). Projected increases in soil temperatures as a result of climate change could cause an increase in seeds from the seed bank losing dormancy outside of optimal conditions (Ooi *et al*., 2009 & 2012). For species whose dormancy is broken by a specific environmental trigger, such as gaps or a fire, loss of dormancy due to increased soil temperatures (resulting from climate change) between events could reduce the magnitude of successful recruitment after the next specific recruitment event. For example, *A. suaveolens* and *Dillwynia retorta* seeds from the fire-prone sclerophyllous woodland of eastern Australia did not lose dormancy when exposed to projected future average temperatures for the area, but under projected heat-wave conditions there was a significant increase in the loss of PY (Ooi *et al.*, 2012). For these species, whose adult plants are killed by fire and which rely on the seed bank for ensuring a post-fire generation, germination outside of the optimal post-fire environment may result in a net loss to the seed bank if these plants are unable to reproduce before the next fire event. This would reduce the number of seeds available to respond to the next fire event.

While in fire-prone regions germination outside of the post-fire environment is likely to result in a net loss to the seed bank, many species with PY in temperate areas also have physiological dormancy (PD) as an additional mechanism to control germination timing and subsequently recruitment success (Van Assche & Vandelook, 2010; Baskin & Baskin, 2014). In these situations (known as combinational dormancy) the embryo can become dormant,

preventing germination, even if PY has been broken. For example, Van Assche & Vandelook (2010) found that in a number of winter annual Fabaceae, including *Vicia sativa, V. hirsuta* and *Medicago arabica,* PD was present in fresh seeds when held at 23°C, but not at temperatures lower than 15°C. For PD to be broken, dry storage for three months was required, although for *V. sativa* this treatment also broke PY (Van Assche & Vandelook, 2010). For these species, this may act as an additional buffering mechanism against the impacts of climate change compared to their PY-only counterparts.

Although several suggestions have been made regarding the mechanisms that determine PY break for individual species, e.g. Jayasuriya *et al.*, (2009) for *Ipomoea*, the mechanisms for the majority of species remain unclear (Van Assche & Vandelook, 2010). Interestingly, however, Zeng *et al.* (2005) analysed the fatty acid composition in the seed coats of *T. subterraneum* and *Trifolium spumosum* from Western Australia, and found that the fatty acids within the seed coat had melting points ranging from 22-85°C. This mirrors the temperatures seeds would be exposed to in the soil seed bank during the summer period. If dormancy loss in PY seeds is related to the fatty acid composition of the seed coat, then prediction of the conditions required for PY breakdown may be possible. Further research into correlations between fatty acid composition, seed dormancy and dormancy-breaking requirements could be of great benefit to understanding PY and related seed bank dynamics.

2.8. Physical dormancy response to climate change and its implications

Across the literature reviewed here, a number of broad generalisations can be made with regard to physically dormant species. Firstly, evidence from heritability studies, based primarily on agricultural species, suggests that heritability (narrow-sense) for initial PY is high (generally greater than 80%, Table 2.2), with a few major genes involved and a number

of minor ones. Secondly, seeds produced under a higher maternal temperature, and low water availability, seem to produce seed lots with a higher initial percentage PY. The duration for which seeds are able to remain dormant is also affected by high maternal temperatures, although consensus on the direction of this effect is lacking, potentially reflecting species-specific variation (e.g. Argel & Humphreys, 1983a; Hill *et al.,* 1986a & b; Piano *et al.,* 1996; Keigley & Mullen, 1986; Norman *et al.,* 2006; Jayasuriya *et al.,* 2008a). Thirdly, the humidity experienced by the maternal plant, and in some cases by the seeds post dispersal, determines PY onset (Tozer & Ooi, 2014) and, finally, the exact causes of the variation observed at the intra-specific level, genetically or environmentally, are not clear.

Although the genetic influence on all the PY traits is far from understood, initial PY does seem to be dominant and heritable, at least in agricultural species. There is also some support for this from studies of species in natural ecosystems. For example, Lacerda *et al.* (2004) compared PY (after one year of storage) in *Senna multijuga* and *Plathymenia reticulata* from individual maternal plants from two populations per species. Although collections were done over multiple years for *S. multijuga,* introducing maternal and non-maternal environmental differences, the coefficient of genotypic determination suggests that a large amount (0.91-0.97 and 0.86-0.95, respectively) of the trait variation is due to genetically based differences. However, while studies show a high genetic contribution to PY (at least initial PY), large variation in many other PY traits within a given seed lot is also often found (e.g. Salisbury & Halloran, 1983), suggesting that some PY traits display phenotypic plasticity. This may be the result of past selection to cope with the stochastic climates under which PY species often occur.

Phenotypic plasticity may buffer some PY species against increasingly variable climatic conditions project for the future (IPCC, 2014), providing a mechanism for coping with changing environmental conditions, at least in the short term. The level of buffering will be dependent on the extent of the increase in climatic variation. If the traits are not plastic, or only slightly plastic, then adaptation will depend on whether PY change can keep pace with the projected rate of climate change. For example, Nichols *et al.* (2009) showed that selection for changes to PY can occur over a relatively short time period in annuals. Within the 16-year time frame of their study, clear changes in initial PY and the ability of seeds to maintain PY had occurred, suggesting that relatively rapid changes to PY in annuals species may result from selection pressures induced under future climate change. However, the ability of many perennial PY species to adapt to such rapid change is likely to be much lower, particularly in natural ecosystems where generational turnover is long or driven by intermittent processes, such as fire, which can occur only once every 10 to 30 years.

For 'wild' species in natural habitats, initial levels of PY, in particular, are less important, and understanding the impacts of climate change on dormancy-breaking requirements and the resulting population dynamics are of much greater significance for predicting species' persistence. Temperature, rainfall and humidity have all been shown to alter the ability of a seed in storage to maintain dormancy, and there is a significant chance that dormancy loss outside of optimal recruitment conditions may result. For example, an increase in the intensity and duration of heat-wave events, as suggested by Ooi *et al.* (2012), may deplete the seed bank during the inter-fire period. This could result in a much smaller seed reserve available for post-fire recruitment. Understanding the impacts of climate change on PY species in natural ecosystems will therefore depend on knowing if the variation in the conditions required to break dormancy is determined by a predominantly plastic response, and if so, how

plastic the response is. Just as importantly, understanding seedling survival after dormancy loss is necessary, particularly due to the likely effects of changing rainfall regimes on germination from the seed bank and recruitment success.

2.9. Future directions

Variation exists in the dormancy-breaking criteria for PY species, both intra- and inter-specifically. Environmental conditions appear to play a major role in PY determination, particularly initial PY, although whether this is purely environmental or the result of phenotypic plasticity is uncertain. The PY literature is currently dominated by studies based on agricultural species, resulting in many of the genetic studies being based on inbred lines that have been subjected to years of artificial selection. There is also a strong bias towards annual species in the Fabaceae family (in particular the Papilinoideae clade), and a focus on the initial PY trait. While certain information can be transferred to natural ecosystems from these studies, there are limits. We therefore suggest that a key requirement of future study is to focus on species from natural ecosystems, with the aim to establish how PY traits, beyond that of simply initial levels of dormancy, vary in response to the different factors projected to change in the future. In particular, understanding how PY changes will affect natural seed bank dynamics is critical for accurate modelling of population persistence in the future.

In addition to this broad suggestion, a number of more specific factors related to understanding PY and its variation would benefit from additional study. For example, it is often assumed in the current literature that paternal effects on PY are negligible. However, given the influence that paternal effects can have on seed size and other seed traits (e.g. Lacey *et al.*, 1997; Galloway, 2001; House *et al.*, 2010), it is possible that it may also influence PY, particularly with regard to morphological traits and control of dormancy-breaking conditions.

Further investigation of this relationship would have clear consequences for the development of future experiments investigating the inheritance of PY and parental environment effects.

Studying the genetic variation in PY traits within natural ecosystems, particularly on perennial species, is inherently difficult, given the often long generation times and expense. However, without understanding the natural genetic intra-specific variation in PY traits, it will not be possible to properly account for the impact a new climate regime may have on their selection. With genomic methods this may be possible (see Storz, 2005 for discussion), allowing for a better understanding of natural selection on PY traits. Investigation into the genotypic variation of PY traits within populations could also be approached by conducting experiments on seed collected from multiple individual plants within a population. This will enable a better understanding of the proportion of maternal environmental, versus maternal genetic, contributions to PY. We recognise that seed numbers from individual maternal plants can be limiting, so this may be more difficult for some species. However, using methods like those highlighted in Hoyle *et al.* (2011), where lower seed numbers are used to conduct germination experiments, may offer one way in which this can be achieved for as broad a range of species as possible. Isolation of maternal environmental and maternal genetic effects will greatly add to the robustness of climate change response projections, by improving our understanding of population dynamics.

Finally, our ability to make generalisations from the literature reviewed, regarding the mechanisms underlying PY variation, was hindered by a lack of comparability between studies. We suggest that the use of a framework for studies of PY traits, where factors are delimited temporally from the stages of embryo formation to dormancy break, would improve comparability. This could be based around: 1) the initial proportion of seeds dormant at

release from the maternal plant; 2) the conditions required to break dormancy; and 3) changes to the conditions required to break dormancy over time. Furthermore, it is important to report the time frames for all the stages studied, such as storage durations prior to dormancy tests, to get a clearer understanding of the processes determining PY trait variation, adaptation potentials of species and future population dynamics under a changing climate.

Chapter 3. SPATIAL AND TEMPORAL VARIATION IN PHYSICAL DORMANCY CHARACTERISTICS: CAUSES AND CONSEQUENCES.

3.1. Introduction

In-situ seed banks provide an important mechanism that plants can use to cope with environmental variability (Fenner & Thompson, 2005). A seed bank enables partial control over the timing of germination, improving the chance of successful recruitment (Baskin & Baskin, 2014), as well as storing a new generation that contributes to population persistence upon the death of the standing generation (Fenner & Thompson, 2005). Phenotypic variation in the dormancy breaking, or germination requirements, of a population's seed bank provides resilience against recruitment failure by ensuring not all seeds germinate in response to one environmental stimulus (Fenner & Thompson, 2005). This can be viewed as a form of environmental buffering, and is particularly important in areas where environmental conditions are highly stochastic, such as deserts and fire-prone environments. In these situations, understanding phenotypic variation in the response of seed traits to environmental stimuli is critical for understanding population dynamics and, therefore, enabling prediction of species responses to future change (Walck *et al.*, 2011; Hudson *et al.*, 2015). Moreover, with increased emphasis being placed on *ex-situ* seed banks for conservation (e.g. Hoban & Strand, 2015; Visscher *et al.* 2016), understanding dormancy variation is essential for designing suitable seed collection strategies and efficient usage of stored seed.

Physical dormancy (PY) is one of the most common types of seed dormancy in fire-prone environments. It is present when the seed coat (testa), or endocarp, of a seed is impermeable, preventing water from reaching the embryo (Baskin & Baskin, 2014). In order for germination to be possible, the seed coat must be cracked allowing water to reach the embryo.

In fire-prone environments, it is usually heat from a fire that causes the seed coat to crack (Baskin *et al.,* 2000; Baskin & Baskin, 2014). Physical dormancy in fire-prone systems is commonly associated with the obligate seeding life-history strategy, present in many perennial species, whereby the fire kills the parental generation. Consequently, stands of these species effectively have one reproductive event within a fire cycle and generations have limited overlap due to the fire killing the parental generation.

Without successful germination post fire, the populations of obligate seeding species face decline or local extinction. Population maintenance in these species is, therefore, dependent upon both successful germination post-fire and the survival of seedlings to reproductive age before the next fire event. The former can occur by maximising the chance of successful germination after a fire event (i.e. loss of seed dormancy), to counter the death of the standing generation; whilst the latter can be aided by retaining a portion of seed dormant within the seed bank until a subsequent fire event, buffering against potential seedling death.

In order to replace the parental generation lost during a fire event (thereby ensuring population persistence), dormancy loss must occur in at least a proportion of the seed bank irrespective of the fire conditions experienced. However, the temperatures reached in the soil during a fire are highly variable both spatially (within a habitat and through the soil profile), and temporally (during a fire) (Whelan, 1995). For example, across 40 locations and three separate fires in south-eastern Australia soil temperatures ranged from 17°C to over 100°C within the top 5 cm (Penman & Towerton, 2008). Consequently, position within the soil profile, position in the environment, fire severity, as well as, fire residence time will all influence the temperature a seed is exposed to (Auld, 1986c). By producing a seed lot with a

range of dormancy breaking requirements, a population would be able to respond to the range of possible soil temperatures that could be experienced during a fire.

Recent evidence has suggested that the conditions needed to break PY in fire-prone species may vary intra-specifically (e.g. Auld & O'Connell, 1991; Bolin, 2009; Ooi *et al.,* 2012 & 2014; Liyanage & Ooi, 2015). Moreover, evidence from studies on predominantly agricultural species has suggested that environmental conditions (e.g. in rainfall or temperature) experienced by parental plants during seed production can modify the characteristics of PY (alongside genetic influence) (e.g. Argel & Humphreys, 1983a; Norman *et al.,* 2006). For example, Argel & Humphreys (1983a) found that plants of *Stylosanthes hamata* grown under higher temperatures produced more dormant seeds than those produced under cooler temperatures. In addition, Michael *et al.* (2006) found that the water availability at a site influenced the ability of *Malva parviflora* seeds to retain their dormancy over time. However, the impacts of environmental factors on PY characteristics appear to be variable (see Hudson *et al.*, 2015 and Jaganathan, 2016 for reviews). Moreover, few studies have investigated this in non-agricultural species, particularly across multiple populations, to account for potential local adaptation (Hudson *et al*., 2015 Chapter 2; although see Michael *et al.,* 2006 and Ooi *et al.,* 2012). Yet, should environmental conditions experienced by parental plants during seed development influence PY traits, then there is the potential for future climate change to modify the seed bank dynamics of species with PY in fire-prone environments.

In this study we set out to quantify the variation occurring in the PY characteristics of an obligate seeding species with a broad distribution from a fire-prone environment. Specifically, we will focus on three characteristics of PY: proportional dormancy loss, the proportion of seeds remaining dormant and the proportion of seeds inviable. Until now, the majority of

investigations into seed bank dynamics in fire-prone environments have focused on the variation in seed dormancy within seed lots collected over a single year. However, in reality, for perennial species, seed rain typically enters the seed bank over multiple years, with adult plants contributing to the seed bank until the next fire event. Consequently, we collected seed rain produced over three successive years from a range of populations, before heat treating the seeds at a range of possible fire-related heat treatments and observing germination (i.e. dormancy loss). By including parental plants exposed to a range of climatic conditions (temperature and rainfall), this allows us to address the following questions:

1. Do the three characteristics of PY vary among years or populations after a range of fire-related temperature treatments?
2. Can the variation in the three PY characteristics after the range of fire-related temperature treatments be explained by the temperature or rainfall conditions experienced by the parental plants during seed development?
3. Can the variation in time to germination within a seed lot after a range of fire-related heat treatments be explained by the maximum site temperatures?

Overall, these three questions allow us to ask, can the variation in PY characteristics be explained by characteristics of the developmental environment? We predict that the warmer the climatic conditions are likely to be post-fire, the larger a residual seed bank will be, and the greater the variation in germination timings post dormancy loss.

3.2. Methods

3.2.1. Species and habitat

We used the commonly occurring species *Acacia suaveolens* Sm. (Willd.) from dry sclerophyllous woodland of eastern New South Wales, Australia. This habitat is characterised by nutrient poor, sandstone soils with a flora dominated by the Fabaceae, Mrytaceae, Proteaceae, Epacridaceae and Rutaceae families (Keith, 2004). *Acacia suaveolens* has a wide distributional range, extending from Tasmania in the south to the Hervey Bay area of Queensland, covering approximately a 13° latitudinal span (Fig. 1.1B; Morrison, 1986a). The species can also be found along altitudinal gradients from 0 m asl to 950 m asl (Morrison, 1986a). *Acacia suaveolens* flowers during the autumn, with seed dispersal occurring during the spring and summer (Morrison, 1986a). The seeds of the species are physically dormant upon dispersal and are reportedly long lived; Ewart (1908) reported viable seeds of *A. suaveolens* after 51 years, while Auld (1986b) estimated a half-life of 10.7 years for *A. suaveolens* seeds within the soil seed bank, suggesting that the seeds are able to remain dormant in the soil seed bank for decades.

3.2.2. Field sites and seed collection

We collected seeds from a total of eighteen sites over two climatic gradients, one altitudinal and one latitudinal, over three successive years (2012-2014). The altitudinal gradient extends from 200 m asl to 903 m asl whilst the latitudinal gradient spans six degrees of latitude. Both gradients cover a 3-5°C change in average temperature (Table 3.1). The average fire-return intervals (area weighted) for the altitudinal sides is 21 years, and 14-21 years for the coastal locations (Enright *et al*., 2012). Seed collection took place between October and December

2012 to 2014. We only collected mature, dormant seeds at their point of natural dispersal. Dormant seeds of *A. suaveolens* are dry and easily distinguished from non-dormant seeds by their black colour and hard seed coats (Auld, 1986a). We randomly collected seeds from at least 25 maternal plants per site and bulked all seeds to give a final seed lot per site for each year. By collecting from at least 25 maternal plants per site, we ensured representation of population diversity. We collected the seeds from the same group of maternal plants each year, although the numbers of seeds per plant contributing to the bulked seed lot did vary. After collection, we stored seeds from each site under constant laboratory conditions (approximately 23°C) until April of the following year when dormancy trials began. Seeds from some populations could not be collected for each year (Table 3.1).

Table 3.1. Seed collection sites for *Acacia suaveolens* seeds. Sites are arrayed along intersecting altitudinal and latitudinal temperature gradients.

Site	Abb.	Latitude (°S)	Longitude (°E)	Elevation (m asl)	Years seed collected
Narooma	Nar	36°14'56"	150°08'16"	9	2012,2014
Potato Point Road	PPrd	36°05'38"	151°05'31"	106	2012,2013
Camel Rock	CR	36°22'48"	150°04'22"	18	2013,2014
Garie Trig	GT	34°42'36"	151°03'18"	211	2012-2014
Heathcote	Hea	34°04'05"	150°59'44"	55	2012-2014
Temptation Creek	TC	34°03'36"	151°04'01"	113	2012-2014
Diamond Head	DH	31°40'37"	152°48'03"	19	2012-2014
Crescent Head	CH	31°13'0"8	152°57'28"	5	2012-2014
Hat Head	HH	30°59'52"	153°01'30"	28	2013,2014
Angourie	Ang	29°28'48"	153°21'21"	18	2013,2014
Evans Head	EH	29°04'15"	153°25'01"	17	2013,2014
Lennox Head	LH	28°46'58"	153°35'17"	*	2013,2014
Faulconbridge	Faulc	33°41'56"	150°31'30"	461	2012-2014
Lawson	Cem	33°43'40"	150°25'55"	690	2012-2014
Mount Hay 1	MtH1	33°39'03"	150°22'01"	875	2012-2014
Mount Hay 2	MtH2	33°39'18"	150°22'04"	906	2012-2014
Mount Tomah	MtT	33°32'56"	150°22'58"	867	2012-2014

** no data available*

3.2.3. Dormancy assessment

We conducted the germination trials over three years (2013-2015), in the April following seed collection. Prior to each year's germination trials, we weighed 30 randomly selected seeds from each seed lot. Seed weight is known to influence the time to germination, as well as seedling survival (Moles & Westoby, 2004), so we included it in the analysis as a co-variate. After weighing, we returned all weighed seeds to the bulk seed lot, before splitting the seed lots randomly into 15 groups of 15-20 seeds (three replicate groups for each of the five treatments). We subjected each seed lot to dry heat at 40°C, 60°C, 80°C or 100°C for 10 minutes, while three groups remained under laboratory conditions as a control. This method ensures all seeds receive an even heat treatment. The control group reflected the proportion of seeds dormant in the seed bank at the point of our 'fire'. These temperatures cover a range that seeds in the soil are commonly exposed to during fire events, therefore representing a range of possible fire conditions that the seed bank could experience (Penman & Towerton, 2008). We heat-treated each replicate separately. In some cases, we did not have enough seeds for a population / year to run all the fire-related temperature treatments (identified as such in the figure legends).

Following heat treatment, we placed seeds on moist filter paper in sealed petri dishes, incubating the dishes at a 25/18°C 12 hour cycling temperature regime. We followed germination for 40 days after the dishes were placed into the incubator, classing seeds as germinated once the radicle of the emerging seedling could be seen. Our incubator temperature regime reflected average summer conditions experienced in the Sydney region, as this represents the peak fire season for eastern Australia (NSW RFS, 2016). Seeds that have lost dormancy during fires over this period would therefore germinate primarily during the

summer months (Ooi *et al.*, 2012). At the end of the 40-day trial we manually scarified any seeds that had not germinated, before returning them to the incubator under the same conditions. We monitored their germination for a further four weeks to check for viability. We assumed, that because these seeds had not had their dormancy broken by their respective heat treatments, under natural situations they would remain dormant in the seed bank until the next fire event.

3.2.4. Climatic data

For each collection site and year of seed collection, we collated climatic data (rainfall and temperature) for the period of flowering and pod formation (autumn (March-May) and winter (June-August)) from the closest Bureau of Meteorology (BoM) weather stations (BoM, 2015; Appendix 2). Where there was no suitable nearby weather station, we extrapolated site-specific data from the BoM data based on the altitudinal / latitudinal position of the seed collection site and the latitudinal / altitudinal difference between the two closest weather stations. In these situations the data is identified in Appendix 2. We selected climatic data covering the period of flowering and pod formation, as this reflects the period when environmental variables are most likely to influence seed dormancy characteristics (Donohue, 2009). In order to test the relationship between the proportion of seeds remaining dormant in the seed bank and the maximum temperatures each collection site experienced, we collated mean maximum daily summer temperatures between 2000 and 2015 in the same manner as above (except for one weather station (Springwood) where climate data was only available from 2006). We selected maximum summer temperatures, as this is likely to reflect the most extreme climatic conditions the seed bank and emerged seedlings are likely to experience.

3.2.5. Statistical analysis

We conducted all statistical analyses using R v.3.2.1 (R Core Development Team, 2015). We analysed the results from each temperature treatment separately, using a generalised linear model (GLM) with a binomial error function to correct for the error distribution associated with proportional data (seed germination, seed mortality and seeds remaining dormant) (Crawley, 2013). We analysed each temperature separately, to represent the potential response of the seed bank to different possible fire severities, allowing us to compare across the seed collection sites and years. Where the data were overdispersed, we included a quasibinomial error function to account for this. In certain cases, the assumptions for a binomial / quasibinomial GLM were not met. In these cases we arcsine square root transformed the proportion of seeds that germinated, and, subsequently analysed the transformed data with a GLM and a Gaussian error distribution. Due to a technical failure, we were unable to collect seed inviability data for the 2013 seed lot. Consequently, we only included in the inviability analysis those sites where 2012 and 2014 seed were available. In all analyses, all seed source sites were analysed together, representing a single temperature gradient. We included seed weight, and seed storage time as co-variates in the GLM analyses. Seed weight was included to control for potential maternal effects (Monty & Mahy, 2009), whilst storage time was used to account for differences in the time from seed collection to heat treatments among populations and years.

To investigate the relationship between the observed variation in the physical dormancy traits and the conditions experienced by the parental plants during seed production, we used regression analysis. We specifically focused on mean maximum daily autumn and winter

temperatures and mean rainfall as potential explanatory variables, given that this is when the seeds usually develop (Morrison, 1986a).

We calculated the time to 50% germination (T_{50}) for each replicate by plotting cumulative germination over time, and fitting a polynomial model through zero. The equation of the line was then used to calculate T_{50}. Where fewer than four seeds within a replicate germinated we were unable to reliably fit a polynomial to calculate T_{50}. Consequently, we could only calculate T_{50} for the 80°C treatment. We analysed the T_{50} values using a GLM with a Poisson error structure. The Poisson error structure accounted for the error distribution associated with count data (Crawley, 2013).

If the climatic conditions experienced by the parental plants during the reproductive phase indeed influence the traits of PY, we might expect to see wide variation between seed collection sites and years of seed collection. To address this, we compared the variability in germination, both among years for sites, and between sites over years, using the co-efficient of variation (CV). We calculated CV for the germination after the 80°C and 100°C temperature treatments, and for the proportion of seeds inviable after the 100°C temperature treatment. To test for statistical significance in the constancy of the CV values between sites and across years we used Bartlett's tests.

3.3. Results

3.3.1. Proportion of seeds germinating

Germination (proportion) within the control seeds ranged between 0.07–0.28 in 2012, 0.02–0.23 in 2013 and 0–0.42 in 2014 across source populations, indicating wide variation both among populations and between years in the proportion of dormant seeds within the seed

bank (Fig. 3.1A). For the initial level of germination after five months of laboratory storage, there was a significant interaction between population and year of seed collection (Gaussian GLM (Arcsin squareroot transformation): $\chi^2 = 2.10$, $P = 0.016$).

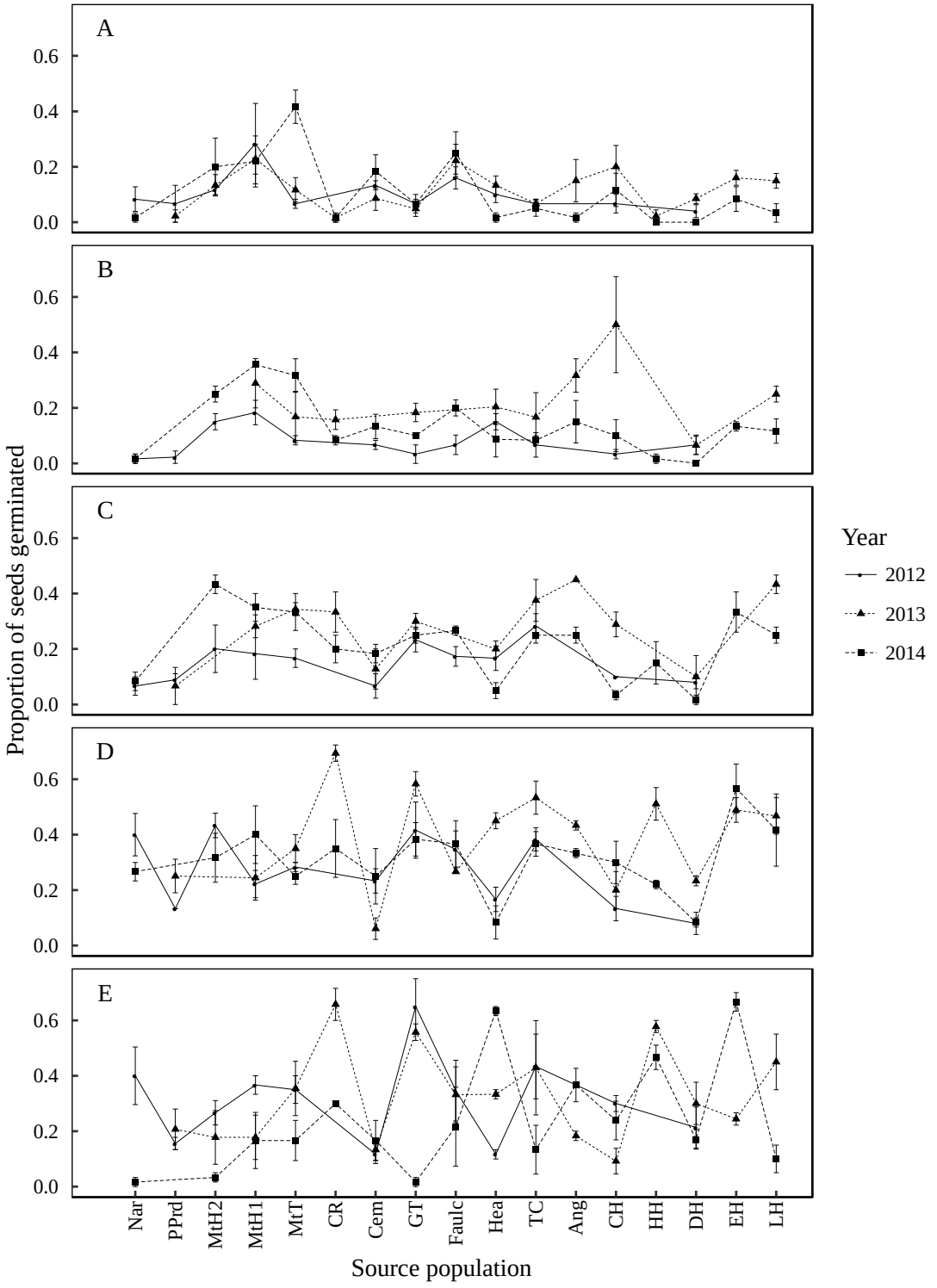

Figure 3.1. The proportion of *Acacia suaveolens* seeds germinating in response to: A) control conditions; B) 40°C; C) 60°C; D) 80°C, and E) 100°C fire-related temperature treatments (±SE). Source populations are listed in ascending order of mean daily maximum summer temperatures (not to scale).

The proportion of seeds germinating in response to the 40°C and 60°C temperature treatments was 0.14 (range = 0–0.50; Fig. 3.1B) and 0.21 (range = 0.17–0.45; Fig. 3.1C), respectively, when averaged across populations and years, indicating minimal dormancy loss. Germination was significantly influenced by the interaction between source population and year of seed collection for both temperature treatments (40°C - Gaussian GLM (Arcsin squareroot transformation): χ^2 = 1.64, P = 0.016; 60°C - Binomial GLM: χ^2 = 88.05, P = 0.01 respectively).

For the majority of source populations, seed germination (and therefore dormancy loss) was greatest after the 80°C, or 100°C, temperature treatments. After the 80°C treatment, the germination proportion across all years and source populations averaged 0.33 (range = 0.06–0.69) (Fig. 3.1D). The proportion of seeds germinating showed wide variation between the individual populations across years, with CV values ranging from 0.16 (Ang) to 0.77 (Hea), although there was no evidence for significant differences in variances among the populations (Bartlett's Test: K = 20.1, DF = 16, P = 0.2). Variation among populations across the three study years was more consistent (CV: 2012 = 0.53, 2013 = 0.47, 2014 = 0.51; Bartlett's Test: K = 2.2, DF = 2, P = 0.3). For the proportion of seeds germinating after the 80°C treatment, there was a significant interaction between the source population and year (Binomial GLM: χ^2 = 98.48, P < 0.0001) (Fig. 3.1D).

Germination in response to the 100°C treatment showed the greatest degree of variation, both among populations and across years, with germination proportions ranging from 0.02 to 0.67 (Fig. 3.1E). Across all source populations, the proportion of seeds germinating after the 100°C treatment was significantly influenced by the interaction between year of seed production and source population (Gaussian GLM: χ^2 = 2.29, P < 0.0001) (Fig. 3.1E). The

CV values ranged from 0.16 to 1.15 for source populations over the three years of collection, which were significantly different (Bartlett's Test: K = 32.4, DF = 16, P = 0.01). The between population variation for a given year was CV = 0.58 in 2012, CV = 0.61 in 2013 and CV = 0.90 in 2014, although this was not significant across the three years (Bartlett's Test: K = 1.4, DF = 2, P = 0.5).

3.3.2. Seed dormancy retention and inviability

Dormancy retention after all heat treatments was high, except after the 100°C heat treatments, indicating an ability of the seed bank to maintain a portion of dormant seeds irrespective of the fire conditions experienced. Levels of dormancy retention were greater than 40% for the control, 40°C, 60°C and 80°C treatments (except MtH1 2014 seed) (Fig. 3.2A-E), with levels of seed inviability not exceeding 25% (except MtH1 2014 seed) for these treatments (Fig. 3.3A-D). This indicates that seeds were still viable after the heat treatments, but had not had their dormancy broken. In contrast, after the 100°C heat treatment, dormancy retention varied from 0% to 48%. Dormancy retention after the 100°C heat treatment was significantly influenced by the interaction between year and populations (Gaussian GLM (Arcsine squareroot transformed): χ^2 = 2.67, P < 0.0001). However, after the 100°C heat treatment, seed inviability was high, reaching almost 100% in some population year combinations (range 7-98%) indicating high seed mortality (Fig. 3.3E). The proportion of seeds that were inviable was significantly influenced by the interaction between collection year and population (Quasibinomial GLM: F = 11.28, P < 0.0001). Interestingly, for all populations (except Hea), inviability was greater in 2014 than 2012 (Fig. 3.3).

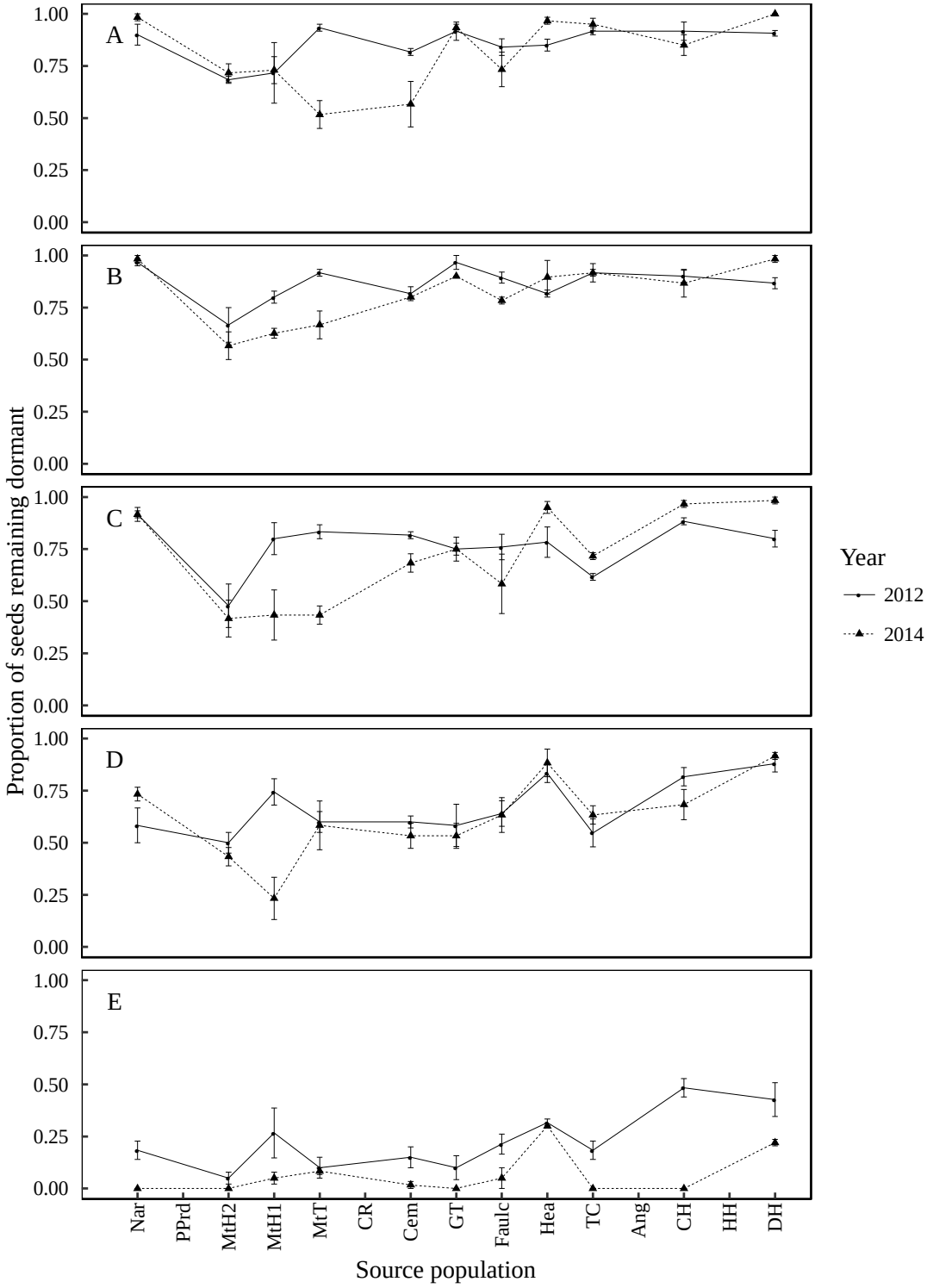

Figure 3.2. The proportion of *Acacia suaveolens* seeds remaining dormant in response to: A) control conditions; B) 40°C; C) 60°C; D) 80°C, and E) 100°C fire-related temperature treatments (±SE). Source populations are listed in ascending order of mean daily maximum summer temperatures (not to scale).

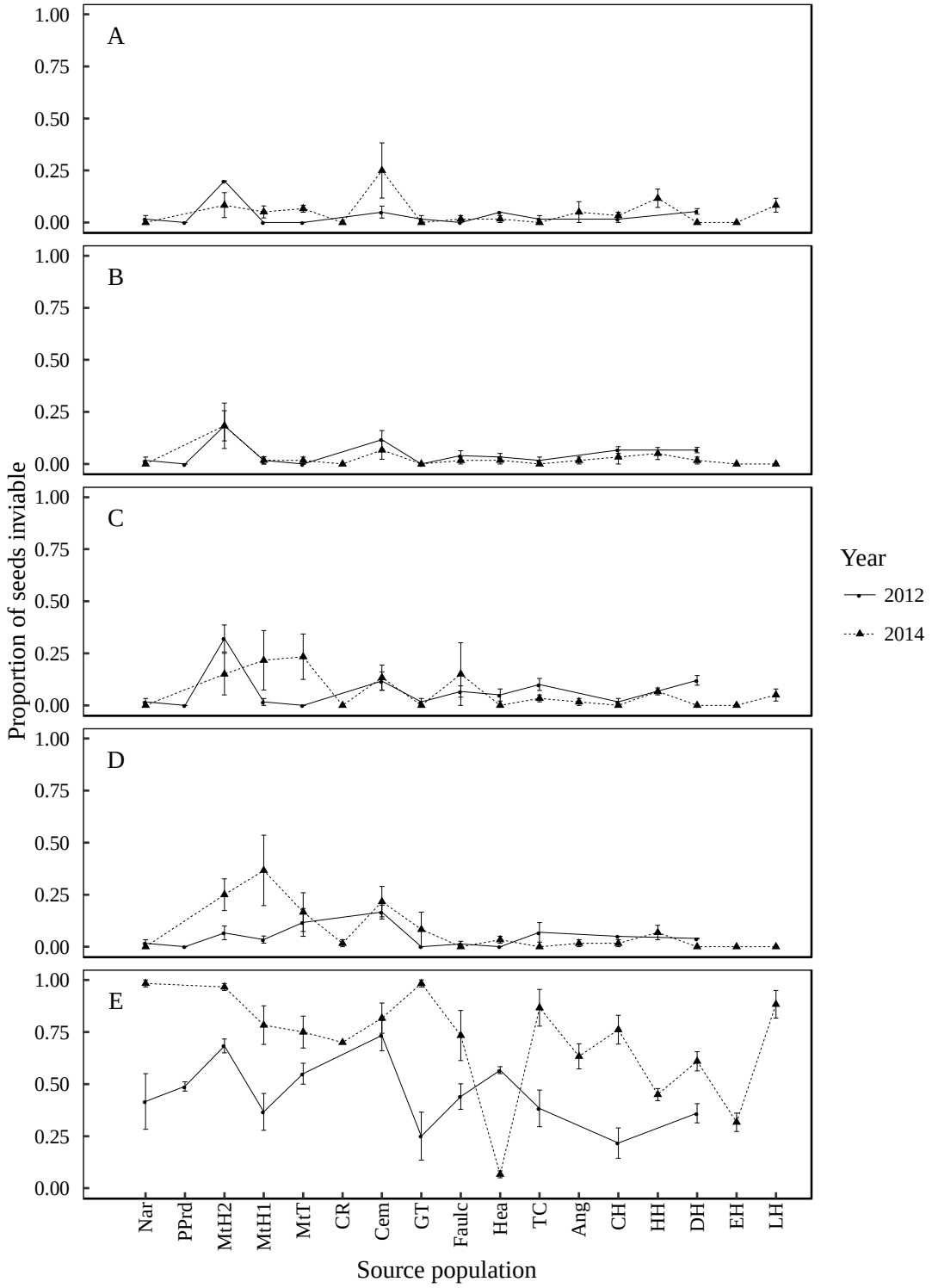

Figure 3.3. The proportion of *Acacia suaveolens* seeds inviable in response to: A) control conditions; B) 40°C; C) 60°C; D) 80°C, and E) 100°C fire-related temperature treatments (±SE). Source populations are listed in ascending order of mean daily maximum summer temperatures (not to scale).

3.3.3. Time to 50% germination

Across all populations T_{50} after the 80°C treatment averaged 16.4 days, but ranged from 7.3 to 29.5 days (Fig. 3.4). T_{50} was significantly influenced by the interaction between site and year of seed collection (Poisson GLM: $\chi^2 = 52.07$, $P = 0.0005$).

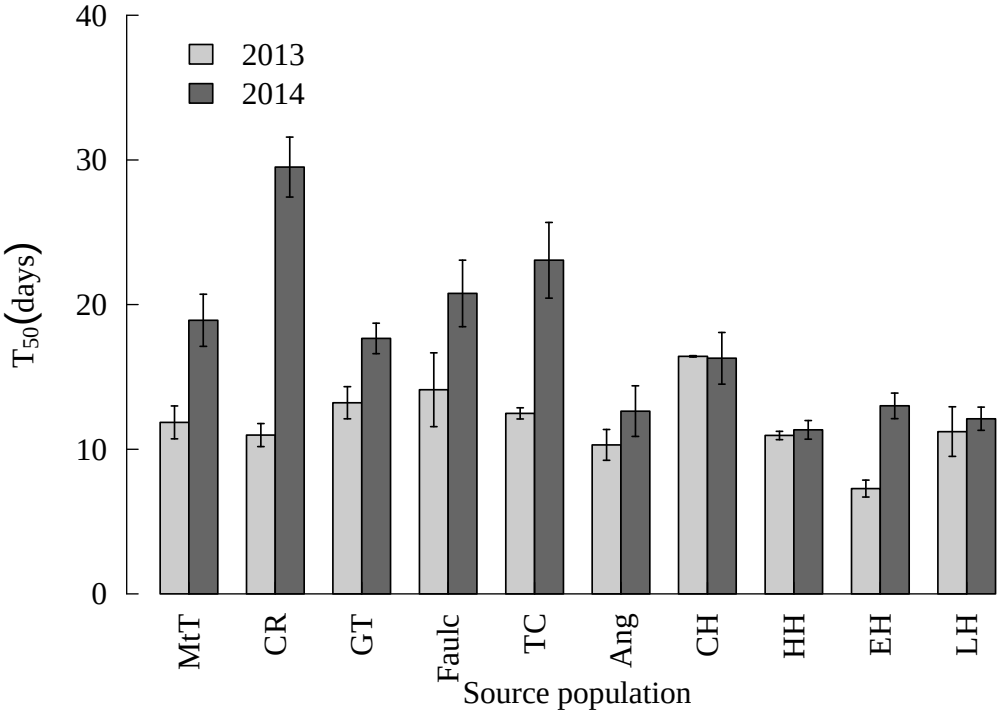

Figure 3.4. Time to 50% germination of *Acacia suaveolens* seeds after an 80°C fire-related temperature treatment (±SE). Source populations are listed in ascending order of mean daily maximum summer temperature (not to scale).

3.3.4. Environmental correlations

Of the climatic variables tested, mean maximum daily temperatures experienced by maternal plants during the winter months explained 16% of the variation in initial dormancy ($P < 0.0001$, data not shown). Seeds from populations experiencing warmer maximum daily temperatures during the winter had a higher proportion of seeds initially dormant than seeds

from populations experiencing lower maximum temperatures. All other variables tested (autumn temperature and, autumn and winter rainfall) were not significantly correlated with initial dormancy. No relationships were found between the climatic variables and the proportion of seeds germinating, or the proportion of seeds inviable, in response to the 40°C to 100°C fire-related temperature treatments.

The proportion of seeds remaining dormant after the high fire-related temperature treatments (80°C and 100°C) showed a strong positive correlation with the long-term mean daily maximum summer temperature of the source site, explaining 39% (P = 0.001) and 19% (P = 0.03) of the variation, respectively (Fig. 3.5). Seeds from source populations experiencing more extreme, hotter summer temperatures had a greater proportion of seeds able to remain dormant in the seed bank even after a high temperature fire.

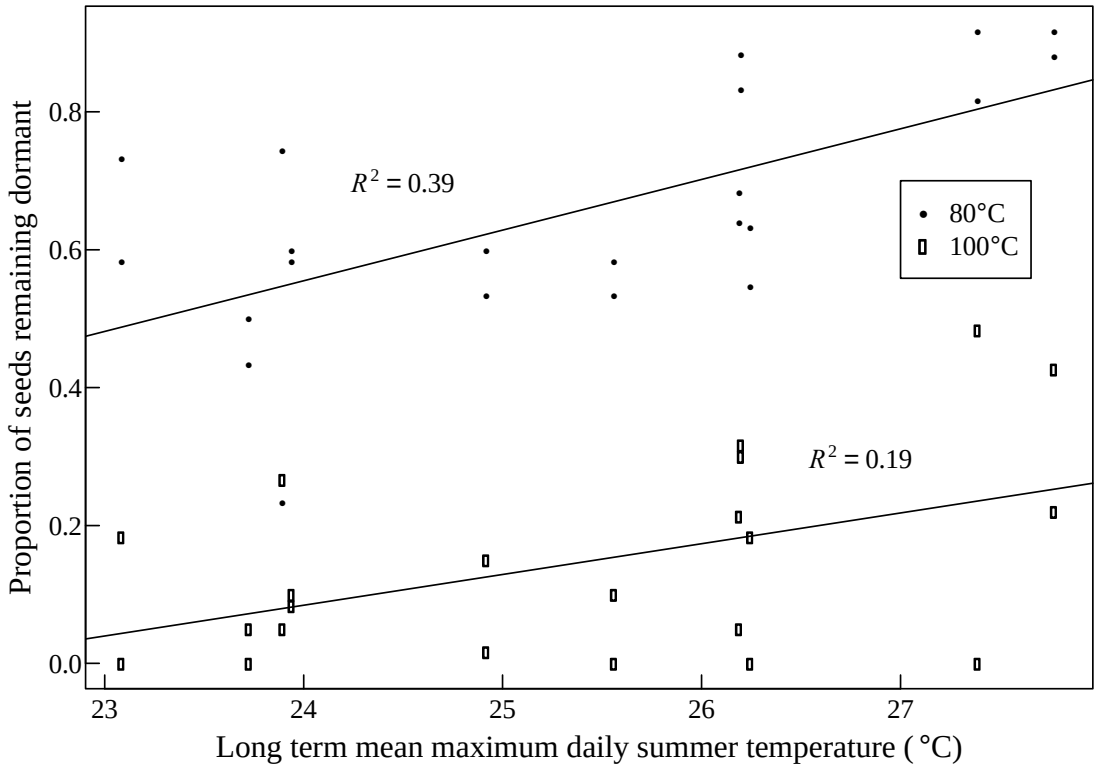

Figure 3.5. The relationship between the long-term daily mean maximum summer temperature of the source site and the proportion of *Acacia suaveolens* seeds remaining dormant after an 80°C and 100°C fire-related temperature treatment.

When correlated against the environmental variables, T_{50} showed a significant relationship with both mean autumn and winter daily temperature maximums. Mean maximum daily autumn temperature explained 22% of the variation in T_{50} ($P < 0.0001$) (Fig. 3.6A), while mean maximum daily winter temperature explained 27% of the variation ($P < 0.0001$) (Fig. 3.6B). In all cases the relationship was negative, indicating that seeds produced under warmer autumn and winter conditions germinated quicker than those produced under cool autumn and winter conditions. However, maximum daily autumn and winter temperatures also explained 15% and 21%, respectively, of the variation in seed weight (data not shown), and seed weight in turn explained 15% of the variation in T_{50} ($P < 0.0001$) (Fig. 3.7). Heavier seeds took longer to germinate after the fire-related temperature treatment than lighter seeds. Thus, warmer autumn and winters result in generally lighter seeds that tend to germinate quicker than heavier seeds.

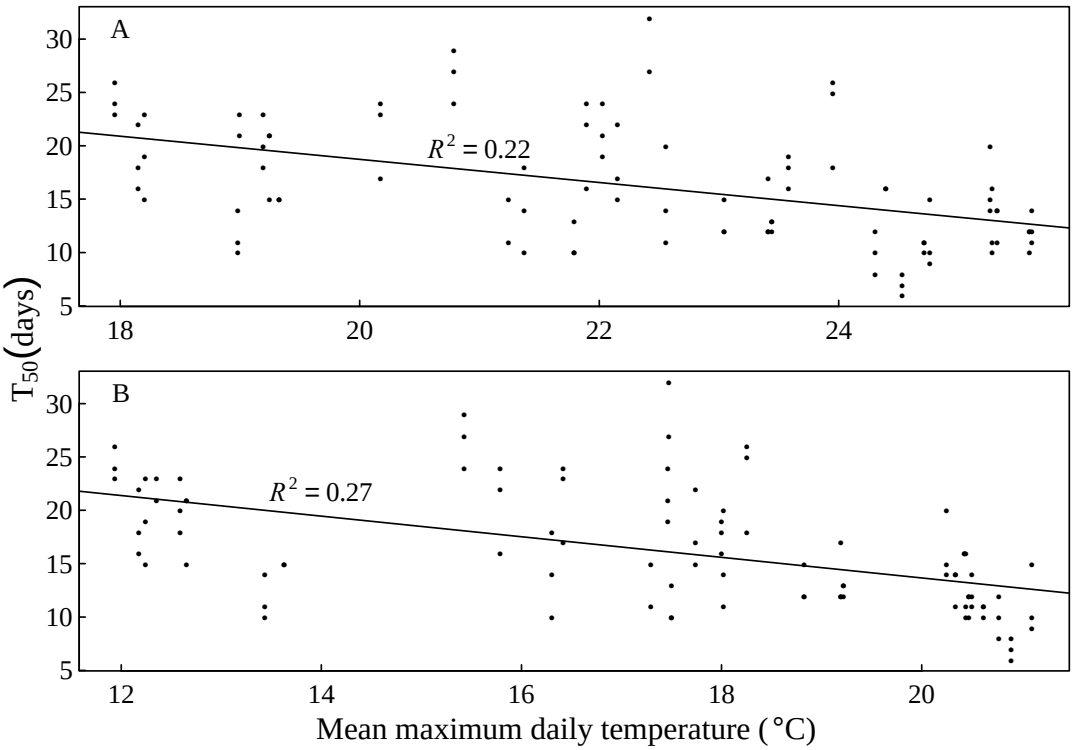

Figure 3.6. The relationship between mean maximum daily temperature of the source site during: A) autumn, and B) winter with the time to 50% germination in *Acacia suaveolens* seeds after an 80°C fire-related temperature treatment.

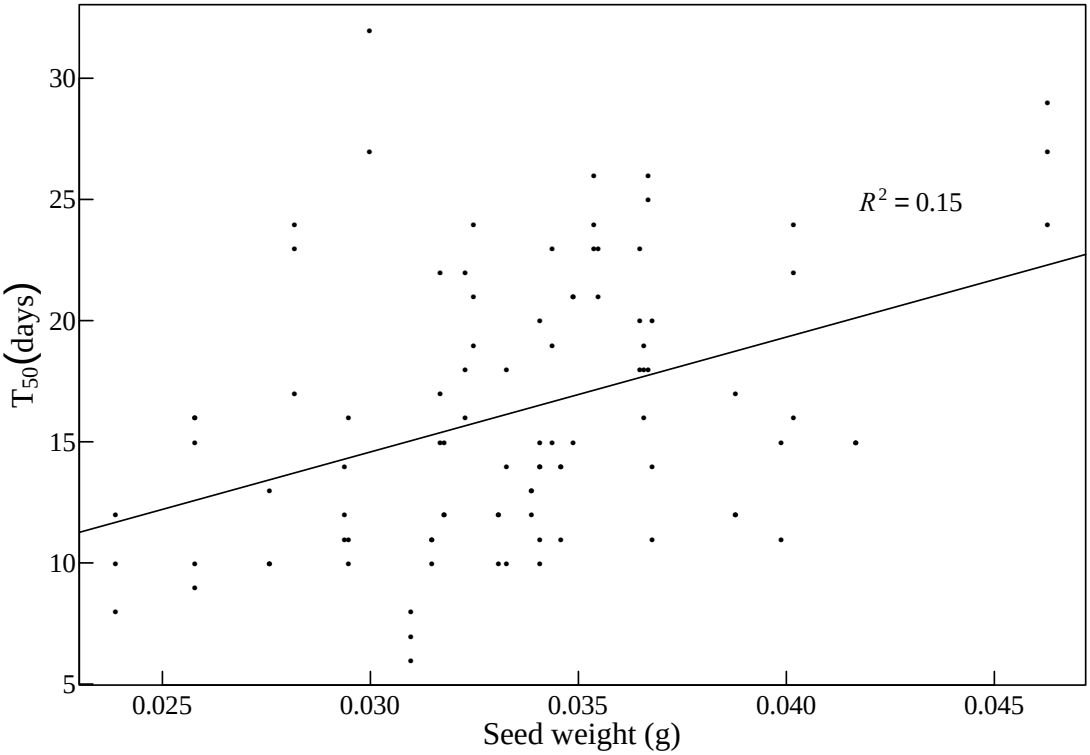

Figure 3.7. The relationship between seed weight and the time to 50% germination in *Acacia suaveolens* seeds after an 80°C fire-related temperature treatment.

3.4. Discussion

Overall, our results provide evidence that the temperatures experienced by the parent plants during reproduction influence the characteristics associated with physical dormancy in *Acacia suaveolens.* We found wide variation, both between years and across populations, in the temperatures needed to break PY, in dormancy retention, and in germination timing after dormancy loss. We also found evidence to suggest that variation in the rate of germination, and the size of the residual seed bank, are linked to the temperature conditions experienced by the parental plants during the reproductive phase and the summer months, respectively.

Germination occurred after all fire-related temperature treatments. However, we found significant differences in the proportion of seeds able to germinate, both among source

populations and years of seed collection, at each of the fire-related temperature treatments. This implies that dormancy loss is possible under a wide variety of fire-related conditions for all populations investigated. This supports the results of Moreira & Pausas (2012) who found wide variation in the germination percentages (and therefore PY loss) in response to a 120°C temperature treatment among populations of five different Mediterranean species. Similarly, Segura *et al.* (2015) found initial dormancy (PY) to vary between 13% and 97% depending on the year of seed collection in *Astragalus nitidiflorus.* Consequently, it may be that in natural populations of species with PY, variation is common. In our fire-prone system, such variation would maximise the chance of some degree of germination after any fire event, irrespective of the soil temperature experienced, or seed placement within the soil profile, thereby ensuring replacement of the lost parental generation.

Despite the wide variation in PY characteristics we recorded, only dormancy levels in the control showed a correlation with mean maximum daily temperatures during seed development. Similar trends for reduced dormancy in seeds produced under cooler maternal environments have been found for *Stylosanthes hamata* cv. Verano and *Trifolium subterraneum* lines (dormancy at the point of seed release - Argel & Humphrys, 1983a; Piano *et al.,* 1996, respectively). Indeed, Ooi *et al.* (2012), studying *A. suaveolens*, also found similar trends along an altitude gradient. However, here only 16% of the variation in dormancy was explained by maximum daily winter temperature, implying that additional factors are involved in driving the among site PY variation identified. It is possible that genetic variation between populations also has a role in determining dormancy levels. To date, in non-agricultural species, there are few studies of genetic variation in PY. Lacerda *et al.* (2004) estimated the co-efficient of genetic determination (CoGD) for variation in PY in two populations each of *Plathymenia reticulata* and *Senna multijuga.* They found CoGD

values to be greater than 0.85 indicating a large proportion of PY variation to be of genetic origin. This suggests that genetic variation among populations may be more important in driving the PY variation we identified, as compared to environmental factors. Genetic differentiation among populations of *A. suaveolens* identified by Hudson *et al.* (Chapter 7) was found to be high. It is also possible that the wide variation identified may be the result of the limited seed numbers used during the experiment. Overall however, irrespective of cause, the large variation among sites highlights the need to account for range wide trait variation in species ecological studies and conservation applications (Valladares *et al.*, 2014; Cochrane *et al.*, 2015a).

We found strong evidence for dormancy retention (rather than seed mortality) within seed lots after the fire-related temperature treatments for all source populations, especially for treatments 80°C and below. Hence, a proportion of seeds are likely to remain dormant in the seed bank until a subsequent fire, irrespective of the fire conditions experienced during the first fire. Our dormancy retention figures for the 80°C heat treatment are slightly higher than those estimated by Auld & Denham (2006) for *A. suaveolens* after three bush fires around the Sydney region (Australia), however the 100°C figures are within their range estimates. This highlights that our lab-based study produced comparable results to estimates of seed bank response under natural fire conditions. The dormancy retention demonstrated (i.e. residual seed bank potential), may buffer *A. suaveolens* populations against recruitment failure post-fire. However, the advantages of a residual seed bank for population fitness is dependent upon similar levels of variation remaining in the temperatures needed to break dormancy within the seed lot after the fire, as compared to before it. Flood *et al.* (UOW, Australia, 'unpubl. Res.') found no changes to the average temperature needed to break dormancy of *A. suaveolens* seeds after multiple fire-related heat treatments, implying dormancy breaking requirements

are little changed by fire events. However, Liyanage & Ooi (in press) found significant changes to the dormancy breaking requirements of four Australian legume species with PY after six and eighteen months of field storage. Consequently, evidence suggests, that the temperature range needed to break dormancy of the residual seed bank seeds may not be altered during the fire event, but may be subject to change due to the natural aging of seeds. Thus, even if dormancy-breaking requirements change over time, retaining a proportion of seeds dormant within the seed bank after a fire will buffer the population against recruitment failure of emerging seedlings post-fire.

We predicted that as the post-fire mean maximum daily summer temperatures of a seed source site increased, so too would the proportion of seeds remaining dormant after a fire. We found that seeds from source populations experiencing warmer maximum summer temperatures (i.e. a greater chance of water stress) had greater dormancy retention within seed lots after 80°C and 100°C fire-related temperatures treatments (Fig. 3.5). Similarly, Ooi *et al.* (2012) reported a decrease in the germination percentage among four populations of *A. suaveolens* seeds with increasing mean summer temperatures of the source site under simulated heat wave conditions; implying an increase in the proportion of seeds able to retain dormancy as mean source site temperature increased. This trend of increased dormancy retention at sites with warmer maximum temperatures, would act to reduce the risk of recruitment failure presented by a hotter germination environment. Moreover, under future climatic warming this may be advantageous.

Seeds from populations that had experienced warmer autumn and winter temperatures produced seeds that germinated quicker after dormancy had been broken, as compared to those that had experienced cooler autumn and winter conditions. It is possible the relationship

between germination timing and temperature is the result of changes to maternal resource allocation, as both autumn and winter temperatures and T_{50} showed negative correlations with seed weight (i.e. warmer autumns and winters produced lighter seeds which germinated quicker). Alternatively, MacGregor *et al.* (2015) found that the temperature of the environment maternal *Arabidopsis thaliana* plants were grown under altered gene expression within the seeds, leading to different concentrations of seed coat procyanidins, modifying seed coat permeability to tetrazolium. Thus, given that PY is caused by the impermeable seed coat, it is possible a warmer developmental environment could modify gene expression within the seed altering the seed coat strength, and therefore the rate at which the radicle can emerge. However, whether quicker germination would be detrimental to the species would depend upon the level of competition experienced in the post-fire flush of seedling growth (Daskalakou & Thanos, 2010).

While we have provided evidence for a relationship between the temperatures experienced during seed development, as well as maximum summer source site temperatures, and PY characteristics, the physiological mechanism for the effects remains unclear. As previously mentioned, studies on *A. thaliana* suggest that the developmental environment can modify seed coat features, particularly procyandins influencing seed coat permeability (MacGregor *et al.*, 2015). Similarly, Zeng *et al.* (2005) found that in *T. subterraneum* and *T. spumosum*, the melting points of the fatty acids present within the seed coat reflected the temperatures seeds were likely to be exposed to during the summer months. Thus, while modification of the chemical composition of the seed coat is a possible explanation for the developmental environment effects on PY characteristics, changes to the seed coat thickness or structure is also possible. Meisert (2002) reported an exponential relationship between the thickness of the palisade, and sub-palisade, layers of the seed coat, and the percentage of impermeable

seeds across thirteen *Pelargonium* species (although see Russi *et al.*, 1992; Visscher *et al.*, 2016). Argel & Humphreys (1983b) reported shorter, denser palisade cells within the seed coat of *S. hamata* seeds, with higher PY dormancy levels, compared to those with lower levels of PY. This is an area of research that requires further investigation (Zeng *et al.*, 2005).

The variation we detected acts to buffer populations against fire-variation and post-fire environmental heterogeneity. The presence of such variation, and the ability to buffer environmental stochasticity within a single species, may be advantageous to buffer the species against future climatic change effects. Physical dormancy is believed to have evolved in response to environmental stochasticity (Baskin *et al.*, 2000, although see Paulsen *et al.*, 2013), a phenomenon that is predicted to increase in the future as a result of climatic change (Dowdy *et al.*, 2015). However, fully understanding the environmental limits of the PY variation in the fire-prone system is required to identify if the variability we found is sufficient to deal with the degree of environmental change projected, particularly to the extremes of environmental stochasticity. Moreover, further work is needed to understand how such variation may be modified by additional, interacting climatic changes such as increasing CO_2 levels.

3.5. Acknowledgements

I would like to thank all the volunteers who helped in collecting the seed for this project.

Chapter 4. INTER-POPULATION VARIATION IN THE RESPONSE OF FLOWERING PHENOLOGY, SEED PRODUCTION AND DORMANCY CHARACTERISTICS TO EXPERIMENTAL WARMING IN *ACACIA SUAVEOLENS*.

4.1. Introduction

Donohue (2009) highlighted maternal effects as the 'missing link' in the expression of the plant life cycle, in that they form a cross over between generations, enabling the maternal plant to influence the phenotype of the offspring aside from direct genetic contributions. Perhaps most importantly, maternal environmental effects may influence how the maternal plant alters the phenotype of its offspring in response to the environment in which it is living (Roach & Wulff, 1987; Weiner *et al.*, 1997; Donohue & Schmitt, 1998; Galloway, 2005; Donohue, 2009). These interactions are important in influencing multiple aspects of germination (Roach & Wulff, 1987; Fenner, 1991; Hudson *et al.*, 2015 (Chapter 2)), all of which have a direct impact on the timing, and chance, of successful recruitment from the seed bank (Baskin & Baskin, 2014). However, the expression of traits in the 'seed' generation reflects maternal effects, the seeds genotype and direct interactions between the environment and the developing seed (Roach & Wulff, 1987; Donohue, 2010; Hudson *et al.*, 2015 (Chapter 2)). Therefore, throughout this paper, we use the term 'developmental effects' to encompass maternal effects as defined by Roach & Wulff (1987), reflecting the time from pre-fertilization to the point of seed release from the maternal plant.

In many cases the loss of seed dormancy and subsequent germination is triggered in response to some form of environmental cue, usually experienced post-dispersal (Baskin & Baskin, 2014). For example, temperature stratification (e.g. Schütz & Rave, 1999; Baskin & Baskin, 2014; Mackenzie *et al.*, 2016), light (e.g. Baskin & Baskin, 1983; Roeder *et al.*, 2013), heat

shock (e.g. Auld & O'Connell, 1991; Thanos *et al.*, 1992; Moreira *et al.*, 2010) and smoke (e.g. Moreira *et al.*, 2010; Mackenzie *et al.*, 2016) have all been shown to trigger dormancy loss and / or germination. Consequently, successful seedling recruitment is dependent not only upon the environmental conditions experienced by the maternal plant during seed development, but also on a seed's ability to respond to environmental cues triggering germination post dispersal. This means that in order to predict how plant life cycles will be altered by future climate change, the influence of environmental conditions on seed development, and on a seeds ability to respond to environmental cues, must be understood.

Future climate changes are unlikely to affect seed dormancy and germination in isolation, with changes to both the onset and / or duration of flowering and fruiting of maternal plants also likely (e.g. Fitter & Fitter, 2002; Parmesan & Hanley, 2015). A number of studies have highlighted that to predict climate change impacts on plant reproduction accurately we need to address the entire sequence of reproduction inclusively (Post *et al.*, 2008; Haggerty & Galloway, 2011; Del Cacho *et al.*, 2013). However, what is not clear, is whether these changes to phenological timings have the potential to alter seed characteristics (Post *et al.*, 2008; Haggerty & Galloway, 2011). Therefore, in order to predict how plant life cycles will be altered by future climate change, the influence of changing reproductive phenology and developmental environment conditions on seed development, and on a seed's ability to respond to environmental cues, must be jointly understood.

Seed dormancy, defined as an inability of the seed to germinate under otherwise favourable conditions (Baskin & Baskin, 2014), can control the timing of germination allowing seedling emergence into an environment facilitating their chance of survival (Baskin & Baskin, 2014). Physical dormancy (PY) is one of five types of dormancy, caused by a seed coat (or pericarp)

impermeable to water (Baskin & Baskin, 2014). The seed coat (or pericarp) must be fractured in some way, allowing water to reach the seed embryo initiating germination. In fire-prone ecosystems, it is usually heat generated by fire that fractures the seed coat (Baskin *et al.*, 2000; Baskin & Baskin, 2014). For many of these species, the fire also kills the maternal population (i.e. the obligate seeder fire response), resulting in a high dependence upon successful recruitment from the seed bank for population maintenance (Auld, 1986a; Auld & Myerscough, 1986). Evidence exists for the ability of the developmental environment to alter the temperature requirements for dormancy loss in species with physiological dormancy (e.g. Hoyle *et al.*, 2008; De Vitis *et al.*, 2014). However, most of the research into maternal environment effects on PY has been conducted on agricultural species, focusing on the percentage of seeds dormant at release, rather than changes in seed responses to dormancy-breaking environmental stimuli (see Hudson *et al.* 2015 (Chapter 2) for review, although see Chamorro *et al.*, 2016). In addition, there is no consensus as to the influence of developmental effects on the expression of physical dormancy (Hudson *et al.* 2015 (Chapter 2)). Should maternal environmental effects have the potential to alter the fire-related temperatures needed to break physical dormancy, this would modify not only the proportion of the seed bank germinating after any given fire, but also the conditions into which the seedlings germinate.

Many of the studies into maternal effects have focused on annual species and genetically similar lines to allow for the strict sense of the 'maternal effects' definition. However, it has been noted that it is unclear in many cases whether these results would still be evident at the population level and in out-crossing species, due to population level variation (Germain & Gilbert, 2014). To investigate whether the developmental environment influences inter- and intra-population phenotypic variation in seed production and dormancy characteristics, we grew plants from nine geographically distinct populations across the species range and

examined the response of plants under two different developmental environments during the flowering and seed production phases. This design allowed us to assess whether differences in the two developmental environments alone could produce measurable changes in reproductive phenology characteristics across all populations, or if local adaptation of individual source population's would modify any treatment effects. Because of the broad study, from flowering through to seed ripening, we refer to the impacts of 'developmental environment' rather than 'maternal environment'. Specifically, this paper focuses on a species with seed dormancy release triggered by fire. We ask:

1. Do different developmental temperatures alter flowering phenology in *Acacia suaveolens*?
2. Do different developmental temperatures alter seed pod development in *Acacia suaveolens?*
3. Does the response of seed characteristics to different developmental temperatures vary among source populations or along climatic gradients?
4. Do different developmental environments influence the temperature needed to break physical dormancy?

4.2. Methods

4.2.1. Species and distribution

Acacia suaveolens Sm. (Wlld). is a common, fire sensitive, obligate seeding species with PY from eastern Australia. Its distribution is restricted to predominantly coastal sandstone substrate, extending from Tasmania in the south, to mid-coast Queensland (Morrison *et al.*, 1983; Fig. 1.1B). Flowering occurs in the species between March and August, with seeds

ripening from November to December (Morrison *et al.,* 1983). Seeds of the species are predominantly dormant at the point of dispersal, retaining dormancy until a subsequent fire event (Morrison, 1986a). It is heat from fire that causes dormancy loss within the species (fracturing the seed coat), and the first rainfall event post-fire that triggers germination (providing water required to stimulate embryo growth). Auld & O'Connell (1991) reported highest dormancy loss in the species to occur after a 10 minute heat treatment of between 80°C and 100°C. The primary juvenile period under natural conditions is approximately two years (Morrison *et al*., 1983; Auld & Myerscough, 1986).

Pollination within the species is predominantly mediated by insects (Morrison, 1986a & b), with the introduced European honeybee, *Apis mellifera,* a prominent pollinator in certain areas (A. Gilpin, *pers. comm.*). The species is andromonoecious, producing 13–50% bisexual flowers (Morrison, 1986a & b). There has been no direct investigation into the degree of selfing possible within the species, however observational (Gilpin, *et al*. *in prep*) and genetic (Chapter 7) evidence suggests it is possible.

4.2.2. Seed collection and plant growth

We collected *A. suaveolens* seeds from nine sites distributed along two climatic gradients (one altitudinal and one latitudinal) between October and December of 2011 (Table 4.1). The sites span almost the entire latitudinal and altitudinal range of the species, covering a range of 8.8°C in average maximum temperature and spanning over a 1000 m altitudinal change. All sites have sandy soils, and support dry sclerophyllous woodland dominated by a *Eucalyptus* and *Angophora* canopy, and an understory comprised of predominantly Fabaceae, Proteaceae and Ericaceae (Keith, 2004; Ooi *et al*., 2012).

Table 4.1. Details of the original seed collection sites (source populations) for *Acacia suaveolens.* Sites were arrayed along intersecting altitudinal and latitudinal temperature gradients.

Site	Abbr.	Latitude	Longitude	Altitude (m asl)
Tasmania	Tas	42°33’05”	147°52’04”	*
Narooma	Nar	36°14'56”	150°08'16”	30
Garie Trig	GT	34°42'36”	151°03'18”	235
Diamond Head	DH	31°40'37”	152°48'03”	12
Red Rock	RR	30°00’15”	153°12’45”	23
Faulconbridge	Faulc	33°41'56”	150°31'30”	461
Ingar Camp	IC	33°46’22”	150°27’39”	578
Ingar Trail	IT	33°46’10”	150°25’58”	795
Mount Hay	MtH	33°39'03”	150°22'01”	875

* no data available

At each source site we collected ripe dormant seeds from at least 30 individual maternal plants, bulking them together in order to ensure representation of population diversity. We stored seeds in paper envelopes under laboratory conditions (average 23°C) until November 2012. We used dry heat treatments and manual scarification (using sand paper) to break seed dormancy and obtain germination. We planted 50-55 germinated seeds per source site into 15 cm pots (one seed per pot) containing a vermiculite: sand mixture (ratio 1:5). We added 3 g of Osmocote® Australian native plant fertilizer to each pot. Seedlings were grown outside under common garden conditions at the University of Wollongong’s Ecological Research Centre until early April 2014.

4.2.3. Manipulation of the developmental environments

On 27th March 2014, prior to the onset of flowering, we transferred half of the surviving plants from each population (N = 15-24) to an adjacent greenhouse, whilst half remained outside (hereafter referred to as 'warm' and 'cool' developmental conditions, respectively). This gave a total of 18 source populations x developmental condition levels. Both treatment groups received the same watering and fertilizer regime. We covered the outside plants with a

plastic sheet and shade cloth to protect from rainwater, and to minimise the impact of direct sunlight (to replicate the light levels inside the greenhouse). Using DS1923 iButton Hygrochron data loggers we recorded temperature and relative air humidity in both developmental environments. Prior to analysis, we corrected the raw humidity data according to the methodology of Ashcroft & Gollan (2013). Based on the iButton data, we calculated the daily mean temperature and humidity values (corrected), as well as the average difference in temperature and humidity.

Our experiment aimed to compare the influence of warmer versus cooler developmental conditions on the plants. The two developmental conditions differed significantly in average daily ambient temperatures (ANOVA: $F = 202.97$, $P < 0.0001$). The difference in temperatures experienced between the two treatments was greatest during the warmer months, with the greenhouse plants being on average 3.81°C warmer than those outside during spring (Table 4.2). In contrast, during the autumn months greenhouse plants were on average only 2.4°C warmer than outside plants (Table 4.2). These differences are at the upper limits of the climate change projections for the Sydney region (GT source population), whereby temperatures are expected to be 1.3°C to 4.7°C higher by 2090 depending upon the emissions scenario (Dowdy *et al.*, 2015). The temperatures parental plants were exposed to under both developmental environments (warm and cool) were greater than plants would usually experience at their home sites for the high altitude and high latitude source populations (MtH, IT, IC, Tas, Faulc). However, the developmental environment temperatures were lower than plants would usually experience at the low latitude source populations (RR and DH) for the equivalent times of the year.

Table 4.2. Developmental environment temperature differences throughout the study period. Temperature and humidity values are based on the mean daily recorded values.

Season	Temperature (°C)			Relative Humidity (%)		
	Inside	**Outside**	**Difference**	**Inside**	**Outside**	**Difference**
Autumn	20.99	18.64	2.35	79.48	76.20	3.29
Winter	16.94	13.84	3.08	75.97	73.38	2.63
Spring	23.21	19.40	3.81	73.34	74.51	-1.17
December*	25.48	21.33	3.95	78.98	79.66	-5.15

* experiment finished at the end of December

On average, over the study period, humidity levels within the two developmental environment treatments were not significantly different (ANOVA: $F = 0.3417$, $P = 0.5591$). However, the greenhouse plants experienced a higher humidity during the autumn and winter months, whereas plants outside experienced a higher humidity during the spring (Table 4.2). It is possible that the maximum and minimum humidity's experienced by plants differed between the two developmental environments.

4.2.4. Flowering and pod formation

Once flower buds had begun to form, we covered all plants within each source population x developmental treatment combination with frost-cloth to prevent crosspollination between populations and developmental treatments. We conducted hand pollination randomly between plants within each population x treatment combination to control for potential inter-population paternal effects.

We checked plants weekly for the presence of flowers in bloom, continuing monitoring until all flowering had ended. We were therefore able to calculate the length of flowering span for each plant.

Once pods had started to form, we tagged five pods (less than five millimetres long) from five plants per population x treatment combination, recording pod length weekly until ripening.

Once pod ripening had started (identified by the pods turning brown in colour and splitting open), we collected ripened pods daily. For each pod collected that contained fully formed seeds, we recorded the number of fully formed seeds and the number of aborted seeds (not fully formed). We stored seeds in paper envelopes under standard laboratory conditions (23°C) until February 2015 when dormancy trials began.

4.2.5. Seed dormancy trials

Due to the low seed numbers per individual plant, we pooled seeds across individuals per population x developmental treatment combination, providing a minimum of 300 seeds per combination. In order to maintain consistency, where more than 12 plants in a population / treatment had produced seed, only 12 were used, excluding plants that produced fewer than five seeds. We randomly selected thirty seeds from each bulk seed lot to be weighed prior to heat treatments.

We exposed three replicates of 15-20 seeds to one of five fire-related temperatures (control, 40°C, 60°C, 80°C and 100°C) for 10 minutes, with each replicate being heat-treated separately. We heated each seed lot within an open glass petri dish using a drying oven. These heat treatments reflect a range of conditions that may be experienced by seeds stored in the soil seed bank during any given fire event (Penman & Towerton, 2008). The control seeds

received no heat treatment, and were left under the standard laboratory conditions (23° C). Following heat treatment, we placed seeds on moistened filter paper in sealed petri dishes and incubated them at mean summer temperatures for the Sydney region (25/18°C). We selected summer temperatures as the peak fire season for the area runs over this period (NSW RFS, 2016), and therefore seeds are most likely to germinate during this time. We monitored germination every other day for 40 days. We classified seeds as germinated once the radicle could be observed protruding from the seed coat, once this had occurred seeds were removed from the petri dish. After 40 days, we scarified those seeds that remained hard (neither germinated nor imbibed) using sandpaper. Post-scarification, we monitored germination for an additional 10 days to assess seed viability.

4.2.6. Source site temperature data

For each of the source sites, we collated annual mean maximum daily temperatures from the nearest Australia Bureau of Meteorology (BoM) weather station (BoM, 2015). Where there was no BoM station close to the source site, we extrapolated maximum temperatures based on the altitudinal / latitudinal position of the two nearest weather stations and the altitude / latitude of the source site (Appendix 2). Due to differences between the weather stations in timespans of data available, we averaged over the years 2000-2014 for all stations except for one (Appendix 2) where data was only available from 2006.

4.2.7. Statistical analysis

We used the R statistical platform for data analysis (R Development Core Team, 2015). We analysed seed weight using a two-way ANOVA, whilst we used generalised linear models (GLM) with a Poisson error structure for all count data (seed pod variables, number of days to

first flowering and pod ripening, and flowering duration). Where appropriate, we used a quasipoisson error structure to account for overdispersion of the data.

Analysis of differences in the proportion of seed germinating between source populations and developmental environments was only possible for the 80°C treatment due to low levels of germination in the remaining treatments. We analysed the 80°C treatment data with a GLM including a quasibinomial error structure to account for overdispersion. Across the different source populations, developmental environments and fire-related heat treatments, we found very little variation in the proportion of a seed lot inviable and therefore did not analyse it statistically. In the control through to 80°C degree heat treatments, inviability was very low (< 7%). After the 100°C heat treatment inviability reached almost 100% across all replicates.

We analysed the mean time from fire-related heat treatment to seed germination by GLM using a quasipoisson error structure to account for overdispersion. We did this for each fire-related temperature treatment separately. This was to assess differences in the germination speed between source populations and developmental environments. This was only possible for the 80°C treatment, due to low germination after the other fire-related heat treatments.

We calculated standard effect sizes to assess the influence of average maximum daily source site temperature on flowering phenology and seedpod variables, using the following calculation:

$$\ln(X_{warm} / X_{cool})$$

where X_{warm} and X_{cool} represent the variable mean from the greenhouse and outside temperature treatments, respectively. A positive value represents an increase in the response

variable due to warming. We plotted effect sizes against average maximum daily source site temperatures, assessing the relationship with a linear model.

4.3. Results

4.3.1. Flowering phenology

Warmer environmental conditions resulted in a delayed onset of flowering and a shorter flowering span than cooler environmental conditions, but the effect varied significantly with source population (Table 4.3). For eight of the nine populations, flowering occurred earlier for plants under cooler parental conditions than those in the warmer environment (Fig. 4.1A), where day of first flowering was on average 33 days later (range = 7-66 days). The flowering period for seven of the nine populations was on average 26 days shorter under the warmer environment than under the cooler environment (Fig. 4.1B). For the warmest source population plants (RR) the flowering period was 39 days longer under the warmer conditions whereas, for the coolest source population (MtH), the flowering period was an average of 37 days longer for plants exposed to the cooler environment (Fig. 4.1B).

There was no significant difference in the proportion of plants flowering between the two environment treatments when bulked over the populations (range: 0.45-1.00 warm, 0.69-1.00 cool; $\chi^2 = 11.66$, DF = 8, $P > 0.05$, data not shown). However, there was a slight positive trend between the effect size for percentage of surviving plants that flowered and the average maximum daily home site temperature, although the relationship was not statistically significant ($R^2 = 0.16$, $P = 0.09$). This suggests that plants from warmer home sites may be able to flower better under the warmer conditions, compared to plants from cooler home sites.

Table 4.3. Results of models for seven reproductive attributes of *Acacia suaveolens* measured in response to warming (treatment) for the multiple source populations (source) studied.

Measure	Analysis Method	Treatment			Home			Interaction		
		F	**DF**	***P***	**F**	**DF**	***P***	**F**	**DF**	***P***
Days to first flowering plant	GLM (quasipoisson)	65.40	1	***	12.92	8	***	3.51	8	***
Number of days spent flowering	GLM (quasipoisson)	15.55	1	**	2.03	8	*	5.79	8	***
Days to first pod ripening	GLM (poisson)	137.49[a]	1	***	109.12[a]	6	***	NS	NS	NS
Number of days from tagging to ripening	GLM (quasipoisson)	97.68	1	***	1.85	6	NS	2.45	6	*
Number of number of fully formed seeds per pod	GLM(quasibinomial)	75.98	1	***	8.39	7	***	3.28	7	**
Number of inviable seeds per pod	GLM (quasibinomial)	79.05	1	***	11.50	7	***	5.03	7	***
Seed weight	2-way ANOVA	27.90	1	***	115.10	7	***	24.20	7	***

[a] Chi-squared value; * *P < 0.05; ** P < 0.001; *** P < 0.0001*

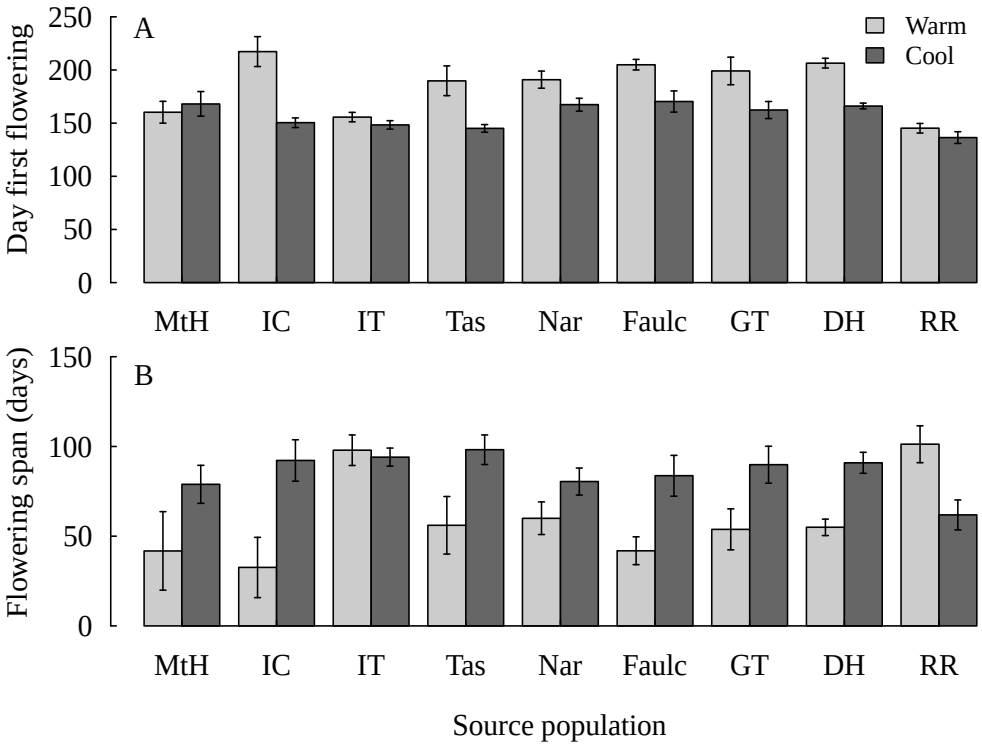

Figure 4.1. The effect of warm versus cool developmental environments on: A) mean day of first flowering, and B) mean number of days spent flowering (±SE) across multiple populations of *Acacia suaveolens.*

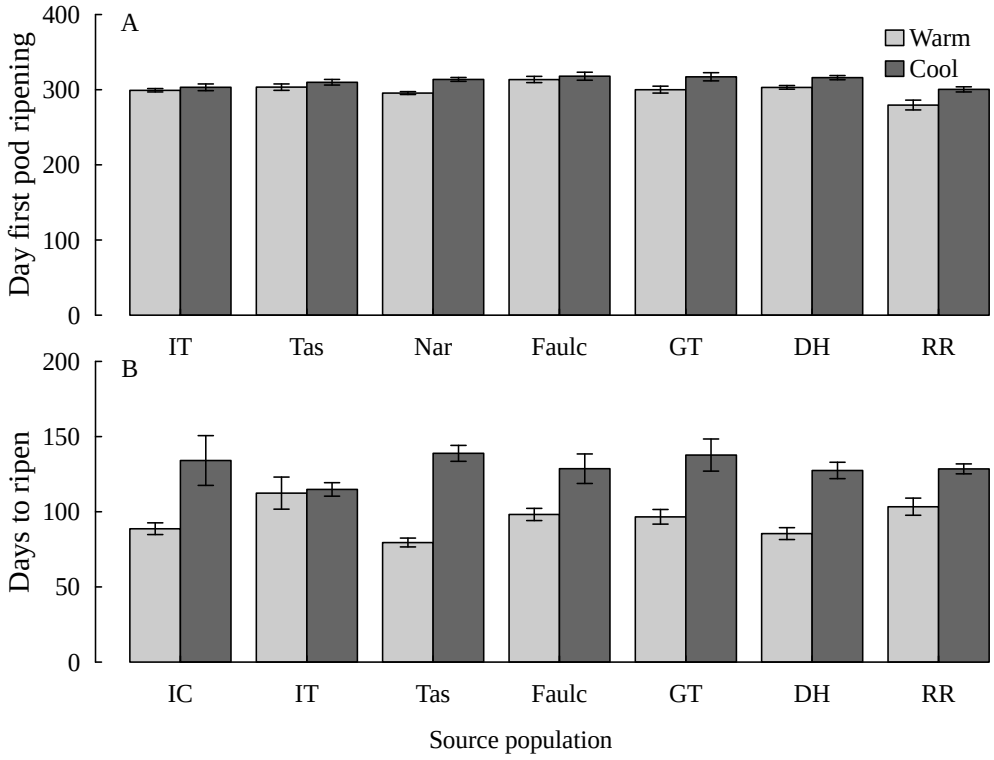

Figure 4.2. The effect of warm versus cool developmental environments on pod ripening characteristics: A) mean day of first pod ripening, and B) mean number of days for pods to ripen (±SE) across multiple populations of *Acacia suaveolens.*

4.3.2. Pod growth and ripening

On average, first pod ripening was 12 days earlier on plants under warmer conditions than under cooler conditions (range: 4–21 days; Fig. 4.2A). Both source population and developmental environment significantly influenced the day of first pod ripening, however the interaction between the two was not significant (Fig. 4.2A; Table 4.3). The effect of the warmer maternal environment seemed to be greater on plants originating from warmer home sites than those from cooler home sites, with average maximum home site temperature explaining around 23% of the variation in effect size, however this was not statistically significant ($P = 0.15$; data not shown).

Only seven of the nine source populations produced sufficient pods to enable a comparison of performance under both developmental environments. For six of the seven source populations, pods took longer to develop and ripen under the cooler developmental environment than under the warmer developmental environment (Fig. 4.2B). Whereas the date of first pod ripening was not significantly influenced by the interaction between source population and maternal environment, the average time taken for pods to develop and ripen was significantly affected by the interaction (Table 4.3). However, while home site and parental growing environment significantly interacted to influence the number of days from tagging to ripening (Table 4.3), there was very little variation in the time from tagging to ripening between the populations within each maternal growing environment (Fig. 4.2B). For all source populations the warmer parental environment produced a response shift in the same direction, indicating that warmer developmental conditions are related to a reduction in the number of days for pods to ripen irrespective of any local home site adaptations.

4.3.3. Seed pods

For six of the populations, seeds produced under greenhouse conditions were on average lighter than seeds produced under the cooler outside conditions. Source population and developmental environment significantly influenced seed weight, and the interaction between these two factors was significant (Fig. 4.3; Table 4.3).

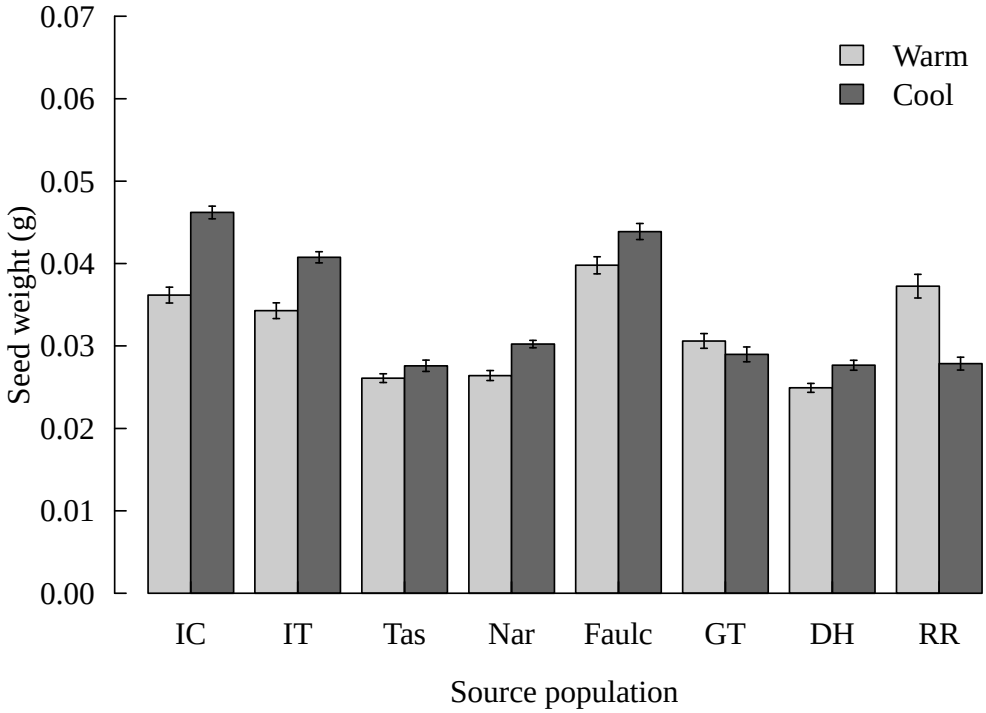

Figure 4.3. The effect of warm versus cool developmental environments on mean seed weight (±SE) across multiple populations of *Acacia suaveolens.*

Pods produced under warmer developmental environments contained fewer fully formed seeds than those produced under cooler developmental environments across seven of the eight study populations (Fig. 4.4A). This effect was significant, with the interaction between source site and developmental environment influencing the number of fully formed seeds (Table 4.3). In seven of the eight source populations, the number of seeds per pod aborted during development was greater under warmer developmental environments than cooler ones,

although the source population did significantly influence the level of abortion induced by the developmental environment (Fig. 4.4B, Table 4.3). When focusing on the stage at which seed abortion occurred, irrespective of developmental environment or source population, most seed abortion occurred during the seed growth stage prior to seed filling (data not shown).

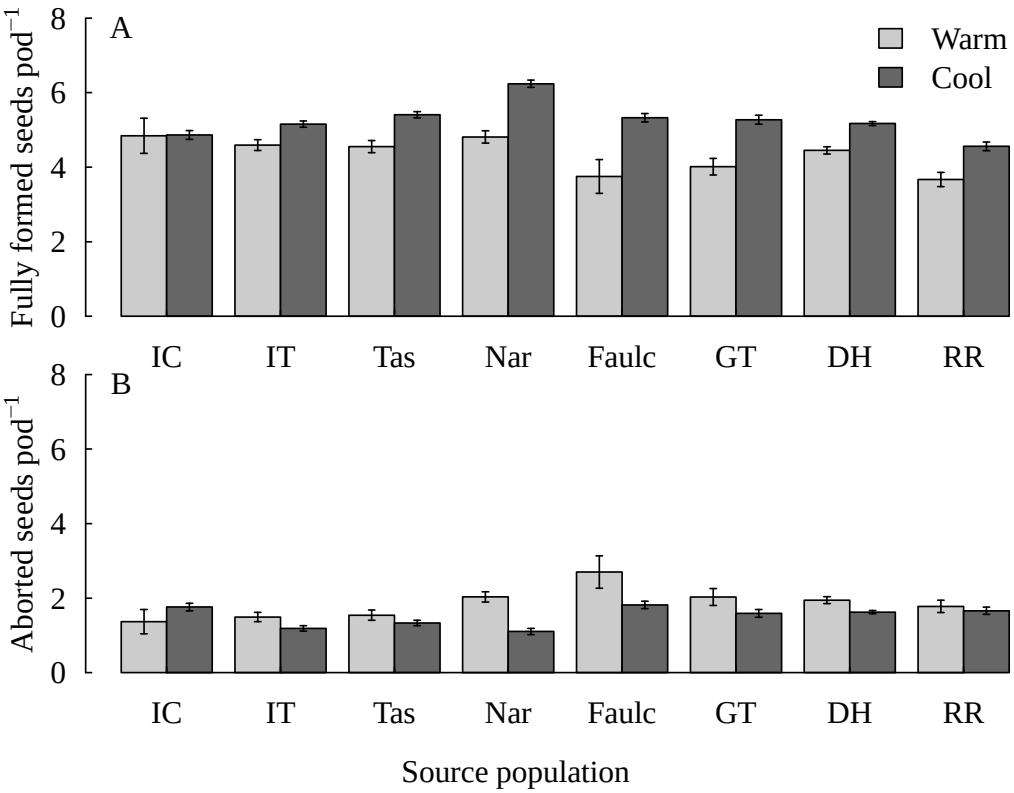

Figure 4.4. The effect of warm versus cool developmental environments on seed production: A) mean number of fully formed seeds per pod, and B) mean number of seeds aborted during development (±SE) across multiple populations of *Acacia suaveolens.*

Due to the changes in the onset of flowering and pod formation, we tested if the changes to the duration of the reproductive period can influence reproductive performance. The average number of days taken for the entire reproductive phase (average number of days flowering + average number of days for pods to ripen) was positively correlated with the average number of fully formed seeds per pod (R^2 = 0.24, P = 0.04, Fig. 4.5). Conversely, the average duration of the entire reproductive phase explained only 7% (P = 0.18) of the variation in average

number of seeds aborted during fill. There was no significant correlation between average number of days for the entire reproductive phase and average seed weight (R^2 = -0.012, P = 0.38).

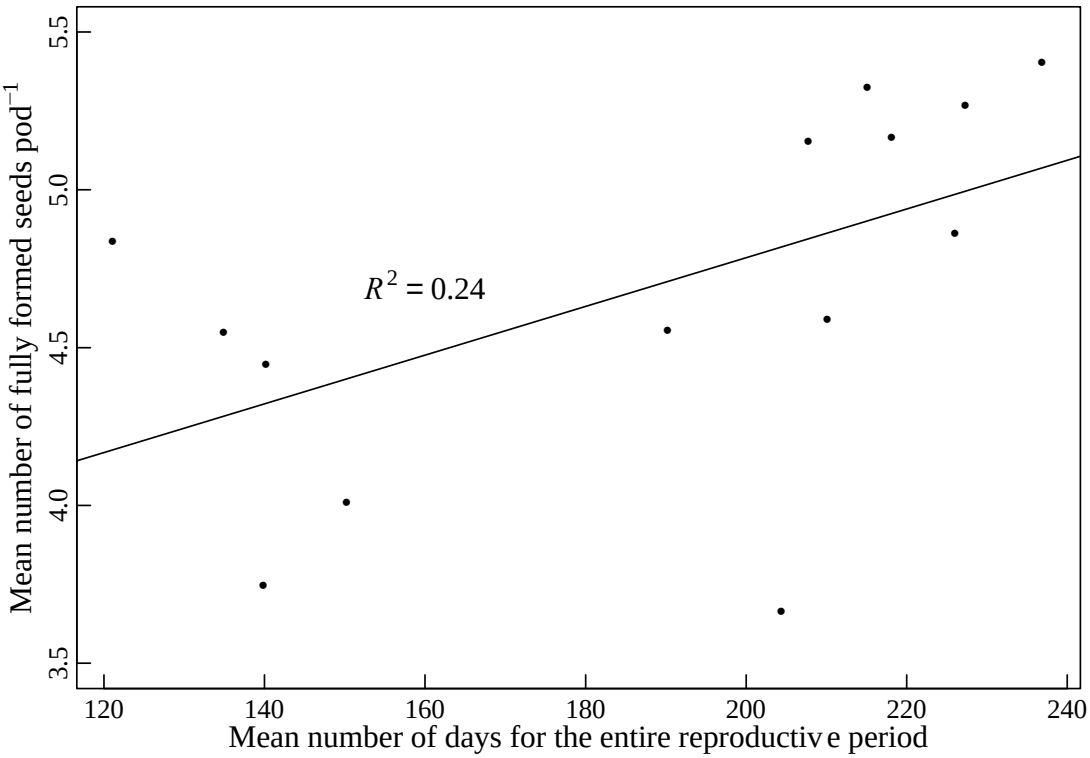

Figure 4.5. The relationship between the average number of days for the entire reproductive period and the mean number of fully formed seeds per pod for *Acacia suaveolens.*

4.3.4. Seed dormancy trials

Only six of the nine populations produced enough seeds under the warm and cool developmental environments to allow for comparisons of the temperature required to break dormancy. Under control conditions (representing the level of dormancy within the seed lot at the point of seed release from the maternal plant), germination was less than 20% for all source populations x developmental environment combinations. This suggests a high level of

dormancy within all seed lots produced (Fig. 4.6A), implying a low ability of seed to germinate during the inter-fire years.

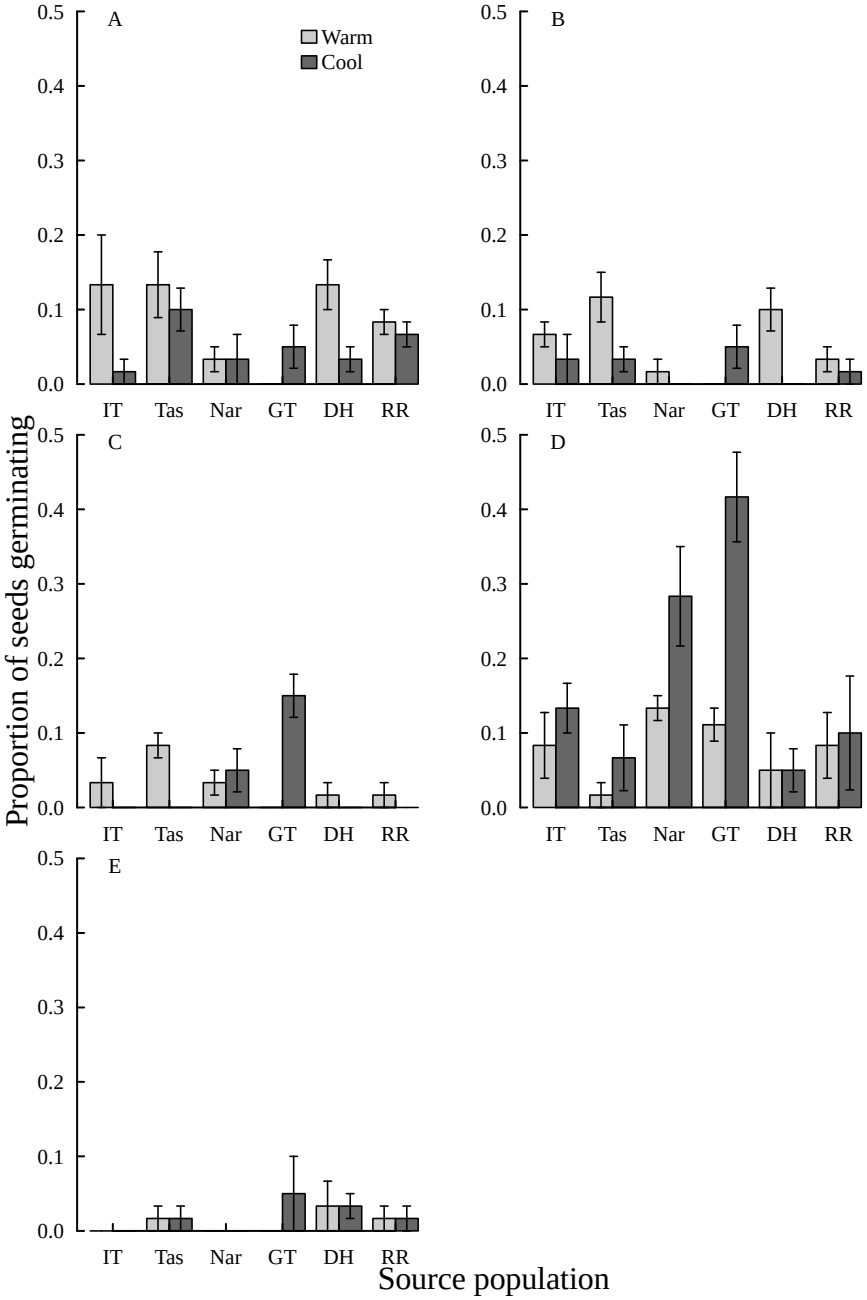

Figure 4.6. The effect of warm versus cool developmental environments on seed dormancy loss after heat treatments: A) control conditions; B) 40°C; C) 60°C; D) 80°C, and E) 100°C (±SE) for multiple populations of *Acacia suaveolens.*

Seed germination was highest after the 80°C fire-related heat treatment (Fig. 4.6D). At this fire-related heat treatment, germination was significantly affected by both source population (quasibinomial GLM: $F = 5.81$, $P = 0.0008$), and developmental environment (quasibinomial GLM: $F = 10.05$, $P = 0.004$), but not the interaction between the two. For seeds grown under the cooler developmental environment, dormancy loss was lower than for those grown under the warmer developmental environment for five of the six populations tested (Fig. 4.6D). Germination was low for the 40°C, 60°C and 100°C heat treatments. Germination over all the experiments showed a high degree of variation. Seed viability analysis showed greater than 95% viability of ungerminated seeds for all seed lots at the control, 40°C, 60°C and 80°C treatments, implying the low germination at the 40°C and 60°C fire-related temperature treatments was caused by dormancy maintenance rather than seed mortality. In contrast, the 100°C temperature treatment caused almost 100% mortality of seeds irrespective of source site or parental environmental conditions, meaning the low germination was the result of seed mortality rather than dormancy maintenance.

The variation in the time taken for seeds to germinate between source populations and developmental treatments could only be analysed for the 80°C fire-related temperature treatment. The number of days to germinate post heat treatment was significantly affected by source population (Poisson GLM: $F = 2.46$, $P = 0.04$), but not by parental environment or the interaction between parental environment and source site. Across both parental environments, seeds from plants of cooler sites germinated more quickly in response to the 80°C degree heat treatment than those of plants from warmer sites ($R^2 = 0.52$, $P = 0.06$). Moreover, this variation in days to germination was not significantly correlated with seed weight ($R^2 = 0.16$, $P = 0.24$).

4.4. Discussion

Under future climate scenarios, south-eastern Australia is projected to experience warming and drying (Dowdy *et al.*, 2015). Our results show that under warmer conditions, annual periods of flowering and reproduction are reduced in *Acacia suaveolens* populations and this, in combination with an increased level of seed abortion during development, could result in fewer seeds entering the seed bank. These changes generally occurred independently of source population, suggesting that climatic warming could drive such phenological changes irrespective of any local adaptation existing within source populations. In addition, to our knowledge, this is the first demonstration that temperature of the environment experienced during seed development has the potential to alter the temperatures required to break PY across multiple populations, controlling (at least in part) for the influence of between population genetic variation of a wild species.

In order to understand the impacts of projected climatic changes on plant populations, increased focus needs to be placed on linking consequences across multiple life-history stages (e.g. Post *et al.*, 2008; Haggerty & Galloway, 2011). Here we have been able to show that, in general, a warmer developmental environment caused a delay in flowering onset and a reduction in length of the whole reproductive process in *A. suaveolens.* Other studies on Australian species have shown delayed flowering (e.g. *Eucalyptus leucoxylon* (Keatley *et al.*, 2002), *E. polyanthemos, E. obliqua* and *E. radiata* (Rawal *et al.*, 2015)) or a contraction of the flowering period (e.g. *Wurmbea dioica* and *Hypoxis vaginata* (Hovenden *et al.*, 2008)) under climatic warming, and when addressed alone such results imply a flexibility of reproductive phenology to match shifting climatic niches in the short term (Amano *et al.*, 2014). However, we found that the length of the reproductive phase overall showed a positive

correlation with the number of fully formed seeds per pod. This correlation suggests that a shortening of the reproductive phase, due to environmental conditions, results in a higher seed abortion rate. It is possible that this is caused by insufficient time for complete seed development to occur prior to pod ripening. From a population fitness perspective, this shorter reproductive phase would likely have a direct negative effect, due to reduced seed entering the seed bank. Hence the negative impacts on reproductive output, and therefore population fitness, due to climatic warming do occur, but are manifested at a later stage in the reproductive cycle. Similarly, Del Cacho *et al.* (2013) found significant links among different reproductive stages under experimental warming in *Dorycnium pentaphyllum,* as did Liu *et al.* (2012) studying multiple species on the Tibetean plateau. Consequently, studying the response of isolated reproductive stages to climatic warming (such as purely flowering phenology) has the potential to result in erroneous conclusions regarding a species adaptability to climate change (Liu *et al.,* 2012; Del Cacho *et al.,* 2013).

Models predicting climate change responses of species frequently assume all populations throughout a species range respond equally to climatic pressures (Valladares *et al.,* 2014; Parmesan & Hanley, 2015). Given the wide variation in phenotypic responses to experimental warming across the populations here, our study adds empirical support to suggestions that variation among populations due to phenotypic plasticity and local adaptation should be accounted for in species distribution models under future climate change (e.g. Valladares *et al.,* 2014). Across the seven variables we monitored, six were influenced by a significant interaction between developmental environment and source population (Table 4.3). Similarly, Cochrane *et al.* (2015b) showed that plants from different populations of each of two *Banksia* species along a climatic gradient showed different seedling growth responses to warming treatments, however they found no correlation between climatic gradient and inter-population

variation. In our study, only one of the seven variables showed a moderate correlation between effect size and mean maximum source site temperature. This may suggest that local adaptation to source site temperature is important for controlling at least part of the response of these variables to developmental environment. Inter-population genetic variation is also likely to have contributed to the variation in phenotypic responses seen here, in that the range of possible phenotypes a genotype can display has a genetic basis. This source of inter-population variation can be influenced by factors such as gene flow or genetic drift (Kawecki & Ebert, 2004; Gonzalo-Turpin & Hazard, 2009; Cochrane *et al.*, 2015a). To dissect the relative influence of local adaptation to temperature versus inter-population genetic variation on responses to experimental warming would require reciprocal transplant experiments.

A number of previous studies have compared the differences in fire-related temperatures required to break PY between different populations of a species (e.g. Moreira & Pausas, 2012; Ooi *et al.*, 2012; Chapter 3) showing considerable inter-population variation, but investigation into the causes of this variation has been limited. In our study, all source seed lots germinated best at 80°C. However, germination was on average 42.6% lower for seeds from warmer developmental environments, a response particularly evident for the mid-latitude populations. This suggests that seeds from the warmer developmental environments require a higher temperature (but still less than 100°C, as indicated by the high mortality), or longer periods of heating to break dormancy. It should be noted however, that seeds produced in this experiment showed a higher degree of dormancy, and reduced variation in dormancy than wild collected seeds from along the same climatic gradients (Chapter 3). Despite the higher overall degree of dormancy in the seeds produced, modification of the temperatures required to break dormancy will inherently alter post-fire recruitment dynamics within the species. Requiring higher temperatures or longer heating durations to break dormancy would reduce

recruitment success under less severe fires, or within cooler burning areas of the fire, by limiting the number of seedlings able to emerge.

Within this study we delimited maternal influence based on the definition of Roach & Wulff (1987), whereby maternal effects occur from pre-fertilization to the point of seed release from the maternal plant. However, from seed formation to seed dispersal, the environmental conditions experienced by the seed and the maternal plant are not independent. Determining if the environmental effects on the developing seed are through the maternal plant (thereby making them strictly maternal environmental effects), or are acting directly on the seed, was not possible here. Under natural situations, the warmer temperatures expected under projected climatic change will affect all stages with interactions acting across the divisions implying that our first assumption is valid.

While our results suggest that under warmer climatic conditions significant changes to flowering and reproduction are likely to occur, it is possible that additional factors varied between the two developmental environment treatments which may have influenced our results. Firstly, humidity is known to particularly influence initial dormancy in *Acacia*, with increasing humidity resulting in fewer dormant seeds (Tozer & Ooi, 2014). Although we found no statistical differences in average humidity, it is possible the extremes did significantly vary. Secondly, a number of papers have suggested a role for water availability in controlling PY and phenological traits (e.g. Norman *et al.,* 2006; Segura *et al.,* 2015; Chamorro e*t al.,* 2016). In our study, all plants were watered equally to capacity, but it is possible that plants under the warmer developmental environment were more water limited than those in the cool developmental environment due to increased evaporation. Morrison & Myerscough (1989) found that rainfall during March and April significantly influenced the

number of buds *A. suaveolens* produces but did investigate the relationship with the variables investigated here. Chamorro *et al.* (2016) investigated the influence of drought during seed development in *Cistus ladanifer* (PY species) finding no significant impact upon seed weight or initial dormancy, however drought during seed development did significantly influence the germination response after a fire-related heat treatment. Thirdly, it is possible that light levels differed between the two treatments, however Argel & Humphreys (1983c) found no significant effect of shade on the development of PY in *Stylosanthes hamata.*

Overall, our results imply that projected temperature increases due to climate change are likely to have significant but mixed impacts upon the reproduction of *A. suaveolens*. The greatest negative impact upon the population dynamics of the species may be through an increase in the seed abortion rate during pod formation under warmer developmental environments, resulting in part from a reduction in the length of the reproductive period of the maternal plant. This research focused on temperature as the key variable of interest, however in reality interacting effects or corresponding changes to other climatic variables or CO_2 levels will occur. Therefore, further research into how these additional climatic changes may influence reproduction in the species is required.

4.5. Acknowledgements

I would like to thank Juana Correa-Hernandez, Isuru Koongahagoda, Sean Williamson, and Ken and Sue Hudson for help with the experiment.

Chapter 5. VARIATION IN THE EMERGENCE, SURVIVAL AND GROWTH AT EARLY LIFE HISTORY STAGES OF *ACACIA SUAVEOLENS* ALONG AN ATITUDINAL GRADIENT.

5.1. Introduction

Modelling the response of species to climate change is believed to be one of the best methods for predicting the consequences of anthropogenic climatic change on species distributions (Thuiller *et al.,* 2005; McLaughlin & Zavaleta, 2011). However, all models are based on assumptions that limit their accuracy (see Pearson & Dawson, 2003 and Hampe, 2004 for discussion). One particular limitation of modelling studies is how differences in environmental tolerances (biotic and abiotic) between seedling and adult life-history stages, and / or among populations throughout species ranges are accounted for (McLaughlin & Zavaleta 2011; Stevens *et al.*, 2014; Valladares *et al.,* 2014; Koide *et al.,* 2016). For example, Koide *et al.* (2016) projected distributional changes to *Fagus crenata* with and without accounting for the environmental limits of juveniles. They concluded that the inclusion of juvenile niche conditions altered the rate of predicted regional species loss (Koide *et al.,* 2016). While Valladares *et al.* (2014) found that when inter-population variation in trait responses to climate change were included in models for *Pinus sylvestris*, projections of regional species loss changed. Combining the conclusions of these two studies highlights the importance of characterising variation in environmental tolerances at juvenile plant life-history stages, as well as adult life-history stages, to accurately predict distributional changes.

It has long been recognized that in many plant species the environmental conditions limiting plant survival differ between seedlings and adults (Grubb, 1977; Becker *et al.,* 2006; Jackson *et al.*, 2009, Walck *et al.,* 2011; Bell *et al.,* 2014). Consequently, the response of a species

adult and seedling stages to future environmental changes are likely to differ (Becker *et al.*, 2006; Donohue *et al.*, 2010; Walck *et al.*, 2011). Seedlings are generally considered to have a narrower tolerance to environmental variables than adult plants because of their limited nutrient storage abilities and smaller root networks (Jackson *et al.*, 2009; Walck *et al.*, 2011; although see Bertrand *et al.*, 2011). The seedling stage is therefore likely to be the most limiting to the ability of a species to cope with environmental change, or successful establishment at new sites (Becker *et al.*, 2006; Donohue *et al.*, 2010; Walck *et al*., 2011). For example, Black & Bliss (1980) showed that decreased water availability within the soil increased seedling mortality, but had little effect on adult trees of *Picea mariana*. As anthropogenic climate change continues we need to better describe the factors controlling and limiting seedling survival in order to robustly, and accurately, predict future changes to population survival and species migration (Walck e*t al.*, 2011; De Frenne *et al.*, 2012).

The environmental (biotic and abiotic) limits influencing seedling survival may vary throughout a species range governed by adaptive or random differentiation of individual populations (Bell *et al.*, 2014). If the environmental conditions limiting seedling survival vary among populations, then the range of potential seedling establishment sites will likely be greater than if the environmental limits of a single population were considered. However, a number of factors are likely to influence the degree of inter-population variation in environmental limits on seedling survival; including the species distribution, gene-flow among populations, fine-scale environmental heterogeneity, as well as effective population sizes (Kawecki & Ebert, 2004; Bowman *et al.*, 2008; Leimu & Fisher, 2008). By conducting a reciprocal transplant between Italian and Swedish populations of *Arabidopsis thaliana*, Ågren & Schemske (2012) showed that Swedish accessions of *A. thaliana* had a greater tolerance of low minimum winter temperatures as compared with Italian ones. Hence, projections of future

species distributions based purely on environmental tolerances of the Italian populations would be very different to if the tolerances of Swedish populations were also included.

The environmental constraints limiting plant survival include biotic as well as abiotic factors. For example, the growth of seedlings, or local ecotypes, outside of their current range can result in exposure to different abundances and types of predators or parasites (Rasmann *et al.*, 2014). *Pinus sylvestris* populations were shown to experience greater levels of herbivory at lower altitudinal limits as compared to the upper limits of the species range, which is expected to compound the effects of climate warming at low altitudes increasing the rates of population decline in these areas (Matías & Jump, 2015). Therefore including the effects of trophic interactions, alongside the influence of abiotic variables, on seedling survival will increase the accuracy with which potential changes to distribution can be made (Garrido *et al.*, 2012).

Reciprocal transplants are one of the most common methods used to investigate local adaptation of plant traits and the environmental factors important in driving the adaptation (Kawecki & Ebert, 2004). Seedlings, or plants, from a range of source sites differing in environmental variables are transplanted back into their source site, and to each of the other source sites (Kawecki & Ebert, 2004). By comparing performance at their source site with performance at the additional sites, it is possible to infer the environmental variables important in controlling survival or specific traits, as well as if populations are locally adapted (providing genetic differences are controlled for) (Hereford *et al.*, 2009). Many transplant experiments have been conducted investigating inter-population performance at seedling, as well as at adult, life-history stages along environmental gradients (see Leimu & Fischer, 2008; Hereford *et al.*, 2009), however, few have been conducted on species where recruitment dynamics are dependent upon fire (Bieger *et al.*, 2014; although see Tozer & Bradstock,

1997; Montalvo & Ellstrand, 2000; Lloret *et al*., 2004). Fire-prone environments are often highly stochastic, and the conditions into which the seedlings recruit can be very different to those experienced by the adult plants (Whelan, 1995). For obligate seeding species the difference in environmental conditions experienced by adults and seedlings is particularly distinct, as seedlings emerge into a post-fire environment with minimal generational overlap (Whelan, 1995; Keith & Myerscough, 2016). For these species in particular, the differences in the adult survival niche and the seedling survival niche (post seed dispersal) are likely to be great (Keith & Myerscough, 2016). In addition, for obligate seeding species, the parental generation is often killed by the fire, meaning that population persistence is reliant upon the soil seed bank and the survival to reproduction of emerging seedlings to replace the lost generation (Auld & O'Connell, 1991). Consequently, we aimed to investigate the environmental factors limiting survival and growth of an obligate seeding species through two transplant experiments into recently burnt areas across an altitudinal gradient. However, the fire-dynamic makes replicated reciprocal transplants inherently difficult as the sporadic nature of fires limits the availability of burnt sites. Because of this, we focused on emergence, survival, and growth characteristics of seedlings at a range of transplant sites only (with no home site transplant), rather than the classic 'home' and 'away' reciprocal transplant design. Using *Acacia suaveolens*, a commonly occurring obligate seeding species from the fire-prone sclerophyllous woodland of south-eastern Australia, we asked the following questions:

1. Does the survival of transplanted *Acacia suaveolens* seedlings depend upon the altitude of origin or of the transplant site?

2. Is the development and growth of *Acacia suaveolens* seedlings influenced by the altitude of origin or of the transplant site?

3. Do levels of herbivory vary between seedlings of different origins and between different transplant sites?

4. Do seed emergence and seedling survival respond in the same way to transplantation into sites of differing altitude from the site of origin?

5. Are rainfall or temperature variables of the transplant sites important in explaining any trends observed across the transplant gradient?

5.2. Methods

5.2.1. Species and habitat

We selected *Acacia suaveolens* (Sm.) Willd. as the study species due to its broad distribution along an altitudinal gradient extending from the Blue Mountains National Park (~1000 m elevation) to the coastal regions around Sydney (~150 m elevation), in south-eastern Australia (Morrison, 1986a). The species is a perennial shrub, with physically dormant seeds. Seed dormancy is broken by heat produced during a fire event, allowing germination to occur after the next rainfall event. Parental plants are generally killed during fire events, meaning that population persistence is dependent upon the survival of the seedlings germinating from the seed bank post-fire. Plants are reported to produce seeds after reaching one-and-a-half to two years of age in the field (Morrison, 1986a; Auld, 1987).

We conducted a transplant experiment within the Blue Mountains, Royal and Heathcote National Parks around the Sydney region of New South Wales, Australia, at locations all within the Sydney coastal and Sydney hinterland dry sclerophyllous woodland habitats. This

habitat is characterised by the presence of *Angophora costata, Eucalyptus haemostoma, Lambertia formosa, Banksia* species and *Dillwynia* species (Keith, 2004).

5.2.2. Seed collection

We collected seeds at nine sites along the altitudinal gradient, specifically at three sites from each of three altitudinal ranges (50– 250 m asl; 450–700 m asl; 850-950 m asl) between September and December 2012 (Table 5.1). We collected seeds from a minimum of 20 adult plants per site of origin, selecting maternal plants at least five metres apart and bulking seed collected from each individual to produce one seed lot per site of origin. We germinated and grew the collected seed under common garden conditions at the Ecological Research Centre at the University of Wollongong, Australia, until they were approximately eight months old. We grew the seedlings in a coarse river sand and vermiculite medium at a 5:1 ratio, in 5 cm forestry stock tubes until transplantation. All seedlings received an equal dose of Osmocote® native plant fertilizer (3 g) at the time of seed planting. We grew the seedlings under common garden conditions prior to transplant to minimise the chance of transplant shock as a cause of death.

Table 5.1. Initial seed collection (source) sites and transplant (transp.) sites for *Acacia suaveolens*

Site	Abbr.	Source / Transp.	Latitude	Longitude	Altitude (m asl)
Garie Trig	GT	Source	34°42'36"	151°03'18"	235
Temptation Creek	TC	Source	34°03'36"	151°04'01"	113
Heathcote	Hea	Source	34°04'05"	150°59'44"	55
Faulconbridge	Faulc	Source	33°41'56"	150°31'30"	461
Park Road	Prk	Source	33°44'29"	150°28'50"	593
Lawson	Cem	Source	33°43'40"	150°25'55"	690
Mount Hay 1	MtH1	Source	33°39'03"	150°22'01"	875
Mount Hay 2	MtH2	Source	33°39'18"	150°22'04"	906
Mount Tomah	MtT	Source	33°32'56"	150°22'58"	867
Temptation Creek	TC	Transp.	34°03'30"	151°04'10"	111
Garrawarra	Garr	Transp.	34°09'32"	150°58'24"	266
Sebastapol	HWY	Transp.	34°06'58"	150°59'16"	230
St. Georges Crescent	StG	Transp.	33°41'26"	150°31'31"	458
Podgers Glen	Podg	Transp.	33°42'30"	150°25'15"	752
Talbot Road	Talb	Transp.	33°42'36"	150°28'90"	619
Katoomba 1	Kat1	Transp.	33°43'40"	150°17'22"	949
Katoomba 2	Kat2	Transp.	33°43'22"	150°19'31"	932
Mount Wilson	MtW	Transp.	33°31'15"	150°22'14"	1013

5.2.3. Experiment 1: seedling transplant

Under natural field conditions *A. suaveolens* seedlings germinate into the post fire environment. Due to the random nature of wildfires, only one of the seed collection sites contained an area burnt within the year prior to seedling transplant (Temptation Creek, TC). Consequently, we selected a further eight sites along the same altitudinal gradient to be transplant sites. The selection criteria we used were based on a similarity in species composition, proximity to the seed collection sites and altitude (transplant sites; Table 5.1). Due the locations of recently burnt sites, this study is not a classical reciprocal transplant design. However, by using altitude as a proxy, and investigating linked environmental changes along the gradient, we believe using sites at similar altitudes will still allow assessment of the influences on early seedling recruitment within the species.

We transplanted seedlings into the burnt sites in August / September 2013, in a randomised block design, consisting of 16 blocks per site. Each block contained nine seedlings (one from each seed collection site), randomly positioned in a 3 x 3 grid layout. We randomly distributed the blocks throughout the burnt sites. Seedlings had their height, the number of pinnate leaves and the number of adult leaves recorded prior to transplantation.

For the first two months following transplant, we monitored seedling survival fortnightly. Height and growth measurements were undertaken monthly for the first four months, then bimonthly until the conclusion of the experiment (16 months). The cause of seedling death was attributed to one of three factors; drought (plants were observed dry and withered but intact), herbivory (only a stump of the seedling stem remained) or unidentified (no trace of the seedling could be found). Due to unforeseen circumstances we could not undertake measurements at all transplant sites at two of the set recording times (October 2013 and April 2014). Based on the height measurements, we calculated absolute growth rates (AGR) for each plant using the following equation:

$$AGR = \frac{\log(Final\ plant\ height) - \log(Initial\ plant\ height)}{T_2 - T_1}$$

following the methodologies of Eckhart *et al.* (2004), Baraloto *et al*. (2005) and Sage (2011), where T_2 is the time of the final height census and T_1 is the time of transplant (for plants surviving to the end of the experiment only). We selected this method of calculating AGR as destructive harvest would have severely limited our replication. Due to the influence of herbivory, a minority of the growth rate values were negative. We removed these values from the data set prior to analysis.

During the first three months following the seedling transplant, the region experienced a severe drought (BoM, 2016). To prevent total seedling death, we watered all seedlings equally on two occasions (censuses 4 and 5).

5.2.4. Experiment 2: seed transplant

In April 2014 (seven months after the initial seedling transplant) we planted seeds from each origin site into the transplant sites. Prior to planting, we broke the dormancy on all the seeds (using sandpaper scarification), monitoring them to ensure radicle emergence. By only planting seeds that showed radicle emergence we ensured that all seeds were viable prior to planting. Therefore any failure to emerge could not be linked to seed dormancy or embryo inviability. We planted seeds in a randomised block design of a 3 x 3 grid, with two seeds per origin site per grid and five grids per transplant site (2 x origin x 9 origins = 18 seeds per grid x 5 = 90 seeds per site). Due to limited seed numbers, we were unable to plant seeds at one of the mid-altitude transplant sites (StG). We monitored seeds for emergence and mortality monthly for the first two months and then bimonthly. We were unable to conclusively identify cause of death for the emerged seedlings.

5.2.5. Weather data

We recorded the soil temperature at a 1 cm depth at each transplant site using three DS1923 iButton thermochron data loggers per site. Due to iButton failures, not all sites had a complete data set for the entire study period. In these cases, we collected maximum daily temperature records from the two nearest Bureau of Meteorology (BoM) recording stations and extrapolated transplant site temperatures based on the altitudinal position of the site relative to the two nearest BoM stations (BoM, 2016). We fitted linear models using maximum daily soil

temperatures recorded at the transplant sites (based on the iButton data) and the extrapolated BoM data during an overlap period using R (R Core Development Team, 2016). We found strong significant correlations between the iButton data and the extrapolated BoM temperatures, exceeding $R^2 = 0.45$ in all cases (Mean = 0.67; Appendix 3). We then used the generated linear models to predict the missing transplant site temperature data based on the extrapolated BoM temperatures for the missing time periods (Appendix 3). We extrapolated site rainfall for each transplant site based on the altitudes of the two nearest BoM weather stations and the altitude of the transplant site.

5.2.6. Statistical analysis

We conducted all statistical analyses in R (R Core Development Team, 2016). We found generally low correlations between altitude and the rainfall and temperature variables for the transplant sites ($R^2 < 0.29$). Consequently, we included temperature and rainfall variables in our analysis.

As low seedling survival resulted in limited levels of replication per transplant site, we combined data from the transplant sites into those at low-altitude (TC, HWY and Garra), mid-altitude (StG, Talb, Podg) and high-altitude (Kat1, Kat2, MtW) groupings. In order to investigate the factors influencing seedling survival, development and growth, we used a combination of generalised linear mixed models (GLMMs) and linear mixed models (LMMs). We analysed the proportional seedling survival, and the number of weeks to first adult leaf with generalised linear mixed models (GLMMs), while we analysed height at the final census and AGR with linear mixed models (LMMs). All models included the fixed effects altitudinal position of origin, altitudinal position of transplant site, transplant site total rainfall over the study period, mean transplant site daily maximum temperature, initial plant height (not for

seedling emergence) and mean seed weight. For the analysis of seedling survival we used environmental variables for the first six months following seedling transplant, as this was the peak period of mortality. For analysis of the number of weeks to first adult leaf, we only used environmental variables for the first month following seedling transplant as the majority of plants developed their first adult leaf within this timeframe. We included initial plant height and average seed weight as co-variates to account for potential maternal effects, given that we used wild collected seed (Monty & Mahy, 2009). All models also included transplant site (categorical) as a random effect to account for any variation between sites that was not explained by the altitudinal gradient or the environmental variables used (Zuur *et al.,* 2005). We used the lme4 package in R to conduct all mixed model analyses (Bates *et al*., 2015). For the GLMMs we selected the appropriate error structures and link functions based on the data type (count data = poisson error structure, proportional data = binomial error structure (Crawley, 2013)). Where the data were overdispersed, an observation level random effect was added in to the model, preventing violation of the GLMM assumptions (Bolker *et al.,* 2011). Due to the low survival of seedlings emerging from the transplanted seeds, model fit for survival data was poor and has therefore not been included.

To establish whether the level of herbivory seedlings were exposed to varied between sites, we analysed seedling death using a GLMM, including altitudinal position of transplant site and origin, cause of death, transplant site rainfall and mean maximum transplant site daily temperature. We included environmental variables in the analysis to investigate if the level of herbivory was dependent upon site temperature or water availability.

For all GLMMs and LMMs, we used AIC stepwise regression to find the best fitting model for the data, following the methodology of Bolker *et al.* (2011).

5.3. Results

5.3.1. Experiment 1: seedling transplant

Proportional seedling survival over the 16 months of the experiment ranged from 0.05 ± 0.02 (SE) to 0.53 ± 0.03 across the transplant sites (mean survival = 0.17 ± 0.02). At only one transplant site did survival exceed 0.40 (TC). The proportion of seedlings surviving differed significantly depending on the altitudinal position of the transplant site (P = 0.004; Table 5.2) (Fig. 5.1A), and the altitudinal position of origin (P = 0.04; Table 5.2) (Fig. 5.1B), but the effects of environmental variation (rainfall and mean maximum daily temperature during the first six months of the experiment), and the interaction between altitudinal position of origin and of transplant were not significant. Seedling survival was particularly high at the low-altitude transplant sites (0.34 ± 0.04), and decreased as altitude increased (0.11 ± 0.02 and 0.06 ± 0.01 for mid- and high-altitude transplant sites, respectively) (Fig. 5.1A). When focused on the altitude of origin, there was a slight, but significant, trend for increased seedling survival in those originating from high-altitude sites (Fig. 5.1B). The initial height of seedlings at the time of transplant had no significant effect on the proportion of seedlings that survived to the end of the experiment.

Table 5.2. GLMM and LMM results for *Acacia suaveolens* seed and seedlings following transplant. R = total rainfall, T = mean maximum daily temperature.

	Seedling survival			**Weeks to first adult leaf**			**Final plant height**		**Absolute growth rate**			**Seedling emergence**	
	X^2	*P*		X^2	*P*		X^2	*P*	X^2	*P*		X^2	*P*
Transplant position*Origin position	NS		Transplant position*Origin position	NS		Transplant position*Origin position	NS		NS		Transplant position*Origin position	NS	
Transplant position	11.2	**	Transplant position	15.7	***	Transplant position	5.3	0.07	5.6	0.06	Transplant position	13.5	**
Origin position	6.6	*	Origin position	11.7	**	Origin position	7.3	*	5.7	0.06	Origin position	NS	
R*T (6 months post transplant)	NS		R*T (1 month post transplant)	NS		R*T (entire study period)	NS		NS		R*T (1 month post transplant)	4.9	*
R (6 months post transplant)	NS		R (1 month post transplant)	NS		R (entire study period)	5.6	*	5.6	*	R (1 month post transplant)	NC	
T (6 months post transplant)	NS		T (1 month post transplant)	NS		T (entire study period)	2.8	NS	2.4	NS	T (1 month post transplant)	NC	
Initial plant height	0.1	NS	Initial plant height	3.7	0.06	Initial plant height	29.1	***	7.5	**	Initial plant height	-	
Seed weight	1.2	NS	Seed weight	0.8	NS	Seed weight	0.06	NS	0.02	NS	Seed weight	0.7	NS

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS = non-significant (if with no X^2 value was removed from the model), NC = significance not calculated as interaction was significant.

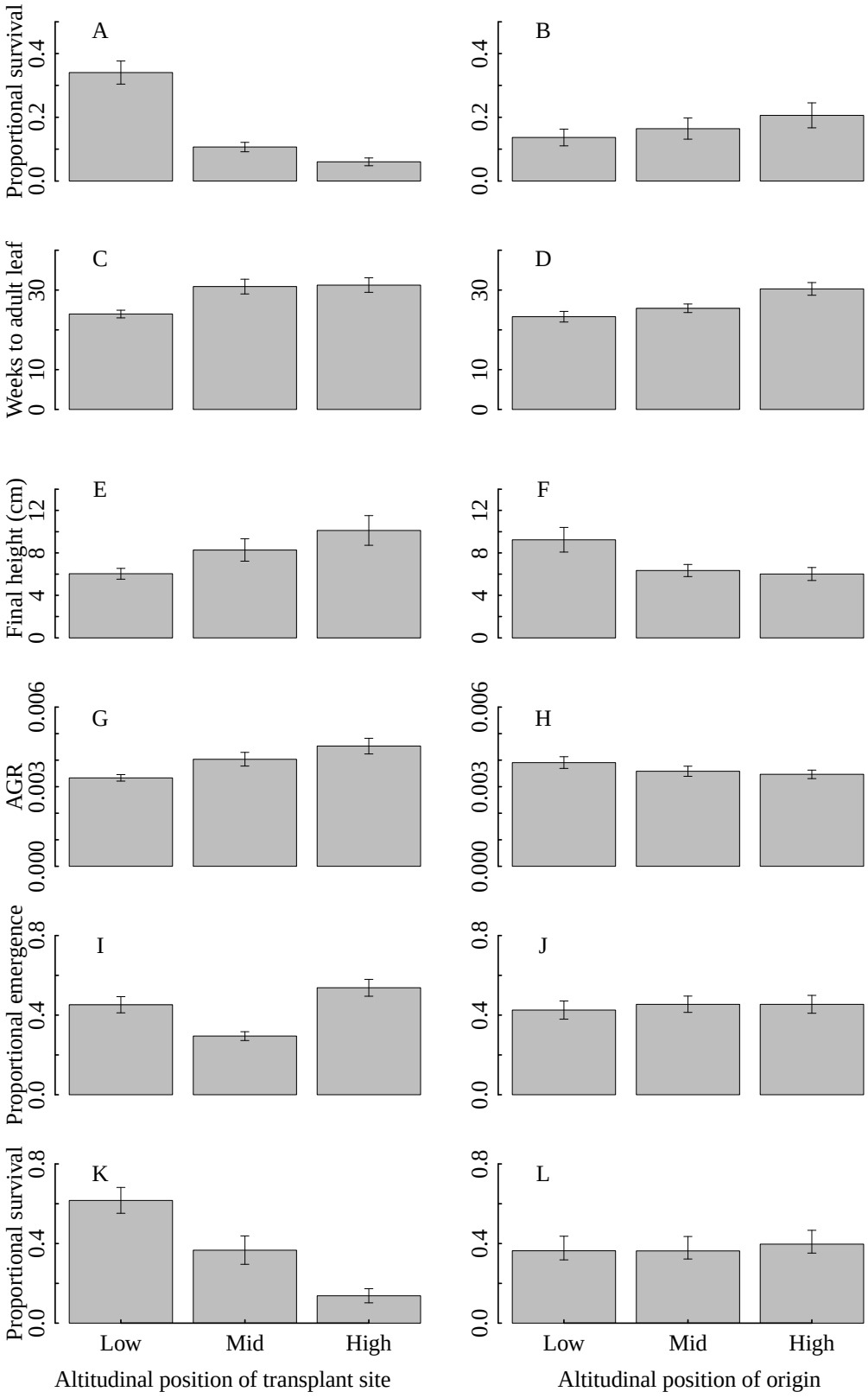

Figure 5.1. The effects of altitudinal position of transplant site and of source site (origin) on; A & B) transplant seedling survival; C & D) weeks to first adult leaf; E & F) height at the final census; G & H) absolute growth rate (AGR); I & J) emergence from seed, and K & L) survival post emergence of *Acacia suaveolens*.

Seedlings transplanted into the mid- and high-altitude transplant sites, and those originating from high-altitude sites, took the longest time for first adult leaves to emerge (30.9 ± 1.9 weeks, 31.2 ± 1.8 weeks and 30.3 ± 1.6 weeks, respectively) (Fig. 5.1C & D). The number of weeks taken for first adult leaf to emerge was significantly influenced by altitudinal position of transplant (P = 0.0004; Table 5.2) and of origin (P = 0.003; Table 5.2). The height of seedlings at the time of transplant had a marginally non-significant impact upon the time taken for the emergence of first adult leaf (P = 0.06; Table 5.2). None of the environmental variables were found to have a significant influence upon the time taken for the transplanted seedlings to develop their first adult leaf.

At the final plant census (13th) seedlings transplanted into the mid- and high-altitude sites (8.3 ± 1.1 cm and 10.1 ± 1.4 cm, respectively) were generally taller than those at the low-altitude transplant sites (6.0 ± 0.5 cm; Fig. 5.1E). Yet, plants originating from low-altitude sites were taller overall (9.2 ± 1.2 cm compared to 6.34 ± 0.6 cm (mid) and 6.0 ± 0.6 cm (high); Fig. 5.1F). Altitudinal position of origin, and mean total transplant site rainfall over the study period, significantly influenced final plant height (P = 0.03 and P = 0.02 respectively; Table 5.2), as did seedling height at the time of transplant (P < 0.0001; Table 5.2). There was, however, no clear directional trend in the impact of total rainfall on plant height. This height result is the absolute height at the end of the experiment, irrespective of any herbivory plants may have experienced.

Of the those seedlings which survived to the end of the experiment, seedling growth rate generally increased with increasing altitude of transplant site, but decreased as altitude of origin increased (Fig. 5.1G). Seedling growth rate was significantly influenced by the total rainfall transplant sites received over the entire study period (P = 0.018; Table 5.2), and the

initial height of seedlings at the time of transplant ($P < 0.006$; Table 5.2). Although both the altitudinal position of transplant and of origin were marginally non-significant (both $P = 0.06$, Table 5.2), seedlings originating from high-altitude sites had a significantly lower growth rate than those originating from the low-altitudinal sites (Fig. 5.1H). As total site rainfall increased over the study period, growth rate of the seedlings decreased.

At the low-altitude transplant sites, herbivory was the predominant cause of death, accounting for an average proportion of 0.51 of all deaths, compared to just 0.25 and 0.13 at mid- and high-altitude transplant sites, respectively (Fig. 5.2). In contrast, lack of water was the predominant cause of death at mid- and high-altitude sites, accounting for an average proportion of 0.60 and 0.75 of deaths, respectively, compared to just 0.22 at low-altitude sites. The interactions between cause of death and total rainfall, and cause of death and mean daily maximum temperature, were significant in influencing seedling death (both $P < 0.0001$; Table 5.3). As the total rainfall for the transplant site increased, the proportion of seedlings dying due to drought decreased, and the proportion of seedlings dying due to herbivory increased. As mean maximum daily temperature of the transplant site increased, the proportion of seeds dying due to drought decreased.

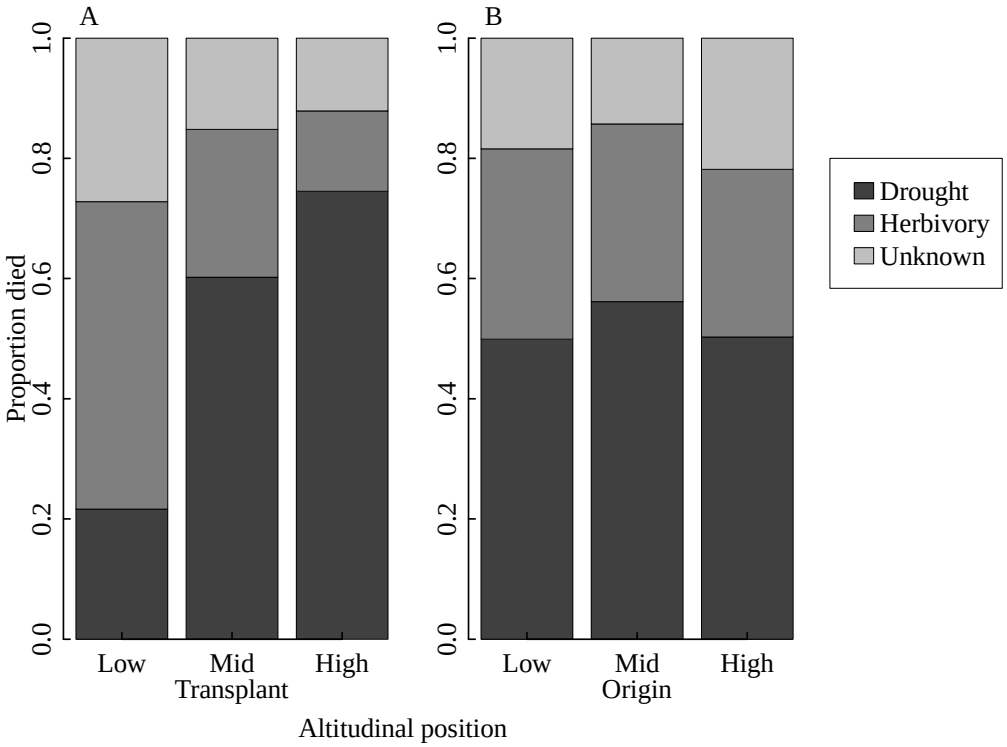

Figure 5.2. The proportional cause of death for *Acacia suaveolens* seedlings following transplantation depending upon altitudinal position of: A) transplant site, or B) origin.

Table 5.3. GLMM results for the cause of *Acacia suaveolens* seedlings death (COD) following transplant. R = total rainfall, T = mean maximum daily temperature.

	Proportional cause of death	
	X^2	*P*
Transplant position*Origin position	NS	
Transplant position*COD	166.6	***
Origin position*COD	12.9	***
Transplant position	NC	
Origin position	NC	
COD	NC	
R*T (over entire experiment)	NS	
R*COD (over the entire study period)	70.4	***
T*COD (over the entire study period)	37.8	***
R (over entire experiment)	NC	
T (over entire experiment)	NC	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS = non-significant (if with no X^2 value was removed from the model), NC = significance not calculated as interaction was significant.

5.3.2. Experiment 2: seed transplant

Of the seeds buried, seedling emergence was greatest at the high-altitude position (proportional emergence 0.54 ± 0.04) and lowest at the mid-altitude position (0.29 ± 0.02) (Fig. 5.1I). Seedling emergence was significantly influenced by altitudinal position (P = 0.002; Table 5.2). The interaction between total rainfall in the first month after burial and the mean maximum daily temperature also had a significant impact upon seedling emergence (P = 0.03; Table 5.2). Seedling emergence was rapid across all transplant sites, with the majority of seedlings emerging within the first month following planting. There was a slight trend for decreasing seedling emergence as mean maximum daily site temperature increased.

The rainfall experienced during the first month following seed planting differed significantly from that experienced by the transplanted seedlings in their first month (paired T-test: t = 2.6, DF = 7, P = 0.035), while mean maximum daily temperatures experienced showed no significant difference between the two time periods (paired T-test: t = -1.9, DF = 7, P = 0.09).

Of the seedlings emerging from transplanted seeds, survival was highest at the low-altitude position and lowest at the high-altitude position, mirroring the result from the transplanted seedlings (Fig. 5.1K). Proportional seedling survival averaged 0.62 ± 0.07 at the low-altitude position as compared with only 0.14 ± 0.04 at the high-altitude position.

5.4. Discussion

Our transplant experiment showed no evidence for altitudinal adaptation in *A. suaveolens* seedlings, however there was evidence for the dominance of seedlings originating from a single altitudinal position in certain traits. We found that the most important explanatory variable differed depending upon the stage, or process, within the seedling life history.

However, we found survival of transplanted seedlings, and seedlings emerging from transplanted seeds, to be highest at the low-altitude position. In particular, we observed that seedlings originating from higher altitude sites showed higher survival but were generally shorter than their low origin counterparts with slower development times. We demonstrated that herbivory pressures had a significant impact upon the survival of seedlings, but in general we found limited evidence for the influence of temperature and rainfall on seedling performance (except growth and height).

We found no evidence in this study to support the idea that seedlings show a higher survival rate at transplant sites with a similar altitude to that from which they originate. This was true for both transplanted seedlings, and for seedlings emerging from transplanted seed. Indeed, seedling survival (of transplanted seedlings) was greatest in those originating from the high-altitude position at each of the transplant positions. This is in contrast to the results of Byars *et al.* (2007) who found that seedlings of the Australian alpine species *Poa hiemata* tended to exhibit higher survival when transplanted into sites of similar altitude to their origin (including non-origin sites of a similar altitude to the origin), as compared with those transplanted to sites at a different altitude. While a number of authors, including Byars *et al.* (2007), have reported altitudinal or latitudinal adaptation in early life history stages (e.g. Ågren & Schemske, 2012; De Frenne *et al*., 2012; Kim & Donohue, 2013), a number of studies have also reported that during the early life history stages, local environmental factors at transplant sites have a greater influence over survival (Antonovics & Primack, 1982; Van Tienderen & Van der Toorn, 1991; Gordon & Rice, 1998; Becker *et al.*, 2006). Becker *et al.* (2006) found a significant individual plot effect on the survival of *Carlina vulgaris* within a transplant site, thus inferring fine scale environmental influences to be important.

Consequently, it is possible that fine scale environmental differences at the transplant sites had a greater influence over survival, than any altitudinal adaptation.

Interestingly, we found no significant relationship between the climatic variables tested and seedling survival. This is somewhat surprising given that desiccation was a primary cause of seedling death (particularly at high-altitudes (Fig. 5.2)), a similar finding to that of Auld (1987) who reported moisture stress to be a prominent cause of *A. suaveolens* seedling death following natural emergence after a wildfire. The lack of any clear effect of the climatic variables investigated on seedling survival is likely the result of the high mortality and the relatively small range of difference in rainfall across the sites. Giménez-Benavides *et al.* (2007) attained only 10% survivorship in their study of *Silene ciliata* seedlings following transplant due to a severe drought, and similarly Montalvo & Ellstrand (2000) reported seedling survival of 3.6–50% in *Lotus scoparius,* with particularly low survival in the first year due to drought. Both of these studies were conducted in Mediterranean type ecosystems, where rainfall is expected to strongly limit the seedling survival niche (Lloret *et al.*, 2004). In our study, during the first month following seedling transplant, a number of the transplant sites experienced drought with less than 10 mm of rain, therefore it is possible that this was beyond the survival limits of most seedlings, obscuring any observable relationship between rainfall and seedling survival.

Despite finding the highest seedling survival at the low-altitude position, we also found the herbivore pressure to be strongest there. Our results indicate that across the altitudinal gradient, *A. suaveolens* seedling survival is constrained to a greater degree by herbivory at low-altitude, as compared too high-altitude. Similar evidence for gradients in herbivory have been reported by Rasmann *et al.* (2014) who found levels of insect herbivory to decrease as

altitude increased; while in a study of two species, Matías & Jump (2015) found that browser herbivory on *Pinus sylvestris* decreased as altitude increased, but that the pattern was reversed for *Juniperus communis*. Our observations suggest that deer browsing was the most prevalent form of herbivory at the low altitude sites. Keith & Pellow (2005) highlighted the negative impact of the non-native deer, C*ervus timorensis*, on native Australian plants within the Royal National Park, NSW (where one of low-altitude study sites was located). They found *C. timorensis* showed a preference for young foliage, in particular of the Fabaceae family (Keith & Pellow, 2005).

While the level of herbivory experienced by seedlings in this study was high, the level of seedling survival reported here (5–53%) is comparable with that reported in other similar studies from Mediterranean-type climates. Tozer & Bradstock (1997) reported approximately 0-50% survival of transplanted *Eucalyptus luehmania* seedlings, while Pausas *et al.* (2004) reported survival of over 40% for *Pinus halepensis* and over 35% for *Quercus ilex* 20 months after transplant and Daskalakou & Thanos (2010) reported 30-50% survival of natural *P. halepensis* seedlings. However, Auld (1987) reported survival of 48% and 65% for *A. suaveolens* seedlings naturally emerging at two sites following bush fires, despite lower than average rainfall at one site following the bush fire. Our results are therefore similar to those of transplanted seedlings in other studies, but lower than may occur naturally for the species.

For seedling survival, height and AGR we found seedlings originating from one position to perform the best across all transplant positions (exception being AGR at high altitude) (Fig. 5.1). This would seem to suggest some degree of genetic influence on these traits, or greater acclimation ability, in plants originating from certain altitudes. An investigation into the population genetics of seven of the nine *A. suaveolens* origin populations used in this

experiment reported high levels of genetic differentiation among the populations (Chapter 7), providing some support to the theory. However, it is also possible that this result could be the influence of maternal effects, particularly as the seedlings were grown from wild collected seed (Roach & Wulff, 1987; Kawecki & Ebert, 2004; Monty & Mahy, 2009). This was partially accounted for by growing the seedlings under the common garden conditions for the first six months, and by including seed weight as a co-variate in our statistical models (a metric commonly used to account for maternal effects (e.g. Monty & Mahy, 2009; Garrido *et al*., 2012)). Including seed weight in the statistical models had no effect on the outcome for any of the seedling variables.

Across the different stages and processes we recorded, we found the influence of environmental variables to differ. For example, we found rainfall to be significant in influencing growth, whilst there was no evidence of either rainfall or temperature significantly impacting the timings to first adult leaf, but the interaction between rainfall and temperature significantly influenced emergence from seed. This highlights the importance of focusing on multiple measures of fitness / plant performance to investigate the limits to successful seedling performance. Benavides *et al.* (2016) also reported differences in environmental controls on seedling performance in *Pinus nigra*. They found that annual rainfall had a significant impact upon the number of seedlings, whereas temperature was more important in controlling growth (Benavides *et al.,* 2016). Cochrane *et al*. (2014) also reported different trait responses to water and temperature manipulations in both *Banksia baxteri* and *B. media,* in particular for seedling percentage emergence and above ground leaf biomass. Although under future climate change these environmental variables will change together understanding how individual aspects modify seedling performance is still important.

When comparing the emergence and survival from transplanted seeds with the survival of transplanted seedlings, we found survival to be highest at the low-altitude position for both. This is despite the highest seedling emergence occurring at the high-altitude position and significantly different rainfall in the month following seedling transplant and the month following seed planting across the study sites. It is possible that the high grazing pressure at the low-altitude transplant sites may have resulted in lower recorded seedling emergence at these sites than would occur had herbivores been excluded. Combined, these results suggest that the low-altitude position is more suitable for *A. suaveolens* survival. The distributional range of *A. suaveolens* is primarily coastal, extending up altitudinal gradients at only three locations (Morrison, 1986a). Therefore, it is possible, that the species as a whole is primarily adapted to warmer coastal conditions. Kim & Donohue (2013) found a similar, but reversed, result for the alpine species *Erysimum capitatum.* They found survival to be reduced at the low-altitude position as compared to the high-altitude position, inferring low-altitude environments to exert greater stress on the species (Kim & Donohue, 2013). Consequently, under future climate change, warmer conditions at high-altitudes may enhance seedling survival particularly if herbivore levels do not increase.

The results presented here, highlight the importance of addressing different traits and potential environmental drivers in assessing the controls on early life history stages in plants. Although we were unable to isolate specific environmental drivers behind the significant altitudinal effects influencing some traits, we did show rainfall and temperature as important in influencing different traits. Under future climate change, the study region is expected to see an increase in temperature and extreme weather events (including drought) (Dowdy *et al.*, 2015). Under such a scenario, regeneration of *A. suaveolens* populations after fire may be particularly effected through the influence of drought on seedling survival, but favoured by

warmer conditions in high-altitude areas. Further investigation into identifying genetic versus environmental controls on traits associated with early life history stages within the species would greatly improve the ability to predict the impact of climate change on the species.

5.5. Acknowledgements

I would like to thank all the volunteers who help with the transplanting of all the seedlings, and Juana Correa-Hernandez for her help in the field. I would also like to thank Leonie Gough for her statistical advice.

Chapter 6. MICROSATELLITE PRIMERS FOR THE AUSTRALIAN NATIVE *ACACIA SUAVEOLENS* (FABACEAE)

6.1. Introduction

Acacia suaveolens (Sm.) Willd. (Fabaceae) is a commonly occurring species within dry sclerophyllous woodland along coastal regions of eastern Australia. It occurs on sandy soils, only extending inland at three locations (Morrison, 1986a). The broad distribution of this fire sensitive species with physically dormant seed (latitudinal range 24°26'S to 43°15'S; altitudinal range 0 – 950 m asl (Morrison, 1986a)) means it spans a wide range of climatic conditions thus providing a good case study for the impacts of climatic change on population dynamics, and particularly the effects of temperature variation on germination. To date, much work has been done on various aspects of the species life history such as seed dormancy (e.g. Ooi *et al.*, 2012; Chapters 3 & 4), pollination (e.g. Morrison & Myerscough, 1989) and plant demographics (e.g. Auld, 1986b), but the genetic structure and diversity of populations has not been investigated. Therefore, we sought to develop microsatellite primers for *A. suaveolens* to fill these knowledge gaps.

6.2. Methods and results

All DNA was extracted from silica-dried phyllodes, following a modified version of the Doyle & Doyle (1987) protocol using less than 20 mg of dried sample. We sent five samples of DNA to Macrogen (Seoul, Korea) for GS-FLX Titanium emPCR 1/8 region and GS-FLX Titanium Shotgun library construction. The returned DNA library contained 135,570 reads, with an average read length of 433 bp. This library was constructed from an individual of *A. suaveolens* grown at the Ecological Research Centre (ERC) at the University of Wollongong

(UoW), in New South Wales, Australia, from seed collected at Temptation Creek within Royal National Park, NSW, Australia (TC, 34°03'33"S 151°03'59"E, Voucher specimen WOLL#11296, Appendix 4). We ran the returned sequence library through the MSATCOMMANDER programme v.0.8.1 (Faircloth, 2008) to identify possible microsatellite regions and primers, using the following criteria: 1) product size of 100-450 bp; 2) minimum repeat lengths of seven for dinucleotides, five for tetranucleotides and four for pentanucleotides; 3) primer size range of 18-20 (optimum 20); 4) a primer T_m range of 58-72°C (optimum 68°C), and 5) a primer GC content of 40–70%. We also specified the addition of an M13R-21 tag (5'-GTAAAACGACGGCCAGT-3') and pigtailing (5'-GTTT-3' added).

MSATCOMMANDER produced 264 possible tagged microsatellite primer pairs. We selected forty of these primer pairs for trialling based on the left and right T_m's being within 1°C of each other. The selected primers for trialling were synthesized by Sigma-Aldrich (Sydney, Australia). We conducted the primer trials on a range of plants grown at UoW from seed collected at Narooma (36°14'54"S 150°08'27"E), Faulconbridge (33°42'00"S 150°31'34"E), Garie (34°10'16"S 151°02'57"E) and Diamond Head (31°40'42"S 152°48'06"E). We selected these sites as they span a large proportion of the species geographic range therefore ensuring amplification throughout the species range.

Following initial PCR trials, we discarded 12 primer pairs due to inconsistent PCR amplification. We assigned the remaining 28 primer pairs one of four fluorescently labelled M13R-21 tags (6-FAM, VIC, NED or PET, Applied Biosystems' (Carlsbad, California, USA)), and trialled each primer pair using three to four individuals from the same stands through to sequencing. We used MyTaq^{TM} HS DNA Polymerase (Bioline) for the PCR reactions, and sequencing was conducted on an ABI 3730 Sequencer, at UoW. We used

GeneMapper v3.7 software (Applied Biosystems') for allele scoring. Of the 28 primer pairs sequenced, we discarded 18 due to low variability or difficulty in the scoring process, leaving 10 successful primers (Table 6.1). The exact PCR concentrations and protocols varied between the primers (Table 6.2). We undertook further testing of the 10 remaining primers on larger sample sizes from the GT (N = 13) and TC (N = 12) stands (Appendix 4). We lodged voucher specimens within the JCH herbarium at UoW (Appendix 4).

Table 6.1. Characteristics of ten microsatellite loci developed for *Acacia suaveolens.*

Repeat motif	Allele size range (bp)	Ta (°C)	PCR protocol
$(AG)_9$	173-193	64	ARH63FP1
$(AC)_7$	321-331	64	ARH63FP1
$(AAAT)_5$	220-240	64	ARH63FP1
$(AC)_9$	424-434	58	ARH58FP1
$(AG)_9$	174-188	58	ARH58FP1
$(AT)_8$	300-346	63	ARH63FP1
$(AG)_9$	374-430	58	ARH58FP3
$(AC)_7$	454-472	58	ARH58FP3
$(AC)_8$	290-308	58	ARH58FP3
$(AC)_7$	309-331	64	ARH65FP1

Table 6.2. Specific PCR reagent volumes and cycling conditions used for the ten microsatellites developed for *Acacia suaveolens.* Superscripts link the reagent volumes with the appropriate PCR conditions for each primer pair.

	Volume (µL)									
Reagent	**CD4[a]**	**CD5[a]**	**CD9[b]**	**GH8[b]**	**AB9[b]**	**GH1[c]**	**AB7[c]**	**CD1[c]**	**EF2[c]**	**EF8[d]**
5x MyTaq™ reaction buffer	1.00	2.00	1.00	1.00	1.50	1.50	1.00	1.00	1.00	1.50
DNA	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Primer master mix*	0.10	0.20	0.10	0.10	0.15	0.15	0.10	0.10	0.10	0.15
MyTaq™ HS DNA polymerase	0.12	0.12	0.12	0.12	0.12	0.20	0.12	0.12	0.12	0.12
Flurotag**	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
H_20	7.70	6.60	7.70	7.70	7.15	7.07	7.70	7.70	7.70	7.15

	PCR Conditions											
	[a]ARH58FP1			**[b]ARH58FP3**			**[c]ARH63FP1**			**[d]ARH65FP1**		
PCR stage	**Temp.**	**Time**	**Cycles**	**Temp.**	**Time**	**Cycles**	**Temp.**	**Time**	**Cycles**	**Temp.**	**Time**	**Cycles**
Initial denaturation	95°C	01:00	1	95°C	05:00	1	95°C	01:00	1	95°C	05:00	1
Denaturing	95°C	00:15	35	95°C	00:20	28	95°C	00:15	35	95°C	00:20	28
Annealing	58°C	00:20		58°C	00:40		63°C	00:20		65°C	00:40	
Extension	72°C	00:10		72°C	00:10		72°C	00:10		72°C	00:10	
Denaturing	95°C	00:20	10	95°C	00:20	8	95°C	00:20	10	95°C	00:20	10
Annealing	54°C	00:40		54°C	00:40		54°C	00:40		54°C	00:40	
Extension	72°C	01:00		72°C	01:00		72°C	01:00		72°C	01:00	
Final elongation	72°C	10:00	1	72°C	10:00	1	72°C	10:00	1	72°C	10:00	1

* Primer master mix contained concentrations of 1,000 nm labelled and 100,000 nm unlabelled primers

**Flurotag stock concentration of 16667 nm

We tested for all loci for deviation from Hardy-Weinberg equilibrium (HWE) using exact tests in GENEPOP (Raymond & Rousset, 1995), while we calculated observed and expected heterozygosity (H_o and H_e respectively), the number of alleles (A), and the fixation index (F) using GenAlEx (Peakall & Smouse, 2012). As evidence suggests *A. suaveolens* has the potential for selfing (Morrison & Myerscough, 1989), we tested the microsatellite loci for the presence of null alleles using INEst2, which does not assume HWE (Chybicki & Burczyk, 2009). All loci displayed heterozygote deficits, with five loci showing significant deviation from HWE for the TC stand and four in the GT stand (Table 6.3). Estimated frequency of null alleles was less than 5% for all loci across both stands. The heterozygote deficits identified, combined with the low estimations for null allele presence, suggests evidence of moderate inbreeding levels within the GT and TC stands. The number of alleles per locus averaged 4.2 and 4 for the TC and GT stands respectively across all loci, ranging from 3 to 7 for both stands. H_o ranged from 0.083 to 0.583 for the TC stand, averaging 0.354, while H_e ranged from 0.288 to 0.750, averaging 0.549, over all loci. For the GT stand, H_o was slightly greater ranging from 0.231 to 0.615, averaging 0.454, while H_e ranged from 0.370 to 0.775, averaging 0.618, over all loci. F values ranged from -0.190 to 0.858, with an average of 0.318, for the TC stand over all loci, and -0.248 to 0.405, averaging 0.238, for all loci at the GT stand.

Table 6.3. Results of primer screening in two stands of *Acacia suaveolens*. *Na* = number of alleles, H_o = Observed heterozygosity, H_e = Expected heterozygosity, *F* = F statistic.

Locus	TC (N = 12)				GT (N = 13)			
	Na	***H_o***	***H_e***	***F***	***Na***	***H_o***	***H_e***	***F***
AB7	5	0.500	0.420	-0.190	4	0.385	0.618	0.378
CD1	5	0.583	0.726	0.196	4	0.538	0.627	0.142
EF2	5	0.333	0.684	0.513	3	0.538	0.601	0.103
CD4	3	0.333	0.531	0.373	3	0.462	0.615	0.250
CD5	4	0.333	0.538	0.381	5	0.385	0.71	0.458
GH1	3	0.455	0.574	0.209	3	0.462	0.521	0.114
GH8	7	0.333	0.75	0.556	5	0.615	0.722	0.148
CD9	3	0.333	0.392	0.150	3	0.231	0.624	0.630
AB9	3	0.250	0.288	0.133	3	0.462	0.370	-0.248
EF8	4	0.083	0.587	0.858	7	0.462	0.775	0.405

We tested an additional five *Acacia* species for cross-amplification of the primers (Appendix 4; Table 6.4). Four of the loci amplified in additional species within the expected size range.

Table 6.4. Results of primer screening for cross-species amplification. x = successful amplification, - = no amplification.

Species	N	Locus									
		CD5	**AB9**	**GH8**	**CD9**	**EF2**	**EF8**	**CD4**	**CD1**	**AB7**	**GH1**
Acacia falcata	2	x	x	-	-	-	-	-	x	-	-
A. cultriformis	2	x	x	-	-	-	-	-	x	-	-
A. linifolia	2	x	x	-	-	-	x	-	x	-	-
A. penninervis	2	x	x	-	-	-	x	-	x	-	-
A. caesiella	3	x	x	-	-	-	x	-	x	-	-

6.3. Conclusion

The 10 microsatellite loci developed in this study will enable investigation into the genetic diversity and population structure of *A. suaveolens.* This will add to ecological data for the species, helping to build up a case study of an Australian native plant as a model species, on which the impacts of climate change can be investigated. The loci also showed cross species amplification for a number of other native Australian *Acacias.* These loci will continue to be used to describe the genetic variation across the geographic and altitudinal range of *A. suaveolens* (Chapter 7).

Chapter 7. A GENETIC TEST OF THE ASSUMPTIONS UNDERLYING THE ECOLOGICAL 'SPACE-FOR-TIME' APPROACH TO CLIMATE CHANGE STUDIES.

7.1. Introduction

Predicting the response of species to future climate change is a challenge that varies in complexity. From an ecological perspective, the length of a species life span, the time to first reproduction or the number of offspring produced can all generate experimental difficulties. One method used to deal with some of these ecological difficulties is the so-called 'space-for-time' (SFT) approach (Pickett, 1989; Dunne *et al.,* 2004; Blois *et al.,* 2013). One variation of the SFT approach makes use of naturally occurring variation in climatic conditions across geographic gradients as a proxy for future temporal climatic change (Dunne *et al.,* 2004; Fukami & Wardle, 2005; Blois *et al.,* 2013). By identifying associations between trait changes and climatic changes along the spatial gradient, projections of trait responses to similar future climatic changes can be made, based on these associations (Dunne *et al.,* 2004; Fukami & Wardle, 2005). The main assumption of the SFT approach is that species will show the same response to future climatic changes, as they currently do to equivalent climatic changes over geographic gradients (Dunne *et al.,* 2004; Fukami & Wardle, 2005). This assumption could be met through either, 1) the migration of individuals, or the transfer of useful alleles, among populations along the gradient, or 2) by populations at, for example, the cooler sites adapting to the warmer conditions as the populations at the warmer sites did in the past. Across SFT studies, there is wide variation in the types of spatial gradients that are used (see Koch *et al.,* 1995; Dunne *et al.,* 2004). Population connectivity, reproductive mode and the genetic diversity of populations will all vary depending upon the nature of the spatial

gradient selected (Hamrick & Godt, 1996; Whitlock & McCauley, 1999; Ohsawa & Ide, 2008; Frankham *et al.*, 2010; Jay *et al*., 2012), and so too will the likelihood of the SFT assumption being met.

In addressing the main SFT assumption, it is important to focus on the cause of trait changes observed over the geographic gradients. Trait changes within populations in response to climatic variability over spatial scales could be due to phenotypic plasticity or local adaptation (Jump & Peñuelas, 2005; Valladares *et al*., 2014), however, the proportional contribution of these two factors to future climate change response is likely to differ (Jump & Peñuelas, 2005). Current responses to climatic variability, and those over the short term, are more likely to be driven by phenotypic plasticity, whereas adaptation and migration are expected to be more important in long-term responses to climatic change (Jump & Peñuelas, 2005). Therefore, in relation to the SFT approach, local adaptation and migration are expected to be of greater importance. The degree of local adaptation a population displays, and the potential for the migration of individuals or genes, are a result of demographic history and environmental drivers, as well as evolutionary processes, all of which vary along and between spatial gradients (Hamrick & Godt, 1996; Whitlock & McCauley, 1999; Frankham *et al.*, 2010). Theoretically, whilst gene flow should help transfer potentially useful traits throughout a species distribution, it also has the potential to hinder local adaptation if the homogenising effects of gene flow opposes selection for local or even regional adaptation (Kawecki & Ebert, 2004). Consequently, when using SFT gradients, without characterising the potential gene flow among populations along the SFT gradient, it is not possible to establish if either of the two ways in which the main SFT assumption can be met could occur at a rate equivalent to that needed to keep pace with future climatic changes.

Few ecological SFT studies account for the possibility of variation within the mating system of the focal species, despite the prevalence of mixed mating systems in plants, and the influence this can have on gene flow. In a meta-analysis of 345 plant species Goodwillie *et al.* (2005) found that 42% had mixed mating systems. Differences in the mating system along or between spatial gradients will also alter patterns of genetic structure (broad and fine scale) and diversity, as well as gene flow (Neel *et al.*, 2001; Barrett, 2003; Charlesworth, 2006). Differences in the mating system between populations can result from genetic bottlenecks reducing population size, gene-flow barriers preventing outcrossing, different pollinator abundances or flowering asynchrony (Charlesworth, 2006), all of which can vary between and along spatial and climatic gradients. Besides influencing the transfer of climate adapted traits, variation in the mating system among populations will also modify population susceptibility to the effects of deleterious alleles or inbreeding depression (Frankham *et al.*, 2010), as well as altering the levels of standing variation upon which selection can act (Halbritter *et al.*, 2015). Consequently, if the mating system of a species varies along an SFT gradient then it has the potential to invalidate both SFT assumptions.

For commonly occurring species with broad distributions, predicting temporal response to climate change from a single spatial gradient is assuming that all spatial gradients will display similar genetic connectivity and adaptive variation to the one studied. For example, one key driver of differences between spatial gradients is geographic scale, evident in altitude versus latitude gradient comparisons. Equivalent changes in temperature generally occur over much greater geographic distances along latitudinal gradients than altitudinal gradients (Körner, 2007; Nardin *et al.*, 2015). For the south-eastern coast of Australia, a 4°C change in average temperature occurs over approximately a 900 km, or six degree of latitude span, whereas the same temperature change occurs over just 84 km with a 700 m elevation increase west of

Sydney, Australia. Consequently, thermal adaptation, through gene flow carrying potentially adaptive genotypes or alleles, may be more realistic along the altitudinal gradient compared to the latitudinal gradient due to the shorter geographic distances involved (Fukami & Wardle, 2005; Jump *et al.*, 2009; Loarie *et al.*, 2009). Thus, for species with a broad distribution, covering multiple climatic gradients, assuming trends observed over one gradient can be used to predict trends observed over another may be invalid (Ohsawa & Ide, 2008).

Acacia suaveolens has been used for a number of SFT studies (e.g. Chapters 3-5; Warton & Wardle, 2003) using both an altitudinal and a latitudinal gradient making it an ideal species to test the genetic assumptions underlying the SFT approach. The species occurs within dry-sclerophyllous woodland of south-eastern Australia, a habitat in which population processes are largely driven by fire (Whelan, 1995). The fire dynamic can significantly influence genetic diversity and structure, in part dependent upon the fire adaptations a species has (e.g. England *et al.*, 2003; Ayre *et al.*, 2009; Bradbury *et al.*, 2016a & b). In addition, fires are patchy over space and time, likely increasing genetic differentiation among populations, by introducing different selection pressures, along with different rates of population turnover depending upon fire interval length (Pausas & Keeley, 2014; Bradbury *et al.*, 2016a). However, fire variability may also act to reduce genetic diversity within the standing population due to the influence of genetic drift resulting from changes to the adult population size (Honnay *et al.*, 2008; Bradbury *et al.*, 2016a & b).

We surveyed genetic variation at putatively selectively neutral microsatellite loci for populations distributed along two 5°C temperature gradients (one altitudinal and one latitudinal) within the study habitat. We specifically set out to address the following questions:

1. Does the level and pattern of fine-scale genetic diversity within sites vary along or between spatial gradients?
2. Do the breeding systems inferred from adult genotypes vary along or between spatial gradients?
3. Does the degree of genetic structure and inferred gene flow along the two spatial gradients vary?

7.2. Methods

7.2.1. Study species

Acacia suaveolens is a commonly occurring species within the fire-prone dry sclerophyllous woodland of eastern Australia. It has a wide latitudinal distribution, extending from Tasmania to mid-coast Queensland, Australia, covering approximately 19° latitude (Fig. 1.1B), but is restricted to sandstone soils (Morrison, 1986a). It is known to extend inland at three locations covering an altitudinal range of 950 m (Morrison, 1986a). The species is pollinated by a broad range of insects, including the introduced honeybee (*Apis mellifera*) (Morrison, 1986b). Seed dispersal is primarily by ants and has been shown to have a restricted distance (Auld, 1986a). The species is classified as an obligate seeder (Morrison, 1986a), whereby parental plants are killed during a fire event, and replaced by germination from a seed bank (often long lived) following the fire, replacing the lost parental generation (Auld & Myerscough, 1986; Whelan, 1995).

7.2.2. Sampling

The ecological SFT studies using *A. suaveolens* focused primarily on one latitudinal, and one altitudinal, gradient within NSW, Australia (Chapters 3-5). The latitudinal gradient spanned approximately 7° of latitude and approximately 870 km (direct distance), whilst the altitudinal gradient covered a 796 m altitude change over approximately 85 km (direct distance) (Fig. 7.1). We sampled ten sites along the latitudinal gradient and seven sites along the altitudinal gradient (two sites common to both gradients) in common with the ecological SFT studies (Chapters 3-5). At each site, we collected phyllode samples from 12 to 15 individual plants, sampling a total of 331 individuals (Table 7.1), preserving the samples in silica gel until DNA extraction. The individual plants we sampled were at least five metres apart to reduce the chance of sampling half-siblings or clonemates.

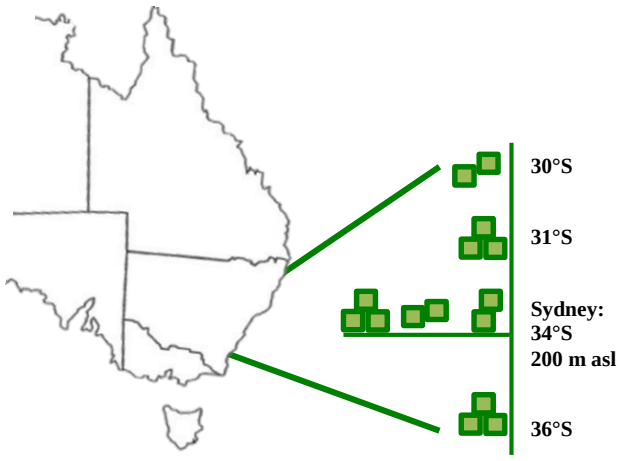

Figure 7.1: Schematic map of the gradients and stand locations in NSW eastern Australia.

Table 7.1. Details of stand locations for *Acacia suaveolens* used for DNA extraction.

Stand	Abbr.	Gradient	N	Latitude	Longitude	Altitude (m asl)
Mount Hay 1	MtH1	Altitude	25	33°39'03"	150°22'01"	888
Mount Hay 2	MtH2	Altitude	25	33°39'18"	150°22'04"	904
Mount Tomah	MtT	Altitude	25	33°32'56"	150°22'58"	862
Faulconbridge	Faulc	Altitude	25	33°41'56"	150°31'30"	468
Lawson	Cem	Altitude	24	33°43'40"	150°25'55"	670
Garie Trig	GT	Altitude / Latitude	13	34°42'36"	151°03'18"	210
Temptation Creek	TC	Altitude / Latitude	12	34°03'36"	151°04'01"	108
Potato Point	PPrd	Latitude	24	36°05'38"	151°05'31"	83
Narooma	Nar	Latitude	20	36°14'56"	150°08'16"	7
Camel Rock	CR	Latitude	25	36°22'48"	150°04'22"	18
Diamond Head	DH	Latitude	25	31°40'37"	152°48'03"	12
Crescent Head	CH	Latitude	25	31°13'08"	152°57'28"	2
Hat Head	HH	Latitude	24	30°59'52"	153°01'30"	*
Angourie	Ang	Latitude	20	29°28'48"	153°21'21"	18
Evans Head	EH	Latitude	20	29°04'15"	153°25'01"	17

* no data available

7.2.3. DNA extraction and amplification

We extracted total genomic DNA from the silica-dried phyllodes using a modified CTAB method (Doyle & Doyle, 1987). We used ten pairs of microsatellite primers identified for *A. suaveolens* in Hudson *et al.* (Chapter 6). Further testing highlighted one locus (AB9) as difficult to reliably score and was therefore omitted from this study, leaving nine reliable loci. We followed the PCR protocols for the nine loci from Hudson *et al.* (Chapter 6, Table 6.2).

7.2.4. Genetic diversity and mating system assessment

We compared levels of genetic diversity among stands using estimates of rarefied allelic richness (*AR*), private allelic richness (*pAR*), number of effective alleles (N_e) and expected heterozygosity (H_e). We estimated N_e and H_e using GenAlEx (Peakall & Smouse, 2006 &

2012), and *AR* and *pAR* using the programme HP-Rare (Kalinowski, 2005). HP-Rare accounts for among stand variation in sample sizes, using rarefaction to give corrected estimates of allelic variables (Kalinowski, 2005). As previous studies have shown the level of self-compatibility to be variable within Australian *Acacias* (Kenrick & Knox, 1989), we estimated inbreeding co-efficients for each stand using INEst (Chykicki & Burczyk, 2009). INEst allows users to calculate inbreeding co-efficients by generating models for each stand with and without taking account for potential null alleles. A deviance information criterion (DIC) score for each model (with and without accounting for null alleles) is returned, allowing model comparison to find the best fit for the data (Chykicki & Burczyk, 2009). The results from the model with the lowest DIC represent the best fit to the data and are presented. We used t-tests to test for significant differences in the genetic parameters between the altitude and latitude gradient stands using R (R Core Development Team, 2016).

We used GENEPOP v.4.4.3 to check if the proportion of heterozygotes within our stands were equivalent to those expected under Hardy-Weinberg Equilibria (HWE) (Raymond & Rousset, 1995; Rousset, 2008). We checked for linkage disequilibria (LD) between all pairs of loci within each stand using GENEPOP v.4.4.3 (Raymond & Rousset, 1995; Rousset, 2008). We checked the loci for the presence of null alleles with the programme INEst using 50,000 iterations (Chykicki & Burczyk, 2009). We selected INEst over other programmes, as it checks for the presence of null alleles while not assuming HWE (Chykicki & Burczyk, 2009). We ran the analysis using the full model (including null alleles and inbreeding) to gain probabilities of null allele occurrence across all stands and loci.

We found a low probability of null alleles being present across all nine loci and 15 stands, at less than 0.17 for all loci by stand combinations (Appendix 5). Only one locus showed a

probability of null allele occurrence greater than 0.1 (Locus GH1: MtH1 = 0.11, Faulc = 0.14 and PPrd = 0.17). Due to the high amplification success across all loci and individuals, and the low estimated probabilities of null allele occurrences within stands (Table 7.2), we retained all loci. Averaged across loci, we found all stands to deviate significantly from HWE. Following sequential Bonferroni corrections, only one of the 540 locus-by-locus LD comparisons showed deviations from HWE.

We estimated the degree of selfing (*s*) within stands using the RMES programme (David *et al.,* 2007). RMES estimates *s* based on the presence of multiple homozygous genotypes, a method independent from F_{IS} based estimates (David *et al.,* 2007). We estimated the number of unique genotypes per stand (N_g) using GenAlEx (Peakall & Smouse, 2006 & 2012). Because N_g revealed evidence of clonality in some stands, we used the programme GenClone 2.0 (Arnaud-Haond & Belkhir, 2007) to calculate adapted Shannon-Wiener genotypic diversity and evenness indices. Due to our stands having different sample sizes we also calculated the normalised Shannon-Wiener index to allow comparison among populations (Grünwald *et al.,* 2003). The evenness index reports the contributions of different genotypes to the population. An index of 1 suggests all genotypes contribute equally to the population, whereas an index of 0 indicates that the population is dominated by a single genotype (Grünwald *et al.,* 2003). This is calculated as the Shannon-Wiener index divided by the log of the sample size (Grünwald *et al.,* 2003). Due to GenClone 2.0 not accepting missing data, we removed individuals from six stands prior to analysis. Across the stands this totalled no more than 16% of individuals (range = 5-16%; mean = 12%).

Table 7.2. Mean genetic diversity characteristics and mating assessments of *Acacia suaveolens* stands (based on genotypes of all plants sampled).

Stand	N[1]	*A*[1]	*Ae*[1]	*AR*[1]	*pAR*[1]	H_o[1]	H_e[1]	F_{IS}[1]	*s*[1]	*NE*[1]	*Ng*[1]	*H*[1]	*NH*[1]	*VH*[1]
Ang	20	2.00	1.39	1.84	0.11	0.08	0.20	0.61	0.60	0.00	14	2.43	1.90	0.95
Cem	24	4.00	2.27	3.51	0.10	0.28	0.54	0.50	0.56***	0.00	24	3.18	2.30	1.00
CH	24	2.44	1.70	2.32	0.13	0.19	0.31	0.46	0.65**	0.00	22.00	3.06	2.22	0.99
CR	25	2.11	1.35	1.99	0.16	0.04	0.20	0.84	0.96***	0.00	10	1.61	1.15	0.70
DH	25	2.00	1.74	1.97	0.00	0.10	0.35	0.73	0.86***	0.00	24.00	3.16	2.26	1.00
EH	20	2.11	1.68	2.00	0.21	0.09	0.26	0.65	0.72*	0.02	19	2.81	2.24	0.99
Faulc	25	5.56	3.25	4.65	0.34	0.33	0.62	0.41	0.27	0.05	25.00	3.00	2.31	1
GT	13	4.11	2.96	4.06	0.25	0.45	0.65	0.32	0.14	0.01	13	2.57	2.31	1
HH	24	1.78	1.15	1.61	0.00	0.04	0.10	0.60	0.00	0.00	11.00	1.88	1.36	0.79
MtH1	25	4.89	2.43	4.04	0.20	0.20	0.52	0.61	0.71***	0.03	25.00	3.05	2.31	1
MtH2	25	4.89	2.85	4.16	0.18	0.40	0.55	0.31	0.13	0.01	25	3.22	2.30	1
MtT	25	4.33	2.84	3.88	0.33	0.25	0.51	0.54	0.64***	0.01	25	3.22	2.30	1
Nar	20	1.67	1.35	1.59	0.06	0.07	0.20	0.64	0.70	0.00	13	2.39	1.84	0.93
PPrd	24	2.44	1.40	2.17	0.09	0.07	0.21	0.63	0.70*	0.02	18	2.69	2.03	0.97
TC	12	4.33	2.58	4.36	0.39	0.37	0.58	0.40	0.49**	0.03	12	2.40	2.30	1

[1]*N* = number of individuals; *A* = Number of different alleles; *Ae* = Effective number of alleles; *AR* = Rarefied allelic richness; *pAR* = Private allelic richness; H_o = Observed heterozygosity; H_e = Expected heterozygosity; F_{IS} = Inbreeding co-efficient; *s* = estimated selfing rate and significance values (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$); NF = Null allele frequencies; *Ng* = Number of unique genotypes; *H* = Shannon index of genotypic diversity; NH = normalised Shannon index; VH = Shannon index of genotypic diversity evenness index

As we identified putatively clonally replicated genotypes within a number of stands, we conducted all analyses twice (expect spatial auto-correlation analysis, genetic diversity analysis and InStruct analysis), once using the genotypes of all sampled plants, and a second time including only the unique genotypes. As this did not modify the outcome of significance tests between the two gradients, only the results without clonal individuals are presented. We have highlighted this where appropriate.

Gene flow within the species is predicted to occur predominantly through insect mediated pollen transfer due to limited seed dispersal (less than 10 m (Auld 1986b)) and a 16-grain pollen polyad too heavy for wind dispersal (Auld, 1986a; Morrison, 1986a & b). This may result in fine-scale genetic structuring within stands, dependent upon the efficiency of the insect pollinators (Loveless & Hamrick, 1984). Therefore, we tested for fine-scale spatial autocorrelation using GenAlEx (Peakall & Smouse, 2006 & 2012), using 999 permutations and bootstraps, and even distance classes of 10 m (5 m for TC and MtH2).

7.2.5. Genetic differentiation along the gradients

First, to estimate the level of genetic differentiation between each pair of sampled stands we used pairwise F_{ST} calculations in GenAlEx (Peakall & Smouse, 2006 & 2012). We also used a Baysian approach to identify discrete genetic clusters (*K*) in the programme INSTRUCT, without any predefined population specification (Gao *et al.,* 2007). In contrast to some other packages, INSTRUCT does not assume HWE within populations (Gao *et al.,* 2007). Following initial investigation, we ran INSTRUCT using Mode 2 with 1,000,000 iterations, a burn-in phase of 500,000 and a thinning interval of ten, for ten chains each using *K'*s between 8 and 15. We selected the optimal number of discrete genetic units (*K*) based on the reported DIC scores, visualising the results using the DISTRUCT software (Rosenberg, 2004). We ran

the INSTRUCT programme with clonal individuals removed. We used the same methodology to run the programme for individuals along the altitude and latitude gradients independently.

Second, given that we are using two gradients spanning different geographic distances, to assess if the genetic variation we identified among stands is influenced by the differences in geographic distances, we conducted Isolation by Distance (IBD) analysis. We first created an individual genetic distance matrix in GenAlEx (Peakall & Smouse, 2006 & 2012), which we then used to conduct IBD Mantel tests for all 15 stands together, and then individually across the two gradients using $F_{ST}/(1-F_{ST})$. We repeated this analysis for each gradient using only stands that were 20-150 km apart. In addition, we used the individual genetic distance matrix for principal co-ordinate analysis (PCoA) using GenAlEx (Peakall & Smouse, 2006 & 2012) PCoA provides a clear way to visualise the patterns of genetic differentiation among individuals and stands.

To estimate gene flow among stands, we calculated the number of migrants (N_m) moving between stands per generation based on Shannon's information index of population subdivision ($^{s}H_{UA}$), calculated in GenAlEx (Peakall & Smouse, 2006 & 2012). A number of papers have argued that this approach is more effective than F_{ST} based estimates of N_m due its greater sensitivity to rare alleles and ability to deal with uneven population numbers (Sherwin *et al.,* 2006; Rossetto *et al.,* 2008 & 2011). However, both methods assume an island model of gene flow, rather than the stepping stone model.

7.3. Results

7.3.1. Within stand genetic diversity

A total of 83 alleles across the nine loci were detected, however, while the number of alleles detected ranged from 15 to 50 alleles per stand (Appendix 5), the diversity of alleles and private alleles was significantly greater in stands along the altitudinal gradient (*AR*: t = -9.36, DF = 5.08, $P < 0.001$; *pAR*: t = -6.68, DF = 5.52, $P < 0.001$). When corrected for sample size, across all stands, *AR* ranged from 1.59 to 4.65 alleles per locus (mean = 2.94 ± 0.30 SE) (Table 7.2), while *pAR* was generally low, ranging from 0.00 to 0.39 (mean = 0.17 ± 0.03) (Table 7.2). Across all stands H_e was generally low (H_e = 0.10–0.65; mean = 0.39 ± 0.05) (Table 7.2), and was also significantly greater for stands along the altitudinal gradient as compared to those along the latitudinal gradient (t = -9.6, DF = 11, $P < 0.0001$).

7.3.2. *Inferred breeding systems and fine-scale stand structure*

Across all stands we found significant evidence of large heterozygote deficiencies ($P < 0.0001$), which are indicative of substantial selfing or inbreeding. F_{IS} ranged from 0.31 for MtH2 to 0.84 for CR (mean = 0.55 ± 0.04) (Table 7.2). On average, F_{IS} values were significantly greater for stands along the latitude gradient (mean = 0.59 ± 0.05) as compared with the altitude gradient (mean = 0.44 ± 0.04) (t-test: t = 2.64, DF = 8.16, $P = 0.03$). The higher level of selfing / inbreeding within the latitudinal populations, as implied by the F_{IS} results, was supported by the test for fine-scale spatial autocorrelation. All six of the latitudinal stands we tested (excluding the intersect stands) showed evidence of fine-scale spatial auto-correlation within a 50 m radius, as compared with only two of the five altitudinal

stands tested (excluding intersect stands), indicating a greater degree of restriction in gene dispersal within stands along the latitudinal gradient (Table 7.3).

Table 7.3. Auto-correlation co-efficient (*r*) results for *Acacia suaveolens* stands, evidence for fine-scale genetic structuring (based on genotypes of all plants sampled).

Distance Class (m) / **Population**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
PPrd	0.19*	0.10	0.07	0.11	-0.05	-0.13	-0.14	-0.21	-0.01	0.21*
Nar	0.51*	0.41*	0.44*	0.47*	0.00	-0.01	-0.16	-0.44	1.05	0.05
CR	0.55*	0.30*	0.46*	0.69*	0.81*	0.04	0.18	0.06	-0.09	-0.19
GT	-0.08	0.28	-0.05	0.00	0.06	0.10	0.17*	0.10	-0.18	0.10
TC	0.07	-0.04	0.26	0.15	0.05	-0.04	-0.08	-0.08	-0.02	-0.09
DH	0.35*	0.30*	0.05	0.11	-0.06	0.02	-0.04	-0.12	-0.25	-0.17
CH	0.31*	-0.01	-0.02	0.04	0.08	0.00	-0.14	0.02	-0.30	-0.04
HH	-0.11	0.10*	-0.06	0.04	-0.02	-0.07	0.18	-0.03	-0.13	0.05
Faulc	0.02	0.02	0.00	-0.02	0.10	0.20*	-0.20	0.03	-0.05	-0.04
Cem	0.06	0.07*	-0.03	0.00	-0.06	-0.04	0.01	0.03	-0.04	-0.01
MtH1	0.01	0.01	-0.02	0.07*	0.02	0.02	-0.05	-0.02	-0.09	0.04
MtH2	0.03	-0.01	0.01	-0.04	0.02	-0.01	0.02	NA	NA	NA
MtT	0.08	0.05	0.02	-0.04	-0.01	0.01	-0.04	0.00	0.00	-0.26

* Significant at $P < 0.05$

We found evidence of clonal reproduction within all eight non-intersect stands along the latitudinal gradient, but none within stands along the altitudinal gradient (Table 7.2). Although the normalised *H"* (indicating genotypic diversity) was significantly lower for the latitudinal stands (*H"*= 1.96 ± 0.13) as compared to the altitudinal stands (*H"* = 2.31 ± 0.002) (t-test: t = -2.64, DF = 9, *P* = 0.03), the diversity within the latitudinal stands was still moderately high (despite the clonality). This was supported by the corresponding Shannon genotypic evenness index which ranged from V'H" = 0.7 to 1 (mean = 0.93 ± 0.03) among stands along the latitudinal gradient (including intersect sites), indicating little evidence for the dominance of a single clonal genotype in any of the stands (Table 7.2).

7.3.3. *Geographic structure*

We assessed the geographic genetic structure of *A. suaveolens* stands using Bayesian and F_{ST} based methods. Based on F_{ST} methods, we found evidence of greater genetic differentiation, and inferred lower levels of gene flow, among stands along the latitude gradient as compared with the altitude gradient. Pairwise F_{ST} scores were significantly higher (t-test: t = 13.1, DF = 64.0, $P < 0.001$) among stands from the latitude gradient (mean = 0.38 ± 0.02) (Table 7.4) as compared with the altitude gradient (mean = 0.13 ± 0.01) (Table 7.5). We detected a significant IBD signal for all stands studied (R^2 = 0.44, P = 0.001) (Fig. 7.2A), and those along the latitude gradient (R^2 = 0.31, P = 0.002) (Fig. 7.2B), but not for the altitude gradient stands (R^2 = 0.0007, P = 0.45) (Fig. 7.2C). Given the differences in geographic separation among sites along the latitudinal gradient as compared with the altitudinal gradient, we repeated the IBD analysis for both gradients including only sites that were 20–150 km apart. Using only the sites 20–150 km apart we found no evidence for significant IBD along either gradient. This suggests that the greater geographic distances among stands along the latitudinal gradient is reflected by greater genetic differentiation along the entire gradient. However, the pairwise F_{ST} scores for latitudinal stands 20–150 km apart (Mean = 0.43 ± 0.03) were still significantly greater than for altitudinal stands 20-150 km apart (Mean = 0.12 ± 0.01) (t-test: t = 8.6, DF = 7.8, $P < 0.001$). The PCoA plot shows the patterns of genetic differentiation described among individuals and stands (Fig. 7.3).

Table 7.4. Pairwise F_{ST} scores (below the line) and their significance values (above the line) between stands of *Acacia suaveolens* along the latitudinal gradient (based on unique genotypes only).

	CH	**DH**	**GT**	**TC**	**Ang**	**EH**	**HH**	**Nar**	**PPrd**	**CR**
CH		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
DH	0.236		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
GT	0.310	0.260		0.008	0.001	0.001	0.001	0.001	0.001	0.001
TC	0.339	0.293	0.034		0.001	0.001	0.001	0.001	0.001	0.001
Ang	0.350	0.426	0.371	0.420		0.001	0.001	0.001	0.001	0.001
EH	0.409	0.409	0.363	0.426	0.397		0.001	0.001	0.001	0.001
HH	0.404	0.425	0.397	0.413	0.295	0.448		0.001	0.001	0.001
Nar	0.265	0.180	0.319	0.352	0.455	0.568	0.529		0.001	0.001
PPrd	0.383	0.354	0.352	0.406	0.524	0.635	0.621	0.364		0.001
CR	0.403	0.316	0.285	0.367	0.457	0.495	0.498	0.367	0.349	

Table 7.5. Pairwise F_{ST} scores (below the line) and their significance values (above the line) between stands of *Acacia suaveolens* along the altitudinal gradient (based on unique genotypes only).

	GT	**TC**	**Faulc**	**Cem**	**MtH1**	**MtH2**	**MtT**
GT		0.015	0.001	0.001	0.001	0.001	0.001
TC	0.034		0.001	0.001	0.001	0.001	0.001
Faulc	0.095	0.082		0.001	0.001	0.001	0.001
Cem	0.172	0.195	0.102		0.001	0.001	0.001
MtH1	0.131	0.080	0.104	0.233		0.001	0.001
MtH2	0.154	0.134	0.143	0.193	0.130		0.001
MtT	0.172	0.113	0.060	0.166	0.148	0.153	

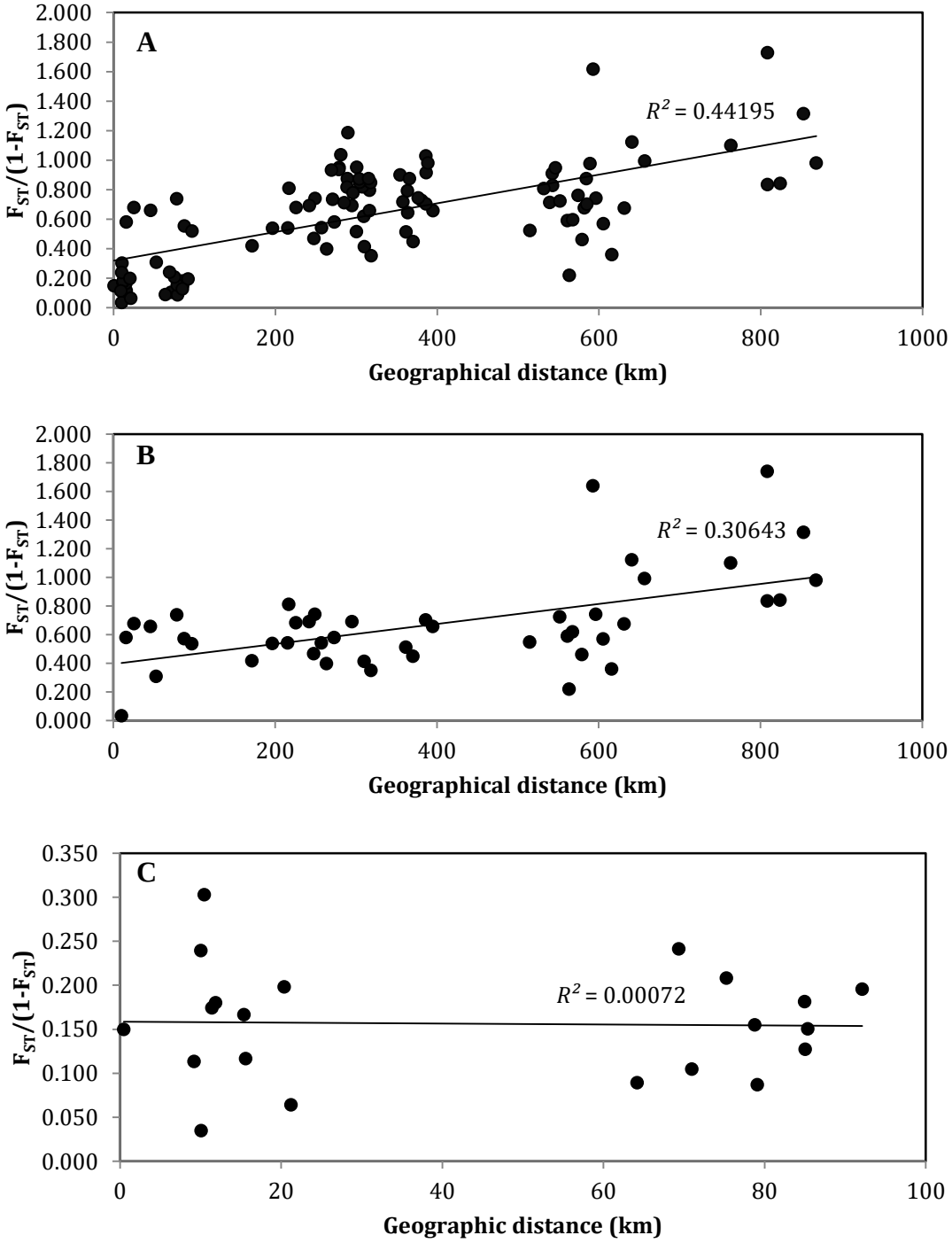

Figure 7.2. Mantel test results for isolation by distance effects: A) among all stands; B) among latitudinal stands only, and C) among altitudinal stands only (based on unique genotypes only).

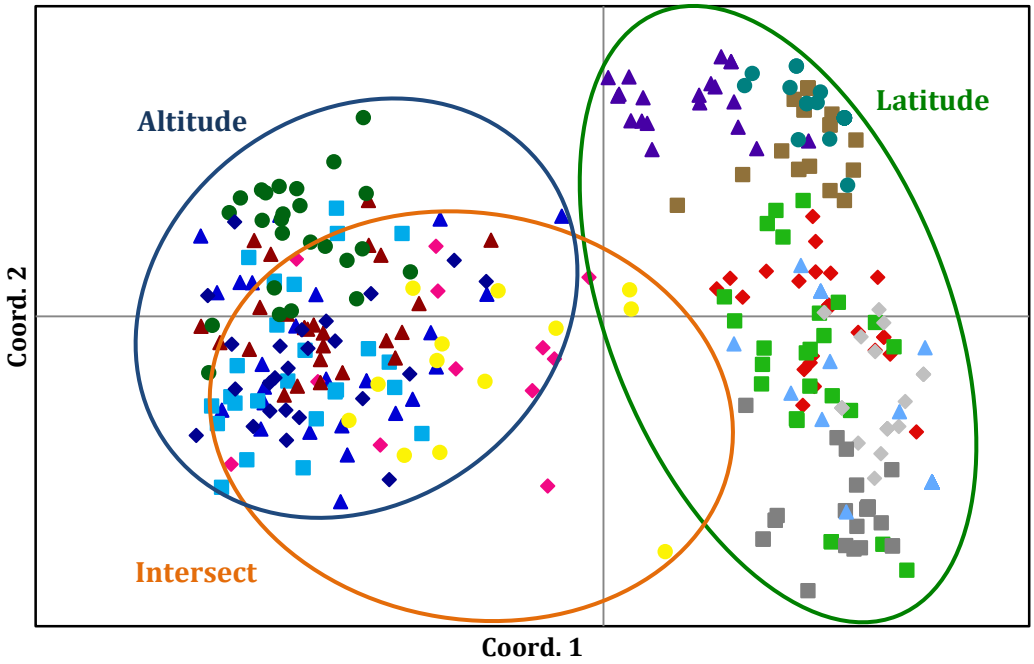

Figure 7.3. Principle co-ordinate analysis of the genetic differences between all unique genotypes. The same colour and style points identify the different source stands. Individuals from the altitudinal, latitudinal and intersect stands are circled (blue, green and orange, respectively).

Bayesian analysis (InStruct) revealed a similar pattern of genetic structure to the F_{ST} approach. Analysis of the entire data set revealed $K = 10$ to be the optimal number of genetic clusters, as compared with the initial 15 geographic stands samples were collected from. We found strong evidence for a genetic split between individuals from the latitude and altitude gradients (Fig. 7.4A). Individuals from the two intersecting stands showed a closer genetic association with individuals from the altitudinal gradient than the latitudinal gradient (Fig 7.4B). Along the latitude gradient, $K = 8$ was identified as the optimal number of genetic clusters (Fig. 7.4B), however, there was minimal difference between $K = 8$ and $K = 9$ (in both DIC scores and log likelihood values), with $K = 8$ fitting the data marginally better (average DIC = 2800 vs. 2801; average log likelihood = -1400 vs. -1401, respectively). The majority of individuals from two of the southernmost stands clustered into one genetic grouping (CR and PPrd), despite these stands being separated by the Nar stand. Individuals from the Nar stand clustered more strongly with the northern stands (DH and CH) despite a geographic separation of over 550 km. Along the altitude gradient $K = 9$ was the optimal fit, despite the individuals originating from seven stands (Fig. 7.4C). Individuals from the Cem stand were found to be genetically distinct from individuals of the other altitudinal stands (Fig 7.4C). There is some evidence of segregation of individuals from the Faulc and MtH1 stands (Fig. 7.4C).

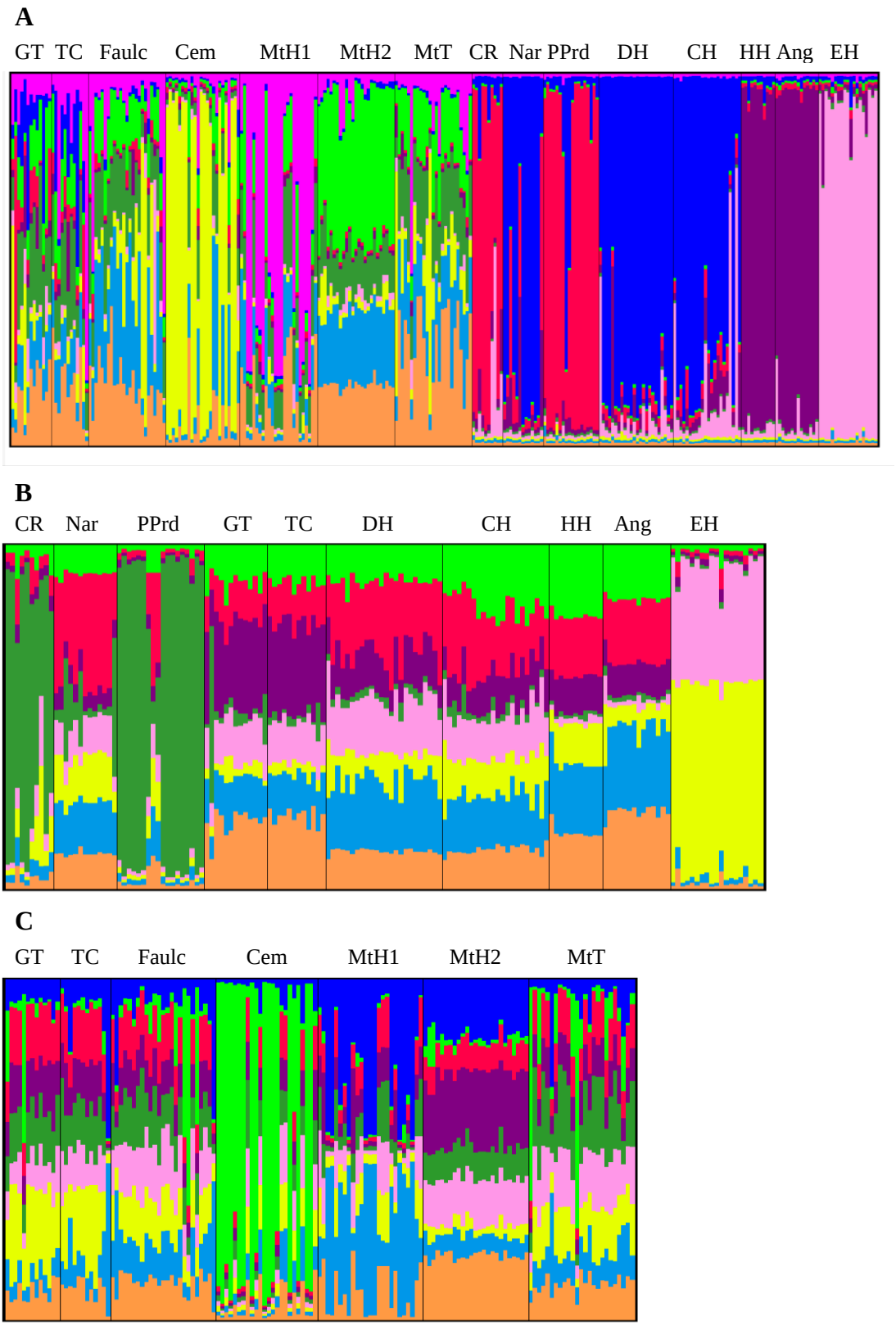

Figure 7.4. InStruct Bayesian analysis of proportion membership to identified clusters for unique genotypes from: A) all stands (*K* = 10 clusters); B) latitudinal stands only (*K* = 8 clusters), and C) altitudinal stands only (*K* = 9 clusters).

The estimated number of migrants per generation (N_m) was significantly lower (t-test: t = -2.11, DF = 49.8, P = 0.04) for the latitudinal gradient (mean = 0.34 generation^{-1} ± 0.05) as compared to the altitudinal gradient (mean = 0.49 generation^{-1} ± 0.05) (Table 7.6).

Table 7.6. Estimated number of migrants per generation (N_m) between stands of *Acacia suaveolens*, based on an island model (includes unique genotypes only).

	CH	DH	Faulc	GT	TC	MtH1	MtH2	MtT	Cem	Ang	EH	HH	Nar	PPrd	CR
CH	0.00														
DH	1.11	0.00													
Faulc	0.07	0.08	0.00												
GT	0.18	0.22	0.40	0.00											
TC	0.17	0.21	0.68	0.99	0.00										
MtH1	0.07	0.07	0.58	0.32	0.77	0.00									
MtH2	0.08	0.08	0.39	0.29	0.40	0.40	0.00								
MtT	0.06	0.06	1.12	0.26	0.52	0.43	0.46	0.00							
Cem	0.06	0.06	0.66	0.26	0.32	0.24	0.26	0.56	0.00						
Ang	0.33	0.19	0.08	0.11	0.09	0.07	0.08	0.08	0.07	0.00					
EH	0.28	0.22	0.08	0.11	0.10	0.07	0.09	0.08	0.06	0.35	0.00				
HH	0.47	0.43	0.10	0.11	0.12	0.08	0.08	0.12	0.09	0.78	0.33	0.00			
Nar	0.88	1.61	0.10	0.17	0.15	0.09	0.08	0.07	0.06	0.24	0.12	0.28	0.00		
PPrd	0.41	0.39	0.09	0.16	0.13	0.08	0.07	0.06	0.07	0.17	0.08	0.14	0.74	0.00	
CR	0.40	0.51	0.15	0.19	0.13	0.09	0.08	0.08	0.10	0.21	0.18	0.26	0.43	0.54	0.00

7.4. Discussion

Acacia suaveolens has been used for multiple SFT studies but the underlying assumptions have not been tested. Across the two spatial gradients studied, we found significant differences in the level of neutral genetic diversity within stands, inferred breeding systems and inferred gene flow. Overall, stands along the latitudinal gradient showed significantly greater genetic differentiation, higher levels of heterozygous deficiencies and greater inferred inbreeding, as compared to those along the altitudinal gradient. Crucially, if trait changes along these gradients, identified through the SFT methodology, are to be used to infer the

capacity of *A. suaveolens* to respond to future climate change, our data suggests that the levels of gene flow among stands along both spatial gradients are too low to allow the transfer of useful alleles for climate change.

Few SFT studies account for possible variation in the breeding and mating systems of populations along climatic gradients, despite the potential this has to influence gene flow and population resilience to environmental perturbations (although see Halbritter *et al.*, 2015). In this study, stands of *A. suaveolens* along the latitudinal gradient showed significantly greater levels of inbreeding and clonal reproduction as compared to stands along the altitudinal gradient. In line with these results, Roberts *et al.* (2016) reported levels of clonality in *A. loderi* populations to vary along an aridity gradient. In assessing the main assumption of the SFT approach, these results are likely to most strongly influence gene flow and dispersal (Cheptou & Donohue, 2011). High inbreeding, and therefore low outcrossing, will likely reduce gene flow among populations (Lloyd, 1992; Barrett, 2003), and therefore reduce the transfer of useful alleles for climate change adaptation along the SFT gradient, one of the two ways in which the main SFT assumption can be met.

Many plant species ranges span climatic gradients. For species with wide geographic ranges, they are likely to cover different climatic gradients e.g. altitudinal and latitudinal gradients. If predictions based on one SFT gradient are applied to other types of gradients within the species range, it is important the main SFT assumption also holds over the additional gradients. In SFT studies, this is rarely tested. Loarie *et al.* (2009) and Anderson *et al.* (2012), amongst others, predicted that rates of gene flow are likely to be greater along altitudinal gradients than along latitudinal gradients. We found significant differences in the average levels of inbreeding, clonality, genetic differentiation and inferred gene flow among stands

along the altitudinal as compared to the latitudinal gradient. Even when we considered only those sites that are 20-150 km apart along both gradients, stand genetic differentiation was still significantly greater among latitudinal stands as compared to the altitudinal stands, supporting the hypotheses of Loarie *et al.* (2009) and Anderson *et al.* (2012); as well as the results of other genetic studies finding significant genetic differences among populations over different geographic gradients (e.g. Quiroga & Premoli, 2007; Butcher *et al.*, 2009; Halbritter *et al.*, 2015). However, when the generally low genetic diversity, high stand differentiation and low gene flow estimates are considered, it implies that in *A. suaveolens*, even over the altitudinal gradient, gene flow is unlikely to be sufficient to transfer alleles at a pace equivalent to that of climate change.

As well as significant variation between the two gradients in genetic factors, we observed differences along the gradients. Along the altitudinal gradient, our results imply uneven genetic stand differentiation, and therefore inferred gene flow levels. For example, as altitude increased, stand heterozygosity decreased and inbreeding increased. Along the latitudinal gradient, InStruct results highlighted stronger genetic connections among certain latitudinal stands than others (Fig. 7.4B), which were supported with lower pF_{ST} values and higher N_m values. Hensen *et al.* (2011) also reported a significant decrease in genetic diversity with increasing altitude in *Polylepis incana*, whilst van Rossum *et al.* (2016) found heterozygosity to decrease as latitude increased in *Silene nutans.* Therefore, gene flow is unlikely to be equal among populations along SFT gradients. With increasing levels of habitat fragmentation in many areas, this is likely to further impede gene flow (Anderson *et al.*, 2012; Hand *et al.*, 2016). Consequently, if gene flow rates differ along a climatic gradient, assuming the even transfer of climate-adapted alleles along a spatial gradient would not be valid.

Of the two ways in which the main SFT assumption could be met, within this study we have primarily dealt with first (gene flow / migration). However the main SFT assumption could also be valid if populations at, for example, the cooler end of the climatic gradient can adapt to deal with warmer climatic conditions as those at the warmer end already have. Adaptation can occur through selection on standing (neutral) genetic variation, or on new genetic mutations (Barrett & Schluter, 2008). Standing genetic variation is defined as 'a gene (or a locus) that has no (or almost no) effect on fitness' (Holderegger *et al.,* 2006 pp. 798). However, under new environmental conditions associated with climate change, what was previously neutral variation may become advantageous, and therefore selected for (Barrett & Schlutter, 2008; Jay *et al.,* 2012). In relation to climate change, adaptation from standing variation is believed to be preferable for a number of reasons, namely due to a quicker rate of evolution (as the mutations already exist and are likely to be more prevalent in the population than a single new mutation (see Barrett & Schluter, 2008 for discussion)). Therefore, low neutral genetic diversity within stands, as we found here for *A. suaveolens*, may imply limited standing variation, reducing the chance of potentially useful alleles in climate change adaptation being present. However, the neutral variation present may still be adaptive under future environments, and neutral genetic data gives limited information on the quantitative genetic variation important for trait adaptation (Reed & Frankham, 2001). To accurately address the adaptation assumption of the SFT approach would require investigation into the adaptive genetic variation of the species. Particularly the adaptive genetic variation associated with the phenotypic traits identified through the SFT study as important in coping with climate change.

For *A. suaveolens* specifically, it is possible that a portion of each stands genetic diversity is contained within the soil seed bank, given that the species is known to have a long-lived soil

seed bank (Auld, 1986b; Morrison, 1986a). As generational overlap in obligate seeding species is limited, the seed bank may contain additional genetic variation not evident within the standing population. For example, Dolan *et al.* (2008) reported changes in the genetic structure of *Hypericum cumulicola* populations after wild fires, with expected heterozygosity increasing post-fire. However, both Ayre *et al*. (2009) and Roberts *et al.*, (2014) found no evidence for changes in the genetic structure of *Persoonia mollis* ssp. *mollis* and *Grevillea macleaya* populations, respectively, after one or more successive fires. A literature review by Honnay *et al.* (2008) found no evidence to support the retention of higher levels of genetic diversity within the seed bank than observed in adult populations. Whilst it is possible that this study only captures part of the true genetic diversity within the species, there is no reason to suspect that the proportion of a stands diversity held within seed banks should vary over the climatic gradients. Moreover, it is possible that any genetic diversity held within the seed bank may contain genotypes adapted to past climates, and therefore of limited advantage under future climate change (Anderson *et al.,* 2012). Further studies into the genetics of seedling cohorts across the gradients following fire would help to answer this question.

Our results highlight that for *A. suaveolens* gene flow within the species is unlikely to be sufficient to aid climate change adaptation. Evidence based on stand genetic diversity, stand genetic differentiation and estimations of migrants per generation among stands suggest low inferred gene flow. Given that our results reflect past population processes, and that habitat fragmentation is likely to increase within the study area further limiting gene flow, in this species, the SFT gene flow assumption is likely violated. However, we did find significant differences in population genetic factors between the two spatial gradients. Thus, despite the two spatial gradients spanning approximately equal changes in mean annual temperatures, the mating systems in particular were significantly different. It is therefore likely that the

mechanisms by which climate change adaptation occurs along the two gradients will differ. We recommend that where possible the ecological SFT approach should be used in tandem with population genetics or quantitative genetic studies to assess the validity of the SFT assumptions.

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Chapter 8. GENERAL DISCUSSION

8.1. Introduction

This thesis developed a detailed case study of the reproduction and early life-history stages of *Acacia suaveolens,* to enable prediction of the potential impacts of future climate change. This thesis also provides a test of the assumptions and validity of the 'space-for-time' (SFT) approach. To date, the majority of case studies developed with the aim of predicting responses to future climate change on early life history stages, have focused on northern hemisphere species (e.g. *Arabidopsis thaliana* (Donohue, 2009), *Anemone nemorosa* (De Frenne *et al.*, 2011), *Campanula americana* (Galloway, 2005), *Chamaecrista fasiculata* (Etterson, 2004a & b), *Quercus lobata* (Grivet *et al.*, 2008; Sork *et al.*, 2010)). However, there are few detailed case studies of the effect climate change may have on early life-history stages of southern hemisphere species (although see *Poa hiemata* (Byars *et al.*, 2007 & 2009)), particularly from sclerophyllous and fire-prone environments (Walck *et al.*, 2011; Bieger *et al.*, 2014). Whilst the existing studies provide vital information, it is unclear how their results can be applied to species in less studied ecosystems or those with different forces driving population dynamics. The results presented here, combined with the work of Morrison (1986a), Auld (1986a-c), Auld & Myerscough (1987), Morrison & Myerscough (1989), Warton & Wardle (2003), Auld & Denham (2006) and Ooi *et al*. (2012) form an in depth study of a southern hemisphere model species with physical dormancy that can be used for comparison with similar species in dry sclerophyllous woodland or Mediterranean-type ecosystems. Importantly, this thesis provides evidence from ecological and genetic surveys, climate manipulation and transplant experiments to address multiple aspects of reproduction and early seedling growth across multiple populations throughout the species range. The use of different experimental

methodologies enables the accuracy of the assumptions underlying the SFT approach to be investigated for this case study. This conclusion will be separated into two sections. Firstly, an assessment of accuracy of the SFT assumptions for this case study, and, secondly, a prediction of the future for *A. suaveolens* reproduction under projected climatic changes for the south-eastern coast of Australia and further research recommendations.

8.2. The 'space-for-time' approach

The SFT approach is one of the key ways in which a mechanistic understanding of species response to climate change can be achieved in natural settings (Dunne *et al.*, 2004; Fukami & Wardle, 2005; Blois *et al.*, 2013). However, the key assumption is that trait variation observed over spatial climatic gradients is equivalent to changes that could occur in response to future climatic change (Pickett, 1989; Dunne *et al.*, 2004; Fukami & Wardle, 2005). This assumption can be met through the migration and / or transfer of useful alleles along the gradient or, if climate induced selection on standing variation (or new mutations) in populations at cooler sites, for example, occurs as it did in the past at populations at the warmer sites. However, multiple factors are known to influence the validity of these assumptions, including the rapid pace of predicted climatic change, co-occurring changes to biotic interactions (e.g. herbivore interactions), the interacting effects of multiple changing environmental variables (see Pickett, 1989; Dunne *et al.*, 2004; Fukami & Wardle, 2005; De Frenne *et al.*, 2013), as well as genetic influences (e.g. gene flow rates, variation in the breeding system).

The change in traits of a species observed over spatial climate gradients is likely the result of local adaptation, genetic differentiation of populations (Dunne *et al.*, 2004), or phenotypic plasticity. If it is local adaptation that is important, then it is likely that the adaptive changes

have developed over multiple generations, at a much slower rate than is expected to be required to keep pace with projected rates of future climate change (Jump & Peñuelas, 2005; Lusk *et al.,* 2008). In the short-term however, plastic responses to changing climate are likely to be more important (although it should be noted that the degree of plasticity a given trait shows is under genetic control) (Jump & Peñuelas, 2005). It has been suggested that if SFT studies and climate manipulation experiments (looking at short term responses) both imply the same capacity to respond to climatic variables, then increased confidence can be placed in the likelihood of the response occurring under future climate change (Dunne *et al.,* 2004; De Boeck *et al.,* 2015), and therefore the assumption met.

I found a significant negative correlation between developmental environment temperature and seed weight along the climatic gradient (Chapter 3), and generally smaller seeds produced under warm developmental environments (Chapter 4). This indicates that the production of smaller seeds under warmer climatic conditions is able to occur within a short time frame (one reproductive season), as well as potentially being the result of local adaptation. Because both experiments showed that warmer developmental environment conditions result in the production of smaller seeds, I can place increased confidence in the prediction that under future climate change seeds of *A. suaveolens* will likely become smaller. In this case meeting the SFT assumption.

The finding of similar trait responses to warmer conditions between different types of SFT investigations was however dependent upon the trait investigated. In Chapter 4 seeds produced on plants growing under warmer parental environments showed significantly reduced dormancy loss (i.e. reduced germination) after the 80°C fire-related heat treatment as compared to those seeds developed under cooler parental conditions. In contrast, in Chapter 3,

there was no relationship between dormancy loss after the 80°C fire-related heat treatment and the temperatures experienced by the parental plants during the seed development period. Hence, whilst seed weight showed similar responses in both investigations, the loss of PY did not. De Frenne *et al.* (2011), found that plant height and reproductive success responded in the same manner across a latitudinal temperature gradient and after artificial warming, however specific leaf area and seed production showed different responses. This highlights the need to combine experimental evidence with observational type SFT studies to prevent over simplistic conclusions being drawn, which do not necessarily meet the SFT assumption. In this case, results from Chapter 3 imply no link between the temperature parental plants experience during seed development and dormancy loss, whereas results from Chapter 4 do. Moreover, the causes of differences in response between types of experiments could be important in understanding the influence of climate change on traits (Parmesan & Hanley, 2015). The difference between traits in response to artificial warming versus spatial gradients may depend upon the proportional genetic versus environmental control of traits, epigenetic effects, or the number of environmental variables important in controlling the trait. For example, in many alpine species flowering phenology is known to be closely linked with the timing of snow melt (Iler *et al.,* 2013). Indeed, flowering phenology responded similarly along a climatic gradient and to experimental warming in *Delphinium nuttallianum* (Dunne *et al.,* 2003 & 2004). In contrast, seedling survival is dependent upon numerous biotic and abiotic variables (Walck *et al.,* 2011), and distinct differences were found in seedling survival along a rainfall gradient as compared to a water manipulation experiment in *Sarcopoterium spinosum* (Rysavy *et al.,* 2014).

Over SFT gradients, localised site factors, including fine-scale environmental heterogeneity, are liable to alter climate interactions with traits (as well as site-specific evolutionary

histories) (Dunne *et al.*, 2004; De Frenne *et al.*, 2013). Specifically, this would influence the chance that populations at the cooler end of the climatic gradient would adapt in the same way that those at the warmer end have done in the past (one of the two ways in which the main SFT assumption can be met). For example, if the environmental factors predominantly limiting growth or reproduction differ among populations, then how a population responds to climatic warming will depend upon whether or not temperature is a predominantly limiting factor or not. When focused on the response of seeds to a 100°C fire-related heat treatment, I found evidence to suggest additional environmental influence/s, besides temperature, on the high temperature tolerance of seeds, either directly or through a phenotypically plastic response. For the three populations studied in Chapters 3 and 4, for seeds developed under natural conditions and then exposed to a 100°C fire-related temperature treatment, proportional mortality ranged from 0.05 to 1 across the two years of study (Chapter 3). In comparison, equivalent mortality rates for seeds developed under common garden conditions (including the warm and cool treatments) ranged from 0.85 to 1 (Chapter 4). Hence, mortality variation in response to high fire temperatures was much greater in wild grown than common garden grown seed, implying additional environmental variables experienced by parental plants at their home sites modifies the high temperature tolerance in seeds. Possible candidates for this include water availability (Hill *et al*., 1986b), humidity (Tozer & Ooi, 2014) or substrate nutrient status (Norman *et al*., 2002). Consequently, if the environmental factors, or site-specific evolutionary histories involved in controlling the tolerance of seeds to high fire-related temperatures differ along the SFT gradient, then it is possible that the main assumption of the SFT approach may not be met through adaptation.

Besides fine-scale environmental variation, biotic interactions are likely to vary along climatic gradients and among sites (MacColl, 2011; Rysavy *et al.*, 2014; Matías & Jump, 2015). If

biotic selection pressures differ along climatic gradients, then this may influence the response of populations to environmental selections pressures, and therefore impact upon whether or not populations will respond in the future, in the same way that they do to current environmental variation. In Chapter 5, sites differed in the level of herbivory seedlings experienced along the altitudinal gradient. This variation in the levels of herbivory may potentially obscure any relationships between climatic variables and seedling traits, due to the additional selection pressure exerted on the seedlings at the low-altitude sites. Similarly, Rysavy *et al.* (2014) found that as the harshness of the parent site increased, the role of adult shrubs in modifying seedling micro-environment became more important in *Sarcopoterium spinosum.* In this case, adult shrubs ameliorated the negative consequences of the increasing environmental harshness, at least to some degree (Rysavy *et al.,* 2014). Thus, although climatic variables along SFT gradients provide important information for predicting response to climate change, we highlight that the influence of biotic interactions cannot be neglected, and should be investigated where possible.

Whether the key assumption of the SFT approach holds is partially influenced by a number of genetic factors. For example, the level of gene flow will influence the likelihood of traits useful in climate change adaptation being transferred between populations, whilst the level of standing genetic variation within a population may influence the chance of local adaptation occurring (Hereford, 2010). The breeding system of a species, will in part, influence gene flow (Charlesworth, 2006) and the level of standing variation (Charlesworth & Charlesworth, 1995; Hereford, 2010). However, few studies account for possible breeding system differences within populations along SFT gradients (Anderson *et al.,* 2012). Results from Chapter 7 suggest that the breeding system of *A. suaveolens* varies throughout the species range, particularly between the altitudinal and latitudinal gradients. Levels of clonality and

inbreeding were significantly greater (reduced heterozygosity) among populations along the latitudinal gradient as compared to the altitudinal gradient (Chapter 7). Consequently, levels of genetic diversity and inferred levels of gene flow were significantly different among populations along the two gradients. As a result, populations along the two gradients may respond differently to new selection pressures associated with climate change, as well as differ in the rate at which alleles for climate adapted traits can spread along the gradient (Reed & Frankham, 2003; Charlesworth, 2006; Anderson *et al.,* 2012). While a number of studies have shown intraspecific variation in the breeding system (e.g. Winn et al., 2011; Halbritter *et al.,* 2015; Roberts *et al.,* 2016), we highlight the need for this to be addressed in SFT studies.

Overall, the approach taken to climate change study in this thesis, of combining SFT analysis with a climate manipulation study, a transplant study and a population genetic investigation, builds on the suggestions of Dunne *et al.* (2004) and De Boeck *et al.* (2015) that localised site factors can alter climate interactions with traits. The combination of different methodologies increased the confidence in inferences made based on certain results (e.g. that seed weight is dependent upon the temperature experienced by parental plants during reproduction), whilst highlighted areas where further research is needed (e.g. the role of environmental factors in controlling high temperature fire resilience). In particular, by including a population genetics component, we partially overcame the issue of not being able to use controlled genetic or maternal lines (due to insufficient seed set per plant). We recommend the use of this methodology, however we highlight the need to investigate the assumptions underlying the approach, in particular the impact that differences in breeding system may have.

8.3. The future for *Acacia suaveolens* and potential research directions

The region under investigation is predicted to see a 2.7°C to 4.7°C temperature rise by 2090 and an increase in rainfall variability (Dowdy *et al.*, 2015). Under this scenario, the results gained throughout this thesis suggest that *A. suaveolens* displays a number of traits that may be advantageous to its survival, but also highlights potential limits to the species ability to respond. Wide variation in PY characteristics within and among populations suggests *A. suaveolens* produces a seed bank able to respond to a broad range of potential fire conditions which may buffer it against future increases in fire severity (Chapter 3). In addition, the ability of individuals to flower and produce seeds up to 2-3°C above their home site temperature (not accounting for any interacting climatic changes), as well as the wide distribution of the species (Morrison, 1986a) suggest the potential to cope with temperature increases (Chapter 4). However, the moderate levels of genetic population differentiation, along with inbreeding and clonal reproduction imply low levels of gene flow among populations, and therefore limited population connectivity (Chapter 7). Moreover, when combined with a predicted reduction in flowering span and higher seed abortion rates under warmer parental environments (Chapter 4), and potential drought susceptibility of seedlings (Chapter 5), recruitment potential within the species may be limited.

This thesis provides a first attempt at predicting how reproduction in a native Australian species with physical dormancy from dry sclerophyllous woodland may alter under future climate change, in particular under warmer environmental conditions. However, there are also a number of questions raised by the investigations conducted that, if answered, could improve the value of the predictions made here.

Firstly, how will a warmer climate alter the herbivore–plant interactions associated with *A. suaveolens*? Previous research by Auld & Myerscough (1986) indicated herbivory levels of between 10% and 61% on *A. suaveolens* seed crops by the weevil *Melanterius corosus*. In addition, a key finding within this thesis was the high level of herbivory induced seedling mortality (although this did vary depending upon site; Chapter 5). When combined with the prediction that under warmer developmental environments seed production in *A. suaveolens* may decrease (Chapter 4), any co-occurring increase in herbivore pressure may negatively impact upon population persistence. For example, Auld & Myerscough (1986) hypothesized that a factor within the life history of *M. corosus* currently limits the degree of herbivory on *A. suaveolens* seeds, given that a 100% seed crop loss was never observed. However, it is possible that the life history factor limiting the degree of *M. corosus* herbivory may well alter under a warmer climate, given the importance of temperature in controlling insect development and growth (Bale *et al.,* 2002). Indeed, historic evidence from past warming events suggests that the intensity of insect herbivory increased (see Bale *et al.,* 2002). Therefore, further research into the interaction between herbivory, particularly by *M. corosus*, and climatic change will enable a more detailed prediction as to the impact of a warmer climate on population persistence in natural settings.

Secondly, does seed longevity in *A. suaveolens* vary depending upon developmental environment during seed production or among populations? Previous research has suggested that *A. suaveolens* seeds stored under laboratory and field conditions show no significant difference in dormancy (Auld, 1986b). Indeed, Auld (1986b) estimated the half-life of *A. suaveolens* seeds in the soil to be 10.7 years, whilst Ewart (1908) estimated that the seeds may be able to survive over 50 years (Ewart, 1980; Auld, 1986b). However, studies on additional species with PY, such as Liyanage & Ooi (in press), challenge this claim. Moreover, Mondoni

et al. (2011) found that for a number of species, seeds collected from alpine populations (non-PY) had a shorter period of viability than those collected from lowland populations, implying intra-specific variation in seed longevity. Whilst, Mondoni *et al.* (2014) showed seed longevity in *Silene vulgaris* ssp. *vulgaris* was under both genetic and environmental control (although *S. vulgaris* does not have PY). Hence, it is possible that trends in the loss of seed viability may vary intra-specifically or with environmental factors. In the experiments described within this thesis, seeds were stored in the same place, under standard laboratory conditions, until they were used for experimentation. Given the broad range of populations used throughout this thesis (Appendix 1), and the strong population genetic differentiation identified (Chapter 7), it is possible that the storage conditions used may have impacted seed dormancy of populations differently. However, the results of Auld (1986b) support the assumption within this thesis that seeds from all sources were affected to a similar degree by the seed storage conditions used. Without further research into the variation and controlling factors influencing the longevity of *A. suaveolens* seeds the assumption cannot be fully supported. In addition, if the developmental environment does influence seed longevity, further research into this would aid in the predictions of net seed bank losses during the inter-fire period.

Thirdly, what are the mechanical or hormonal changes to *A. suaveolens* seeds (due to differences in the developmental environment) that result in changes to PY characteristics (e.g. the temperature needed to break dormancy (Chapter 4))? Currently, multiple theories exist as to how changes to PY characteristics may occur, including through changes to seed coat thickness (although see Chapter 2 for discussion), changes to the fatty acid composition of the seed coat (Zeng *et al.,* 2005), changes to seed procyanidin concentrations (through altered gene expression, MacGregor *et al.,* 2015) and alterations to the water-gap region

(Gama-Arachechige *et al.,* 2013). Within agricultural settings a large amount of research has been conducted on species with PY, given the economic importance of many PY species (e.g. *Trifolium, Stylosanthes, Cicer*). However, this research is often conducted on recombinant inbred lines, or varieties resulting from selective breeding (Chapter 2). This makes applying the wealth of PY knowledge within the agricultural literature to wild species with PY difficult. If the mechanisms by which alteration of the parental environment changes PY characteristics can be identified, then it may offer a link by which the knowledge on PY within the agricultural literature could be applied to ecological research.

Fourthly, what is the relative contribution of phenotypic plasticity versus local adaptation of populations to the reproductive responses identified within *A. suaveolens* after warming of the parental environment? Within this thesis, the intra-population variability of PY characteristics among years seems to suggest high phenotypic plasticity (Chapter 3), and the correlation between long-term summer temperatures and dormancy retention seems to indicate local adaptation within PY characteristics (Chapter 3). However, these relationships are purely correlative. The importance of accounting for phenotypic plasticity and local adaptation in how a species responds to climate change when modelling was highlighted by Valladares *et al.* (2014), who found this to alter the model outcomes. However, methodologically for many species this is difficult, particularly for fire-related PY characteristics. Hoyle *et al.* (2011) provided a framework for reducing the seed numbers required in dormancy trials based on limited replication, but for *A. suaveolens* given the wide intra-population variation in PY characteristics across years documented here (Chapter 3), limiting replication may not capture this variation. Therefore, we suggest the best way to proceed within the species would be through development of the genetic microsatellite work presented here (Chapter 7), heritability studies, or making use of new genomic methods to isolate genotypes of

individuals. For example, Steane *et al*. (2014) used genomic methods to identify strong adaptation among *Eucalyptus tricarpa* populations to temperature and moisture availability. This shows that such methods can be used for non-model species and provides a suggested methodology for such work.

In addition, does the level of inbreeding identified within *A. suaveolens* populations have a fitness related consequence? For example, Dudash (1990) found that self-progeny of *Sabatia angularis* showed reduced survival rates and lower fruit masses per plant when compared to outcrossed-progeny under both field and garden settings. Thus, if the differences in inbreeding identified here (Chapter 7) between the two gradients results in observable differences in fitness related traits, such as seed weight, then the susceptibility of populations along the two gradients to climatic warming is likely to differ. Moreover, a meta-analysis by Armbruster & Reed (2005) found that generally the impacts of inbreeding depression were greater when a species was within a relatively stressful environment as compared to a neutral environment (Cheptou & Donohue, 2011; although see Sander & Matthies, 2016). If the changing climate creates a relatively more stressful environment for *A. suaveolens* then any inbreeding depression effects may be magnified.

Finally, tying a number of the above questions together, how will predicted changes to fire activity under future climate change influence the recruitment dynamics of *A. suaveolens*? Climate change predictions for the region indicate an increase is likely in fire activity, due to warming and drying of the region increasing the number of 'very high' and 'extreme' fire days expected (Hennessy *et al.,* 2005; Lucas *et al.,* 2007; Clarke *et al.,* 2011). This thesis showed that seeds developed under a warmer parental environment require a higher fire temperature, or longer fire duration, to lose dormancy (Chapter 4). This may act to buffer the

population against as increase in fire severity or fire duration to a certain degree, however, seed mortality was still high in response to a 100˚C fire-related heat treatment. What remains unclear is how a shorter fire-return interval will influence the seed bank dynamics. The ability of *A. suaveolens* to cope with a shorter fire-return interval will depend upon the proportion of seeds remaining dormant in the seed bank after each fire, the number of new seeds entering the seed bank during the inter-fire period, and any changes to the net loss of seeds from the seed bank during the inter-fire period. Evidence presented in this thesis suggests, under warmer climatic conditions seed input into the seed bank may decrease due to higher seed abortion rates (Chapter 4), whilst evidence from Ooi *et al.* (2012) suggests the proportion of *A. suaveolens* seeds losing dormancy during the inter-fire period may increase as the frequency of heat-wave conditions increases. As highlighted above, however, it is still uncertain how changes in seed longevity or herbivore interactions will impact seed bank dynamics of the species, without which it is not possible to accurately predict how changes to fire activity will alter the recruitment dynamics of *A. suaveolens.*

REFERENCES

Ågren, J. and Schemske, D.W. (2012) Reciprocal transplants demonstrate strong adaptive differentiation of the model organism *Arabidopsis thaliana* in its native range. *New Phytologist*. **194**: 1112-1122.

Alexander, L.V. and Arblaster, J.M. (2009) Assessing trends in observed and modelled climate extremes over Australia in relation to future projections. *International Journal of Climatology*. **29**: 417-435.

Amano, T., Freckleton, R.P., Queenborough, S.A., Doxford, S.W., Smithers, R.J., Sparks, T.H. and Sutherland, W.J. (2014) Links between plant species' spatial and temporal responses to a warming climate. *Proceedings of the Royal Society B.* **281**: e20133017.

Anderson, J.T., Panetta, A.M. and Mitchell-Olds, T. (2012) Evolutionary and ecological responses to anthropogenic climate change. *Plant Physiology*. **160**: 1728-1740.

Antonovics, J. and Primack, R.B. (1982) Experimental ecological genetics in *Plantago*: VI. The demography of seedling transplants of *P. lanceolata. Journal of Ecology*. **70**: 55-75.

Argel, P.J. and Humphreys, L.R. (1983a) Environmental effects on seed development and hardseededness in *Stylosanthes hamata* cv. Verano. I. Temperature. *Australian Journal of Agricultural Research.* **34**: 261-270.

Argel, P.J. and Humphreys, L.R. (1983b) Environmental effects on seed development and hardseededness in *Stylosanthes hamata* cv. Verano. III. Storage humidity and seed characteristics. *Australian Journal of Agricultural Research.* **34**: 279-287.

Argel, P.J. and Humphreys, L.R. (1983c) Environmental effects on seed development and hardseededness in *Stylosanthes hamata* cv. Verano. II. Moisture supply and illuminance. *Australian Journal of Agricultural Research.* **34**: 271-277.

Armbruster, P. and Reed, D.H. (2005) Inbreeding depression in benign and stressful environments. *Heredity.* **95**: 235-242.

Arnaud-Haond, S. and Belkhir, K. (2007) GENCLONE: a computer program to analyse genotypic data, test for clonality and describe spatial clonal organization. *Molecular Ecology Notes.* **7**: 15-17.

Ashcroft, M.B. and Gollan, J.R. (2013) Moisture, thermal inertia, and the spatial distributions of near-surface soil and air temperatures: understanding factors that promote microrefugia. *Agricultural and Forest Meteorology.* **176**: 77-89.

Auld, T.D. (1986a) Dormancy and viability in *Acacia suaveolens* (Sm.) Willd.. *Australian Journal of Botany.* **34**: 463-472.

Auld, T.D. (1986b) Population dynamics of the shrub *Acacia suaveolens* (Sm.) Willd.: dispersal and the dynamics of the soil seed-bank. *Australian Journal of Ecology*. **11**: 235-254.

Auld, T.D. (1986c) Population dynamics of the shrub *Acacia suaveolens* (Sm.) Willd.: fire and the transition to seedlings. *Australian Journal of Ecology.* **11**: 373-385.

Auld, T.D. (1987) Population dynamics of the shrub *Acacia suaveolens* (Sm.) Willd.: survivorship throughout the life cycle, a synthesis. *Australian Journal of Ecology*. **12**: 139-151.

Auld, T.D. (1996) Ecology of the Fabaceae in the Sydney region: fire, ants and the soil seedbank. *Cunninghamia*. **4**: 531-551.

Auld, T.D. and Denham, A.J. (2006) How much seed remains in the soil after a fire? *Plant Ecology*. **187**: 15-24.

Auld, T.D. and Myerscough, P.J. (1986) Population dynamics of the shrub *Acacia suaveolens* (Sm.) Willd.: seed production and predispersal seed predation. *Australian Journal of Ecology*. **11**: 219-234.

Auld, T.D. and O'Connell, M.A. (1991) Predicting patterns of post-fire germination in 35 Australian Fabaceae. *Australian Journal of Ecology*. **16**: 53-70.

AVH (2016) *Australia's Virtual Herbarium,* Council of Heads of Australasian Herbaria. http://avh.chah.org.au.

Ayre, D.J., Ottewell, K.M., Krauss, S.L. and Whelan, R.J. (2009) Genetic structure of seedling cohorts following repeated wildfires in the fire-sensitive shrub *Persoonia mollis* ssp. *nectans*. *Journal of Ecology*. **97**: 752-760.

Bale, J.S., Masters, G.J., Hodkinson, I.D., Awmack, C., Bezemer, T.M., Brown, V.K., Butterfield, J., Buse, A., Coulson, J.C., Farrar, J., Good, J.E.G., Harrington, R., Hartley, S., Jones, T.F., Lindroth, R.L., Press, M.C., Symrnioudis, I., Watt, A.D.

and Whittaker, J.B. (2002) Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology*. **8**: 1-16.

Baraloto, C., Forget, P.M. and Goldberg, D.E. (2005) Seed mass, seedling size and neotropical tree seedling establishment. *Journal of Ecology*. **93**: 1156-1166.

Barrett, S.C.H. (2003) Mating strategies in flowering plants: the outcrossing-selfing paradigm and beyond. *Philosophical Transactions of the Royal Society of London B.* **358**: 991-1004.

Barrett, R.D.H. and Schluter, D. (2008) Adaptation from standing genetic variation. *Trends in Ecology and Evolution.* **23**: 38-44.

Baskin, J.M. and Baskin, C.C. (1983) Germination ecology of *Veronica arvensis. Journal of Ecology*. **71**: 57-68.

Baskin, C.C. and Baskin, J.M. (2014) *Seeds: ecology, biogeography, and evolution of dormancy and germination.* 2nd Ed. New York, USA, Academic Press.

Baskin, J.M., Baskin, C.C. and Li, X. (2000) Taxonomy, anatomy and evolution of physical dormancy in seeds. *Plant Species Biology*. **15**: 139-152.

Bates, D.M., Maechler, M., Bolker, B. and Walker, S. (2015) *lme4*: *Linear mixed-effects models using **Eigen** and S4*. R package version 1.1-10, http://CRAN.R-project.org/packge=lme4.

Becker, U., Colling, G., Dostal, P., Jakobsson, A. and Matthies, D. (2006) Local adaptation in the monocarpic perennial *Carlina vulgaris* at different spatial scales across Europe. *Conservation Ecology.* **150**: 506-518.

Bell, D.M., Bradford, J.B. and Lauenroth, W.K. (2014) Early indicators of change: divergent climate envelopes between tree life stages imply range shifts in the western United States. *Global Ecology and Biogeography.* **23**: 168-180.

Benavides, R., Escudero, A., Coll, L., Ferrandis, P., Ogaya, R., Gouriveau, F., Peñuelas, J. and Valladares, F. (2016) Recruitment patterns of four tree species along elevation gradients in Mediterranean mountains: not only climate matters. *Forest Ecology and Management.* **360**: 287-296.

Bertrand, R. Gégout, J.C. and Bontemps, J.D. (2011) Niches of temperate tree species converge towards nutrient-richer conditions over ontogeny. *Oikos.* **120**: 1479-1488.

Bieger, A., Rajakaruna, N. and Harrison, S. (2014) Little evidence for local adaptation to soils or microclimate in the post-fire recruitment of three Californian shrubs. *Plant Ecology and Diversity.* **7**: 411-420.

Black, R.A. and Bliss, L.C. (1980) Reproductive ecology of *Picea mariana* (Mill.) BSP. at a tree line near Inuvik, northwest territories, Canada. *Ecological Monographs.* **50**: 331-354.

Blois, J.L., Williams, J.W., Fitzpatrick, M.C., Jackson, S.T. and Ferrier, S. (2013) Space can substitute for time in predicting climate-change effects on biodiversity. *PNAS.* **110**: 9374-9379.

Bolin, J.F. (2009) Heat shock germination responses of three eastern North American temperate species. *Castanea.* **74**:160-167.

Bolingue, W., Vu, B.L., Leprince, O. and Buitink, J. (2010) Characterisation of dormancy behaviour in seeds of the model legume *Megicago truncatula. Seed Science Research.* **20**: 97-107.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H. and White, J.S.S. (2011) GLMMs in action: gene-by-environment interaction in total fruit production of wild populations of *Arabidopsis thaliana,* revised version, part 1. www.cell.com/cms/attachment/601623/4742453/mmc1.pdf

BoM (2015) http://www.bom.gov.au/climate/data/

Bowman, G., Perret, C., Hoehn, S., Galeuchet, D.J. and Fischer, M. (2008) Habitat fragmentation and adaptation: a reciprocal replant-transplant experiment among 15 populations of *Lychnis flos-cuculi. Journal of Ecology.* **96**: 1056-1064.

Bradbury, D., Tapper, S.L., Coates, D., Hankinson, M., McArthur, S. and Byrne, M. (2016a) How does the post-fire facultative seeding strategy impact genetic variation and phylogeographical history? The case of *Bossiaea ornata* (Fabaceae) in a fire-prone, Mediterranean-climate ecosystem. *Journal of Biogeography.* **43**: 96-110.

Bradbury, D., Tapper, S.L., Coates, D., McArthur, S., Hankinson, M. and Byrne, M. (2016b) The role of fire and a long-lived soil seed bank in maintaining persistence, genetic diversity and connectivity in a fire-prone landscape. *Journal of Biogeography.* **43**: 70-84.

Broadhurst, L.M., Young, A.G. and Forrester, R. (2008) Genetic and demographic responses of fragmented *Acacia dealbata* (Mimosaceae) populations in southeastern Australia. *Biological Conservation.* **141**: 2843-2856.

Burrows, G.E., Virgona, J.M. and Heady, R.D. (2009) Effect of boiling water, seed coat structure and provenance on the germination of *Acacia melanoxylon* seeds. *Australian Journal of Botany.* **57**: 139-147.

Butcher, P.A., McDonald, M.W. and Bell, J.C. (2009) Congruence between environmental parameters, morphology and genetic structure in Australia's most widely distributed eucalypt, *Eucalyptus camaldulensis. Tree Genetics and Genomes*. **5**: 189-210.

Byars, S.G., Papst, W. and Hoffmann, A.A. (2007). Local adaptation and cogradient selection in the alpine plant, *Poa hiemata,* along a narrow altitudinal gradient. *Evolution.* **61**: 2925-2941.

Byars, S.G., Parsons, Y. and Hoffmann, A.A. (2009) Effect of altitude on the genetic structure of an Alpine grass, *Poa hiemata. Annals of Botany.* **103**: 885-599.

Chamorro, D., Parra, A. and Moreno, J.M. (2016) Reproductive output, seed anatomy and germination under water stress in the seeder *Cistus ladanifer* subjected to experimental drought. *Environmental and Experimental Botany*. **123**: 59-67.

Charlesworth, D. (2006) Evolution of plant breeding systems. *Current Biology*. **16**: R726-R735.

Charlesworth, D. and Charlesworth, B. (1995) Transposable elements in inbreeding and outbreeding populations. *Genetics.* **140**: 415-417.

Cheptou, P.O. and Donohue, K. (2010) Environment-dependent inbreeding depression: its ecological and evolutionary significance. *New Phytologist.* **189**: 395-407.

Chykicki, I.J., and Burczyk, J. (2009) Simultaneous Estimation of Null Alleles and Inbreeding Coefficients. *Journal of Heredity.* **100**: 106-113.

Clarke, H.G., Smith, P.L. and Pitman, A.J. (2011) Regional signatures of future fire weather over eastern Australia from global climate models. *International Journal of Wildland Fire.* **20**: 550-562.

Cochrane, A.J., Hoyle, G.L., Yates, C.J., Wood, J. and Nicotra, A.B. (2014) Climate warming delays and decreases seedling emergence in a Mediterranean ecosystem. *Oikos.* **124**: 150-160.

Cochrane, A.J., Yates, C.J., Hoyle, G.L. and Nicotra, A.B. (2015a) Will among-population variation in seed traits improve the chance of species persistence under climate change? *Global Ecology and Biogeography.* **24**: 12-24.

Cochrane, A.J., Hoyle, G.L., Yates, C.J., Wood, J. and Nicotra, A.B. (2015b) The phenotypic response of co-occurring *Banksia* species to warming and drying. *Plant Ecology.* **216**: 27-39.

Cowling, R.M., Rundel, P.W., Lamont, B.B., Arroyo, M.K. and Arianoutsou, M. (1996) Plant diversity in Mediterranean-climate regions. *Trends in Ecology and Evolution.* **11**: 362-366.

Crisp, M.D., Laffan, S., Linder, H.P. and Monro, A. (2001) Endemism in the Australian flora. *Journal of Biogeography.* **28**: 183-198.

Crawley, M.J. (2013) *The R Book.* 2nd Ed. UK, Wiley.

Daskalakou, E.N. and Thanos, C.A. (2010) Postfire seedling dynamics and performancy in *Pinus halepensis* Mill. populations. *Acta Oecologica.* **36**: 446-453.

David, P., Pujol, B., Viard, F., Castella, V. and Goudet, J. (2007) Reliable selfing rate estimates from imperfect population genetic data. *Molecular Ecology.* **16**: 2474-2487.

Davis, M.B., Shaw, R.G. and Etterson, J.R. (2005) Evolutionary responses to changing climate. *Ecology.* **86**: 1704-1714.

Dawson, T.P., Jackson, S.T., House, J.L., Prentice, I.C. and Mace, G.M. (2011) Beyond predictions: biodiversity conservation in a changing climate. *Science.* **332**: 53-58.

De Boeck, H.J., Vicca, S., Roy, J., Nijs, I., Milcu, A., Kreyling, J., Jentsch, A., Chabbi, A., Campioli, M., Callaghan, T., Beierkuhnlein, C. and Beier, C. (2015) Global change experiments: challenges and opportunities. *BioScience.* **65**: 922-931.

De Frenne, P., Brunet, J., Shevtsoval, A., Kolb, A., Graae, B.J., Chabrerie, O., Cousins, S.A., Decocq, G., De Schrijver, A., Diekmann, M., Gruwez, R., Heinken, T., Hermy, M., Nilsson, C., Stanton, S., Tack, W., Willaert, J. and Verheyen, K. (2011) Temperature effects on forest herbs assessed by warming and transplant experiments along a latitudinal gradient. *Global Change Biology.* **17**: 3140-3253.

De Frenne, P., Graae, B.J., Brunet, J., Shevtsova, A., Schrijver, A.D., Chaberie, O., Cousins, S.A.O., Decocq, G., Diekmann, M., Hermy, M., Heinken, T., Kolb, A., Nilsson, C., Stanton, S. and Verheyen, K. (2012) The response of forest plant

regeneration to temperature variation along a latitudinal gradient. *Annals of Botany*. **109**: 1037-1046.

De Frenne, P., Graae, B.J., Rodríguez-Sánchez, F., Kolb, A., Chabrerie, O., Decocq, G., De Kort, H., De Schrijver, A., Diekmann, M., Eriksson, O., Gruwez, R., Hermy, M., Lenoir, J., Plue, J., Coomes, D.A. and Verheyen, K. (2013) Latitudinal gradients as natural laboratories to infer species' responses to temperature. *Journal of Ecology*. **101**: 784-795.

Del Cacho, M., Peñuelas, J. and Lloret, F. (2013) Reproductive output in Mediterranean shrubs under climate change experimentally induced by drought and warming. *Perspectives in Plant Ecology, Evolution and Systematics*. **15**: 319-327.

De Souza, F.H.D. and Marcos-Filho, J. (2001) The seed coat as a modulator of seed-environment relationships in Fabaceae. *Revista Brasilivia de Botânica*. **24**: 365-375.

De Vitis, M., **Seal, C.E., Ulian, T., Pritchard, H.W., Magrini, S., Fabrini, G. and Mattana, E.** (2014) Rapid adaptation of seed germination requirements of the threatened Mediterranean species *Malcolmia littorea* (Brassicaceae) and implications for its reintroduction. *South African Journal of Botany*. **94**: 46-50.

D'hondt, B., Brys, R. and Hoffmann, M. (2010) The incidence, field performance and heritability of non-dormant seeds in white clover (*Trifolium repens* L.). *Seed Science Research*. **20**: 169-177.

Dolan, R.W., Quintana-Ascencio, P.F. and Menges, E.S. (2008) Genetic change following fire in populations of a seed-banking perennial plant. *Oecologia*. **158**: 355-360.

Donnelly, E.D., Watson, J.E. and McGuire, J.A. (1972) Inheritance of hard seed in *Vicia*. *Journal of Heredity*. **63**: 361-365.

Donohue, K. (2009). Completing the cycle: maternal effects as the missing link in plant life histories. *Philosophical Transactions on the Royal Society B.* **364**: 1059-1074.

Donohue, K. and Schmitt, J. (1998) Maternal environmental effects: adaptive plasticity? In. *Maternal effects as adaptations* Mousseau, T.A. and Fox, C.W. (Eds.). Oxford, UK, Oxford.

Donohue, K., Dorn, L., Griffith, C., Kim, E., Aguilera, A., Polisetty, C.R. and Schmitt, J. (2005) The evolutionary ecology of seed germination of *Arabidopsis thaliana*: variable natural selection on germination timing. *Evolution.* **59**: 758-770.

Donohue, K., Rubio de Casas, R., Burghardt, L., Kovach, K. and Willis, C.G. (2010) Germination, postgermination adaptation, and species ecological ranges. *Annual Reviews of Ecology and Evolutionary Systematics*. **41**: 293-319.

Dowdy, A., Abbs, D., Bhend, J., Chiew, F., Church, J., Ekström, M., Kirono, D., Lenton, A., Lucas, C., McInnes, K., Moise, A., Monselesan, D., Mpelasoka, F., Webb, L. and Whetton, P. (2015) East Coast Cluster Report. In. *Climate Change in Australia Projections for Australia's Natural Resource Management Regions: Cluster Reports* Ekström, M., Whetton, P., Gerbing, C. Grose, M., Webb, L. and Risbey, J. (Eds.) Australia, CSIRO and Bureau of Meteorology.

Doyle, J.J., and Doyle, J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin.* **19**: 11-15.

Dudash, M.R. (1990) Relative fitness of selfed and outcrossed progeny in a self—compatible, protandrous species, *Sabatia angularis* L. (Gentianaceae): a comparison in three environments. *Evolution.* **44**: 1129-1139.

Dunne, J.A., Harte, J. and Taylor, K.J. (2003). Subalpine meadow flowering phenology responses to climate change: integrating experimental and gradient approaches. *Ecological Monographs.* **73**: 69-86.

Dunne, J.A., Saleska, S.R., Fischer, M.L. and Harte, J. (2004) Integrating experimental and gradient methods in ecological climate change research. *Ecology.* **85**: 904-916.

Eckhart, V.M., Geber, M.A. and McGuire, C.M. (2004) Experimental studies of adaptation in *Clarkia xantiana.* I. Sources of trait variation across a subspecies border. *Evolution.* **58**: 59-70.

England, P.R., Whelan, R.J. and Ayre, D.J. (2003) Effects of seed bank disturbance on the fine-scale genetic structure of populations of the rare shrub *Grevillea macleayana. Heredity.* **91**: 475-480.

Enright, N.J., Keith, D.A., Clarke, M.F. and Miller, B.P. (2012) Fire regimes in Australian sclerophyllous shrubby ecosystems: heathlands, heathy woodlands and mallee woodlands. In. *Flammable Australia: Fire Regimes, Biodiversity and Ecosystems in a Changing World.* Bradstock, R.A., Gill, A. M. and Williams, R.J. (Eds.) Australia, CSIRO Publishing.

Etterson, J.R. (2004a) Evolutionary potential of *Chamaecrista fasiculata* in relation to climate change I. Clinal patterns of selection along an environmental gradient in the Great Plains. *Evolution.* **58**: 1446-1458.

Etterson, J.R. (2004b) Evolutionary potential of *Chamaecrista fasiculata* in relation to climate change. II. Genetic architecture of three populations reciprocally planted along an environmental gradient in the Great Plains. *Evolution.* **58**: 1459-1471.

Etterson, J.R. and Shaw, R.G. (2001) Constraint to adaptive evolution in response to global warming. *Science.* **294**: 151-154.

Evenari, M., Koller, D. and Gutterman, Y. (1966) Effects of the environment of the mother plant on germination by control of seed-coat permeability to water in *Ononis sicula.* Guss. *Australian Journal of Biological Sciences.* **19**: 1007-1016.

Ewart, A.J. (1908) On the longevity of seeds. *Proceedings of the Royal Society of Victoria.* **21**: 1-210.

Faircloth, B.C. (2008) MSATCOMMANDER: detection of microsatellite repeat arrays and automated, locus-specific primer design. *Molecular Ecology Resources*. **8**: 92-94.

Falconer, D.S. (1989). *Introduction to quantitative genetics*. 3rd Ed. USA, Longman Scientific and Technical.

Farrell, T.P. and Ashton, D.H. (1978) Population studies on *Acacia melanoxylon* R. Br. I. Variation in seed and vegetative characteristics. *Australian Journal of Botany.* **26**: 365-379.

Fenner, M. (1991) The effects of the parent environment on seed germinability. *Seed Science Research.* **1**: 75-84.

Fenner, M. and Thompson, K. (2005) *The ecology of seeds.* 2nd Ed. Cambridge, UK, Cambridge University Press.

Fitter, A.H. and Fitter, R.S.R. (2002) Rapid changes in flowering time in British plants. *Science.* **296**: 1689-1691.

Frankham, R., Ballou, J.D., Briscoe, D.A. and McInnes, K.H. (2010) *Introduction to conservation genetics.* 2nd Ed. Cambridge, UK, Cambridge University Press.

Fukami, T. and Wardle, D.A. (2005) Long-term ecological dynamics: reciprocal insights from natural and anthropogenic gradients. *Proceedings of the Royal Society B.* **272**: 2105-2115.

Furbeck, S.M., Bourland, F.M. and Watson, C.E.Jr. (1993) Inheritance of resistance to seed deterioration in cotton. *Euphytica*. **69**: 203-209.

Galloway, L.F. (2001) The effect of paternal and maternal environments on seed characters in the herbaceous plant *Campanula americana* (Campanulaceae). *American Journal of Botany*. **88**: 832-840.

Galloway, L.F. (2005) Maternal effects provide phenotypic adaptation to local environmental conditions. *New Phytologist.* **166**: 93-100.

Gama-Arachchige, N.S., Baskin, J.M., Geneve, R.L. and Baskin, C.C. (2010) Identification and characterization of the water gap in physically dormant seeds of

Geraniaceae, with special reference to *Geranium carolinianum*. *Annals of Botany*. **105**: 977-990.

Gama-Arachchige, N.S., Baskin, J.M., Geneve, R.L. and Baskin, C.C. (2011) Acquisition of physical dormancy and ontogeny of the micropyle-water-gap complex in developing seeds of *Geranium carolinianum* (Geraniaceae). *Annals of Botany*. **108**: 51-64.

Gama-Arachchige, N.S., Baskin, J.M., Geneve, R.L. and Baskin, C.C. (2013) Identification and characterization of ten new water gaps in seeds and fruits with physical dormancy and classification of water-gap complexes. *Annals of Botany*. **112**: 69-84.

Gao, H., Williamson, S. and Bustamante, C.D. (2007) A markov chain monte carlo approach for joint inference of population structure and inbreeding rates from multilocus genotype data. *Genetics*. **176**: 1635-1651.

Garrido, J.L., Rey, P.J., Herrera, C.M. and Ramírez, J.M. (2012) Negative evidence of local adaptation to the establishment conditions in a perennial herb. *Plant Ecology*. **213**: 1555-1569.

Germain, R.M. and Gilbert, B. (2014) Hidden responses to environmental variation: maternal effects reveal species niche dimensions. *Ecology Letters*. **17**: 662-669.

Giménez-Benavides, L., Escudero, A. and Iriondo, J.M. (2007) Local adaptation enhances seedling recruitment along an altitudinal gradient in a high mountain Mediterranean plant. *Annals of Botany*. **99**: 723-734.

Gonzalo-Turpin, H. and Hazard, L. (2009) Local adaptation occurs along altitudinal gradient despite the existence of gene flow in the alpine species *Festuca eskia*. *Journal of Ecology*. **97**: 742-751.

Goodwillie, C., **Kalisz, S. and Eckert, C.G.** (2005) The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. *Annual Review of Ecology and Evolutionary Systematics.* **36**: 47-79.

Gordon, D.R. and Rice, K.J. (1998) Patterns of differentiation in Wiregrass (*Aristida beyrichiana*): implications for restoration efforts. *Restoration Ecology*. **6**: 166-174.

Gresta, F., Avola, G. and Abbate, V. (2007) Germination ecology of *Scorpiurus subvillosus* L. seeds: the role of temperature and storage time. *Plant Ecology*. **190**: 123-130.

Grivet, D., Sork, V.L., Westfall, R.D. and Davis, F.W. (2008) Conserving the evolutionary potential of California valley oak (*Quercus lobata* Née): a multivariate genetic approach to conservation planning. *Molecular Ecology*. **17**: 139-156.

Grubb, P.J. (1997) The maintenance of species-richness in plant communities: the importance of the regeneration niche. *Biological Reviews.* **52**: 107-145.

Grünwald, N.J., Goodwin, S.B., Milgroom, M.G. and Fry, W.E. (2003) Analysis of genotypic diversity data for populations of microorganisms. *Analytical and Theoretical Plant Pathology.* **93**: 738-746.

Haggerty, B.P. and Galloway, L.F. (2011) Response of individual components of reproductive phenology to growing season length in a monocarpic herb. *Journal of Ecology*. **99**: 242-253.

Halbritter, A.H., Billeter, R., Edwards, P.J. and Alexander, J.M. (2015) Local adaptation at range edges: comparing elevation and latitudinal gradients. *Journal of Evolutionary Biology.* **28**: 1849-1860.

Hampe, A. (2004) Bioclimate envelope models: what they detect and what they hide. *Global Ecology and Biogeography.* **13**: 469-476.

Hamrick, J.L. and Godt, M.J.W. (1996) Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions of the Royal Society of London B.* **351**: 1291-1298.

Hand, B.K., Muhfeld, C.C., Wade, A.A., Kovach, R.P., Whited, D.C., Narum, S.R., Matala, A.P., Ackerman, M.W., Garner, B.A., Kimball, J.S., Stanford, J.A. and Luikart, G. (2016) Climate variables explain neutral and adaptive variation within salmonid metapopulations: the importance of replication in landscape genetics. *Molecular Ecology.* **25**: 689-705.

Heisler-White, J.L., Blair, J.M., Kelly, E.F., Harmoney, K. and Knapp, A.K. (2009) Contingent productivity responses to more extreme rainfall regimes across a grassland biome. *Global Change Biology.* **15**: 2894-2904.

Hennessy, K.J., Lucas, C., Nicholls, N., Bathols, J.M., Suppiah, R. and Rickets, J.H. (2005) *Climate change impacts on fire-weather in south-east Australia.* Report C/1061. Australia, CSIRO.

Hensen, I., Cierjacks, A., Hirsch, H., Kessler, M., Romoleroux, K., Renison, D. and Wesche, K. (2011) Historic and recent fragmentation coupled with altitude affect the

genetic population structure of one of the world's highest tropical tree line species. *Global Ecology and Biogeography*. **21**: 455-464.

Hereford, J. (2010) Does selfing or outcrossing promote local adaptation? *American Journal of Botany*. **97**: 298-302.

Herranz, J.M., Ferrandis, P. and Martinez-Sanchez, J.J. (1998) Influence of heat on seed germination of seven Mediterranean Leguminosae species. *Plant Ecology*. **136**: 95-103.

Hill, H.J., West, S.H. and Hinson, K. (1986a) Soybean seed size influences expression of the impermeable seed-coat trait. *Crop Science*. **26**: 634-637.

Hill, H.J., West, S.H. and Hinson, K. (1986b) Effect of water stress during seedfill on impermeable seed. *Crop Science*. **26**: 807-812.

Hoban, S. and Strand, A. (2015) Ex situ collections will benefit from considering spatial sampling design and species' reproductive biology. *Biological Conservation*. **187**: 182-191.

Hof, C., Levinsky, I., Araújo, M.B. and Rahbek, C. (2011) Rethinking species' ability to cope with rapid climate change. *Global Change Biology*. **17**: 2987-2990.

Holderegger, R., Kamm, U. and Gugerli, F. (2006) Adaptive vs. neutral genetic diversity: implications for landscape genetics. *Landscape Ecology*. **21**: 797-807.

Honnay, O., Verheyen, K., Butaye, J., Jacqumyn, H., Bossuyt, B. and Hermy, M. (2002) Possible effects of habitat fragmentation and climate change on the range of forest plant species. *Ecology Letters.* **5**: 525-530.

Honnay, O., Bossuyt, B., Jacquemyn, H., Shimono, A. and Uchiyama, K. (2008) Can a seed bank maintain the genetic variation in the above ground plant population? *Oikos.* **117**: 1-5.

House, C.R., Roth, C., Hunt, J. and Kover, P.X. (2010) Paternal effects in *Arabidopsis* indicate that offspring can influence their own size. *Proceedings of the Royal Society B-Biological Sciences.* **277**: 2885-2893.

Hovenden, M.J., Williams, A.L., Kongstad Pedersen, J., Vander Schoor, J.K. and Willis, K.E. (2008) Elevated CO_2 and warming impacts on flowering phenology in a southern Australian grassland are related to flowering time but not growth form, origin or longevity. *Australian Journal of Botany.* **56**: 630-643.

Hoyle, G.L., Steadman, K.J., Daws, M.I. and Adkins, S.W. (2008) Pre- and post-harvest influences on seed dormancy status of an Australian Goodeniaceae species *Goodenia fasicularis*. *Annals of Botany.* **102**: 93-101.

Hoyle, G.L., Kravchuk, O.Y., Daws, M.I., Adkins, S.W. and Steadman, K.J. (2011) Replicated versus un-replicated factorial experiments for preliminary investigation of seed germination and dormancy: alternative approaches using fewer seeds. *Seed Science and Technology.* **39**: 93-111.

Hudson, A.R., Ayre, D.J. and Ooi, M.K.J. (2015) Physical dormancy in a changing climate. *Seed Science Research.* **25**: 66-81.

Humphrey, M.E., Lambrides, C.J., Chapman, S.C., Aitken, E.A.B., Imrie, B.C., Lawn, R.J., McIntyre, C.L. and Liu, C.J. (2005) Relationships between hard-seededness and seed weight in mungbean (*Vigna radiata*) assessed by QTL analysis. *Plant Breeding.* **124**: 292-298.

IPCC (2014) Summary for policymakers. In. *Climate change 2014: impacts, adaptation and vulnerability. Part A: Global and sectoral aspects. Contribution of working group II to the 5th assessment report of the Intergovernmental Panel on Climate Change* Field, C.B.; Barros, V.R.; Dokken, D.J.; Mach, K.J.; Mastrandrea, M.D.; Bilir, T.E.; Chatterjee, M.;. Ebi, K.L; Estrada, Y.O.; Genova, R.C.; Girma, B.; Kissel, E.S.; Levy, A.N.; MacCracken, S.; Mastrandrea, P.R.; White L.L. (Eds.) Cambridge, UK, Cambridge University Press.

Iler, A.M., Høye, T.T., Inouye, D.W. and Schmidt, N.M. (2013) Nonlinear flowering responses to climate: are species approaching their limits of phenological change? *Philosophical Transactions of the Royal Society B.* **368**: 20120489.

Isemura, T., Kaga, A., Tomooka, N., Shimizu, T. and Vaughan, D.A. (2010) The genetics of domestication of rice bean, *Vigna umbellata*. *Annals of Botany*. **106**: 927-944.

Isemura, T., Kaga, A., Tabata, S., Somata, P., Srinives, P., Shimizu, T., Uken, J., Vaughan, D.A. and Tomooka, N. (2012) Construction of a genetic linkage map and genetic analysis of domestication related traits in Mungbean (*Vigna radiata*). *PLOS One*. **7**: e41304.

Jackson, S.T., Betancourt, J.L., Booth, R.K. and Gray, S.T. (2009) Ecology and the ratchet of events: climate variability, niche dimensions, and species distributions. *PNAS.* **106**: 19685-19692.

Jaganathan, G.K. (2016) Influence of maternal environment in developing different levels of physical dormancy and its ecological significance. *Plant Ecology*. **217**: 71-79.

James, A.T., Lawn, R.J., Williams, R.W. and Lambrides, C.J. (1999) Cross fertility of Australian accessions of wild Mungbean (*Vigna radiata* ssp. *sublobata*) with green Gram (*V. radiata* ssp. *radiata*) and black Gram (*V. mungo*). *Australian Journal of Botany*. **47**: 601-610.

Jay, F., Manel, S., Alvarez, N., Durand, E.Y., Thuiller, W., Holderegger, R., Taberlet, P. and Francois, O. (2012) Forecasting changes in population genetic structure of alpine plants in response to global warming. *Molecular Ecology.* **21**: 2354-2368.

Jayasuriya, K.M.G.G., Baskin, J.M. and Baskin, C.C. (2008a) Cycling of sensitivity to physical dormancy-break in seeds of *Ipomoea lacunose* (Convolvulaceae) and ecological significance. *Annals of Botany.* **101**: 341-352.

Jayasuriya, K.M.G.G., Baskin, J.M., Geneve, R.L., Baskin, C.C. and Chien, C.T. (2008b) Physical dormancy in seeds of the holoparastic angiosperm *Cuscuta australis* (Convolvulaceae, Cuscuteae): dormancy-breaking requirements, anatomy of the water gap and sensitivity cycling. *Annals of Botany.* **102**: 39-48.

Jayasuriya, K.M.G.G., Baskin, J.M., Geneve, R.L. and Baskin, C.C. (2009) A proposed mechanism for physical dormancy break in seeds of *Ipomoea lacunosa* (Convolvulaceae). *Annals of Botany.* **103**: 433-445.

Jeffrey, D.J., Holmes, P.M. and Rebelo, A.G. (1988) Effects of dry heat on seed germination in selected indigenous and alien legume species in South Africa. *South African Journal of Botany.* **54**: 28-34.

Jump, A.S., Junt, J.M., Martínez-Izquierdo, J.A. and Peñuelas, J. (2006) Natural selection and climate change: temperature-linked spatial and temporal trends in gene frequency in *Fagus sylvatica. Molecular Ecology.* **15**: 3469-3480.

Jump, A.S., Mátyás, C. and Peñuelas, J. (2009) The altitude-for-latitude disparity in the range retractions of woody species. *Trends in Ecology and Evolution.* **24**: 694-701.

Jump, A.S. and Peñuelas, J. (2005) Running to stand still: adaptation and the response of plants to rapid climate change. *Ecology Letters.* **8**: 1010-1020.

Kaga, A., Isemura, T., Tomooka, N. and Vaughan, D.A. (2008) The genetics of domestication of the Azuki bean (*Vigna angularis*). *Genetics.* **178**: 1013-1035.

Kalinowski, S.T. (2005) HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. *Molecular Ecology Notes.* **5**: 187-189.

Kawecki, T.J. and Ebert, D. (2004) Conceptual issues in local adaptation. *Ecology Letters.* **7**: 1225-1241.

Keatley, M.R., Fletcher, T.D., Hudson, I.L. and Ades, P.K. (2002) Phenological studies in Australia: potential application in historical and future climate analysis. *International Journal of Climatology*. **22**: 1769-1780.

Keigley, P.J. and Mullen, R.E. (1986) Changes in soybean seed quality from high temperature during seed fill and maturation. *Crop Science.* **26**: 1212-1216.

Keim, P., Diers, B.W. and Shoemaker, R.C. (1990) Genetic analysis of soybean hard seededness with molecular markers. *Theoretical and Applied Genetics*. **79**: 465-469.

Keith, D. (2004) *Ocean shores to desert dunes: the native vegetation of New South Wales and the ACT*. Hurstville, Australia, NSW Department of Environment and Conservation.

Keith, D.A. and Myerscough, P.J. (2016) Population variation in germination traits and its implications for responses to climate change in a fire-prone plant species complex. *Plant Ecology.* **217**: 781-788.

Keith, D.A. and Pellow, B. (2005) Effects of Javan rusa deer (*Cervus timorensis*) on native plant species in the Jibbon-Bundeena area, Royal National Park, New South Wales. *Linnaean Society of New South Wales.* **126**: 99-110.

Kenrick, J. and Knox, R.B. (1989) Quantitative analysis of self-incompatibility in trees of seven species of *Acacia. Journal of Heredity*. **80**: 240-245.

Kilen, T.C. and Hartwig, E.E. (1978) An inheritance study of impermeable seed in soybeans. *Field Crops Research.* **1**: 65-70.

Kim, E. and Donohue, K. (2013) Local adaptation and plasticity of *Erysimum capitatum* to altitude: its implications for responses to climate change. *Journal of Ecology.* **101**: 796-805.

Kimball, S., Angert, A.L., Huxman, T.E. and Venable, D.L. (2010) Contemporary climate change in the Sonoran Desert favours cold-adapted species. *Global Change Biology.* **16**: 1555-1565.

Koch, G.W., Vitousek, P.M., Steffen, W.L. and Walker, B.H. (1995) Terrestrial transects for global change research. *Vegetatio.* **121**: 53-65.

Kochanek, J., Buckley, Y.M., Probert, R.J., Adkins, S.W. and Steadman, K.J. (2011) Pre-zygotic parental environment modulates seed longevity. *Austral Ecology.* **35**: 837-848.

Koide, D., Higa, M., Nakao, K., Ohashi, H., Tsuyama, I., Matsui, T. and Tanaka, N. (2016) Projecting spatiotemporal changes in suitable climate conditions to regenerate trees using niche differences between adult and juvenile trees. *European Journal of Forest Research.* **135**: 125-136.

Körner, C. (2007) The use of "altitude" in ecological research. *Trends in Ecology and Evolution.* **22**: 569-574.

Lacerda, D.R., Filho, J.P.L., Goulart, M.F., Ribeiro, R.A. and Lovato, M.B. (2004) Seed-dormancy variation in natural populations of two tropical leguminous tree species: *Senna multijuga* (Caesalpinoideae) and *Plathymenia reticulata* (Mimosoideae). *Seed Science Research.* **14**: 127-135.

Lacey, E.P., Smith, S. and Case, A.L. (1997) Parental effects on seed mass: seed coat but not embryo/endosperm effects. *Australian Journal of Botany*. **84**: 1617-1620.

Lee, J.A. (1975) Inheritance of hard seed in cotton. *Crop Science.* **15**: 149-152.

Leimu, R. and Fischer, M. (2008) A meta-analysis of local adaptation in plants. *PLOS One.* **3**: e4010.

Li, X., Baskin, J.M. and Baskin, C.C. (1999a) Pericarp ontogeny and anatomy in *Rhus aromatica* Ait. and *R. glabra* L. (Anacardiaceae). *Journal of the Torrey Botanical Society.* **126**: 279-288.

Li, X., Baskin, J.M. and Baskin, C.C. (1999b) Anatomy of two mechanisms of breaking physical dormancy by experimental treatments in seeds of two north American *Rhus* species (Anacardiaceae). *American Journal of Botany.* **86**: 1505-1511.

Li, X., Buirchell, B., Yan, G. and Yang, H. (2012) A molecular marker linked to the *mollis* gene conferring soft-seediness for marker-assisted selection applicable to a wide range of crosses in lupin (*Lupinus angustifolius* L.). *Molecular Breeding.* **29**: 361-370.

Liu, Y., Mu, J., Niklas, K.J., Li, G. and Sun, S. (2012) Global warming reduces plant reproductive output for temperate multi-inflorescence species on the Tibetan plateau. *New Phytologist.* **195**: 427-436.

Liyanage, G.S. and Ooi, M.K.J. (2015) Intrapopulation level variation in physical dormancy breaking temperature thresholds. *Annals of Botany.* **116**: 123-131.

Liyanage, G.S. and Ooi, M.K.J. (*in press*) Do dormancy-breaking temperature thresholds change as seeds age in the soil seed bank? *Seed Science Research.*

Lloyd, D.G. (1992) Self- and cross-fertilization in plants. II. The selection of self-fertilization. *International Journal of Plant Sciences.* **153**: 370-383.

Loarie, S.R., Duffy, P.B., Hamilton, H., Asner, G.P., Field, C.B. and Ackerly, D.D. (2009) The velocity of climate change. *Nature.* **462**: 1052-1055.

Loveless, M.D. and Hamrick, J.L. (1984) Ecological determinants of genetic structure in plant populations. *Annual Reviews in Ecology and Systematics.* **15**: 65-95.

Lucas, C., Hennessy, K.J., Mills, G.A. and Bathols, J.M. (2007) *Bushfire weather in southeast Australia: recent trends and projected climate change impacts.* Australia, Australian Bureau of Meterology and CSIRO Marine and Atmospheric Research.

Lusk, C.H., Reich, P.B., Montgomery, R.A., Ackerly, D.D. and Cavender-Bares, J. (2008) Why are evergreen leaves so contrary about shade? *Trends in Ecology and Evolution.* **23**: 299-303.

MacColl, A.D.C. (2011) The ecological causes of evolution. *Trends in Ecology and Evolution.* **26**: 514-522.

MacGregor, D.R., Kendall, S.L., Florance, H., Fedi, F., Moore, K., Paszkiewicz, K., Smirnoff, N. and Penfield, S. (2015) Seed production temperature regulation of primary dormancy occurs through control of seed coat phenylopropanoid metabolism. *New Phytologist.* **205**: 642-652.

Mackenzie, B.D.E., Auld, T.D., Keith, D.A., Hui, F.K.C. and Ooi, M.K.J. (2016) The effect of seasonal ambient temperatures on fire-stimulated germination of species with physiological dormancy: a case study using *Boronia* (Rutaceae). *PLOS One.* **11**: e0156142.

Majd, R., Aghaie, P., Monfared, E.K. and Alebrahim, M.T. (2013) Evaluation of some treatments on breaking seed dormancy in Mesquite. *International Journal of Agronomy and Plant Production.* **4**: 1433-1439.

Martin, R.E., Miller, R.L. and Cushwa, C.T. (1975) Germination response of legume seeds subjected to moist and dry heat. *Ecology.* **56**: 1441-1445.

Matías, L. and Jump, A.S. (2015) Asymmetric changes of growth and reproductive investment herald altitudinal and latitudinal range shifts of two woody species. *Global Change Biology.* **21**: 882-896.

McLaughlin, B.C. and Zavaleta, E.S. (2012) Predicting species responses to climate change: demography and climate microrefugia in California valley oak (*Quercus lobata*). *Global Change Biology.* **18**: 2301-2312.

Meisert, A. (2002) Physical dormancy in Geraniaceae seeds. *Seed Science Research.* **12**: 121-128.

Michael, P.J., Steadman, K.J. and Plummer, J.A. (2006) Climatic regulation of seed dormancy and emergences of *Malva parviflora* populations from a Mediterranean-type environment. *Seed Science Research.* **16**: 273-281.

Moles, A.T. and Westoby, M. (2004) Seedling survival and seed size: a synthesis of the literature. *Journal of Ecology*. **92**: 372-383.

Mondoni, A., Probert, R.J., Rossi, G., Vegini, E. and Hay, F.R. (2011) Seeds of alpine plants are short lived: implications for long-term conservation. *Annals of Botany*. **107**: 171-179.

Mondoni, A., Orsenigo, S., Donà, M., Balestrazzi, A., Probert, R.J., Hay, F.R., Petraglia, A. and Abeli, T. (2014) Environmentally induced transgenerational changes in seed longevity: maternal and genetic influence. *Annals of Botany*. **113**: 1257-1263.

Montalvo, A.M. and Ellstrand, N.C. (2000) Transplantation of the subshrub *Lotus scoparius*: testing the home-site advantage hypothesis. *Conservation Biology*. **14**: 1034-1045.

Monty, A. and Mahy, G. (2009) Clinal differentiation during invasion: *Senecio inaequidens* (Asteraceae) along altitudinal gradients in Europe. *Oecologia*. **156**: 305-315.

Moreno, J.M. and Oechel, W.C. (1991) Fire intensity effects on germination of shrubs and herbs in southern California chaparral. *Ecology*. **72**: 1993-2004.

Moreira, B. and Pausas, J.G. (2012) Tanned or burned: the role of fire in shaping physical seed dormancy. *PlosOne*. **7**: e51523.

Moreira, B., Tormo, J., Estrelles, E. and Pausas, J.G. (2010) Disentangling the role of heat and smoke as germination cues in Mediterranean Basin flora. *Annals of Botany*. **105**: 627-635.

Morin, X., Viner, D. and Chuine, I. (2008) Tree species range shifts at a continental scale: new predictive insights from a process-based model. *Journal of Ecology.* **96**: 784-794.

Morley, F.H.W. (1958) The inheritance and ecological significance of seed dormancy in subterranean clover (*Trifolium subterraneum* L.). *Australian Journal of Biological Science.* **11**: 261-274.

Morrison, D.A. (1986a) A review of the biology of *Acacia suaveolens* (Smith) Willd. (Mimosaceae). *Proceedings of Linnaean Society of NSW.* **109**: 271-292.

Morrison, D.A. (1986b). A demographic analysis of reproduction in *Acacia suaveolens* (Smith) Willd. (Mimosaceae), Sydney: University of Sydney, Ph.D. thesis, unpubl.

Morrison, D.A. and Myerscough, P.J. (1989) Temporal regulation of maternal investment in populations of the perennial legume *Acacia suaveolens. Ecology.* **6**: 1629-1638.

Morrison, D., Auld, T.D. and Gallaher, K. (1983) The distribution ecology and conservation status of *Acacia suaveolens. The Victorian Naturalist.* **100**: 140-145.

Nair, R.M., Craig, A.D., Rowe, T.D., Biggins, S.R. and Hunt, C.H. (2004) Genetic variability and heritability estimates for hardseededness and flowering in balansa clover (*Trifolium michelianum* Savi) populations. *Euphytica.* **138**: 197-203.

Nardin, M., Musch, B., Rousselle, Y., Guérin, V., Sanchez, L., Rossi, J.P., Gerber, S., Marin, S., Pâques, L.E., and Rozenberg, P. (2015) Genetic differentiation of European larch along an altitudinal gradient in the French Alps. *Annals of Forest Science.* **72**: 517-527.

Neel, M.C., Ross-Ibarra, J. and Ellstrand, N.C. (2001) Implication of mating patterns for conservation of the endangered plant *Erigonum ovalifolium* var. *vineum* (Polygonaceae). *American Journal of Botany.* **88**: 1214–1222.

Nichols, P.G.H., Cocks, P.S. and Francis, C.M. (2009) Evolution over 16 years in a bulk-hybrid population of subterranean clover (*Trifolium subterraneum* L.) at two contrasting sites in south-western Australia. *Euphytica.* **169**: 31-48.

Nicotra, A.B., Atkin, O.K., Bonser, S.P., Davidson, A.M., Finnegan, E.J., Mathesius, U., Poot, P., Purugganan, M.D., Richards, C.L., Valladares, F. and van Kleunen, M. (2010) Plant phenotypic plasticity in a changing climate. *Trends in Plant Science.* **15**: 684-692.

NSW RFS (2016) Bush fire danger period and fire permits. http://www.rfs.nsw.gov.au/fire-information/BFDP

Norman, H.C., Cocks, P.S., Smith, F.P. and Nutt, B.J. (1998) Reproductive strategies in Mediterranean annual clovers: germination and hardseedness. *Australian Journal of Agricultural Research.* **49**: 973-982.

Norman, H.C., Cocks, P.S. and Galwey, N.W. (2002) Hardseededness in annual clovers: variation between populations from wet and dry environments. *Australian Journal of Agricultural Research.* **53**: 821-829.

Norman, H.C., Smith, F.P., Nichols, P.G.H., Si, P. and Galwey, N.W. (2006) Variation in seed softening patterns and impact of seed production environment on hardseededness

in early-maturing genotypes of subterranean clover. *Australian Journal of Agricultural Research.* **57**: 65-74.

Ohsawa, T. and Ide, Y. (2008) Global patterns of genetic variation in plant species along vertical and horizontal gradients on mountains. *Global Ecology and Biogeography.* **17**: 152-163.

Ooi. M.K.J. (2012) Seed bank persistence and climate change. *Seed Science Research.* **22**: 53-60.

Ooi, M.K.J., Auld, T.D. and Denham, A.J. (2009) Climate change and bet-hedging: interactions between increased soil temperatures and seed bank persistence. *Global Change Biology.* **15**: 2375-2386.

Ooi, M.K.J., Auld, T.D. and Denham, A.J. (2012) Projected soil temperature increase and seed dormancy response along an altitudinal gradient: implications for seed bank persistence under climate change. *Plant Soil.* **353**: 289-303.

Ooi, M.K.J., Denham, A.J., Santana, V.M. and Auld, T.D. (2014) Temperature thresholds of physically dormant seeds and plant functional response to fire: variation among species and relative impact of climate change. *Ecology and Evolution.* **4**: 656-671.

Parmesan, C. (2006) Ecological and evolutionary responses to recent climate change. *Annual Reviews of Ecology, Evolution and Systematics.* **37**: 637-669.

Parmesan, C. and Hanley, M.E. (2015) Plants and climate change: complexities and surprises. *Annals of Botany.* **116**: 849-864.

Paulsen, T.R., Colville, L., Kranner, I., Daws, M.I., Högstedt, G., Vandvik, V. and Thompson, K. (2013) Physical dormancy in seeds: a game of hide and seek? *New Phytologist*. **198**: 496-503.

Pausas, J.G., Bladé, C., Valdecantos, A., Seva, J.P., Fuentes, D., Alloza, J.A., Vilagrosa, A., Bautista, S., Cortina, J. and Vallejo, R. (2004) Pines and oaks in the restoration of Mediterranean landscapes of Spain: new perspectives for an old practice – a review. *Plant Ecology*. **171**: 209-220.

Pausas, J.G. and Keeley, J.E. (2014) Evolutionary ecology of resprouting and seeding in fire-prone ecosystems. *New Phytologist*. **204**: 55-65.

Peakall, R. and Smouse, P.E. (2006) GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*. **6**: 288-295.

Peakall, R. and Smouse, P.E. (2012) GenALEx 6.5: genetics analysis in Excel. Population genetic software for teaching and research – an update. *Bioinformatics*. **28**: 2537-2539.

Pearson, R.G. and Dawson, T.P. (2003) Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? *Global Ecology and Biogeography*. **12**: 361-371.

Penman, T.D. and Towerton, A.L. (2008) Soil temperatures during autumn prescribed burning, implications for the germination of fire responsive species. *International Journal of Wildland Fire*. **17**: 572-578.

Pérez-García, F. and Escudero, A. (1997) Role of the seed coat in germination of *Cistus populifolius* L. *Israel Journal of Plant Sciences.* **45**: 329-331.

Piano, E., Pecetti, L. and Carroni, A.M. (1996) Climatic adaptation in subterranean clover populations. *Euphytica.* **92**: 39-44.

Pickett, S.T.A. (1989) Space-for-time substitution as an alternative to long-term studies. In. *Long-term studies in ecology: approaches and alternatives* Likes, G.E. (Ed.). New York, USA, Springer-Verlag.

Pollard, D.A. (2012) Design and construction of recombinant inbred lines. *Methods in Molecular Biology.* **871**: 31-39.

Post, E.S., Pedersen, C., Wilmers, C.C. and Forchhammer, M.C. (2008) Phenological sequences reveal aggregate life history response to climatic warming. *Ecology.* **89**: 363-370.

Probert, R.J. (2000) The role of temperature in the regulation of seed dormancy and germination. In. *Seeds: The ecology of regeneration in plant communities* (2nd Ed.) Fenner, M. (Ed.). Wallingford, UK, CABI.

Quinlivan, B.J. and Nicol, H.I. (1971) Embryo dormancy in subterranean clover seeds. *Australian Journal of Agricultural Research.* **22**: 599-606.

Quiroga, M.P. and Premoli, A.C. (2007) Genetic patterns in *Podocarpus parlatorei* reveal the long-term persistence of cold-tolerant elements in the southern Yungas. *Journal of Biogeography.* **34**: 447-455.

R Core Development Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

R Core Development Team. (2016) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Ramsay, G. (1997) Inheritance and linkage of a gene for testa-imposed seed dormancy and faba bean (*Vicia faba* L.). *Plant Breeding.* **116**: 287-289.

Rasmann, S., Pellissier, L., Defossez, E., Jactel, H. and Kunstler, G. (2014) Climate-driven change in plant-insect interactions along elevation gradients. *Functional Ecology*. **28**: 46-54.

Rawal, D.S., Kasel, S., Keatley, M.R. and Nitschike, C.R. (2015) Herbarium records identify sensitivity of flowering phenology of eucalypts to climate: implications for species response to climate change. *Austral Ecology*. **40**: 117-125.

Raymond, M. and Rousset, F. (1995) GENEPOP (Version 1.2): Population genetics software for exact tests and ecumenicism. *Journal of Heredity*. **86**: 248-249.

Reed, D.H. and Frankham, R. (2003) Correlation between fitness and genetic diversity. *Conservation Biology*. **17**: 230-237.

Rix, K.D., Gracie, A.J., Potts, B.M., Brown, P.H., Spurr, C.J. and Gore, P.L. (2012) Paternal and maternal effects on the response of seed germination to high temperatures in *Eucalyptus globulus. Annals of Forest Science.* **69**: 673-679.

Roach, D.A. and Wulff, R.D. (1987) Maternal effects in plants. *Annual Review of Ecology and Systematics.* **18**: 209-235.

Roberts, D.G., Ottewell, K.M., Whelan, R.J. and Ayre, D.J. (2014) Is the post-disturbance composition of a plant population determined by selection for outcrossed seedlings or by the composition of the seedbank? *Heredity.* **112**: 409-414.

Roberts, D.G., Forrest, C.N., Denham, A.J. and Ayre, D.J. (2016) Varying levels of clonality and ploidy create barriers to gene flow and challenges for conservation of an Australian arid-zone ecosystem engineer, *Acacia loderi*. *Biological Journal of the Linnaean Society.* **118**: 330-343.

Roeder, M., Ferraz, I.D.K. and Hölscher, D. (2013) Seed and germination characteristics of 20 Amazonian liana species. *Plants.* **2**: 1-15.

Rosenberg, N.A. (2004) DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes.* **4**: 137-138.

Rossetto, M., Kooyman, R., Sherwin, W. and Jones, R. (2008) Dispersal limitations, rather than bottlenecks or habitat specificity, can restrict the distribution of forest trees. *American Journal of Botany*. **95**: 321-329.

Rossetto, M., Thurlby, K.A.G., Offord, C.A., Allen, C.B. and Weston, P.H. (2011) The impact of distance and a shifting temperature gradient on genetic connectivity across a heterogeneous landscape. *BMC Evolutionary Biology.* **11**: 126-127.

Rousset, F. (2008) GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources.* **8**: 103-106.

Royer, P.D., Cobb, N.S., Clifford, M.J., Huang, C.Y., Breshears, D.D., Adams, H.D. and Villegas, J.C. (2011) Extreme climatic event-triggered overstorey vegetation loss increases understorey solar input regionally: primary and secondary ecological implications. *Journal of Ecology*. **99**: 714-723.

Russi, L., Cocks, P.S. and Roberts, E.H. (1992) Coat thickness and hard-seededness in some *Medicago* and *Triflium* species. *Seed Science Research.* **2**: 243-249.

Rysavy, A., Seifan, M., Sternberg, M. and Tielbörger, K. (2014) Shrub seedling survival under climate change – comparing natural and experimental rainfall gradients. *Journal of Arid Environments.* **111**: 14-21.

Sage, R.D., Koenig, W.D. and McLaughlin, B.C. (2011) Fitness consequences of seed size in the valley oak *Quercus lobata* Née (Fagaceae). *Annals of Forest Science.* **68**: 477-484.

Sakamoto, S., Abe, J., Kanazawa, A. and Shimamoto, Y. (2004) Marker-assisted analysis for soybean hard seededness with isozyme and simple sequence repeat loci. *Breeding Science*. **54**: 133-139.

Salisbury, P.A. and Halloran, G.M. (1983) Inheritance of rate of breakdown of seed coat impermeability in subterranean clover (*Trifolium subterraneum* L.) *Euphytica.* **32**: 807-819.

Sander, T.M. and Matthies, D. (2016) The effects of stress intensity and stress type on inbreeding depression in *Silene vulgaris*. *Evolution.* **70**: 1225-1238.

Savolainen, O., Pyhäjärvi, T. and Knürr, T. (2007) Gene flow and local adaptation in trees. *Annual Reviews in Ecology, Evolution and Systematics*. **38**: 595-619.

Schütz, W. and Rave, G. (1999) The effect of cold stratification and light on the seed germination of temperate sedges (*Carex*) from various habitats and implications for regenerative strategies. *Plant Ecology*. **144**: 215-230.

Segura, F., Vicente, M.J., Franco, J.A. and Martínez-Sánchez, J.J. (2015) Effects of maternal environmental factors on physical dormancy of *Astragalus nitidflorus* seeds (Fabaceae), a critically endangered species of SE Spain. *Flora.* http://dx.doi.org/10.1016/j.flora.2015.09.001

Sherwin, W.B., Jabot, F., Rush, R. and Rossetto, M. (2006) Measurement of biological information with applications from genes to landscapes. *Molecular Ecology*. **15**: 2857-2869.

Skelly, D.K., Joseph, L.N., Possingham, H.P., Freidenburg, L.K., Farrugia, T.J., Kinnison, M.T. and Hendry, A.P. (2007) Evolutionary responses to climate change. *Conservation Ecology*. **21**: 1353-1355.

Slattery, H.D. (1986) Physiological and genetic control of seed coat impermeability in subterranean clover (*Trifolium subterraneum* L.) *The Journal of the Australian Institute of Agricultural Science.* **52**: 58.

Smith, F.P., Cocks, P.S. and Ewing, M.A. (1998) Seed production in cluster clover (*Trifolium glomeratum* L.) 1. Flowering time, abortion, seed size, and hardseededness along branches. *Australian Journal of Agricultural Research*. **49**: 961-964.

Sork, V.L., Davis, F.W., Westfall, R., Flint, A., Ikegami, M., Wang, H. and Grivet, D. (2010) Gene movement and genetic association with regional climate gradients in California valley oak (*Quercus lobata* Née) in the face of climate change. *Molecular Ecology*. **19**: 3806-3823.

Specht, R.L. (1970) Vegetation. In. *The Australian Environment* Leeper G.W. (Ed.). Australia, CSIRO.

Sriphadet, S., Lambrides, C.J. and Srinives, P. (2007) Inheritance of agronomic traits and their interrelationship in Mungbean (*Vigna radiata* (L.) Wilczek). *Journal of Crop Science and Biotechnology*. **10**: 249-256.

Steane, D.A., Potts, B.M., McLean, E., Prober, S.M., Stock, W.D., Vaillancourt, R.E. and Byrne, M. (2014) Genome-wide scans detect adaptation to aridity in a widespread forest tree species. *Molecular Ecology.* **23**: 2500-2513.

Stevens, N., Seal, C.E., Archibald, S. and Bond, W. (2014) Increasing temperatures can improve seedling establishment in arid-adapted savannah trees. *Oecologia.* **175**: doi.10.1007/s00442-014-2958-y.

Storz, J.F. (2005) Using genome scans of DNA polymorphism to infer adaptive population divergence. *Molecular Ecology.* **14**: 671-688.

Thanos, C.A., Georghiou, K., Kadis, C. and Pantazi, C. (1992) Cistaceae: a plant family with hard seeds. *Israel Journal of Botany.* **41**: 251-263.

Thomas, C.D., Cameron, A., Green, R.E., Bakkenes, M., Beaumont, L.J., Collingham, Y.C., Erasmus, B.F.N., de Siqueira, M.F., Grainger, A., Hannah, L., Hughes, L.,

Huntley, B., van Jaarsveld, A.S., Midgley, G.F., Miles, L., Ortega-Huerta, M.A., Peterson, A.T., Phillips, O.L. and Williams, S.E. (2004) Extinction risk from climate change. *Nature.* **427**: 145-148.

Thuiller, W., Lavorel, S. and Araújo, M.B. (2005) Niche properties and geographical extent as predictors of species sensitivity to climate change. *Global Ecology and Biogeography.* **14**: 347-357.

Tozer, M.G. and Bradstock, R.A. (1997) Factors influencing the establishment of seedlings of the Mallee *Eucalyptus luehmanniana* (Myrtaceae). *Australian Journal of Botany.* **45**: 997-1008.

Tozer, M.G. and Ooi, M.K.J. (2014) Humidity-regulated dormancy-onset in the Fabaceae: a conceptual model and its ecological implications for the Australian wattle *Acacia saligna* (Fabaceae (Labill.) H.L.Wendl). *Annals of Botany.* **114**: 579-590.

Trabaud, L. and Oustric, J. (1989) Heat requirements for seed germination of three *Cistus* species in the garrigue of southern France. *Flora.* **183**: 321-325.

Valladares, F., Matesanz S., Guilhaumon, F., Araújo, M.B., Balaguer, L., Benito-Garzón, M., Cornwell, W., Gianoli, E., van Kleunen, M., Naya, D.E., Nicotra, A.B., Poorter, H. and Zavala, M.A. (2014) The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. *Ecology Letters.* **17**: 1351-1364.

Van Assche, J.A. and Vandelook, F.E.A. (2006) Germination ecology of eleven species of Geraniaceae and Malvaceae, with special reference to the effects of drying seeds. *Seed Science Research.* **16**: 263-290.

Van Assche, J.A. and Vandelook, F.E.A. (2010) Combinational dormancy in winter annual Fabaceae. *Seed Science Research.* **20**: 237-242.

Van der Veken, S., Bellemare, J., Verheyen, K. and Hermy, M. (2007) Life-history traits are correlated with geographical distribution patterns of western European forest herb species. *Journal of Biogeography.* **34**: 1723-1735.

Van Klinken, R.D. and Flack, L. (2005) Wet heat as a mechanism for dormancy release and germination of seeds with physical dormancy. *Weed Science.* **53**: 663-669.

Van Rossum, F., Weidema, I.R., Martin, H., Le Cadre, S., Touzet, P., Prentice, H.C. and Philipp, M. (2016) The structure of allozyme variation in *Silene nutans* (Caryophyllaceae) in Denmark and in north-western Europe. *Plant Systematics and Evolution.* **302**: 23-40.

Van Tienderen, P. and Van der Toorn, J. (1991) Genetic differentiation between populations of *Plantago lanceolata.* 1. Local adaptation in three contrasting habitats. *Journal of Ecology.* **79**: 27-42.

Vázquez-Yanes, C. and Orozco-Segovia, A. (1982) Seed germination of a tropical rain forest pioneer tree (*Heliocarpus donnell-smithii*) in response to diurnal fluctuation of temperature. *Physiologia Plantarum.* **56**: 295-298.

Veasey, E.A. and Martins, P.S. (1990) Variability in seed dormancy and germination potential in *Desmodium* Desv. (Leguminosae). *Brazilian Journal of Genetics*. **14**: 527-545.

Venable, D.L. (2007) Bet-hedging in a guild of desert annuals. *Ecology*. **88**: 1086-1090.

Visscher, A.M., Seal, C.E., Newton, R.J., Frances, A.L. and Pritchard, H.W. (2016) Dry seeds and environmental extremes: consequences for seed lifespan and germination. *Functional Plant Biology*. **43**: 656-668.

Walck, J.L., Hidayati, S.N., Dixon, K.W., Thompson, K. and Poschlod, P. (2011) Climate change and plant regeneration from seed. *Global Change Biology*. **17**: 2145-2161.

Warton, D.I. and Wardle, G.M. (2003) Site-to-site variation in the demography of a fire-affected perennial, *Acacia suaveolens*, at Ku-ring-gai Chase National Park, New South Wales, Australia. *Austral Ecology*. **28**: 38-47.

Weiner, J., Martinez, S., Muller-Scharer, H., Stoll, P. and Schimd, B. (1997) How important are environmental maternal effects in plants? A study with *Centaurea maculosa*. *Journal of Ecology*. **85**: 133-142.

Whelan, R.J. (1995) *The ecology of fire.* Cambridge, UK, Cambridge University Press.

Whitlock, M.C. and McCauley, D.E. (1999) Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm+1)$. *Heredity*. **82**: 117-125.

Winn, A.A., Elle, E., Kalisz, S., Cheptou, P.O., Eckert, C.G., Goodwillie, G., Johnston, M.O., Moeller, D.A., Ree, R.H., Sargent, R.D. and Vallejo-Marin, M. (2011)

Analysis of inbreeding depression in mixed-mating plants provides evidence for selective interference and stable mixed mating. *Evolution.* **65**: 3339-3359.

Wulff, R.D. (1995) Environmental maternal effects on seed quality and germination. In. *Seed development and germination* Kigel J., Galili, G., (Eds.). New York, USA, Marcel Dekker.

Zeng, L.W., Cocks, P.S., Kailis, S.G. and Kuo, J. (2005) The role of fractures and lipids in the seed coat in the loss of hardseededness of six Mediterranean legume species. *Journal of Agricultural Science.* **143**: 43-55.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A. and Smith, G.M. (2005) *Mixed effects models and extensions in ecology with R.* New York, USA, Springer.

APPENDIX 1: Summary of the chapters each study site was used for.

Table S1.1. Overview of the study sites used for each chapter.

Study site	Ch. 3	Ch. 4	Ch. 5	Ch. 6	Ch.7
Garie Trig	X	X	X	X	X
Temptation Creek	X	-	X	X	X
Heathcote	X	-	X	-	-
Narooma	X	X	-	X	X
Potato Point Road	X	-	-	-	X
Camel Rock	X	-	-	-	X
Tasmania	-	X	-	-	-
Diamond Head	X	X	-	X	X
Crescent Head	X	-	-	-	X
Hat Head	X	-	-	-	X
Red Rock	-	X	-	-	-
Angourie	X	-	-	-	X
Evans Head	X	-	-	-	X
Lennox Head	X	-	-	-	-
Faulconbridge	X	X	X	X	X
Park Road	-	-	X	-	-
Lawson	X	-	X	-	X
Ingar Camp	-	X	-	-	-
Ingar Trail	-	X	-	-	-
Mount Hay 1	X	X	X	-	X
Mount Hay 2	X	-	X	-	X
Mount Tomah	X	-	X	-	X
Garrawarra	-	-	X	-	-
Sebastopol	-	-	X	-	-
St. Georges Crescent	-	-	X	-	-
Talbot Road	-	-	X	-	-
Podgers Glen	-	-	X	-	-
Katoomba 1	-	-	X	-	-
Katoomba 2	-	-	X	-	-
Mount Wilson	-	-	X	-	-

APPENDIX 2: Details of the BoM weather stations used to calculate study site temperature and rainfall data.

TABLE S2.1. Seed collection sites and the corresponding BoM weather stations used*.

Field Site	Weather Station	
	Temperature	**Rainfall**
Tas	Orford	NA
CR	Narooma	Bermagui
	Bega	
PPrd	Moruya Head	Tuross Head
	Narooma	
Nar	Narooma	Narooma
GT	Sydney	Audley
	Bellambi	Bellambi
Hea	Sydney	Audley
	Bellambi	Bellambi
TC	Sydney	Audley
	Bellambi	
DH	Port Macquarie	Laurieton
	Taree	
CH	South West Rocks	Crescent Head
	Port Macquarie	
HH	South West Rocks	South West Rocks
	Port Macquarie	
RR	Coffs Harbour	NA
Ang	Yamba	Yamba
EH	Evans Head	Evans Head
LH	Ballina Airport	Ballina Airport
Faulc	Katoomba	St. Georges Crescent
	Springwood	
Cem	Katoomba	Wentworth Falls
	Springwood	St. Georges Crescent
IC	Katoomba	NA
	Springwood	
IT	Katoomba	NA
	Springwood	
MtH1	Katoomba	Wentworth Falls
	Springwood	
MtH2	Katoomba	Katoomba
	Springwood	

MtT	Katoomba Springwood	Berambing

* Where two weather stations are listed, weather data for the field site was extrapolated based on altitude or latitude position of the field site relative to the two weather stations.

TABLE S2.2. Weather station geographical location details

Station	Station ID	Latitude*	Longitude*	Elevation (m asl)
Orford	092027	42.55	147.88	14
Bega	069139	36.67	149.82	41
Bermagui	069005	36.43	150.08	15
Narooma	069022	36.21	150.14	25
Tuross Head	069067	36.06	150.13	20
Moruya Head	069018	35.91	150.15	17
Bellambi	068228	34.37	150.93	10
Audley	066176	34.06	151.06	120
Sydney	066037	33.95	151.17	6
Taree	060141	31.89	152.51	8
Laurieton	060022	31.64	152.79	12
Port Macquarie	060139	31.43	152.87	4
Coffs Harbour	059040	30.31	153.12	5
Crescent Head	059047	31.18	152.97	8
Kempsey	059017	31.08	152.82	10
South West Rocks	059030	30.92	153.09	117
Yamba	058012	29.43	153.36	27
Evans Head	058212	29.18	153.40	63
Ballina Airport	058198	28.84	153.56	1
Springwood	063077	33.71	150.58	320
St. Georges Crescent	063028	33.69	150.53	460
Berambing	063013	33.54	150.44	792
Wentworth Falls	063227	33.70	150.37	898
Katoomba	063039	33.71	150.31	1015

* in decimal degrees S and E

Appendix 3: Details of the weather stations and linear regression models used for calculating missing temperature data.

Table S3.1. Weather stations details used to extrapolate missing temperature data

Station	Station ID	Latitude (° S)	Longitude (° E)	Elevation (m asl)
Bellambi	068228	34.37	150.93	10
Sydney	066037	33.95	151.17	6
St. Georges Crescent	063028	33.69	150.53	460
Katoomba	063039	33.71	150.31	1015

Table S3.2. Results of linear regression between iButton temperatures from the transplant sites and extrapolated site temperature (based on extrapolation from the nearest two BoM weather stations) over the same time periods.

Transplant site	R^2	P
Garra	0.45	***
TC	0.57	***
HWY	0.45	***
StG	0.79	***
Podg	0.81	***
Talb	0.72	***
Kat 1	0.69	***
Kat 2	0.79	***
MtW	0.76	***

*** $P < 0.001$

Appendix 4: Details of the voucher specimens lodged within the Janet Cosh Herbarium (JCH) at the University of Wollongong, Australia.

Table S4.1. Details of the voucher specimens of *Acacia* spp. Lodged within the Janet Cosh Herbarium

Species	Location	Geographic Co-ordinates		Collectors	Voucher Numbers
Acacia suaveolens	Temptation Creek, Royal National Park, NSW, Australia	34°03'33"S	151°03'59"E	A.R.Hudson & M.K.J. Ooi	WOLL#11296
Acacia suaveolens	Garie, Royal National Park, NSW, Australia	34°10'16"S	151°02'57"E	A.R.Hudson & M.K.J. Ooi	WOLL#11298
					WOLL#11325
Acacia suaveolens	Temptation Creek, Royal National Park, NSW, Australia	34°03'37"S	151°03'59"E	A.R.Hudson & M.K.J. Ooi	WOLL#11297
					WOLL#11324
Acacia falcata	Grown at the University of Wollongong Ecological Research Facility	NA	NA	A.R.Hudson & M.K.J. Ooi	WOLL#11312
					WOLL#11347
Acacia cultiformis	Grown at the University of Wollongong Ecological Research Facility	NA	NA	A.R.Hudson & M.K.J. Ooi	WOLL#11313
					WOLL#11348
Acacia linifolia	Grown at the University of Wollongong Ecological Research Facility	NA	NA	A.R.Hudson & M.K.J. Ooi	WOLL#11314
					WOLL#11349
Acacia penninveris	Warrumbungles National Park, NSW, Australia	31°17'49"S	149°02'02"E	A.Denham & I. Jansens	WOLL#11352
					WOLL#11353
Acacia caesiella	Warrumbungles National Park, NSW, Australia	31°17'49"S	149°02'02"E	A.Denham & I. Jansens	WOLL#11315
					WOLL#11350
					WOLL#11351

Appendix 5: Raw genetic diversity characteristics for each loci for each *Acacia suaveolens* populations sampled in Chapter 7.

Table S5.1. Raw genetic diversity characteristics for each stand by loci (based on all plants genotyped).

	N[1]	*A*[1]	*Ae*[1]	*AR*[1]	*pAR*[1]	*Ho*[1]	*He*[1]	NF[1]
Ang								
AB7	20	1.00	1.00	1.00	0.00	0.00	0.00	0.01
CD1	20	2.00	1.34	2.00	0.00	0.10	0.26	0.00
EF2	20	1.00	1.00	1.00	0.00	0.00	0.00	0.00
EF8	19	4.00	1.31	3.09	0.02	0.16	0.24	0.03
CD9	20	1.00	1.00	1.00	0.00	0.00	0.00	0.01
GH8	20	3.00	2.95	3.00	0.00	0.30	0.66	0.00
CD4	20	2.00	1.60	2.00	1.00	0.10	0.38	0.01
CD5	20	2.00	1.05	1.55	0.00	0.05	0.05	0.00
GH1	20	2.00	1.22	1.97	0.00	0.00	0.18	0.01
Cem								
AB7	24	4.00	1.78	3.42	0.00	0.25	0.44	0.00
CD1	24	3.00	1.61	2.91	0.00	0.21	0.38	0.00
EF2	24	3.00	2.33	2.96	0.00	0.25	0.57	0.00
EF8	24	4.00	1.54	3.16	0.26	0.25	0.35	0.00
CD9	24	4.00	2.59	3.77	0.15	0.33	0.61	0.00
GH8	24	7.00	2.52	5.05	0.46	0.21	0.60	0.00
CD4	24	4.00	2.25	3.56	0.00	0.25	0.56	0.00
CD5	24	3.00	2.99	3.00	0.00	0.33	0.67	0.00
GH1	24	4.00	2.80	3.71	0.00	0.42	0.64	0.00
CH								
AB7	24	1.00	1.00	1.00	0.00	0.00	0.00	0.00
CD1	24	3.00	2.74	3.00	0.00	0.29	0.64	0.00
EF2	24	2.00	1.55	2.00	0.00	0.29	0.35	0.00
EF8	24	4.00	2.87	3.46	0.25	0.38	0.65	0.00
CD9	24	1.00	1.00	1.00	0.00	0.00	0.00	0.00
GH8	24	5.00	2.34	4.72	0.92	0.25	0.57	0.00
CD4	24	2.00	1.13	1.85	0.00	0.04	0.12	0.00
CD5	24	2.00	1.49	2.00	0.00	0.25	0.33	0.00
GH1	24	2.00	1.18	1.92	0.00	0.17	0.15	0.00
CR								
AB7	25	1.00	1.00	1.00	0.00	0.00	0.00	0.00
CD1	25	3.00	2.27	3.00	0.00	0.00	0.56	0.00
EF2	25	2.00	1.13	1.83	0.00	0.04	0.11	0.00
EF8	25	3.00	1.23	2.96	0.44	0.20	0.18	0.00
CD9	25	1.00	1.00	1.00	0.00	0.00	0.00	0.00

GH8	25	3.00	1.87	2.83	1.00	0.08	0.47	0.00
CD4	25	2.00	1.13	1.83	0.00	0.04	0.11	0.00
CD5	25	2.00	1.47	2.00	0.00	0.00	0.32	0.00
GH1	25	2.00	1.04	1.44	0.00	0.04	0.04	0.00
DH								
AB7	25	1	1.00	1.00	0.00	0.00	0.00	0.00
CD1	25	2	2.00	2.00	0.00	0.12	0.50	0.00
EF2	25	2	1.99	2.00	0.00	0.12	0.50	0.00
EF8	25	3	2.90	3.00	0.00	0.16	0.66	0.00
CD9	25	2	1.77	2.00	0.00	0.08	0.44	0.00
GH8	25	3	2.02	2.91	0.00	0.24	0.51	0.00
CD4	25	2	1.81	2.00	0.00	0.12	0.45	0.00
CD5	25	2	1.13	1.83	0.00	0.04	0.11	0.00
GH1	25	1	1.00	1.00	0.00	0.00	0.00	0.00
EH								
AB7	20	1.00	1.00	1.00	0.00	0.00	0.00	0.01
CD1	20	2.00	2.00	2.00	0.00	0.20	0.50	0.01
EF2	20	2.00	1.22	1.97	0.00	0.00	0.18	0.01
EF8	19	5.00	4.43	4.93	1.93	0.05	0.77	0.08
CD9	19	1.00	1.00	1.00	0.00	0.00	0.00	0.05
GH8	20	3.00	1.49	2.55	0.00	0.30	0.33	0.01
CD4	20	1.00	1.00	1.00	0.00	0.00	0.00	0.01
CD5	20	2.00	1.05	1.55	0.00	0.05	0.05	0.01
GH1	20	2.00	1.92	2.00	0.00	0.20	0.48	0.01
Faulc								
AB7	25	5	1.23	3.01	0.01	0.20	0.19	0.02
CD1	24	5	3.08	4.41	0.14	0.29	0.68	0.08
EF2	25	4	2.427	3.52	0.12	0.44	0.59	0.02
EF8	25	6	3.93	5.69	0.02	0.20	0.75	0.07
CD9	24	6	2.776	4.55	1.46	0.38	0.64	0.06
GH8	24	12	7.529	9.25	1.06	0.58	0.87	0.04
CD4	25	4	2.129	3.64	0.21	0.36	0.53	0.02
CD5	25	4	3.141	3.83	0.00	0.28	0.68	0.04
GH1	23	4	3.023	3.94	0.00	0.26	0.67	0.14
GT								
AB7	13	4.00	2.62	3.98	0.09	0.38	0.62	0.01
CD1	13	4.00	2.68	3.98	0.00	0.54	0.63	0.01
EF2	13	3.00	2.50	3.00	0.00	0.54	0.60	0.01
EF8	13	7.00	4.45	6.79	0.98	0.46	0.78	0.01
CD9	13	3.00	2.66	3.00	0.00	0.23	0.62	0.02
GH8	13	5.00	3.60	4.84	1.14	0.62	0.72	0.01
CD4	13	3.00	2.60	3.00	0.02	0.46	0.62	0.01
CD5	13	5.00	3.45	4.98	0.00	0.38	0.71	0.01
GH1	13	3.00	2.09	2.98	0.00	0.46	0.52	0.01

HH								
AB7	24	1.00	1.00	1.00	0.00	0.00	0.00	0.00
CD1	24	1.00	1.00	1.00	0.00	0.00	0.00	0.00
EF2	24	2.00	1.18	1.92	0.00	0.17	0.15	0.00
EF8	24	3.00	1.19	2.42	0.00	0.00	0.16	0.00
CD9	24	1.00	1.00	1.00	0.00	0.00	0.00	0.00
GH8	24	3.00	1.83	2.96	0.01	0.08	0.45	0.01
CD4	24	1.00	1.00	1.00	0.00	0.00	0.00	0.00
CD5	24	2.00	1.04	1.46	0.00	0.04	0.04	0.00
GH1	24	2.00	1.09	1.71	0.00	0.08	0.08	0.00
MtH1								
AB7	25	4.00	1.59	3.50	0.08	0.12	0.37	0.01
CD1	25	3.00	1.73	2.69	0.00	0.08	0.42	0.01
EF2	25	4.00	1.58	3.26	0.00	0.12	0.37	0.01
EF8	25	6.00	3.17	5.03	0.00	0.24	0.68	0.01
CD9	25	5.00	0.65	3.57	0.04	0.28	0.39	0.01
GH8	23	10.00	4.90	7.74	1.49	0.26	0.80	0.07
CD4	25	4.00	3.23	3.69	0.21	0.36	0.69	0.01
CD5	25	5.00	2.54	3.96	0.01	0.36	0.61	0.01
GH1	23	3.00	1.43	2.87	0.00	0.00	0.30	0.11
MtH2								
AB7	25	7.00	3.51	5.52	0.09	0.60	0.72	0.00
CD1	25	4.00	2.44	3.52	0.37	0.36	0.59	0.01
EF2	25	3.00	2.18	2.99	0.00	0.52	0.54	0.00
EF8	25	5.00	1.61	4.03	0.69	0.28	0.38	0.01
CD9	25	2.00	1.04	1.44	0.00	0.04	0.04	0.01
GH8	25	9.00	5.21	7.14	0.06	0.36	0.81	0.02
CD4	25	3.00	1.71	2.44	0.00	0.32	0.41	0.01
CD5	25	5.00	3.07	4.59	0.30	0.56	0.67	0.01
GH1	25	6.00	4.92	5.74	0.07	0.52	0.80	0.01
MtT								
AB7	25	2.00	1.13	1.83	0.01	0.12	0.11	0.00
CD1	25	4.00	1.72	3.33	0.00	0.28	0.42	0.00
EF2	25	3.00	2.24	2.83	0.26	0.36	0.55	0.00
EF8	25	4.00	2.42	3.35	0.00	0.16	0.59	0.01
CD9	25	3.00	1.23	2.52	0.00	0.12	0.18	0.00
GH8	25	9.00	7.02	7.90	1.04	0.60	0.86	0.00
CD4	25	3.00	1.52	2.91	0.00	0.24	0.34	0.00
CD5	25	6.00	4.63	5.59	0.69	0.24	0.78	0.01
GH1	25	5.00	3.62	4.66	0.98	0.12	0.72	0.02
Nar								
AB7	20	1.00	1.00	1.00	0.00	0.00	0.00	0.00
CD1	20	2.00	1.92	2.00	0.00	0.20	0.48	0.00
EF2	20	1.00	1.00	1.00	0.00	0.00	0.00	0.00

EF8	20	3.00	1.43	2.79	0.00	0.05	0.30	0.00
CD9	20	1.00	1.00	1.00	0.00	0.00	0.00	0.00
GH8	20	2.00	1.84	2.00	0.00	0.10	0.46	0.00
CD4	20	2.00	1.88	2.00	0.00	0.25	0.47	0.00
CD5	20	2.00	1.05	1.55	0.55	0.05	0.05	0.00
GH1	20	1.00	1.00	1.00	0.00	0.00	0.00	0.00
PPrd								
AB7	24	1.00	1.00	1.00	0.00	0.00	0.00	0.01
CD1	24	3.00	2.30	2.99	0.00	0.25	0.57	0.00
EF2	24	1.00	1.00	1.00	0.00	0.00	0.00	0.00
EF8	24	4.00	2.27	3.91	0.00	0.25	0.56	0.00
CD9	24	2.00	1.28	1.98	0.00	0.00	0.22	0.00
GH8	24	4.00	1.19	2.63	0.00	0.04	0.16	0.01
CD4	24	1.00	1.00	1.00	0.00	0.00	0.00	0.01
CD5	24	3.00	1.18	2.31	0.00	0.13	0.16	0.00
GH1	21	3.00	1.34	2.74	0.78	0.00	0.25	0.17
TC								
AB7	12	5.00	1.73	4.83	0.92	0.50	0.42	0.01
CD1	12	5.00	3.65	4.92	0.56	0.58	0.73	0.01
EF2	12	5.00	3.17	5.00	1.00	0.33	0.68	0.03
EF8	12	4.00	2.42	3.91	0.00	0.08	0.59	0.05
CD9	12	3.00	1.65	2.92	0.00	0.33	0.39	0.02
GH8	12	7.00	4.00	6.83	0.00	0.33	0.75	0.02
CD4	12	3.00	2.13	3.91	1.00	0.33	0.53	0.02
CD5	12	4.00	2.17	3.91	0.00	0.33	0.54	0.02
GH1	11	3.00	2.35	3.00	0.00	0.45	0.57	0.07

[1]N = number of individuals; *A* = Number of different alleles; *Ae* = Effective number of alleles; *AR* = Rarefied allelic richness; *pAR* = Private allelic richness; H_o = Observed heterozygosity; H_e = Expected heterozygosity; NF = Null allele frequencies.

CERTIFICATION

I, Alice Rose Hudson, declare that this thesis, submitted in partial fulfilment of the requirements for the award Doctor of Philosophy, in the School of Biological Sciences, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. This document has not been submitted for qualifications at any other academic institution.

A.R. Hudson

Alice Rose Hudson

14th December 2016

As the primary supervisor, I, Professor David Ayre, declare that the greater part of the work in each article listed is attributed to the candidate, Alice Rose Hudson. In each of the above manuscripts, Alice led the study design and was primarily responsible for data collection, analysis and interpretation. The first draft of each manuscript was written by the candidate, who was then responsible for responding to comments made by her co-authors. The co-authors, Professor David Ayre and Doctor Mark Ooi, were responsible for assisting with study design, interpreting data and editing of the manuscripts where necessary. Alice has been solely responsible for submitting relevant manuscripts for publication to appropriate journals, and she has been in charge of responding to reviewers' comments, with assistance from her co-authors.

Alice Rose Hudson	Professor David Ayre
PhD Candidate	Principal Supervisor
14th December 2016	14th December 2016