A multi-scale exploration of the drivers and implications of germination strategy in Australian alpine plants

Annisa Satyanti

A thesis submitted for the degree of Doctor of Philosophy of the
Australian National University
December 2017

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Declaration

The research presented in this thesis is my original work. All of the chapters are co-authored. The authorship order indicates the intellectual input and workload. No part of this thesis has been submitted for any previous degree.

Annisa Satyanti

19 December 2017

Acknowledgements

Doing a PhD together with raising a family in a foreign country was, so far, the biggest adventure of my life. It started with a mixture of feelings when I flew solo to Canberra to chase my dream but which might means leaving my family behind. I am so grateful to have my children and husband around me again soon after I arrived in Canberra. In the end, what I found to be the hardest challenge, juggling between PhD and looking after young children, indeed turned into the most rewarding journey of my life. I would like to conclude this chapter by acknowledging the people who have crossed path with me and made this journey meaningful.

First of all, I would like to thank my chair of panel: Adrienne Nicotra. Thanks for taking me as your student, help me to get the ANU scholarship, and being an awesome supervisor. I would not have made it without your keen and continuous support throughout this work. Thanks for your generosity and endless motivations, and being so optimistic. I really admire your knowledge and great insight on plant ecology. You will always be my inspiration in academic life as well as a role model in running a family.

Lydia Guja, my co-supervisor, for being there all the time more than I could have hoped for. Thanks for being so patient and kind to me and for keeping a trust and confidence in me that I can finish well. Thanks for the constructive comments and endless support. I won't make it without you! Also, thanks for keeping me in the seed circle, it was really meaningful.

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A PhD is about persistence and determination, but I would not make it without endless love, encouragement, and prayers from my parents. I dedicate this work for my parents. *Ayah, ibuk, matursuwun sanget pangestunipun. Karya tulis ini kupersembahkan untuk ayah dan ibuk. Terimakasih, Ayah dan Ibuk.*

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Abstract

Alpine ecosystems are particularly sensitive to the effects of global warming because they are characterized by species adapted to low temperatures. Although adapted to low temperatures, alpine plants may still be capable of persisting under a changing climate and this will largely be dependent on the ability of their seeds to germinate, establish and reproduce *in situ*, or after dispersal to new sites. The effects of climate change on regeneration from seeds may be influenced by intrinsic factors such as seed longevity (how long seeds remain viable and able to germinate after dispersal), or germination success (the proportion of a seed population that will germinate following exposure to future climate scenarios), and the seasonal timing of germination. Germination strategy, among the earliest life history traits expressed, reflects germination timing and pattern across the progression of seasons after seed dispersal and I therefore expected germination strategy would be a key trait affecting the responses of species to climate change.

To examine seed longevity, which reflects the potential for survival in a soil seed bank or in *ex situ* conservation, I explored the seed intrinsic lifespan (longevity) and its correlates across 56 species (Chapter 2). I showed that Australian alpine seeds are short-lived, similar to alpine seeds elsewhere and relatively shorter-lived compared to non-alpine Australian seeds. Although relatively short-lived, the seeds do survive long enough to form persistent soil seed banks. However, it is unclear whether the conditions required for germination will still be present in future climate scenarios. Thus, I conducted experimental studies that investigated how germination success in future climate scenarios will differ from germination in ambient climate scenarios, and whether germination strategy moderates the germination responses across 39 species (Chapter 3). This study highlights that when comparing across species, germination strategy moderates the effect of changing climate on germination success such that species with immediate germination strategy that germinate readily after dispersal are less sensitive to changing temperature and winter duration compared to species with dormant seed components (staggered and postponed strategies).

Since germination strategy is a significant factor in determining the responses of species to climate change I also examined how within-species variation in germination strategy might affect the adaptability or plasticity of species in response to climate change. I

examined the germination strategy variation within-species to specify the climate variables driving variation in germination strategy in wild populations (Chapter 4). I found that within the alpine herb *Oreomyrrhis eriopoda*, variation in germination strategies is mainly determined by temperature variability in the climate of origin. I also examined the consequences of germination strategy on not just germination but the whole plant life cycle, including early establishment, vegetative and reproductive traits and phenology, under ambient and future climate scenarios (Chapter 5). Under warmer soil temperatures, survival was reduced, and lifetime (time to senesce) and reproductive period were both condensed. Several vegetative and reproductive traits were affected by warming and the response indeed differed across germination strategies. This reveals that within-species variation in germination strategy may also enable species to express different patterns in life history traits across development, not just germination timing. Lastly, I also tested whether germination strategy is plastic across generations. Intraspecific germination strategies in *O. eriopoda* were not plastic across generations, even though labile in the species.

The results highlight that across scales immediate species and immediate populations are less constrained by changing climate. What is interesting is the staggered germination strategy that maintained seasonal variations in seedling emergence, at current climate but also under future climate scenarios. Postponed strategy also tend to maintain germination under warmer germination temperatures, and hence, no shift in germination as reported in alpine seed elsewhere is observed across Australian alpine seeds. The germination strategy within species that is observed to be a conserved trait and did not change with maternal condition raises some questions. If germination strategy is not plastic across generations, then why does recent climate matter? Will germination strategy change when maternal conditions they are exposed to repeated over a few more generations? And how do the adult traits differ among the mothers?

Together, the findings of this thesis contribute to improved understanding of alpine seed and seedling ecology, in particular how among- and within-species variation in germination strategy may influence species’ responses to climate change by affecting germination success and contributing to the persistence of species and/or populations. These findings have applied and pure ecological and evolutionary relevance to Australia and will make a substantive contribution to our global understanding of alpine plants.

Table of Content

Design by Afiat Sukmaraga

Chapter 1

Introduction

The humble seed is not a passive entity, but rather an individual constantly interacting with its environment. In tropical rainforests, seeds germinate readily even if conditions might not be advantageous for germination and many seedlings are well equipped to survive very low light on the forest floor for years. Conversely, in a seasonal environment like an alpine system where the growing season is short, part of the year is spent under snow, and there are extremely heterogeneous temporal and spatial environments – a successful transition from seeds to new individuals is also possible. In a seasonal environment, seed dormancy, an evolutionary mechanism that enables seed germination to be delayed or synchronised with changes in environmental conditions (Baskin & Baskin, 2014, Fenner & Thompson, 2005) seems to be the key. For me, coming from a tropical country where seed germination can happen in the blink of an eye at any time of the year, the variability among biomes is genuinely intriguing and inspired me to investigate the scientific facets of this phenomenon. In this thesis, I aim to better understand how and why alpine seeds do what they do (germinate or not germinate) and when they do it. I set forth to explore Australian alpine seed and seedling ecology to better document the drivers and implications of seed dormancy and germination timing on plant life history in the Australian Alps, now and under future climate scenarios.

The Australian Alps, like many alpine regions worldwide, are rich in biodiversity (Costin *et al.*, 2000, Costin *et al.*, 2002), and is identified as one of the centres of plant endemism (Crisp *et al.*, 2001). In Australia, only 0.15% (11500 km^2) of the landmass is alpine and subalpine (Figure 1), but this area is home to 212 plant species, including 30 exclusively alpine and 21 species endemic to the Kosciuszko alpine zone (Costin *et al.*, 2000). Alpine plant communities contribute largely to the structure of the alpine system and associated ecosystem services, including retention and provision of water across the catchment. Alpine ecosystems and their plant communities worldwide have been identified as threatened or vulnerable to a changing climate (Beniston, 2003, Beniston *et al.*, 1997, Diaz & Bradley, 1997, Hughes, 2011). The Australian Alps bioregion is characterised by snow that currently may persist for 4 to 6 months on the higher peaks or only a few weeks at elevations of < 1400 m, and frost that can occur throughout the year (Edmonds *et al.*, 2006, Green & Pickering, 2009, Whetton *et al.*, 1996). The current mean annual temperatures are of 3 – 12 °C, minimum air temperatures of -7 °C and maximum air temperature of 29.5 °C (NSW National Parks and Wildlife Service, 2003). Future climate predictions for the Australian Alps suggest that snow depth will decrease and there will be a ~3 °C temperature increase and a further decrease in snow depth up to 99% relative to 1990 by 2050 (Hennessy *et al.*, 2007). Indeed, snowpack duration has already been shortened by ~9 days since 1954 (Hennessy *et al.*, 2003, Sánchez-Bayo & Green, 2013).

In Australia, where the alpine region extends across an elevational range of just 400 m and some plant communities depend on snowdrifts (Costin *et al.*, 2002), conserving alpine flora and understanding the scope and potential of alpine plant establishment under changing climate conditions is crucial. Plants, as sessile organisms, must be able to respond to novel conditions to persist. Alpine plant responses to a changing climate and the disturbances associated with it will reflect their potential to recruit *in situ* or migrate to higher elevations (track climate). Both processes are inherently dependent on establishment from seed (Lenoir *et al.*, 2008, Parolo & Rossi, 2008, Vittoz *et al.*, 2009, Walck *et al.*, 2011) from either dispersed seeds, or those present in the sizeable soil seed banks found in the Australian Alps (Hoyle *et al.*, 2013, Venn, 2010, Venn & Morgan, 2009).

The Australian alpine region and the services it provides are considered to be particularly vulnerable to global change, due to its small vertical range, limited extent and the relative isolation of high-elevation habitats (Hennessy *et al.*, 2007, La Sorte & Jetz, 2010, Laurance *et al.*, 2011, Reisinger *et al.*, 2014, Steffen *et al.*, 2009). Since upward migration will be impeded by habitat fragmentation and limited by the availability of suitable sites, alpine plant species may be particularly dependent on tolerance, plasticity and adaptation through modification of physiological processes at seeds and seedling life stages. Indeed, climate change may impact plant population dynamics by influencing seed germination via seed maturation or seed traits (e.g. seed mass) or via seed persistence in the soil (Walck *et al.*, 2011).

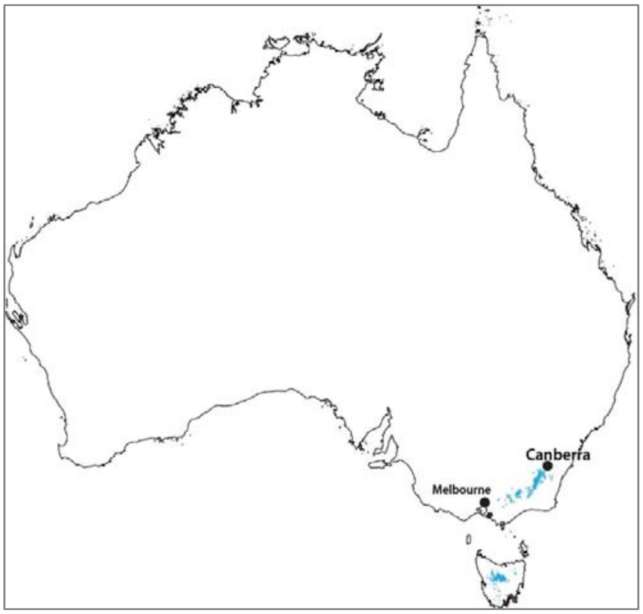


Figure 1. Outline of the Australian alpine region. Climate zones defined as alpine (Australian Alpine and Tasmania Central Highlands, based on Interim Biogeographic Regionalisation for Australia – IBRA version 7) are shaded in blue.

Thus, in this study I aimed to examine many features of Australian alpine seeds, encompassing seed longevity, germination strategies and seedling growth under future climate scenarios, across numerous species as well as within-species, and how these features may be associated with variations in germination strategy. My study extended beyond the earliest phenological transition, germination, through to adult plant development and reproductive phenology, as well as transgenerational effects on progeny.

Quantifying variation in seed and germination traits (seed longevity and germination strategy) among species

Plant population dynamics and future distributions of species are significantly affected by the ability of seeds and seedlings to establish and recruit successfully under novel environmental conditions (Briceño *et al.*, 2015, Pearson, 2006, Thuiller *et al.*, 2008). In alpine ecosystems, although clonal (vegetative) regeneration is thought to be dominant, recruitment via seeds is often evident (Briceño *et al.*, 2015, Venn & Morgan, 2009). Seeds are an important aspect of alpine plant regeneration, indicated by a high rate of seedling establishment (Erschbamer *et al.*, 2001, Forbis, 2003, Körner, 2003, Schlag & Erschbamer, 2000), the contribution seeds make to high gene flow (Pluess & Stöcklin, 2004), and the corresponding presence of diverse genotypes (Gabrielsen & Brochmann, 1998, Jonsson *et al.*, 1996). Alpine soils contain a sizeable amount (approximate range of 150 up to > 3500 seeds per m^2) of viable and persistent seeds (Arroyo *et al.*, 1999, Hoyle *et al.*, 2013, Marcante *et al.*, 2009, Schwienbacher *et al.*, 2010, Venn & Morgan, 2010, Walck *et al.*, 2005). Further, half of the alpine species in the standing vegetation may also be present in the soil seed bank (McGraw & Vavrek, 1989).

Plants are equipped with various strategies to help them disperse across space and time (Fenner & Thompson, 2005). There are two key elements that enable plants to persist; first, their inherent seed longevity which affects how long they may survive – *in situ* and *ex situ* – and second, the germination strategy that represents the degree of seed dormancy as well as resultant germination timing and pattern (Hoyle *et al.*, 2015). Seed longevity and germination responses of species with varying strategies under future climates are examined across a wide range of Australian alpine species in the first half of this thesis (Chapter 2 and 3).

Seed longevity

Seed longevity, seed dispersal, and generation time are complex functional traits that could influence plant responses to changing environments (Anderson *et al.*, 2012) . The inherent seed longevity of a species is crucial to enable dispersal over time (Long *et al.*, 2015) and is associated with persistence in the soil which spreads the risk of recruitment failure in a variable and changing environment (Ooi *et al.*, 2009, Thompson, 2000). Hence, seed longevity might be a significant aspect of the evolution of a species' germination strategy. Seed longevity is associated with many factors (Box 1) and contributes to seed persistence in the soil together with other variables such as soil humidity, temperature, microbial activity, and predation (Long *et al.*, 2015). The effect of some variables on Australian alpine seeds are known, for example, soil warming reduces germination from the soil seed bank, but increases the diversity of species that germinate (Hoyle *et al.*, 2013). Thus, warming will potentially change community composition as mediated by direct effects on germination from the soil seed bank. Seed longevity has been associated with many plant and seed traits as well as environmental factors, but there is not a clear pattern of which intrinsic seed and plant traits or climate aspects drive seed longevity across different scales and biomes, let alone in the Alps (Box 1). Understanding seed longevity will provide important insight on seed persistence *in situ*, as well for *ex situ* seed conservation and seed bank management (Box 1). Seed longevity is related to survival *ex situ*, which depends on the temperature and relative humidity in *ex situ* storage (Ellis & Roberts, 1980). In Chapter 2, across a wide range of Australian alpine species, I explored the seed traits and environmental variables putatively associated with seed longevity and performed model selection to determine the seed and ecological correlates of longevity.

Germination strategy

Seed dormancy is a common trait among Australian alpine plants (Hoyle *et al.*, 2015). Dormancy prevents germination during times when suitable germination conditions are sporadic and there is a low probability of seedling survival (Fenner & Thompson, 2005), such as in autumn or early spring when frost may occur. Dormancy is generally investigated by describing the fraction of seeds that will germinate at a point in time and under a given set of conditions (e.g. Schütz, 2002, Schwienbacher *et al.*, 2011). Rarely is the impact of changes in the dormant fraction throughout seasonal progression considered, despite the significance of season for germination and establishment of alpine plants. Therefore, a method of describing and categorizing the multidimensional changes in dormant fractions, as well as seasonal germination timing, was developed to summary species germination strategy (Hoyle *et al.*, 2015).

Across Australian alpine seeds, three germination strategies have been described: immediate, staggered and postponed (Hoyle *et al.*, 2015). Species with non-dormant seeds germinate immediately after natural dispersal in autumn. The postponed strategy is comprised of species with dormant seeds that germinate after winter. The final category is species with seed germination that is staggered over time. Australian alpine celery (*Aciphylla glacialis*), which exhibits a postponed germination strategy, requires at least six weeks of winter to germinate (Hoyle *et al.*, 2014), suggesting that climatic cues and seasonal changes could affect the germination pattern in these and other alpine species. Seed germination is therefore likely to be affected by climate change as many species have specific dormancy alleviation and germination requirements associated with climatic variables (reviewed in Donohue *et al.*, 2010, Walck *et al.*, 2011).

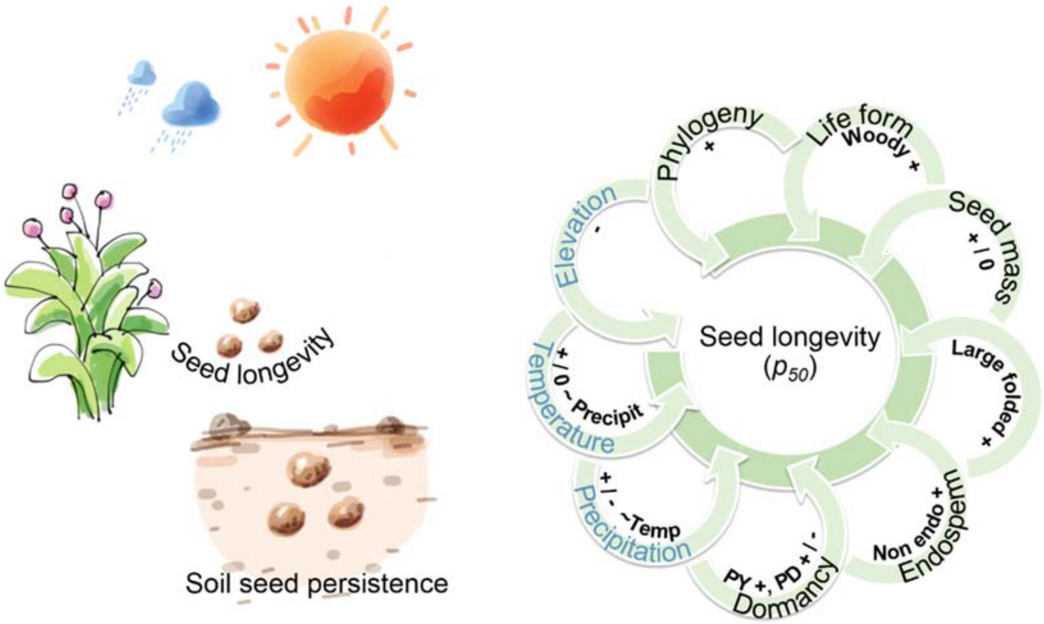
Seed germination of alpine (and sub-arctic) species vary considerably in response to climate warming and seasonal change that either increase, decrease, or shift germination time (Bernareggi *et al.*, 2016, Graae *et al.*, 2008, Hoyle *et al.*, 2014, Hoyle *et al.*, 2013, Milbau *et al.*, 2009, Milbau *et al.*, 2017, Mondoni *et al.*, 2015, Mondoni *et al.*, 2012, Shevtsova *et al.*, 2009). This is of great significance in the Alps where it means that seedlings will be exposed to one of two very different thermal extremes, either they will need to persist in snow or frost conditions, or they will need to tolerate the warm dry summer conditions in the Australian Alps early in their life cycle. Furthermore, seedling size and development relative to the growing season is also vastly different (established versus emergent seedling).

Under future climate scenarios, species with non-dormant seeds will likely have an advantage over species with dormant seeds as the growing season becomes longer and seeds can germinate readily. However, we might expect that species with an immediate germination strategy that emerge in autumn will be exposed to harsh conditions if snow insulation during winter disappears. Conversely, species with a postponed germination strategy may survive winter better as they stay as dormant seeds in the soil, but dormancy alleviation can be disrupted, for example, by shorter snow duration (cold stratification) (Hoyle *et al.*, 2014). Soil warming reduces seedling emergence (germination) from alpine soil seed banks but germination responses to warming are species-specific as indicated by a higher diversity of species emerging after under warming (Hoyle *et al.*, 2013). How seed germination of Australian alpine species may respond to the projected changes and to what extent responses may differ across species and across germination strategies, is not yet known. A study to better understand the relationships among germination strategy and germination characteristics, and the consequences of those traits now and under future climate scenarios, was conducted across 39 alpine species (Chapter 3).

Determinants of a species' capacity to respond to climate change (within-species)

In alpine regions, a substantial variation of thermal micro-environments is ubiquitous even over short spatial scales (Scherrer & Körner, 2010). Such a highly variable environment could drive diversification of life history and functional traits within an alpine species (Briceño *et al.*, 2015), including the evolution of diverse germination traits as a form of adaptation to the heterogeneous alpine environment (Wagner & Simons, 2009). Alpine regions typically contain a high proportion of endemic, cold-adapted species but their capacity to respond to climatic change is largely unknown. Nevertheless, species with high levels of phenotypic variation within and among populations may have greater capacity to respond to changing climate (Nicotra *et al.*, 2010).

Within-species variation in germination traits (including the degree of dormancy) may be one such example of variation that could affect responses to climate change. Within-species variation in germination traits can be due to genetics, parental environment (including epigenetic effects) or the interaction thereof (Baskin & Baskin, 2014, Donohue, 2005, Herman & Sultan, 2011). Climatic variables such as maternal and seed development temperature (Baskin & Baskin, 2014, Donohue *et al.*, 2008, Fenner, 1991, Gutterman, 2000), as well as local temperature (Donohue *et al.*, 2005, Zettlemoyer *et al.*, 2017) affect within-species variations in seed and germination traits.



Box 1. Seed longevity index (p_{50}) can be used to estimate how long seed can remain viable in the soil (seed persistence *in situ*) as well as in the *ex situ* storage. Here, I synthesised the plant, seed, and environmental correlates of the p_{50} values (measured in days in controlled ageing) across studies that examined seed longevity using a standard controlled seed ageing method (Newton *et al.*, 2009). Generally, seeds with impermeable seed coat (physical dormancy) or seeds with deep physiological dormancy are longer lived (Long *et al.*, 2015). Longer-lived seeds tend to be non-endospermic (Merritt *et al.*, 2014), and have an embryo type that is typically large, e.g. folded, investing, bent embryo (Merritt *et al.*, 2014, Probert *et al.*, 2009). Seed longevity increases with seed mass across Australian species (Merritt *et al.*, 2014), but no correlation is found across 195 species worldwide (Probert *et al.*, 2009). Woody and serotinous perennials tend to have longer lived seeds (Merritt *et al.*, 2014). Mean annual temperature is not significant (Merritt *et al.*, 2014, Mondoni *et al.*, 2010) or positively related to p_{50} (Probert *et al.*, 2009). The association between temperature and seed longevity is dependent on humidity. Across global scale and Italian flora, seeds from hot-dry were longer-lived than those from cool-wet environment (Mondoni *et al.*, 2010, Probert *et al.*, 2009), but for Australian species, seeds from hot-wet were longer-lived seeds than those from cool-drier regions (Merritt *et al.*, 2014). Seed longevity decreased with elevation, p_{50} from alpine seeds was fifty percent lower than lowland seeds with mean p_{50}values of 28.2 and 56.1, respectively (Mondoni *et al.*, 2010). Note: The association between each variable to p_{50} is given in 0 (non-significant), + (positive relationship), +/- or +/0 (inconsistent depending on biomes or scales), - (negative association).

A mixed germination pattern within a single species is apparent for numerous Australian alpine species, whereby both dormant and non-dormant seed characteristics and consequently autumn and spring germination were exhibited within the same species, and even at times within a population collection (Hoyle *et al.*, 2015). *Oreomyrrhis eriopoda*, for example, was observed by Hoyle *et al.* (2015) and seed banks to demonstrate extreme within-species germination timing and pattern variations. Germination timing defines the post-germination environment and ultimately affects seedling establishment, survival, phenology, and has long-term consequences over a plant's life cycle (Cleland *et al.*, 2007, Donohue, 2002, Inouye, 2008). However, much remains to be understood about the drivers and consequences of that within-species variation and to what extent it may enable alpine species to adapt to changes in climate. To address this question, I performed germination assays across 29 populations of *Oreomyrrhis eriopoda* (Apiaceae), a widespread Australian herb (Figure 2), to determine the climate determinants of germination strategy as well as whether subsequent establishment traits are affected by germination strategy (Chapter 4).

Phenology shifts

During a plant's life, not only germination (the transition from seed to seedling stage) but also the transition from vegetative to reproductive stage, determines their fate (Elzinga *et al.*, 2007, Venable & Brown, 1988). Flowering time is central to plant reproductive success. Flowering time dictates plant reproductive success as it influences flower production (Saavedra *et al.*, 2003), the environmental condition when seed develops (Giménez-Benavides *et al.*, 2007, Lacey *et al.*, 2003), and the life history of their offspring, including germination phenology (Galloway & Etterson, 2007). Under future climate scenarios, reproductive phenology shifts are projected, flowering time in particular has already advanced (Parmesan & Hanley, 2015, Parmesan & Yohe, 2003).

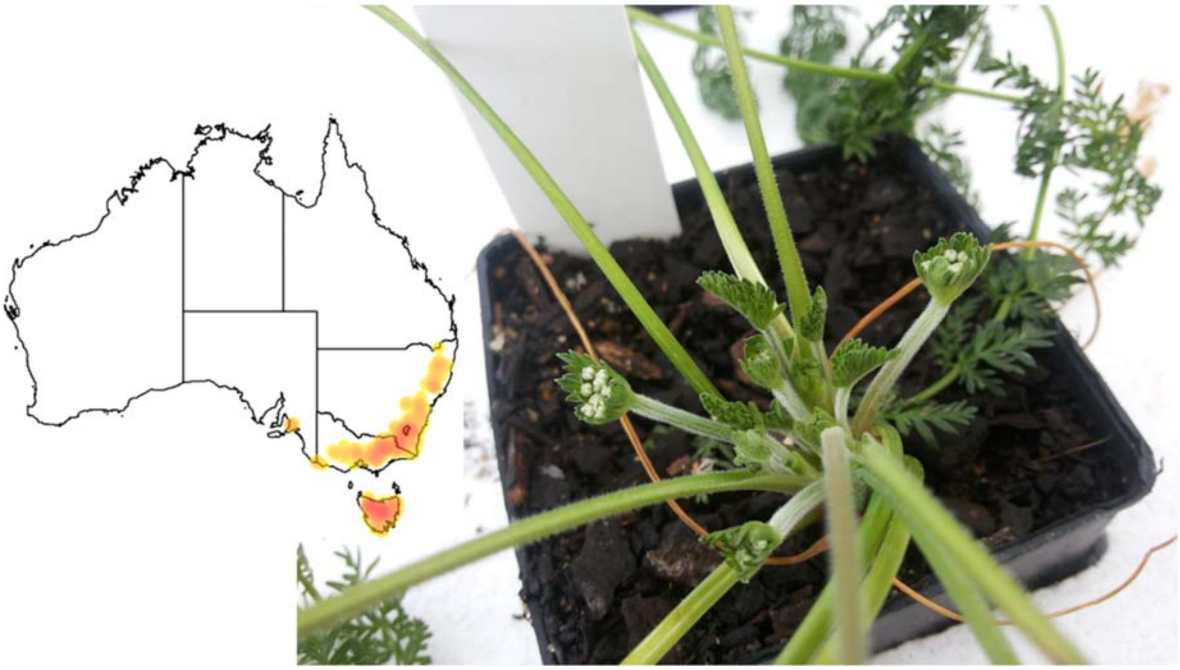


Figure 2. *Oreomyrrhis eriopoda* (Apiaceae) is an endemic Australian herb with a main distribution in the alpine bioregion of south-eastern Australia and Tasmania (yellow and orange shaded area). This species exhibits variations in germination strategy across geographic distribution.

Besides altering phenology, global warming is known to affect vegetative and reproductive traits, such as leaf length, biomass, number of leaves, leaf mass, seed yield, seed mass and number of fruits (Arft *et al.*, 1999, Debouk *et al.*, 2015, Hedhly *et al.*, 2009, King *et al.*, 1999, Liu *et al.*, 2012), and survival (Bernareggi *et al.*, 2016, Milbau *et al.*, 2017, Mondoni *et al.*, 2015). Under warming, not only is flowering time advanced but the whole reproductive period could be condensed or expanded (CaraDonna *et al.*, 2014, Menzel *et al.*, 2006, Root *et al.*, 2003, Taylor & Garbary, 2003). Little is known on how the effect of climate change on reproductive phenology, including timing to seed maturation, and the duration of seed production, might also vary with germination strategy. Given the effect of germination strategy on the timing of establishment these variations in germination strategy may also have differing long-term effects on the plant's life cycle (Donohue *et al.*, 2010). I examined the effect of soil warming (to simulate a warmer alpine establishment scenario) across the lifetime of *O. eriopoda* plants, considering aspects of seed, vegetative and reproductive traits, survival, and the reproductive phenology and explored how variation in germination strategy affected that response (Chapter 5).

Transgenerational effects

Conditions during flowering and seed development are known to affect seed traits, such as longevity (Bernareggi *et al.*, 2015, Kochanek *et al.*, 2011), the degree of dormancy (Mondoni *et al.*, 2012), and germination (Fernández-Pascual & Jiménez-Alfaro, 2014). However, to what extent plant species respond to changes in the environment via rapid evolution or plasticity is not clear (Franks *et al.*, 2014). Understanding the factors that affect seed morphological and physiological characteristics, including seed dormancy, and germination plays an important role to forecast the future plant dynamics in warmer climate. Offspring phenotypes are determined by genetic inheritance and by non-genetic influences of their environments, and adjusting phenotypes through plasticity can be exhibited within-and across-generations (Auge *et al.*, 2017). I examined the occurrence of transgenerational plasticity in *O. eriopoda* to assess the effects of maternal environment on the offspring's germination strategy. Additionally, I looked at how the patterns of maternal environment (pre-dispersal) vs progeny environment (post-dispersal) influence the offspring's germination characteristics (Chapter 5).

Thesis aims and structure

As described above, the main aim of this thesis was to explore regeneration via seeds in Australian plants, with a special focus on seed germination among species and within-species, as a basis for understanding alpine plant dynamics, including their responses to environmental change. As regeneration via seeds is determined by vegetative traits and reproductive phenology and is carried over generations, these aspects were also covered.

This thesis is presented as a series of papers each exploring a different aspect of the ecology of Australian alpine seed germination, other reproductive aspects, plasticity and maternal effects, in the context of climate change. Chapters 2 to 5 are written in the form of manuscripts, with Chapter 2 and 4 under review and the others to be submitted to peerreviewed academic journals. As such, there is some repetition in introduction or discussion sections between chapters, and some variation in formatting.

Chapter 1: “Introduction”

Chapter 2: “Patterns and drivers of seed longevity across Australian alpine plants”

(Annisa Satyanti, Adrienne B. Nicotra, Thomas Merkling, Lydia K. Guja)
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The seed longevity of Australian alpine seed is determined using controlled accelerated ageing and the seed traits and environmental correlates are then analysed. Identifying seed longevity is important to reveal the significance of being relatively short- or long-lived in determining germination strategy, seed persistence in the soil seed bank, and management of seed collections in *ex situ* conservation.

I designed, carried out, analysed and wrote this chapter. ABN and LKG advised on design and substantial contributions on the draft. LKG & TM advised on statistical analyses. All authors commented on drafts.

Chapter 3: “Future climate effects on alpine plant regeneration via seed are modulated by the species’ germination strategy”

(Annisa Satyanti, Toton Liantoro, Lydia Guja, Susanna Venn, Terry Neeman, Adrienne Nicotra)
In preparation for submission to Oikos.

Australian alpine species with various germination strategies are investigated to determine, for the first time, how projected future climate may influence the germination response of these species, a trait that will be crucial for species persistence and community composition into the future.

I designed, analysed and wrote this chapter. ABN and LKG advised on design and provided substantial feedback on the draft. AS and TL carried out the experiment. TN advised on statistical analyses. All authors commented on drafts.

Chapter 4: "Temperature variability drives within-species variation in germination strategy and establishment characteristics of an alpine herb"

(Annisa Satyanti, Lydia K. Guja, Adrienne B. Nicotra)

Manuscript under review, AoB Plants.

An exploration of within-species variation in germination strategy and investigation of the determinants, as well as associated seed traits and consequences on seedling growth, was carried out. This study provides evidence that within-species variations potentially contribute to species persistence in a novel environment.

I designed, carried out, analysed and wrote this chapter. ABN advised on design and analysis. ABN and LKG advised on the draft and contributed in the revisions.

Chapter 5: "Effects of warming on development and phenology traits depend on germination strategies in the alpine herb *Oreomyrrhis eriopoda*, and vary during different parts of the life cycle"

(Annisa Satyanti, Lydia K. Guja, Toton Liantoro, Morgan Thomas, Adrienne B. Nicotra)

Manuscript prepared for submission to Global Change Biology.

An experiment involving manipulation of soil temperature was conducted to examine the effect of warming on the plant life cycle and how germination strategy moderates vegetative, reproductive and phenological responses to warming within a single species. I also examined the transgenerational effect of maternal warming on seed and germination traits of the progeny.

I designed, carried out, analysed and wrote this chapter. ABN advised on design and analysis. ABN and LKG contributed substantially in the drafts. AS and TL performed the phenological observations and periodic plant monitoring and measurement. MT and TL assisted in the trans-generation experiment. All authors commented on drafts.

Chapter 6: "Synthesis: Regeneration from seed across Australian alpine plants with varying germination strategies under changing climate"

This chapter summarises and synthesises the results of the preceding chapters, and provides a discussion of future research directions.

Appendix: "An Australian view on current directions in alpine seed and seedling ecology"

(Annisa Satyanti, Lydia K. Guja, Adrienne B. Nicotra)

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Chapter 2

Seed mass and elevation explain variation in seed longevity of Australian alpine plants

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Abstract

Conserving alpine ecosystems and the plant communities they contain using *ex situ* conservation requires an understanding of seed longevity. Knowledge of seed longevity may determine the effectiveness of *ex situ* seed banking for alpine plant conservation, and may provide insight into plant recruitment in situ. We sought to determine the influence of elevation and climatic variables, as well as plant and seed traits, on the seed longevity of 57 species inhabiting a unique biome, (sub-) alpine regions of mainland Australia. Seed longevity was estimated using controlled accelerated ageing tests to determine the time taken for seed viability to fall by 50%. We found that, across the study species, like alpine seeds elsewhere in the world, Australian alpine seeds are relatively short-lived and overall shorter-lived than Australian plants in general. Seed mass and elevation explained most of the variation in seed longevity among the Australian alpine species considered. Species with larger seed mass, and collections made at higher elevations, were found to have relatively short-lived seeds. Phylogeny, however, explained very little of the variation in longevity. Our results suggest that viability testing for Australian alpine seeds in *ex situ* seed banks should be conducted with shorter intervals than for the non-alpine flora. This study highlights how seed longevity in the Australian Alps is not dictated primarily by evolutionary lineage but rather by a complex combination of environmental variables and intrinsic seed characteristics. Potential implications for conservation *ex situ* and *in situ* in the context of climate change are discussed.

Keywords

Alpine seeds, controlled ageing, comparative longevity, ecological correlates, elevation, lifespan, p_{50}*, seed traits*

Introduction

The potential for many plant species to respond to climate change and other disturbances depends heavily on seeds. Seeds enable plants to regenerate and re-establish either in their current site or elsewhere and also to disperse germination over time. Seeds can persist in the soil for thousands of years (Shen-Miller *et al.*, 1995; Sallon *et al.*, 2008; Yashina *et al.*, 2012) and this dispersal over time assists plants to spread the risk of recruitment failure (Thompson, 2000; Ooi *et al.*, 2009). This realised persistence *in situ* is a result of intrinsic seed longevity in concert with other biotic and abiotic factors (e.g. microclimatic conditions, granivory, disturbance). Measures of seed longevity therefore describe the potential lifespan of a mature seed underlain by complex interactions of physiological and physical traits (Long *et al.*, 2015).

Understanding variation in and drivers of longevity is important for effective *ex situ* seed banking because it underpins the selection of viability re-test intervals and re-collection strategies (Probert *et al.*, 2009). Direct measures of seed longevity and persistence, however, are hard to obtain in real time as it may take decades or even centuries (Walters *et al.*, 2005). The p_{50} value, which is the time taken for seed viability to decline to 50% in artificial ageing, is now a well-established proxy for seed longevity in storage (Newton *et al.*, 2009) and in particular in conservation many studies have been undertaken at 45 °C and 60% relative humidity (RH) to allow comparative ranking of species (Probert *et al.*, 2009; Merritt *et al.*, 2014). P_{50} serves as an index, although an approximate storage lifespan/longevity can be calculated using viability equations (if storage conditions and species constants are available) (http://data.kew.org/sid/viability/). A p_{50} value might also be somewhat related to persistence *in situ*, for example Long *et al.* (2008) demonstrate that a p_{50} of 20 days in their study species and conditions is likely to indicate persistence of several seasons *in situ*. However, multiple factors can interact with longevity to affect persistence *in situ* (see Long *et al.*, 2015). The controlled accelerated ageing test is a valuable technique because by applying a standard set of protocols it yields comparable results for understanding relative longevity across multiple species and biomes.

The longest-lived seeds tested thus far are Australian, with a maximum p50 of 771 days and an average of 202 days under accelerated ageing conditions, which is much greater than longevity of seed from other biomes and regions (Probert *et al.*, 2009). Merritt *et al.* (2014) confirm the relatively long lifespan of Australian species with an investigation of 172 species, which shows that p50 ranges from 3 up to 588 days revealing that although high on average, not all Australian species have long-lived seeds. Alpine seeds in general are known to be short-lived. Mondoni *et al.* (2010) report a mean p50 of 20.5 days across 36 Italian alpine species. From the 36 species, six accessions were compared to lowland accessions of the same species and alpine seed was significantly shorter-lived than the lowland pairs, with mean p50 values of 28.2 and 56.1, respectively (Mondoni *et al.*, 2010).

Previous studies (Probert *et al.*, 2009; Mondoni *et al.*, 2010; Merritt *et al.*, 2014; Long *et al.*, 2015) show that p_{50} is associated with other seed or plant traits including seed mass, dormancy, embryo type, endospermy, and life-form, and with environmental variables such as elevation and climatic variables. Maternal conditions can also affect seed longevity (Kochanek *et al.*, 2010; Kochanek *et al.*, 2011; He *et al.*, 2014; Righetti *et al.*, 2015; Bernareggi *et al.*, 2016). However, correlations or significant factors determining seed longevity vary depending on the species, biomes, the scale of study, as well as the combinations of variables included. Indeed, the only consistent pattern found multiple times across these studies is that non-endospermic seeds tend to be longer-lived. From a plant evolutionary-biology perspective, endospermy, seed mass, embryo, dormancy type (Finch-Savage and Leubner-Metzger, 2006), and the environment of origin (Feild and Arens, 2005) would be expected to be related to longevity. But intriguingly, the variation of p_{50} seems to often be independent of these traits and to change across environments and biomes. P_{50} might also be expected to be correlated with phylogeny, as more closely related species could share mechanisms of longevity (Walters *et al.*, 2005; Probert *et al.*, 2009; Mondoni *et al.*, 2010; Merritt *et al.*, 2014). Lastly, given the significance of regeneration by seed for species' persistence, p_{50} might reflect differences in germination strategy but this is yet to be investigated. Thus, in a search of predictors of p_{50} one must consider a broad range of seed, plant, and environmental traits.

Alpine ecosystems and alpine plant communities worldwide have been identified as particularly vulnerable to global warming (Coyne, 2001; Parolo and Rossi, 2008; Thuiller *et al.*, 2008). Local extinction of plant taxa formerly restricted to high elevations has already been reported (IPCC, 2001). In Australia, the alpine region is distributed across just 0.15% of the continent's landmass but because of its high level of endemism is recognized as an alpine biodiversity hotspot, and is distinctive in many ways in relation to mountain areas worldwide (Myers *et al.*, 2000; Kirkpatrick, 2002; Hopper and Gioia, 2004). The Australian Alps region maintains an assemblage of vegetation communities found nowhere else in the world (Costin *et al.*, 2002), harbouring unique ecological communities (Department of Environment, 2015) and 212 plant species that include 30 exclusively alpine and 21 species endemic to Mt. Kosciuszko (Costin *et al.*, 2000). In the face of climate change, the limited capacity of alpine plants to migrate to higher elevations may increase their extinction risk.

To ensure meaningful *ex situ* conservation of Australian alpine seeds, seed conservation programs must be underpinned by data on seed longevity. This study aims to investigate whether Australian alpine seeds perform like other alpine species and are short-lived, or more like other Australian species and are relatively long-lived. Secondly, we aim to understand important variables that underlie the variation of p_{50} for Australian alpine seeds. Because previous studies have shown that correlations between p_{50} and seed or plant traits and environmental variables vary, we took a comprehensive approach taking account of as many plausible intrinsic and extrinsic variables as we could. In addition, ours is the first study to examine potential associations between germination strategy and p_{50}. The germination strategy of Australian alpine plants is associated with seed mass and endospermy (Hoyle *et al.*, 2015), hence, germination strategy (or the associated traits mass and endospermy) could be expected to be related to longevity as well. We set out to determine the relative importance of both seed/plant traits and environmental variables in explaining variation in p_{50} values, and predicted that Australian alpine seeds would be short-lived (mean p_{50} values ~ 20 days) like alpine seeds elsewhere.

Materials and methods

Study species

Species of Australian native plants that occur predominantly in the alpine bioregion on the mainland (from 1330 – 2228 m elevation), and in any alpine vegetation communities, were selected for this study. The Australian Alps, based on the bioregion's physiographic elements, can be categorized as alpine (> 1850 m), subalpine (1400 – 1850 m), montane (1100 – 1400 m), and tableland (< 1100 m) (NSW NPWS, 1988). In this study, we considered alpine broadly, therefore seeds that were collected at elevations above 1330 m were termed alpine. Most seeds were collected from Kosciuszko National Park between 2007 – 2013 and were retrieved from the National Seed Bank, Australian National Botanic Gardens (ANBG) Canberra, Australia. These seeds were stored according to international genebank standards at approximately -20 °C after drying at 15 °C and 15% RH (Table 1, supplementary Fig. S1).

Germination assays

Assays were carried out to determine whether a species had high enough germination (> 75% viability adjusted) to be included in the controlled ageing tests. The assays is also used to determine the species germination strategy. Germination of 121 accessions of 96 species was investigated following the methodology published by (Hoyle *et al.*, 2015). Species known to have immediate germination (Hoyle *et al.*, 2015) were germinated on 1% water agar at 25/15 °C. Species identified as postponed, staggered, or not listed in Hoyle *et al.* (2015) were germinated on both 1% water agar and 1% water agar infused with gibberrelic acid (GA_3) to achieve a final concentration of 200 mg L^{-1} GA_3 at 25/15 °C. If germination was low in both agar and GA_3 assays under summer temperatures (25/15 °C) then stratification was applied to mimic the natural progression of seasons. For the stratification steps, a further three replicates of 25 seeds each were exposed to 6 weeks at 25/15 °C, followed by 8 weeks at 5/5 °C, and returned to 25/15 °C for at least 6 weeks, to mimic seasonal change in the field (see Hoyle *et al.*, 2015). These temperatures mimic *in situ* soil temperatures and the main seasonal changes alpine seeds are exposed to, and respond to, in Australia (Hoyle *et al.*, 2015).

Table 1. Details of 56 species analysed for their seed longevity (p_{50}) and the correlates used in the analysis

Species	Family	Locality	p_{50}	Germination Strategy	Life form	Endosperm Presence	Embryo type	Seed mass (mg)	Collection Elevation (m)	Elevation Range (m)	Precipitation - annual (mm)	Radiation - annual mean	Temperature - annual mean (°C)	Temperature - seasonality
*Gingidia algens**	Apiaceae	KNP	2.28	Postponed	Herb	Yes	Axile	3.189	1799	642	1764	14.3	5.4	1.71
Poa costiniana	Poaceae	KNP	3.85	Immediate	Monocot	Yes	Basal	0.246	2109	1578	2282	13.7	3.7	1.76
*Cardamine robusta**	Brassicaceae	KNP	4.1	Immediate	Herb	No	Axile	0.463	2083	632	2326	13.7	3.5	1.76
Ranunculus clivicola	Ranunculaceae	KNP	4.16	Staggered	Herb	Yes	Basal	2.838	1742	530	1890	14.2	5.2	1.71
Astelia alpina var. *novae-hollandiae*	Liliaceae	KNP	4.94	Postponed	Herb	Yes	Axile	1.067	2008	733	2170	13.9	4.2	1.76
*Ranunculus acrophilus**	Ranunculaceae	KNP	5.3	Staggered	Herb	Yes	Basal	1.514	2039	184	1419	15.2	7.3	1.76
Australopyrum velutinum	Poaceae	KNP	5.45	Immediate	Monocot	Yes	Basal	3.875	1401	1244	1420	15.1	8.2	1.77
Ranunculus lappaceus	Ranunculaceae	NNP	6.48	Staggered	Herb	Yes	Basal	2.895	1592	1853	2073	14	4.6	1.76
Gingidia harveyana	Apiaceae	KNP	6.63	Postponed	Herb	Yes	Axile	6.288	1704	743	2287	13.8	3.7	1.69
*Craspedia leucantha**	Asteraceae	KNP	7.15	Immediate	Herb	No	Axile	0.829	1996	512	2143	13.9	4.2	1.75
Carpha nivicola	Cyperaceae	KNP	7.51	Postponed	Herb	Yes	Basal	3.407	1924	1646	2073	14	4.6	1.75
Carex cephalotes	Cyperaceae	KNP	7.55	Postponed	Monocot	Yes	Basal	0.628	2095	1001	1074	15.8	9	1.76
Celmisia costiniana	Asteraceae	KNP	7.95	Staggered	Herb	No	Axile	3.086	2046	2019	2196	13.9	4.1	1.77
Richea continentis	Ericaceae	NNP	9.59	Staggered	Herb	Yes	Axile	0.06	1588	754	1419	15.2	7.3	1.76
Psychrophila introloba	Ranunculaceae	KNP	9.61	Postponed	Herb	Yes	Basal	0.707	1941	2126	1863	14.2	5.3	1.75
Wahlenbergia ceracea	Campanulaceae	KNP	9.77	Immediate	Herb	Yes	Axile	0.032	1974	1354	680	16.9	13.3	1.76
Aciphylla glacialis	Apiaceae	KNP	9.84	Postponed	Herb	Yes	Axile	5.761	2164	954	2368	13.7	3.4	1.76
Plantago glacialis	Plantaginaceae	KNP	9.99	Immediate	Herb	Yes	Axile	0.279	1957	487	2196	13.9	4.1	1.75
Poa hiemata	Poaceae	KNP	10.4	Immediate	Monocot	Yes	Basal	0.314	2042	1762	1363	15.2	8.5	1.77
Ranunculus muelleri	Ranunculaceae	KNP	10.8	Staggered	Herb	Yes	Basal	1.356	1955	1758	2167	13.9	4.2	1.75
*Erigeron setosus**	Asteraceae	KNP	10.9	Immediate	Herb	No	Axile	0.229	1928	1062	1831	14.3	5.5	1.75
Oreomyrrhis ciliata	Apiaceae	KNP	12	Staggered	Herb	Yes	Axile	1.926	1748	942	1903	14.2	5.1	1.71
Erigeron paludicola	Asteraceae	KNP	12	Immediate	Herb	No	Axile	0.022	1989	1204	2105	14	4.4	1.76
Luzula acutifolia subsp. *nana**	Juncaceae	KNP	12	Immediate	Monocot	Yes	Basal	0.285	1957	668	1074	15.8	9	1.75
Aciphylla simplicifolia	Apiaceae	KNP	12.3	Postponed	Herb	Yes	Axile	4.188	1743	1135	1863	14.2	5.3	1.71
Austrodanthonia alpicola	Poaceae	KNP	12.4	Immediate	Monocot	Yes	Basal	0.793	2052	1387	2190	13.9	4	1.76
Stylidium graminifolium	Stylidiaceae	KNP	15.7	Postponed	Herb	Yes	Axile	0.137	1745	2006	1850	14.2	5.4	1.71
Olearia algida	Asteraceae	NNP	16.4	Staggered	Shrub	No	Axile	0.192	1636	1935	1890	14.2	5.2	1.76
*Ranunculus dissectifolius**	Ranunculaceae	KNP	17.2	Postponed	Herb	Yes	Basal	2.346	1752	338	2000	14	4.8	1.71
Ranunculus graniticola	Ranunculaceae	KNP	18	Staggered	Herb	Yes	Basal	2.766	1734	1946	1419	15.2	7.3	1.73
Ozothamnus cupressoides	Asteraceae	NNP	18.5	Staggered	Shrub	No	Axile	0.163	1599	1084	2105	14	4.4	1.76
Oreomyrrhis eriopoda	Apiaceae	KNP	20.4	Postponed	Herb	Yes	Axile	3.969	1743	1766	1419	15.2	7.3	1.72
Erigeron bellidioides	Asteraceae	KNP	20.6	Immediate	Herb	No	Axile	0.022	1730	919	2170	13.9	4.2	1.71
Carex hebes	Cyperaceae	KNP	21.2	Postponed	Monocot	Yes	Basal	1.098	2153	1941	2282	13.7	3.7	1.76
Craspedia alba	Asteraceae	KNP	21.5	Immediate	Herb	No	Axile	0.705	1855	495	2073	14	4.6	1.75
Rytidosperma pumilum	Poaceae	KNP	21.6	Immediate	Monocot	Yes	Basal	0.165	2115	960	2215	13.8	4	1.76
Rytidosperma nudiflorum	Poaceae	KNP	21.9	Immediate	Monocot	Yes	Basal	0.709	2105	2147	2190	13.9	4	1.76
Epacris paludosa	Ericaceae	NNP	22.4	Postponed	Shrub	Yes	Axile	0.039	1636	2089	1456	15.1	7.2	1.76
Scleranthus singuliflorus	Caryophyllaceae	KNP	22.9	Staggered	Herb	Yes	Periphera	1.248	2042	480	2199	13.9	4.1	1.76
*Brachyscome stolonifera**	Asteraceae	KNP	24.3	Staggered	Herb	No	Axile	0.264	1941	406	2287	13.8	3.7	1.76
Deschampsia cespitosa	Poaceae	KNP	24.4	Staggered	Monocot	Yes	Basal	0.3	1743	2069	1903	14.2	5.1	1.72
Baeckea utilis	Myrtaceae	NNP	27.1	Staggered	Shrub	No	Axile	0.091	1603	2082	1419	15.2	7.3	1.76
Leucochrysum alpinum	Asteraceae	KNP	27.4	Immediate	Herb	No	Axile	1.275	2131	1651	2105	14	4.4	1.76
Luzula modesta	Juncaceae	KNP	27.7	Immediate	Monocot	Yes	Basal	0.435	1340	1451	680	16.9	13.3	1.74
Senecio gunnii	Asteraceae	KNP	27.8	Immediate	Herb	Yes	Axile	0.328	1735	1638	2167	13.9	4.2	1.71
Brachyscome spathulata	Asteraceae	KNP	29.1	Immediate	Herb	No	Axile	0.322	2090	2110	2237	13.8	3.9	1.77
Epilobium gunnianum	Onagraceae	KNP	29.3	Immediate	Herb	No	Axile	0.039	1339	2147	1863	14.2	5.3	1.74
Neopaxia australasica	Portulacaceae	KNP	29.3	Postponed	Herb	No	Periphera	0.533	2028	2137	1456	15.1	7.2	1.76
Craspedia lamicola	Asteraceae	KNP	32.7	Immediate	Herb	No	Axile	0.623	1986	877	2215	13.8	4	1.76
Colobanthus affinis	Caryophyllaceae	KNP	33.2	Immediate	Herb	Yes	Periphera	0.149	1736	1562	2014	14	4.7	1.74
*Craspedia costiniana**	Asteraceae	KNP	34.7	Immediate	Herb	No	Axile	0.66	1986	705	2215	13.8	4	1.76
Carex gaudichaudiana	Cyperaceae	KNP	39.3	Postponed	Monocot	Yes	Basal	2.193	1330	2110	2368	13.7	3.4	1.74
Baeckea gunniana	Myrtaceae	NNP	45.3	Staggered	Shrub	No	Axile	0.077	1592	2034	1419	15.2	7.3	1.76
Carex blakei	Cyperaceae	KNP	50.4	Postponed	Monocot	Yes	Basal	2.329	1683	1349	1831	14.3	5.5	1.71
Poa phillipsiana	Poaceae	KNP	71.2	Immediate	Monocot	Yes	Basal	0.346	1399	1342	2073	14	4.6	1.77
Epacris celata	Ericaceae	KNP	81.6	Postponed	Shrub	Yes	Axile	0.022	1604	941	1419	15.2	7.3	1.76

Further details, including geographic coordinates, dates of collection, germination treatment and categorisation of germination strategy is presented in supplementary Table S1.

Germination assays were also used to determine the germination substrate and dormancy alleviation methods. Following controlled seed ageing, each species was germinated either on water agar, water agar with GA_3 infusion, or with stratification. The selection was based on the substrate/treatment that gave the highest germination percentage for each species. For staggered and postponed strategy, If GA_3 or stratification increased germination compared to germination on water agar then the most successful treatment was selected to be applied to that species throughout the ageing experiment (supplementary Table S1). If GA_3 and stratification produced similar results then stratification was selected. At the end of the germination assay, we performed cut-tests to determine seed fill and death for ungerminated seeds. There were 57 species which had germination > 75% and thus were amenable to Probit analyses (see below) and were included in the controlled ageing test (Table 1, supplementary Table S1).

Controlled ageing test

Seed longevity represented by a p_{50} value, is determined using a controlled artificial ageing test (also called a standard rapid ageing protocol, Newton *et al.*, 2009; Probert *et al.*, 2009). The basic principle of the controlled ageing protocol is to expose seeds to warm and humid conditions in order to decrease seed viability rapidly. The controlled artificial ageing test consists of two steps: rehydration and ageing. To first hydrate the seeds, 11 samples of 50 seeds for each species were placed in open glass vials for 14 days in air-tight polycarbonate electrical enclosure boxes (28 x 28 x 14 cm; NHP Fibox, Australia) above a non-saturated solution of LiCl (370 g L^{-1}; anhydrous, Sigma®, Australia) in an illuminated incubator (TRIL 396-1-SD, Thermoline Scientific, NSW, Australia), creating a relative humidity (RH) of 47% at 20 °C (Hay *et al.*, 2008). Overall, the design consisted of six boxes of seed vials. Each box contained a stratified random sample of multiple species with representatives of each of the different germination strategies. The 11 vials of a given species were kept in a single box.

To ensure seeds had equilibrated to 47% RH by the end of seed rehydration period their relative humidity was checked using a reference sample of *Eucalyptus macrorhyncha* seeds that accompanied the study species (supplementary Table S2). Unlike the study species, *E. macrorhyncha* seeds are relatively large and were available in sufficient quantity to fill in a hygrometer chamber for measurement of humidity. The seed equilibrium relative humidity was measured using a water activity measuring instrument which comprised a hygrometer sensor housed in an AW VC-DI0 water activity probe, used in conjunction with a HygroPalm PD 2 dock-station display unit (Rotronic, Bassersdorf, Switzerland).

Following rehydration, the solution in the polycarbonate electrical enclosure boxes was replaced with a non-saturated solution of LiCl (this time 300 g L^{-1}) at 45 °C which results in 60 % RH in the incubator (Oven model TO-500F, Thermoline Scientific, NSW, Australia) for seed ageing. The relative humidity was checked at 6 week intervals using a combined temperature and humidity data logger (T-TEC 7-1C, Temperature Technology, Adelaide) and the bulk solution adjusted as necessary to maintain the 60% RH, by adding distilled water (Hay *et al.*, 2008).

One vial of 50 seeds for each species was removed for germination testing after each of 11 times (1, 7, 14, 21, 28, 35, 42, 50, 75, 156, and 250 days). Seeds were sown on agar in 90 mm diameter Petri dishes and placed in an incubator (TRIL 396-1-SD, Thermoline Scientific, NSW, Australia) under the germination treatment previously demonstrated in the germination assays to be suitable for germination of each species (supplementary Table S1). Species that required stratification to germinate were subjected to stratification steps following removal from the electrical boxes. Germination was checked weekly and seeds scored as germinated once the radicle was visible, approximately > 1 mm. Observation for each plate was considered completed when there were no further germinants for 6 weeks.

To ensure that conditions were homogeneous across boxes over the course of the experiment we placed seeds of *Ficinia nodosa* in each box and retrieved and germinated them at the same ageing time as the study species (supplementary Table S2). The variation of p_{50} values of the *Ficinia nodosa* were small across boxes (mean p_{50} = 60.61 ± 3.56 days). To ensure the experimental procedures were comparable to other longevity studies we included seeds from four of the same accessions used in the Merritt *et al.* (2014) longevity study: *Brachyscome tenuiscapa* var. *pubescens*, *Poa hiemata*, *Streptoglossa macrocephala*, and *Wurmbeia dioica* (supplementary Table S2). There were no significant differences in mean p_{50} values for these species (paired t-test, df = 3, p = 0.442) indicating that the conditions, and thus results of the two studies, are comparable.

Potential correlates of seed longevity

Seed and plant traits and environmental data were gathered for the 57 study species (Table 1, supplementary Table S1). The plant and seed trait variables were: seed mass, embryo type, endosperm presence, germination strategy, plant life-form. Environmental data were: long term mean annual temperature, temperature seasonality, mean annual precipitation, and mean radiation. We also assessed elevation of collection site, average elevation for the species, and species elevation range. Specifics of each variable are discussed below.

Seed mass was measured on three replicates of 50 seeds per species, and average individual seed mass was calculated from this. Embryo type and endosperm presence were determined by dissecting the seeds, assessing presence/absence of endosperm, and categorizing embryo type. There were 38 species with endospermic seed and 18 species with non-endospermic seed. Embryo type was categorized as axile (31 species), basal (22 species), or peripheral (3 species) following the classification system of Martin (1946).

Previously, three germination strategies (immediate, staggered, and postponed) were identified among Australian alpine species (Hoyle *et al.*, 2015). Germination strategy represents germination timing and pattern over time and seasons (Hoyle *et al.*, 2015). Based on the germination results we categorise species as having a particular germination strategy under the conditions tested (see germination assays above). Non-dormant seeds germinate immediately once the germination requirement is met. Seeds with staggered and postponed strategies require certain dormancy alleviation cues (e.g. cold stratification) and the presence of favourable germination conditions before germination will occur. Staggered and postponed strategies differ in the timing of germination. Species with a staggered strategy have seeds that germinate both before and after winter, whereas seeds of postponed species germinate almost entirely after winter. Of the 57 species, 28 species were also tested in Hoyle *et al.* (2015) and almost all of these exhibited the same germination strategy as in our assays. *Ranunculus acrophilus* and *Wahlenbergia ceracea* were exceptions to this (details in supplementary Table S1). In total 24 species were categorized as immediate, 15 species as staggered, and 17 species as having a postponed strategy. Details on the germination strategy categorisation of the 57 species can be found in supplementary Table S1. Finally, plant life-form was categorized as monocotyledonous herbs (14 species), other herbs (36 species), and woody shrubs (6 species) to follow the plant functional types described by Costin *et al.* (2000) for the alpine flora (Table 1, supplementary Table S1).

For seed collection and environmental variables, we retrieved the collection elevation data from the ANBG living collection database. Mean, minimum, and maximum elevation of the species in Australia were gathered from the Australian National Herbarium Specimen Information Register (http://www.anbg.gov.au/cgi-bin/anhsir) or from Australia's Virtual Herbarium (http://avh.ala.org.au) to determine the elevation range spanned by each species. Mean temperature, temperature seasonality, precipitation, and mean radiation were bioclimatic values estimated from interpolation of long-term climate data, 75-years of data for temperature and precipitation and 25-year for solar radiation, at a resolution of 0.01 degrees, and were imported from the Atlas of Living Australia (http://spatial.ala.org.au).

Data analyses

Probit analysis of seed longevity

Probit analysis was carried out on the germination data for the study species as a function of ageing days using GenStat Release 17.1 (VSN International Ltd, Oxford, UK). P_{50} was estimated by fitting viability equations of Ellis and Roberts (1980).

$$v = K_i - (p/\sigma)$$

where v is the viability in normal equivalent deviates (NED) at time p days in storage, K_i is the estimated initial viability, and σ is the standard deviation of the normal distribution of seed deaths over time.

Drivers of seed longevity

First, we checked for collinearity among explanatory variables using correlation tests, ANOVAs or Chi-square tests, depending on the nature of the data (supplementary Table S3). Some variables were very strongly correlated ($r > 0.85$; supplementary Table S3), so we kept only one from each group. We kept collection elevation and elevation range but removed mean elevation. We kept mean temperature and temperature seasonality but removed mean precipitation and radiation. Finally, we kept endospermy type but removed embryo type and life form. These selections were made because mean annual temperature has previously been shown to be positively related to p_{50} (Probert *et al.*, 2009), and longer-lived seeds to be non-endospermic (Probert *et al.*, 2009; Merritt *et al.*, 2014). After removing those variables, we performed preliminary explorations with all-subset regressions and found that seed mass and collection elevation were consistently significant predictors of p_{50} (results not shown). We also tested family and genus as fixed factors and confirmed that the variation in p_{50} was not related to family ($p = 0.13$) or genus ($p = 0.37$). Similarly, family and genus did not explain much variation of p_{50} when assigned as random factor (supplementary Table S4).

To formally test which explanatory variable(s) best explained p_{50}, we performed model selection based on the goodness-of-fit of the data quantified by the corrected or second order Akaike Information Criterion (AICc) (Burnham and Anderson, 2003; Johnson and Omland, 2004; Bolker *et al.*, 2009; Grueber *et al.*, 2011) in R 3.2.4 (R Core Team, 2016). All continuous and binary explanatory variables were standardized to facilitate model convergence and comparison of effect sizes between models (Gelman, 2008; Merkling *et al.*, 2017). Standardization was achieved by subtracting the mean of the all data points and dividing by 2 standard deviations (Gelman, 2008). Standardization does not influence the correlations of variables with the response variable and is undertaken to facilitate comparisons between the effects of variables that are on different scales. The p_{50} values were log-transformed in all analyses to meet model assumptions. Following our data exploration (above), we also included the interaction term between seed mass and each of the two elevation variables in our list of models (supplementary Table S5). To avoid over-fitting and to limit the number of models in our list, each model contained a maximum of two explanatory variables and we did not consider models containing weakly correlated explanatory variables (supplementary Table S5).

We did not have access to a phylogeny of the 56 species considered, so we accounted for the effect of relatedness by running linear mixed models with family or genus as a random effect. There were no significant effects of the random terms, therefore showing that phylogeny explained a negligible amount of the variance in p_{50} (Supplementary Table 5). Although we could probably have discarded the random effects and run linear models (Supplementary Table 5), we decided to retain genus as a random effect to get more conservative estimates of the factors influencing p_{50}. Genus was selected as random because the AICcs were smaller compared to when we used family, or genus nested in family, as the random factors (supplementary Table S5).

Model selection was carried out using the *lme4* package (Bates *et al.*, 2015; R Core Team, 2016). For each model, we calculated its AICc and its AICc difference (ΔAICc). ΔAICc provides a measure of information lost between the best model and a given model of a candidate set. We selected models with ΔAICc < 5 following Bolker *et al.* (2009) and calculated a weight (ωAICc) for each model using the *AICcmodavg* package (Mazerolle, 2016) based on maximum likelihood estimation (supplementary Table S6). The calculated weight (ωAICc) represents the probability that a given model is the best of the subset of models. We then computed model-averaged parameter estimates, standard errors, and confidence intervals to take model selection uncertainty into account.

Comparisons of seed longevity within Australia and between alpine regions

In order to determine whether Australian alpine longevity is similar to other alpine seeds or more similar to the seed longevity of Australian plants, we did two comparisons: Australian alpine vs alpine elsewhere (European species) and Australian alpine vs Australian non-alpine. To enable international and continental scale comparisons we selected families that were shared across the different studies. We compared the p_{50} for the available paired families, i.e. families that were included in the present study as well as in the Italian alpine study of Mondoni *et al.* (2010). Likewise, we paired among families of Australian non-alpine species using data from Merritt *et al.* (2014). The Italian alpine p_{50} values were selected from those species specifically indicated as alpine in Mondoni *et al.* (2010). For Australian seed longevity comparison we excluded five species from the Merritt study (*Brachyscome tenuiscapa* subsp. *pubescens*, *Epilobium gunnianum, Poa hiemata*, *Podolepis robusta*, *Xerochrysum subundulatum*) to ensure the analysed data were exclusively non-alpine accessions (collected at elevations < 1300 m) (Merritt *et al.*, 2014). We used linear mixed models, assigning p_{50} as a response variable, the region of origin (either alpine vs non-alpine in Australia, or Australian alpine vs Italian alpine) as an explanatory variable, and paired family (six families for Australian alpine versus Italian alpine and 10 families for Australian alpine versus non-alpine comparisons) to control for potential effects of relatedness among species.

Results

Australian alpine seed longevity

Across the 57 species considered, all but one (*Melaleuca pityoides*) declined in viability such that p_{50} could be calculated (supplementary Table S1). Hereafter, we will discuss the 56 species for which p_{50} could be calculated. Our index of longevity (p_{50}) ranged from 2.28 days for *Gingidia algens* (Apiaceae) to 81.59 days for *Epacris celata* (Ericaceae), with a mean of 19.8 ± 15.5 days. Nearly 60% of the species tested had seeds with p50 values < 13.7 days (< 13.7 days = short-lived; Newton *et al.*, 2009; Probert *et al.*, 2009), and so can be considered to be relatively short-lived. Four species had p_{50} > 50 days, relatively long lived compared to other floras. The seeds of seven of the nine Kosciuszko alpine zone endemic plants tested were short-lived (p_{50} values < 13.7 days), whereas *Ranunculus dissectifolius*, *Brachyscome stolonifera* and *Craspedia costiniana* had relatively intermediate p_{50} values of 17.17, 24.3 and 34.7 days, respectively.

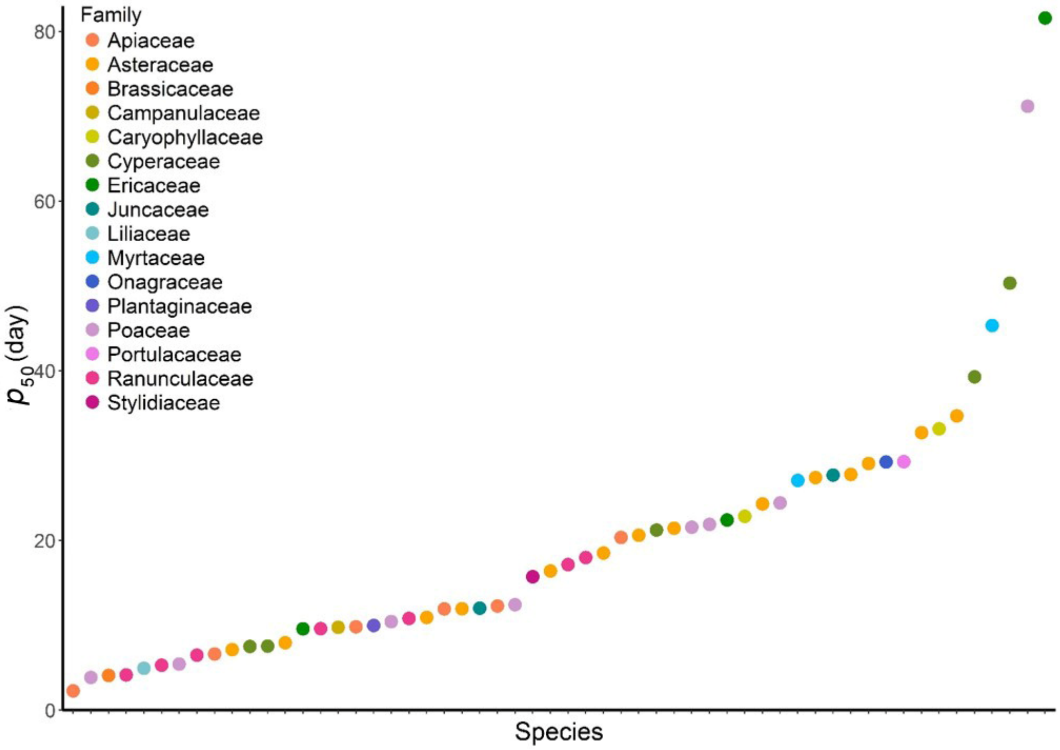

Figure 1. Variation in seed longevity index, p_{50}, across 56 Australian alpine species from 16 families. Each dot represents one species, colours indicate family.

Among Australian alpine species, the variation of seed longevity among species and across families was large (Fig. 1). Sometimes, the variation within a single genus was larger than variation between genera within a family (Fig. 2). For example, within Ericaceae, the p_{50} values of two *Epacris* species differed by 59.19 days (*Epacris celata* and *E. paludosa,* Fig. 2). Among the ecologically dominant Poaceae species we found most were short- to intermediate-lived (7 species), with the exception of one, *Poa phillipsiana*. Apiaceae (5 of 6 species short-lived, 1 intermediate) and Ranunculaceae (5 of 7 species short-lived, 2 intermediate) were shown to have consistently short-lived seeds compared to other Australian alpine families (Fig. 2). Conversely, Myrtaceae had relatively intermediate- to long-lived seeds (p_{50} 45.34 and 27.08 for *Baeckea gunniana* and *B. utilis*, respectively); germination of *Melaleuca pityoides* did not decline sufficiently over the 250 days of ageing time and therefore we could not calculate a p_{50} value for this species (supplementary Table S1).

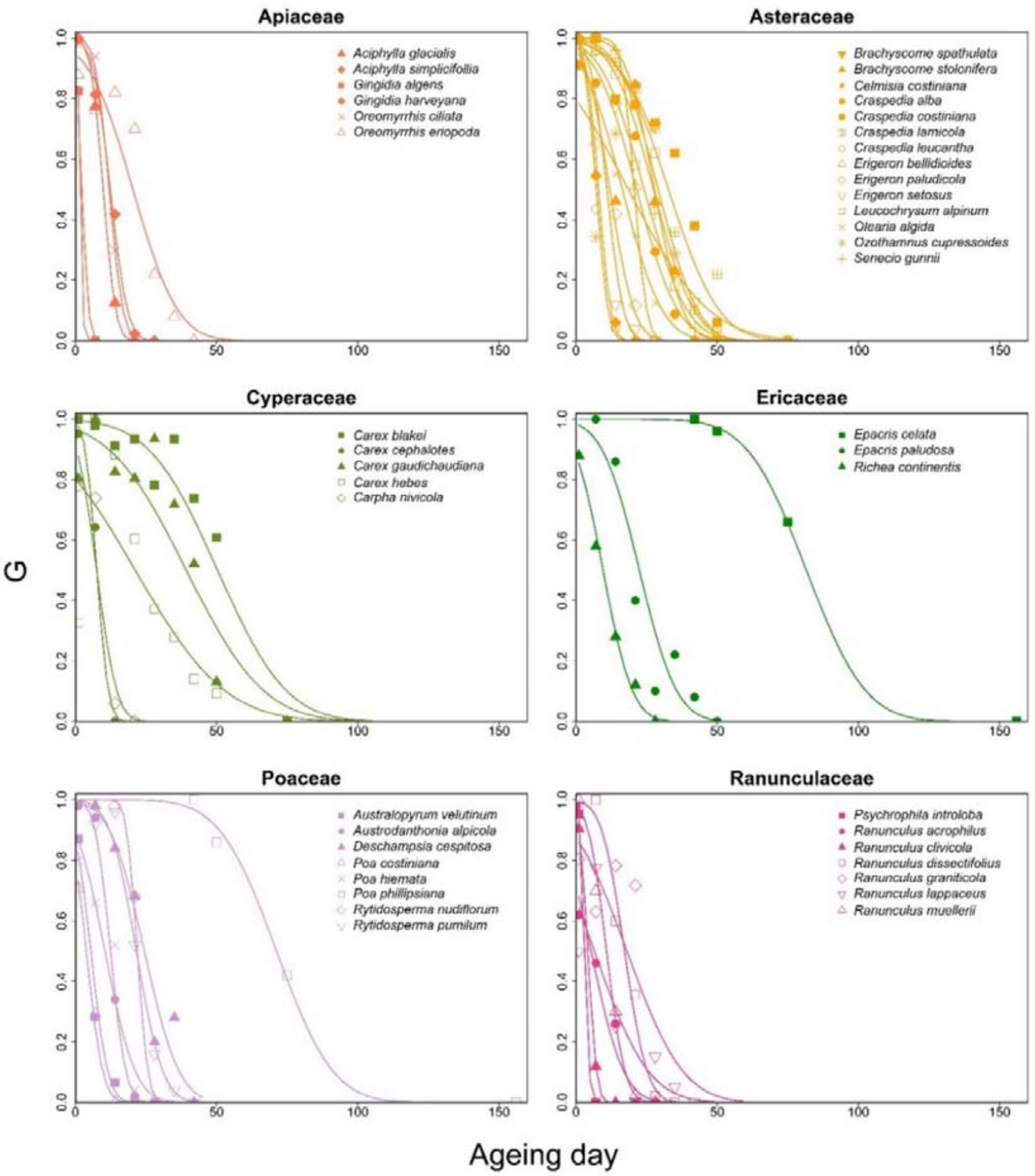

Figure 2. Seed longevity varies among families and among genera within a family. G (y-axis) is germination proportion at an aging day (x-axis). For every species (each represented by a different line), p_{50} is the day when G was 0.5. Families shown here are those for which more than two species were included in our study, family colour matched to Fig. 1.

Correlates of Australian alpine seed longevity

Seed mass and elevation were the only two of the ten variables tested in this study that were in all the top-ranked models of our model list and therefore explained most of the variance of p_{50} for the Australian alpine species considered (supplementary Table S6). Across species, seeds with lower seed mass were longer-lived than seeds with higher seed mass (Fig. 3a, Table 2), and seeds collected at lower elevation were longer-lived than those from higher elevation (Fig. 3b). The model with the interaction between seed mass and elevation was the second best of the models considered (supplementary Table S6). However, its AICc was only 0.59 higher than the best (additive) model, indicating that the interaction had a very low explanatory power. The interaction suggests that if anything, at higher elevation seed mass was more negatively correlated with p_{50} than at lower elevation (Fig. 3d). Also, species elevation range had a positive effect on p_{50}, but the relationship was much weaker as compared to the effects of seed mass and collection elevation, as the confidence interval did slightly overlap with zero (Table 2, Fig. 3c).

The climatic variables and other seed traits such as germination strategy, endospermy and embryo type, did not explain significant variation in p_{50}; these other variables only appeared in a top model also containing seed mass and elevation or they did not appear in any of the best models (supplementary Table S6). Most of the models containing just one of these variables were actually worse than the null model (supplementary Table S5).

Table 2. Model-averaged estimates of the variables explaining the seed longevity of 56 Australian alpine species (p_{50}, log-transformed). Continuous and binary input variables were standardized to facilitate model comparisons. In bold are estimates in which CI (confidence interval) does not overlap with zero and are therefore significant.

Parameter	Estimate	Standard Error	Lower CI	Upper CI
Intercept	1.178	0.0392	1.1011	1.2548
Elevation of collection	**-0.2145**	**0.0777**	**-0.3668**	**-0.0621**
Seed mass	**-0.2313**	**0.078**	**-0.3843**	**-0.0784**
Species elevation range	0.1527	0.0785	-0.0011	0.3065
Seed Mass*Elevation of collection	0.2633	0.1513	-0.0332	0.5598

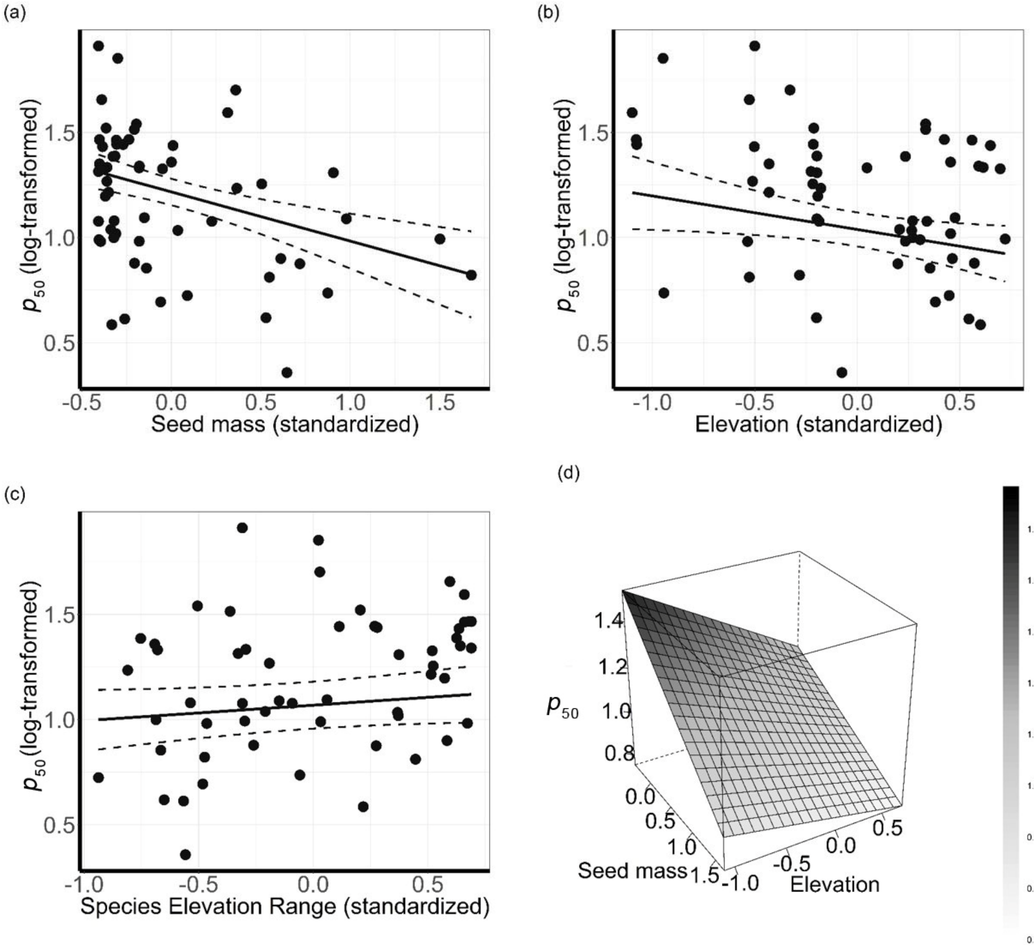

Figure 3. (a) Seeds with lower seed mass were longer-lived than seeds with higher seed mass, (b) Seeds collected at higher elevation are shorter-lived than seeds collected from lower elevation, (c) Species with wide elevation range are longer-lived, but the significance was borderline. For a, b, and c, solid line represents model-averaged predictions from the model, dash lines are the model-averaged standard errors, points are standardized raw data, (d) 3-D graph depicting the interaction of seed mass (standardized) and collection elevation (standardized), the plane represented the p_{50} value predictions from the interaction model.

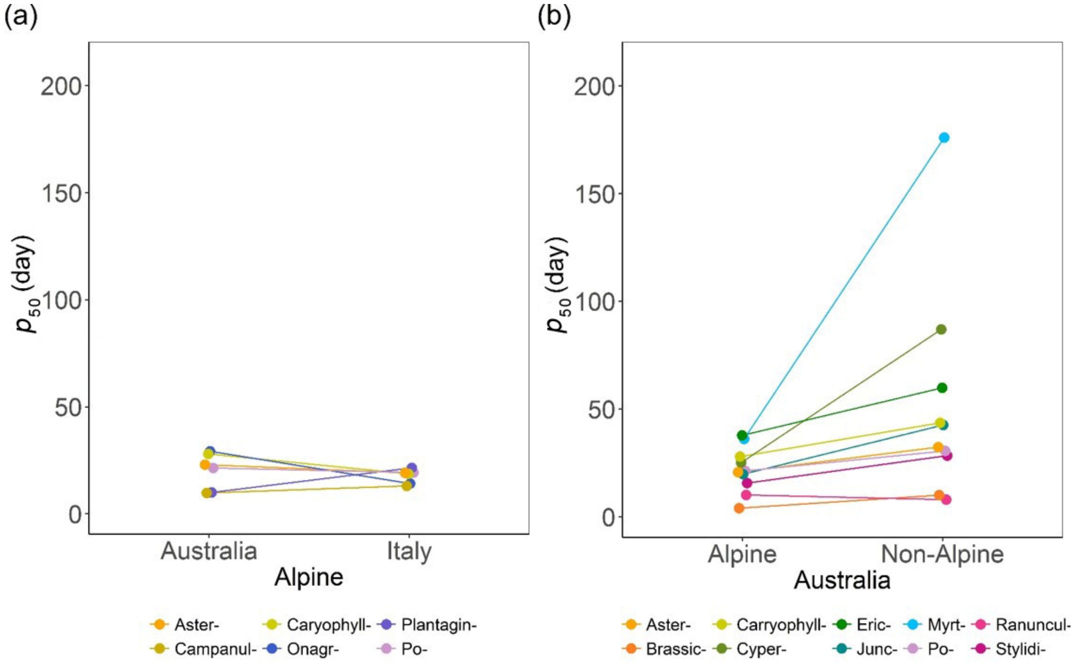

Figure 4. Comparison of longevity (p_{50}) of Australian alpine seeds with a) Italian alpine species (Mondoni *et al.*, 2010) and b) and (b) Australian non-alpine seeds (Merritt *et al.*, 2014). The comparisons were made across paired families; family colour matched to Fig. 1.

Comparisons of the Australian alpine flora to other floras

The longevity of Australian alpine seeds was relatively similar to alpine seeds in Italy, across the six families, i.e. 19.8 and 19 days, respectively ($p = 0.741$, Fig. 4a). In contrast, Australian alpine seeds were relatively short-lived compared to non-alpine Australian flora ($p < 0.000$, Fig. 4b). The difference in p_{50} between alpine and non-alpine families was large and varied across the ten families. For example, the difference between mean p_{50} values for alpine and non-alpine Australian Poaceae was 9 days, whereas for Myrtaceae the difference was 139.8 days. Despite large geographic separation there was a greater similarity among species and families from the same bioregion (i.e. alpine) than there was among species that occur within Australia in different bioregions (alpine or not).

Discussion

We aimed to understand patterns of seed longevity among Australian alpine flora and the important variables influencing it. Our index of seed longevity (p_{50}) for 56 Australian alpine species ranged from ~ 2 days to more than 81 days with a mean of 19.8 days; these values were relatively small compared to the wider Australian flora in which the p_{50} can exceed 750 days and averages 105 days, and comparable to other alpine flora. The present results indicate that seed mass and collection elevation are important drivers of seed longevity in the Australian Alps. Germination strategy and endospermy, although predicted to be important intrinsic traits, were not associated with longevity of Australian alpine seeds. Here we explore intrinsic traits and environmental drivers of Australian alpine seed longevity, as well as the significance longevity may hold for *ex situ* plant conservation and *in situ* seed bank persistence, particularly in the context of a changing climate.

Comparative longevity and drivers thereof

The p_{50} variation across the 56 species considered here is rather large (2.28 to 81.59 d), and twice that reported for another alpine region (Mondoni *et al.*, 2010). This might be attributed to the diverse combinations of plant and seed traits, and collection locations or habitats that we sampled in our study. In contrast to some previous works (Probert *et al.*, 2009; Mondoni *et al.*, 2010; Merritt *et al.*, 2014), we found that the p_{50} of Australian alpine species was positively associated with seed mass. Merritt *et al.* (2014) also found that Australian seed longevity generally increased with seed mass (Merritt *et al.*, 2014), but no correlation was found across 195 species worldwide (Probert *et al.*, 2009). Although seed mass is often a significant factor in longevity studies the direction of the relationship seems to vary across biomes and geographic scales. Seed mass is dependent on environmental variables. For instance, larger seeds are generally found at lower latitudes and this relationship co-varies with higher long-term temperature (Moles *et al.*, 2005; Moles *et al.*, 2007; Soper Gorden *et al.*, 2016). Furthermore, within the Australian Alps, the seed mass of *Aciphylla glacialis*, *Oreomyrrhis eriopoda*, *Ranunculus gunnianus*, *Richea continentis*, and *Wahlenbergia ceracea*, increased with elevation (Segal et al., unpublished data). Such within-species variation may potentially have a counter-balancing effect on the p_{50} decline among species at higher elevation. Some of the species in our study (e.g. *Carex gaudichaudiana*, *Neopaxia australasica*, *Oreomyrrhis eriopoda, Ranunculus lappaceus*, *Stylidium graminifolium*) have cosmopolitan distributions, and thus one could examine whether the effect of elevation and seed mass on seed longevity is consistent across regions.

Endosperm has been shown to have a consistent positive association with p_{50} in other species elsewhere, but was not directly related to the p_{50} of the species tested here. Across Australian alpine plants, non-endospermic species have a low seed mass, are non-dormant at dispersal, and thus have an immediate germination strategy, but endospermy has not been shown to be associated with Specific Leaf Area (SLA), plant height or elevation (Sommerville *et al.*, 2013; Hoyle *et al.*, 2015). We found the same correlation patterns among non-endospermy, seed mass, dormancy class, and germination strategy as in the previous studies (Sommerville *et al.*, 2013; Hoyle *et al.*, 2015) yet endospermy and germination strategy were not associated with longevity. Endospermic seeds in Australian alpine species are generally associated with dormant seed and a postponed germination strategy (Hoyle *et al.*, 2015). One can expect that for a dormant seed, high longevity is more relevant than for a non-endospermic seed with an immediate germination strategy that, if the required conditions are met, presumably germinates soon after natural dispersal. These patterns may indicate that across Australian alpine plants seed longevity, though influenced by some of the same traits as germination strategy, may be an ecologically important trait that evolves somewhat independently of germination strategy.

Seed longevity has also previously been shown to be associated with phylogeny (Probert *et al.*, 2009; Merritt *et al.*, 2014), particularly when a given family possesses a distinctive character e.g. hard-seeded-ness (physical dormancy) in Fabaceae. However, this pattern was not detected among the alpine species studied here. A more thorough analysis applying actual phylogenetic data would be needed to formally assess the relationship between p_{50} and phylogeny of Australian alpine seeds. Variation in seed longevity at family and genus level has previously been demonstrated in the Asteraceae (Walters *et al.*, 2005), Australian Cyperaceae (Merritt *et al.*, 2014), and the genus *Plantago* (Plantaginaceae; Mondoni *et al.*, 2010). We suggest that the results presented here, in conjunction with the results of other studies that show high levels of variation within phylogenetic groups (and even species), phylogenetic data seem unlikely to reveal strong associations. Rather, longevity appears to be a fairly labile trait.

Mondoni *et al.* (2010) demonstrated that seeds of alpine plants are short-lived compared to seeds of the same species collected from lowland plants. Our study is the first to provide evidence that even within the higher elevation (sub-alpine and alpine), elevation still affects seed longevity; even though the climate gradient was relatively narrow and did not itself directly explain variation in p_{50}. Interestingly, climate was a significant correlate of seed longevity in other studies (Probert *et al.*, 2009; Mondoni *et al.*, 2010; Merritt *et al.*, 2014) but was not significant in this study, possibly indicating that climate affects longevity at bioregional scales but that other drivers may be more important within a region. That elevation matters for p_{50} but climate variables do not in our study may seem contradictory, but most likely indicates that a narrow amplitude of long term climate variability recorded by alpine climate stations may not reflect conditions at plant or soil level. The long-term temperature (used to model bioclimate values), for instance, are calculated from temperature data measured at 1.2 m above the ground and there could be as much as a 7 °C discrepancy between the temperatures measured at this height and at the ground (www.bom.gov.au). Seed development (post-zygotic and pre-zygotic) conditions, instead of long-term mean climate variables, were the significant factor explaining p_{50} variation in Italian flora (Mondoni *et al.*, 2010), and maternal conditions may increase and decrease the p_{50}, again depending on the species and origin (Kochanek *et al.*, 2010; Kochanek *et al.*, 2011; Bernareggi *et al.*, 2015). Hence, in addition to broad-scale elevation patterns, microclimatic and maternal conditions may explain subtler patterns of variation in p_{50} and deserve further investigation.

Ex situ and in situ conservation implications in a climate change context

Of the species assessed here, nearly half had seeds with p_{50} values lower than 13.7 days, a commonly applied threshold to define seeds to be short-lived (Newton *et al.*, 2009; Probert *et al.*, 2009), or if we refer to the classification by Mondoni *et al.* (2010), 18 species would be considered short-lived (p_{50} < 10 days). The low p_{50} values might mean years of viability under *ex situ* conditions, where relative humidity and temperature are kept low, but this still needs to be empirically proven.

Climate model studies have demonstrated that alpine plants are unlikely to be able to adapt or disperse quickly enough to keep up with rapid climate change, and thus there is a high risk of local extinction (Cotto *et al.*, 2017). Our data provide some indication that longevity was higher for species with wide elevation ranges than for species with short elevation ranges, such a patterns could indicate an advantage for these species compared to those with restricted alpine distribution, and this effect thus may warrant further consideration.

The Australian alpine region is relatively low-lying and permanent snow is absent, leading to an even more constrained potential for upward migration. Thus, *ex situ* plant conservation might be the best option to provide a safeguard for many alpine plants, particularly in the Australian context. From an *ex situ* conservation perspective, p_{50} values provide a convenient and reliable proxy to estimate the storage life. For native species where seed availability is often limited, determination of species constants and calculation of a full viability equation to determine storage lifespan, which requires many more seeds, cannot be performed. Thus p_{50} derived from controlled ageing is the most practical approach to determine seed management in *ex situ* storage. *Ex situ* seed bank collections consist of 'active' collection (10 – 20 years term storage) for research use, and planting materials and 'base' collections (long-term storage, FAO/IPGRI, 1994). The original FAO/IPGRI (1994) genebank standards recommend for 5 to 10 years interval for viability testing but the more recent FAO (2013) protocol advised for an interval one third of the time predicted for viability to fall from its initial value. Our finding advocates for a more considered handling of alpine seeds in *ex situ* seed conservation – alpine seeds should be stored and utilized relatively quickly (i.e. base may be only 10-20 years) with ~ 3 year intervals of viability monitoring (see Hay and Probert, 2013).

The relatively short longevity of Australian alpine could also influence *in situ* persistence. Australian alpine soils contain a substantial seed bank of viable seeds that germinate well (Venn and Morgan, 2010; Hoyle *et al.*, 2013). Alpine communities elsewhere also have sizeable soil seed banks (Arroyo *et al.*, 1999; Arroyo *et al.*, 2004), and can survive five years in a burial experiment *in situ* (Schwienbacher *et al.*, 2010). More than 50% of alpine species present in the standing vegetation may exist in the soil seed bank (McGraw and Vavrek, 1989). Australian alpine seeds in contrast are relatively small (1.25 ± 0.2 mg across our study species which was small compared to Australian seed mass tested in Merritt *et al.* (2014), 8.84 ± 1.29 mg). Small size makes them more likely to be buried and incorporated into the soil. Seeds that are small and round underlie the strategy for longer persistence and a form of seed predator avoidance (Moles *et al.*, 2000). Further, Mondoni *et al.* (2010) suggested that seeds buried in the substrate at alpine sites are likely to deteriorate more slowly in alpine compared to lowland sites because of the low temperatures. Small seeded-species usually persist longer (e.g. Thompson *et al.*, 1993; Cerabolini *et al.*, 2003; Funes *et al.*, 2003; Peco *et al.*, 2003).

Soil seed banks are likely to be important in maintaining the alpine communities in changing and variable climatic conditions and potentially can act as a buffer against variable weather conditions, and extreme fluctuations plants are not adapted to. Our data at least indicate that the seed longevity of many Australian alpine species may facilitate soil seed bank formation and persistence, but how seed longevity may interact with other biotic and abiotic factors to affect soil seed bank dynamics remains unknown. Actual persistence in the soil depends upon not only potential longevity but other factors (Long *et al.*, 2015). In the Australian Alps, soil warming reduces germination from the soil seed bank, but increases the diversity of species and thus alters species composition of germinants (Hoyle *et al.*, 2013). None-the-less, Long *et al.* (2008) show that p_{50} values are positively correlated to realized seed persistence for 27 north-western European species. Long *et al.* (2008) also estimate the association between p_{50} and seed persistence for 13 Australian weed species, and suggest that p_{50} values < 20 days are equivalent to approximately 1-year persistence *in situ* and p_{50} values of 20 to 50 days correspond to 1 to 3 years field persistence in their study region. Further efforts to refine estimates of *in situ* seed persistence from p_{50} in different regions will further assist in developing approaches for habitat management and species conservation.

Conclusions

Recent comparative longevity studies using controlled accelerated ageing protocols show that the Australian flora generally has long-lived seed. Nonetheless, our study reveals that Australian alpine seeds are relatively short-lived just like alpine seeds elsewhere. Our study demonstrated that elevation combined with seed mass were the strongest determinants for Australian alpine seed longevity (p_{50}) and suggest that even within the relatively narrow topographic range, the elevation of collection location is a strong determinant of seed longevity, although, at this scale we found little indication that specific climate variables were directly associated with elevation. This study is also the first study of longevity, globally, to examine the patterns and drivers of seed longevity by including regeneration processes such as germination strategy as explanatory variables. Germination strategy is particularly important for alpine plant recruitment. Interestingly, we found that seed longevity was not associated with germination strategies or with other seed traits known to be ecologically important for alpine plants. This suggests that although longevity may be influenced by some of the same seed traits that influence germination strategy, longevity may be an ecologically important process that operates independently from germination. The variables shown to be important for p_{50} in Australian alpine species differed from other comparative longevity studies. In part, this reflects differences in approach. For example, we analysed 56 species which all had relatively low seed mass in a global context and were collected in one bioregion, and we took into account 12 potential correlates (including 5 seed/plant traits, see supplementary Table S1). Other studies have included more species with broader variation in seed mass and climatic gradients or bioregions, but often fewer correlates. Because the controlled ageing protocol has been shown to be robust and is a standardized method, a study looking at global p_{50} is highly feasible and would bring even more comprehensive insights into the role of plant traits, seed structure and climate in explaining the evolution of seed longevity. Seed persistence and seed burial studies in the field, to complement the p_{50} data, would also increase our confidence to use p_{50} to predict seed persistence in various habitats and may be useful for informing *in situ* and *ex situ* conservation of priority species. Australian alpine regions are valuable yet vulnerable, and the success of *ex situ* as well as *in situ* plant conservation efforts will in part rely on information on seed longevity, soil seed bank persistence and germination of the alpine flora; the present study is an important step toward that knowledge.

Supplementary material

Details of Australian alpine species collection, p_{50} values, plant and seed traits as well as climate variable used in the analysis, and model averaging results can be found in the supplementary material online.

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Conflict of interest

None.

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Supplementary information

Supplementary Table S1. Collection information, details of ageing test (box number and germination treatment), p50 result, and intrinsic and environmental variables for each of the 57 accessions, ordered by p50 values. Asterisk (*) indicates species endemic to the Australian Alps. Accession numbers are preceded by herbarium code CANB. KNP= Kosciuszko National Park, NSW; NNP= Namadgi National Park, ACT. During the controlled ageing each box contained at least one representative of each germination strategy (Immediate, Staggered, Postponed (Hoyle *et al.*, 2015). Treatment is the germination condition used for each germination screen where S = stratification (12/12 hours photoperiod, 6 weeks 25/15 °C, 8 weeks 5/5 °C, 6 weeks 25/15 °C), G = 1% water agar with 200 mg L^{-1} GA_3 (12/12 hours photoperiod 25/15 °C), and A= 1% water-agar (12/12 hours photoperiod 25/15 °C). Embryo type follows Martin (1946). Details on climate data can be accessed from http://spatial.ala.org.au/layers. *Melaleuca pityoides* was not included in the probit or further analyses and hence the environmental correlates were not shown.

(Supplementary Table S1, please turn over)

SI Chapter 2

Supplementary Table S1

Species	Author	Family	Accession	Latitude	Longitude	Collection date	Locality	Box nr	Treatment	p50	Germination Strategy	Life form	Endosperm Presence	Embryo type	Seed mass (mg)	Collection Elevation (m)	Elevation Average (m)	Elevation Range (m)	Precipitation - annual (mm)	Radiation - annual mean (MJ/m2/d)	Temperature - annual mean (°C)	Temperature - seasonality
*Gingidia algens**	(F.Muell.) J.W.Dawson	Apiaceae	792231	-36.3669	148.3713	30-Mar-10	KNP	3	G	2.28	Postponed	Herb	Yes	Axile	3.189	1799	1579	642	1764	14.3	5.4	1.71
Poa costiniana	Vickery	Poaceae	798459	-36.475	148.2716	30-Mar-11	KNP	1	A	3.85	Immediate	Monocot	Yes	Basal	0.246	2109	1356	1578	2282	13.7	3.7	1.76
*Cardamine robusta**	I.Thomps.	Brassicaceae	771040	-36.3916	148.3197	2-Apr-08	KNP	1	A	4.1	Immediate	Herb	No	Axile	0.463	2083	1802	632	2326	13.7	3.5	1.76
Ranunculus clivicola	B.G.Briggs	Ranunculaceae	792145	-36.4202	148.3511	29-Jan-10	KNP	3	A	4.16	Staggered	Herb	Yes	Basal	2.838	1742	1793	530	1890	14.2	5.2	1.71
Astelia alpina var. novae-hollandiae	Skottsb.	Liliaceae	792193	-36.4486	148.2708	15-Mar-10	KNP	5	S	4.94	Postponed	Herb	Yes	Axile	1.067	2008	1766.5	733	2170	13.9	4.2	1.76
*Ranunculus acrophilus**	B.G.Briggs	Ranunculaceae	786947	-36.4069	148.2963	6-Jan-10	KNP	3	S	5.3	Staggered	Herb	Yes	Basal	1.514	2039	2105.5	184	1419	15.2	7.3	1.76
Australopyrum velutinum	(Nees) B.K.Simon	Poaceae	783423	-35.88	148.5016	3-Feb-09	KNP	4	A	5.45	Immediate	Monocot	Yes	Basal	3.875	1401	1392	1244	1420	15.1	8.2	1.77
Ranunculus lappaceus	Sm.	Ranunculaceae	813613	-35.5208	148.7813	16-Jan-13	NNP	1	A	6.48	Staggered	Herb	Yes	Basal	2.895	1592	927.5	1853	2073	14	4.6	1.76
Gingidia harveyana	(F.Muell.) J.W.Dawson	Apiaceae	792215	-36.3913	148.4566	29-Mar-10	KNP	6	S	6.63	Postponed	Herb	Yes	Axile	6.288	1704	1332.5	743	2287	13.8	3.7	1.69
*Craspedia leucantha**	F.Muell.	Asteraceae	783441	-36.3563	148.3536	25-Feb-09	KNP	6	A	7.15	Immediate	Herb	No	Axile	0.829	1996	1836	512	2143	13.9	4.2	1.75
Carpha nivicola	F.Muell.	Cyperaceae	748568	-36.4583	148.2969	5-Mar-07	KNP	1	S	7.51	Postponed	Herb	Yes	Basal	3.407	1924	1247	1646	2073	14	4.6	1.75
Carex cephalotes	F.Muell.	Cyperaceae	770501	-36.4755	148.2716	14-Mar-08	KNP	1	A	7.55	Postponed	Monocot	Yes	Basal	0.628	2095	1621.5	1001	1074	15.8	9	1.76
Celmisia costiniana	M.Gray & Given	Asteraceae	798295	-36.4288	148.2769	2-Mar-11	KNP	4	A	7.95	Staggered	Herb	No	Axile	3.086	2046	1130.5	2019	2196	13.9	4.1	1.77
Richea continentis	B.L.Burtt	Ericaceae	865439	-35.5213	148.7822	20-Feb-13	NNP	2	G	9.59	Staggered	Herb	Yes	Axile	0.06	1588	1717	754	1419	15.2	7.3	1.76
Psychrophila introloba	(F.Muell.) W.A.Weber	Ranunculaceae	792160	-36.4586	148.2972	5-Feb-10	KNP	6	G	9.61	Postponed	Herb	Yes	Basal	0.707	1941	1138	2126	1863	14.2	5.3	1.75
Wahlenbergia ceracea	Lothian	Campanulaceae	792185	-36.455	148.2883	3-Mar-10	KNP	3	A	9.77	Immediate	Herb	Yes	Axile	0.032	1974	1530	1354	680	16.9	13.3	1.76
Aciphylla glacialis	(F.Muell.) Benth.	Apiaceae	782521	-36.4555	148.2611	11-Mar-09	KNP	1	S	9.84	Postponed	Herb	Yes	Axile	5.761	2164	1753	954	2368	13.7	3.4	1.76
Plantago glacialis	B.G.Briggs, Carolin & Pulley	Plantaginaceae	797747	-36.4122	148.3072	16-Feb-11	KNP	1	A	9.99	Immediate	Herb	Yes	Axile	0.279	1957	1990.5	487	2196	13.9	4.1	1.75
Poa hiemata	Vickery	Poaceae	798300	-36.4269	148.2783	2-Mar-11	KNP	6	A	10.44	Immediate	Monocot	Yes	Basal	0.314	2042	1241	1762	1363	15.2	8.5	1.77
Ranunculus muelleri	Benth.	Ranunculaceae	786950	-36.4572	148.2991	28-Jan-10	KNP	5	G	10.82	Staggered	Herb	Yes	Basal	1.356	1955	1256	1758	2167	13.9	4.2	1.75
*Erigeron setosus**	(Benth.) M.Gray	Asteraceae	792158	-36.4111	148.3083	4-Feb-10	KNP	2	A	10.94	Immediate	Herb	No	Axile	0.229	1928	1591	1062	1831	14.3	5.5	1.75
Oreomyrrhis ciliata	Hook.f.	Apiaceae	792149	-36.4319	148.3455	27-Jan-10	KNP	1	G	11.95	Staggered	Herb	Yes	Axile	1.926	1748	1376	942	1903	14.2	5.1	1.71
Erigeron paludicola	S.J.Forbes	Asteraceae	792191	-36.4491	148.2736	15-Mar-10	KNP	5	A	11.96	Immediate	Herb	No	Axile	0.022	1989	1421	1204	2105	14	4.4	1.76
*Luzula acutifolia subsp. nana**	Edgar	Juncaceae	797746	-36.4122	148.3072	16-Feb-11	KNP	5	A	12.02	Immediate	Monocot	Yes	Basal	0.285	1957	1831	668	1074	15.8	9	1.75
Aciphylla simplicifolia	(F.Muell.) Benth.	Apiaceae	792163	-36.4272	148.355	16-Feb-10	KNP	6	S	12.28	Postponed	Herb	Yes	Axile	4.188	1743	1459.5	1135	1863	14.2	5.3	1.71
Austrodanthonia alpicola	(Vickery) H.P.Linder	Poaceae	771036	-36.3944	148.3202	2-Apr-08	KNP	3	A	12.43	Immediate	Monocot	Yes	Basal	0.793	2052	1496.5	1387	2190	13.9	4	1.76
Stylidium graminifolium	Sw.	Stylidiaceae	792181	-36.4302	148.3572	2-Mar-10	KNP	5	S	15.74	Postponed	Herb	Yes	Axile	0.137	1745	1008	2006	1850	14.2	5.4	1.71
Olearia algida	N.A.Wakef.	Asteraceae	813614	-35.5222	148.7725	17-Jan-13	NNP	6	S	16.41	Staggered	Shrub	No	Axile	0.192	1636	1043.5	1935	1890	14.2	5.2	1.76
*Ranunculus dissectifolius**	F.Muell. ex Benth.	Ranunculaceae	792148	-36.4319	148.3455	27-Jan-10	KNP	3	G	17.17	Postponed	Herb	Yes	Basal	2.346	1752	1856	338	2000	14	4.8	1.71
Ranunculus graniticola	Melville	Ranunculaceae	786945	-36.4636	148.3213	6-Jan-10	KNP	3	G	18	Staggered	Herb	Yes	Basal	2.766	1734	1134	1946	1419	15.2	7.3	1.73
Ozothamnus cupressoides	Puttock & D.J.Ohlsen	Asteraceae	866370	-35.5227	148.7819	17-Apr-13	NNP	4	S	18.53	Staggered	Shrub	No	Axile	0.163	1599	1580	1084	2105	14	4.4	1.76
Oreomyrrhis eriopoda	(DC.) Hook.f.	Apiaceae	783437	-36.4269	148.3677	24-Feb-09	KNP	4	S	20.36	Postponed	Herb	Yes	Axile	3.969	1743	1276	1766	1419	15.2	7.3	1.72
Erigeron bellidioides	(Hook.f.) S.J.Forbes & D.I.Morris	Asteraceae	792150	-36.4291	148.3597	29-Jan-10	KNP	6	A	20.63	Immediate	Herb	No	Axile	0.022	1730	1662.5	919	2170	13.9	4.2	1.71
Carex hebes	Nelmes	Cyperaceae	808766	-36.4591	148.2655	22-Mar-12	KNP	2	S	21.24	Postponed	Monocot	Yes	Basal	1.098	2153	1233.5	1941	2282	13.7	3.7	1.76
Craspedia alba	J.Everett & Joy Thomps.	Asteraceae	770451	-36.4588	148.2972	30-Jan-08	KNP	6	S	21.46	Immediate	Herb	No	Axile	0.705	1855	1887.5	495	2073	14	4.6	1.75
Rytidosperma pumilum	(Kirk) Clayton & Renvoize ex Connor & Edgar	Poaceae	770497	-36.4188	148.2855	13-Mar-08	KNP	6	A	21.58	Immediate	Monocot	Yes	Basal	0.165	2115	1680	960	2215	13.8	4	1.76
Rytidosperma nudiflorum	(P.Morris) Connor & Edgar	Poaceae	770475	-36.4036	148.3013	21-Feb-08	KNP	3	A	21.9	Immediate	Monocot	Yes	Basal	0.709	2105	1176.5	2147	2190	13.9	4	1.76
Epacris paludosa	R.Br.	Ericaceae	813615	-35.5222	148.7725	17-Jan-13	NNP	2	S	22.43	Postponed	Shrub	Yes	Axile	0.039	1636	1077.5	2089	1456	15.1	7.2	1.76
Scleranthus singuliflorus	(F.Muell.) Mattf.	Caryophyllaceae	808772	-36.4063	148.2969	26-Mar-12	KNP	6	S	22.86	Staggered	Herb	Yes	Peripheral	1.248	2042	1938	480	2199	13.9	4.1	1.76
*Brachyscome stolonifera**	G.L.Davis	Asteraceae	792161	-36.45	148.28	5-Feb-10	KNP	5	S	24.32	Staggered	Herb	No	Axile	0.264	1941	1933	406	2287	13.8	3.7	1.76
Deschampsia cespitosa	(L.) P.Beauv.	Poaceae	783436	-36.4269	148.3677	24-Feb-09	KNP	1	G	24.43	Staggered	Monocot	Yes	Basal	0.3	1743	1072.5	2069	1903	14.2	5.1	1.72
Baeckea utilis	F.Muell. ex Miq.	Myrtaceae	866372	-35.5236	148.7819	17-Apr-13	NNP	2	A	27.08	Staggered	Shrub	No	Axile	0.091	1603	1081	2082	1419	15.2	7.3	1.76
Leucochrysum alpinum	(F.Muell.) R.J.Dennis & N.G.Walsh	Asteraceae	792171	-36.4566	148.27	17-Feb-10	KNP	5	A	27.42	Immediate	Herb	No	Axile	1.275	2131	894.5	1651	2105	14	4.4	1.76
Luzula modesta	Buchenau	Juncaceae	866326	-35.5355	148.908	11-Dec-12	KNP	4	A	27.72	Immediate	Monocot	Yes	Basal	0.435	1340	1335.5	1451	680	16.9	13.3	1.74
Senecio gunnii	(Hook.f.) Belcher	Asteraceae	792165	-36.4252	148.3525	16-Feb-10	KNP	1	A	27.79	Immediate	Herb	Yes	Axile	0.328	1735	1214	1638	2167	13.9	4.2	1.71
Brachyscome spathulata	Gaudich.	Asteraceae	797826	-36.4461	148.2683	2-Mar-11	KNP	5	A	29.08	Immediate	Herb	No	Axile	0.322	2090	1228.5	2110	2237	13.8	3.9	1.77
Epilobium gunnianum	Hausskn.	Onagraceae	866335	-35.5363	148.9063	12-Feb-13	KNP	2	A	29.27	Immediate	Herb	No	Axile	0.039	1339	1133.5	2147	1863	14.2	5.3	1.74
Neopaxia australasica	(Hook.f.) O.Nilsson	Portulacaceae	746148	-36.4072	148.3011	24-Jan-07	KNP	4	G	29.3	Postponed	Herb	No	Peripheral	0.533	2028	1071.5	2137	1456	15.1	7.2	1.76
Craspedia lamicola	J.Everett & Joy Thomps.	Asteraceae	808041	-36.408	148.3055	6-Mar-12	KNP	3	A	32.71	Immediate	Herb	No	Axile	0.623	1986	1761.5	877	2215	13.8	4	1.76
Colobanthus affinis	(Hook.) Hook.f.	Caryophyllaceae	786943	-36.4302	148.3244	6-Jan-10	KNP	1	A	33.17	Immediate	Herb	Yes	Peripheral	0.149	1736	1401	1562	2014	14	4.7	1.74
*Craspedia costiniana**	J.Everett & Joy Thomps.	Asteraceae	808040	-36.408	148.3055	6-Mar-12	KNP	3	A	34.7	Immediate	Herb	No	Axile	0.66	1986	1847.5	705	2215	13.8	4	1.76
Carex gaudichaudiana	Kunth	Cyperaceae	866336	-35.5358	148.9077	12-Feb-13	KNP	2	S	39.3	Postponed	Monocot	Yes	Basal	2.193	1330	1067	2110	2368	13.7	3.4	1.74
Baeckea gunniana	Schauer	Myrtaceae	866373	-35.5225	148.7816	18-Apr-13	NNP	2	G	45.34	Staggered	Shrub	No	Axile	0.077	1592	1043	2034	1419	15.2	7.3	1.76
Carex blakei	Nelmes	Cyperaceae	792229	-36.3683	148.375	30-Mar-10	KNP	2	S	50.35	Postponed	Monocot	Yes	Basal	2.329	1683	675.5	1349	1831	14.3	5.5	1.71
Poa phillipsiana	Vickery	Poaceae	783421	-35.8786	148.5036	3-Feb-09	KNP	2	A	71.2	Immediate	Monocot	Yes	Basal	0.346	1399	1241	1342	2073	14	4.6	1.77
Epacris celata	Crowden	Ericaceae	866367	-35.523	148.7822	17-Apr-13	KNP	2	G	81.59	Postponed	Shrub	Yes	Axile	0.022	1604	1472.5	941	1419	15.2	7.3	1.76
Melaleuca pityoides	(F.Muell.) Craven	Myrtaceae	813602	-35.5672	148.7836	15-Jan-13	NNP	4	A	NA	Immediate	Shrub	No	Axile	0.004	1692	NA	NA	NA	NA	NA	NA

Supplementary Table S2. Additional seed accessions used as box-effect controls for rehydration and ageing and to ensure results of this study were comparable with (Merritt *et al.*, 2014).

Species	**Family**	**Accession**	**Control type**	**Boxes**	**Treatment**
Eucalyptus macrorhyncha	Myrtaceae	NA	Across boxes	All rehydration	NA
Ficinia nodosa	Cyperaceae	NA	Across boxes	All ageing	A
Brachyscome tenuiscapa var. *pubescens*	Asteraceae	Merrit 2014	Across studies	5 ageing	A
Poa hiemata	Poaceae	Merrit 2014	Across studies	2 ageing	A
Streptoglossa macrocephala	Stackhousiaceae	Merrit 2014	Across studies	4 ageing	A
Wurmbea dioica	Colchicaceae	Merrit 2014	Across studies	6 ageing	A

Supplementary Table S3. Correlations among the variables considered (seed/plant traits, climate variables and elevations).

	Statistics	**Elevation Range (m)**	**Average Elevation (m)**	**Precipitation - annual (Bio12)**	**Radiation - annual mean (Bio20)**	**Temperature - annual mean (Bio01)**	**Temperature seasonality (Bio04)**	**Elevation of collection**	**Seed mass**	**Endospermy**	**Embryo type**	**Life form**	**Germination Strategy**
Elevation Range (m)	ρ / F / X^2	1	$-.891^{**}$	-0.146	0.136	0.127	0.125	-0.156	-0.129	0.03	0.349	2.96	0.3
	p		**0**	0.281	0.316	0.353	0.358	0.25	0.342	0.87	0.707	0.06	0.742
Average Elevation (m)	ρ / F / X^2		1	0.181	-0.183	-0.169	0.011	$.307^{*}$	0.014	0.078	0.456	3.091	1.29
	p			0.182	0.18	0.214	0.935	**0.021**	0.918	0.782	0.636	0.054	0.284
Precipitation - annual (Bio12)	ρ / F / X^2			1	$-.991^{**}$	$-.995^{**}$	0.107	$.395^{**}$	0.188	2.52	0.439	2.277	0.13
	p				**0**	**0**	0.432	**0.003**	0.165	0.12	0.647	0.113	0.879
Radiation - annual mean (Bio20)	ρ / F / X^2				1	$.988^{**}$	-0.093	$-.403^{**}$	-0.182	2.29	0.434	2.246	0.038
	p					**0**	0.497	**0.002**	0.18	0.14	0.65	0.116	0.963
Temperature - annual mean (Bio01)	ρ / F / X^2					1	-0.096	$-.411^{**}$	-0.182	2.62	0.563	1.881	0.015
	p						0.479	**0.002**	0.18	0.11	0.573	0.163	0.985
Temperature seasonality (Bio04)	ρ / F / X^2						1	$.350^{**}$	-0.219	4.88	0.202	2.485	3.351
	p							**0.008**	0.105	**0.03**	0.818	0.093	**0.043**
Elevation of collection	ρ / F / X^2							1	0.147	0.47	0.316	3.754	0.945
	p								0.28	0.5	0.731	**0.03**	0.395
Seed mass	ρ / F / X^2								1	6.59	0.517	2.853	7.763
	p									**0.01**	0.599	0.067	**0.001**
Endospermy	ρ / F / X^2									1	16.408	10.661	7.862
	p										**0**	**0.005**	**0.02**
Embryo type	ρ / F / X^2										1	31.029	0.225
	p											**0**	0.994
Life form	ρ / F / X^2											1	9.709
	p												**0.046**
Germination Strategy	ρ / F / X^2												1
	p												

Supplementary Table S4. Family (n = 16), genus (n = 33), and genus nested within family were assigned as either fixed term or random term, with p50 (log-transformed) as response variable to tease apart the effect of phylogeny on p50 values. F-statistics (fixed term) and estimated variance component (random term) values indicate the absence of phylogeny signal in the variation of our alpine seed longevity.

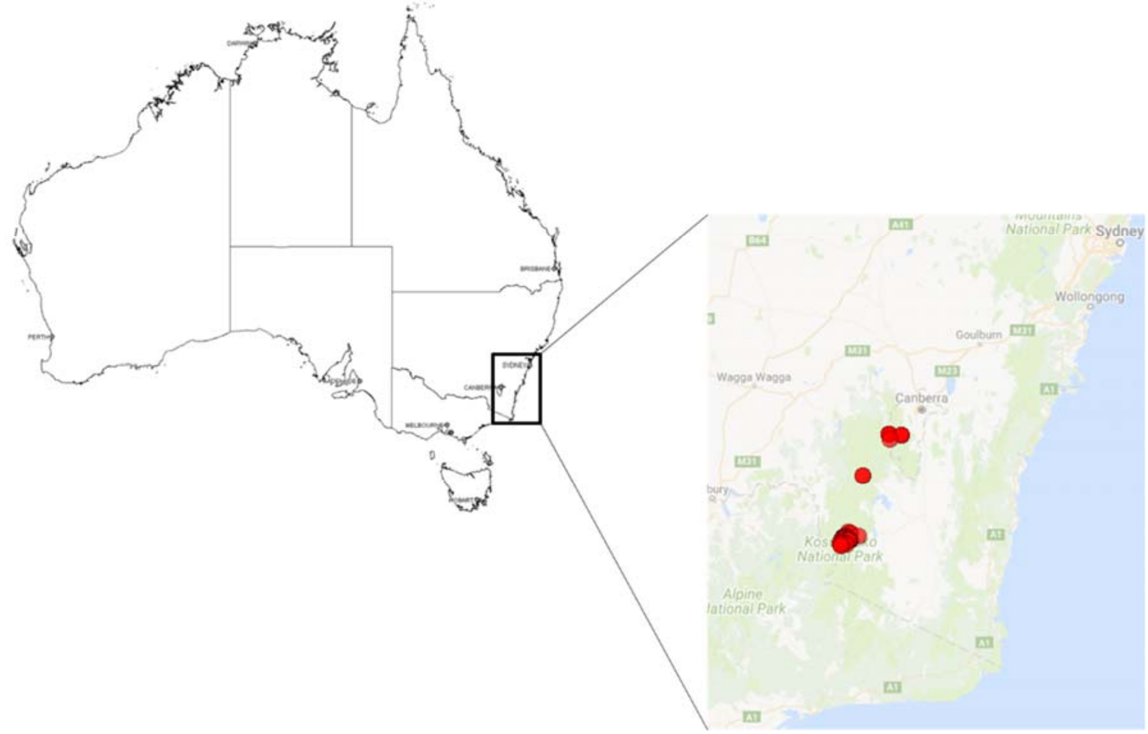
Model	**Test for fixed effect**			**Residual variance model**	
	Fixed term	**F statistic**	**F pr**	**Estimate**	**SE**
log(p50) ~ Family	Family	1.56	0.13	0.096	0.021
log(p50) ~ Genus	Genus	1.15	0.37	0.101	0.03
log(p50) ~ Family/Genus	Family	1.47	0.2	0.101	0.03
	Family.Genus	0.86	0.62		
Model	**Estimated Variance Component**			**Residual variance model**	
	Random term	**component**	**SE**	**Estimate**	**SE**
log(p50) ~ (1| Family)	Family	0.015	0.018	0.097	0.021
log(p50) ~ (1| Genus)	Genus	0.017	0.021	0.093	0.024
log(p50) ~ (1| Family/Genus)	Family	0.013	0.203	0.094	0.025
	Family.Genus	0.005	0.024		

Supplementary Table S5. List of model selections ordered by AICc values – better models are indicated by smaller AICc. Response variables p50 was log-transformed, explanatory variables were standardized, and random variable was genus. Within a model, explanatory variables were independent from one another.

Model	**AICc**	AICc	AICc	AICc
	(mixed model, random = genus)	(mixed model, random = fam)	(mixed model, random = fam/genus)	(linear model)
p50 ~ Seed Mass + Collection Elevation + Elevation Range	**28.4**	28.51	31.02	25.99
p50 ~ Seed Mass * Collection Elevation	**28.99**	29.28	31.6	26.76
p50 ~ Seed Mass + Collection Elevation	**29.42**	29.66	31.93	27.25
p50 ~ Seed Mass + Elevation Range	**32.13**	32.13	34.64	29.71
p50 ~ Seed Mass + Collection Elevation + Elevation Range	**32.89**	33.5	35.26	32.54
p50 ~ Seed Mass + Elevation Range + Temperature Seasonality	**33.74**	33.74	36.35	31.22
p50 ~ Collection Elevation	**33.82**	35.52	36.2	34.82
p50 ~ Collection Elevation + Endospermy	**33.88**	35.96	36.39	33.54
p50 ~ Seed Mass + Elevation Range + Mean Temperature	**34.36**	34.36	36.98	31.85
p50 ~ Seed Mass * Elevation Range	**34.53**	34.53	37.14	32.01
p50 ~ Seed Mass	**35.15**	35.15	37.57	32.83
p50 ~ Elevation Range	**36.61**	36.19	38.6	34.62
p50 ~ Elevation Range + Endospermy	**36.83**	36.84	39.35	34.42
p50 ~ Seed Mass + Temperature Seasonality	**37.06**	37.06	39.58	34.65
p50 ~ Seed Mass + Mean Temperature	**37.46**	37.46	39.97	35.04
p50 ~ Collection Elevation + Germination Strategy	**37.93**	39.61	40.54	38.05
p50 ~ Elevation Range + Mean Temperature	**39.01**	38.6	41.12	36.93
p50 ~ Elevation Range + Temperature Seasonality	**39.02**	38.52	41.03	36.91
p50 ~ 1	**39.99**	39.95	42.18	38.61
p50 ~ Endospermy	**40.35**	40.75	42.76	38.42
p50 ~ Elevation Range + Germination Strategy	**40.83**	40.61	43.23	38.4
p50 ~ Temperature Seasonality	**42.31**	42.25	44.57	40.62
p50 ~ Mean Temperature	**42.31**	42.26	44.59	40.83
p50 ~ Mean Temperature + Endospermy	**42.64**	42.95	45.15	40.53
p50 ~ Germination Strategy	**44.46**	44.49	46.94	42.68
p50 ~ Mean Temperature + Temperature Seasonality	**44.73**	44.65	47.07	42.94
p50 ~ Germination Strategy + Endospermy	**44.97**	45.18	47.59	42.66
p50 ~ Mean Temperature + Germination Strategy	**46.97**	46.99	49.55	45.08

Supplementary Table S6. AICc model selection tables for models explaining p50 in relation to the variables. 'AICc' is the AIC corrected for finite sample size, 'ΔAICc' is the difference between AICc of a given model to that of the best model, 'ωAICc' is the probability of each model being the best model given the data and the model set. These are models with the lowest ΔAICc (ΔAICc < 5) among all possible models listed in Supplementary Table 5.

Models (with genus as random factor)	**AICc**	**ΔAICc**	**Log Likelihood**	**ωAICc**
P50 ~ seed mass + collection elevation + species elevation range	28.4	0	1	0.33
P50 ~ seed mass * collection elevation	28.99	0.58	0.75	0.25
P50 ~ seed mass + collection elevation	29.42	1.02	0.6	0.2
P50 ~ seed mass + species elevation range	32.13	3.73	0.16	0.05
P50 ~ collection elevation + species elevation range	32.89	4.49	0.11	0.04



Supplementary Figure S1. Map of the collection sites of the 56 species.

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Design by Afiat Sukmaraga

Chapter 3

Future climate effects on alpine plant regeneration via seed are modulated by the species' germination strategy

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Abstract

Specific germination strategies have evolved in extreme environmental condition, such as in alpine system. Germination strategy is expressed in the germination timing and the proportion of germination across seasons and is affected by seed dormancy and germination requirement. A relatively high temperatures and certain winter duration are crucial for alpine seed dormancy alleviation and to initiate germination. Warmer temperatures and change of seasonal pattern will thus, potentially alter alpine seed germination responses, including success, timing, and distribution of germination across seasons. The objective of this study was to better understand the consequences of germination strategy (immediate, staggered, or postponed) on seed recruitment in the Australian Alps, both now and under future climate scenarios. We found that germination occurred faster under warmer temperature treatment for species with a staggered strategy, and a shorter winter accelerated the germination time for species with both staggered and postponed strategies. Warmer temperatures and a shorter winter reduced germination across all strategies, but species characterised by immediate germination were much less sensitive and hence retained high germination proportion under the future climate scenario. The winter duration effect was stronger than temperature in decreasing germination of seeds from species with other germination strategies – those that stagger germination across seasons or postpone until after winter. We concluded that regeneration from seed will be more successful for species with an immediate germination strategy than staggered and postponed strategies, and that there will be an increased proportion of autumn germination for species with a staggered strategy under a predicted warmer climate.

Keywords

Adaptation, Australian Alps, autumn germination, cold stratification, dormancy cycling, global warming, non-dormant, seed germination, shorter winter, spring germination.

Introduction

Alpine ecosystems contain unique biodiversity, and important in providing ecological and economic services. Alpine plants are characterised by their adaptation to low temperatures and hence, under global climate change alpine ecosystem is among the most vulnerable regions under global climate change (Beniston, 2003, Beniston *et al.*, 1997, Diaz & Bradley, 1997, Hughes, 2011). Alpine plant responses to a changing climate will depend on their potential to recruit *in situ* (adaptation, tolerance and phenotypic plasticity) or move to follow more favourable climates (migration); both process are inherently dependent on seed (Lenoir *et al.*, 2008, Parolo & Rossi, 2008, Vittoz *et al.*, 2009, Walck *et al.*, 2011). In alpine ecosystems recruitment via seed is evident, indicated by high rates of seedling establishment and genotypic diversity (Forbis, 2003, Gabrielsen & Brochmann, 1998, Jonsson *et al.*, 1996, Schlag & Erschbamer, 2000, Schönswetter *et al.*, 2003, Venn & Morgan, 2009b). Thus, sexual reproduction and regeneration via seed will inevitably become more important in determining alpine plant population dynamics and distributions in the future (Briceño *et al.*, 2015, Pearson, 2006, Thuiller *et al.*, 2008).

Alpine and other-cold adapted seeds generally possess physiological dormancy at dispersal (e.g. Hoyle *et al.*, 2015, Schwienbacher *et al.*, 2011, Shimono & Kudo, 2005). Physiologically dormant seeds usually require a certain length of cold stratification (winter temperature) before dormancy can be relieved and germination starts (Hoyle *et al.*, 2014, Hoyle *et al.*, 2015). Alpine seed relies on special cues including a relatively warm temperature as a germination requirement (Billings & Mooney, 1968, Körner, 2003). Projected future warming could therefore promote recruitment of these cold-adapted species (Mondoni *et al.*, 2012). In a changing climate, certain temperature thresholds and seasonal (winter) patterns which are required to alleviate dormancy and trigger germination, may be altered and potentially disrupting plant regeneration via seed (reviewed in Donohue *et al.*, 2010, Walck *et al.*, 2011). Warmer temperatures can either reduce or increase germination as well as shift germination time (Bernareggi *et al.*, 2016, Milbau *et al.*, 2009, Mondoni *et al.*, 2015, Mondoni *et al.*, 2012, Shevtsova *et al.*, 2009). For example, +4 °C warmer summer and autumn increases germination of several alpine plants of Italian Alps, particularly increasing the proportion of seeds that germinate in autumn (Mondoni *et al.*, 2015, Mondoni *et al.*, 2012). On the other hands, a moderate +2.5 °C increase in summer temperature is found to reduce overall germination but reduced mean germination time of sub-arctic plants (Milbau *et al.*, 2009, Shevtsova *et al.*, 2009). Thus, for sub-arctic species, future warmer temperature leading to earlier germination could be an advantage in this system where growing season is short (Milbau *et al.*, 2009). However, winter is also predicted to be shorter and colder leading to a reduced germination in sub-arctic and Italian alpine seeds from snow bed community (Milbau *et al.*, 2009, Mondoni *et al.*, 2015). The results from the aforementioned future climate experiments indicate that seed germination response to changed climate variables is not unidirectional, likely owing to the habitat, species, or seed and germination characteristic. For Australian alpine seeds, seedling emergence is reduced but intriguingly a more diverse species emerge from the soil under warming (Hoyle *et al.*, 2013).

Australian alpine species generally display one of three main germination strategies: 1) *immediate germination* of seeds that are non-dormant at dispersal and readily germinate in autumn; 2) *postponed germination* after alleviation of dormancy by exposure to winter temperatures (cold stratification) and hence, germination occurs in the spring of subsequent year; and 3) *staggered strategies* where both immediate and postponed strategies are observed and thus, both autumn and spring germination present among seed of a single dispersal event (Hoyle *et al.*, 2015). Staggered germination strategy is quite common in alpine species, in which germination of the seeds dispersed in autumn occurs intermittently started immediately after dispersal and continue over time until spring/summer (Hoyle *et al.*, 2015, Mondoni *et al.*, 2012). The diversification of germination strategy within a single dispersal event as exemplified by species with staggered germination strategy is often associated with evolutionary bet hedging (Simons, 2011). A staggered strategy may buffer the effect of climate anomalies and increase the likelihood for seedling growth under favourable conditions (Akiyama & Ågren, 2014, Shimono & Kudo, 2003). Not only staggering germination within one year, germination can be spread over a longer time scale (beyond the subsequent spring/summer) through seed persistence in the soil (Adams *et al.*, 2005, Long *et al.*, 2015). In an unpredictable environment, the soil seed bank is another important component to maintain long-term survival and viability (Cohen, 1966, Cohen, 1967). Thus, understanding the fate of the un-germinated seed fraction, whether seeds are dead or alive and dormant, under future climate condition is also crucial.

In Australia, where the alpine region extends across an elevational range of just 400 m, understanding the scope and potential for alpine plant recruitment, particularly the interactions between temperature and winter duration effect, is crucial for forecasting alpine plant populations and community assemblages under future climate scenarios. A 3 °C temperature increase relative to 1990 is projected across the Australian Alps as soon as 2050 (Hennessy *et al.*, 2003). Snowpack duration has also decreased by ~9 days since 1954 (Hennessy *et al.*, 2003, Sánchez-Bayo & Green, 2013). Given the significance that winter has for different germination strategies, winter cues that change rapidly under future climate scenarios will likely disrupt the germination of alpine species (Hoyle *et al.*, 2015). For example, a winter duration that is shorter than six weeks is too brief to alleviate physiological dormancy of *Aciphylla glacialis*, an Australian alpine species with a postponed strategy (Hoyle *et al.*, 2014, Venn & Morgan, 2009a). However, little is known about the relative importance of warmer temperatures and an altered winter duration on the germination of Australian alpine seed, nor whether the response to a future climate scenario will be constant across germination strategies.

In order to understand Australian alpine plant establishment via seed, we conducted a germination experiment under conditions that mimic current and future climates. Specifically, we asked: How will a future climate including warmer temperature and reduced winter duration affect germination responses such as mean time of germination, autumn germination, final germination, and non-germination proportion, compared to the current climate? Are both autumn and spring germination present in species with a staggered germination strategy under warmer temperatures? Whether germination reduction as exhibited by reduced seedling emergence from alpine soil seed bank under soil warming can be explained by germination strategy? We predicted that: 1) Germination is accelerated by warmer temperature and is not affected by winter duration in all strategies, and hence a decreased in mean germination time in all strategies; 2) Warmer temperature will also lead to an increase in autumn germination in staggered and postponed strategies; 3) Final germination in all strategies is reduced under warmer temperature and shorter winter; 4) Shortened winter duration particularly leads to a reduction of final germination in strategies with dormant-seed (staggered and postponed strategy); 5) Under future climate scenarios, where winter duration is too brief for dormancy alleviation, the un-germinated seeds of the staggered and postponed strategies are still viable and dormant.

Methods

Experimental set-up

The experiment involved exposing seeds of 39 Australian alpine species with varying germination strategies (13 species per strategy: immediate, staggered, postponed; Table 1) from a range of alpine families and life forms to modified climatic seasonal cycles. The seeds were collected from the Australian Alps bioregion, Kosciuszko National Park, NSW and Namadgi National Park, ACT between 2009 – 2013 (Table 1). Seeds were stored at the National Seed Bank, Australian National Botanic Gardens in Canberra under - 20 °C or under 15 °C and 15% RH for year 2013 collections. Species' germination strategy was defined using germination assays described in Hoyle *et al.* (2015). The species germination strategies in our assays matched to those listed in Hoyle *et al.* (2015), except for *Richea continentis* and *Ranunculus acrophilus* that we defined to have staggered strategy (same accessions but categorised as postponed and immediate, respectively, in Hoyle *et al.*, 2015). The germination strategies for *Baeckea gunniana*, *B. utilis*, *Epacris celata*, *Epilobium gunnianum*, *Luzula modesta*, *Olearia algida*, *Poa philipsiana*, *Ranunculus pimpinellifolius,* and *Stylidium graminifolium* were determined here for the first time (Table 1).

Table 1. The species and family for the three germination strategies, immediate, staggered, and postponed, tested in this study. Asterisk indicates endemic species. Locality is the seed collection origin – Kosciuszko National Park (KNP) and Namadgi National Park (NNP), Australia.

Germination Strategy	Species	Family	Life form	Collection Elevation (m)	Latitude	Longitude	Collection date	Locality
Immediate	*Colobanthus affinis*	Caryophyllaceae	Herb	1736	-36.4302	148.3244	6-Jan-10	KNP
Immediate	*Craspedia costiniana**	Asteraceae	Herb	1986	-36.408	148.3055	6-Mar-12	KNP
Immediate	*Erigeron setosus**	Asteraceae	Herb	1928	-36.4111	148.3083	4-Feb-10	KNP
Immediate	*Epilobium gunnianum*	Onagraceae	Herb	1339	-35.5363	148.9063	12-Feb-13	NNP
Immediate	*Leucochrysum alpinum*	Asteraceae	Herb	2131	-36.4566	148.27	17-Feb-10	KNP
Immediate	*Luzula acutifolia subsp. nana**	Juncaceae	Monocot	1957	-36.4122	148.3072	16-Feb-11	KNP
Immediate	*Luzula modesta*	Juncaceae	Herb	1340	-35.5355	148.908	11-Dec-12	NNP
Immediate	*Plantago glacialis*	Plantaginaceae	Herb	1957	-36.4122	148.3072	16-Feb-11	KNP
Immediate	*Poa costiniana*	Poaceae	Monocot	2109	-36.475	148.2716	30-Mar-11	KNP
Immediate	*Poa phillipsiana*	Poaceae	Monocot	1399	-35.8786	148.5036	3-Feb-09	KNP
Immediate	*Ranunculus pimpinellifolius*	Ranunculaceae	Herb	1640	-36.3727	148.4822	5-Jan-10	KNP
Immediate	*Rytidosperma nudiflorum*	Poaceae	Monocot	2105	-36.4036	148.3013	21-Feb-08	KNP
Immediate	*Wahlenbergia ceracea*	Campanulaceae	Herb	1974	-36.455	148.2883	3-Mar-10	KNP
Staggered	*Astelia psychrocharis*	Liliaceae	Herb	1702	-36.4294	148.3586	28-Feb-12	KNP
Staggered	*Baeckea gunniana*	Myrtaceae	Shrub	1592	-35.5225	148.7816	18-Apr-13	NNP
Staggered	*Baeckea utilis*	Myrtaceae	Shrub	1603	-35.5236	148.7819	17-Apr-13	NNP
Staggered	*Brachyscome stolonifera**	Asteraceae	Herb	1941	-36.45	148.28	5-Feb-10	KNP
Staggered	*Deschampsia cespitosa*	Poaceae	Monocot	1743	-36.4269	148.3677	24-Feb-09	KNP
Staggered	*Olearia algida*	Asteraceae	Shrub	1636	-35.5222	148.7725	17-Jan-13	NNP
Staggered	*Oreomyrrhis ciliata*	Apiaceae	Herb	1748	-36.4319	148.3455	27-Jan-10	KNP
Staggered	*Oreomyrrhis pulvinica*	Apiaceae	Herb	1733	-36.4294	148.3202	4-Feb-10	KNP
Staggered	*Ozothamnus cupressoides*	Asteraceae	Shrub	1599	-35.5227	148.7819	17-Apr-13	NNP
Staggered	*Ranunculus acrophilus**	Ranunculaceae	Herb	2039	-36.4069	148.2963	6-Jan-10	KNP
Staggered	*Ranunculus graniticola*	Ranunculaceae	Herb	1734	-36.4636	148.3213	6-Jan-10	KNP
Staggered	*Ranunculus muelleri*	Ranunculaceae	Herb	1955	-36.4572	148.2991	28-Jan-10	KNP
Staggered	*Richea continentis*	Ericaceae	Herb	1588	-35.5213	148.7822	20-Feb-13	NNP
Postponed	*Aciphylla glacialis*	Apiaceae	Herb	2164	-36.4555	148.2611	11-Mar-09	KNP
Postponed	*Aciphylla simplicifolia*	Apiaceae	Herb	1767	-36.43	148.3447	17-Mar-10	KNP
Postponed	*Astelia alpina var. novae-hollandiae*	Liliaceae	Herb	2008	-36.4486	148.2708	15-Mar-10	KNP
Postponed	*Carex echinata*	Cyperaceae	Herb	1959	-36.4097	148.3055	22-Feb-07	KNP
Postponed	*Carex gaudichaudiana*	Cyperaceae	Monocot	1330	-35.5358	148.9077	12-Feb-13	KNP
Postponed	*Carpha nivicola*	Cyperaceae	Herb	1924	-36.4583	148.2969	5-Mar-07	KNP
Postponed	*Chionogentias muelleriana* ssp. *alpestris*	Gentianaceae	Herb	1741	-36.43	148.3586	17-Mar-10	KNP
Postponed	*Epacris celata*	Ericaceae	Shrub	1604	-35.523	148.7822	17-Apr-13	KNP
Postponed	*Gingidia algens**	Apiaceae	Herb	1799	-36.3669	148.3713	30-Mar-10	KNP
Postponed	*Oreomyrrhis eriopoda*	Apiaceae	Herb	1744	-36.4294	148.3586	3-Feb-10	KNP
Postponed	*Psychrophila introloba*	Ranunculaceae	Herb	1941	-36.4586	148.2972	5-Feb-10	KNP
Postponed	*Ranunculus dissectifolius**	Ranunculaceae	Herb	1752	-36.4319	148.3455	27-Jan-10	KNP
Postponed	*Stylidium graminifolium*	Stylidiaceae	Herb	1745	-36.4302	148.3572	2-Mar-10	KNP

Future climate scenarios

The climate scenarios comparing current and future regimes were arranged in a fully factorial design consisting of two factors; temperature (ambient and warmer) and winter duration (current and shorter) (Table 2). The ambient temperature and current winter duration scenario was selected to mimic the seasonal progressions in the field based on current mean temperatures (for more detail and justification see Hoyle *et al.*, 2015). The modified future climate seasons represented a five week (33 days) shorter winter and a 4 °C increase of temperature compared to ambient climate scenarios (Table 2). A 4 °C temperature increase for future climates versus ambient temperature that reflect seasonal changes was selected for the experiment based on extensive consultation and research, as follows: Australia alpine temperatures are projected to rise 5.1 °C by 2090, which is higher than the global average (CSIRO and Bureau of Meteorology, 2015). Hence, an increase of 4 °C was selected for the future temperature scenario. Warmer temperature is followed by a decline in snow depth (Fiddes & Pezza, 2015), and the average length of the snow season in the Australian Alps by 2050 is predicted to be reduced by 20 days in the best case scenario, and by 100 days in the worst case scenario (Hennessy *et al.*, 2008, Pickering & Venn, 2013). Already, climatic changes have shortened the snow season by ~9 days since 1954 (Hennessy *et al.*, 2003, Sánchez-Bayo & Green, 2013). Thus, in the experimental design for this study we selected an intermediate level of reduction in winter duration – the snow season was reduced by 33 days (with winter duration in current scenario being 74 days). Winter temperature was kept constant at 5 °C across all treatments reflecting the stability of temperature under snow, and the logistical constraints of our growth cabinets. The models and conventional wisdom suggest commencement of the winter season is unlikely to change even when snowmelt occurs sooner (Ken Green & Rebecca Harris, personal communication). Therefore, the timing of the onset of winter was not changed but the warm conditions was extended on the spring and summer side in our future climate scenario (Table 2).

Table 2. Experiment design implemented in controlled environment facility to mimic ambient and future climate scenarios.

Current Winter (74 days)	Season	Duration (days)	Temperature (°C)	
			Ambient	Warm by 2050 (+4)
	summer (2nd half)	24	25/15	29/19
	early autumn	17	20/10	24/14
	late autumn	15	10/5	14/9
	winter	74	4 – 5	4 – 5
	early spring	15	10/5	14/9
	late spring	17	20/10	24/14
	summer	48	25/15	29/19
	early autumn	15	20/10	24/14
	late autumn	17	10/5	14/9
Shortened winter (41 days)	Season	Duration (days)	Temperature (°C)	
			Ambient	Warm by 2050 (+4)
	summer (2nd half)	24	25/15	29/19
	early autumn	17	20/10	24/14
	late autumn	15	10/5	14/9
	winter	41	4 – 5	4 – 5
	early spring	15	10/5	14/9
	late spring	17	20/10	24/14
	summer	81	25/15	29/19
	early autumn	15	20/10	24/14
	late autumn	17	10/5	14/9

Germination

The four factorial combinations of the climate scenarios were achieved using controlled environment chambers (Thermoline Twin Stack Plant Growth Chamber Model TPG 2x450-TO-Spec, Thermoline Scientific NSW). Seeds of the 39 species (13 species for each germination strategy) were germinated in petri dishes on agar. For each species, four replicate dishes of 25 seeds per dish was assigned to each climate scenario. The seeds were assigned to one of four controlled environment chambers to accommodate the two temperature regimes and the two winter duration regimes with seasonal cycles (Table 2). The autumn, summer and spring photoperiod was 16/8 hours day/night and winter photoperiod was 12/12 hours day/night. In each chamber, daylight was provided by fluorescent lights emitting ~350µE. Chamber conditions were monitored throughout to maintain consistent light regimes. Limitations in chamber space and availability precluded replicating at the chamber level. Each replicate was stacked together in a group (block) and the position of the petri-dish within the block was randomly rotated after each germination scoring. Germination was checked and scored twice a week until the end of week four, by which point many species of the immediate strategy reached > 80% germination. From week five onwards germination was checked weekly. Seeds were considered to have germinated when the radicle protruding from the seed coat was visible.

Germination scoring was performed until the end of the experiment (week 35) and subsequently cut-tests were performed to determine whether the un-germinated seeds were empty, filled but mushy (dead seed), or filled and alive. The germination data from weekly scoring were used to determine the proportions of autumn germination, and final germination. The actual filled seed number (total sown minus empty seed) was used to adjust autumn and final germination values. The deep-dormant seed fraction was indicated by un-germinated alive seed at the end of the experiment. In addition, we calculated the Mean Germination Time $MGT = \sum_{i}^{n}(n_i t_i)/\ N$ where n_i was the number of newly germinated seeds at week t_i, t was time from the beginning of germination test in weeks, and N the total number of seeds that germinated. We excluded from the statistical analyses two species from that we had classified as having a postponed germination strategy because both had final germination lower than 10%: *Carpha nivicola, Chionogentias muelleriana* ssp. *alpestris*.

Statistical analysis

The proportion of germination, including final germination, non-dormant fraction, and un-germinated viable seed fraction were analysed using generalized linear mixed models (Bolker *et al.*, 2009). Analysis of each of the germination proportions when all three germination strategies were included in the model resulted in convergence failure. The convergence failure source was that the intrinsic germination value of each strategy differed markedly, immediate reached nearly 100% germination with very little variations among species in contrast to the staggered and postponed strategies. Hence, we here present results of the analysis performed separately for each germination strategy and make general inference across them within reason. We analysed final germination, autumn germination and un-germinated viable seed proportions, using generalized linear mixed models, using binomial distribution and link logit, assigning temperature and winter duration and their interaction as fixed effects and species and block as random effects. For autumn germination, generalized linear mixed models were also used and the fixed effect assigned was only temperature. For mean germination time, the same fixed and random factors were used except that linear mixed-effects models were used. The mixed-effects models were performed using *lme4* package (Bates *et al.*, 2015) in R 3.2.4 (R Core Team, 2016). Multiple comparisons was performed using *lsmeans* package (Lenth, 2016) in R 3.2.4 (R Core Team, 2016).

Results

Mean Germination Time

As predicted, there were significant reductions of mean germination time in response to warming, except for species with immediate strategy. Contrary to our prediction, winter duration significantly reduced mean germination time for species with staggered strategy. For species with a postponed strategy mean germination time was shorter under a shorter winter, but only for those species in which dormancy had been alleviated (Fig. 1, Table 3). For species with an immediate strategy there was not a statistically significant effect of temperature and winter duration on mean germination time.

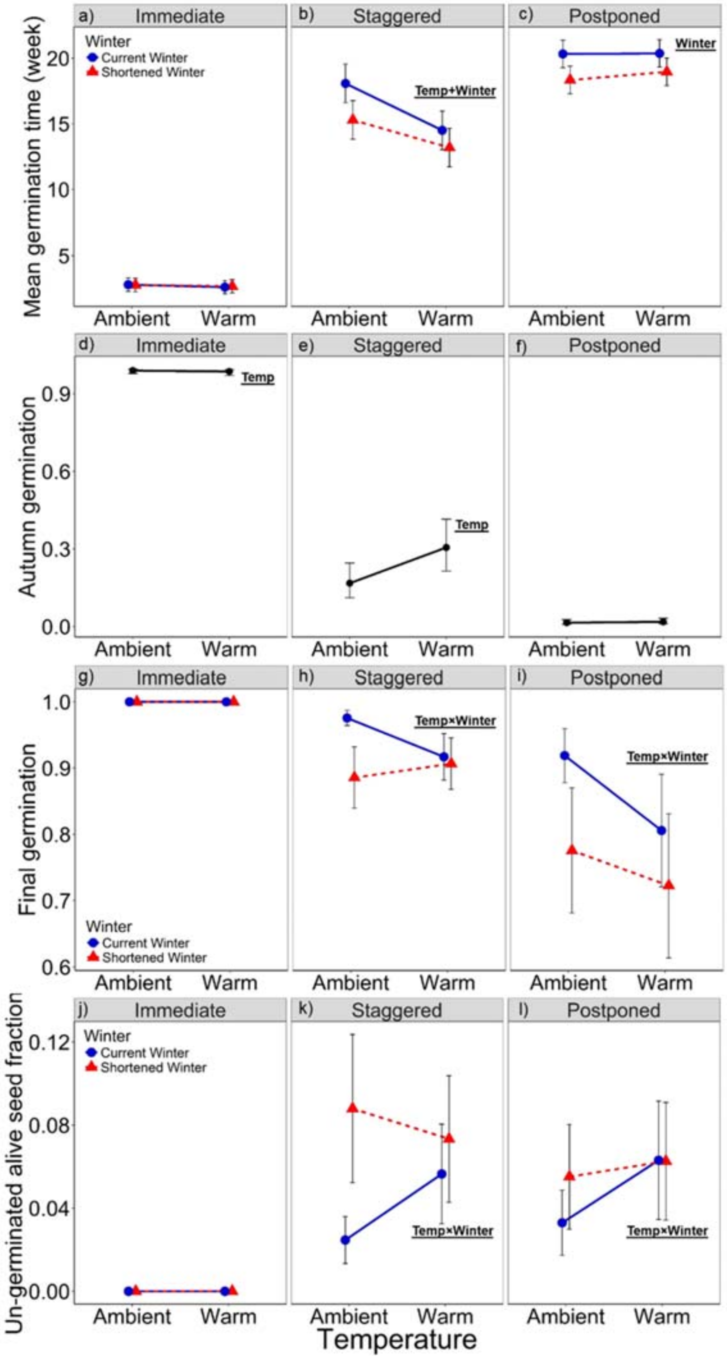

Figure 1. Treatment effects presented for each germination strategy. The effects of temperature (Ambient and Warm) and winter duration (Current vs and Shortened) on mean germination time (a, b, c; Table 3). The effect of temperature on autumn germination (before winter commences) indicated by black line because this is for non-dormant seed measures and thus, only temperature matters (d, e, f; Table 4). The effects of temperature and winter duration on the final germination (g, h, i; Table 3), and on un-germinated alive seed fraction (j, k,l). Significant interaction, additive or single treatment effect are indicated by the bold and underlined variables. Temp stands for temperature and Winter for winter duration; × indicates interaction effect, and + indicates additive effect (Table 3).

Autumn germination

We expected that species with a staggered germination strategy would show a significant increase in the proportion of seeds germinating in autumn under the warmer scenario and this prediction was supported by our data (Fig. 1e, Table 4). There was approximately 13% increase in autumn germination for staggered germination strategy indicating a shift in germination timing in response to warming, but the shift was not seen for species with postponed strategy (Fig. 1f). Contrary to our expectation, however, the autumn germination for the immediate strategy species was also marginally reduced under warmer scenario. The reduced autumn germination of immediate strategy was marginal because it was only occurred for a few species (e.g. *Craspedia costiniana* and *Colobanthus affinis* (Fig. 1d, SI Fig. 2, Table 4). The seeds of these two species were then germinated later, and thus, leading to the diminished effect of temperature on the final germination of immediate strategy.

Final germination

Despite germination acceleration, we predicted that final germination would be negatively affected by increased temperature and particularly by shortened winter duration for species with staggered and postponed germination strategy (dormant seeds). Overall, we found a high final germination for species with immediate strategy with no significant effect of temperature or winter duration (Fig. 1g) despite a marginal but significant reduction of autumn germination under warmer temperature (Fig. 1d). This indicated that a minor fraction (< 20% of the seeds) from the species with immediate germination strategy could overwinter as seed, and this minor seed fraction might continue germination in spring until it reaches almost 100% regardless of the temperature and winter duration regimes (SI Fig. 2 and SI Fig. 3). We expected that a shorter winter would significantly reduce germination of the staggered and postponed strategies, and we found that the interaction effect between winter duration and temperature reduced the final germination of species with staggered strategy such that warmer temperatures significantly reduced final germination under current winter, but not under short winter (Fig. 1h, Table 3). For species with postponed strategy, the interaction effect was also significant to reduce final germination where under ambient temperature shorter winter duration significantly reduced final germination but under warmer temperature, the effect of winter duration was not significant (Fig. 1i, Table 3).

Un-germinated viable seed fraction

We predicted that under future climate scenarios, there would be an increase in viable but un-germinated seed fractions in species with staggered and postponed strategy at the end of the experiment. We found that the un-germinated viable seed fractions were on average 5% of the total seed for either staggered or postponed strategy (Fig. 1k and 1l, SI Fig. 4) and in fact present in all germination strategies, although for species with immediate strategy the un-germinated seeds was nearly zero (Fig. 1j, SI Fig. 4). For the staggered and postponed strategies, the temperature and winter duration effects interacted, whereby the un-germinated viable seed fraction was significantly higher under shortened winter as predicted, but the effect of winter duration was only significant under ambient temperatures (Fig 1k and 1l, Table 3).

Table 3. Summary of the mixed models for each germination strategy – immediate, staggered and postponed, for the mean germination time, final germination, and un-germinated viable seed fractions. Note: ‡Relative to ambient temperature; †Relative to future shortened winter.

Response	Germination Strategy	Fixed effect	Estimate	SE	Pr(>|z|)
Mean germination time	Immediate	Intercept	2.771	0.507	**<0.001**
		Warm Temperature ‡	-0.092	0.180	0.609
		Short Winter†	0.029	0.180	0.873
		Warm Temperature‡ : current winter†	-0.097	0.255	0.704
	Staggered	Intercept	15.297	1.472	**<0.001**
		Warm Temperature ‡	-2.110	0.583	**<0.001**
		Current Winter†	2.782	0.583	**<0.001**
		Warm Temperature‡ : current winter†	-1.460	0.824	0.078
	Postponed	Intercept	18.340	1.044	**<0.001**
		Warm Temperature ‡	0.625	0.577	0.281
		Current Winter†	1.987	0.577	**<0.001**
		Warm Temperature‡ : current winter†	-0.585	0.816	0.474
Final germination	Immediate	Intercept	56.07	59.14	0.343
		Warm Temperature ‡	-44.80	59.14	0.449
		Current Winter†	-13.73	61.06	0.822
		Warm Temperature‡ : current winter†	14.10	61.06	0.817
	Staggered	Intercept	2.047	0.457	**<0.001**
		Warm Temperature ‡	0.227	0.140	0.102
		Current Winter†	1.644	0.202	**<0.001**
		Warm Temperature‡ : current winter†	-1.515	0.250	**<0.001**
	Postponed	Intercept	1.240	0.543	**0.022**
		Warm Temperature ‡	-0.283	0.110	**0.010**
		Current Winter†	1.188	0.125	**<0.001**
		Warm Temperature‡ : current winter†	-0.722	0.166	**<0.001**
Un-germinated viable seed fractions	Immediate	Intercept	56.07	59.14	0.343
		Warm Temperature ‡	-44.80	59.14	0.449
		Current Winter†	-13.73	61.06	0.822
		Warm Temperature‡ : current winter†	14.10	61.06	0.817
	Staggered	Intercept	-2.339	0.445	**<0.001**
		Warm Temperature ‡	-0.198	0.152	0.195
		Current Winter†	-1.338	0.207	**<0.001**
		Warm Temperature‡ : current winter†	1.059	0.265	**<0.001**
	Postponed	Intercept	-2.841	0.484	**<0.001**
		Warm Temperature ‡	0.135	0.153	0.378
		Current Winter†	-0.537	0.175	**0.002**
		Warm Temperature‡ : current winter†	0.545	0.229	**0.018**

Table 4. Summary of the mixed models for each germination strategy for autumn germination. The fixed effect for autumn germination was temperature (‡ Relative to ambient temperature).

Germination strategy	**Fixed effect**	**Estimate**	**SE**	**Pr(>|z|)**
Autumn germination				
Immediate	Intercept	4.525	0.745	**<0.001**
	Warm Temperature‡	-0.289	0.143	**0.042**
Staggered	Intercept	-1.602	0.480	**<0.001**
	Warm Temperature‡	0.782	0.084	**< 0.001**
Postponed	Intercept	-4.179	0.583	**<0.001**
	Warm Temperature‡	0.191	0.148	0.198

Discussion

Under future climate scenarios our results indicate that the total germination of Australian alpine seeds will likely decrease. This result concurs with studies investigating seedling emergence from the Australian alpine soil seed bank under a soil warming scenario (Hoyle *et al.*, 2013) and also germination studies of sub-arctic species (Milbau *et al.*, 2009, Shevtsova *et al.*, 2009). Seed germination of (sub-) arctic and alpine species, however, has been shown to vary considerably in response to warmer mean temperatures as well as winter duration and temperature (e.g. Bernareggi *et al.*, 2016, Graae *et al.*, 2008, Milbau *et al.*, 2009, Milbau *et al.*, 2017, Mondoni *et al.*, 2015, Mondoni *et al.*, 2012, Shevtsova *et al.*, 2009). Functional groups, e.g. herb, monocot, shrub, and tree, could not explain these variations in species germination response (e.g. Milbau *et al.*, 2009). In this study, we investigated the germination response of a wide range of Australian alpine species under future seasonal changes and thermal regime, by also including the germination characteristic of the species, i.e. ‘germination strategy’. Perhaps, the most striking of our results was that the germination strategy employed was robust in describing the germination responses under climate change, at least across 39 Australian alpine species tested. Here we discuss the salient findings regarding shifts in germination timing and the fraction of viable non-dormant seed across germination strategies and their implications for alpine plant community composition in the future.

Germination shift

There was a significant increase in autumn germination exhibited by species with staggered strategies under warmer temperatures but the shift was not complete and hence, both forms, autumn and spring seedlings of species with staggered strategy, would still be present under warmer temperatures. On contrary, we found no evidence of substantial increase of autumn germination for species with a postponed germination strategy, which has been observed elsewhere (Mondoni *et al.*, 2015, Mondoni *et al.*, 2012).

The adjustment of germination phenology or a shift in germination timing are suggested to be the major strategies for alpine plants to increase seedling success in their current sites (Mondoni *et al.*, 2015, Mondoni *et al.*, 2012). Shifting germination timing (autumn versus winter) could lead to either mortality or increased fitness (Donohue, 2002). Shifting germination earlier to autumn could be advantageous for plants, particularly in Australian alpine regions where warmer temperature and earlier snowmelt is associated with a warmer soil temperature (Slatyer, 2016). Early snowmelt in spring leading to a longer growing season was also found to enhance recruitment success in glacier foreland herbs (Mondoni *et al.*, 2015), subarctic grasses and shrubs (Milbau *et al.*, 2009). Germinating early is generally favoured for reproductive success, but does not necessarily guarantee high seedling survival (Rathcke & Lacey, 1985). Warming increase seedling emergence but also increase mortality of common subarctic plants (Milbau *et al.*, 2017).

Un-germinated seeds as potential buffer

A low total germination proportion under warming, exhibited by staggered and postponed strategies, does not necessarily indicate a detrimental situation because a large proportion of un-germinated seed remains viable. Indeed, even non-dormant seeds can enter secondary or conditional dormancy if germination cues are absent, for example germination temperature is beyond the thermal window (Baskin & Baskin, 2014). The un-germinated viable seeds may therefore accumulate in the soil seed bank over time (Adams *et al.*, 2005). For some species, seed banks serve as a bet-hedging strategy against inter-annual climate variation and offer re-establishment opportunities following disturbance, and provide a seed reserve when immediate recruitment fails or seed quality and production are poor (reviewed in Hoyle *et al.*, 2013), a feature that particularly important in a heterogeneous environment.

Alpine soil seed banks are mainly identified as persistent, indicated by > 2 years viability in the soils (Arroyo *et al.*, 1999, Schwienbacher *et al.*, 2010, Venn & Morgan, 2010, Walck *et al.*, 2005). As future soil temperatures increase, seedling emergence from soil seed bank is expected to be reduced eventually leading to reductions in regeneration capacity (Hoyle *et al.*, 2013). Whether the reduction of seedling emergence is caused by repeated dormancy cycling, seed mortality, or the conditions required for germination are simply no longer met, requires further investigation. Seed persistence *in situ* is determined not only by soil temperature, moisture, or microbial activity but also by inherent seed longevity (Long *et al.*, 2015). Like other mountain species, Australian alpine seeds are relatively short-lived (Chapter 2), attempts to quantify Australian alpine seed persistence (e.g. through seed burial experiments) are important because they may provide insight into plant recruitment *in situ* and whether, under future climate, regeneration from soil seed bank will be sufficient for alpine system.

Which germination strategy will be the most susceptible under future climate conditions?

The response of germination traits to warming and winter duration was not unidirectional and could indicate the potential for germination strategy variations to buffer the impact of the changing climate at a species or community level. The experiment is a simplified version of real world and other factors such as more Growing Degree Days (GDD) and differences of soil temperature across season (from the timing of snow melt) that potentially affect the germination responses cannot be all fully captured. However, a strong pattern emerged indicate that germination strategy here apparently reflected different outcomes of selection on seedling survival and growth across different environmental drivers. Species with immediate germination strategy, for instance, may germinate without being disrupted by novel climate regimes and potentially optimize seedling growth over a longer growing seasons, but the effect may depend on whether the seedlings can survive winter (Donohue, 2002). Species with a staggered strategy would have both autumn and spring germination, and the ratio of autumn germination may become higher under warming scenario which may increase proportion of seedlings to potentially grow under longer growing season. For species with postponed strategy, and to a lesser extent the staggered ones the greater impact is likely to be duration of winter which may contribute to build up on the viable fraction in the soil seed bank.

In some alpine species a persistent soil seed bank is suggested to be an adaptation to low chance of establishment (Schwienbacher *et al.*, 2011). The un-germinated viable seeds fraction were mainly composed by species with staggered and postponed strategies. Proportionally, one would expect the composition of the soil seed bank to be dominated by seeds with a postponed strategy. However, Hoyle *et al.* (2013) reported that seedling emergence from the alpine soil seed bank in Australia was predominantly from Poaceae, Asteraceae *Epilobium*, *Cardamine,* and *Neopaxia,* families and species with immediate strategies, rather than species from staggered or postponed. Some discrepancies between our results and the findings in Hoyle *et al.* (2013) is likely owing to the dormancy alleviation used. Hoyle *et al.* (2013) used GA_3 is used for alleviating dormancy because physiologically dormant seeds was predicted to be prominent across the species composing the soil seed bank. Germination assays of 145 seed accessions across 99 Australian alpine species revealed that warm-cold stratification in general resulted in a higher final germination percentage than GA_3 application (unpublished data). Thus, germination or seedling emergence of the species found could be confounded by the methods used.

The Australian Alps are dominated by the Poaceae family, which mainly consists of species with an immediate strategy which showed little impact of warming and were unaffected by the changed seasonality. In alpine region elsewhere, the germination of graminoids (*Poa* and *Luzula*) is also not affected by warming (Mondoni *et al.*, 2015). Further, alpine and arctic graminoids will likely obtain increased growth under warmer climate scenarios (Dormann & Woodin, 2002). Thus, our results could indicate that graminoids in particular Poaceae will continue to dominate or even increase in alpine regions in the future Poaceae will still likely be a dominant family in the future Australian alpine landscape together with other species from the immediate strategy, if it were solely dependent on number of germinating seeds.

Future directions

Under future climate scenarios, one of the main questions for debate in the seed ecology world is whether changes in dormancy and germination strategy can keep up with the rapid rate of environmental change. In conjunction with previous work, our results provide is a strong indication that Australian alpine seed germination will decrease under future climate scenarios, particularly for staggered and postponed strategies, however, several other factors remain to be investigated. For example, seeds used in this study here were developed under ambient temperature. We have not a clear sense of how for example warmer maternal condition could "acclimate" the seeds to warmer germination condition. Warmer maternal condition and seed development temperature reduce the intensity of seed dormancy which in turn may (Fernández-Pascual & Jiménez-Alfaro, 2014, Fernández-Pascual *et al.*, 2013, García-Fernández *et al.*, 2015, Hoyle *et al.*, 2008) or may not (Bernareggi *et al.*, 2016) alter autumn germination fraction and hence, the species germination phenology. To have a better overview on whether changing climate in the future will really matter for Australian alpine plant regeneration from seed, we need to have a better understanding of whether seeds under future climate are adapted or acclimated in such a way by the parent as to be advantaged in the new climatic condition (Bernareggi *et al.*, 2016). Further study should therefore focus on the role of maternal effect, whether seeds developed in warmer temperature grow better in that warmer world particularly, to what extent germination strategy and germination niche is changed (or not changed) by maternal and germination temperature at dispersal.

Plant species persistence and survival under future climate may also be facilitated by the fine-grain heterogeneity across alpine landscapes which provides pockets of suitable microclimate, termed as 'microrefugia' (Hannah *et al.*, 2014, Rull, 2009). Indeed, the alpine landscape contains a mosaic of microclimates (Scherrer & Körner, 2010, Scherrer & Körner, 2011), which also associated with snow-melting patterns and exert a great influence on species composition (Körner, 2003, Schöb *et al.*, 2009). The strong correlation between climate and plant regeneration from seeds has resulted in the evolution of specific germination requirement, and phenology (Baskin & Baskin, 2014, Carta *et al.*, 2014, Fenner & Thompson, 2005). Populations of a single species may exhibit different germination strategies over microclimate (Fernández-Pascual *et al.*, 2012). Examination of variation in within-species germination strategy in germination responses and the subsequent seedling establishment traits may reveal further the potential for species to cope with, and adapt to, changes in climate (Chapter 4). To better understand the impact of warming and altered seasonality on seeding establishment and growth I conducted an experiment using the germinants of this present experiment. Those data will be the subject of a subsequent paper.

Conclusions

Our approach has revealed that species germination strategy is robust to predict germination responses in the Australian alpine flora under future climate conditions. However, to what extent intraspecific variation in germination strategy occurs across Australian alpine species in not completely known and hence, further study on to what extent germination strategy varies within species is crucial. Investigating the combined effect of warmer temperature, change in winter duration, and species germination strategy is novel and may provide a more comprehensive and inclusive overview on the future of alpine plant communities. When we better understand the effect of multiple interacting elements of climate change on plant germination and recruitment we will be in a much stronger position to predict impacts of warming climates on alpine plant communities.

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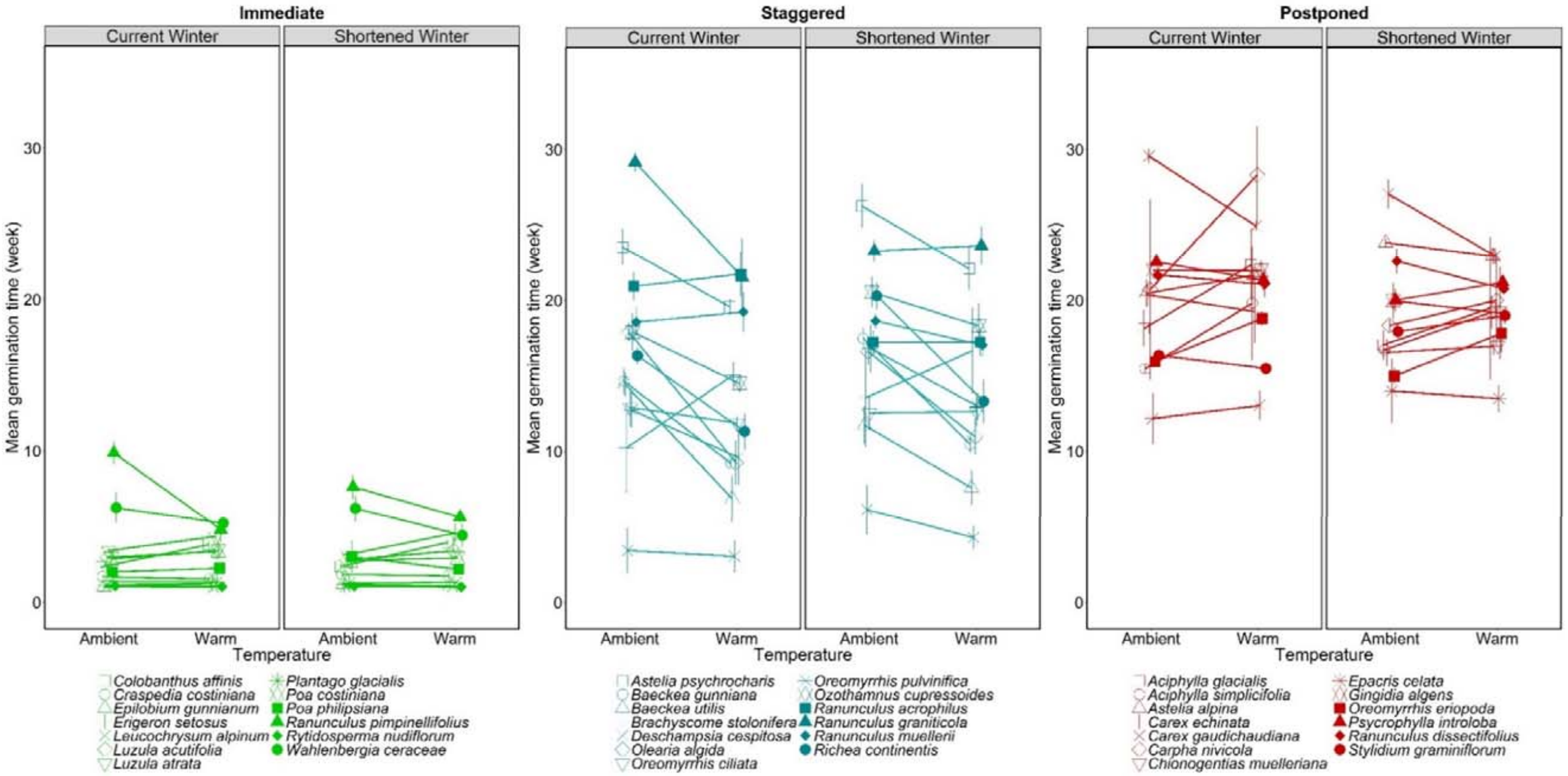
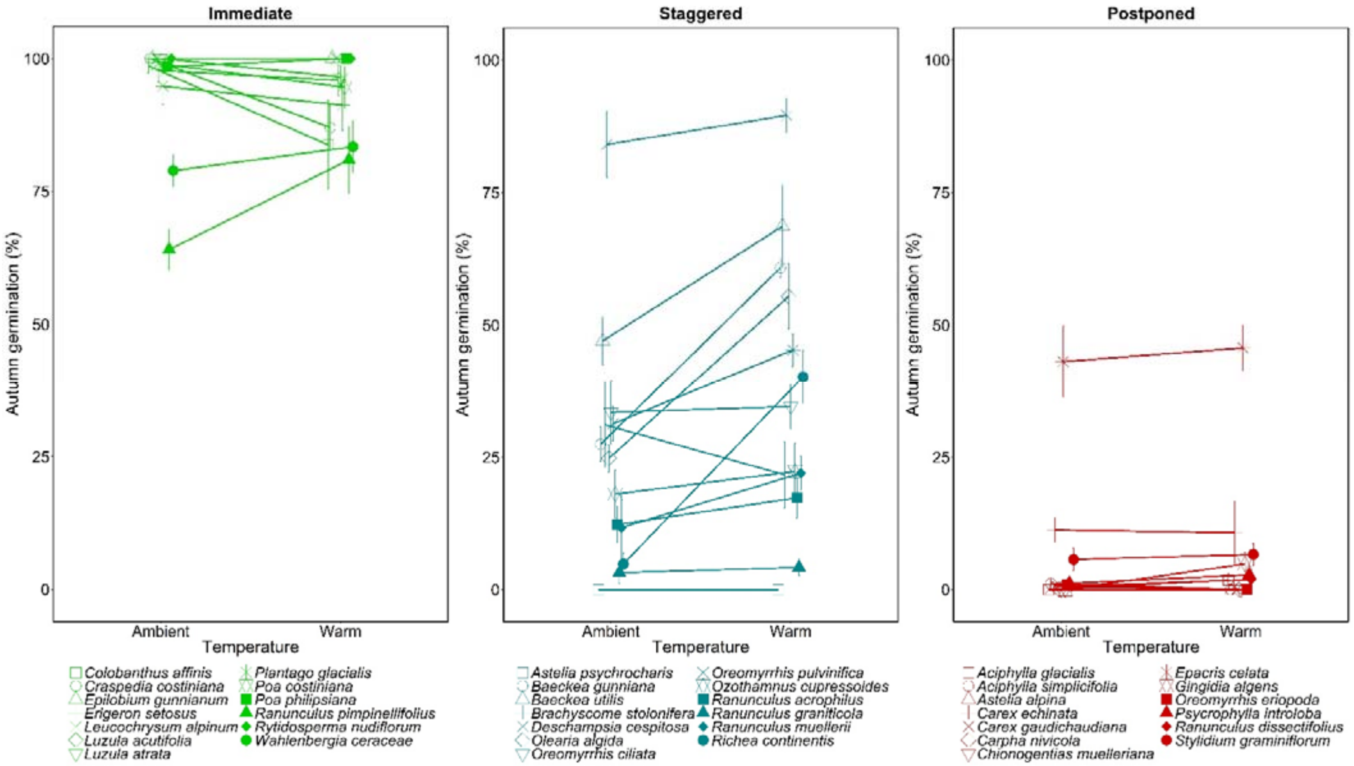
Supplementary Information

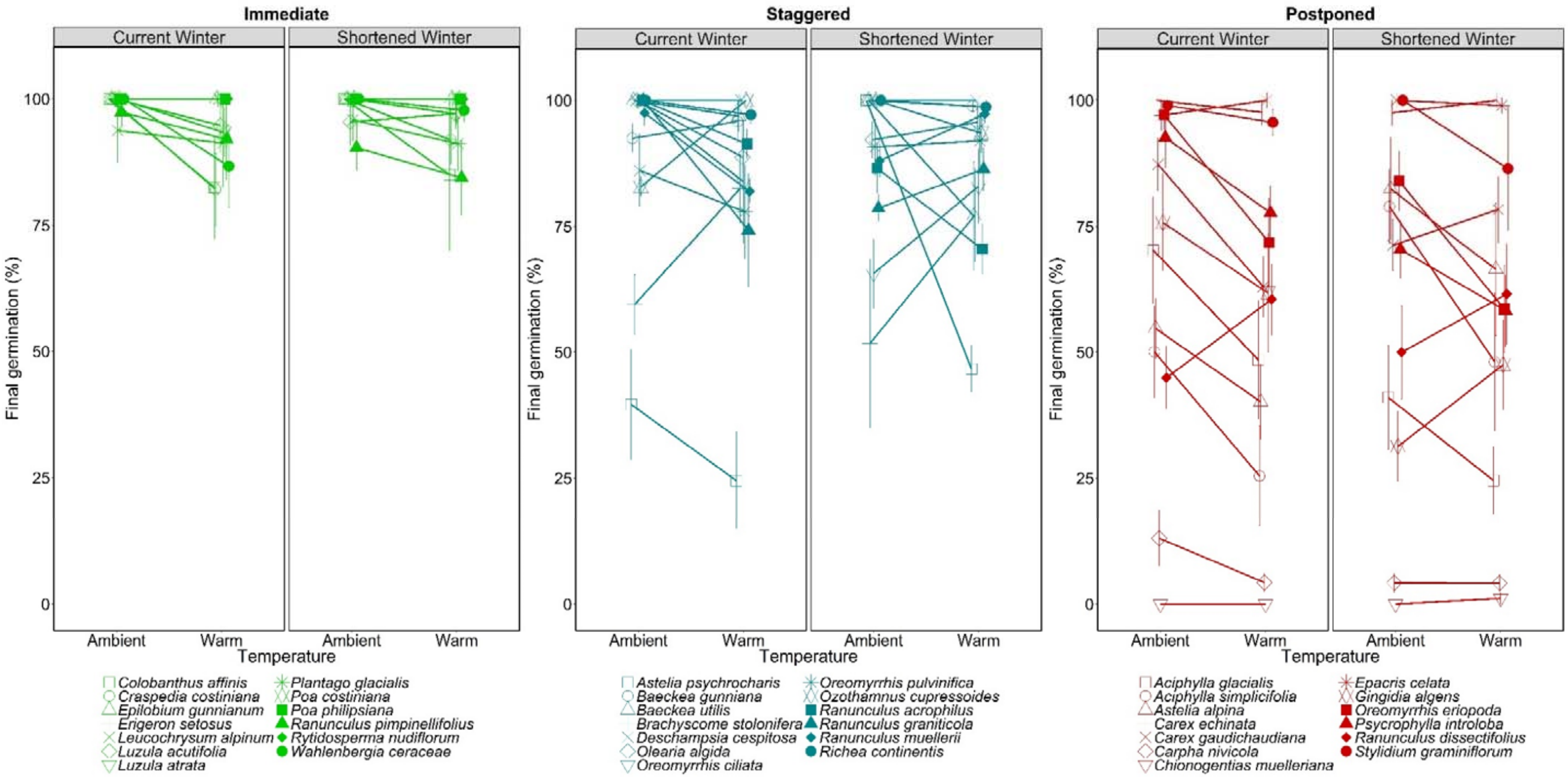


SI Figure 1. Mean germination time in week across species under different temperatures (ambient and warm) and winter duration (current and shorter winter), presented for each germination strategy – immediate, staggered, and postponed.

SI Chapter 3

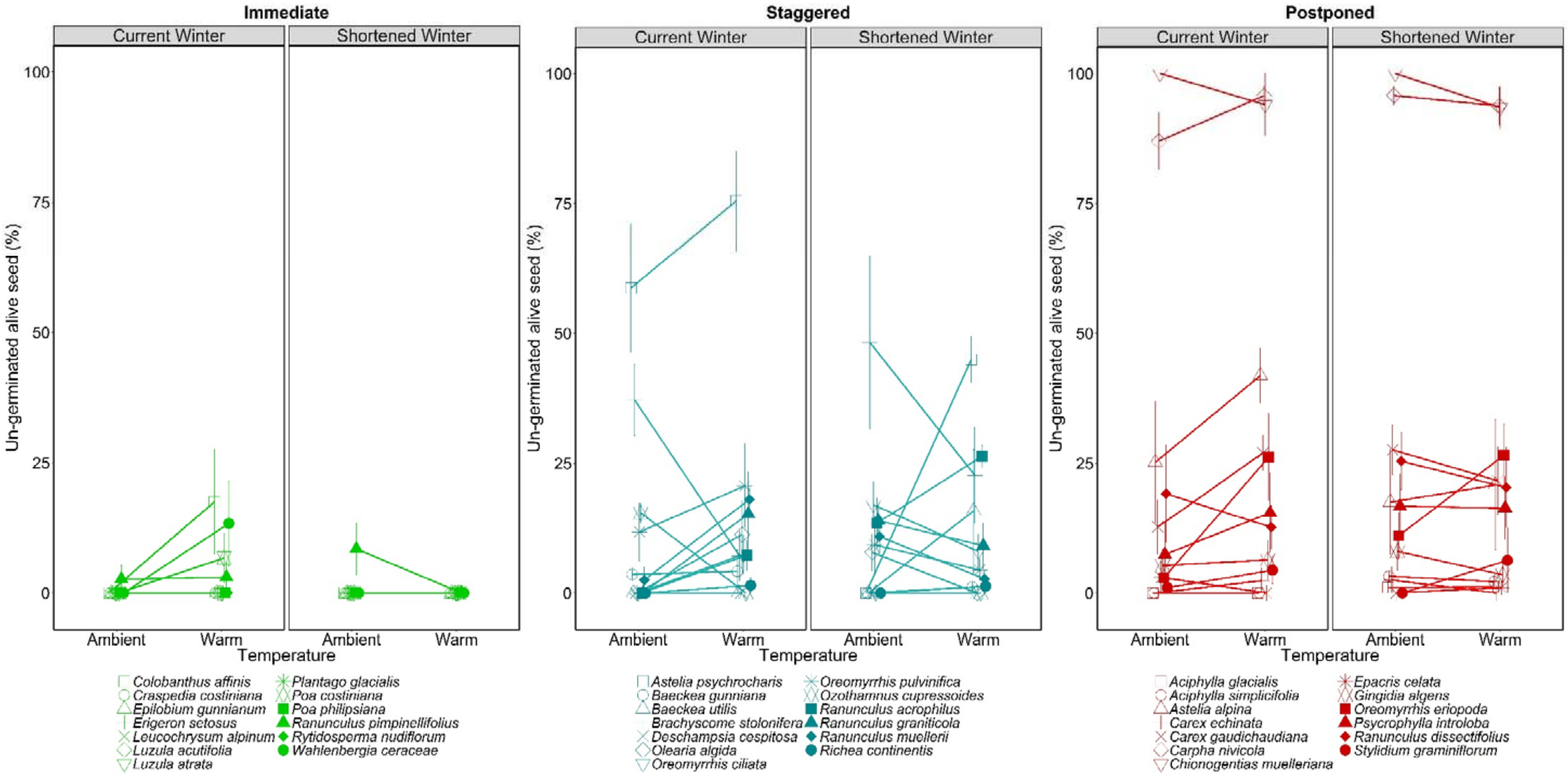


SI Figure 2. Autumn germination in percent under ambient and warm temperature scenario across species, presented for each germination strategy. Autumn germination was measured before winter and hence, temperature was the only fixed factor.



SI Figure 3. The final germination at the end of the experiment across species under two temperature scenarios (ambient and warm) and two winter durations (current and shortened winter), presented for each germination strategy.

SI Chapter 3



SI Figure 4. The un-germinated alive seed fraction at the end of the experiment across species under two temperature scenarios (ambient and warm) and two winter durations (current and shortened winter), presented for each germination strategy.

Design by Afiat Sukmaraga

Chapter 4

Temperature variability drives within-species variation in germination strategy and establishment characteristics of an alpine herb

Annisa Satyanti, Lydia K Guja, Adrienne B Nicotra

Abstract

Plant establishment and subsequent persistence are strongly influenced by germination strategy, especially in temporally and spatially heterogeneous environments. Germination strategy determines the plant's ability to synchronise germination timing and seedling emergence to a favourable growing season and thus variation in germination strategy within species may be key to persistence under more extreme and variable future climates. However, the determinants of variation in germination strategy are not well resolved. To understand the variation of germination strategy and the climate drivers, we assessed seed traits, germination pattern, and seedling establishment traits of *Oreomyrrhis eriopoda* from 29 populations across its range. Germination patterns were then analysed against climate data to determine the strongest climate correlates influencing the germination strategy. *Oreomyrrhis eriopoda* exhibits a striking range of germination strategies among populations: varying from immediate to staggered, postponed, and postponed-deep. Seeds from regions with lower temperature variability were more likely to exhibit an immediate germination strategy, however those patterns depended on the time scale of climatic assessment. In addition, we show that these strategy differences extend to seedling establishment traits: Autumn seedlings (from populations with an immediate or staggered germination strategy) exhibited a higher leaf production rate than spring seedlings (of staggered or postponed strategy). Our results not only confirm that there is substantial within-species variation in germination strategy across the species distribution range but that this variation correlates with environmental drivers. Given that these differences also extend to establishment traits they may reflect a critical mechanism for persistence in changing climate. Further examination of the generality of these results is necessary to better predict effects of climate change on alpine species worldwide.

Keywords

local adaptation, maternal environment, microclimate, plastic variation, recent climate change, seed dormancy, seedling growth, reproductive ecology

INTRODUCTION

Diversification in the timing of germination regulates the seasonal emergence of seedlings and is described as a species' 'germination strategy' (Donohue, 2005, Hoyle et al., 2015, Simons and Johnston, 2000). Germination strategies reflect patterns in the dormant fraction of seeds, germination speed and germination timing into simple groups and time early seedling growth to coincide with favourable environmental and microclimatic conditions for establishment, flowering, length of seed maturation, and ultimately, are presumed to have evolved to increase survival (Simons and Johnston, 2000, Donohue, 2002, Donohue et al., 2005). Variation in germination strategy occurs not only among species, but within (reviewed in Baskin and Baskin, 2014), and yet much remains to be understood about the drivers and consequences of that variation (Cochrane et al., 2015).

Germination strategy has the potential to determine how a population might respond to climate change (Hoyle et al., 2015). Variation in germination strategy is thought to be particularly relevant for plants inhabiting unpredictable environments or where climate change projections are most extreme, such as in alpine environments (Parolo and Rossi, 2008, Thuiller et al., 2008, Beniston, 2003, Wagner and Simons, 2009). Alpine habitats provide an ideal setting in which to examine drivers and associations of germination strategy (Hoyle et al., 2015). For example, germination strategy in Australian alpine species has been shown to vary from immediate germination strategies to postponed strategies (Hoyle et al., 2015). In addition, staggered strategies have been identified in which some seeds of a given seed collection germinate early in autumn and others late in spring (Hoyle et al., 2015). In this temporally and spatially heterogeneous environment, postponing germination until spring may increase seedling survival by reducing exposure to random mortality events such as warm, dry spells or severe frost in autumn (Mercer et al., 2011). However, spring seedlings are likely to compete with already established autumn seedlings such that selection may favour either accelerated germination or larger seed size relative to autumn germinants (Rathcke and Lacey, 1985). Within-species diversification of germination timing or germination strategies may provide a mechanism to overcome the risk of failed recruitment and may be an advantage for survival in unpredictable conditions (Walck et al., 2011).

Intraspecific variation in germination may be due to genetics, parental environment (including epigenetic effects) or the interaction thereof (Baskin and Baskin, 2014, Herman and Sultan, 2011). Seed dormancy can vary among populations of a single species with respect to elevation gradients (Nishitani and Masuzawa, 1996, Barclay and Crawford, 1984, Meyer and Kitchen, 1994), with local long-term temperature and precipitation (Carta et al., 2016, Schütz and Milberg, 1997, Wagmann et al., 2012), or be associated with other forms of habitat variability (Meyer and Kitchen, 1994, Meyer et al., 1995, Fernández-Pascual et al., 2013, Andersson and Milberg, 1998, Hoyle et al., 2014). For example, seeds from populations with warmer and more humid climate have a wider thermal niche for germination than those from colder and drier bioclimatic zones (Skordilis and Thanos, 1995, Schütz and Milberg, 1997, Wagmann et al., 2012, Torres-Martínez et al., 2017). Such variation in germination strategy may reflect sustained local climate differences among sites that lead to local adaptation to geographic location (Montesinos-Navarro et al., 2011, Fernández-Pascual et al., 2013, Zhou et al., 2013). Intraspecific variation in germination may also reflect the conditions to which the parent plant is exposed during seed development (Donohue et al., 2008, Fenner, 1991, Finch-Savage and Leubner-Metzger, 2006, Baskin and Baskin, 2014, Gutterman, 2000). For example, a lower degree of dormancy can be associated with high temperature and shorter day length during seed development (Fenner, 1991), or autumn precipitation variability (Torres-Martínez et al., 2017). Finally, seed mass and dormancy are reproductive traits that potentially interact, both reflecting adaptations to environmental conditions (Volis and Bohrer, 2013), but there is no universal consensus on the direction of the relationship between seed mass and dormancy or germination strategy.

To date, studies on drivers of variation in germination strategy have primarily focused on the effects of mean climate variables (e.g. temperature, precipitation) and overlooked the relative importance of climate variability though climate variability and extremes are important for many biological processes (Vasseur et al., 2014, Reyer et al., 2013, Vázquez et al., 2015). Further, only a few of these studies separate the pre-zygotic (before seed set) and post-zygotic (after seed set) developmental stages (e.g. Bernareggi et al., 2016, Kochanek et al., 2011). Indeed, despite numerous studies on the variation of dormancy and germination within species (see Baskin and Baskin, 2014), none has simultaneously examined the potential climatic drivers and correlations with seed traits, like embryo:seed ratio and seed mass, with extension to the potential effects on seedling establishment.

Here, we aim to quantify the extent of germination variation among populations of *Oreomyrrhis eriopoda*, a widespread Australian (sub-) alpine herb. *Oreomyrrhis eriopoda* occupies a wide range of micro-environments and is found in heath, herb fields and surrounding alpine bogs and fens at higher elevations, as well as in forests and shrub lands at lower elevations (Costin et al., 2000, Jobson, 1999, South Australian Seed Conservation Centre-Botanic Gardens of South Australia). *Oreomyrrhis eriopoda* seed, like many Apiaceae, has been reported to have morpho-physiological dormancy (Sommerville et al., 2013) and considerable variation in strategy is documented among populations (Hoyle et al., 2015, Wood, 2014, South Australian Seed Conservation Centre-Botanic Gardens of South Australia). Thus, the species provides an ideal case for examining how contrasting selection pressures associated with sites and climate could drive variation in germination strategy and seedling traits.

We made three main predictions: 1) that we would confirm that variation in germination syndromes previously reported from scattered populations of O. eriopoda is a general pattern found across the species distribution; 2) that germination strategy will be correlated with key climate variables, and particularly that long-term and short-term temperature would be key; 3) that germination strategy will be associated with seed and seedling traits e.g. populations with smaller seeds and a larger embryo to seed ratio will germinate earlier and have lower seedling growth rates, and 4) that seedlings with an immediate germination strategy (germination in autumn) will grow faster than those with a postponed germination strategy because they must reach near-mature size before winter. By extension, we predicted that for populations with a staggered germination strategy, the seedlings that germinate in autumn would also grow faster than those that germinate in spring.

MATERIALS AND METHODS

Seed accessions

Database searches revealed that seed accessions of 29 populations were available in sufficient numbers for investigation, from four seed banks in Australia. Seed had been collected from across four states and territories spanning a latitudinal distance of 770 km and at elevations between 650 to 2159 m asl, covering much of the distribution range of this species **[see Supporting Information** Fig. S4 and Table S1], between the years 1977 and 2013, with 2009 being the modal year. Seeds had been stored at either -18 °C or 5 °C. Prior to germination testing seeds were kept in a controlled environment room (15% relative humidity and 15 °C) for 1 – 2 weeks. We used only seed bank collections because they represent a wide range of the species geographic distributions, ensure consistent protocols for collection and storage and the species identifications have been validated by the respective herbaria. The IDs of the seedlings were again verified against a species description from relevant Floras, including Australia's Virtual Herbarium http://avh.chah.org.au, New South Wales Flora http://plantnet.rbgsyd.nsw.gov.au, Flora of Victoria http://vicflora.rbg.vic.gov.au, Flora of South Australia http://flora.sa.gov.au, Kosciuszko Flora (Costin et al., 2000), and by native plant expert at the Australian National Botanic Gardens (Joe McAuliffe, pers. comm.).

Germination assay

To assess the germination strategy of the populations we carried out a germination assay under a set of conditions that mimicked the diurnal temperature at the time of seed dispersal (autumn) in Kosciuszko NP, the central region of the Australian Alps (method followed Hoyle et al., 2015), and then applied a transition from autumn to winter to spring. Three replicates of 25 seeds per population were placed on 1% water-agar in sealed petri dishes in a germination chamber (Model TRIL-120-1-VW/ BMS, Serial 30455, Thermoline Scientific, NSW, Australia). Assays started at 25/15 °C, 12/12 hours light/dark, for 9 weeks; were moved to 5 °C constant temperature and 12/12 hours light/dark for 8 weeks; and then returned to 25/15 °C, 12/12 hours light/dark, for 27 weeks. These conditions were previously demonstrated to be effective at germinating a wide range of Australian alpine and montane species (Hoyle et al., 2015). The germination assay ended at 34 weeks when there had been no further germination for four weeks. Each of the three replicates per accession was placed on a different shelf within the same germination chamber and within a given shelf the position of petri dishes was randomised every week. Photoperiods were provided throughout by fluorescent lamps (c. 35 μmolm^{-2}s^{-1}).

Germination, defined as radicle emergence of more than 1 mm, was recorded every week. Cut-tests were conducted to determine whether non-germinated seeds were empty, filled and alive (likely dormant), or filled and dead. The final germination percentage was calculated based on the number of filled seeds (empty seeds were subtracted from each replicate).

Most (21 of the 29) populations had viable seeds (seed viability was determined by cut-test and a result of germination > 30% after 34 weeks). The seeds from eight non-viable populations were consistently the older collections or those that had been stored at 5 °C rather than -18 °C. These were thus excluded from any further analyses [**see Supporting Information** Table S1]. Prior work demonstrated that storage at -18 °C for 3 years or more did not alter the germination strategy or viability of seed of several Australian alpine species (Hoyle et al., 2015), thus a combination of inherent longevity and suboptimal storage conditions seem the likely cause of loss of viability in five collections. We assessed whether age of collection affected viability of the remaining accessions by regression against final total percent germination and found no correlation. To assess the potential confounding effect of seed age on germination strategy, we tested the association between them and found non-significant association (ANOVA, $p = 0.3556$), [**see Supporting Information** Fig. S5].

Germination strategy cluster analysis

We categorised germination strategy using cluster analysis using the time (weeks) to specific cumulative germination percentiles (10, 20, 30, 40, 50, 60, 70, 80, and 95%) plus final germination percentage, and final percentage of non-germinated viable seeds. All data were the mean of three replicate dishes. The calculation is based on Z-standardized values using the Euclidean distance matrix clustering method and a nearest neighbour joining algorithm. We determined germination strategy using this approach because in addition to considering final germination values, or germination timing (e.g. T_{50}) this approach allows us to dissect the differences of germination strategy represented by the germination curve shape or patterns among populations (Hoyle et al., 2015), and also potentially indicate the season when the germination is most likely to happen. Cluster analysis was done in SPSS version 12 for Windows.

Climatic analyses

We analysed possible association of the germination strategy categories with a) long-term climate b) short-term maternal climate (pre-zygotic, 1 year prior to seed collection) and c) seed development conditions (post-zygotic, 3 months prior to seed collection). Long-term climate data (27 layers from temperature, precipitation and solar exposure) were imported from the Atlas of Living Australia (http://spatial.ala.org.au/) based on population geographic coordinates. Bioclimate values are estimations from a spline surface fitted to weather station data in ANUCLIM 6.1 (Xu and Hutchinson, 2011). These climate layers were derived from interpolation of 75-years of data for mean rainfall and temperature and 25-year means for solar radiation at a resolution of 0.01 degree (~ 1 km).

We assessed short-term climate at two scales to distinguish between pre- and post-zygotic environmental effects, e.g. the maternal plant (1 year) and seed development (3 months) conditions respectively. We extracted daily minimum and maximum temperatures, monthly rain, and daily solar radiation from the closest weather station from BOM records (http://www.bom.gov.au/climate/data/). We refer to the 75 year averaged data as 'long-term climate' and the conditions to which the maternal plants were exposed immediately prior to and during seed development as 'maternal' and 'seed-development' conditions, respectively.

We used canonical variate analysis (CVA) to understand which climate variables – temperature, precipitation, and solar radiation, had strong associations with the germination strategies. We included measures of the mean, minimum, maximum, and seasonality of temperature, precipitation and radiation to compare which were strongest. We first performed CVA for long-term, maternal and seed development conditions separately. For long-term climate, twelve variables i.e. mean, maximum, minimum, and seasonality (variability) of temperature, precipitation and radiation were included in the analysis [**see Supporting Information** Table S2]. We subsequently calculated analogues of the same 12 long-term climate values for maternal and seed development conditions.

Seasonality was calculated as the coefficient of variation (CV) for the respective climate variable, which indeed represents the variability of the climate variable. To assess the maternal and seed development conditions semi-independently of the long-term trends we regressed the 1 year and 3 months data against the respective long-term values and obtained the residuals of each variable. These residuals were then used in the CVA for maternal and seed development CVA. Data for one site/year combination (Kos6, 1988) from the nearby climate station were not available, hence, we did not include this population in the short-term climate analyses.

Initially, we included all twelve variables in each analysis for long-term climate, maternal, and seed development conditions. Many of the variables were correlated, and hence, we performed several CVA with different combinations of variables that had low collinearity (Pearson coefficient < 0.8) for long-term climate, maternal environment and seed development conditions. We found that the results were robust such that the same climate variables had strong associations with germination strategies regardless of the combination of variables included. Therefore, we only report the analysis that included the complete set of twelve variables. Finally, we ran another CVA comprising the climatic indicator with the highest loading for each temperature, precipitation and radiation respectively and for long-term, maternal, and seed development conditions to determine the relative importance of long-term or short-term conditions for variation in germination strategy. CVA was carried out in Genstat for Windows 17th Edition (VSN International, UK).

Trait measurement

Seed traits: To determine whether seed traits were associated with germination strategy we measured seed mass, length, and embryo length for each population. Seed mass was measured on five replicates of five seeds per population. Seed length and embryo length were measured at the full imbibition state (determined by imbibition test, data not shown), 24 h after seeds were irrigated with distilled water. For seed and embryo length measurement, five seeds per population were cut vertically in a plane estimated to best display the embryo using a scalpel under a microscope (Olympus SZ40, Olympus, Japan). Embryo and seed length were subsequently measured from digital images taken using a microscope (Nikon SMZ25, Nikon, NSW, Australia) with attached camera (Nikon

Digital Sight DS-U3 Nikon, NSW, Australia) and computer with ND2-NIS Nikon software. As stated above, preliminary analyses demonstrated there was no association between collection age and germination strategy, thus collection date was not included in subsequent analyses.

Seedling traits: All germinating seeds from the 21 populations were transplanted to soil 1-2 weeks after germination when the radicle was > 1 cm. Seedlings were grown in 4 × 4 × 10 cm pots (T40S, Garden City Plastics, NSW, Australia), one seedling per pot, with a mixture of Martins mix potting-soil (Martins Fertilizer, NSW, Australia) plus 10% steamed river sand and c. 4 gram/pot of slow release fertilizer (Osmocote Exact Patterned Release Fertilizer Standard Blue 15% N: 4% P: 7.5% K, Scotts International BV, Heerlen, The Netherlands). The glasshouse environment was kept at 25/15 °C (day/night) with a natural spring/ summer photoperiod of Canberra, ACT, Australia, selected to mimic the natural spring/ summer temperature in the Australian Alps (see Hoyle et al., 2015). Seedling position was randomised within and across glasshouse benches every 1-2 weeks. To determine whether seedling traits were associated with germination strategy, five seedlings were randomly selected and leaf number and length were measured at four and eight weeks after potting. SLA was also measured at 4 and 8 weeks after potting, on the youngest fully expanded leaf, from five additional seedlings each. SLA was derived by harvesting and scanning the leaf on a flatbed scanner and images were analysed using ImageJ 1.49. After scanning, leaves were oven-dried (60 °C) for 4 days and weighed. SLA was then calculated by dividing the leaf area (mm^2) by its dry weight (mg). Two (Kos12 and Nam1) of the four populations with staggered germination strategy had sufficient seed for germination of an additional set so that germination of early (autumn) and late (spring) seedlings coincided and they were measured after growth under common glasshouse conditions. We were only able to measure SLA of Kos12 and Nam1 at week 4 due to limited numbers of seedlings.

Statistical analysis of traits: We used Residual Maximum Likelihood (REML) Mixed Models and Least Significant Differences (LSD) post-hoc tests, if applicable, to test for the statistical significance of differences in all seed and seedling traits. We tested whether seed traits (seed mass, embryo length, seed length and the embryo to seed length ratio (hereafter E:S) differed significantly with germination strategy. Germination strategy (immediate, staggered, postponed and postponed-deep) was included in the model as a fixed term and population as a random factor. Five replicate seeds per population were used for seed length, embryo, E:S measurements, and seed mass (five replicates of the average, see above). Immediate, postponed and postponed-deep seedlings were represented by five replicates per population. There were ten replicates per population for those with a staggered strategy, five each for autumn and spring seedlings. We tested whether seedling traits (leaf number, leaf length, and SLA) differed significantly with germination strategy, seedling age, or the interaction thereof. For analysis of leaf number and length the interaction term of germination strategy and time was included as the fixed factor with individual seedling nested within population as a random factor because both measurements were done within an individual plant. The same analysis applied for SLA, except that the random factor was population (individuals were only measured once due to the destructive harvesting method). All seed and seedling traits, except E:S, were log-transformed prior to model fitting based on the examination of the residual plots. For each experiment a full model including all main factors, and all interactions where applicable, was computed. All seed and seedling growth analyses were carried out in Genstat for Windows 17th Edition (VSN International, UK).

RESULTS

Variation in germination strategy in *O. eriopoda*

Variation in germination strategy among populations of *O. eriopoda* was broad, ranging from completely non-dormant populations that germinated comprehensively within a few days of sowing, to populations possessing dormant seeds that started to germinate only 14 weeks after sowing (Fig. 1a). Four germination strategies, described as immediate, staggered, postponed, and postponed-deep were identified via cluster analysis (Fig. 1b). Populations in the immediate category (n = 5) reached almost full germination within three weeks. Seeds from postponed populations (n = 9) started germination during or after cold-stratification (14 or more weeks after sowing), a pattern consistent with physiological or morpho-physiological dormancy. Four populations included almost equal numbers of early germinating seeds and late germinating seeds and are therefore described as having a staggered strategy. Finally, three populations exhibited a postponed-deep germination strategy. In these populations most of the seeds (> 80%) were viable, but only approximately half of these viable seeds germinated within a single simulated year, suggesting a deeper dormancy, or that the conditions for germination were not met.

Associations between germination strategies and climate

The cluster analysis of germination patterns revealed that within a given geographic region (e.g. Kosciuszko National Park) all four germination strategies can occur, thus there is no geographic separation of the strategies. However, there were significant differences in the elevation at which the germination strategies were found. Populations with immediate germination strategy were found at lower elevations, whereas populations with the staggered and postponed-deep germination strategies were found at mid-elevations, and the postponed strategy was found at the highest elevations ($p < 0.001$, Fig. 2). Therefore, it is valuable to elucidate which feature of elevation is responsible for the impact of elevation on germination strategy.

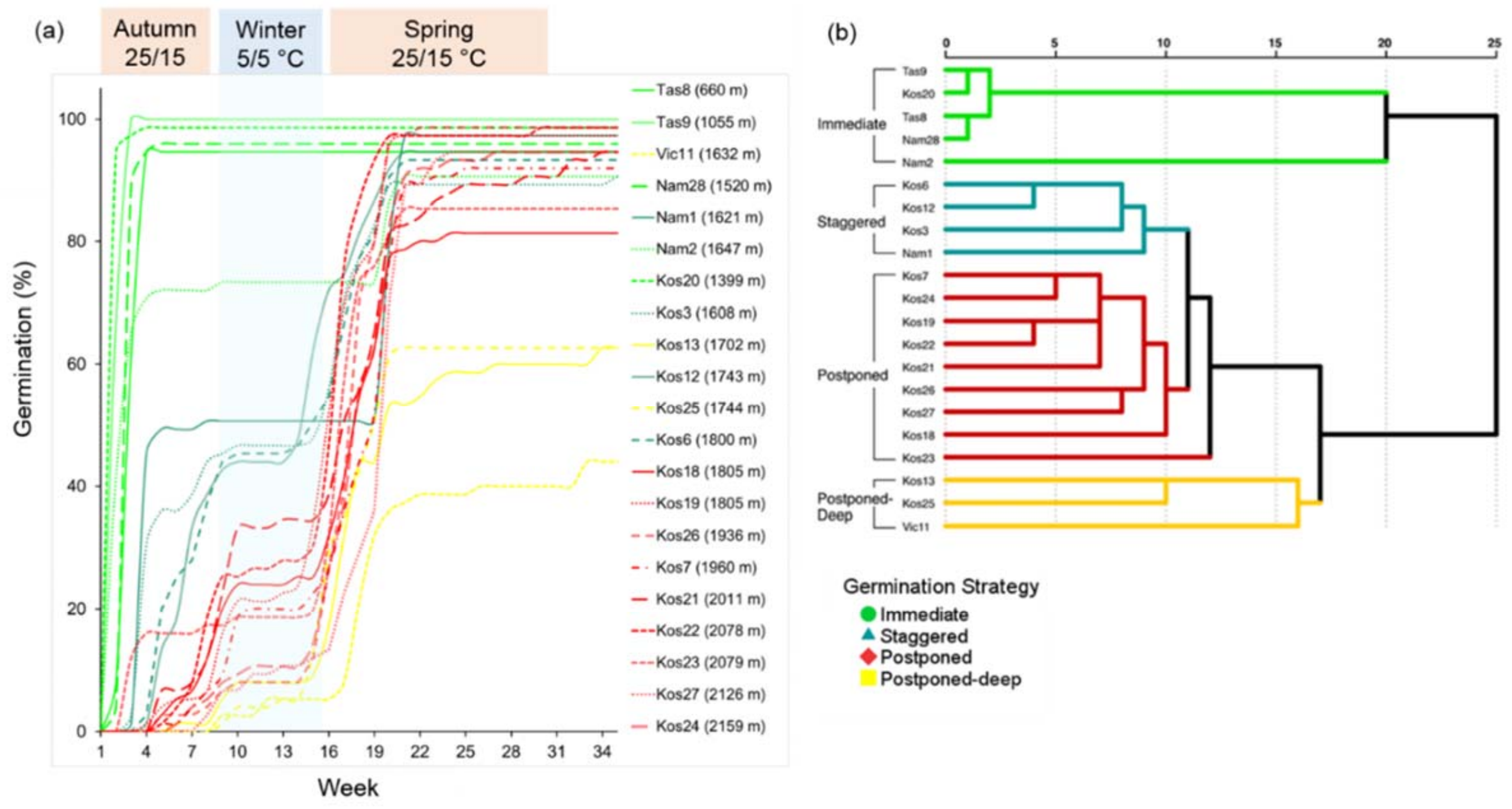


Figure 1. (a) Germination strategies of 21 viable populations of *O. eriopoda*. Codes indicate region (Kos = Kosciuszko National Park, Nam = Namadgi National Park, Tas = Tasmania, Vic = Alpine National Park Victoria) followed by population number, and in brackets are the elevation of the seed collection; (b) Cluster analysis dendrogram based on time to cumulative germination percentiles (10, 20, 30, 40, 50, 60, 70, 80, 95), final germination percentage, and number of viable but un-germinated seeds.

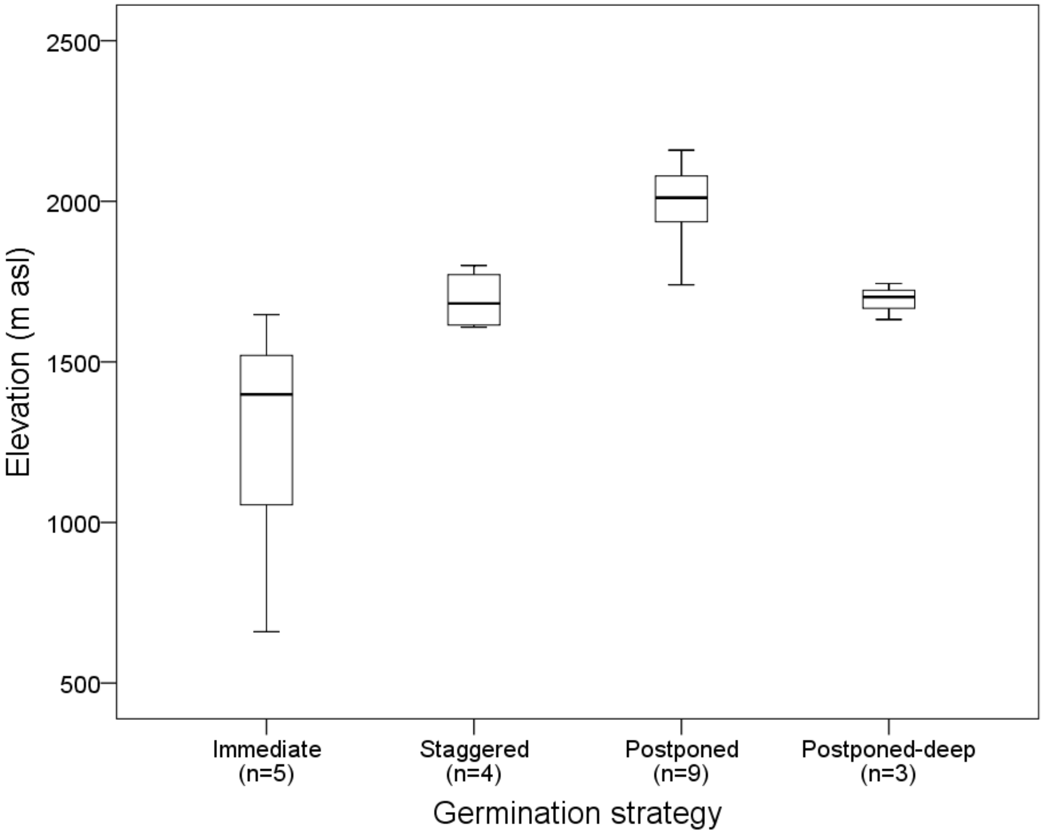


Figure 2. The distribution of germination strategy among *O. eriopoda* populations (n) by elevation.

Our examination of the relationship between germination strategy and climate showed that temperature variability was the strongest correlate of differentiation in germination strategy, regardless of time scale (long-term climate, maternal or seed-development conditions, shown by higher latent vectors (Fig. 3), [**see Supporting Information** Table S3]. The immediate germination strategy was associated with populations that come from locations with low temperature variability and additionally, warmer mean temperature compared with populations in other strategies [**see Supporting Information** Fig. S6]. Axis 2 and 3 differ slightly between time scales and represent a relatively small portion of the variance, but these axes are important to separate the staggered and postponed-deep strategies from the other germination strategies. For long-term climate, the separation between the populations with staggered versus other strategies is driven by minimum and mean radiation as well as temperature variability. For maternal conditions, the populations with postponed-deep and staggered germination strategies (Fig. 3b) were distinguished along an axis of minimum radiation as well as temperature variability. For seed development conditions, the populations with postponed-deep strategy were distinguished along an axis of minimum radiation as well as temperature variability (Fig. 3c). Overall, under maternal time scale, the separation pattern of germination strategies on axis 2 was not as strong as the pattern for long-term climate or the seed development scale.

When the strongest drivers of variation in germination strategy (long-term temperature variability, mean temperature, minimum, and mean radiation and residuals of their short-term analogues) from each separate time scale were combined in a single analysis, we found long-term and seed-developmental temperature variability are the most influential variables for axis 1 and axis 2 (Fig. 3d). Climatic conditions in the year preceding seed collection were found to be less important than long-term climate and seed-development conditions. Radiation had a weaker association than temperature, and precipitation had minimal influence on germination strategies.

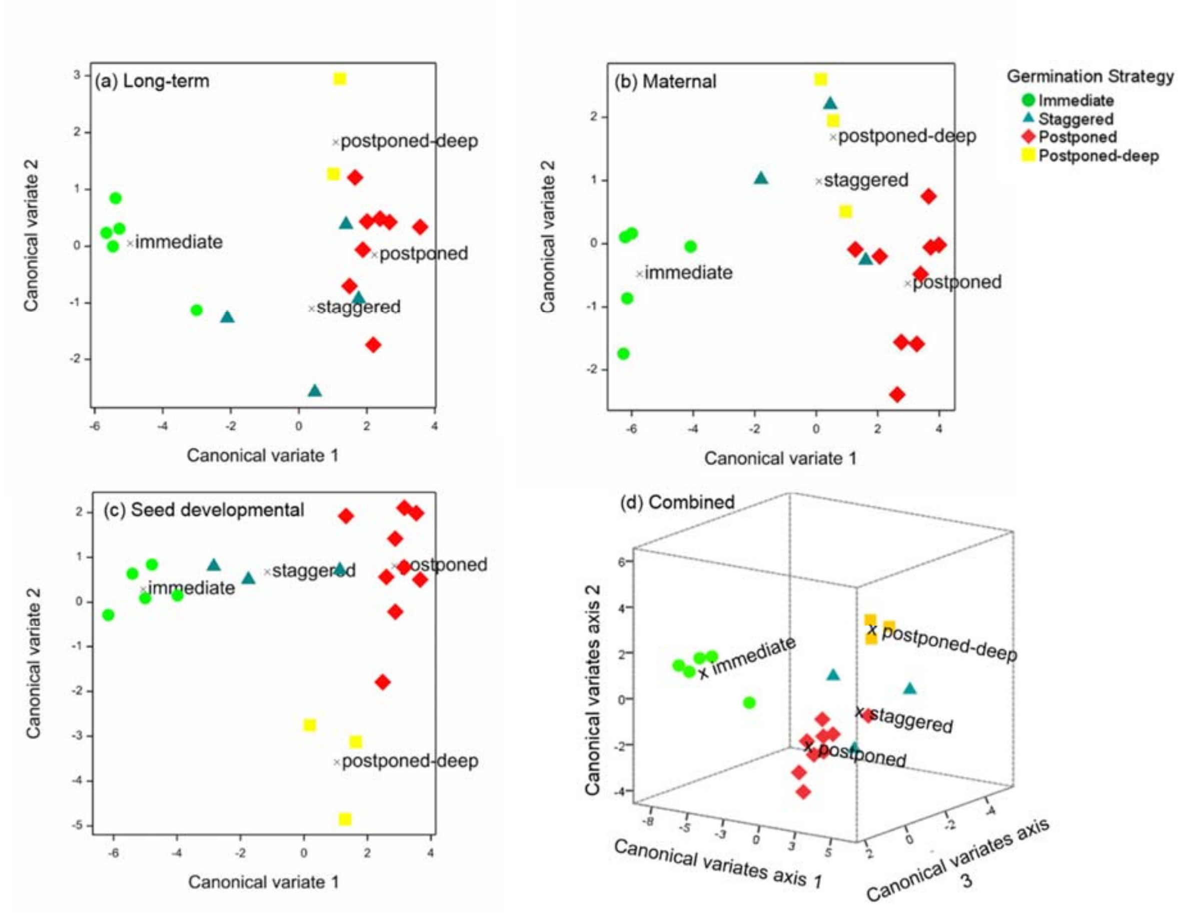


Fig. 3. Canonical variate means of (a) long-term, (b) maternal, (c) seed-developmental, and (d) the three time scales combined in 3-D on the germination strategies. Population means are indicated with green circles (immediate), blue triangles (staggered), red diamonds (postponed) and yellow squares (postponed-deep) and germination strategy means by an X of the same colour. Axes indicate variables that have the strongest association with germination strategy. Climate variables for Fig 3a, axis 1: temperature variability and minimum radiation, axis 2: temperature variability and minimum radiation. Kos13 and Kos25 (postponed-deep) overlapped. Fig 3b, axis 1: temperature variability and mean radiation, axis 2: temperature variability and mean radiation. Fig 3c, axis 1: temperature variability and mean radiation, axis 2: temperature variability and minimum radiation. Fig 3d, axis 1, 2, 3: temperature variability of long-term, seed development conditions, and maternal condition, respectively. Kos6 (staggered) short-term climate data were not available and hence Kos6 was excluded from Figs 3 b, c, and d.

Associations between germination strategy and seed and seedling traits

We examined whether the differences in germination strategy that were underlain by climatic drivers were also associated with variation in seed and seedling traits. Seeds from populations with immediate germination strategy were markedly, and significantly lighter, nearly 50%, than those of the other germination strategies (Fig. 4a). Populations with staggered germination strategy had greater seed length than other germination strategies, whereas, the embryos of the seeds from populations with postponed-deep germination strategy were shorter than those of other germination strategies (Fig. 4b). However, regardless of these variations in overall seed and embryo size, all strategies showed signs of under-developed embryos, as indicated by the ratio of embryo to seed length (E:S). Although the E:S was significantly higher for populations with immediate and postponed strategies (both 0.15 ± 0.01 SE) than for populations with staggered and postponed-deep germination strategies (both 0.123 ± 0.01, Fig. 4b), the extent of E:S ratio variation was small.

Finally, as predicted, autumn seedlings (early germinating seeds), whether from populations with immediate or staggered germination strategy, had greater rates of leaf production than spring seedlings (late germinating seeds). For populations with an immediate germination strategy higher leaf production (leaf number) in comparison to staggered, postponed, and postponed-deep strategies was observed for seedlings grown under common conditions (Fig. 5a) at the two common time points post-germination/potting (4 and 8 weeks). Within the staggered germination strategy autumn seedlings (those that germinated earliest) produced more leaves than spring seedlings (Fig. 5b, Table 1) and the difference was a function of measurement time point, in which leaf number differences between autumn and spring seedling of populations with staggered strategy were more apparent in week 8 than week 4. Leaf lengths did not differ among the four germination strategies (Fig. 5c, Table 1). However, within the staggered strategy autumn seedlings had shorter leaves than spring seedlings (Fig. 5d, Table 1). There were significant differences in SLA among germination strategies, though the patterns were less distinct (Fig. 5e, Table 1). Of the four populations with staggered germination there were insufficient seedlings to measure SLA and hence, we could not get an inference on whether SLA of autumn and spring seedlings might differ within staggered strategy.

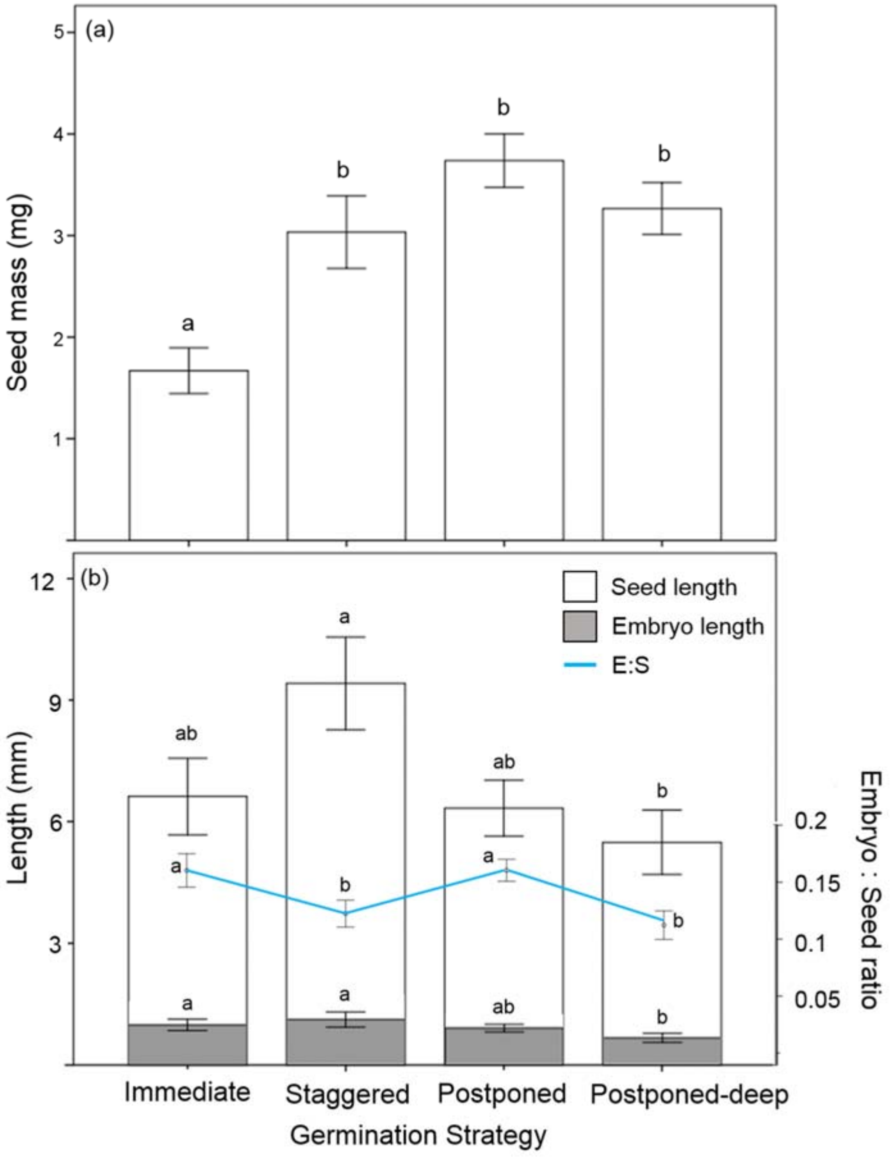


Fig. 4. (a) Seed mass and (b) seed length, embryo length, and E:S ratio of *O. eriopoda*. Letters show significant differences between strategies based on LSD post-hoc tests on log-transformed values of mean seed mass (mg), embryo length (mm), seed length (mm) and actual value of E:S (Table 1).

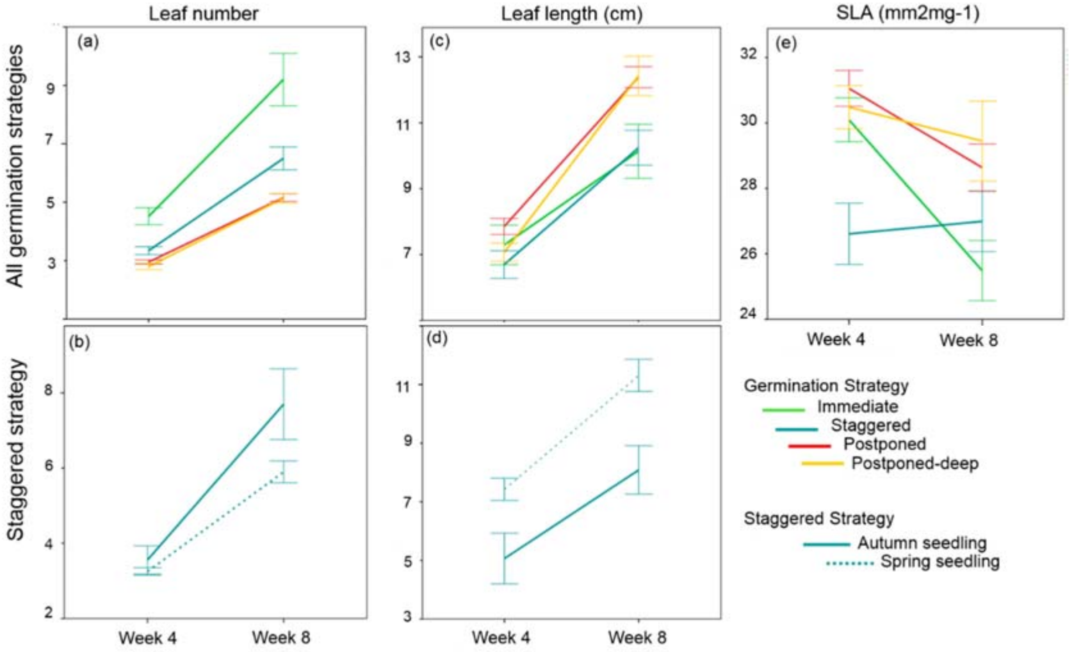


Fig. 5. Leaf number (a, b), length (c, d) and SLA (e) across germination strategies (top row) and within the staggered germination strategy (bottom row). Leaf number (a, b) and leaf length (c, d) for staggered strategy were not available for autumn seedlings of Kos3 and Kos6. Note that due to the destructive nature of SLA measurement and low seedling availability SLA difference between autumn and spring seedling within staggered strategy was not measured. Staggered strategy in Fig 5e consisted of incomplete sets of autumn seedlings (week 4: Kos12, Nam1; no week 8 measurement) and spring seedlings (week 4 and 8: Kos3, Kos6, Kos12, and Nam1).

DISCUSSION

Our results demonstrate that the striking variation in germination strategy among populations of a *O. eriopoda* reflect both long-term climate and seed development conditions, particularly temperature variability and further, that seed and seedling traits differed among strategies. To our knowledge, this is the first experiment that investigated the consequences of intraspecific variation in seed dormancy and germination strategy on seedling growth. Parmesan and Hanley (2015) highlights that a focus on early plant life-history state is crucial to better understand community response to climate change variables. Below we discuss the physiological and evolutionary implications of this variation in germination strategy, and how they might be relevant for species' recruitment, survival and persistence under changing climate.

Table 1. Significance values for seed and seedling traits across germination strategies.

	Trait	**Fixed term**	**df**	**F statistic**	**F pr**
All germination strategies (21 populations)	**Seed**				
	Seed mass	Germination strategy	3	8.53	**0.001**
	Seed length	Germination strategy	3	4.26	**0.007**
	Embryo length	Germination strategy	3	5.5	**0.011**
	E:S (raw data)	Germination strategy	3	4.71	**0.004**
	Seedling				
	Leaf number	Time	1	967.66	**<0.001**
		Germination strategy	3	28.68	**<0.001**
		Time × Germination strategy	3	1.87	0.139
	Leaf length	Time	1	694.16	**<0.001**
		Germination strategy	3	6.02	0.086
		Time × Germination strategy	3	6.35	**<0.001**
	SLA	Time	1	15.77	**<0.001**
		Germination strategy	3	3.39	**0.043**
		Time × Germination strategy	3	4.01	**0.008**
Staggered strategy (4 populations‡)	Leaf number	Time	1	470.96	**<0.001**
		Seedling (autumn or spring)	1	8.17	**0.009**
		Time × Seedling	1	11.8	**0.002**
	Leaf length	Time	1	134.03	**<0.001**
		Seedling (autumn or spring)	1	45.79	**<0.001**
		Time × Seedling	1	1.81	0.189

‡ Leaf number and length of the staggered populations (Kos3, Kos6, Kos12, and Nam1) consisted of autumn and spring seedlings at week 4 and 8, autumn seedlings of Kos3 and Kos6 were not available.

Germination strategy associations

Variation of germination strategy was associated with variation in several seed traits. Seed mass differed among germination strategies: seeds from populations with a postponed germination strategy had significantly larger seed mass than those from populations with immediate germination strategies. This result echoes prior cross-species results for Australian alpine species (Hoyle et al., 2015). Seed mass for many alpine species is positively correlated with elevation, likely because the environmental conditions for seedling establishment are more adverse at higher elevations and larger seeds and therefore seedlings are at an advantage (Pluess et al., 2005). Thus, the larger seed mass of the postponed populations may reflect that these were also found at high elevation. We also found significant differences in embryo size and E:S ratio among germination strategies. Small embryos and low E:S ratios are consistent with morphological or morpho-physiological dormancy (Forbis et al., 2002, Baskin and Baskin, 2014, Mattana et al., 2014). Interestingly, even seeds from the immediate populations had very low E:S ratios, and these germinated immediately regardless, which is a contrary to the general rule that morphologically dormant seeds exhibit a non-immediate germination. We did not directly assay embryo growth to confirm that the seeds are morpho-physiologically dormant. It is however plausible that the germination, particularly for autumn germinating seeds, is constrained by physiological rather than morpho-physiological constraints.

Amongst the climate and distribution characteristics considered, temperature variability strongly explained germination strategy and elevation was also associated with germination strategy. At lower elevation, where the climate is supposedly more benign and temperature variability is low, immediate germination strategies were found, perhaps reflecting lack of selection for delayed germination. At the highest elevations, where snow cover insulates and minimises danger of freeze-induced damage (Fenner and Thompson, 2005) a postponed strategy was evident. At mid-elevation, where the growing season can start early after snow melts, non-dormant seeds might germinate and start growing in advance of dormant seeds, but they might be at risk as frosts might still occur. Indeed in this range we found populations that exhibit either staggered or postponed-deep germination strategy.

The staggered and postponed-deep strategies may both indicate plant bet-hedging strategy (Cohen, 1966, Simons, 2014, Simons and Johnston, 2006). Populations with a postponed-deep strategy allow a persistent seed bank *in-situ* that may buffer seedling loss in the first spring, whereas populations with a staggered strategy distributes seedling germination timing across the year, either growing fast as autumn seedlings or germinating in safer spring conditions. What remains to be determined is whether the two groups within staggered (non-dormant vs dormant seeds) and postponed-deep (dormant vs deeply-dormant seeds) were produced by different plants, or whether one individual can produce seeds with different types and degree of dormancy.

Unlike the staggered and postponed-deep strategy where germination is staggered and somewhat intermittent, populations with the postponed strategy rely on cold stratification (constant winter temperature under snow) and germination started almost simultaneously across the accession at the end of cold stratification. Hence, a rapid environmental change, such as warmer temperature and earlier spring, may disrupt the germination of the populations with a postponed strategy. We do not know the minimum length of cold stratification required to alleviate dormancy in most Australian alpine plants. In *Aciphylla glacialis*, a co-occurring herb from the same family, winter durations as short as six weeks were adequate to alleviate dormancy (Hoyle et al., 2014). Alternatively, warming may lead to changes in alpine seed dormancy status and a phenological shift from spring to autumn germination (Mondoni et al., 2012).

Early germinating seeds of both immediate and staggered strategy differed in establishment traits from spring-germinating seeds. Seeds that germinated in autumn (immediate strategy) produced more leaves than seedlings that germinated in spring (postponed strategy) when compared at a common time and under common conditions. Our results suggest that differentiation in germination strategy is associated with a suite of traits that together affect seedling establishment. Previous work has shown that across Australian alpine species germination strategy does not correlate with adult vegetative traits (Hoyle et al., 2015), thus it is striking that the differences we find are apparent in early growth stages.

The inherent variation in growth rate between autumn and spring seedlings may be related to germination timing and plant growth trade-offs in alpine seasonal environments where seeds that germinate early have lower probabilities of survival than those germinating later, but the few that do survive may have increased fitness (Rathcke and Lacey, 1985, Verdú and Traveset, 2005, Kimball et al., 2011). Mondoni et al. (2015) show that a high proportion (up to 75%) of autumn seedlings survive winter, however, survival over winter involves high energy consumption by seedlings which may reduce the growing capacity in spring (Maruta, 1994). Autumn germinating seeds of *Isatis violascens*, a cold desert annual from NW China, produce taller plants with higher vegetative allocation and lower reproductive allocation therefore require a longer time to flower (Lu et al., 2016). However, the lower fitness shown by the autumn seedling of *I. violascens* might be confounded by season. The seedlings were not grown under common conditions – autumn seedlings grew under winter conditions, while spring seedlings were exposed to conditions suited to fast growth immediately after germination.

Evolutionary trajectory and mechanisms

Our results as well as previous studies indicate that the evolution of germination strategy reflects long-term selection significantly driven by local climate, but that there is also evidence of plastic mechanisms that fine-tune that strategy in response to conditions during seed development. In our study system, temperature variability and mean radiation emerged as the best predictors of germination strategy. We are particularly interested in the element of variability given the strong association it had with germination strategy. While mean temperature is known to be one of the most significant factors for alleviation of physiological dormancy (Pearson, 2006, Thuiller et al., 2008), less attention has been placed on variability. The specific climatic drivers of germination strategy are likely to vary among systems. For example long-term precipitation (Wagmann et al., 2012), summer precipitation (Fernández-Pascual et al., 2013), or winter precipitation (Carta et al., 2016) might be more important in other systems. Regardless, our results lead us to urge future work to focus not just on mean climatic measures, but on variability therein, at both long and short term scales.

Another interesting evolutionary element of our results was the documentation of a staggered germination strategy in several populations that could be indicative of a bet-hedging strategy for germination (Starrfelt and Kokko, 2012, Venable, 2007), as populations contained almost equal numbers of non-dormant and dormant seeds. The variation in dormancy degree within populations exhibiting staggered and postponed-deep strategy could indeed reflect bet hedging and not just a purely plastic response (Starrfelt and Kokko, 2012, Simons, 2014). In staggered populations where both autumn and spring indeed present, the variation is indeed a bet-hedging when the source of variation lies within an individual – an individual plant produced both non-dormant and dormant seed with varying proportions (Starrfelt and Kokko, 2012). Our experiment used seed collected in the field and bulked across plants within the same population, thus we cannot conclusively demonstrate whether a single plant produced seeds that are substantially different in the degree of dormancy. Further investigation to identify the mechanisms of within-strategy variation in dormancy and germination at the individual plant level would answer this question and also allow genetic, developmental and environmental signals to be identified.

CONCLUSIONS

Within-species variation in germination strategies could be critical to survival and persistence in the context of rapid climate and other changes. We have shown that the germination strategies exhibited in *O. eriopoda* populations may reflect long-term patterns of climatic conditions, in particular temperature variability, but that there is also a much finer time signal. Species like *O. eriopoda* that are geographically constrained and not equipped with special appendages for rapid and long-range dispersal are unlikely to track rapid climate change geographically, consequently, plasticity or between-population variation in seed traits such as germination strategy may play a key role defining species response to climate change.

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Supporting Information

Table S1. *Oreomyrrhis eriopoda* seed accessions. BG refers to the botanic gardens from which we retrieved the seeds. Population code indicates locality and population number in this experiment. Climate station n/a indicate that either no data present or the corresponding population did not have germination strategy to assign for.

(continued)

Accession number	**Latitude**	**Longitude**	**Alt (m)**	**Collection date**	**Localities**	**Long-term mean tempera-ture (°C)**	**Long-term mean precipita-tion (mm)**	**Final germination (%)**	**BG**	**Climate station and distance from population**	**Population code**
CANB 813617	-35.5219	148.7719	1621	17-Jan-13	Ginini West *Sphagnum* peat bog southern part, 0.5 km W from the Mt Ginini carpark on the Mt Franklin Road towards Bulls Head, and c. 0.4 km N of road.	7.2	1456	97.33	ANBG	Mt Ginini AWS, 37 km	Nam1
CANB 866345	-35.568	148.7844	1647	19-Feb-13	Namadgi National Park: Snowy Flat, southern end; Mt Franklin Road, c. 5 km S of locked gate near Mt Ginini.	7.2	1421	90.67	ANBG	Mt Ginini AWS, 34 km	Nam2
NSW 618325	-36.3706	148.4761	1608	10-Feb-04	Rainbow Lake Track, off Kosciuszko Road between Perisher Valley and Guthega turn-off.	5.4	1759	90.67	RBG Mt Annan	Thredbo Village, 31 km	Kos3

(continued)

Accession number	Latitude	Longitude	Alt (m)	Collection date	Localities	Long-term mean tempera-ture (°C)	Long-term mean precipita-tion (mm)	Final germination (%)	BG	Climate station and distance from population	Population code
					Kosciuszko NP Southern Tablelands.						
NSW 4120705	-36.8833	148.45	1470	11-Apr-86	Three Mile Dam, Kiandra-Cabramurra Road, Southern Tablelands.	7.9	1596	1.33	RBG Mt Annan	n/a	Kos4
NSW 4154320	-36.5667	148.2667	715	13-Feb-85	Bob's Ridge, Cascade Trail.	5.9	1788	0	RBG Mt Annan	n/a	Kos5
NSW 4154437	-36.45	148.3167	1800	1-Mar-88	1 km along the summit walking track to Mt Stillwell.	4	2178	93.33	RBG Mt Annan	n/a	Kos6
NSW 617524	-36.4911	148.285	1960	24-Feb-04	Approximately 400 m NW of the top of Crackenback chairlift, on walking track to Mt Kosciuszko. Kosciuszko NP Southern Tablelands	4.6	2066	92	RBG Mt Annan	Thredbo AWS, 3 km	Kos7
TSCC 0009097	-42.1509	146.4604	660	16-Feb-09	Nive Plains. Lyell Highway, 500 m NW of Nive River Bridge	8.9	1004	94.67	RBG Tasmania	Lake St Claire National Park, 24 km	Tas8
TSCC 0005951	-41.9577	146.6786	1055	24-Jan-08	Great Lake. Cameroon lagoon, small wetland on west side of Lake Highway;	6.6	953	100	RBG Tasmania	Liawenee, 7 km	Tas9

(continued)

Accession number	**Latitude**	**Longitude**	**Alt (m)**	**Collection date**	**Localities**	**Long-term mean temperature (°C)**	**Long-term mean precipitation (mm)**	**Final germination (%)**	**BG**	**Climate station and distance from population**	**Population code**
					7 km S of Liawenee. Subalpine health, associated with *Ozothamnus* spp; *Poa* spp.						
MEL (donation from John Morgan)	unknown	unknown	na	1996 or 1997	Falls Creek, Bogong High Plains	n/a	n/a	n/a	RBG Melbourne	n/a	Kos10
MEL 2338193	-37.0601	147.092	1632	25-Jan-11	Alpine NP, beside the Dargo High Plains Road about 16.1 km from the Alpine Road.	6.7	1750	44	RBG Melbourne	Mt Hotham, 10 km	Vic11
CANB 783437	-36.4269	148.3677	1743	24-Feb-09	Kosciuszko NP, IBRA region AA; in fens in and along Spencers Creek – 790 m NE from the Spencers Creek bridge on Kosciuszko Road.	5.1	1903	94.67	ANBG	Thredbo, 10 km	Kos12
CANB 807987	-36.4294	148.3586	1702	28-Feb-12	Kosciuszko NP; on the Kosciuszko Road at the Spencers Creek bridge, c. 50 m W of bridge on S side of road.	5.3	1863	62.67	ANBG	Perisher Valley AWS, 5 km	Kos13

(continued)

Accession number	Latitude	Longitude	Alt (m)	Collection date	Localities	Long-term mean tempera-ture (°C)	Long-term mean precipita-tion (mm)	Final germination (%)	BG	Climate station and distance from population	Population code
CBG 9604859	-35.6608	148.788	1900	5-Mar-96	Namadgi NP, c. 150 m direct NW of the summit of Bimberi Peak.	6.7	1496	0	ANBG	n/a	Nam14
CBG 8001425	-36.45	148.26	2000	19-Feb-80	Kosciuszko NP, 1.2 km E of Mt Kosciuszko summit.	4.1	2210	0	ANBG	n/a	Kos15
CBG 64941	-35.5833	148.7833	1850	23-Feb-76	Mt Gingera, Brindabella Range, 42 km SW of Canberra.	7.2	1410	0	ANBG	n/a	Nam16
CBG 67614	-36.45	148.3333	1800	15-Mar-77	Kosciuszko NP, Charlottes Pass.	4.6	2041	9.33	ANBG	n/a	Kos17
CANB 783604	-36.4338	148.3066	1805	5-Feb-09	Kosciuszko NP, IBRA region AA; 1.4 km SW from where the Main Range walking track to Blue Lake crosses the Snowy River.	5.3	1998	81.33	ANBG	Thredbo AWS, 7 km	Kos18
CANB 782508	-36.4302	148.3583	1740	10-Mar-09	Kosciuszko NP: 215 m NW from Spencers Creek, growing close to bogs.	4.9	1850	97.33	ANBG	Thredbo AWS, 9 km	Kos19
CANB 749000	-35.8263	148.4927	1399	2-Mar-07	Kosciuszko NP; roadside at Old Kiandra Goldfields, 3 km N of turn-off to Cabramurra and Khancoban.	5.4	1396	98.67	ANBG	Cabramurra SMHEA AWS, 16 km	Kos20

(continued)

Accession number	Latitude	Longitude	Alt (m)	Collection date	Localities	Long-term mean tempera-ture (°C)	Long-term mean precipita-tion (mm)	Final germination (%)	BG	Climate station and distance from population	Population code
CANB 748561	-36.4394	148.2702	2011	7-Mar-07	Kosciuszko NP; 2 km N of Mt Kosciuszko summit, on Main Range track.	8.5	2228	94.67	ANBG	Thredbo AWS, 6 km	Kos21
CANB 770116	-36.4522	148.2747	2078	5-Mar-08	Kosciuszko NP: Kosciuszko summit road c. 1 km from Seaman's Hut. Small stream running nearby.	3.7	2287	98.67	ANBG	Thredbo AWS, 5 km	Kos22
CANB 747602	-36.4625	148.2677	2079	14-Feb-07	Kosciuszko NP; 800 m ~NE from Lake Cootapatamba, on margin of bog.	3.6	2311	85.33	ANBG	Thredbo AWS, 4 km	Kos23
CANB 792219	-36.4525	148.265	2159	30-Mar-10	Kosciuszko NP; Lake Albina Track	3.4	2368	94.67	ANBG	Thredbo AWS, 5 km	Kos24
CANB 792151	-36.4294	148.3586	1744	3-Feb-10	Kosciuszko NP; S side of Spencers Creek bridge; roadside and water side.	5.3	1863	62.67	ANBG	Thredbo AWS, 9 km	Kos25
CANB 792159	-36.4161	148.3111	1936	4-Feb-10	Kosciuszko NP; N side of path, overlooking Headley Tarn.	4.8	2006	98.67	ANBG	Thredbo AWS, 9 km	Kos26
CANB 798446	-36.4533	148.2788	2126	16-Mar-11	Kosciuszko NP; Aspect S; moderate to steep boulder strewn slope; granite. Tall Alpine herbfield, found with *Craspedia maxgrayi*,	3.7	2287	94.67	ANBG	Thredbo AWS, 4 km	Kos27

(continued)

Accession number	Latitude	Longitude	Alt (m)	Collection date	Localities	Long-term mean temperature (°C)	Long-term mean precipitation (mm)	Final germination (%)	BG	Climate station and distance from population	Population code
					C. costiniana, *Celmisia costiniana*, *Poa* sp.						
CBG 9604821	-35.6347	148.78	1520	5-Mar-96	Namadgi NP, Leura Gap, ca 3 km direct NNW of Bimberi Peak.	7.6	1329	96	ANBG	Tidbinbilla Nature Reserve, 26 km	Nam28
CBG 9704595	-36.4172	148.3097	1880	13-Apr-97	600 m SW of Hedley Tarn, Kosciuszko NP.	4.4	2105	2.67	ANBG	n/a	Kos29

Table S2. Long-term climate associations with germination strategy were assessed using the following bioclimatic values. Values were drawn from Xu and Hutchinson (2011).

Climate variable (long-term)	**Variable description**	**Bioclimatic code**
Mean Annual Temperature	The mean of all the period maximum and minimum temperature (Celsius)	Bio01
Temperature Seasonality	The temperature coefficient of variation as the standard deviation of the period mean temperatures expressed as a percentage of the mean of those temperature (i.e. the annual mean). For this calculation, the mean in degrees Kelvin is used to avoid the possibility of having to divide by zero	Bio04
Temperature Warmest Period	The highest temperature of any period maximum temperature	Bio05
Temperature Coldest Period	The lowest temperature of any period minimum temperature	Bio06
Mean Annual Precipitation	The sum of all the monthly (period) precipitation estimates	Bio12
Precipitation of Wettest Period	The precipitation of the wettest month (period)	Bio13
Precipitation of Driest Period	The precipitation of the driest month (period)	Bio14
Precipitation Seasonality	The precipitation coefficient of variation, defined as the standard deviation of the period precipitation estimates expressed as a percentage of the mean of those estimate (annual mean)	Bio15
Mean Annual Radiation	The mean of all the period maximum and minimum radiation (MJ/m2/day)	Bio20
Radiation Highest Period	The highest temperature of any period maximum solar radiation	Bio21
Radiation of Lowest Period	The lowest temperature of any period minimum solar radiation	Bio22
Radiation Seasonality	The radiation coefficient of variation as the standard deviation of the period mean radiation expressed as a percentage of the mean of those radiation (i.e. the annual mean)	Bio23

Table S3. Loadings from CVA for (a) long-term climate (b) maternal conditions (c) seed development conditions and (d) combined variables absolute values. Temperature (Celsius), Precipitation (mm), Solar radiation (MJ/m^2/day). Bolded cells indicate the variates with the highest loadings for each axis in each analysis.

(continued)

Variate	**a. Long-term climate‡**		
	Axis 1	**Axis 2**	**Axis 3**
Latent roots	10.09	0.89	0.47
Percentage variation	88.15	7.76	4.09
Mean Temperature	**2.71**	0.16	5.71
Mean Precipitation	0.01	0.02	0.02
Mean Radiation	1.49	**24.62**	6.79
Seasonality Temperature	**9.18**	**96.28**	28.11
Seasonality Precipitation	0.25	0.15	2.12
Seasonality Radiation	**2.83**	6.24	2.69
Minimum Temperature	2.14	3.15	9.92
Minimum Precipitation	0.29	0.48	0.26
Minimum Radiation	**7.44**	**31.51**	13.73
Maximum Temperature	0.29	0.60	1.02
Maximum Precipitation	0.16	0.15	0.04
Maximum Radiation	**3.09**	3.65	1.89
Variate	**b. Maternal Condition‡**		
	Axis 1	**Axis 2**	**Axis 3**
Latent roots	15.33	1	0.61
Percentage variation	90.49	5.94	3.57
Mean Temperature	**2.44**	1.01	0.09
Mean Precipitation	0.01	0.01	0
Mean Radiation	**4.06**	**1.5**	4.58
Seasonality Temperature	**5.26**	**8.67**	4.2
Seasonality Precipitation	0.18	0.19	0.35
Seasonality Radiation	0.9	0.43	1.44
Minimum Temperature	0.22	**1.75**	0.5
Minimum Precipitation	0.33	0.07	0.21
Minimum Radiation	**1.64**	0.38	4.86
Maximum Temperature	0.26	0.62	0.2
Maximum Precipitation	0.08	0.02	0.01
Maximum Radiation	1.64	0.42	2.98
Variates	**c. Seed-development condition‡**		
	Axis 1	**Axis 2**	**Axis 3**
Latent roots	13.06	2.88	0.63

Table S3. Loadings from CVA for (a) long-term climate (b) maternal conditions (c) seed development conditions and (d) combined variables absolute values. Temperature (Celsius), Precipitation (mm), Solar radiation (MJ/m^2/day). Bolded cells indicate the variates with the highest loadings for each axis in each analysis.

(continued)

Percentage variation	78.83	17.37	3.8
Mean Temperature	1.84	0.4	0.74
Mean Precipitation	0.01	0.01	0
Mean Radiation	**4.53**	**5.23**	10.96
Seasonality Temperature	**27.87**	**63.55**	17.25
Seasonality Precipitation	0	0.61	0.08
Seasonality Radiation	0.55	2.86	1.9
Minimum Temperature	0.64	0.58	0.09
Minimum Precipitation	0.01	0	0.03
Minimum Radiation	**2.19**	**5.94**	2.47
Maximum Temperature	0.32	0.18	0.23
Maximum Precipitation	0.01	0.01	0.01
Maximum Radiation	1.24	0.28	4.35
Variates	**d. Combined‡**		
	Axis 1	**Axis 2**	**Axis 3**
Latent roots	19.54	4.16	2.11
Percentage variation	75.73	16.11	8.16
Long term Mean Temperature	2.51	0.14	4.73
Long term Mean Radiation	**21.33**	**29.17**	3.4
Long term Seasonality Temperature	**62.52**	**123.53**	**9.69**
Long term Minimum Radiation	0.95	1.53	7.42
Maternal Mean Temperature	1.13	2.09	0.32
Maternal Mean Radiation	1.73	1.6	0.35
Maternal Seasonality Temperature	8.97	2.59	**17.23**
Maternal Minimum Radiation	4.37	5.89	3.32
Seed Development Mean Temperature	4.36	2.74	3.48
Seed Development Mean Radiation	**10.52**	**23.27**	**10.24**
Seed Development Seasonality Temperature	**85.34**	**127.32**	**10.68**
Seed Development Minimum Radiation	**11.67**	**14.85**	0.02

‡CVA carried out for (a) long-term climate consisted of bioclimatic values from 21 populations. CVA for (b) maternal, (c) seed-developmental and (d) combined consisted of data from 20 populations (all populations minus Kos6).

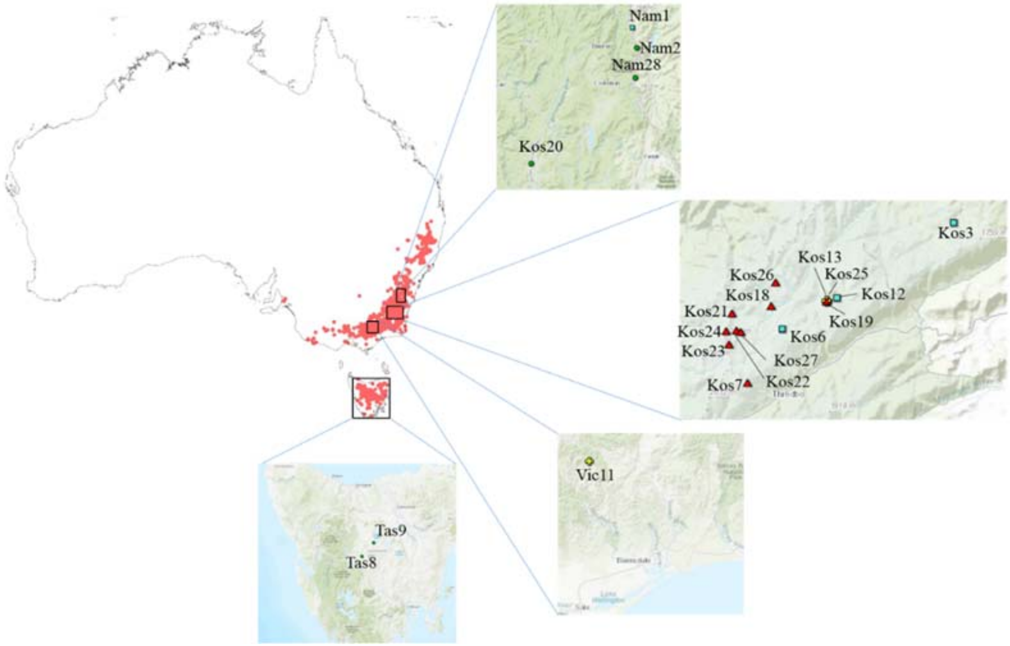

Figure S4. Distribution map of *O. eriopoda* and the locality of the populations we used for in the clustering analysis of germination strategy.

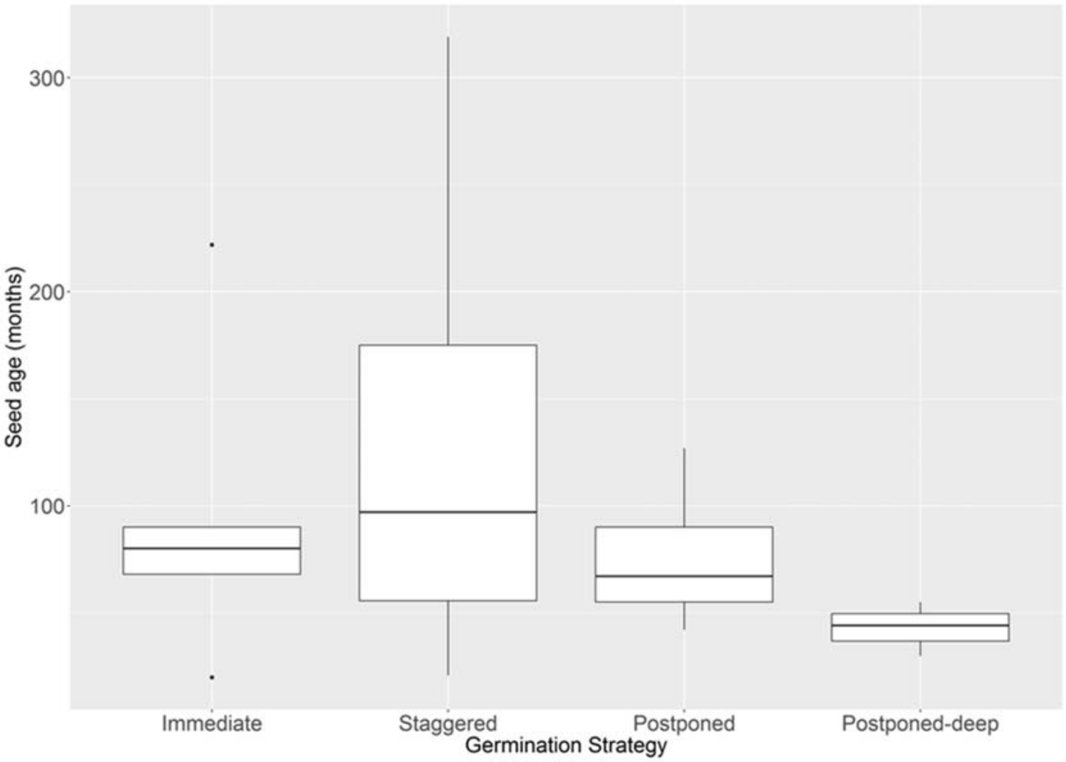

Figure S5. Box plots illustrating variation of germination strategy across seed age (immediate = 5 populations, staggered = 4, postponed = 9, postponed-deep = 3). There was no significant association between the age of the collection and germination strategy ($p = 0.3556$).

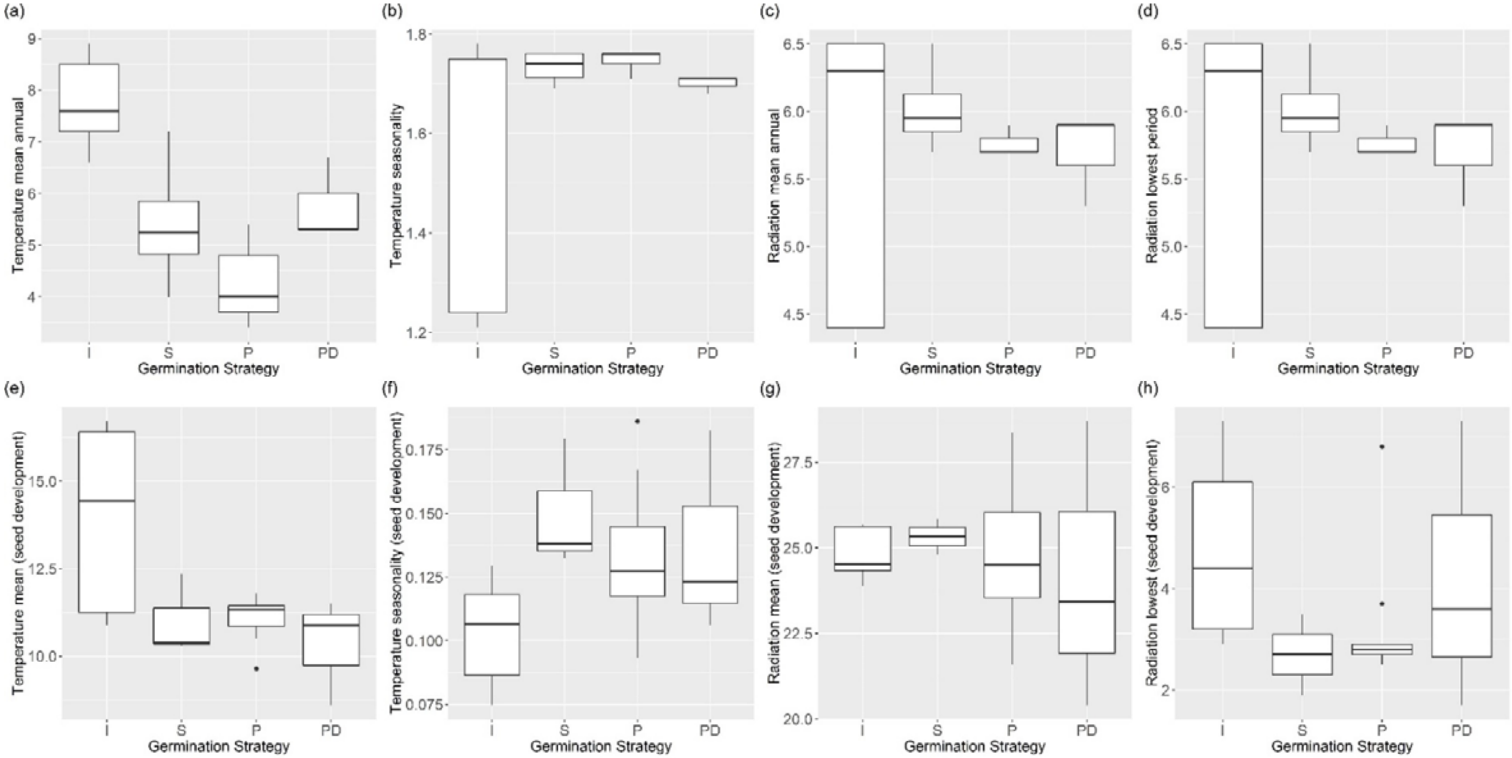

Figure S6. Box plots illustrating variation as a function of germination strategy for climate variables that had the highest latent vectors in CVA analyses, a) long-term mean temperature, b) long-term mean temperature seasonality, c) long-term mean radiation, d) long-term minimum radiation, e) seed-developmental mean temperature, f) seed developmental temperature seasonality, g) seed-developmental mean radiation, and h) seed developmental minimum radiation. Germination strategy: I = immediate, S= Staggered, P = Postponed, and PD = Postponed-deep.

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Design by Afiat Sukmaraga

Chapter 5

Effects of warming on development and phenology traits depend on germination strategies in the alpine herb *Oreomyrrhis eriopoda,* and vary during different parts of the life cycle

Annisa Satyanti, Lydia K Guja, Toton Liantoro, Morgan Thomas, Adrienne B Nicotra

Abstract

Global climate change is predicted to have large impacts on the phenology and reproduction of alpine plants. Warming experiments have been used to assess plant responses to global climate change. Despite the wide range of evidence that warming responses in either reproductive and vegetative characters or phenology will vary within as well as between species, we still lack knowledge on how important within species variation will be under future warming scenarios. Using *Oreomyrrhis eriopoda*, a montane herb native to Australia, in which within-species variation in germination strategy and growth characteristics has been demonstrated, we assessed variation in plastic response to soil warming, and assessed developmental trajectories which may be determined by patterns of germination timing. Further, we looked at how the next-generation seed and germination traits are altered by the maternal soil environment. We found that soil warming effects did not occur constantly throughout the plant's life, but mainly during the active developmental stage of each organ. The effect of warming also varied as a function of germination strategies, particularly for plant growth during the vegetative stage, the number of inflorescences, proportion of viable infructescences, and proportion of plants surviving. Interestingly, germination strategy was conserved across generation, indicating a low plasticity in germination strategy itself, despite extensive plasticity in thermal response of other traits. Our study is novel in revealing substantial within-species variation and how these variations affect a plant's responses to a warming environment.

Keywords

Germination temperature, intraspecific variation, maternal effects, seed development temperature, transgenerational effects.

Introduction

Warming as associated with climate change has been documented to exhibit a strong effect on plant life cycles, both in vegetative growth and reproductive phases (Arft *et al.*, 1999, Debouk *et al.*, 2015, Hedhly *et al.*, 2009, King *et al.*, 1999, Liu *et al.*, 2012). Alpine plants that are adapted to low temperature and a short growing season are particularly responsive to warming (Anadon-Rosell *et al.*, 2014, Arft *et al.*, 1999, Bjorkman *et al.*, 2015, Cao *et al.*, 2016, Kudernatsch *et al.*, 2008, Kudo & Suzuki, 2003, Oberbauer *et al.*, 2013). Warming is associated with shifts in phenology of bud burst, flowering, and senescence (Arft *et al.*, 1999, Cleland *et al.*, 2007, Farnsworth *et al.*, 1995, Körner & Basler, 2010, Moore & Lauenroth, 2017, Munson & Sher, 2015, Peñuelas & Filella, 2001, Rathcke & Lacey, 1985, Sherry *et al.*, 2007, Walther *et al.*, 2002). Warming has also been shown to lead to an extension of flowering time (Taylor & Garbary, 2003), delayed last flowering (CaraDonna *et al.*, 2014), as well as delayed fruiting (Menzel *et al.*, 2006, Peñuelas *et al.*, 2002, Root *et al.*, 2003). Early flowering in response to warming for example, is not always followed by a shift of last flowering and thus may result in the expansion of flowering period (CaraDonna *et al.*, 2014). Substantial plasticity in the duration of the phenology event in response to warming and cooling was also reported in six alpine meadow species (Li *et al.*, 2016). Flowering time also determines subsequent life history characteristics, particularly germination phenology of the seeds produced (Galloway & Etterson, 2007). The germination timing determines the seasonal conditions experienced by seedlings and all subsequent stages, including flowering time (Donohue, 2009). In some species warming also increases plant mortality from early ontogeny (Bernareggi *et al.*, 2016, Milbau *et al.*, 2017, Mondoni *et al.*, 2015) to adult stages (Allen *et al.*, 2010, van Mantgem *et al.*, 2009).

Indeed, the effect and direction of response to warming is also dependent on biome, functional groups, stage of life, and therefore may differ across plant species. Similar to biome effects, warming effects on reproductive phenology are dependent on other phenology traits. For instance, tallgrass prairie species that flower early (before mid-summer), start reproduction early, but conversely, species that flower late (after mid-summer), delay their reproduction when exposed to warming treatments (Dunnell & Travers, 2011, Sherry *et al.*, 2007). Hoffmann *et al.* (2010) demonstrated that the effects of warming on alpine plant phenology can be immediate, however, sometimes may accumulate across years. If we look at the plant community level, the variation of response may promote co-existence among species (CaraDonna *et al.*, 2014, Sherry *et al.*, 2007). However, studies often focus on particular suites of traits (vegetative growth vs reproduction vs phenology) separately, which may lead to incomplete assessments of the effects of warming on the entire plant life history.

Alpine regions are recognized as one of the most vulnerable ecosystems under warming climates; impacts are forecast to be pronounced and detectable earlier than in other biomes (Grabherr *et al.*, 2010). Alpine plant growth is characterized mainly by adaptation to low temperature and the short growing season (Körner, 2003). In this strongly seasonal and unpredictable environment, plants often evolved specific seed dormancy that could drive variation in germination strategy (Willis *et al.*, 2014). Germination strategy, hence, reflects across species pattern of germination timing and proportion of germination over the progression of seasons (Hoyle *et al.*, 2015). But more strikingly, intraspecific (within-species) variation of the germination strategy is also quite common in alpine species, mainly controlled by the degree of seed dormancy (e.g. Hoyle *et al.*, 2015, Wagner & Simons, 2009). For alpine plants, a strikingly high soil temperature is needed to trigger germination (Schütz, 2000, Schütz & Milberg, 1997). Warmer germination temperatures indeed accelerate germination timing of alpine and sub-arctic species (Milbau *et al.*, 2009, Mondoni *et al.*, 2012). Seed developmental temperature may also effect germination responses of alpine plants. The interplay between pre- (maternal) and post-dispersal (germination) temperatures is shown to drive physiological dormancy levels and a transgenerational plastic adjustment of seed germination and dormancy across three snowbed species (Bernareggi *et al.*, 2016). For alpine communities, warmer growing seasons have similarly been shown to increase production of vegetative ramets and increase reproductive growth in adult plants *in situ* (Anadon-Rosell *et al.*, 2014, Kudernatsch *et al.*, 2008, Kudo & Suzuki, 2003). Phenological shift of alpine plant communities is mediated by earlier snowmelt (Price & Waser, 1998). The absence of snow in alpine systems increases soil temperatures (Slatyer, 2016). We therefore might expect a phenological shift in alpine plants due to warmer soils as well.

While research on the adaptive capacity of plants under future climate is increasing, we still lack understanding of plant plasticity and their capacity to evolve when challenged by new environmental conditions, particularly for germination and early life history traits

(Parmesan & Hanley, 2015). We know little about the extent of within-species variation in these responses to future climate scenarios. Malyshev *et al.* (2016) demonstrated that variation in vegetative and physiological responses to climatic extremes within-species (summer drought, spring frost, winter warming) could be as high as the variation of among species and across functional groups. Such substantial variation within a species (among populations) could facilitate species to persist in changing climate (Walck *et al.*, 2011). Intraspecific variation of germination strategy may help to facilitate the regeneration and survival of plants in short growing seasons, and highly variable environments, like that of alpine habitats (Chapter 3). Germination strategy is considered an adaptation to local climate and has consequences for post-germination traits, natural selection on them, and determines the ecological range of a species (Donohue *et al.*, 2010). Within the cold desert annual *Isatis violascens*, autumn germinating plants also had larger plant size, produced more seeds, and had a longer lifespan, than that of the spring germinating plants (Lu *et al.*, 2016). Likewise, maternal condition has a carry-over effect across generations and could determine the characters and life history of the progeny (Donohue, 1998, Donohue, 2009). For example, maternal environment, particularly light, nutrients and flowering phenology, determine the germination season of *Campanula americana* and its germination season subsequently determines whether its offspring will become annual or biennial (Galloway, 2001, Galloway, 2002, Galloway, 2005). We, however, lack the information on whether variation in germination strategy within species contributes to plant life history traits in alpine species. For such species, which are likely sensitive to warming, the effects of a warmer maternal environment may consequently effect germination strategy and dormancy of seed progeny.

Oreomyrrhis eriopoda (Apiaceae), an Australian native herb commonly known as alpine caraway, was identified to have four germination strategies, identified as immediate, staggered, postponed, and postponed-deep (Chapter 3). Plants from some populations produce non-dormant seeds at dispersal and hence germinate immediately, whereas other populations produce dormant seeds that require winter (cold) cues to germinate in spring. Interestingly, some populations produce seeds with varying dormancy degrees, leading to a staggered germination strategy. Hoyle *et al.* (2015) suggests that a staggered germination strategy expressed in the diversified patterns in germination characteristics within a species is underlined by an evolutionary bet-hedging mechanism. The germination strategy of *O. eriopoda*, besides being closely linked to long-term temperature variability, also shows signatures of effects of temperature variability during seed production: the immediate strategy was associated with lower temperature variability (Chapter 3). This intraspecific variation in germination strategy has some important consequences for the vegetative growth of offspring. Early germinating seedlings (autumn seedling) from the immediate germination strategy and a fraction of staggered strategy were found to grow faster than the late germinating seedlings (spring seedling) of the postponed, postponed-deep, and the late-germinating fraction of staggered strategies (Chapter 3).

Using *O. eriopoda*, we aim to understand the effect of warming on the vegetative growth, reproductive output, phenology and the germination traits of the next generation, and to explore whether populations of different germination strategies show variation in those warming responses. Many of the experimental warming studies apply active heating, using overhead infrared lamps, or passive heating, which uses the open-top chamber method, or an undersoil heating mat or cable. Both active and passive experimental designs tend to reduce total irradiance and soil moisture, which can delay plant phenology (reviewed in Wolkovich *et al.*, 2012), and might not be representative of the actual warming effect. Alpine plants are of low stature and likely decoupled from overall air temperatures (Körner, 2003). Soil temperatures are important parameters for alpine life conditions because they influence plant growth and phenology (Higgins & Spomer, 1976). However, soil temperatures match the microclimate that alpine plants experience better than the air temperatures (Reinhardt & Odland, 2012, Scherrer *et al.*, 2011). Hence, we focused on the effect of soil warming on the functional plant traits.

In this study, we intended to extract the effect of soil warming in the absence of water and accordingly, we exposed seedlings of Australian alpine herb *Oreomyrrhis eriopoda* from four germination strategies to contrasting warm and ambient soil temperatures. Specific research questions were: (i) How do soil warming and germination strategy affect vegetative, reproductive, and phenological traits, from early growth to senescence? (ii) Do maternal condition (soil temperatures) and post-dispersal germination treatments influence the germination strategy or success of the offspring? We hypothesised that: (i) Warming will affect the vegetative and reproductive capacity and lead to a shift in the phenology traits, likely advancement of reproduction time, as well as increased plant mortality, (ii) Seedling and plant responses to soil temperature will be dependent on the germination strategy, (iii) Germination strategy is a plastic trait that responds to environment, i.e. germination strategy of the offspring will be affected by maternal environment (pre-dispersal temperature) such that response to germination condition (post-dispersal temperature) will change.

Methods

Study species and plant propagation

Oreomyrrhis eriopoda (Apiaceae) is a perennial herb that occupies a wide range of micro-environments and is found in heath, herbfields and surrounding alpine bogs and fens, as well as in forests and shrublands at lower elevations (Costin *et al.*, 2000). *O. eriopoda* umbels (inflorescence) are usually composed of 12 – 35 flowers. In their natural habitats, flowering occurs in late spring with seed ripening and dispersal in early summer (Ainsley et al. 2010). The genus *Oreomyrrhis* is mainly self-pollinated, which in *O. eriopoda* is supported by the fact that the basal umbels are very inconspicuous and nearly sessile during early anthesis (Chung *et al.*, 2005). *O. eriopoda* seed, like many Apiaceae species, has under-developed embryos and is physiologically dormant (Chapter 3).

To test the effect of soil temperature and germination strategy on the plant development and phenology of *O. eriopoda*, we included seedlings from 19 populations (Supplementary Fig. 1 and Supplementary Table 1) that represented the range of germination strategies expressed in this species. We aimed for a balanced representation of germination strategies based on prior assays, and concurrently analysed the germination data. Ultimately we had five populations with an immediate germination strategy, four with a staggered strategy, seven postponed and three with a postponed-deep germination strategy. Seeds from each population were germinated in two batches so that germination of early (autumn) and late (spring) seedlings coincided so that seedling age was relatively equal regardless of germination strategy (Supplementary Material 1). These seedlings were subsequently transferred to the soil warming experimental set-up at 18 – 20 weeks old (Supplementary Materials 1).

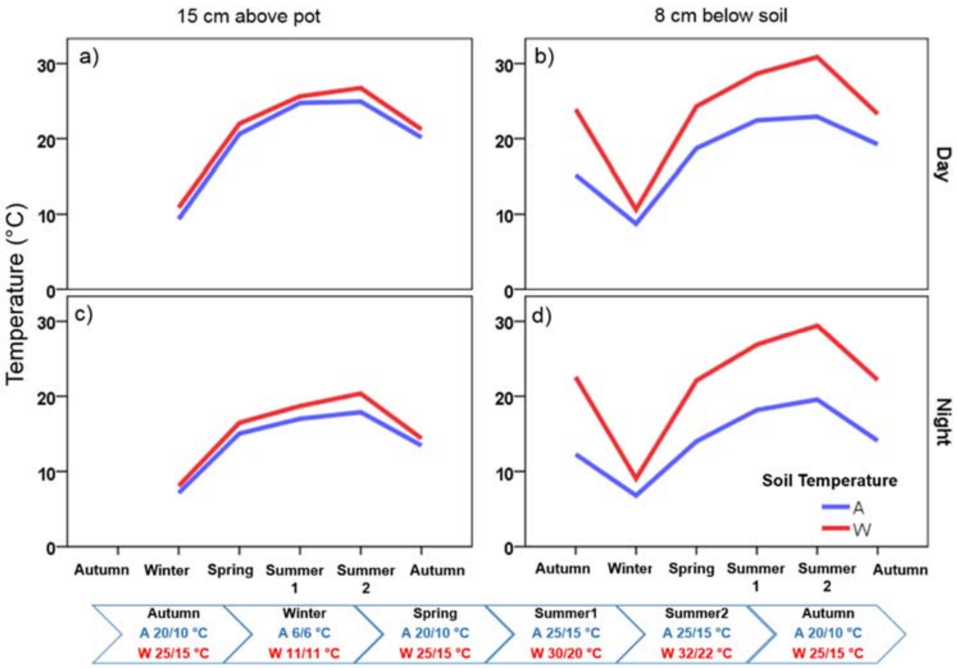

Figure 1. The realised mean temperature in ambient and warm conditions during the day at a) plant level 15 cm above the pot, and b) 8 cm below soil; and during the night, c) plant level 15 cm above the pot, and d) 8 cm below soil. At each season, warm conditions were significantly warmer than ambient ($p < 0.001$, ANOVA). Note: data loggers for plant level temperatures were only started in winter. In the boxes are the target soil temperatures for ambient (A) and warm (W) treatment for each season.

Experimental design

For each population 10 individuals were randomly allocated to each of the two soil warming treatments. We assessed initial seedling size and confirmed that there were no a-priori differences in the allocation of plants between ambient and warm soil treatments (one-way ANOVA, $p = 0.453$ and $p = 0.686$ for leaf number and leaf length, respectively). For the staggered germination strategy, both early (autumn) and late (spring) seedlings, were placed in the respective block, thus, these populations had 20 individuals/soil warming treatments. One representative of each putative germination strategy was assigned to each of the 4 blocks. Subsequent analyses of germination strategy revealed imbalance in numbers of populations per strategy. Specifically, only three populations had a postponed-deep germination strategy. However, the imbalance was not an impediment for the analyses. There were five populations for which we did not have 20 seedlings and for these we assigned half of the available number to each warming temperature. Extra plants were placed in the empty spaces on the bench to maintain homogeneous spacing across the experiment but were not included in analyses (number in grey, Supplementary Fig. 1).

Warm and ambient benches were set up in a glasshouse with air temperature set to follow the seasonal changes and natural photoperiod (Figure 1). The experiment commenced in autumn. The targeted air temperature day/night sequence was 20/10 °C (autumn), 5/5 °C (winter), 20/10 °C (spring), 25/15 °C (summer), and finally 20/10 °C (autumn). Soil warming was achieved by placing a heating mat (Electronic Foil Panel with Thermostat, ADLOHEAT, Victoria) on the bench set to be continuously ~ 5 °C warmer than the set glasshouse air temperature throughout the experimental period, including winter. The 5 °C soil temperature increase is based on Australian alpine mean air temperature predictions for 2050, i.e. an increase of +0.6 to +2.9 °C (Hennessy *et al.*, 2003) and that the maximum soil temperatures in Australia is to increase by almost double that of air temperature by the end of 21st century (Ooi *et al.*, 2009). The 5 °C, thus, fall between the predicted +1.2 to 5.8 °C soil temperature increase by 2050. Under projected future climate, air temperature increases will also lead to snow melt as well as warmer and more variable soil temperatures (Jungqvist *et al.*, 2014, Schuerings *et al.*, 2014). Ambient benches had mats but no heat. A frame of standard 6 mm-thick PVC sheet was placed around the bench, 17 cm above the mat, and a 5 cm thick sheet of polystyrene foam was placed on the top of this to insulate the soil. Square openings in the polystyrene matched the pot size and held these in place in the frame. Temperature at plant level (15 cm above bench; Fig. 1.) and soil (2, 8 (where most roots occurred, Fig 1.), and 14 cm below surface) was monitored during the course of the experiment with i-Button data-loggers (Thermochron DS1921G, Temperature Technology, Adelaide) in each block (32 in total). We analysed the temperatures at plant and soil level to determine efficacy of the design using ANOVA and found significant warming differences for all the measurement levels. The temperature difference between ambient and warm soil at 8-cm below the surface was approximately 6 °C during the day and 9 °C during the night (Fig. 1), in agreement with climate patterns which show that night-time temperatures have increased more than day-time temperatures (Donat & Alexander, 2012, Easterling *et al.*, 1997). With warmer air temperature and reduced snow cover, the soil in the Australian Alps becomes warmer, evidenced by a snow removal experiment (Slatyer, 2016). Thus, we also increased the soil temperature during winter for the warmer soil scenario, which was raised to 35 °C in the summer. This scenario is realistic for the Australian mountains where dark soils can exceed 45 °C on sunny days and the difference to the nearby vegetated soils can be over 30 °C (Slatyer, 2016).

The irrigation system was set to keep plants adequately-watered. We used automatic Water-Pro vapour pressure deficit (VPD) to provide automatic watering controlled system (MicroGrow Green House Systems, Temecula, California), with each plant being watered individually by a dripper at soil level. Drippers were calibrated to a standard flow rate that was checked at the beginning of the experiment. Watering events were triggered when pre-set VPD targets were reached. Plants received ~ 90 ml per watering which was enough to saturate the soil at the start of the experiment. As the plants grew, we adjusted the VPD point, based on the two sensors for ambient and warm soils, so that the drippers sufficiently delivered water more frequently to the plants, in order to keep all plants healthy. To focus on the effect of warming and minimise potential genetic influences that may have occurred from pollination across populations, a structure made of hollow PVC ~110 cm tall was built over the bench and organza sheeting hung to separate the populations to avoid potential cross pollination.

Trait measurement

Leaf number and the length of the longest leaf were recorded for all plants at the start of the experiment, and at the end of autumn (day 38). Those traits were recorded for a subset of plants (4 individuals per soil temperature and (sub-) population) in early spring (day 124). Leaf increment rate at the early vegetative stage was calculated from the total leaf number at the end of autumn divided by 38 (number of days from planting). Leaf increment at the transition to reproductive stage was the difference between total leaf number at day 38 and day 124, divided by 86 (number of days between measurement). Specific Leaf Area (SLA), and ramet number were measured for every individual at the start of winter (day 52). SLA was measured by acquiring the youngest fully expanded leaf for each individual. The leaf was then scanned on a flatbed scanner, dried at 60 °C for 72 hrs and weighed. The SLA was calculated as area/weight (cm^2g^{-1}). In early spring, a subset of plants was photographed with a reference scale over a white Styrofoam board to determine plant area. Each plant image was then converted to an 8-bit graphic file. The threshold was adjusted so that only the actual plant area was detected for selection and using the known distance of the reference scale we calculated the plant area. The image analysis of the plant area was performed in ImageJ (Schneider *et al.*, 2012).

Plants were monitored every 1-2 days for phenology recording. We recorded the date of the first flower when the inflorescence clearly emerged (inflorescence with closed buds emerged) from the plant base. All infructescences were collected at the point of natural dispersal when all seeds were brown or purple-brown and easily dislodged, and the date of collection was recorded for each. Infructescences were stored at 15 % RH and 15 °C. Total number of inflorescences (infructescences) and proportion of viable and aborted infructescences were determined for each plant. An infructescence was recorded as viable if it produced at least one seed. Further, we measured seed mass for a subset of individuals (five per population per soil treatment) by weighing three replicates of 25 seeds. The date of collection of the last infructescence was recorded as the end of seed dispersal, and the time between the first and the last seed harvest was defined as the duration of seed production. Some plants naturally senesced over the course of the experiment and date of senescence (all leaves browned, no new leaves emerging) was recorded. At the end of the experiment aboveground biomass was harvested for all plants, dried at 60 °C to constant weight, and weighed. Those plants that died were sampled for biomass within a week of senescence.

Transgenerational effect on germination traits

Seeds from parent plants of three germination strategies: immediate, staggered, and postponed were used to test whether there was an effect of soil temperature during development on the germination strategy and response to germination temperature of progeny. Seeds from a subset of individuals from twelve populations were selected to represent the three germination strategies (Supplementary Fig. 1). For each population and soil temperature, we selected 50 seeds (from peak seed production period) from each of five fruiting individuals per warming treatment. Twenty five seeds each were sown on each of two petri dishes, and randomly allocated to one of two germination chambers set at 25/15 °C and 30/20 °C 12/12 hours photoperiod, each consisting of five blocks (shelves). For a given population, one plant was represented in each block. Seeds were stratified as above and then placed at 25/15 °C or 30/20 °C. Germination was scored weekly for 34 weeks, and at the end of the experiment a cut-test was performed to determine whether ungerminated seeds were empty, dormant, or dead.

Statistical analyses

Mixed models were selected for the analysis of plant traits. Models included terms for germination strategy and soil temperature and the interaction thereof as fixed factors, and population nested within blocks were assigned as random factors. An exception was made in the random model for the days to first flower, where block was used as the random factor because inclusion of population nested in block resulted in convergence failure. Vegetative and reproductive traits that were discreet (number of leaves, number of ramets, number of inflorescences, day to flower, day to seed dispersal, seeding duration, and day to senesce) were analysed using Generalized Linear Mixed Models (GLMM), setting the distribution family as Poisson and the link function as natural logarithm (Bolker *et al.*, 2009). Leaf number (but not length of the longest leaf) at the start of the experiment significantly varied across germination strategies (Supplementary Table 2). Hence, we used leaf number at the start of the experiment as a covariate for corresponding traits, i.e. leaf number at transition to reproductive stage, leaf increment rate, SLA, plant area, aboveground biomass. For proportion data (survival and proportion of plants producing seed), GLMM were used with the distribution family as binomial, the link function as logit, and the dispersion parameter set to be estimate. Responses that were continuous (longest leaf, leaf increment rate, individual seed mass, SLA, and aboveground biomass) were analysed with Linear Mixed Models (Restricted Maximum Likelihood, REML) and when necessary, the responses were transformed using natural logarithm prior to fitting to REML (Supplementary Table 2).

Repeated measures analyses were run for the leaf number and leaf length using germination strategy, soil temperature and measurement time as fixed factors; and population nested in block as the random model and leaf number at the start of the experiment as covariates the analysis of the leaf number. However, the results were the same as when we performed the analyses for each measurement time point and thus we present the results from the two measurement points as they provide a clearer visual inference.

To assess transgenerational effects on germination strategy, we analysed the final germination, non-dormant seed fraction (germination before spring), and time to reach 50 % germination. The final germination and non-dormant seed fraction were analysed using GLMM, assigning germination temperature, soil temperature, and germination strategy as the fixed factors, population and individual plants nested on the incubator shelf were set as the random factor, and we set the distribution family as binomial and the link function as logit. Time to reach 50% germination was derived by examination of cumulative germination for each dish to the closest 0.25 week and treated as a continuous variable and analysed using Linear Mixed Models (REML) with fixed and random factors as for the non-dormant fraction analyses. GLMM and REML were performed in Genstat 17th Edition. Following a significant effect of fixed effect and/ or the interactions, multiple comparisons of means were performed using *lsmeans* package (Lenth, 2016) in R 3.2.4 (R Core Team, 2016).

Results

The effect of warming was pronounced over the course of the *O. eriopoda* life-cycle, but varied among traits (Fig. 2). We measured 19 traits across 4 different life history stages, vegetative, reproductive, phenological, and transgenerational, for significant effects of warming, germination strategy, and interactions thereof. 16 of the 19 variables showed a significant interaction, additive effect, or main effect of warming and/or germination strategy (Table 1, 2). Overall, the early vegetative stage was mostly influenced by interactions between germination strategy and warming. Some of the reproductive traits were influenced by an interaction between warming and germination strategy while others were influenced by main effects of those variables. Some of the phenological traits, particularly those related to seed set, were influenced by additive effects of warming and germination strategy. Transgenerational seed germination was influenced by germination strategy and responded to germination temperature, but showed no transgenerational effect of maternal warming (Fig. 2).

Table 1. Significance values for selected vegetative, reproductive and phenology traits analyses; full details in SI Table 2. Mixed models were used in which germination strategy (GS) and Soil Temperature (SoilT) were assigned as the fixed factors and population nested in block as random factor.

Response	**GS**	**SoilT**	**GS×SoilT**
Early vegetative stage			
Leaf number ‡	**0.027**	**<0.001**	**0.001**
Leaf increment rate (leaf per day) ‡	**0.037**	**<0.001**	**<0.001**
Specific Leaf Area (cm^2g^{-1}) ‡	0.65	**<0.001**	0.81
Transition to reproductive stage			
Leaf number ‡	0.266	0.645	0.113
Leaf increment rate (leaf per day) ‡	0.652	**0.009**	0.081
Plant area (cm^2) ‡	0.116	0.675	0.144
Reproduction stage			
Proportion of plant flowering	0.569	**0.003**	0.107
Total number of inflorescence	0.279	**<0.001**	**0.029**
Proportion viable infructescence	**0.009**	0.565	**0.038**
Single seed mass	**<0.001**	0.282	0.289
Phenology			
Days to first flower	**<0.001**	0.826	0.307
Days to seed maturity from flowering	**<0.001**	**<0.001**	0.164
Duration of seed production	**0.009**	**<0.001**	0.192
Days to plant death from planting	0.614	**<0.001**	0.091
Final survival and aboveground biomass			
Proportion of plant surviving	0.161	**0.001**	**0.015**
Aboveground biomass ‡	0.751	**0.021**	0.387

‡ Leaf number at the start of the experiment was also added as a covariate in the analysis.

Table 2. Significance values of transgenerational effects on germination strategy. Germination strategy (GS), maternal soil temperature (SoilT), and Germination Temperature (GermT) were assigned as fixed factors, with individual nested in population and shelf blocking as random factor. Generalized Linear Mixed Models were used to analyse the percentage of non-dormant seed fraction and final germination. A Linear Mixed Model was used to analyse the time to reach 50 % of germination. Details in Supplementary Table 3.

Response	GS	SoilT	GermT	GS×SoilT	GS×GermT	SoilT×GermT	GS×SoilT×GermT
Transgenerational seed germination							
Non-dormant seed fraction	**0.004**	0.419	**<0.001**	0.239	0.342	0.477	0.187
Time to reach 50 % of seed germination	**<0.001**	0.526	**<0.001**	0.178	0.432	0.138	0.692
Final germination	0.397	0.623	0.064	0.431	0.52	0.227	0.462

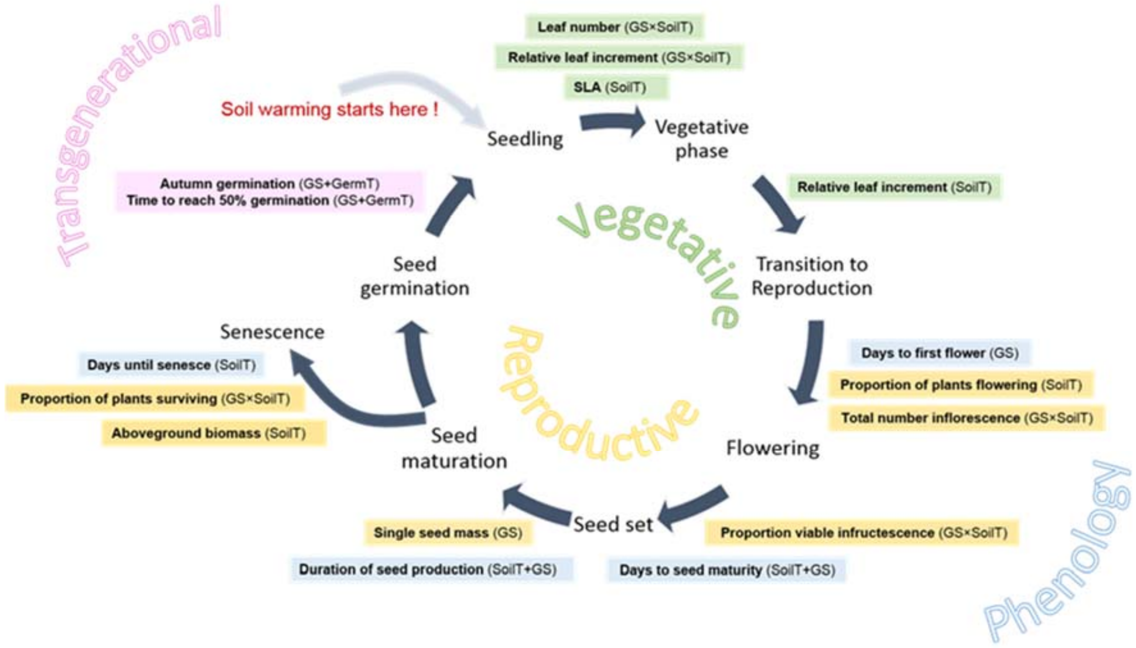

Figure 2. The life cycle of *Oreomyrrhis eriopoda* from early vegetative growth to reproduction and senescence. All traits were influenced by experimental factors: germination strategy (GS), development temperature (SoilT), or their interactions (GS×SoilT), at some stage. Transgenerational seed traits were only affected by germination temperature (GermT) and not maternal SoilT. Box colours indicate traits: vegetative (pale green), reproductive (pale yellow), phenology (pale blue), and the transgenerational effect on seed germination (pink). Significant fixed terms are given in brackets; detailed results are presented in Table 1, Table 2, Figure 3 to 5, and Supplementary Table 2 and 3.

Vegetative

An interaction between warming and germination strategy significantly increased early vegetative growth, (relative leaf increment and leaf number, Fig. 3a and b), but these vegetative effects were no longer apparent at the transition to reproductive stage (Fig. 3 b, d)). Soil warming significantly increased the leaf increment in that first autumn of the immediate, staggered and postponed-deep germination strategies, but not the postponed strategy. The immediate strategy produced leaves at the fastest rate and the postponed strategy was the slowest, which was also reflected by the leaf number (Fig. 3, Table 1). Growth under warm conditions led to increased SLA relative to plants grown in ambient conditions (218.8 vs 231.8 $cm^{-2}g^{-1}$), and did not differ across the germination strategies (Table 1). The plants transitioned to reproductive growth in spring as evidenced by traits measured in the second week of spring conditions. At this point, many individuals had just started to flower. The diminished effect of warming and germination strategies on vegetative growth at this stage coincided with a plateau for vegetative growth and transition to the reproductive stage, as also indicated by the plant area and leaf number (Table 1). Notably, at the transition to reproductive stage the leaf increment rate of all strategies was decreased by warming, contrary to the effect of soil warming during the early vegetative stage (Fig. 3, Table 1). The warming effect on growth returned later, as observed in the final aboveground biomass, although the reduction was only 2 grams (Table 1).

Reproductive

There was a significant reduction in the number of plants flowering under warmed conditions (Fig. 5, Table 1). Total inflorescence production per plant was reduced, but the amount of reduction depended on germination strategy (Fig. 2, Fig. 4a), whereby immediate strategy changed least in response to warming. (Fig. 4a, Table 1). The effect of warming on proportion of viable infructescence (infructescence that contains seed) also depended on germination strategy. Viable infructescence increased with warming in postponed-deep and immediate strategies, but showed a non-significant decrease in staggered and postponed (Fig. 4b). Notably, the postponed-deep strategy that produced total number of infructescence similar to the postponed strategy had the lowest proportion of viable infructescence (around 50% of abortion) compared to other strategies at ambient soil temperatures. The proportion of viable infructescence was much higher at warm soil temperature with almost no change in individual seed mass. Seed mass did vary among germination strategies, but warming did not elicit a significant response in any strategy (Fig. 2, Fig. 4c). Compared to the field collected seed, mass of the seed produced in the soil warming experiment was up to 1.2 mg heavier than the seed collected from the field (parent), except for those exhibiting an immediate germination strategy, where seed mass between field and experiment was constant (Fig. 4c).

Transgenerational effects of warming

There was no effect of maternal warming (maternal soil temperature) on any of the measured germination characteristics (final germination, non-dormant seed fraction, and time to reach 50% germination), which indicated that germination strategy was not a plastic trait as we expected. The transgenerational effects on non-dormant seed fraction, and time to reach 50% germination were affected by germination strategy and germination temperature only, but not by the interaction (Fig. 4d, Table 2). Warmer germination temperature led to longer time to reach 50% germination and reduced germination of the non-dormant seed fraction regardless the germination strategy and maternal warming (Fig. 4d, Table 2, Supplementary Table 3). The absence of interaction effect between germination temperature and maternal warming indicated maternal soil temperatures did not affect the response to germination temperatures, such that seeds produced from warm soil did not do better at higher germination temperatures than seeds produced at ambient soil. Final germination across strategies and temperature treatments was > 90% indicating that all seeds produced were healthy and viable regardless of maternal soil temperature.

Phenology

The number of days to first flowering was only influenced by germination strategy (Fig. 2, Table 1). Early germination (immediate strategy) flowered earlier than other strategies. Although there was no interaction, certain elements of reproductive phenology (days to seed maturity, duration of seed production) were sensitive to both warming and germination strategy. Warming accelerated time to seed maturity and the duration of seed production was overall reduced by warming (Fig. 5, Table 1). The immediate strategy was relatively slower to mature seeds, but because of their earlier flowering date, absolute time to seed maturity was still shorter compared to the other strategies (Fig 5, Table 1). Finally, warming significantly reduced final plant biomass, accelerated time to senescence and thus also reduced lifespan of the plants (Fig. 2, Fig. 5, Table 1).

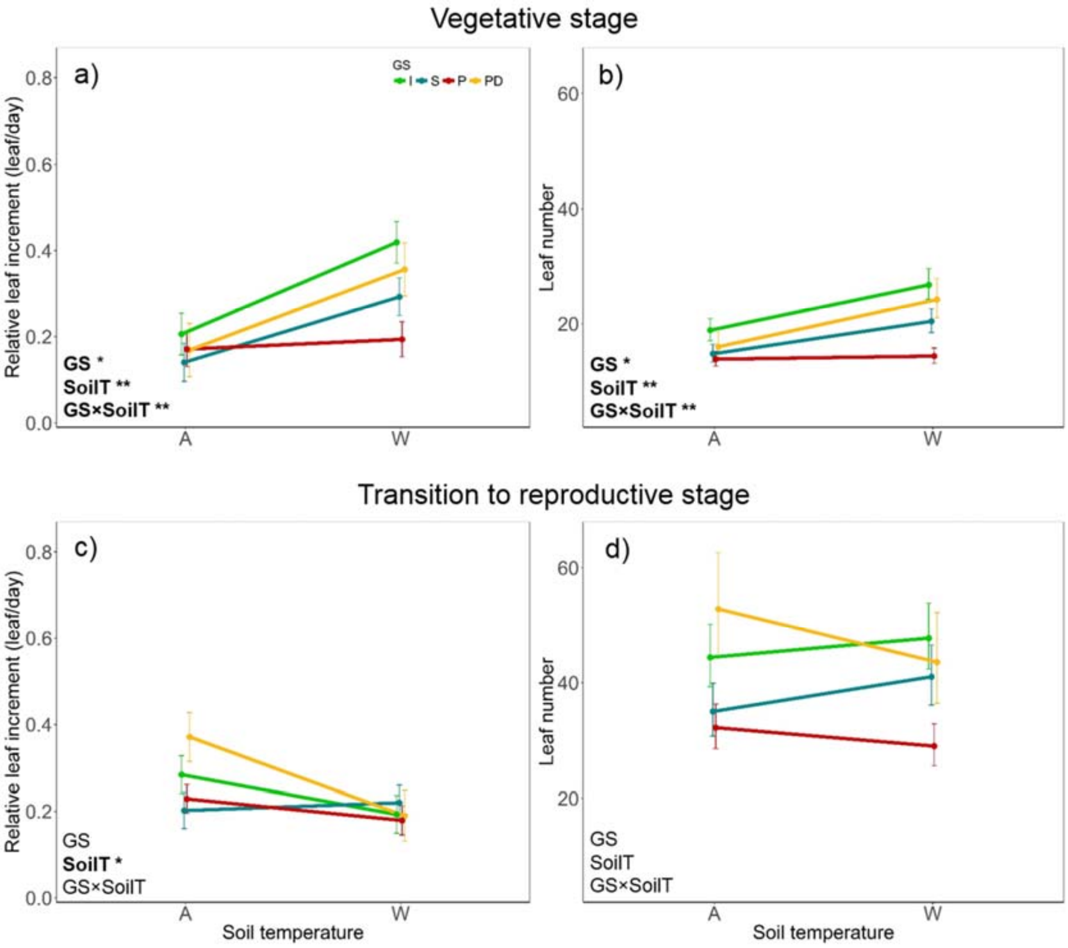

Figure 3a) Relative leaf increment and b) Absolute leaf number at vegetative stage, c) Relative leaf increment, and d) Absolute leaf number at the transition to reproductive stage. At earlier vegetative stage the effect of germination strategy (GS) and soil temperature (SoilT) were more pronounced than at the transition to reproductive stage. Significant terms are indicated by the bolded term and asterisk; * = significant at $p < 0.05$, ** = significant at $p < 0.001$. Line colour represents GS: green (immediate), blue (staggered), red (postponed), yellow (postponed-deep).

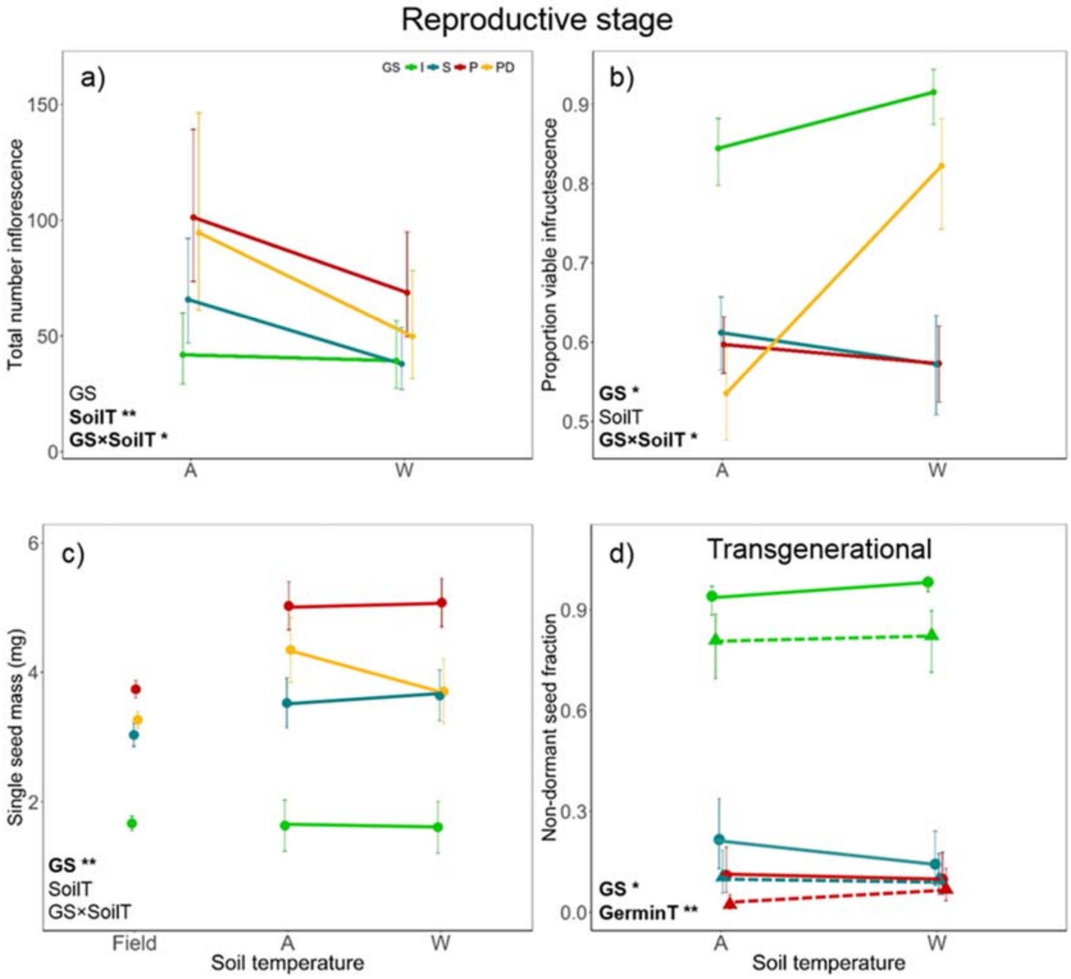

Figure 4. Reproductive stage a) Soil warming reduced the total number of inflorescences produced by an individual for all except the immediate strategy, b) The proportion of viable infructescence significantly differed among strategies, and warming had a positive effect, but only for postponed-deep strategy, and c) Seed mass differed among germination strategy and seeds produced in the experiment had larger seeds than the field-collected seed (mother plant). Transgenerational stage d) Non-dormant seed fraction (autumn germination) was higher when seeds germinated at 25/15 °C (●) than germination at 30/20 °C (▲); germination strategy was conserved indicated by non-significant effect of soil warming. For transgenerational stage, where we had germination strategy, maternal soil temperature, and germination temperature as fixed factors, we only present the significant factors. Significance values are indicated by bolded terms and asterisk. * = significant at $p < 0.05$, ** = significant at $p < 0.001$. Line colour represents GS: green (immediate), blue (staggered), red (postponed), yellow (postponed-deep).

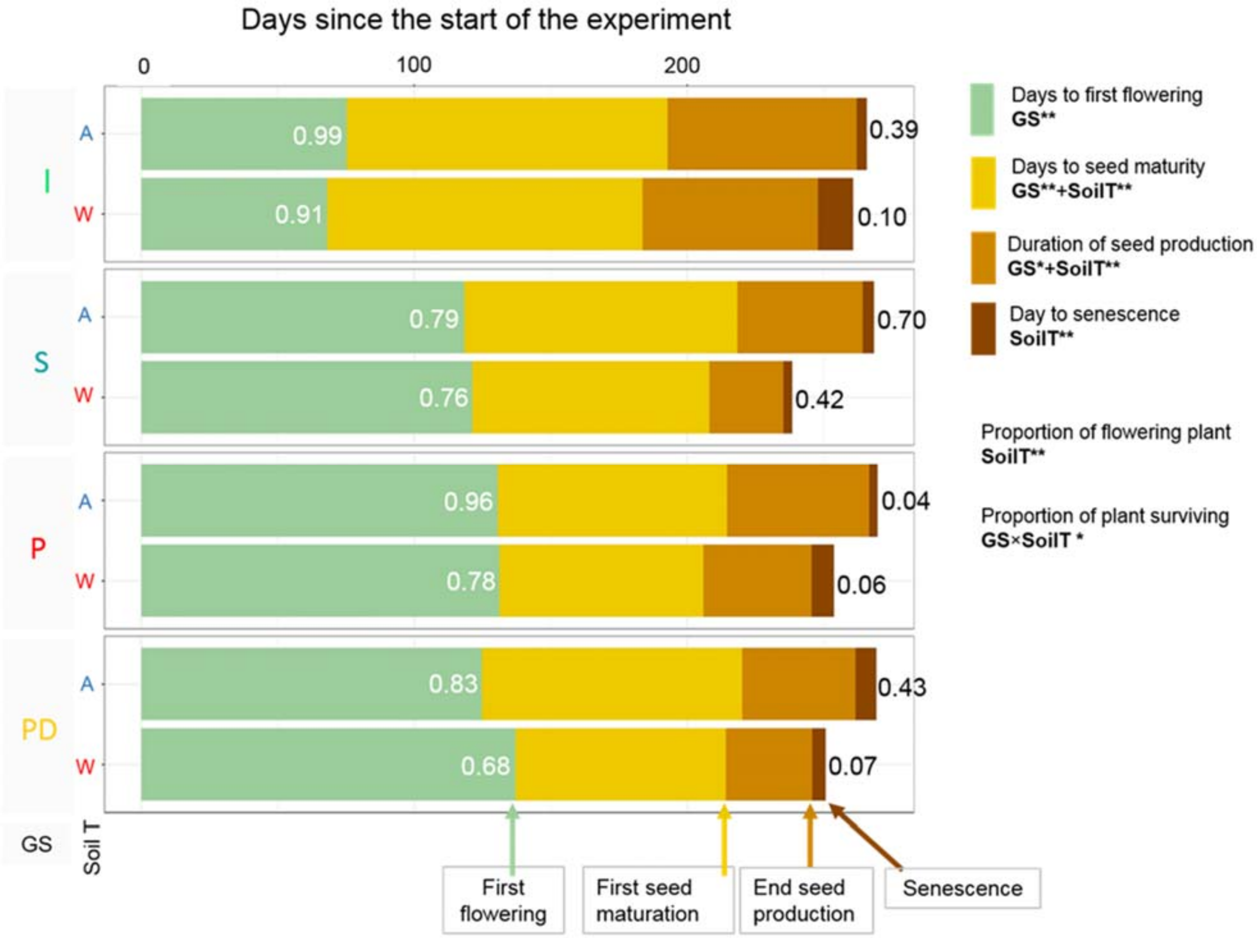

Figure 5. The reproductive phenology and senescence indicated by the coloured bar of the events: day to first flower emergence, day to first seed maturation from flowering, the duration of seed production - first to last seed maturation, and senescence from the last seed maturation. Numbers in white boxes (left) indicate proportion of plants flowering and in black boxes (right) indicate the proportion of plants surviving. In the legend, bolded variable and asterisk indicated significance (Table 1, Supplementary Table 2).

Discussion

We set out to examine the effect of soil warming on the vegetative growth, reproductive output and phenological response, as well as the germination traits of the next generation, and whether these responses were dependent on the germination strategy. Indeed, we found that the vegetative, reproductive and phenological responses of our study species, *Oreomyrrhis eriopoda*, were altered by soil warming. Interestingly, the effect of warming on different traits is most apparent in the active growth stages of the plant life-cycle, and not universally. The pattern and direction of warming effects varied with germination strategy. Notably, the offspring retained their parents' germination strategy regardless of the seed development conditions (maternal soil warming). We consider the diversity of these responses to warming along life-cycle stages, and among germination strategies within a species, to be extraordinary as they highlight the complexity of linkages between the maternal and offspring environment. The consequences of warming effects on adult plant vegetative and reproductive traits, as well as phenological responses, and ultimately, the offspring were all altered by germination strategy.

Plant phenological responses to changes in climate, for example earlier snowmelt in alpine systems, can vary markedly. In the northern hemisphere early snowmelt, associated with soil thaw and freezing and more variable soil temperatures, reduces flower number and increases flower abortion for alpine plants (de Valpine & Harte, 2001, Inouye, 2008, Saavedra *et al.*, 2003, Wipf *et al.*, 2009). Conversely in the Australian alpine system earlier snowmelt, simulated by winter snow removal, increases soil temperature (Slatyer, 2016). Warmer soil temperature may provide plants with an earlier and longer growing season (Crawford, 2008). Our results suggest that with increasing soil temperature, the phenology of this species is negatively affected, as indicated by the a condensed lifespan and reproductive period for all germination strategies except immediate. This could indicate that soil warming across seasons is an important determinant for phenology.

The effects of soil warming on vegetative growth and reproductive output were moderated by germination strategy but showed no universal direction. For example, the immediate strategy was the most responsive to warming during the vegetative stage when compared to the other strategies. This positive effect of soil warming, shown by increased leaf increment at the vegetative stage, is reversed at the commencement of flowering. Conversely, during the reproductive stage, the effect of soil warming on reproductive output and phenology was generally negative, as exhibited by the three strategies, except for the immediate strategy. The interaction effects between soil warming and germination strategy were exhibited not only in early leaf growth and leaf increment, but also in the total number of inflorescences, proportion of viable infructescence, and the proportion of plants surviving. These complex patterns are an indication that multiple sources of differentiation in plasticity of traits can be present, even within a species. The current study contributes significant new information to the field by tracking within-species variation in response to soil warming across all developmental stages through to senescence, and how these are mediated by germination strategy.

The immediate germination strategy might be advantageous under future climate scenarios because soil warming increased vegetative growth, but the effect on reproductive output, phenology, and survival was minimal. Seedlings with an immediate germination strategy seemed to be more tolerant and capable of maintaining fitness under warming. Conversely, non-immediate strategies showed a lower fitness, indicated by, in general, a shorter seed production duration, lower infructescence number, fewer plants surviving, and shorter lifespan in warmer soils, compared to the immediate strategy. In the field, the immediate strategy is found in populations from low elevation (<1400 m), whereas populations from mid and high elevations exhibit either staggered, postponed, or postponed-deep strategies (Chapter 3). This supports a study by Gugger *et al.* (2015), highlighting that across species, lower elevation species seem to shift their reproductive phenology to track ongoing climate changes, whereas high-elevation species were less capable of doing so and appeared more genetically constrained to their specific adaptations to an environment. Our results suggest that shifts in phenology and adaptations to environment may also be present among populations and not necessarily species characteristics. Although non-immediate strategies showed a reduced proportion of flowering plants and fewer inflorescences under warming, seeds had nearly 100% germination regardless of the germination temperature and maternal soil conditions. Thus, it is important to examine the adult traits of offspring to determine whether immediate populations from the lower elevation will replace the other strategies.

Postponed-deep and staggered germination strategies are thought to be a form of bet-hedging (Chapter 4). Notably, for postponed-deep strategies, the proportion of viable infructescences was substantially increased for plants grown in warmer soil conditions, unlike the other strategies that were not responsive to warming. However, the plants of the postponed-deep strategy in this experiment represented the minority of individuals from postponed-deep populations. Less than < 40% of the total seeds sown could be germinated and grown in the experiment so we could not get a true picture of what the remaining majority of seed in these populations would do (Chapter 4). Whether the postponed-deep strategy seeds that were produced would differ by maternal soil temperature is not yet known because we did not include them in the trans-generational experiment. Conversely, for populations with staggered strategies, an individual within a population can produce offspring (seed) with varying germination times – some seeds germinate a few weeks after sowing (in autumn), some in winter and also some seeds only germinate in spring (SI Figure 2). Thus, it is quite likely that the source of variation in staggered populations lies within an individual – an individual plant produced both non-dormant and dormant seed with varying proportions and hence, supports the hypothesis that staggered strategy is a manifestation of bet-hedging (Starrfelt & Kokko, 2012).

Despite the strong warming effects on phenology (except for the time to first flower), the responses were not unidirectional. Contrary to previous studies that suggest warmer climates results in earlier flowering time (e.g. Bradley *et al.*, 1999, Fitter & Fitter, 2002, Gordo & Sans, 2010), soil warming did not shift time to first flower, but advanced seed maturation, and shortened the duration of seed production. Hence, even though warming advanced seed maturation, the whole reproductive period was not automatically lengthened. This echoed previous studies that found phenological response is not reflected by only one event, but may vary based on other life events (CaraDonna *et al.*, 2014, Sherry *et al.*, 2011, Sherry *et al.*, 2007). For *O. eriopoda*, the time to seed maturity and seed production period was also dependent on germination strategy, which indicated that germination timing (and pattern) influences other life history traits (Donohue *et al.*, 2010). Further, the interaction between germination strategy and soil warming that was significant in many vegetative and reproductive traits was not significant for the phenological traits. Our results highlighted that classifying species responses to warming as either positive, negative, or neutral could be an oversimplification when such assessments consider only few populations or are based only on a certain life-stages or traits.

Contrary to our expectation the transgenerational effect of different seed development conditions on germination traits was not significant. Phenotypic plasticity in seed dormancy may help species to adapt to future climates (Walck *et al.*, 2011). However, one cycle of warming did not change seed mass, the fraction of non-dormant seed, or germination time across *O. eriopoda* populations which suggests germination strategy was conserved regardless of seed development temperature. We exposed the plants when they were 18 – 20 weeks old and hence, the effect of warming was mainly pronounced during the seed development period prior to initiation of flowering and not for the entire maternal period. The soil warming that started somewhat late in the development stage within one generation might not be adequate to change the germination strategy. It is also likely that the alteration of seed dormancy could depend on the air more than soil temperature. Warmer temperatures during seed development generally reduce primary seed dormancy intensity (Bernareggi *et al.*, 2016, Gutterman, 2000, Hoyle *et al.*, 2008). There was an increase in temperature elicited by the soil warming treatment, but the difference in air temperature between warm and ambient soil was minor, 1 – 2 °C, which may not be adequate to trigger a signal on the developing seeds. Another possibility is that the soil warming effect on seed and germination traits are buffered rather than immediate. Across some Australian subalpine plants, the cumulative effects of warming on phenological traits can only be detected after several years (Hoffmann *et al.*, 2010). Likewise, a trait microevolution can occur and is reported for the flowering time of *Brassica rapa* in just three generations (Franks *et al.*, 2007).

The effect of soil warming to *O. eriopoda* here is similar to the effect of lower maternal light conditions that accelerated seed maturation and shortened seed production period on *Campanulastrum americanum* (Galloway & Burgess, 2012). Further, timing of seed maturation and season of natural dispersal of *Campanulastrum americanum* influences whether offspring grow as annuals or biennials (Galloway & Burgess, 2012). Interestingly, in the controlled conditions, we observed *O. eriopoda* to exhibit biennial life history regardless of the soil temperatures, contrary to the current literature that defined this species as a perennial herb (Costin *et al.*, 2000). The biennial life history of *O. eriopoda* is indicated by germination occurring in either autumn (immediate and some portions of staggered strategies) or spring (some portions of staggered, postponed and postponed-deep strategies), reproductive events occurring in the following spring, and reproduction lasting until summer, immediately followed by senescence. The majority of plants senesced at the end of the experiment, except for one population, exhibiting a staggered strategy (Nam1), in which the warming effect was also minor for both the autumn and spring seedlings.

Alpine plants can adjust to extreme conditions through modification of their physiology and seasonal behaviour (Beniston, 2003, Körner, 2003). Plants can respond to environmental change by adjusting their life history, including through phenotypic plasticity as an immediate reaction to novel conditions (Thuiller *et al.*, 2008). Indeed, even within-species, we observed remarkable life-history strategy variation across germination strategies in interaction with maternal conditions (soil warming). The warming effect on phenology was non-linear and there was no significant warming and germination strategy interactions to affect phenological traits. Although warming did not affect flowering time, it accelerated time to seed maturity, and reduced the duration of seed production. This indicated several things, a condensed reproduction period under warming and an earlier start of one developmental step (seed maturity) does not mean that the whole reproductive period is automatically lengthened (Chapter 5). Secondly, a shorter lifetime may reduce overall opportunity of reproduction (Bonser, 2013, Fenner & Thompson, 2005). From the *O. eriopoda* example, we saw no apparent trade-off between lifetime and infructescence production, as seen in other systems (Franks, 2011). With a condensed lifetime but still an ability to produce highly viable seeds, *O. eriopoda* is evidenced to have an ability to complete the life cycle and adjust phenology to more favourable times across seasons. This ability is one of the prominent traits proposed to enable persistence under rapid climate change (Aitken *et al.*, 2008, Willis *et al.*, 2008).

Shifts in flowering phenology and selection for earlier flowering are associated with reduced plant fitness (Burgess *et al.*, 2007). A condensed reproductive period that shortened seed maturation time but did not reduce seed mass nor germination success (final germination percentage) may indicate unaffected plant fitness under warmer soils. However, we also found that warmer germination temperature reduced the autumn germination proportion – indicating a slower rate of germination across strategies, but not the final germination proportion. This effect is contrary to the effect of germination temperature across species (Chapter 3), where species with staggered germination strategy have higher autumn germination under warmer temperature although no effect of germination temperature was found for species with postponed strategy. To determine whether the warming effect that on condensed phenology will exhibit an impact on overall fitness of the subsequent generation, monitoring and measurement of subsequent adult plant size at reproduction, seed production, length of seed production, and lifetime are required.

The ability to complete a full life cycle and adjust phenology during more favourable times across seasons are some of the prominent traits to enable persistence under rapid climate change (Aitken *et al.*, 2008, Willis *et al.*, 2008). Divergence of phenological responses among species that compose a plant community can potentially buffer the adverse effect of warming (CaraDonna *et al.*, 2014), and our findings suggested that within-species variations, in this case variations in germination strategy, is potentially as important as between-species variation. Hence, it is also important to incorporate effects of within-species variations in the prediction of species responses to changing climate.

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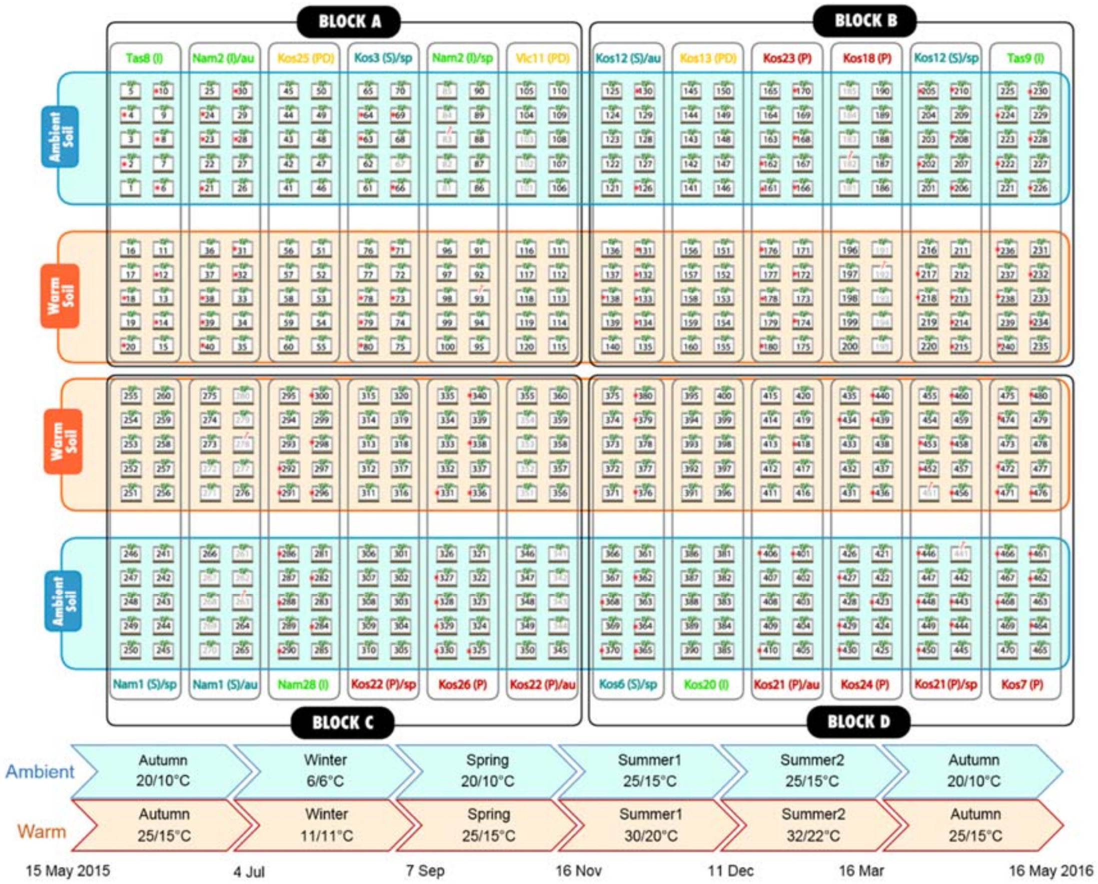
Supplementary Materials 1

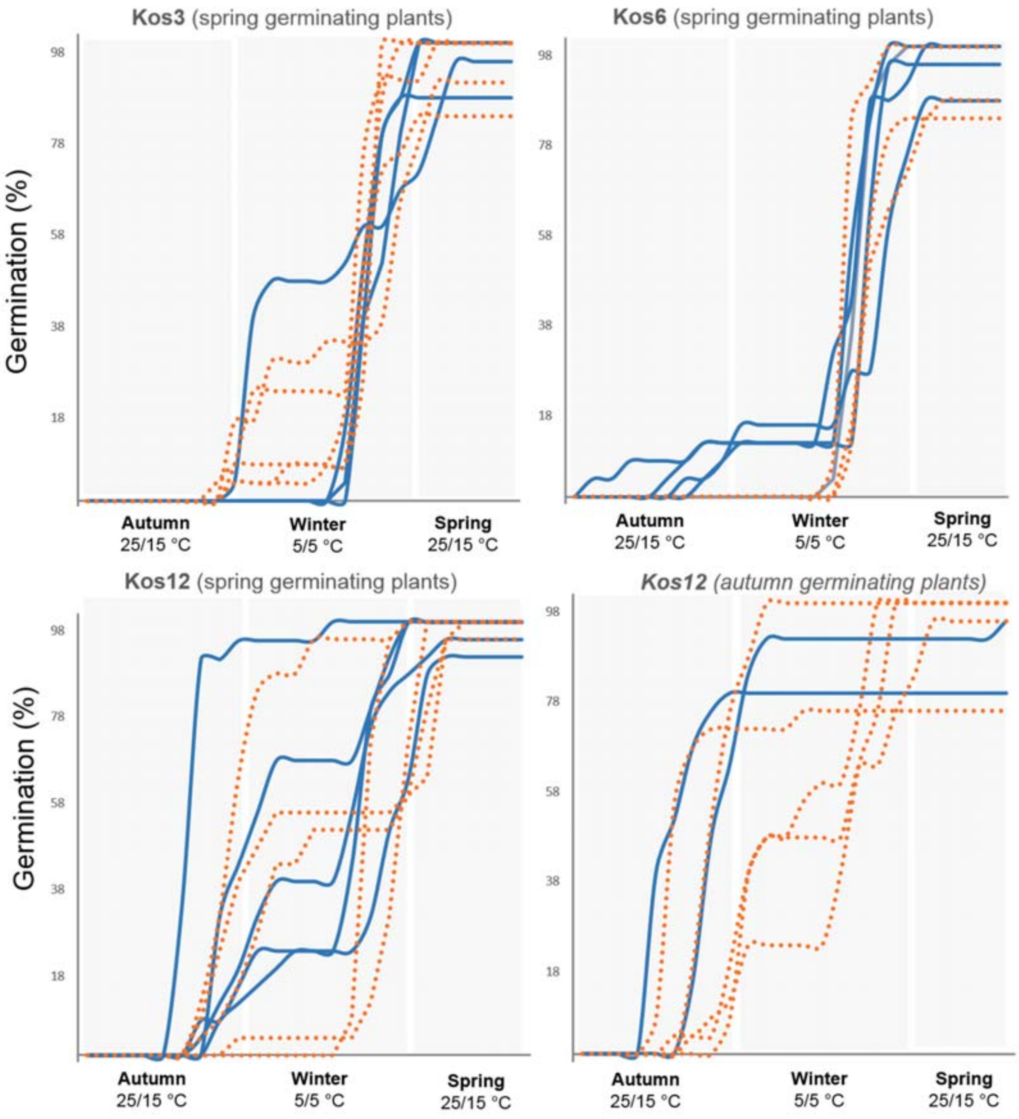
Germination and seedling transplant methods

Seeds from each population were germinated in three replicates of 25 seeds per population and were placed on 1% water-agar in sealed petri dishes in a germination chamber (Model TRIL-120-1-VW/ BMS, Serial 30455, Thermoline Scientific, NSW, Australia). Dishes for populations with the postponed and postponed deep strategy were exposed to at 25/ 15 °C, 12 hours light/ 12 hours dark, for 9 weeks and then were moved to 5 °C constant temperature and 12 hours light/ 12 hours dark for 8 weeks before being returned to 25/ 15 °C, 12 hours light/ 12 hours dark, for 27 weeks. For populations that exhibit immediate or staggered-like strategy (Nam1, Nam2, Kos12, Kos21, and Kos22, Tas8, and Tas9) an additional set of dishes was germinated after the first set had been brought out of ‘winter’ conditions so that germination of early (autumn) and late (spring) seedlings coincided and that seedling age was relatively equal regardless of germination strategy. We did not have enough seeds for staggered populations of Kos3 and Kos6, and hence could only use the late (spring) seedlings for Kos3 and Kos6.

All germinating seeds were transplanted to potting mix when the radicle was just over 1 cm (1 – 2 weeks). Seedlings were grown in 4 × 4 × 10 cm pots (T40S, Garden City Plastics, NSW, Australia), one seedling per pot, with a mixture of Martins mix potting-soil (Martins Fertilizer, NSW, Australia) plus 10% steamed river sand and c. 4 gram/ pot of slow release fertilizer (Osmocote Exact Patterned Release Fertilizer Standard Blue 15% N: 4% P: 7.5% K, Scotts International BV, Heerlen, The Netherlands). The glasshouse environment was kept at 25/15 °C (day/night) with a natural spring/ summer photoperiod of Canberra, ACT, Australia. Seedling position was shuffled within and across glasshouse benches every 1-2 weeks. Immediately before we started the warming experiment, the seedlings were re-potted with the same mixture and fertilizer as above into 7 x 7 x 20 cm pots (T70VINK Garden City Plastics, NSW, Australia). These seedlings were 18 – 20 weeks old when they were transferred to the experimental set-up.



Supplementary Figure 1. The detailed population and germination strategy allocations across blocks with the targeted temperature regime and the seasons. Population names were coloured based on germination strategy and followed by seedling germination season if two germination timings occurred within a population. GS were: Immediate (I), Staggered (S), Postponed (P), and Postponed-deep (PD); autumn or early-germinating seed was coded as (/au) and spring or late-germinating seed as (/sp). Where seedling availability was low, extra plants were placed into the experiment to ensure equal conditions for all pots. Those extra were not part of the analysis and the pots are shown with grey labels. Red asterisks indicate individuals that we used the seeds for the trans-generational experiment. Each pot contained one plant. Pots with orange stick (without plant) were the pots allocated for i-buttons (3 i-buttons below the soil and 1 i-button above the soil) in each block.



Supplementary Figure 2. Offspring germination of the staggered strategy under ambient germination temperature over the progression of time (indicated by x-axis). Lines indicated individual (mother plant) that produced the seeds; each line represented 100 seeds. Line colour and style indicate maternal soil temperature – solid blue for ambient soils and dashed orange for warmer soil temperatures.

SI Chapter 5

Supplementary Table 1. The distribution of population and germination strategies (GS) across the blocks: Immediate (I), Staggered (S), Postponed (P), and Postponed-deep (PD). Population name reflects the collection sites: Namadgi (Nam), Kosciuszko (Kos), Victoria (Vic), and Tasmania (Tas). Seeds for each population were bulk sampled from multiple plants. There was imbalance in the distribution of germination strategies across blocks, but this was not so extensive as to be an impediment to statistical analyses.

(continued)

Block	GS	Population	Accession	Lat.	Long.	Elevation	Collection date	Locality
A	I	Nam2	CANB 866345	-35.568	148.7844	1647	19-Feb-13	Namadgi NP. Snowy Flat, southern end; Mt Franklin Road, c. 5 km S of locked gate near Mt Ginini.
	I	Tas8	TSCC 0009097	-42.151	146.4604	660	16-Feb-09	Tasmania, Nive Plains. Lyell Highway, 500 m NW of Nive River Bridge
	S	Kos3	NSW 618325	-36.371	148.4761	1608	10-Feb-04	Kosciuszko NP. Rainbow Lake Track, off Kosciuszko Road between Perisher Valley and Guthega turn-off; Kosciuszko NP Southern Tablelands.
	P	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	PD	Kos25	CANB 792151	-36.429	148.3586	1744	3-Feb-10	Kosciuszko NP. S side of Spencers Creek bridge; roadside and water side.
	PD	Vic11	MEL 2338193	-37.06	147.092	1632	25-Jan-11	Alpine NP, Victoria. The Dargo High Plains Road about 16.1 km from the Alpine Road.
B	I	Tas9	TSCC 0005951	-41.958	146.6786	1055	24-Jan-08	Tasmania, Great Lake. Cameroon lagoon, small wetland on west side of Lake Highway; 7 km S of Liawenee. Subalpine health, associated with *Ozothamnus* spp; *Poa* spp.
	S	Kos12	CANB 783437	-36.427	148.3677	1743	24-Feb-09	Kosciuszko NP. Fens along Spencers Creek – 790 m NE from the Spencers Creek bridge on Kosciuszko Road.
	P	Kos18	CANB 783604	-36.434	148.3066	1805	5-Feb-09	Kosciuszko NP. The Main Range, about 1.4 km SW from the walking track to Blue Lake crosses the Snowy River.

Supplementary Table 1. The distribution of population and germination strategies (GS) across the blocks: Immediate (I), Staggered (S), Postponed (P), and Postponed-deep (PD). Population name reflects the collection sites: Namadgi (Nam), Kosciuszko (Kos), Victoria (Vic), and Tasmania (Tas). Seeds for each population were bulk sampled from multiple plants. There was imbalance in the distribution of germination strategies across blocks, but this was not so extensive as to be an impediment to statistical analyses.

(continued)

Block	**GS**	**Population**	**Accession**	**Lat.**	**Long.**	**Elevation**	**Collection date**	**Locality**
	P	Kos23	CANB 747602	-36.463	148.2677	2079	14-Feb-07	Kosciuszko NP. Bog’s margin, 800 m ~NE from Lake Cootapatamba.
	PD	Kos13	CANB 807987	-36.429	148.3586	1702	28-Feb-12	Kosciuszko NP. The Kosciuszko Road at the Spencers Creek bridge, c. 50 m W of bridge on S side of road.
C	I	Nam28	CBG 9604821	-35.635	148.78	1520	5-Mar-96	Namadgi NP. Leura Gap, ca 3 km direct NNW of Bimberi Peak.
	S	Nam1	CANB 813617	-35.522	148.7719	1621	17-Jan-13	Namadgi NP. Ginini West *Sphagnum* peat bog southern part, 0.5 km W from the Mt Ginini carpark on the Mt Franklin Road towards Bulls Head.
	P	Kos22	CANB 770116	-36.452	148.2747	2078	5-Mar-08	Kosciuszko NP. Kosciuszko summit road c. 1 km from Seaman's Hut.
	PD	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	P	Kos26	CANB 792159	-36.416	148.3111	1936	4-Feb-10	Kosciuszko NP. N side of path, overlooking Headley Tarn.
D	I	Kos20	CANB 749000	-35.826	148.4927	1399	2-Mar-07	Kosciuszko NP. Roadside at Old Kiandra Goldfields, 3 km N of turn-off to Cabramurra and Khancoban.
	S	Kos6	NSW 4154437	-36.45	148.3167	1800	1-Mar-88	Kosciuszko NP. 1 km along the summit walking track to Mt Stillwell.

Supplementary Table 1. The distribution of population and germination strategies (GS) across the blocks: Immediate (I), Staggered (S), Postponed (P), and Postponed-deep (PD). Population name reflects the collection sites: Namadgi (Nam), Kosciuszko (Kos), Victoria (Vic), and Tasmania (Tas). Seeds for each population were bulk sampled from multiple plants. There was imbalance in the distribution of germination strategies across blocks, but this was not so extensive as to be an impediment to statistical analyses.

(continued)

Block	GS	Population	Accession	Lat.	Long.	Elevation	Collection date	Locality
	P	Kos7	NSW 617524	-36.491	148.285	1960	24-Feb-04	Kosciuszko NP. Kosciuszko NP Southern Tablelands, approximately 400 m NW of the top of Crackenback chairlift, on walking track to Mt Kosciuszko.
	P	Kos21	CANB 748561	-36.439	148.2702	2011	7-Mar-07	Kosciuszko NP. 2 km N of Mt Kosciuszko summit, on Main Range track.
	P	Kos24	CANB 792219	-36.453	148.265	2159	30-Mar-10	Kosciuszko NP. Lake Albina Track.
	PD	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Supplementary Table 2. Significance of the germination strategy (GS) and warming (SoilT) on the vegetative, reproductive traits, and phenology. Generalized Linear Mixed model and Linear Mixed Model were used to analyse the data with fixed term as GS×SoilT. The random term as population nested in block (block/pop). Leaf increment rates, plant area, seed mass, and aboveground biomass were log-transformed.

(continued)

Response	Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Start of the experiment[a]						
Leaf number	GS	21.03	3	6.98	13.7	**0.004**
Longest leaf	GS	9.15	3	3.04	12.9	0.067
Early vegetative stage						
Leaf number [b] ‡	GS	12.8	3	4.24	13.1	**0.027**
	SoilT	83.09	1	83.09	365.6	**<0.001**
	GS×SoilT	15.89	3	5.3	365.1	**0.001**
Leaf increment rate (leaf per day) [b] ‡	GS	11.27	3	3.74	13.7	**0.037**
	SoilT	62.42	1	62.42	408	**<0.001**
	GS×SoilT	33.12	3	11.04	407.3	**<0.001**
Longest leaf	GS	13.13	3	4.3	12.9	**0.026**
	SoilT	1.1	1	1.1	366	0.295
	GS×SoilT	11.31	3	3.77	366	**0.011**
SLA [b] ‡	GS	1.69	3	0.56	12.8	0.65
	SoilT	25.4	1	25.4	363.9	**<0.001**
	GS×SoilT	0.96	3	0.32	362.9	0.81
Transition to Reproductive Stage						
Leaf number ‡	GS	4.44	3	1.47	13.6	0.266
	SoilT	0.21	1	0.21	139.4	0.645
	GS×SoilT	6.08	3	2.03	139.8	0.113
Leaf increment rate (leaf per day) ‡	GS	1.7	3	0.56	14.1	0.652
	SoilT	6.94	1	6.94	162.7	**0.009**
	GS×SoilT	6.85	3	2.28	162.2	0.081
Longest leaf	GS	20.78	3	6.91	12.8	**0.005**
	SoilT	0.3	1	0.3	139.4	0.586
	GS×SoilT	0.23	3	0.08	139.4	0.973
Plant area (cm^2) ‡	GS	6.29	3	2.09	13.7	0.149
	SoilT	0.07	1	0.07	160	0.786
	GS×SoilT	5.11	3	1.7	160.4	0.169
Ramet number ‡	GS	4.17	3	1.39	12.7	0.291
	SoilT	0.01	1	0.01	136.1	0.918
	GS×SoilT	2.46	3	0.82	136.4	0.484

Supplementary Table 2. Significance of the germination strategy (GS) and warming (SoilT) on the vegetative, reproductive traits, and phenology. Generalized Linear Mixed model and Linear Mixed Model were used to analyse the data with fixed term as GS×SoilT. The random term as population nested in block (block/pop). Leaf increment rates, plant area, seed mass, and aboveground biomass were log-transformed.

(continued)

Response	Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Reproductive stage						
Proportion of flowering plant	GS	2.09	3	0.7	13.6	0.569
	SoilT	8.87	1	8.87	368.8	**0.003**
	GS×SoilT	6.15	3	2.05	371.1	0.107
Total number of inflorescence	GS	4.32	3	1.43	12.9	0.279
	SoilT	35.68	1	35.68	288	**<0.001**
	GS×SoilT	9.14	3	3.05	288.1	**0.029**
Proportion viable infructescence	GS	18.36	3	6.11	12.4	**0.009**
	SoilT	0.33	1	0.33	293.2	0.565
	GS×SoilT	8.56	3	2.85	292.3	**0.038**
Single seed mass	GS	111.49	3	37.1	13.2	**<0.001**
	SoilT	1.16	1	1.16	218.1	0.282
	GS×SoilT	3.78	3	1.26	218	0.289
Aboveground biomass final (g)	GS	1.22	3	0.41	13.2	0.751
	SoilT	5.4	1	5.4	324.2	**0.021**
	GS×SoilT	3.04	3	1.01	323.9	0.387
Proportion plants surviving	GS	6.22	3	2.07	11.2	0.161
	SoilT	10.36	1	10.36	371.3	**0.001**
	GS×SoilT	10.61	3	3.54	371.1	**0.015**
Phenology						
Days to first flower †	GS	129.66	3	42.78	88.8	**<0.001**
	SoilT	0.05	1	0.05	318.5	0.826
	GS×SoilT	3.62	3	1.21	318.3	0.307
Days to seed maturity from flowering	GS	35.07	3	11.62	13.1	**<0.001**
	SoilT	11.73	1	11.73	293.2	**<0.001**
	GS×SoilT	5.15	3	1.72	293.6	0.164
Days to seed maturity from planting	GS	4.04	3	1.35	14.2	0.299
	SoilT	5.59	1	5.59	289.9	**0.019**
	GS×SoilT	2.91	3	0.97	290	0.407
Duration of seed production	GS	16.9	3	5.63	15.1	**0.009**
	SoilT	12.15	1	12.15	293.9	**<0.001**
	GS×SoilT	4.77	3	1.59	295	0.192
Days to senesce from last seed production	GS	1.69	3	0.56	12.6	0.653
	SoilT	2.36	1	2.36	222.7	0.126
	GS×SoilT	4.04	3	1.35	223.8	0.26

Supplementary Table 2. Significance of the germination strategy (GS) and warming (SoilT) on the vegetative, reproductive traits, and phenology. Generalized Linear Mixed model and Linear Mixed Model were used to analyse the data with fixed term as GS×SoilT. The random term as population nested in block (block/pop). Leaf increment rates, plant area, seed mass, and aboveground biomass were log-transformed.

(continued)

Response	**Fixed term**	**Wald statistic**	**n.d.f.**	**F statistic**	**d.d.f.**	**F pr**
Days to senesce from planting	GS	1.9	3	0.63	10.5	0.614
	SoilT	12.69	1	12.69	267.1	**<0.001**
	GS×SoilT	6.52	3	2.17	267.1	0.091

a indicates that the fixed factor was GS and random was population as the variables were measured before the experiment started; b indicates the result is also presented in Table 1.

‡ initial number of leaves (at day 0) was added as a covariate; † random factor was only block because the analysis did not work with block/pop and initial leaf number covariate as random.

Supplementary Table 3. The significance of germination strategy (GS), soil temperature as a proxy for seed development temperature (SoilT), and germination temperature (GermT) for transgenerational effects on germination traits. The analyses were performed using mixed models with GS×SoilT×GermT as fixed and population and individual plant nested in shelf as random factors.

Response	Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Final germination	GS	2.04	2	1.02	9.4	0.397
	SoilT	0.24	1	0.24	225.8	0.623
	GermT	3.47	1	3.47	222.6	0.064
	GS×SoilT	1.69	2	0.84	225	0.431
	GS×GermT	1.31	2	0.66	222.6	0.52
	SoilT×GermT	1.46	1	1.46	222.5	0.227
	GS×SoilT×GermT	1.55	2	0.78	222.6	0.462
Non-dormant fraction	GS	21.81	2	10.89	8.9	**0.004**
	SoilT	0.66	1	0.66	223.2	0.419
	GermT	25.71	1	25.71	221.8	**<0.001**
	GS×SoilT	2.88	2	1.44	222.6	0.239
	GS×GermT	2.16	2	1.08	221.5	0.342
	SoilT×GermT	0.51	1	0.51	221.2	0.477
	GS×SoilT×GermT	3.38	2	1.69	221.2	0.187
Time to reach 50% germination	GS	110.11	2	55.05	9.1	**<0.001**
	SoilT	0.4	1	0.4	224.4	0.526
	GermT	19.86	1	19.86	222.6	**<0.001**
	GS×SoilT	3.48	2	1.74	223.8	0.178
	GS×GermT	1.68	2	0.84	222.6	0.432
	SoilT×GermT	2.21	1	2.21	222.6	0.138
	GS×SoilT×GermT	0.74	2	0.37	222.6	0.692

Design by Afiat Sukmaraga

Chapter 6

Synthesis
Regeneration from seed across Australian alpine plants with varying germination strategies under changing climate.

Background

Mountain ecosystems are one of 10 biomes in Australia most vulnerable to reaching a “tipping point", i.e. moderate changes in the environment cause disproportionately large shift in ecosystem properties (Laurance *et al.*, 2011). This designation is due to their small geographic range, narrow climatic envelope and reliance on seasonal snow; climatic changes and habitat fragmentation have been identified as the major threats (Laurance *et al.*, 2011). Regeneration from seed is likely play a role in the response of alpine species to climate change by providing a vehicle for migration and distributing seedling emergence over time (Briceño *et al.*, 2015, Forbis, 2003, Mondoni *et al.*, 2012, Parolo & Rossi, 2008, Vittoz *et al.*, 2009a, Vittoz *et al.*, 2009b). My objective in this thesis was to explore elements of Australian alpine seed and seedling ecology to better document their characteristics and improve understanding of the consequences of dormancy and germination timing on plant life history in the Alps, now and under future climate scenarios.

Key findings

As a starting point, I examined the intrinsic life-span of Australian alpine seeds. This study (Chapter 2) was the first to document seed longevity across a wide range of Australian alpine species. Australian alpine seeds are relatively short-lived and the seed longevity is negatively associated with elevation of collection and seed mass, but not with species germination strategy. What I also found was that for many (40 of 99 species)

Australian native species, we still do not know the germination requirements or process of dormancy alleviation which indeed hinders our ability to determine seed longevity. Using accelerated aging test, Australian seed longevity (p_{50} values) is shown to be relatively low meaning that seeds are short-lived just like European alpine seeds (Mondoni *et al.*, 2010). These low p_{50} values is useful for comparison across, although not necessarily reflect actual storage capacity in real time. P_{50} value of 3 days under accelerated ageing, for instance, could be equal to 20 years of storage life *ex situ*, or a few years in the soil *in situ*. Australian alpine seeds build a persistent soil seed bank like alpine seeds from the European and Andean alpine regions (Arroyo *et al.*, 2004, Schwienbacher *et al.*, 2010, Venn, 2010). Seedling emergence from the Australian alpine soil seed bank is likely to be reduced, but species diversity increased by soil warming (Hoyle *et al.*, 2013). Hence, recruitment from seeds under future climate scenarios is expected to decrease, but the effect of warming could be positive, negative, or nil depending on the species.

Subsequently, to quantify this species specificity, I conducted germination assays across 39 species with varying germination strategies (immediate, staggered, or postponed strategy) to determine germination success under ambient and future climate scenarios (Chapter 3). Both warmer germination temperature and shorter winter duration significantly reduced germination for all strategies, although the effect was small for the immediate strategy. Germination occurred faster under warmer temperature for immediate and staggered strategies, but shorter winter also contributed to faster germination for staggered and postponed strategies. There was a shift in germination timing for the staggered strategy such that autumn germination increased under warmer temperatures. Un-germinated seed fraction in the postponed strategy likely represents deep-dormant seeds that could either enter the soil seed bank or die, we cannot say. These results highlight the importance of considering both duration and temperature change when assessing impacts of climate change on seed ecology.

Although the germination of species may be differentially impacted by future climate, this might be moderated by within-species variation. Populations may be buffered from some of the effects of projected climate change by substantial within-species variation in both genetic and phenotypic plasticity of germination and dormancy (Walck *et al.*, 2012).

This variation could contribute to species persistence in a temporally and spatially heterogeneous environment and I examined this hypothesis in Chapter 4. The alpine herb, *Oreomyrrhis eriopoda*, showed variation in germination strategy which was affected by long-term temperature variability and fine-tuned by temperature variability during the seed development period. The immediate germination strategy was found among populations with relatively low temperature variability. These results present a conundrum: immediate seeds showed less detrimental impacts of warming and shortened winter duration in my cross species study, but this strategy is favoured in climatic regions with historically low temperature variability as shown by some populations of *O. eriopoda*.

Delving further, I found that intraspecific variation in germination strategy was also reflected in seed and seedling growth (Chapter 4), with immediate strategy having relatively higher vegetative growth than the other strategies. Notably, a new strategy, postponed-deep, was documented for the first time in the study species. The postponed-deep germination strategy is indicated by a rapid onset of germination in spring but a substantial fraction of seeds (> 50%) remain dormant and they may germinate in subsequent year if required conditions are met. I followed the life stages of *O. eriopoda* from germination, to vegetative growth, reproduction and senescence and examined how various vegetative, reproductive traits and the reproductive phenology shifted with soil warming and whether this differed among germination strategies. Soil warming mainly affected the most active organs in each developmental stage of the plant, and the direction of soil warming effect on traits and phenology were different across the life cycle. For example, the acceleration of seed maturation was not necessarily associated with a longer mean reproduction period. It is evident that even within a single species, the effect of soil warming across life-history traits and developmental phase also varied across germination strategies.

Lastly, I investigated whether the germination strategy can be altered by seed development conditions as indicated by my cross species study (Chapter 5). I showed that the germination strategy of *O. eriopoda* that varied among populations was not plastic in response to maternal environment. Germination strategy of the offspring inherited the mother germination strategy regardless of seed developmental condition (soil warming).

For *O. eriopoda* populations with staggered strategy, where there seems to be a dormancy continuum within a population, the source of variation differed between populations. For the two populations where we examined seeds from spring individuals only, spring individuals produced predominantly dormant seeds but also a few non-dormant seeds. Whereas for another population for which we had autumn and spring germinating mother plants, we found that each individual produced both non-dormant and dormant seeds (Chapter 5). The source of variation in the staggered strategy thus needs to be further clarified and provides an interesting system in which to explore mechanisms of bet-hedging.

This body of work has added substantially to our understanding of the seed and seedling ecology of Australian alpine species, and I hope it also contributes to our ability to plan for climate change impacts in alpine systems around the world. In the remainder of this synthetic chapter, I will explore the particular elements of these results that I find most intriguing and important and will discuss implications and also identify areas ideal for further study. These are:

- Interspecific germination strategy variation is common among the world's alpine flora but the shift of germination timing under future climate seen elsewhere is not as pronounced for Australian species. Why? And what might the implications be?

- Is winter duration more important than warming itself for Australian alpine seed germination and seedling establishment?

- Germination strategy is particularly important for alpine plant recruitment, so is seed persistence. Why is there no apparent relationship between seed longevity and germination strategy across species? What about within-species?

- Germination strategy affects not just the germination timing but has far reaching consequences for overall fitness. In the future, will species and population with immediate germination strategy replace other strategies?

- What potential does *ex situ* seed bank collections have to predict species persistence under global change?

Across-species germination strategy variation is common among the world's alpine flora but the shift of germination timing under future climate scenarios seen elsewhere is not as pronounced for Australian species. Why? And what might the implications be?

A high germination proportion as well as rapid onset of germination following the winter are observed for the majority of the world's alpine (and sub-arctic) plants (e.g. Graae *et al.*, 2008, Graae *et al.*, 2013, Hoyle *et al.*, 2014, Hoyle *et al.*, 2015, Körner, 2003, Meyer *et al.*, 1995, Milbau *et al.*, 2009, Milbau *et al.*, 2017, Mondoni *et al.*, 2015, Mondoni *et al.*, 2012, Schütz, 2000, Schütz, 2002, Schwienbacher *et al.*, 2011, Shimono & Kudo, 2005). However, another pattern fairly frequently observed is a temporal spreading of germination events (Körner, 2003 based on Soyrinki (1938), Tudela-Isanta *et al.*, 2018), indicating a staggering germination that distributes germination before and after winter. A rapid first wave of germination that is followed by another wave of germination several weeks later is observed from ~25 of 80 species of alpine sub-arctic plants (Körner, 2003 based on Soyrinki (1938)). Across ~50 Australian alpine species tested, 13 species were categorized as staggered strategy (Hoyle *et al.*, 2015). A similar germination pattern extending to a postponed-deep germination strategy which was present for a few *O. eriopoda* populations (Chapter 4), was also reported for six out of ten *Carex* alpine sub-arctic species in which germination takes place in two waves separated by a whole year (Körner, 2003 based on Soyrinki (1938)). Having a postponed-deep strategy is thought to be a strategy to reduce the risk of recruitment failure in a bad year.

Interestingly, no or little pre-winter germination is reported from the early studies of alpine seed germination from the northern hemisphere (Körner, 2003 based on Braun (1913), Ludi (1933), and Soyrinki (1938)). However, subsequent studies (~ 30 years later) suggest a prevalence of non-dormant seed across alpine seeds (Amen, 1966, Bell & Bliss, 1980, Billings & Mooney, 1968, Chabot & Billings, 1972). More recent evidence indeed confirmed that immediate germination (pre-winter germination) can be found across alpine species tested from the central Alps of Austria (~ 2 of 28 species), across alpine systems in Japan (~ 9 of 22 species), and across Australian alpine seeds (~17 of 50 species) (Hoyle *et al.*, 2015, Schwienbacher *et al.*, 2011, Shimono & Kudo, 2005). Whether this prevalence of pre-winter germination has changed due to anthropogenic climate change over these decades is not confirmed. A shift from spring to autumn germination (therefore earlier and increased autumn germination) (Mondoni *et al.*, 2012) and increased germination after winter has been reported for Italian alpine species under simulated climate warming (Mondoni *et al.*, 2015). However, the shift in germination timing found under future climate scenarios for Australian alpine species was not specific to germination strategies. There was no substantial shift in germination timing for the immediate strategy (Chapter 3). The direction and extent of the future climate effect across global alpine seed germination are indeed species and climate scenario dependent (Bernareggi *et al.*, 2016, Graae *et al.*, 2008, Graae *et al.*, 2009, Klady *et al.*, 2011, Milbau *et al.*, 2009, Milbau *et al.*, 2017, Mondoni *et al.*, 2015, Mondoni *et al.*, 2012, Shevtsova *et al.*, 2009). High levels of variation are clearly common among alpine species. Thus, we should not be expecting a single response to climate change. While certain climatic elements are associated with germination strategies, across and within species, there are no strong universal signals. The germination strategies are conserved, within and across small numbers of generations (Chapter 4 and 5). However, the possibility that these strategies might or may not change further down is also tantalising and deserves further consideration. Many alpine species are geographically constrained and not equipped with special appendages for rapid and long-range dispersal, hence, alpine species are unlikely to track rapid climate change geographically. Consequently plasticity or between-population variation in seed traits, such as germination strategy, may play a key role in defining response to climate change. Such high level of plasticity and transgenerational imprinting must be underlain by complex regulatory machines, but is it all shared? Modelling the prediction of alpine plant response in the future, therefore needs to be performed by incorporating across, as well as within, species variations.

Winter duration is more important than warming itself?

Given the language of most studies of climate change impacts on plants, one could be forgiven for assuming that mean temperature change was the most important direct effect. However, given the tight signalling systems plants have to incorporate photoperiod, temperature and moisture availability, is it not surprising that the changes in seasonality are also important. Optimal alpine seed germination requires thermal conditions relatively similar to the temperature that is optimal to species of lower elevation (Körner, 2003). This is thought to be a mechanism to ensure that germination timing coincides with periods adequate for seedling establishment (Körner, 2003 and references therein). Indeed, +4 °C of warmer temperature had a very minimal effect on species with immediate strategies and increased pre-winter germination of species with staggered strategies. The effect of temperature was pronounced for germination strategies that consist of dormant seeds (staggered and postponed strategies), and is moderated by winter duration (Chapter 3).

In earlier studies, the absence of germination in winter (termed as winter quiescence) was thought to suggest that the temperature requirement for germination was not met (Billings & Mooney, 1968). I found that once dormancy is alleviated, winter temperature (at 5 °C in controlled conditions) does not preclude germination (Chapter 3 and 4). Thus, for species and populations with staggered and postponed germination strategy, it is not low temperature that hinders germination but seed dormancy. For species with staggered and postponed strategies, shorter winter alone decreased final germination equal to the combined effect of shorter winter duration and warmer temperature. Under ambient temperature, the shorter winter duration significantly reduced final germination of species with staggered and postponed strategy, confirming the role of winter duration for the majority of Australian alpine flora regeneration from seed.

At mid-elevation areas for temperate mountains (1500 – 2000 m), the impact of global warming on snowpack are felt earlier and more than on the summits and lower elevations (Sproles *et al.*, 2013, Steger *et al.*, 2013). A diversified germination strategy might hold a high significance in regions with particularly variable snowmelt events. An individual plant might produce seeds that are heterogeneous with respect to the extent of dormancy as a strategy to ensure survival of the next generation (Matilla *et al.*, 2007). In mid-elevations, we found *O. eriopoda* populations with mainly staggered and postponed-deep germination strategies (Chapter 4), which indeed indicate the variations of seed dormancy produced by an individual (Chapter 5). However, whether the prevalence of populations with staggered or postponed deep at mid-elevation areas are also pronounced for other species need deserves further investigation.

Projection for future climate at alpine regions include not only warmer mean temperature and change in seasonality but also an increasingly variable climate (Gobiet *et al.*, 2014, Grabherr *et al.*, 2010, Head *et al.*, 2014, Ohmura, 2012). Variability, reflecting fluctuations around the mean and the extremes, is rarely considered in most studies of species response under changing climate; instead mean values are more commonly used (but see Adler *et al.*, 2006, Dalgleish *et al.*, 2010, Knapp *et al.*, 2002, Knapp & Smith, 2001). Species with immediate germination is less sensitive to warmer temperature and winter duration (Chapter 3). This is a paradox when we look at within-species variation where populations with immediate strategy is associated with high temperature variability (Chapter 4). Climate variability is also known to affect population demography of short-lived forbs mainly through recruitment than reducing survival (Dalgleish *et al.*, 2010). Studies on climate variability generally focuses the effects on adult plants (reviewed in Reyer *et al.*, 2013). We lack understanding on how climate variability affect seed and seedling stage (Parmesan & Hanley, 2015). As plants rely on seed dormancy to move through time and space, an experiment looking at how plants hormones in alpine seeds is regulated by temperature variability should be done. Abscisic acid (ABA) and gibberellin (GA) are responsible for physiological dormancy in *Arabidopsis* and indeed vary with temperature variability (Topham *et al.*, 2017). Another example of further seedling study is examining the effect of earlier snowmelts and hence, more variable air and soil temperatures e.g. thaw and freeze events (Milbau *et al.*, 2017), and how their response is regulated by germination timing.

Why there is no strong signal of relationship between the intrinsic seed longevity with germination strategy?

Conventional wisdom holds that germination strategy and seed longevity are related, for example for species with an immediate strategy having seeds that are long-lived is less important than for species with a postponed strategy (Tudela-Isanta *et al.*, 2018). However, we found that there was no significant association between seed longevity and germination strategy across 56 Australian alpine species (Chapter 2). Notably, a fraction of un-germinated seeds from species with staggered and postponed strategies was still viable and dormant – not dead (Chapter 3) and is potentially retained and persist in the soil seed bank. Soil seed bank persistence is very important for alpine plant species (Arroyo *et al.*, 2004, Erschbamer *et al.*, 2001, Marcante *et al.*, 2009). Seed persistence is the survival of seed in the environment after natural dispersal and hence, enable species and populations to survive long after the death of the parents (Long *et al.*, 2015). It is also an ecological adaptation to the low chance of establishment from seeds in the alpine environment (Schwienbacher *et al.*, 2010).

The variation of seed longevity and persistence perhaps lie in the community and habitat associated disturbance levels (Matteodo *et al.*, 2016). Relatively stable alpine grassland has a short-lived or transient seed bank (Cerabolini *et al.*, 2003), but soil seed bank of tall-alpine herbfields habitats across several summits in the Australian Alps is identified as persistent (Venn & Morgan, 2010). Alpine habitats including the Australian tall-alpine herbfields are often exposed to disturbances, such as freeze/thaw events, soil erosion, and even fire (see Billings & Mooney, 1968, Hoyle *et al.*, 2013, McGraw & Vavrek, 1989). Disturbance exerts selection pressures in favour of persistent seed bank (Fenner & Thompson, 2005). Specific habitat type and disturbance level are also to drive variations including seed longevity, seed mass, germination strategy, and even plant longevity (Meyer & Kitchen, 1994, Schippers *et al.*, 2001). Thus, a better approach to understand the relationship between seed persistence, longevity, and germination strategy could be by using multiple populations of a single species with varying germination strategy across habitat types reflecting disturbance gradients. *Oreomyrrhis eriopoda*, for example, is an excellent study species because it has a wide range of geographic distribution and occurs in different habitat types (Costin *et al.*, 2000). This species also has a rapid cycling meaning that reproductive maturity in controlled conditions can be reached within a few months after germination (Chapter 5).

Another possible explanation of the obscure relationship between longevity and germination strategy is likely related to maternal effect, and thus, definitely deserves further investigation. Seeds developed under warmer maternal environment generally have reduced dormancy intensity but also increased longevity (Bernareggi *et al.*, 2015, Coughlan *et al.*, 2017, Kochanek *et al.*, 2010, Kochanek *et al.*, 2011, Mondoni *et al.*, 2014). In a warmer climate, will alpine seeds be provisioned by the parents to be able to be longer-lived and germinate readily? Further study using seeds obtained from soil warming experiment (Chapter 5) can be used to test this hypothesis.

Climate change impacts on plant communities can be buffered by life history divergence across species (Chapter 3). What is somewhat neglected in many studies of climate change is the potential impact of within-species, among populations and individuals. Important within-species variation can also be variation in seed traits and germination strategies, but also further down during seed production and across generations. In fact, studying phenological responses and other traits variation within a single species will eliminate concerns of the effect of phylogeny on the responses seen (Davis *et al.*, 2010).

Germination strategy affects not just the germination timing but has far reaching consequences for overall fitness. In the future, will species and populations with immediate germination strategy replace other strategies?

Germination timing is crucial because it determines the conditions seedlings need to cope with and has consequences until adult stage (Donohue *et al.*, 2010). The combined results of my work show that even within a species, the variation in germination strategy can also be reflected in the seedlings' leaf growth measured at weeks 4 and 8 from germination (Chapter 4), on vegetative measures at week 24, up to the reproductive stage, on the flowering and seed production, phenology, through senescence (Chapter 5). Autumn germinating seeds of *O. eriopoda* produced more leaves than seedlings that germinated in spring when compared at a common time and under common conditions. The inherent variation in growth rate between autumn and spring seedlings may be related to germination timing and plant growth trade-offs in alpine seasonal environments. Here, seeds that germinate early have lower probabilities of survival than those germinating later, but the few that do survive may have increased fitness (Kimball *et al.*, 2011, Rathcke & Lacey, 1985, Verdú & Traveset, 2005). Mondoni *et al.* (2015) show that a high proportion (up to 75%) of autumn seedlings survive winter. However, survival over winter involves high energy consumption by seedlings which may reduce the growing capacity in spring (Maruta, 1994). Similarly, populations of *O. eriopoda* with postponed strategies (seeds germinate in spring) had a lower leaf production compared to populations with immediate strategies, despite the fact that postponed strategy had much greater seed (Chapter 4 and 5). Populations of *O. eriopoda* with postponed strategy also showed a longer time to reach reproductive maturity (time to first flower), but shorter reproductive period compared to immediate strategies.

Within the current species distributions, immediate strategies are generally found in populations from lower elevation and warmer mean temperatures (Chapter 4). Under future soil temperature scenarios, immediate strategy produced leaves at a higher increment rate with soil warming (Chapter 5). Likewise, number of flowering individuals, number of inflorescences, and reproductive period, as well as senescence were not reduced by soil warming for immediate strategy, unlike other strategies. This indicates that immediate strategy exhibited increased fecundity via longer reproduction duration as well as longer lifetime compared to the other strategies (Chapter 5).

So, will populations with immediate strategy replace other strategies? This may depend on: plasticity, high fecundity, rapid generation times, and the capacity of seed to migrate upward and establish at the new sites (Aitken *et al.*, 2008, Alsos *et al.*, 2012). Species like *O. eriopoda* that are not equipped with special dispersal appendages for rapid and long-range dispersal, are likely to migrate and track their climatic niche. Immediate strategy is likely establish better than the other strategies. Although autumn germination has a higher chance of frost-related mortality than spring germination, a high number of autumn germinants of glacier foreland species survive until the subsequent growing season (Marcante *et al.*, 2012, Mondoni *et al.*, 2015). How about for other strategies? For example, *O. eriopoda* populations with postponed strategy occurring at higher elevation sites – can they persist at their current range? The answer may be determined by to what extent these populations can tolerate and adjust the germination and reproductive phenology through plasticity. Under future climate scenarios, final germination of *O. eriopoda* with postponed strategy was reduced under shorter winter duration and warmer temperature (Chapter 3). Maternal effect was not apparent, indicated by the fact that warmer soil effect over one generation did not change germination strategy and seed mass. The number of populations with postpone strategy will perhaps decrease. However, the final germination of the subsequent offspring was nearly 100 % regardless of the maternal soil temperatures. Seeds developed under warmer soil condition also germinated equally well under both ambient and warmer germination temperatures (Chapter 5). Hence, a definitive scenario for each germination strategy is still hard to predict.

Another mechanism to increase likelihood to persist under changing climate is through bet-hedging (Childs *et al.*, 2010, Ooi *et al.*, 2009). Staggered and postponed-deep germination strategy may both indicate plant bet-hedging strategy (Childs *et al.*, 2010, Cohen, 1966, Simons, 2014, Simons & Johnston, 2006). Across *O. eriopoda* populations, staggered or postponed-deep germination strategy is favoured by populations occurring in mid-elevation (Chapter 4). In mid-elevations, where the growing season can start earlier in spring, non-dormant seeds might germinate and start growing when frosts might still occur, which may be fatal, but the seedling may gain an advantage to start germination and grow earlier under the risk of frost. Populations with a staggered strategy distribute seedling germination timing across the year, either growing fast as autumn seedlings or germinating in safer spring conditions I showed that the variation of germination strategy in a population, as exhibited by the staggered strategy, is likely to lie within an individual plant (Chapter 5). An individual plant produced seeds with varying germination strategies – from the same mother, some seeds germinate in autumn and some germinate in spring, despite being grown under the same germination conditions as the mother (Chapter 5). Conversely, populations with a postponed-deep strategy allow a persistent seed bank *in situ* that may buffer seedling loss in the first spring.

Even though germination strategy shows a legacy of long and short term climatic influences (Willis *et al.*, 2014), transgenerational plasticity in germination strategy may not be common. Within-species germination strategy variations across generations could be substantial and heritable but not plastic (Chapter 5). Then why does recent climate matter? This has pros and cons for persistence of species and our ability to project that. An investigation of how the adult plants produced by the seeds developed under warmer soil can adjust and acclimate to warm conditions should be further examined. Future investigations can be performed by rearing the seeds produced by “warmed” mothers over three or more generations and exposing continuous warm scenario on the seedlings. This could enable us to reveal whether germination strategy could evolve rapidly with climate change as demonstrated from other systems (Franks *et al.*, 2007, Nevo *et al.*, 2012, Thompson *et al.*, 2013).

What potential does *ex situ* seed bank collections have to predict species persistence under global change?

In my projects, the majority of seed used (all chapters, except Chapter 5 transgenerational part) were *ex situ* seed bank collections. *Ex situ* seed bank collections represent an array of species' genetic diversity. Species from these seed accessions have been taxonomically confirmed and the collection procedure was validated and uniform. Many Australian alpine species are perennial and hence, seeds of a given species and population collected over multiple years could also enable us to detect shifts in dormancy and germination level and potentially elucidate the genetic basis of further evolutionary shifts driven by climate change (Franks *et al.*, 2007). Examination of variation in germination strategies within species may also reveal the potential for species to cope with and adapt to changes in climate which ultimately determine the species persistence and survival, providing insight for management and conservation (Hoyle *et al.*, 2015). Species information and germination data and that are available from seed banks and botanic gardens, are valuable for ecology and evolutionary study of plants (Donaldson, 2009). The initial idea to look at within-species germination of *O. eriopoda* across its native distribution (Chapter 4 and 5) was mainly based on the knowledge of germination characteristics of this species provided by the seed banks across Australia. In turn, the results also contribute to the conservation work of plant species. The extraordinary variation within-species have implications on the decisions around management and seed banking, such as, how many populations are optimal to capture the life history variations for each species (Guja *et al.*, 2015)? How big should the population size be? And what minimum number of individuals is needed to be representative of the population, and so on. Further work in collaboration with seed banks and botanical gardens should be continued. With the growing number of seed banks worldwide and the seed collections they hold (O'Donnell & Sharrock, 2017), there are many ways to study a species' potential to persist under changing climate.

Concluding remarks

The series of studies presented here approach the challenge of predicting alpine plant regeneration from seed under current and future climate, from the perspectives of both across species' and within species. Seeds can act as "time capsules", providing plants with a mode of dispersal across variable seasons and changing climate. Hence understanding a species' life history strategies, specifically with respect to germination, is particularly important for predicting their survival in the Australian Alps and other mountain regions where snow is currently marginal.

Under future climate scenarios, species with non-dormant seeds will likely have an advantage over species with dormant seeds as the growing season becomes longer. However, we might expect that species with an immediate germination strategy that emerge in autumn will be exposed to harsh conditions if snow insulation during winter disappears; species with a postponed germination strategy may survive winter better as they stay as dormant seeds in the soil. Seedling survival for seedlings with spring emergence may thus be higher but spring germinants may be outcompeted by larger seedlings that emerged in autumn. Thus, staggering germination over time might be the best strategy to maximize highest seedling survival overall. All things considered, my projects have shown that germination strategy variation will play a substantial role in modulating the effects of future climate on seed germination, early establishment and further down to adult life stage of Australian alpine plant.

Overall, this work reveals that across species variation in germination strategy potentially holds significance to species persistence. The substantial variation in germination strategy evidenced within a species also provides the insight that divergence in life history, associated with variation in the expression of germination timing, has far reaching implications on phenology, reproduction and adult vegetative traits through to the end of a plant's life cycle. While we cannot extrapolate directly from these experimental studies, these results can form the basis of listing priority species for conservation and further studies on the impact of changing climatic regimes. In particular, further research should be focused on better understanding the within-species variation, particularly on populations with the staggered germination strategy. Ideally, this future research would identify what mechanisms underlie the variation within an individual, and whether the variation within individuals is further expressed over generations.

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An Australian view on current directions in alpine seed and seedling ecology

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Photo: Annisa Satyanti

An experiment to test the transgenerational effect of soil warming on phenology, seed dormancy, and germination traits of Australian caraway (*Oreomyrrhis eriopoda*, Apiaceae).

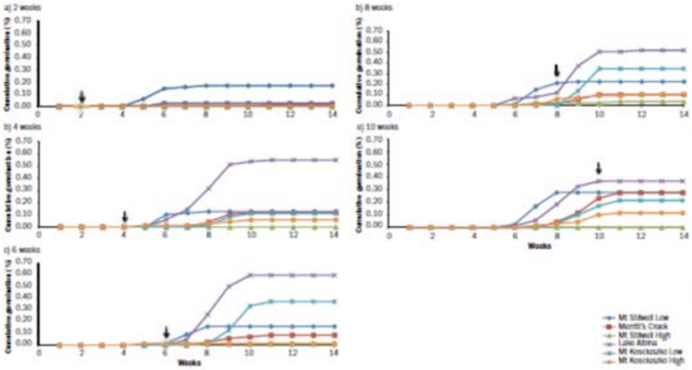
Alpine ecosystems and their plant communities worldwide have been identified as threatened or vulnerable to a changing climate. Future climate predictions for the Australian Alps suggest that snow depth will decrease and by 2050 there will be a 3°C temperature increase relative to 1990. In Australia, only 0.15 per cent of the landmass is alpine, but this area is home to 212 plant species, including 30 exclusively alpine and 21 endemic species.

Plant responses to a changing climate will depend on their potential to recruit *in situ* or migrate upwards, both of which are inherently dependent on establishment from seed. Plant population dynamics and future distributions will therefore be significantly affected by seed and seedling ecology (Briceño et al., 2015). In Australia, where the alpine region extends across an elevational range of just 400 m, understanding the scope and potential of alpine plant migration is crucial.

With our collaborators (The Australian National University ANU, the Australian National Botanic Gardens ANBG and the University of Queensland, funded by the Australian Research Council and supported by the Friends of ANBG) we have established a research programme on Australian alpine plants to address the wide knowledge gaps in alpine seed and seedling ecology while also securing seed through *ex situ* conservation (Briceño et al., 2015; Hoyle et al., 2014; Hoyle et al., 2015; Hoyle et al., 2013; also all references therein). Here we outline several areas of investigation of Australian alpine seed and seedlings currently underway at the ANU and ANBG in Canberra, Australia (a partner in the Australian Seed Bank Partnership).

Seed germination strategies and seedling growth

Seed dormancy is a common trait among Australian alpine species. In general there are three germination strategies that can be observed: immediate, staggered and postponed (Hoyle et al., 2015). Species with non-dormant seeds germinate immediately after natural dispersal in autumn. The postponed strategy is comprised of species with dormant seeds that germinate after winter. The final category are species with seed germination that is staggered over time. Australian alpine celery (*Aciphylla glacialis*), which exhibits a postponed germination strategy, requires at least six weeks of winter to germinate (Figure 1, Hoyle et al., 2014), suggesting that changing climatic cues could affect the germination pattern in these and other alpine species. The combination of colder winter and summer warming is known to accelerate germination and increase seedling survival for many subarctic species. Under future climate scenarios, species with non-dormant seeds will likely have an advantage over species with dormant seeds, as the growing season becomes longer. However, we might expect that species with an immediate germination strategy that emerge in autumn will be exposed to harsh conditions if snow insulation during winter disappears – species with a postponed germination strategy may survive winter better, as they stay as dormant seeds in the soil. Seedling survival for seedlings with spring emergence may thus be higher, but spring germinants may be outcompeted by larger seedlings that emerged in autumn. Thus, staggering germination over time might be the best strategy to maximize highest seedling survival overall. Our current work tests the hypotheses that, 1) decreased winter duration will reduce seed germination and have a greater influence on germination than increased temperature, and 2) decreased winter duration will reduce the germination of species with postponed germination strategies, including the dormant fraction of seeds for species with a staggered germination strategy. Regarding subsequent seedling growth, we hypothesised that warming will improve



Population	Collection date	Elevation (m above sea level)	No. of plants sampled	Seed moisture content (% ± SE, n = 3)	Seed viability (% ± SE, n = 3)
Mt Stilwell-low	29 March 2011	1927	42	64 (±4)	100 (±0)
Merritt's creek	2 March 2011	1944	202	36 (±2)	94 (±3)
Mt Stilwell-high	29 March 2011	2037	50	70 (±1.5)	97 (±3)
Lake Albina	2 March 2011	2046	120 +	34 (±1)	97 (±3)
Mt Kosciuszko-low	2 March 2011	2058	30 +	34 (±1)	88 (±4)
Mt Kosciuszko-high	15 March 2011	2197	89	49 (±5)	94 (±3)

A recent study on the effect of progressively shorter cold duration (constant 5°C, dark; indicated by the black arrow) on germination in *Aciphylla glacialis* (Apiaceae) seed collected from six populations in Kosciuszko National Park (from Hoyle et al., 2014; with permission) demonstrated markedly decreased germination in this morpho-physiologically dormant species.

seedling survival and growth but could alter patterns of temperature-induced mortality and competition among autumn and spring germinants. Our objective is to better understand the consequences of dormancy and germination timing on plant life history in the Alps, now and under future climate scenarios.

Transgenerational effects and phenotypic plasticity of seed germination

Conditions during flowering and seed development are known to affect longevity, dormancy status, and germination plasticity. Thus, as the maternal environment changes, seed characteristics may also change. Plants may respond to environmental changes through phenotypic plasticity (Nicotra et al., 2010). However, we still do not know the potential for alpine species to respond to changes in the environment via rapid evolution or plasticity, or what seed and phenological traits are most responsive to environmental change. Our research examines the prevalence of transgenerational plasticity in alpine plants, to assess how changing climate will affect dormancy, germination strategy, seedling establishment and survival under future climate. The photo on page 10 demonstrates a current experiment assessing transgenerational effects of warming on seed traits. Plants from 19 populations that vary widely in germination strategies are being grown under simulated current and warmed conditions. For this experiment, we hypothesised that soil warming would lead to earlier flowering and higher seed production and reduce physiological dormancy, causing a shift in germination timing for Australian caraway (*Oreomyrrhis eriopoda*, Apiaceae).

Seed longevity and seed bank persistence

Seed bank persistence is crucial for seeds to disperse over time, and spreads the risk of recruitment failure in a variable and changing environment. Increases in air temperature also mean increases in soil temperature, which may affect seed persistence. The persistence of seed in the soil seed bank is an important trait affected by the environment and closely related to the physical and physiological properties of seeds. For an arid system in Australia where the majority of the species are hard-seeded and possess physical dormancy, an increase in soil temperature results in dormancy loss and decreases the soil seed bank. In contrast, for Australian alpine species where seeds frequently exhibit dormancy (Hoyle et al., 2014), soil warming reduces germination from the soil seed bank, but increases the diversity of species that germinate (Hoyle et al., 2013). Thus, warming will potentially change community composition as mediated by direct effects on germination from the soil seed bank. Further research is needed into seed persistence in the soil seed bank particularly for alpine ecosystems.

A controlled ageing protocol, commonly used to estimate lifespan or longevity of seeds in *ex situ* storage, may also enable prediction of *in situ* seed persistence. Australian native species are on average quite long lived, but studies of alpine seed in other regions suggest alpine seed has low longevity. We are currently investigating the longevity of Australian alpine seed in a controlled ageing experiment, to gain an integrated understanding of seed persistence so that we can better estimate how long seed can survive in the soil or in *ex situ* conservation.

Conclusions

Critical gaps in our knowledge of alpine plant seed ecology in Australia and elsewhere are becoming apparent, and have important implications for our ability to predict and manage biodiversity in the face of climate change. A more nuanced understanding of the regeneration stages from seed to seedling in our changing climate is needed. The studies described here will better inform our understanding of the germination niche and requirements of alpine species, and how dormancy and germination will change in a warming climate with altered patterns of seasonality. This knowledge will contribute to improved understanding of alpine seed and seedling ecology with both applied and pure ecological and evolutionary relevance to Australia, and will make a substantive contribution to our global understanding of alpine plants.

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