



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

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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 patterns, 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 timescale 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 demonstrate not only substantial within-species variation in germination strategy across the species distribution range, but also 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.

Keywords Local adaptation · Maternal environment · Plastic variation · Seedling growth · Reproductive ecology

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Introduction

Diversification in the timing of germination regulates the seasonal emergence of seedlings and is described as a species' 'germination strategy' (Simons and Johnston 2000; Donohue 2005; Petru and Tielbörger 2008; Hoyle et al. 2015). 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 (Beniston 2003; Parolo and Rossi 2008; Thuiller et al. 2008; 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 (Herman and Sultan 2011; Baskin and Baskin 2014). Seed dormancy can vary among populations of a single species with respect to elevation gradients (Barclay and Crawford 1984; Meyer and Kitchen 1994; Nishitani and Masuzawa 1996), with local long-term temperature and precipitation (Schütz and Milberg 1997; Wagmann et al. 2012; Carta et al. 2016), or be associated with other forms of habitat variability (Meyer and Kitchen 1994; Meyer et al. 1995; Honěk and Martinková 1996; Andersson and Milberg 1998; Fernández-Pascual et al. 2013; Hoyle et al. 2014). For example, seeds from populations with warmer and more humid climates 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 (Honěk and Martinková 1996; 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 (Fenner 1991; Gutterman 2000; Finch-Savage and Leubner-Metzger 2006; Donohue et al. 2008; Baskin and Baskin 2014). 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 (Reyer et al. 2013; Vasseur et al. 2014; 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. Kochanek et al. 2011; Bernareggi et al. 2016). 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, such as 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 perennial 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 shrublands at lower elevations (South Australian Seed Conservation Centre-Botanic Gardens of South Australia 2018; Jobson 1999; Costin et al. 2000). *Oreomyrrhis eriopoda* seed, like many Apiaceae, has been reported to have morphophysiological dormancy (Sommerville et al. 2013) and considerable variation in strategy is documented among populations (South Australian Seed Conservation Centre-Botanic Gardens of South Australia 2018; Wood 2014; Hoyle et al. 2015). 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 and 2159 m asl, covering much of the distribution range of this species (Supplemental Fig. 4 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 a 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, Thermo-line Scientific, NSW, Australia). Assays started at $25/15^{\circ}\text{C}$, 12/12 h light/dark, for 9 weeks, were moved to 5°C constant temperature and 12/12 h light/dark for 8 weeks and then returned to $25/15^{\circ}\text{C}$, 12/12 h 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 4 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. $35\ \mu\text{mol m}^{-2}\text{s}^{-1}$).

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). For the 21 viable populations, the minimum viability (filled healthy seeds) was 99%. 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 Supplemental Table 1). 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 the age of the 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, $p = 0.3556$; Supplemental Fig. 5).

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-standardised 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 analysis

We analysed the 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° (~ 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 adapted the formula used in long-term climate calculation and subsequently calculated analogues of the same 12 long-term climate values for maternal and seed development conditions using the values extracted from BOM records above. 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. Seasonality was calculated as the coefficient of variation for the mean monthly climate variables—temperature, precipitation, and radiation (Xu and Hutchinson 2011). We first performed CVA for long-term, maternal and seed development conditions separately. For long-term climate, 12 variables, i.e. mean, maximum, minimum, and seasonality (variability) of temperature, precipitation, and radiation were included in the analysis (Supplemental Table 2). 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 12 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 12 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 that 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 $4 \times 4 \times 10$ 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 g/pot of slow release fertiliser (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 4 and 5 weeks after potting. Specific leaf area (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 (mm²) 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 3 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.

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 timescale (long-term climate, maternal or seed development conditions, shown by higher latent vectors (Fig. 3, Supplemental Table 3, Supplemental

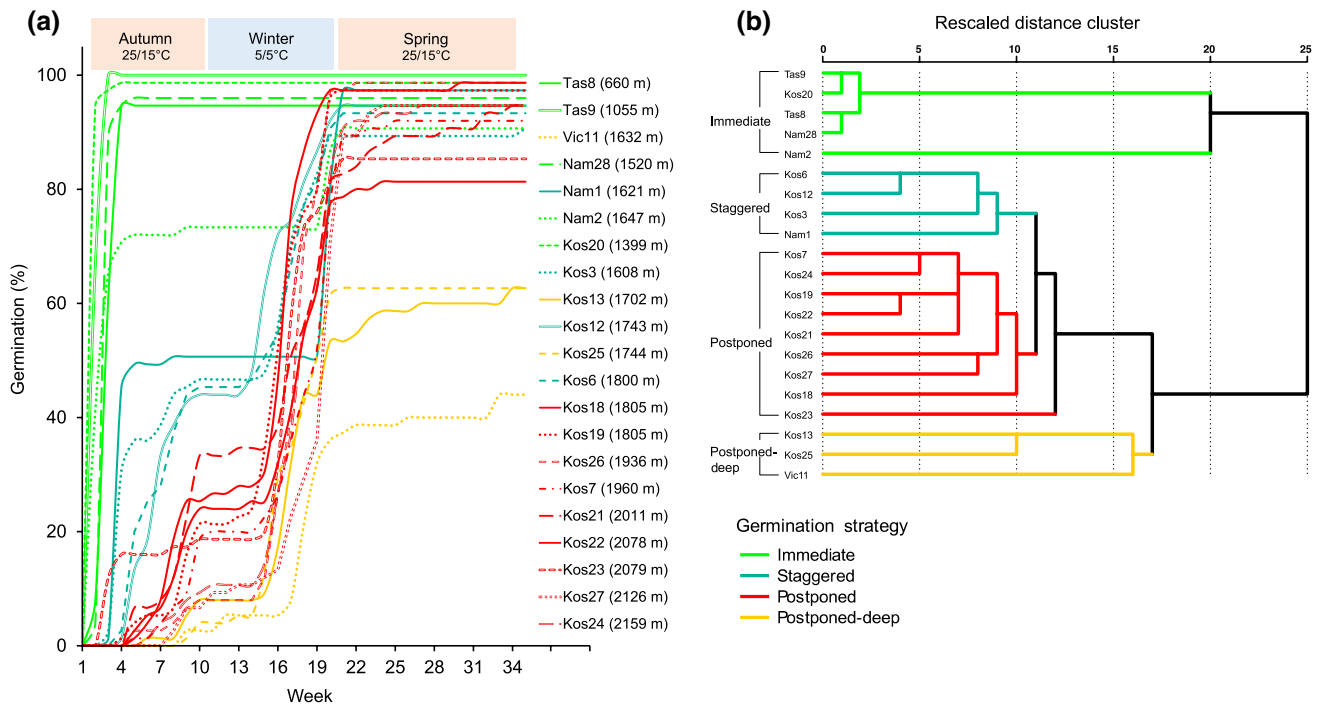


Fig. 1 **a** Germination strategies of 21 viable populations of *O. eriopoda* during a 34 weeks period. Codes indicate region (*Kos* Kosciuszko National Park, *Nam* Namadgi National Park, *Tas* Tasmania, *Vic* Alpine National Park Victoria) followed by population num-

ber, 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

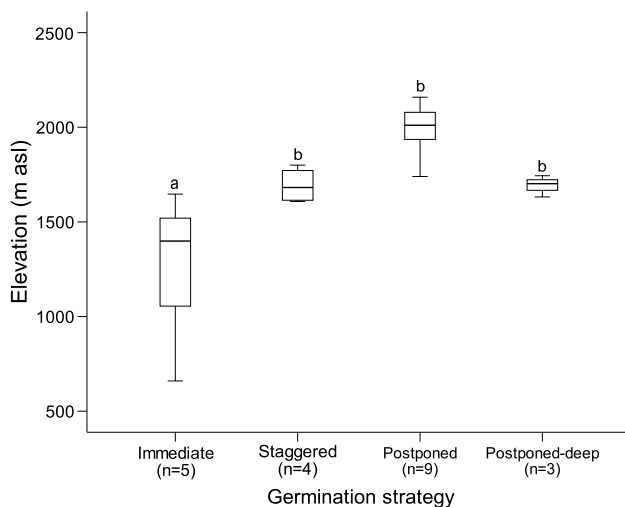


Fig. 2 The distribution of germination strategy among *O. eriopoda* populations by elevation. Letters show significant differences between strategies based on Fisher's LSD post hoc test. The numbers of populations assigned to each strategy by cluster analysis are indicated in parentheses below the strategy name

Fig. 6). *Oreomyrrhis eriopoda* with immediate germination strategy was associated with populations that come from locations with lower temperature variability compared with populations in other strategies. Axes 2 and 3 differ slightly

between timescales 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 populations with immediate germination strategy and the other three strategies was driven by temperature variability (Fig. 3a, Axis 1). Populations with immediate strategy were from locations with lower temperature variability, whereas populations with staggered and postponed-deep strategies were from locations with relatively higher minimum radiation and higher temperature variability (Fig. 3a, Axis 2). For maternal and seed development conditions (Fig. 3b, c), the dominant climate variables were temperature variability and mean radiation. For both maternal and seed development conditions, populations with immediate strategy were characterised by lower maternal temperature variability (Fig. 3b, c, Axis 1). For maternal conditions, the populations with postponed-deep and staggered germination strategies were from locations with higher temperature variability, higher minimum temperature, and minimum radiation (Fig. 3b, Axis 2). For seed development conditions, the populations with postponed-deep strategy were distinguished by higher temperature variability as well as higher mean and minimum radiation (Fig. 3c, Axis 2). Overall, under maternal time-scale, the separation pattern of germination strategies on

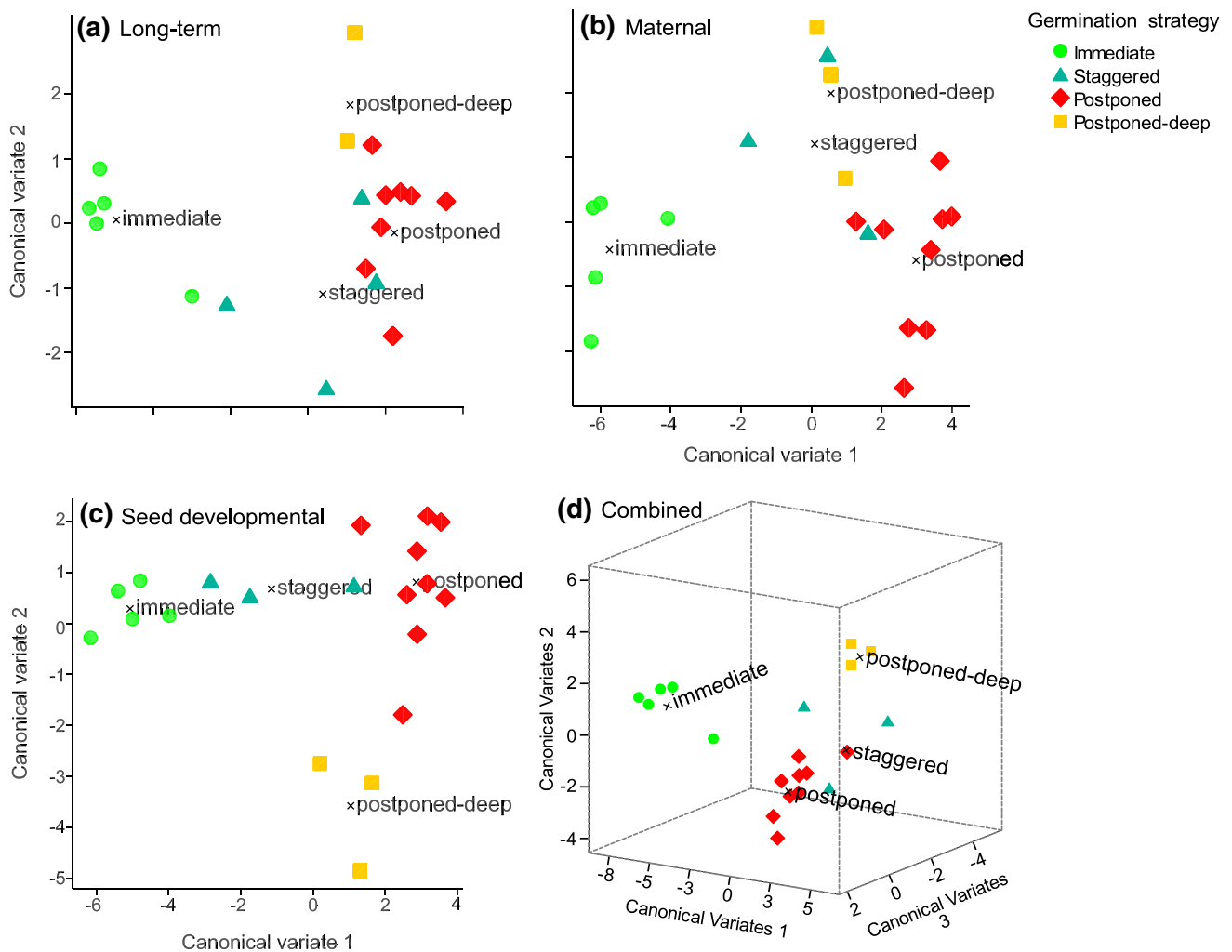


Fig. 3 Canonical variate means of **a** long-term, **b** maternal, **c** seed developmental, and **d** the three timescales combined in 3-D on the germination strategies. Population of *O. eripoda* with different germination strategies are indicated by different shapes. X is the mean for each germination strategy. Axes indicate variables that have the strongest association with the germination strategy. Climate variables for **a** axis 1 (88.15%): temperature variability and minimum radiation, axis 2 (7.76%): temperature variability and minimum radiation. Populations Kos13 and Kos25 (postponed-deep) symbols overlap.

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 timescale were combined in a single analysis, we found long-term and seed developmental temperature variability as 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

For **b** axis 1 (90.49%): temperature variability and mean radiation, axis 2 (5.94%): temperature variability and mean radiation. For **c** axis 1 (78.83%): temperature variability and mean radiation, axis 2 (17.37%): temperature variability and minimum radiation. For **d** axis 1 (75.73%), 2 (16.11%), 3 (8.16%): temperature variability of long-term, seed development conditions, and maternal condition, respectively. Population Kos6 (staggered) short-term climate data were not available and hence Kos6 was excluded from **b–d**

temperature, and precipitation had minimal influence on germination strategies.

Associations between germination strategies 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

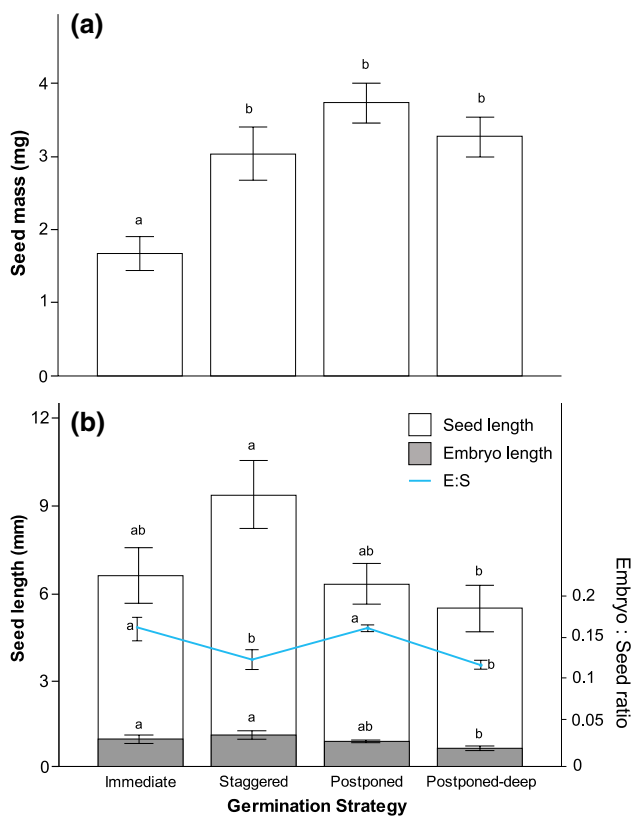


Fig. 4 **a** Seed mass and **b** seed length, embryo length, and embryo:seed ($E:S$) ratio of *O. eriopoda*. Letters show significant differences between strategies based on Fisher's LSD post hoc tests on mean of each variable (Table 1)

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 a staggered strategy.

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) highlight 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.

Germination strategy associations

Amongst the climate and distribution characteristics considered, temperature variability strongly explained germination strategy. Likewise, spring and annual precipitation variability affect intraspecific variation in germination strategy of some arid plants (Barga et al. 2017). In addition to temperature variability, mean and minimum radiations were also dominant. In alpine environments, temperatures are not the only key climate variable; solar radiation, slope, and exposure also define the conditions plants are exposed to (Körner 2003), which may then impose variations in germination strategy. 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 the

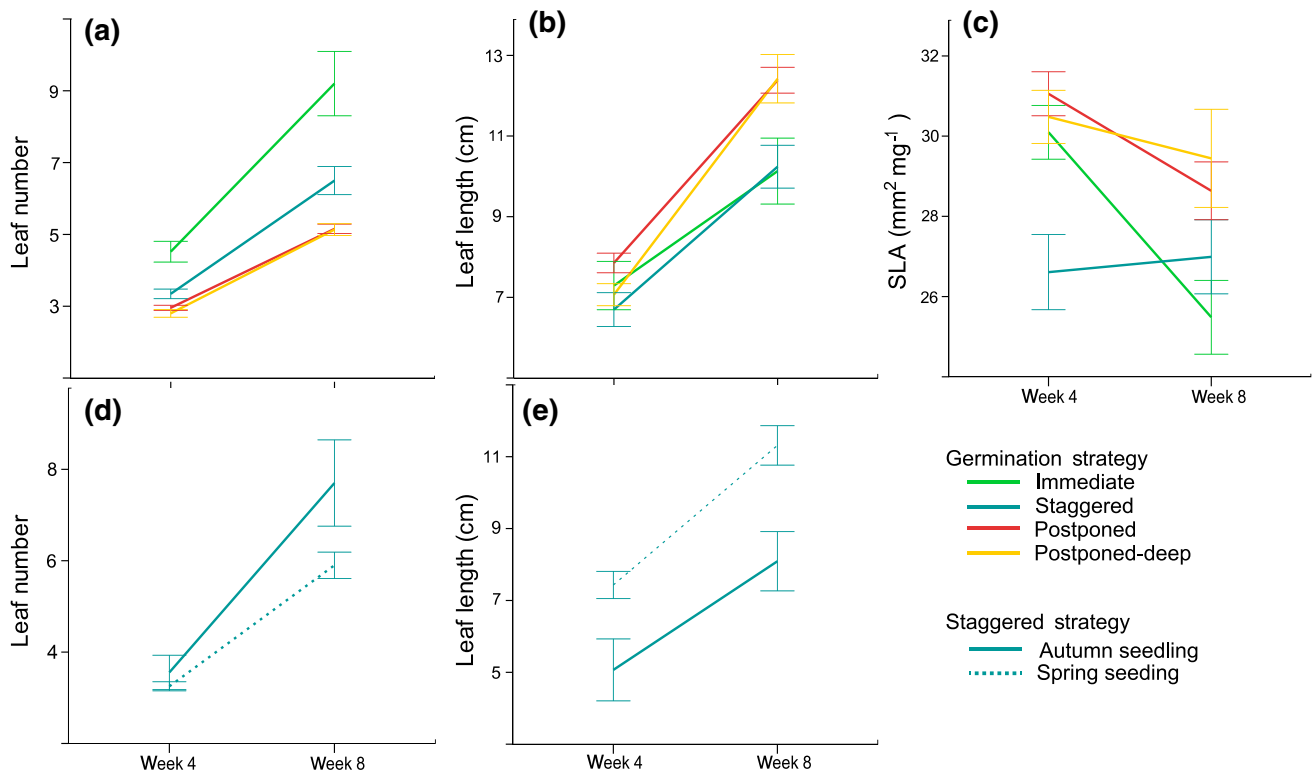


Fig. 5 a, d Leaf number, b, e leaf length, and c specific leaf area (SLA) across germination strategies (top row) and within the staggered germination strategy (bottom row). Leaf number and leaf length for staggered strategy were not available for autumn seedlings

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

Table 1 Results of residual maximum likelihood (REML) mixed models analyses of effects of (a) germination strategy (immediate, staggered, postponed, and postponed-deep) on seed and seedling traits. All seed and seedling traits, except embryo:seed length ratio (*E:S*), were log-transformed prior to model fitting. Population was included as a random factor in all models; (b) differences in leaf traits between autumn and spring-germinating seedlings of the four staggered populations (Nam1, Kos3, Kos6, Kos12) for which both autumn and spring seedlings were available

	Trait	Fixed term	df	F	P
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
	<i>E:S</i>	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
Staggered strategy (four populations)		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
	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

For further details, see “Materials and methods”

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; Venable and Lawlor 1980; Simons and Johnston 2006; Simons 2014). 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.

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 in the seed dormancy.

Unlike the staggered and postponed-deep strategy where dormancy degree within populations varies and 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 6 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 and 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 mechanism

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 (but see Barga et al. 2017). 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 (Venable 2007; Starrfelt and Kokko 2012), 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). It can be assumed that the diverse germination timing exhibited by staggered populations, where both autumn and spring germination occur, may reflect bet hedging. This assumption is correct if the source of variation lies within an individual—an individual plant producing 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.

Most studies on species' regeneration from seeds suggest that, in general, germination will decline under future climates. Across these studies, within-species variation in germination response is rarely considered by studies examining scenarios of species persistence. Our results demonstrate not only substantial within-species variation in germination strategy across the species distribution range, but also that this variation correlates with environmental drivers. Given that these differences also extend to establishment traits, such variation may reflect a critical mechanism for

persistence under novel environmental conditions. Examination of intraspecific variation in germination strategies across species distribution ranges may thus shed light on the potential for species to cope with and adapt to changing climate providing insight for management and conservation.

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Compliance with ethical standard

Conflict of interest The authors declare no conflict of interest.

Research involving human and/or animal participants This article does not contain any studies with human participants or animals performed by any of the authors.

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