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## RESEARCH ARTICLE

# Effects of warming temperatures on germination responses and trade-offs between seed traits in an alpine plant

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## Abstract

1. Climate warming may affect multiple aspects of plant life history, including important factors such as germination responses and the key trade-off between offspring size and number. As a case study to address these concepts, we used an alpine plant (waxy bluebell, *Wahlenbergia ceracea*; Campanulaceae) that shows plasticity to warming in seed traits and in which seed dormancy status regulates germination. We chose an alpine species because alpine environments are ecosystems particularly under threat by climate change.
2. We conducted germination assays under cool and warm temperatures using seeds produced by individuals that were grown under historical (cooler) and future (warmer) temperature scenarios. We assessed the presence of a seed size versus number trade-off, and then examined the effects of seed number and size on germination percentage, the fractions of dormant and viable seeds, and germination velocity. Further, we examined whether warming during parental growth and during germination affected these relationships.
3. We found evidence for a seed size versus number trade-off only under historical parental temperatures. Indeed, under future growth temperatures parental plants produced fewer and smaller seeds and there was no evidence of a trade-off. However, the reductions in both seed traits under warming did not affect germination, despite correlations of seed size and number with germination traits. Warming increased germination, particularly of larger seeds, but overall, it resulted in more than fourfold reductions in parental fitness.
4. *Synthesis.* Our study shows the importance of growth conditions when evaluating the seed size versus number trade-off. Stressful conditions, such as warmer temperatures, can restrain the ability of plants to reach optimal investment in reproduction, masking the trade-off. By analysing responses across the whole life cycle, we show here an overall detrimental effect of warming, highlighting the potential risk of climate change for *Wahlenbergia ceracea*, and, potentially, for alpine plant communities more widely.

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## KEYWORDS

alpine environments, fitness, germination responses, parental effects, phenotypic plasticity, seed dormancy, size vs. number trade-off, warming

## 1 | INTRODUCTION

Offspring size and number are life-history traits assumed to trade-off in any living organism (Smith & Fretwell, 1974). In plants, this trade-off manifests at the seed level, that is, between the size and number of seeds. The production of a progeny of better quality (larger) or larger numbers are both adaptive strategies insofar they increase parental reproductive success (Geritz et al., 1999; Moles & Westoby, 2006; Westoby et al., 2002). Specifically, the production of larger seeds provides the offspring with more resources increasing seedling establishment, particularly during unfavourable conditions; whereas, the production of more seeds allows to build a denser seed bank, therefore increasing seedling recruitment over time (Geritz et al., 1999; Moles & Westoby, 2006; Saatkamp et al., 2009; Westoby et al., 2002). The trade-off arises because access to resources is limited (Smith & Fretwell, 1974) and the outcome of the trade-off varies between species and environmental conditions (Jessup & Bohannan, 2008; Koricheva, 2002). Although the seed size versus number trade-off in plants has been found within fruits, and at the interspecific level, there has been mixed support for this trade-off at the intraspecific level. Species show negative, neutral, and positive relationships between seed size and number (Brancalion & Rodrigues, 2014; Guo et al., 2010; Lázaro & Larrinaga, 2018).

The study of the seed size versus number trade-off is crucial in plants because seed traits, and particularly seed size (the mass of one seed), affect seed germination, one of the most vulnerable stages of a plant's life cycle (Matías et al., 2011; Wong et al., 2012). Larger seeds are generally associated with higher germination rates, higher germination velocity (the reciprocal of mean germination time; Kotowski, 1926; Ranal & Garcia de Santana, 2006), higher seedling growth rate, and higher survival (Benard & Toft, 2007; Domic et al., 2020; Ge et al., 2020; Veselá et al., 2021). However, evidence of a negative relationship between seed size and germination rate has been found in some alpine species (Ge et al., 2020).

Seed size might also affect other aspects of the germination response, such as dormancy levels, which describe the degree to which seeds are responsive to the environmental conditions that trigger germination (Batlla & Benech-Arnold, 2010; Finch-Savage & Leubner-Metzger, 2006; Vleeshouwers et al., 1995). To increase the potential of successful seedling recruitment during favourable times of the year, many alpine plant species stagger their germination across seasons (Hoyle et al., 2015). Alpine ecosystems are unpredictable and heterogeneous environments, where climatic conditions can vary abruptly both spatially and temporally (Körner, 2003), potentially reducing germination success and seedling survival. Therefore, some alpine species produce both seeds that germinate as soon as they are dispersed, and others that remain dormant in

the seed bank until specific environmental requirements are met (usually a period of exposure to low temperatures, the end of which signals snowmelt and the start of the growing season, Hoyle et al., 2015; Satyanti et al., 2019). Because of the differences in size, large and small seeds may have different dormancy levels. For example, Liyanage and Ooi (2018) found that in species that require fire to germinate, larger seeds need lower temperatures to break dormancy. A worldwide survey showed that smaller seeds are more likely to be dormant in legumes (Rubio de Casas et al., 2017). In contrast, for the alpine herb *Oreomyrrhis eriopoda*, populations with a larger average seed mass are associated with a postponed germination strategy, where all seeds were dormant (Satyanti et al., 2019). Thus, there seems to be considerable variation among species in the relationship between seed mass and germination that needs further consideration.

In contrast to the work to date on seed mass and germination, the relationship between seed number and germination responses has seldom been investigated. Seed number might be expected to be positively correlated with dormant seed fractions. Indeed, when dispersal is low, the seedlings originating from large batches of seeds are expected to be under higher competition (Campbell et al., 2017; Lampei et al., 2017). By scattering germination across seasons parental plants may therefore reduce the level of competition among seedlings and increase potential recruitment over time.

Global warming due to climate change will expose plants and their seeds to novel climatic conditions and likely affect seed germination responses (Gremer et al., 2020) with potential impact upon population dynamics and persistence of species. The timing of germination is crucial for survival because it determines the immediate environmental conditions that the seedling will face (Gremer et al., 2020). As such, germination triggered by environmental cues, particularly temperature and precipitation, ensures that seedlings emerge in the appropriate season and under favourable conditions (Fernández-Pascual et al., 2021; Finch-Savage & Leubner-Metzger, 2006; Puglia et al., 2018). Ultimately, the proportion of seeds that survive to germinate and establish determines the fitness of the parents (Campbell et al., 2017; Erb, 2018). However, climate change may decouple the relevant environmental cues from the conditions to which the offspring is exposed at germination, making the cues less reliable (Bonamour et al., 2019; Walck et al., 2011).

Alpine environments are considered particularly threatened by climate change. In these environments, temperatures have increased at a rate twice as fast as lowland ecosystems (Gobiet et al., 2014). As a result, rising temperatures are causing a contraction and an upward shift of the range of alpine species (Bueno de Mesquita et al., 2018; Parolo & Rossi, 2008). However, mountain summits represent physical barriers to further dispersal. The consequences of climate change are already evident in alpine environments, where

warming temperatures are resulting in more frequent heat stress, earlier snowmelt, earlier flowering dates, later snowfall, and longer growing seasons (Carbognani et al., 2016; Harris et al., 2016; Kudo, 2020; Nicotra et al., 2015). Therefore, alpine plants provide a relevant system to explore how novel temperature regimes predicted for the future can affect germination and the relationship between seed traits and germination responses.

Plants can influence germination strategy and dormancy levels of their seeds in response to their environment, where the temperature is one of the most important cues to such parental effects (Lampe et al., 2017; Penfield & MacGregor, 2016). Parental plants may respond to warmer growth conditions by either increasing the germination velocity and reducing the proportion of dormant seeds so that germination occurs in the season of dispersal, or by increasing dormant seed fractions, to avoid heat stress, cold spells, and other unpredictable late autumn weather extremes. Although warmer temperatures during seed development are sometimes associated with decreased dormancy (Bernareggi et al., 2016; Penfield & MacGregor, 2016), Wang et al. (2021) showed an increase in the dormant seed fraction in the Australian alpine plant *Wahlenbergia ceracea* when parents were grown under warm temperatures. There is also evidence that parental individuals can influence seed size in response to warmer growth temperatures (Nakagawa et al., 2020; Penfield & MacGregor, 2016). Therefore, if a relationship exists between seed size and dormancy levels, then parental control over seed dormancy may be exerted through changes in seed size. However, research on parental effects on germination in alpine plants is still scarce and whether parental effects on germination are mediated through changes in seed size is still an open question.

Parental effects that influence seed size should also affect seed number under the assumption of a seed size versus number trade-off (Paul-Victor & Turnbull, 2009; Smith & Fretwell, 1974; Westoby et al., 1992). Investment in either life-history strategy should be accompanied by a change in the opposite direction in the other trait. Whether this has potential repercussions to germination responses has, to our knowledge, never been tested. Alternatively, if responses in both seed traits are in the same direction, due for example, to limiting environmental conditions that affect the optimal investment to reproduction, the trade-off will disappear. Therefore, investigating whether and how warmer temperatures during growth and seed development affect the seed size versus number trade-off in plants is an important question, particularly in the context of a warming climate.

Here, we sought to answer the following questions by using *W. ceracea* as a case study: (1) Is there a seed size versus number trade-off? (2) Do seed size and number influence germination responses, such as germination percentage, germination velocity, dormant seed proportion and the viability of seeds? (3) Does climate warming during parental growth or during germination affect all these relationships? Previous work with *W. ceracea* has shown that individuals decrease both seed size and number and produce higher fractions of dormant seeds when grown under future projected (i.e., warmer) temperatures (Notarnicola et al., 2021; Wang et al., 2021).

To answer our three current questions, we conducted a germination experiment in glasshouses under cool or warm temperatures using seeds developed under temperature conditions that mimicked a historical/cooler (1960–1970) and a future/warmer projected (2090–2100) climate. The focus of this study was on temperature effects on germination, so we therefore did not consider the reductions in precipitation and snow cover that are also projected for many mountain ranges under a changing climate (Hock et al., 2019). We summarise and interpret results to predict fitness consequences of growth under future temperatures for alpine plants.

## 2 | MATERIALS AND METHODS

### 2.1 | Study system

*Wahlenbergia ceracea* Lothian (Campanulaceae; waxy bluebell) is a short-lived perennial/biennial herb, endemic to Australia. The plant is 10–60 cm tall and grows among dense vegetation in moist sites in alpine and sub-alpine environments (Nicotra et al., 2015). *Wahlenbergia ceracea* is hermaphroditic, facultatively autogamous and produces very small seeds (average mass of one seed is 22.5 µg; Notarnicola et al., 2021) in brown cylindrical capsules. Seeds are dispersed by wind at maturity. This species has a staggered germination strategy (Wang et al., 2021) with some seeds germinating in autumn and others requiring a period of low temperatures (cold stratification), to alleviate dormancy. Dormant seeds presumably germinate once snow melts (spring germination) or in subsequent seasons if they are deeply dormant. *Wahlenbergia ceracea* shows plasticity in response to high temperatures for several traits, including seed traits (Nicotra et al., 2015; Notarnicola et al., 2021; Wang et al., 2021). On average, reductions in seed size and number were observed under warmer growth temperatures, but the consequences on germination responses and parental success, as well as whether the two traits trade-off have not been previously examined.

### 2.2 | Experimental conditions to generate seeds

Parental individuals (F1 generation) were obtained from targeted crosses between individuals grown from field collected seeds (F0 generation). The F0 seeds were collected in Kosciuszko National Park (New South Wales, Australia) in March 2015 and April 2016. F0 individuals were grown in the glasshouse under 20/15°C day/night, natural photoperiod, and were crossed to obtain the parental individuals for this study. We refer to each cross as a line. Parental individuals ( $n = 321$ ) from a total of 30 lines were grown in growth chambers under temperature conditions of a historical/cooler (maximum day temperature: 24°C) and a future/warmer (maximum day temperature: 29°C) climate scenario. We will refer to these treatments respectively as parental historical and future temperatures from now on to distinguish from treatments during germination (see below). Growth temperatures and photoperiod during the lifetime

of the parental individuals were changed every 15 days to mimic seasonality (Figure 1) and there was a day/night temperature cycle. All mature capsules were collected over the lifetime of the parental individuals, for a total of 191 days (Figure 1). In total, 236 out of 321 total parental plants produced some seed. We stored the capsules and seed in a desiccator at room temperature (20°C, RH = 15%–20%) for at least 11 weeks. Dry storage can induce after-ripening and reduce dormancy of seeds of some species (Baskin et al., 2006; Baskin & Baskin, 2020), however this procedure was necessary to reduce the excess moisture and humidity from capsules during collection. After this time, we measured *seed size* as the average mass of three lots of 50 seeds divided by 50. We also derived the total seed production (*seed number*) as the ratio between the cumulative mass of the seeds produced by each parent individual and seed size.

### 2.3 | Germination experiment

The germination assays included seeds collected from a sub-sample of 14 lines. We aimed to use six individuals from each line, three from each parental temperature, however, we could not achieve a balanced design because parental individuals grown under future

temperatures produced fewer seeds, with many lines producing no seed or immature seeds. We included lines in which at least one individual produced capsule under future temperatures; however, for three lines all the parents grown under future temperature produced only empty capsules or immature seeds and ultimately could not be used in this experiment. Individuals of one line produced on average more than 20,000 seeds and this line was removed from the analysis because it exerted undue leverage. A list of the lines and the corresponding number of parents are given in Table S1. We used seeds collected when each parent was at its peak of seed production.

Seeds were sown in 1% water agar in 50 mm Petri dishes (25 seeds/dish) and moved into the glasshouse under cool (25/18°C day/night) and warm (30/25°C day/night) germination temperatures (Figure 1). The 25°C of the cool treatment were found to be the optimal temperature for germination in *W. ceracea* (Arnold et al., 2022); we raised the temperature by 5°C (30°C) in the warm treatment. We moved seeds into new Petri dishes whenever dishes started to dry out. We obtained two replicates for each parental individual per germination temperature (the design was 14 lines × 3 parental individuals × 2 parental temperatures × 2 germination temperatures × 2 replicates, however due to some lines and individuals producing no seed the final number of dishes was 200). Dishes were left under these conditions

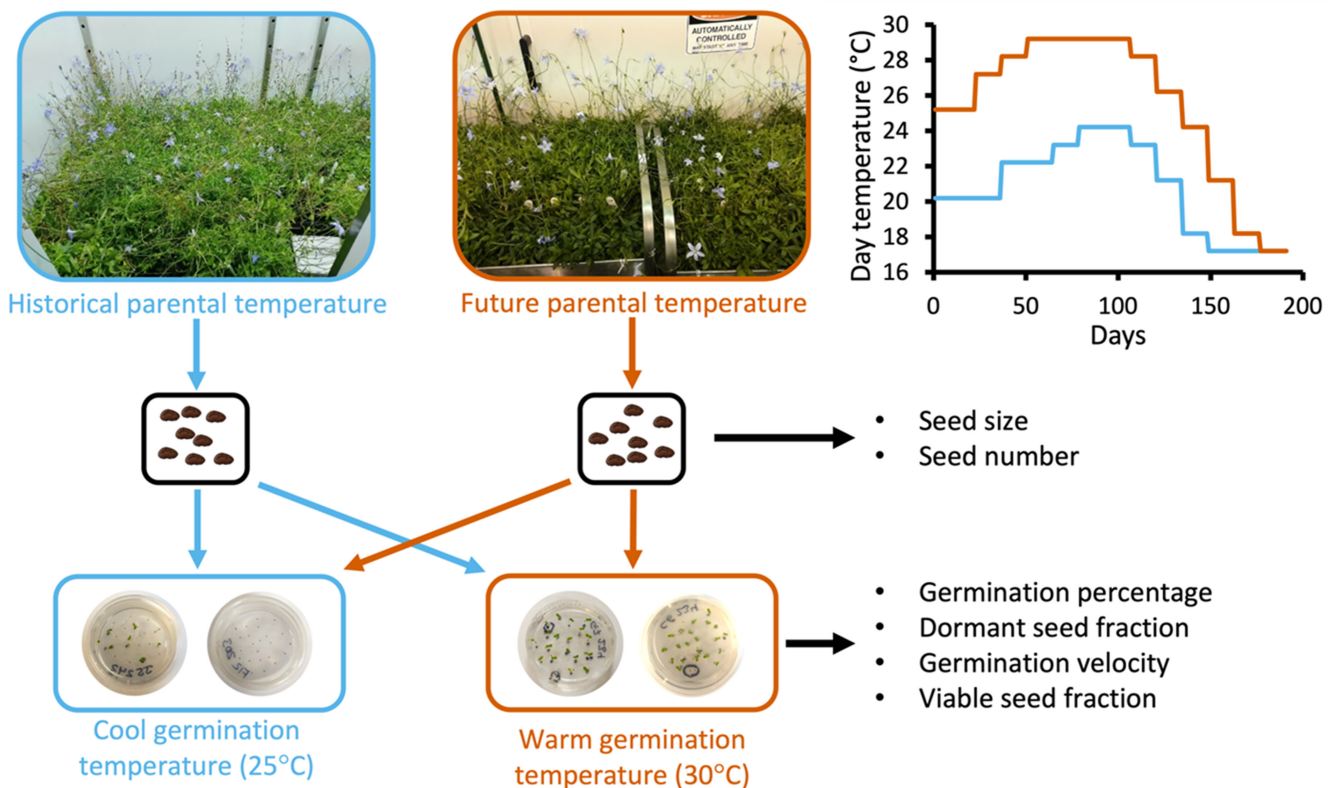


FIGURE 1 Summary of the experimental design. Parental individuals (top) were grown under projected temperatures of a historical/cooler (1960–1970) and a future/warmer (2090–2100) climate. Day and night temperatures were changed fortnightly to mimic seasonality. Day temperatures during parental growth under the two treatments are presented in the graph (top-right). During parental growth, all seeds were harvested to measure seed size (the average weight of one seed,  $\mu\text{g}$ ) and seed number. These seeds were then used in germination assays in the glasshouse under cool (25°C) and warm (30°C) temperatures to measure germination percentage, dormant seed fractions, germination velocity, and viable seed fractions (bottom).



in the glasshouse for 4 weeks to allow germination of the nondormant fraction of the seeds. Germination was checked once per week, where the seed was considered to have germinated once the radicle had emerged. All dishes were then moved to a cold room at 4–5°C in the dark for 4 weeks for cold stratification (after Wang et al., 2021). Seeds that germinated during cold stratification were included in the count of total germination and the fraction of dormant seeds. After cold stratification, seeds were moved back to the glasshouse at the same temperatures as before to allow germination of dormant seeds. Germination was again checked once per week until there was no new germination (8 weeks). Finally, the remaining seeds in each dish that had not germinated were dissected with a scalpel under the microscope to check for viability. Germinated seeds and seeds with an endosperm that did not germinate (deeply dormant seeds) were considered as viable and added to the count of dormant seeds, while empty seeds were considered nonviable. Therefore, we considered as dormant (1) seeds that germinated during 4 weeks of cold stratification; (2) seeds that germinated after cold stratification; and (3) seeds that did not germinate but were viable. The final *germination percentage* (GP) was calculated as the percentage of seeds that successfully germinated out of the initial number of viable seeds.

We calculated *germination velocity* as the reciprocal of the mean germination time (germination velocity (germination [%] week<sup>-1</sup>)  $GV = (G_1 + G_2 + \dots + G_n) / (G_1 \times T_1 + G_2 \times T_2 + \dots + G_n \times T_n)$ , where  $G_n$  is the number of new germinating seeds at each sampling point, and  $T_n$  is the time between each sampling point (= 1 week); Kotowski, 1926; Ranal & Garcia de Santana, 2006). The *fitness index* was calculated as the product of seed number and GP.

## 2.4 | Statistical analysis

We conducted all statistical analyses in R v4.0.2 (R Core Team, 2020). We analysed the seed size versus number trade-off with a nested, linear mixed effects regression model with random intercepts, using the *lmer* function in the 'lme4' R package (Table S2). Seed size was the response variable. To increase the robustness and accuracy of the statistical analysis, we included data from all parents that produced realistic numbers of seeds and of a realistic size ( $n = 229$ ). Fixed effects were parental temperature ('historical' or 'future'), and the two-way interaction between seed number and parental temperature. The random effect was line (14 lines) (Table S2). We considered in the model additional factors that were relevant during parental growth (elevation of origin, a heatwave treatment, and block randomization; Table S3). However, likelihood ratio tests (LRTs) between the models that included these fixed effects and those that did not were never significant (Table S3), and therefore for simplicity and consistency we have excluded these fixed effects from our analyses here.

We analysed the effect of seed size and number on four different measures: (i) germination percentage (GP), (ii) the proportion of dormant seeds, (iii) germination velocity, and (iv) the proportion of viable seeds. To analyse (i), (ii), and (iv) we used generalised linear mixed effect regression models (GLMMs) with random intercepts using the

*glmer* function in the 'lme4' R package (Bates et al., 2015), with a binomial distribution of the data (Table S2); whereas to analyse (iii) we used linear mixed effects regression models (LMMs) with random intercepts with the *lmer* function (Table S2). The total number of trials from which proportions were obtained was the number of viable seeds per dish. The fixed effects were seed size, seed number, germination temperature ('cool' or 'warm'), parental temperature ('historical' or 'future'), and the two-way interactions between seed size and number with germination temperature and parental temperature. Seed size and number were scaled in each of the models considered. For the analysis of seed viability, a quadratic term for seed size was included as fixed effect to meet the assumption of normality of the simulated residuals (*see below*). The random effects were lines and parental IDs nested within germination temperature (2 dishes from each parental individual per temperature treatment) (Table S2). Significant differences in the intercept of plastic responses between lines were tested using an LRT between the full random intercept model and a model without the random effect 'line'. Random slopes for the responses of lines to temperature (germination temperature | line) and to parental temperature (parental temperature | line) were tested but were never significant and we excluded them from the final analyses.

In all cases, we analysed the main effects using models without interactions, and tested for an effect of interactions using the full models with interactions as described above. To determine whether there was statistical support for an effect of factors and interactions on traits, we evaluated *p*-values from each model, using the *summary* function of the 'lmerTest' R package (Kuznetsova et al., 2017).

We verified the assumptions of independence and normality of residuals by plotting residuals from the LMMs. We checked assumptions for GLMMs by plotting simulated residuals (obtained with the function *simulateResiduals* with 500 simulations) with the function *plotResiduals* from the 'DHARMA' R package (Hartig, 2019).

## 3 | RESULTS

### 3.1 | Seed size versus number trade-off in *W. ceracea*

We found only weak statistical evidence for a seed size versus number trade-off in *W. ceracea* (Table 1). The slope of the correlation was negative and significant only under cooler (historical) parental growth temperatures (*p*-value = 0.043; future parental growth temperature: *p*-value = 0.465; Table 1; Figure 2).

### 3.2 | Effects of seed traits on germination responses

Neither seed size nor seed number affected germination percentage (GP) (*p*-values: seed size = 0.929; seed number = 0.233) or dormant seed fraction (*p*-values: seed size = 0.576; seed number = 0.074) in the main effect model (Table 2; Figure 3a–d). Seed number was

**TABLE 1** Regression between seed size and number (seed size vs. number trade-off). Main effects model has no interactions; full nested model has two-way interactions between seed number and the other factors. The intercept corresponds to historical temperature, and other parameter values are relative to this. Significant results, as for  $p$ -value  $< 0.05$  from the `LMERTEST` package in R, are presented in bold. Number, seed number;  $p(\text{ML})$ ,  $p$ -value of the likelihood ratio test between the models with and without the random effect;  $\text{par temp}(\text{F})$ , future parental temperature;  $\text{par temp}(\text{H})$ , historical parental temperature;  $\text{se}$ , standard error;  $\text{var}$ , variance explained by the random effect. Random effects parameters are the variance components associated with that random effect

Seed size			
<b>Main effects:</b>	Estimate	(se)	$p$ -value
Intercept	24.051	0.698	
Seed number	-0.703	0.480	0.144
Parental temperature(future)	<b>-3.188</b>	<b>0.969</b>	<b>0.001</b>
<b>Full nested model:</b>			
<b>Fixed effects</b>	Estimate	(se)	$p$ -value
Intercept	24.096	0.693	
Parental temperature(future)	<b>-2.851</b>	<b>0.987</b>	<b>0.004</b>
Number:par temp(H)	<b>-1.083</b>	<b>0.533</b>	<b>0.043</b>
Number:par temp(F)	0.755	1.032	0.465
<b>Random effects</b>	var		$p(\text{ML})$
Lines	<b>5.1020</b>		<b>0.0161</b>
Residual	45.2510		

positively correlated to the dormant seed fraction in the full model ( $p$ -value = 0.035); because the slope was significant only under historical parental climates this effect only appears significant in the full model (Table S5). Larger seeds germinated more quickly ( $p$ -value = 0.029) and were more viable ( $p$ -value = 0.001) but there was no relationship between seed number and the velocity of germination ( $p$ -value = 0.447) or seed viability ( $p$ -value = 0.162) (Table 2; Figures 3e,f and 4).

### 3.3 | Effects of warming temperatures on the relationships between seed traits and germination responses

Overall, warm temperatures during germination greatly enhanced germination percentages (GP) and increased the velocity of germination (Table 2; Figure 3). Seed size affected GP via interactions with germination temperature (Table 2; Figure 3a). Warm temperatures induced germination mostly of larger seeds ( $p$ -value = 0.028), while cool temperatures resulted in higher germination success of smaller seeds (Table 2; Figure 3a).

We found no evidence of an interaction between seed traits and parental temperature on seed viability ( $p$ -values: seed size = 0.915; seed number = 0.051) (Figure 4), GP ( $p$ -values: seed size = 0.201; seed number = 0.201), dormant seed proportions ( $p$ -values: seed size = 0.389; seed number = 0.220), or germination velocity ( $p$ -values: seed size = 0.692; seed number = 0.661) (Table 2). Therefore, there

was no indication that the reductions in seed size and number under future (warmer) temperatures affected germination responses. The negative correlation between seed number and seed viability under future parental temperatures was marginally not significant (Figure 4b).

As expected, parents grown under future, warmer temperatures produced a higher proportion of dormant seeds than parents grown under historical, cooler conditions (Table 2; Figure 5a). GP, on the other hand, was not affected by parental climate (Table 2; Figure 5b). This means that the fewer seeds produced on average under a future climate will have the same germinability as seeds produced under historical conditions (total germination: parents from a historical climate =  $73.3\% \pm 4.8\%$  SE versus parents from a future climate =  $67.4\% \pm 6.9\%$  SE). Importantly, however, many parents grown under future temperatures failed to produce any seed at all (Table S4; Notarnicola et al., 2021), which drastically reduces their overall mean fitness (Figure 5c).

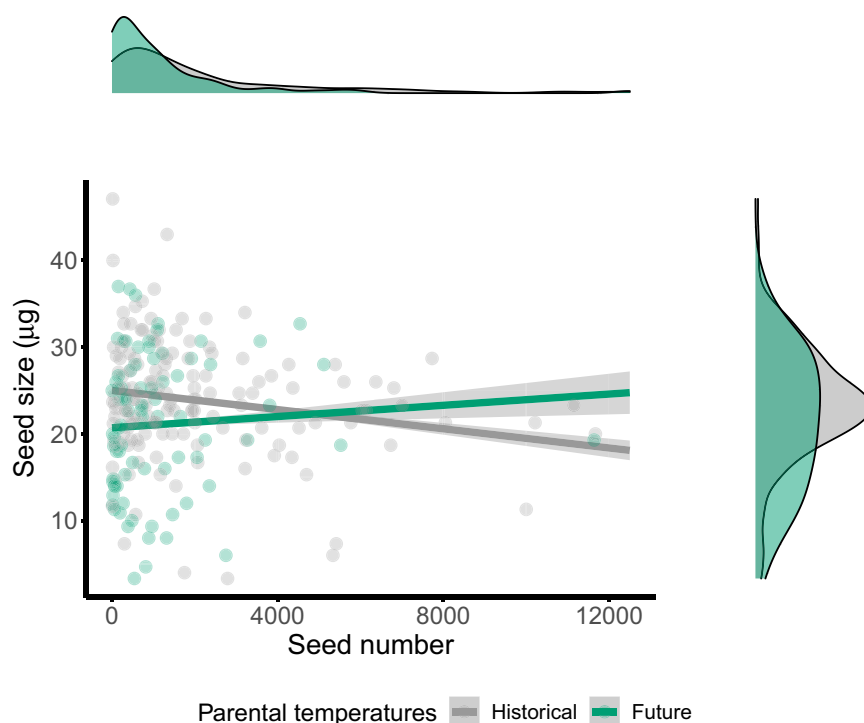
## 4 | DISCUSSION

As the climate changes, the average warmer conditions plants are exposed to will likely impact life-history strategies, with repercussions to reproduction and germination. Application of a whole of life cycle perspective (possibly over more than one generation) on how plant species will respond to changing climatic conditions will enable us to better predict the consequences of climate change on biodiversity, particularly in vulnerable ecosystems (Satyanti et al., 2021). Previously, it was shown that parental individuals of *W. ceracea* grown under warmer (projected future) temperatures produce fewer and smaller seeds (Notarnicola et al., 2021). Here, we sought to determine (1) whether these plastic responses to temperature affected the seed size versus number trade-off; in addition, we deployed those seeds, developed under cooler (historical) and future temperatures, in germination assays to investigate (2) whether seed size and number correlate with germination percentage (GP), dormant seed fractions, germination velocity and viability of the seeds; and, therefore, (3) whether warmer temperatures and plastic responses in seed traits affect all these relationships. We found evidence of a weak seed size versus number trade-off, but only in seeds developed under historical temperatures. Larger seeds germinated faster and were more viable, seed number correlated with dormant seed fractions, and warming induced germination of larger seeds. Below we discuss the implications of correlations between seed and germination traits, and of the effect of future warming on adult plants and seeds to predict future fitness consequences for alpine plants.

### 4.1 | Evidence of a seed size versus number trade-off only under historical (cooler) growth temperatures in *W. ceracea*

We found only weak statistical evidence for a seed size versus number trade-off in *W. ceracea*, and only when parental individuals

**FIGURE 2** Relationships between seed size and seed number under different parental temperatures. Histograms above and on the right of the graph are the distributions of the raw data of the two traits. Grey line and points: Historical (cooler) parental temperature; green line and points: Future (warmer) parental temperature. Points are raw data. Regressions are predictions estimated from model outputs; grey ribbons around regressions are standard errors. Regressions were conducted using linear mixed effects models with the *lmer* function in the *lme4* R package. Predictions were obtained with the *predict* function in base R.



were grown under their historical temperature regimes. An often-overlooked assumption of the Smith and Fretwell (1974) model is that the total investment of parents to the offspring is constant. A growing body of literature is showing negative, positive, and neutral relationships between seed size and number in many species at the intraspecific level (e.g., Brancalion & Rodrigues, 2014; Guo et al., 2010; Lázaro & Larrinaga, 2018), suggesting that this assumption may not always be true in nature. Individuals may differ in the amount of energy they invest in their progeny, particularly when growing under limiting conditions (Liu et al., 2013; Paul-Victor & Turnbull, 2009; Zhang et al., 2021), where the ability to access and allocate resources for reproduction may limit the capacity to reach the optimal energetic investment to the offspring. Other factors that can affect and mask the seed size versus number trade-off are correlations of seed traits with other life-history traits (e.g., flowering time; Gnan et al., 2014), or quantitative trait loci (QTLs) with pleiotropic effects on both seed size and number (Ellis et al., 2021).

The severe reductions in both seed number and size under warmer growth temperatures resulted in the breakdown of the seed size versus number trade-off in *W. ceracea*. The relationship between the two traits was if anything, trending positive, under future, warmer climates. Investment in either seed size or seed number are both adaptive strategies that increase seedling recruitment by provisioning more resources to the offspring or by scattering germination in time and space, respectively. Therefore, reductions in both trait values may have severe consequences for species persistence. The higher production of flowers under a future climate in *W. ceracea* (Notarnicola et al., 2021) suggests an investment of parental individuals in the seed number life-history strategy. Concurrently, we observed a reduction in seed size, confirming the assumption behind

the seed size versus number trade-off (Smith & Fretwell, 1974). However, the limiting conditions of a future climate caused early abortion of many flowers and/or disrupted the correct development of the seeds, which were largely immature or empty. This resulted in reductions in both seed size and number, whose correlation was therefore trending positive, although we do not know whether the trade-off exists when considering only the viable seeds, or at the fruit/capsules and flower levels. There is evidence that both seed traits are positively correlated to seed longevity in the seed bank (Bekker et al., 1998; Saatkamp et al., 2009). Therefore, the relaxation of the trade-off will likely reduce the amount and longevity of the seed reserve available for future recruitment, affecting local species persistence and survival. Future studies should investigate the effect of changes in the seed size versus number trade-off to the persistence in the seed bank, particularly in alpine plants.

#### 4.2 | Seed traits correlate with germination responses

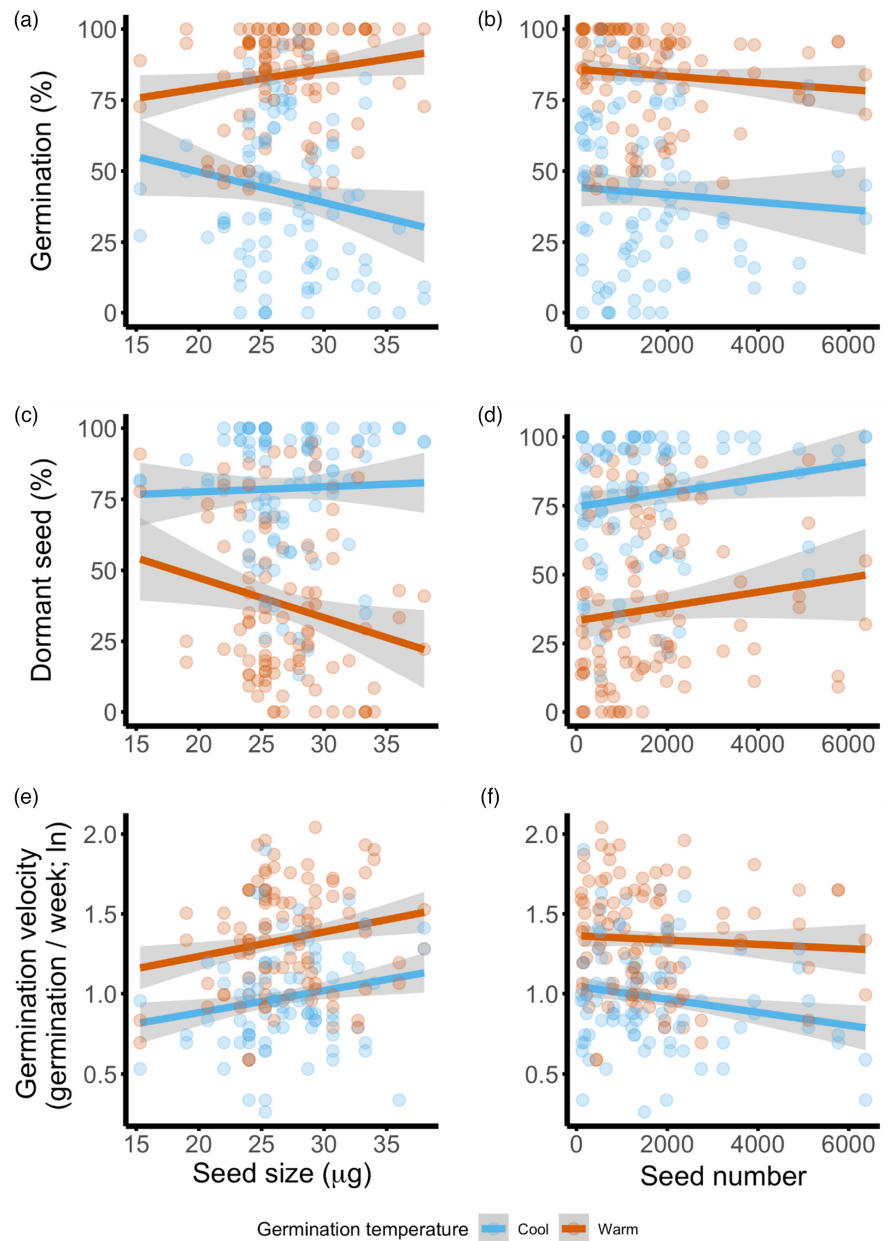
We found that larger seeds were more likely to be viable and germinated faster than smaller seeds in *W. ceracea*. The higher viability of larger seeds may result from a higher investment of mother plants into the embryo, endosperm, and/or seed coat (Counts & Lee, 1991). Indeed, seed size is positively correlated to N and P content inside the seeds (Lamont & Groom, 2013; Vaughton & Ramsey, 1998). The higher viability and vigour of larger seeds may have in turn resulted in the higher germination velocity that we observed. Fast germination and seedling establishment can be vital for persistence in alpine environments, where plants need to accumulate resources before



**TABLE 2** Regressions between seed traits and germination traits: Results from linear random intercept models. The main effects model has no interactions; the full model has all two-way interactions between seed traits and factors. The intercept corresponds to historical parental temperature and cool germination temperatures, and other parameter values are relative to this. Significant results, as for  $p$ -value  $< 0.05$  from the `lmerTest` package in R, are presented in bold. F, future; germ temp, germination temperature; number, seed number;  $p(\text{ML}) = p$ -value of the likelihood ratio test between the models with and without the random effect; par temp, parental temperature; se, standard error; size, seed size; var, variance explained by the random effect; W, warm. Random effects parameters are the variance components associated with that random effect

	Germination percentage			Dormant seed			Germination velocity			Viable seed		
	Estimate	(se)	$p$ -value	Estimate	(se)	$p$ -value	Estimate	(se)	$p$ -value	Estimate	(se)	$p$ -value
<b>Main effects:</b>												
Intercept	-0.276	0.275		1.652	0.286		0.976	0.056		1.680	0.160	
Seed size	0.013	0.142	0.929	-0.090	0.160	0.576	<b>0.069</b>	<b>0.031</b>	<b>0.029</b>	<b>0.309</b>	<b>0.092</b>	<b>0.001</b>
Seed size <sup>2</sup>										-0.082	0.051	0.109
Seed number	-0.153	0.128	0.233	0.264	0.148	0.074	-0.022	0.028	0.447	0.120	0.086	0.162
Germination temperature(warm)	<b>2.573</b>	<b>0.233</b>	<b>&lt;0.001</b>	<b>-2.532</b>	<b>0.264</b>	<b>&lt;0.001</b>	<b>0.375</b>	<b>0.051</b>	<b>&lt;0.001</b>	<b>-0.085</b>	0.154	0.580
Parental temperature(future)	-0.395	0.277	0.154	<b>0.639</b>	<b>0.314</b>	<b>0.042</b>	-0.077	0.062	0.219	-0.188	0.187	0.316
<b>Full model:</b>												
<b>Fixed effects</b>												
Intercept	-0.272	0.264		1.645	0.278		0.977	0.056		1.655	0.163	
Seed size	-0.323	0.202	0.110	0.267	0.237	0.261	0.049	0.047	0.294	0.167	0.140	0.232
Seed size <sup>2</sup>										-0.068	0.055	0.215
Seed number	-0.207	0.174	0.235	<b>0.461</b>	<b>0.219</b>	<b>0.035</b>	-0.048	0.041	0.246	0.204	0.121	0.092
Germination temperature(warm)	<b>2.568</b>	<b>0.225</b>	<b>&lt;0.001</b>	<b>-2.529</b>	<b>0.256</b>	<b>&lt;0.001</b>	<b>0.374</b>	<b>0.051</b>	<b>&lt;0.001</b>	<b>-0.077</b>	0.147	0.601
Parental temperature(future)	-0.284	0.273	0.298	0.550	0.311	0.077	-0.069	0.063	0.274	-0.230	0.182	0.206
Size:germ temp(W)	<b>0.501</b>	<b>0.229</b>	<b>0.028</b>	<b>-0.556</b>	<b>0.264</b>	<b>0.035</b>	0.025	0.053	0.637	0.201	0.150	0.180
Size:par temp(F)	0.347	0.271	0.201	-0.266	0.309	0.389	0.024	0.061	0.692	0.021	0.192	0.915
Number:germ temp(W)	-0.072	0.225	0.748	-0.196	0.265	0.460	0.039	0.052	0.461	0.009	0.152	0.951
Number:par temp(F)	0.441	0.345	0.201	-0.480	0.391	0.220	0.035	0.079	0.661	-0.441	0.226	0.051
<b>Random effects</b>												
germ temp:ID	var		$p(\text{ML})$	var		$p(\text{ML})$	var		$p(\text{ML})$	var		$p(\text{ML})$
line(int.)	<b>0.9449</b>		<b>&lt;0.001</b>	<b>1.3225</b>		<b>&lt;0.001</b>	<b>0.0399</b>		<b>&lt;0.001</b>	<b>0.3794</b>		<b>&lt;0.001</b>
Residuals	<b>0.5862</b>		<b>0.0003</b>	<b>0.5122</b>		<b>0.0028</b>	<b>0.0209</b>		<b>0.001</b>	<b>0.1592</b>		<b>0.003</b>
							0.0443					

**FIGURE 3** Relationships between seed traits and germination traits under different germination temperatures. Germination traits: (a, b) germination percentage; (c, d) percentage of dormant seeds; (e, f) germination velocity (the reciprocal of the mean germination time). Panels on the left: (a, c, e) regressions against seed size; panels on the right: (b, d, f) regressions against seed number. Blue lines and points: Cool germination temperature; red lines and points: Warm germination temperature. Points are raw data. Regressions are predictions estimated from model outputs; grey ribbons around regressions are standard errors.



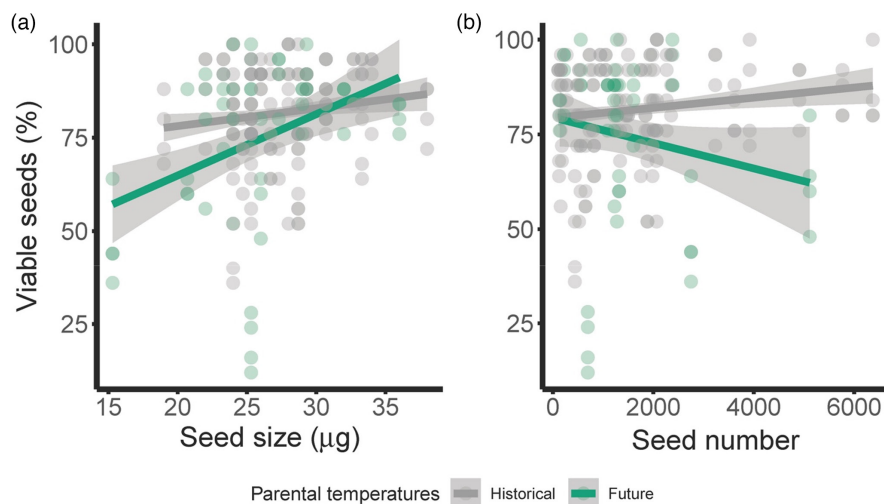
snow deposition (for nondormant seeds) or before the peak of the growing season (for dormant seeds).

On the other hand, seed number correlated with the proportion of dormant seeds in parents grown under historical temperatures. The increase in the dormant seed fraction in larger batches of seeds may represent a mechanism to reduce the competition among siblings during seedling emergence and establishment (Campbell et al., 2017; Grundy et al., 2003; Lampei et al., 2017; Vitalis et al., 2013) and increase the probability of germination success across seasons. This result suggests that there is intraspecific variation in germination strategies in *W. ceracea*. When grown under optimal conditions, individuals that produce larger batches of seeds may adopt a more conservative strategy with seeds persisting in the seed bank. Parents grown under future, warmer temperatures instead increased the dormant fraction (*see below*) independently from how many seeds they produced.

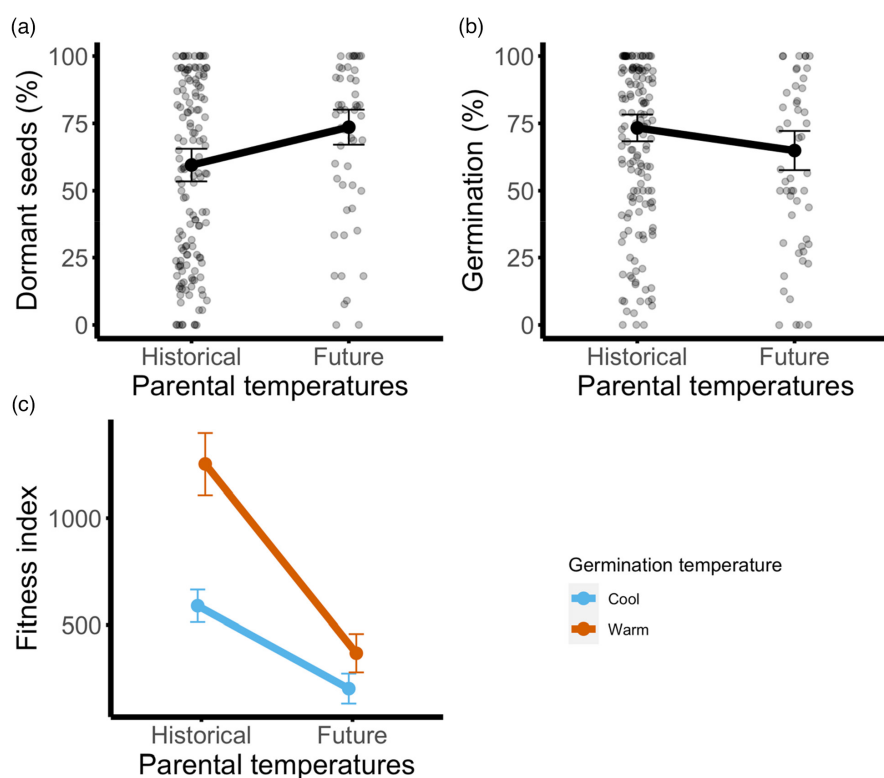
### 4.3 | Warming affects parental success and germination strategies

#### 4.3.1 | Plastic changes in seed traits did not affect germination

Despite the effect of seed size on germination velocity and seed viability, there was no evidence that the reduction in either seed size or number under future temperatures affected any of the germination responses investigated here. Viable seeds were equally likely to germinate regardless of parental growth conditions. Nevertheless, we did find evidence of parental effects in our study; parents grown under future temperatures produced a higher fraction of dormant seeds. It is possible that overall proportions of dormant seeds found in this study may be smaller if dry storage of seeds caused any after-ripening (dormancy alleviation under dry conditions; Baskin &



**FIGURE 4** Relationships between seed viability and (a) seed size or (b) seed number for seeds developed under different parental temperatures. Grey lines and points: Parents under historical (cooler) temperatures; green lines and points: Parents under future (warmer) temperatures. Points are raw data. Regressions are predictions estimated from model outputs; grey ribbons around regressions are standard errors.



**FIGURE 5** Reaction norms for the effect of parental temperatures on (a) dormant seeds (%), (b) germination percentage (GP; %), and (c) a fitness index (calculated as seed number  $\times$  GP). Points in (a) and (b) are raw data. In (c), blue line: Cool germination temperature; red line: Warm germination temperature. Summary values are predictions from the model outputs. Bars are standard errors.

Baskin, 2020). However, given that all seeds were treated equally, storage conditions would not have affected differences in dormancy patterns between parents grown under historical or future temperatures.

In contrast with our result, prior studies have found higher proportions of dormant seeds in parents grown under cool conditions (Bernareggi et al., 2016; Fenner, 1991; Huang et al., 2018; MacGregor et al., 2015). These studies encompassed many experimental approaches and design, including lab and field studies, and thus could be interpreted as suggesting that the reductions in the dormant seed fraction under warmer temperatures could be a general response. But the opposite was found in *W. ceracea*, here and in Wang et al. (2021), suggesting either that this may be a specific

response of this species to warming, that alpine plants differ in the effect of growth temperature on dormancy, or that the pattern is not as consistent as it seems. More studies are needed to investigate parental effects on dormant seed fractions in alpine plants to validate the generality of our result.

#### 4.3.2 | Warming during seed germination increased success of larger seeds

In our study, warming increased germination velocity in addition to inducing GP. This result agrees with a general effect of warmer temperatures in advancing phenology in plants and animals (Cohen et al., 2018;

Gugger et al., 2015). Higher temperatures may increase the rate of the chemical reactions inside seeds that ultimately lead to germination. More studies that directly analyse the effect of temperature on germination velocity are required to assess the generality of these results.

The results of our study suggest that seeds of a different size may have different optima for germination (interaction germination temperature  $\times$  seed size). A worldwide survey on angiosperms and studies on species that require fire to germinate demonstrate that larger seeds need lower temperatures to reach maximum germination (Arène et al., 2017; Hanley et al., 2003; Liyanage & Ooi, 2018). In *W. ceracea*, we found the opposite trend, where warmer temperatures tended increase germination success of larger seeds. There is also a chance that this result reflects different sensitivity of large versus small seeds to after-ripening during dry storage. However, given that after-ripening (if present) tends to be slow in species that require cold stratification to break dormancy (Baskin & Baskin, 2020), such as *W. ceracea*, we suspect this effect is not likely to be large.

Warm-cued germination, that is, the induction of germination at higher temperatures, is well-documented among many alpine species (Billings & Mooney, 1968; Fernández-Pascual et al., 2021). Plants adapted to cold environments possess relatively high temperature optima for germination (Körner, 2003). This is considered a physiological mechanism to reduce germination in autumn or early spring when the probability of frost is high (Billings & Mooney, 1968). Then, exposure to winter temperatures (cold stratification) reduces temperature requirements for later germination (Shimono & Kudo, 2005). Such a response suggests that the temperature window for germination is narrow and there is a fraction of seed that is in effect conditionally dormant when germination temperatures are low. Future climate projections predict that the growing season in alpine ecosystems will lengthen, and autumns will be warmer (Harris et al., 2016). Therefore, autumn germination (soon after dispersal) is more likely to occur in *W. ceracea* (and possibly many other alpine species), which then exposes seedlings to warmer temperatures or weather extremes that might reduce seedling survival. In this context, the seedlings emerging from larger seeds may have greater survival probability until winter. Indeed, seed size is usually positively correlated to seedling size, survival, and competitiveness under limiting conditions, such as nutrient shortage, drought, and shade (Moles & Leishman, 2008; Pivatto et al., 2014; Tungate et al., 2006). Thus, if *W. ceracea* individuals can produce seeds of different sizes within the same reproductive event, then the differential responses of germination to temperature mediated by seed size may represent another case of bet-hedging strategy in this species to overcome environmental or climatic uncertainty at the end of the growing season (Jiang et al., 2019; Scholl et al., 2020).

#### 4.4 | Fitness consequences of a warming climate on alpine plants

Alpine environments are considered particularly threatened by climate change, but research increasingly suggests alpine species may

be resilient to at least occasional warmer seasons. Temperatures in alpine environments are usually below optimum, therefore periodic warmer seasons can stimulate growth and in some species flower production (Frei, Ghazoul, Matter, et al., 2014; Frei, Ghazoul, & Pluess, 2014; Notarnicola et al., 2021). Alpine plants also possess responses to cope with high temperatures. Examples are physiological acclimation to tolerate heat (although studies in alpine environments are lacking; Buchner et al., 2017; Geange et al., 2021; Notarnicola et al., 2021), early flowering onset (shift in phenology; Frei, Ghazoul, Matter, et al., 2014; Frei, Ghazoul, & Pluess, 2014; Gugger et al., 2015; Notarnicola et al., 2021), and germination strategies, where seed dormancy scatters recruitment in different seasons (Hoyle et al., 2015; Satyanti et al., 2019). However, high temperatures also have detrimental effects on plants. Here, we calculated a fitness index for *W. ceracea* ( $\text{Fitness index} = \text{seed number} \times \text{GP}$ ) to summarise the effects of temperature applied on the whole life cycle, from parents to seeds. We found that individuals grown under future, warmer conditions underwent a serious reduction in their overall fitness. The fewer and less viable seeds developed under future temperatures greatly reduced seedling recruitment by more than fourfold. Therefore, a consistently warmer environment due to a sustained rise in temperatures in the long term may benefit those subalpine species that are both better adapted to and better competitors under warmer temperatures, to the potential detriment of alpine species (Seastedt & Oldfather, 2021). Such shifts in vegetation composition have already been documented in the European Alps (Pauli et al., 2012; Steinbauer et al., 2018). While we focused this study on the effect of temperature, we do note that the concurrent reductions in precipitation projected for future climates (Hock et al., 2019), will likely play an important role and intensify effects on fitness in interaction with temperature for alpine plants. Water stress due to decreased transpiration with lower precipitation in the vegetative phase could exacerbate impact of warming from seed and seedling phase through to maturity. Future studies are needed to address how drought and temperature interactions will affect seed traits and germination strategies and how these scale to lifetime performance.

In addition, while the effect of warmer temperatures in increasing germination is often considered positive by the scientific community, it will impact the capacity of alpine plants to scatter germination across seasons and environmental conditions. Plants will need to adapt their germination strategies (as we found in *W. ceracea* that increased the dormant seed fraction). Although there is evidence of higher winter-survival in autumn-emerged seedlings in warmer rather than cooler sites (Mondoni et al., 2015), more studies are needed to investigate the consequences of potentially premature germination on the fate of the early emerging seedlings and on reductions of the soil seed bank.

Despite being part of the equation for adaptive change, intraspecific trait variation is seldom discussed and investigated in plant ecophysiology. Here, we found significant intraspecific variation in all the germination traits considered in this study. Particularly, significant intraspecific variation in GP means that lines differ in their overall fitness. Therefore, selection may act upon this variation in germination traits, if at least part of it is heritable, possibly causing

adaptive switches in the optima for these traits and ultimately leading to changes in seed traits and germination strategies.

## 5 | CONCLUSIONS

Our study demonstrates the importance of seed traits in germination responses and highlights the sensitivity of germination to temperature. We showed here that the seed size versus number trade-off is dependent on growing conditions, where future, warmer growth temperatures limit the optimal investment in reproduction. Indeed, despite the higher investment in flower production, smaller and fewer seeds were produced under these conditions, which broke down the trade-off. Larger seeds were both more viable and germinated faster. Seed number also correlated with proportions of dormant seeds, suggesting a mechanism to reduce competition among siblings in large batches of seeds. Despite these relationships, the reductions of both seed size and number in future climates did not affect germination responses nor percentage. Instead, warming during germination increased germination percentage and velocity. While we observed a drastic reduction in fitness under warming, there is concurrently substantial intraspecific variation in many traits that may hold promise for future adaptive change. For the first time in alpine plants, we show here that larger seeds required higher temperatures to trigger germination than smaller seeds. If this result is representative of other alpine species, then warmer climates will trigger germination in autumn and further studies should investigate whether this will have positive or negative effects on population dynamics.

### AUTHOR CONTRIBUTIONS

All authors contributed to experimental design and rationale. Rocco F. Notarnicola conducted the experiment, the statistical analysis and wrote the manuscript; Loeske E. B. Kruuk and Pieter A. Arnold contributed to statistical analysis; Adrienne B. Nicotra, Loeske E. B. Kruuk and Pieter A. Arnold contributed to revising the manuscript and gave final approval for publication.

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### CONFLICT OF INTEREST

All authors involved in this manuscript have no conflict of interest.

### PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/1365-2745.14014>.

### DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository <https://doi.org/10.5061/dryad.np5hqbzx9> (Notarnicola et al., 2022).

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## SUPPORTING INFORMATION

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