The seed germination spectrum of alpine plants: a global meta-analysis

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Summary

- Assumptions about the germination ecology of alpine plants are presently based on individual species and local studies. A current challenge is to synthesize, at the global level, the alpine seed ecological spectrum.
- We performed a meta-analysis of primary data from laboratory experiments conducted across four continents (excluding the tropics) and 661 species, to estimate the influence of six environmental cues on germination proportion, mean germination time and germination synchrony; accounting for possible effects of seed morphology (mass, embryo:seed ratio) and phylogeny.
- Most alpine plants show physiological seed dormancy, a strong need for cold stratification, warm-cued germination and positive germination responses to light and alternating temperatures. Species restricted to the alpine belt have a higher preference for warm temperatures and a stronger response to cold stratification than species whose distribution extends also below the treeline. Seed mass, embryo size and phylogeny have strong constraining effects on germination responses to the environment.
- Globally, overwintering and warm-cued germination are key drivers of germination in alpine habitats. The interplay between germination physiology and seed morphological traits further reflects pressures

to avoid frost or drought stress. Our results indicate the convergence, at the global level, of the seed germination spectrum of alpine species.

Keywords

alpine; alternating temperature; cold stratification; embryo endosperm ratio; light germination; seed dormancy; seed germination; seed mass

Introduction

Alpine environments occur worldwide above the maximum elevation at which trees can grow naturally (Körner & Paulsen, 2004; Körner *et al.*, 2011; Testolin *et al.*, 2020). These treeless habitats are characterized by low temperatures, unstable substrates and short growing seasons (Körner, 2003). Although most alpine plants can reproduce clonally (Körner, 2003), sexual reproduction by diaspores (i.e. seeds, fruits, or fruits with extra-ovarian structures attached; hereafter 'seeds') is the main strategy to maintain genetic diversity and to colonize suitable new sites in response to environmental changes (Walck *et al.*, 2011). However, the short reproductive season of the alpine habitat constrains the phenological timing of flowering, pollination, seed development and seed dispersal. For regeneration by seed to be successful, dispersed propagules must germinate in the most appropriate period to ensure the survival of seedlings (Chambers *et al.*, 1990; Schütz, 2002; Forbis, 2003). The physiological process of seed germination is therefore an essential life stage that must be timed to occur when the environment is favourable for subsequent seedling survival and growth (Poschlod *et al.*, 2013). In alpine habitats, seed germination will also be a crucial test for plant populations coping with global change, determining whether they will persist or go into extinction.

Natural selection is expected to favour seed germination requirements that reduce the probability of facing environmental conditions which are not appropriate for seedling survival and growth (Angevine & Chabot, 1979). Thus, germination is chiefly regulated by environmental cues related to water availability and soil temperature (Bewley *et al.*, 2013). The propagules of many plant species have also developed seed dormancy, by means of which germination is prevented during periods that are only ephemerally favourable, like a short warm spell in the middle of winter (Baskin & Baskin, 2014). Different degrees of dormancy within a seed population ensure the distribution of seedling emergence across time, a form of bet-hedging offspring survival against unpredictable environments (Venable, 2007). Other germination cues, such as the response to diurnal alternating temperatures (Thompson, 1977) and the need for light or darkness (Carta *et al.*, 2017), allow for a fine-scale detection of germination micro-niches and safe sites (Jumpponen *et al.*, 1999). Given that current assumptions about the germination ecology of alpine plants are mainly based on local studies, a current challenge is to synthesize, at the global level, the seed ecological spectrum of alpine plants. The seed ecological spectrum (Saatkamp *et al.*, 2019) is defined as a set of seed traits that determines the ability of plants to disperse, persist, germinate, and establish in different habitats. A major goal for trait-based seed ecology is to study the relationships and trade-offs between different aspects of the seed ecological spectrum (Saatkamp *et al.*, 2019), such as the physiological drivers of germination versus key morphological traits like seed mass (Moles *et al.*, 2005) and the embryo to seed ratio (Vandelook *et al.*, 2012).

Traditionally, alpine species have been described as having seeds with physiological dormancy, i.e. a type of dormancy that is caused by the seed's internal balance of phytohormones and requires a long exposure to dormancy-breaking treatments to be terminated (Schwienbacher *et al.*, 2011; Sommerville *et al.*, 2013; Baskin & Baskin, 2014). This physiological dormancy would prevent precocious germination of recently dispersed seeds during autumn, when appropriate conditions are not likely to persist for more than a few weeks or days (Meyer & Monsen, 1991). A requirement for cold stratification to break dormancy allows seeds to sense the snow season, thereby postponing germination to a more favourable period for seedling survival and development. Thus, it can be expected that a cold stratification period is a requirement for seed germination in alpine plants. One of the first experiments on this topic was conducted by Söyrinki (1938), who showed how the storage of arctic-alpine species at winter temperatures (i.e. cold stratification) increased seed germination in most species. Nonetheless, Amen (1966) later suggested that cold stratification was not a requirement for seed germination of alpine species from different mountain ranges in the USA, a finding that was supported by subsequent studies (Sayers, 1966; Marchand & Roach, 1980; Kaye, 1997). More recently, research on Australian alpine plants found that a cold stratification period only increased germination in half of the species tested (Sommerville *et al.*, 2013; Hoyle *et al.*, 2015). Cold stratification increased seed germination over a range of temperatures in most of the species studied in the alpine zone of Japan (Shimono & Kudo, 2005). Along an elevational gradient in the central Chilean Andes, cold stratification was an important requirement for seed germination in species from lower elevations, while species from higher sites did not germinate after a single period of cold stratification (Cavieres & Sierra-Almeida, 2018). Finally, in a comparative study on germination of unstratified seeds from different habitats of the Austrian Alps, species from alpine grasslands had the lowest final germination proportion, suggesting that they had the highest level of dormancy (Margreiter *et al.*, 2020).

Seed dormancy is not the only trait that can prevent, in practice, autumn germination. Early studies on the germination ecology of alpine plants demonstrated that, in most species, recently dispersed seeds require relatively high temperatures for germination (Söyrinki, 1938; Bliss, 1958; Amen, 1966; Billings & Mooney, 1968). Warm-cued germination has been considered as an adaptation to prevent seed germination at the time of seed dispersal (autumn) when temperatures are relatively low and there is a high risk of frost (Cavieres & Arroyo, 2000). Indeed, germination of alpine seeds tends to occur after winter, mainly in early summer (Körner, 2003; Mondoni *et al.*, 2015). In recent times, an increasing number of studies reported that fresh seeds of some alpine plants can also germinate at cool incubation temperatures or during cold stratification treatments (Schwienbacher *et al.*, 2011; Hoyle *et al.*, 2015; Fernández-Pascual *et al.*, 2017a; Cavieres & Sierra-Almeida, 2018). This cold-cued germination could be an adaptation to germinate under snow or during snowmelt, which could presumably allow seedlings to develop a deeper root system before topsoil desiccation in summer (Kammer & Möhl, 2002), or to attain an optimal size for overwintering (Billings & Mooney, 1968). Yet, the generality of warm-cued germination across different alpine regions and habitats has never been studied systematically, and the relative importance of cold stratification versus warm-cued germination remains unclear. A central question is whether physiological seed dormancy plays a relevant role in postponing alpine germination during autumn and winter, or whether the warm-cued germination is enough to prevent germination under cool autumn temperatures. The response to this question becomes pertinent under a warming climate, which could result in non-dormant seeds germinating readily in autumn. Non-dormant seeds could possibly have an advantage as the growing seasons become longer, if seedlings have enough time to grow and become sufficiently large to survive the winter snow season. Potentially, having a non-dormant or less dormant seed could be a better strategy for alpine plants to thrive under future climate (Verdú & Traveset, 2005; Mondoni *et al.*, 2015). However, we might also expect that seedlings from these species will be exposed to frost damage if snow starts to melt earlier and insulation during winter disappears (Venn *et al.*, 2013) and therefore mechanisms that enable diverse germination timing (i.e. low germination synchrony) may be favoured.

Besides patterns of seed dormancy and germination temperature, the response of alpine seeds to diurnal alternating temperatures and light can provide further information on their capacity to detect fine-scale environmental cues. Fluctuations of diurnal temperature decrease with increasing burial depths and the depth profile of this decrease depends on soil type, vegetation cover and snow (Thompson, 1977; Van Assche & Vanlerberghe, 1989; Saatkamp *et al.*, 2011). Solar irradiance in physiologically significant quantities penetrates only the first millimetres of the soil (Tester & Morris, 1987). Therefore, the response to alternating temperatures and light can indicate seeds whether they are buried deep in the soil, in rock crevices or under snow. The depth of seed burial is crucial for seedling emergence, as small seeds lack the resources to survive until they reach the soil surface (Bond *et al.*, 1999). A study of 445 species from the Qinghai-Tibet plateau found that species from high elevation alpine meadows did not show a significant response to diurnal temperature oscillations (Liu *et al.*, 2013). This is intriguing given that alpine habitats typically have a strong day-night climatic variation during the growing season. Regarding light and darkness, some local studies have found alpine species to have a preference for germinating in light (Densmore, 1997; Mondoni *et al.*, 2009). This trait is linked with the creation of a persistent soil seed bank (Venn & Morgan, 2010; Jaganathan *et al.*, 2015) which may be advantageous in the temporally and spatially unpredictable alpine environments (Kalin Arroyo *et al.*, 1999; Cavieres & Arroyo, 2001). Indeed, persistent alpine soil seed banks are more frequent than previously supposed, as consistently reported in recent years (Venn & Morgan, 2010; Hoyle *et al.*, 2013; Jaganathan *et al.*, 2015). Nevertheless, other alpine species seem to prefer germination in darkness (Schwienbacher *et al.*, 2011), which may be interpreted as a strategy for detecting safe sites in rock crevices (Arslan *et al.*, 2011). Although some germination studies report laboratory experiments conducted with constant and alternating temperatures, or light and dark conditions, they are very much case-dependent, and therefore it is difficult to evaluate whether these factors show a consistent pattern across global alpine ecosystems.

In this work, we make a global collection of primary germination data from laboratory experiments conducted with seeds of alpine plant species from extratropical regions. The assembled dataset represents eight seasonal alpine regions belonging to four continents. We perform a meta-analysis of this dataset to investigate the germination response of alpine plants to key environmental factors: dormancy-breaking treatments (cold stratification, GA_3 and scarification), average germination temperature, diurnal alternating temperatures and light (Bewley *et al.*, 2013; Baskin & Baskin, 2014). Given the heterogeneity of alpine species lineages adapted to different regions, we account for the shared evolutionary history using a phylogenetic comparative approach (Garamszegi, 2014). We further analyse the relationships between germination ecology and two morphological traits which are key drivers of the seed ecological spectrum: seed mass and the embryo to seed ratio (Saatkamp *et al.*, 2019). Our main aim in conducting this work is to test the consistency of general assumptions about the influence of major environmental cues on seed germination of alpine plants. Specifically, we determine at the global scale whether alpine seeds: (1) have physiological dormancy; (2) require cold stratification to break dormancy; (3) need relatively warm temperatures to germinate; (4) do not require alternating temperatures for germination; and (5) respond positively to light. To understand the ecological constraints of our meta-analysis on alpine species pools, we compare the germination responses of strict alpine species (that occur exclusively above the treeline) and generalist species (that occur above the treeline but also at lower elevations). We can expect that the former group will show a more convergent alpine germination syndrome, while the latter will be more plastic in their response.

Materials and Methods

Data collection

We conducted a meta-analysis of primary data (Mengersen *et al.*, 2013), which consists of pooling together original data from different local studies to synthesize their conclusions at the global level. All data processing, analysis and manuscript production has been performed in *R* (R Core Team, 2020). The original data, plus R code for the analysis and creation of the manuscript using Rmarkdown, can be accessed at the GitHub repository (see Data Availability Statement). To gather data, we contacted research groups which have performed experiments on seed germination of alpine plants, asking them to provide raw data from laboratory germination experiments. The submitted data had to report the studied species, the germination treatments, the number of seeds sown, and the number of seeds germinated. We received data (Table 1) on species from the Cantabrian Mountains of Spain, the European Alps, the Italian Apennines, the Northern Caucasus, the Qinghai-Tibet Plateau of China, the Chilean Andes, and the Australian Alps. We also received data on European alpine species from *Enscobase*, the database of the European Native Seed Conservation Network (http://enscobase.maich.gr/index.tml).

Table 1: Description of the datasets contributing primary data to this meta-analysis. The number of germination records, number of species and number of strict alpine species are given.

Source	Coverage	Records	Species	Strict
Briceño, unpublished	Andes	236	25	5
Bu et al. (2007, 2008)	Qinghai-Tibet	1766	242	68
Cavieres & Arroyo (2000), Cavieres & Sierra-Almeida (2018)	Andes	804	31	6

Source	Coverage	Records	Species	Strict
enscobase.maich.gr	Europe	510	124	75
Fernández-Pascual et al. (2017a)	Cantabrian Mts	792	22	14
Liu et al. (2013)	Qinghai-Tibet	465	151	42
Mondoni, unpublished	Apennines	32	10	7
Mondoni et al. (2009), Mondoni et al. (2012)	European Alps	506	101	74
Rosbakh, unpublished	Caucasus	263	18	9
Rosbakh & Poschlod (2015)	European Alps	971	21	15
Satyanti, unpublished	Australian Alps	632	91	41
Sommerville et al. (2013)	Australian Alps	1395	13	3
Tudela-Isanta et al. (2018)	European Alps	376	47	29
Venn (2007), Venn & Morgan (2009)	Australian Alps	1051	15	5

We standardized the species names according to *The Plant List* (The Plant List, 2013) using the package *Taxonstand* (Cayuela *et al.*, 2019) in *R*. For each species, we recorded its elevation range from local floras (Hegi, 1906; Pignatti, 1982; Castroviejo, 1987; Brach & Song, 2006; Parolly & Rohwer, 2019), catalogues (Rodríguez *et al.*, 2018) and herbaria (*Australian National Herbarium*, https://www.anbg.gov.au/cgi-bin/anhsir). We used the elevation ranges (i) to remove from the dataset outlier species that do not grow above the treeline; and (ii) to classify the rest of the species as *strict alpine* (i.e. species that only grow above the treeline) or *generalist* (i.e. species that can grow above the treeline but also in the lowlands). For each species, we compiled three additional traits: seed dormancy class, seed mass and embryo:seed ratio.

We classified the species in seed dormancy classes following Baskin & Baskin (2014) and Rosbakh et al. (2020a), using five categories: physiological (germination prevented by the seed's internal balance of phytohormones), morphological (germination prevented by an embryo which is underdeveloped at the time of dispersal), morphophysiological (both physiological and morphological dormancy present), physical (germination prevented by a water-impermeable seed coat) or non-dormant. We followed the concept by Vleeshouwers et al. (1995) to define dormancy as an inner seed property (sometimes called 'endodormancy'), which should be differentiated from the absence of appropriate germination cues in the environment ('ecodormancy'); and the classification system of Baskin and Baskin (2004) based on previous work by Nikolaeva et al. (1985) who consider morphological and physical dormancy as classes separated from physiological dormancy.

We collected mean seed mass for each species as provided by the Royal Botanic Gardens Kew Seed Information Database (2017) and additional sources (Bu *et al.*, 2007, 2008; Liu *et al.*, 2013). We recorded the embryo to seed surface area ratio for each species. Embryo to seed surface area values were calculated by dividing embryo area by the surface area of the seed, more specifically embryo plus endosperm and perisperm. Measurements were made using the *ImageJ* software (Schneider *et al.*, 2012) on drawings and photographs of seeds cut in half along the longitudinal axis, retrieved mainly from Martin (1946) and the Royal Botanic Gardens Kew Seed Information Database (2017), supplemented with own photographs (Vandelook, unpublished). For some species that were missing seed mass or embryo values, we calculated genus or family averages. In the case of seed mass, we used species-level values for 473 species, and genus averages for another 188 species. For embryo mass, we used 21 species-level values, 427 genus averages, and 213 family averages. The collected species traits, including information on which taxonomic rank was used to calculate the values for each species, is available in the data folder of the GitHub repository (see Data Availability Statement).

Germination response variables

We used the final germination proportion (i.e. the number of seeds germinated out of seeds sown) at 8 weeks as the main response variable for the germination treatments. Eight weeks was a compromise deadline to unify datasets, as there was a small fraction of trials that had run for longer times. We further calculated the mean germination time and the germination uncertainty index with the package *GerminaR* (Lozano-Isla *et al.*, 2019). Equations for these two germination indices are available in Lozano-Isla et al. (2019). Mean germination time is the time that it takes for half of the seed lot to germinate, indicating the germination speed in each experimental condition. The germination uncertainty index estimates how scattered germination is through time, giving an idea of whether germination is synchronous (i.e. most seeds germinate around the mean germination time) or asynchronous (i.e. germination events are separated through time during the germination experiment). Lower values in the germination uncertainty index indicate higher germination synchrony. The uncertainty index is analogous to the synchrony index and we used it because, unlike the latter, its values are not constrained between 0 and 1 (Lozano-Isla *et al.*, 2019). To calculate these indices, we used only records that met two conditions: (i) more than 50% final germination percentage; and (ii) a mean germination time under 4 weeks. Within these limits, we were able to calculate mean germination time and uncertainty for 293 species. We established this conservative approach to prevent the results being altered by a modification of the seed's dormancy status during the germination incubation (e.g. a fraction of the seed lot is non-dormant at the beginning of the incubation and germinates shortly after the start of the experiment; while another fraction is originally dormant, loses dormancy in response to the incubation conditions, and germinates in a second wave). The primary germination dataset is available in the data folder of the GitHub repository (see Data Availability Statement).

Statistical analyses

We meta-analysed germination data by fitting generalized mixed models with Bayesian estimation (Markov Chain Monte Carlo generalized linear mixed models, MCMCglmms) using the R package *MCMCglmm* (Hadfield, 2010). To model final germination proportion, we used binomial MCMCglmms, while for mean germination time and the germination uncertainty index we used gaussian MCMCglmms. Models had, as fixed effects, the experimental conditions (stratification, GA_3, scarification, temperature, alternating temperature and light), plus their interaction with seed mass and embryo:seed, and the strict alpine or generalist character of the species. Random effects included a reconstructed phylogenetic tree for the 661 species, and species identity, seed lot, lab (i.e. data provider), and alpine region (Table 1). To create the phylogeny, we used the R package *V.PhyloMaker* (Jin & Qian, 2019) which contains an updated mega-tree of the seed plants based on Smith & Brown (2018). We placed taxa absent from the mega-tree at the genus-level basal node. The phylogenetic tree is available in the data folder of the GitHub repository (see Data Availability Statement). In all models, all variables were scaled so their contribution to the effect sizes could be compared. We used weakly informative priors in all models, with parameter-expanded priors for the random effects. Each model was run for 500,000 MCMC steps, with an initial burn-in phase of 50,000 and a thinning interval of 50 (De Villemereuil & Nakagawa, 2014), resulting, on average, in 9,000 posterior distributions. From the resulting posterior distributions, we calculated mean parameter estimates and 95% Highest Posterior Density (HPD) and Credible Intervals (CI). We estimated the significance of model parameters by examining CIs, considering parameters with CIs overlapping with zero as non-significant. To estimate the phylogenetic signal of seed germination over all variables, we used Pagels's lambda ( ) (Pagel, 1999), estimated simultaneously with the models by calculating the mean of the posterior distribution and the 95% CI of as indicated by De Villemereuil et al. (2014). When = 0, related taxa are no more similar than expected by chance, while when = 1, the trait is evolving following a constant variance random walk or Brownian motion model; intermediate values of indicate a phylogenetic correlation in trait evolution that does not fully follow a Brownian motion model (Pagel, 1999). Detailed results of the MCMCglmms are available in the results folder of the GitHub repository (see Data Availability Statement).

Visualization of the alpine seed germination spectrum

To visualize the seed germination spectrum of alpine plants, we employed a Factorial Analysis of Mixed Data (FAMD) as implemented in the package *FactoMineR* (Le *et al.*, 2008). FAMD ordination combines the properties of PCA and MCA to jointly measure the variation of continuous and categorical variables. The ordination was performed at the species level, i.e. calculating a series of continuous and categorical traits for each species. We transformed the final germination proportions to create a continuous variable for each germination cue (i.e. stratification, GA_3, scarification, temperature, alternating temperatures and light). To do so, for each cue and species, we calculated a weighted average of the cue levels (in the case of temperature, cue levels were the temperature treatments; for the other cues the levels were 0 = absence and 1 = presence), weighting by the germination proportion at each level. This approach underrepresents the importance of the levels that were not tested for a given species, but can serve as a proxy of the response to the germination cues when visualized across the whole dataset; it must be stressed that this stage of the analysis serves only for visualization and not for hypothesis testing. We also included germination timing traits by calculating single values for the mean germination time (the minimum, i.e. the time taken at the most favourable treatment) and the germination uncertainty index (the mean). We conducted the FAMD with the 293 species for which we had mean germination time and germination uncertainty index values. We finally included the continuous variables seed mass and embryo:seed, as well as the categorical variables dormancy class (physiological, morphophysiological, morphological, physical or non-dormant) and distribution (strict alpine vs. generalist species).

Results

Description of the dataset

The final dataset used in our meta-analysis contained 9,799 records (i.e. germination proportions for a given seed lot of a species, recorded in a set of experimental conditions) contributed by 12 research groups, representing 62 seed plant families and 661 species. From these, 291 were categorized as strict alpine species and 370 as generalist. Seed lots were originally sampled in 8 alpine regions of the world (Table 1), at middle latitudes of both hemispheres.

Most species in the dataset were reported as having dormant seeds (**Fig. 1a**), but the frequency of the dormancy classes did not differ between generalist and strict alpine species. Among those species with dormant seeds, physiological dormancy was the most common dormancy class, followed by morphophysiological and then physical. Both strict alpine and generalist species had a similar range of values for seed mass (**Fig. 1b**) and embryo:seed ratio (**Fig. 1c**). The values of seed mass ranged from 0.02 mg (*Calceolaria purpurea*) to 58 mg (*Vicia orobus*), with a median value of 0.56 mg. Embryo:seed ratio encompassed the full range of potential values, from endospermic species with very small embryos (0.001) to non-endospermic seeds that store nutrients in the cotyledons (1). The median value was 0.34.

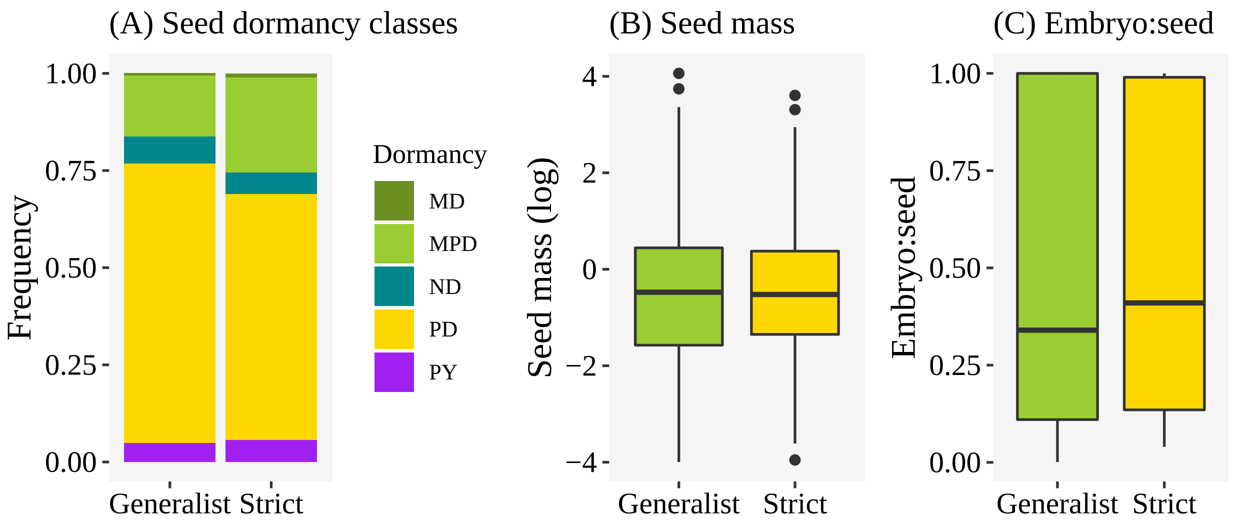


Figure 1: Seed dormancy classes, seed mass and embryo to seed ratio in the subsets of strict alpine and generalist species analysed in this study. Strict species are restricted to the alpine belt, generalist species can be found in the alpine belt but also below the treeline. Seed dormancy classes are abbreviated as follows: MD (morphological), MPD (morphophysiological), ND (non-dormant), PD (physiological), PY (physical).

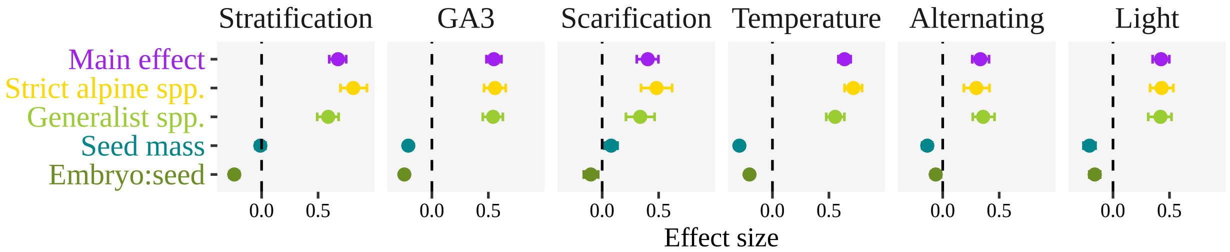
The total number of seeds used in the experiments was 365,808. Germination temperatures (i.e. the weighted average of the day and night temperatures, weighted by the duration of each phase) ranged from 0 to 36 ºC, with 7,521 records of constant temperatures (i.e. experiments that used the same temperature during all their duration) and 2,278 of alternating temperatures (i.e. experiments where different temperatures were applied during the day and the night, in diurnal cycles). Seeds were exposed to light during some part of the diurnal cycle in 8,933 records and kept in total darkness in 866 records. The experiments were performed with untreated seeds in 7,297 records, and of the rest, the majority (2,436) went through cold stratification (< 5 ºC, in darkness). Scarification (i.e. an abrasion of the seed coat to allow water imbibition) was performed in 119 records, and GA_3 (a plant hormone that promotes embryo growth and stimulates germination) in 651 records.

Dormancy breaking treatments

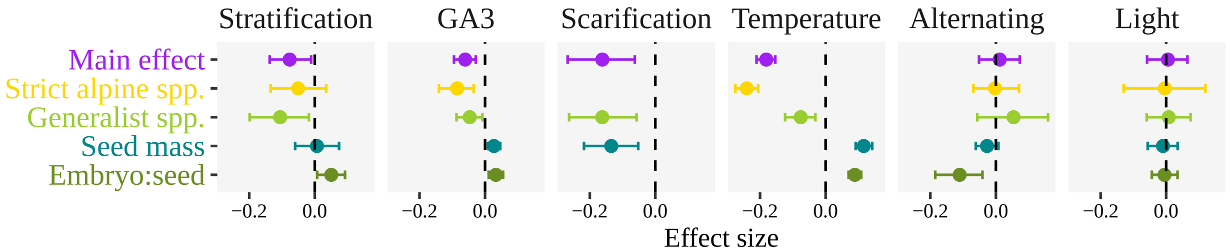
In general, stratification significantly increased the final germination proportion (**Fig. 2a**), as shown by a credible interval not overlapping zero. This positive effect occurred in both generalist and strict alpine species, although the effect size was larger in the strict alpine group. Stratification slightly reduced the mean germination time (**Fig. 2b**), which was evident in the generalist species, but not in the strict alpine species. Further, stratification reduced the germination uncertainty index as a general response (**Fig. 2c**), and slightly more so in generalist species. Seed mass did not interact with stratification, but species with larger embryos had a smaller response to stratification in their final germination proportion and reduced less markedly their mean germination time and germination uncertainty index. GA_3 had similar effects to stratification, with the main difference being that seed mass affected the response to GA_3, whereas it did not interact with the response to stratification.

Scarification promoted the germination proportion (**Fig. 2a**) and reduced the mean germination time (**Fig. 2b**), but it did not affect the germination uncertainty index (**Fig. 2c**). Strict alpine species were slightly more responsive to scarification than generalist species. Heavier seeds were more responsive to scarification, while seeds with larger embryos responded less.

(A) Effect on final germination proportions



(B) Effect on mean germination time



(C) Effect on germination uncertainty

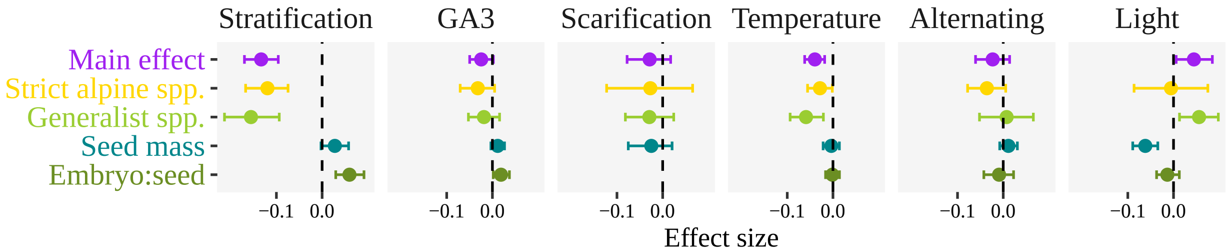


Figure 2: Effect of the germination environment on germination, according to the MCMC meta-analysis of primary data. The dots indicate the posterior mean of the effect size, and the brackets its 95 credible interval. The line of zero effect is shown. When the credible intervals overlap with the zero-effect line, the effect is not significant. Some effects that overlapped with zero (i.e. had no effect) and whose credible intervals were excessively wide are not shown for clarity's sake. The figure shows first the main effect, then the effects for the strict alpine and generalist groups, and then the interaction with seed mass and embryo:seed. A negative interaction (e.g. seed mass and light, in the case of the germination proportion) indicates that, as the interacting factor increases (e.g. seed mass) the response to the germination cue (e.g. light) decreases, in comparison with the main effect.

Germination temperature

Temperature had a positive significant effect on the final germination proportion (**Fig. 2a**), indicating a preference for warmer temperatures. Warmer temperatures also significantly decreased mean germination time (**Fig. 2b**) and the germination uncertainty index (**Fig. 2c**). The effect of warmer temperatures was slightly higher in strict alpine than in generalist species, although the credible intervals of the two groups of species do overlap. Seed mass and embryo:seed had a negative interaction with temperature, reducing the response to temperature. In other words, heavier and less endospermic seeds had a smaller increase in germination proportion and a smaller decrease in mean germination time when germinating in warmer temperatures. The germination uncertainty index did not show an interaction with seed mass or embryo:seed.

Alternating temperatures

Alternating temperatures had a positive effect on the final germination proportion (**Fig. 2a**), but not on the mean germination time (**Fig. 2b**) or the germination uncertainty index (**Fig. 2c**). The positive effect on the germination proportion was slightly higher in the generalist species. As in the case of the mean germination temperature, higher seed mass and embryo:seed values reduced the positive effect of alternating temperatures on the final germination proportion. Alternating temperatures resulted in faster germination (reduced the mean germination time) for seeds with low values of embryo:seed.

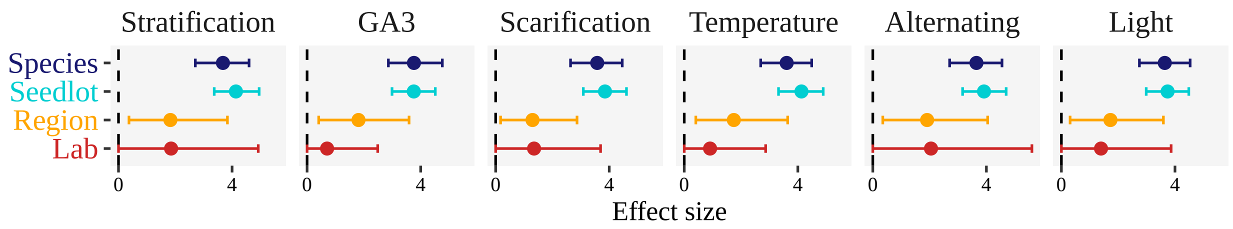
Light

Light increased the final germination proportion (**Fig. 2a**), with no differences between strict alpine and generalist species. It did not affect the mean germination time (**Fig. 2b**), but it did result in more variable germination (increased the germination uncertainty index) of generalist species (**Fig. 2c**). Heavier and less endospermic seeds responded less to light.

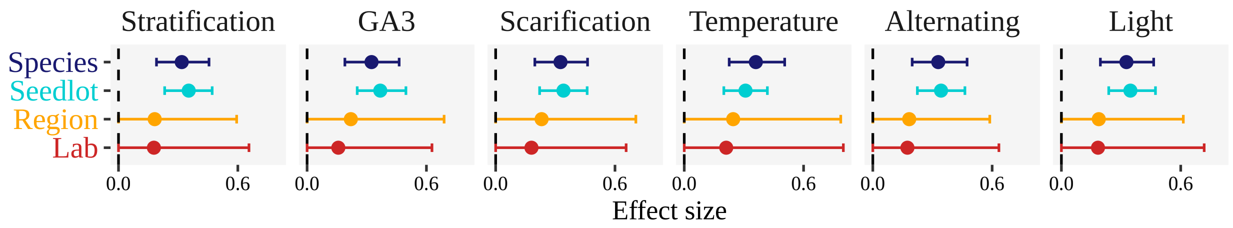
Random effects and phylogenetic signal

Random factors had a significant effect in the MCMCglmm models (**Fig. 3**). The final germination proportion and the mean germination time were affected more strongly, on average, by the seed lot and the species identity, suggesting that these traits are subject to intra- and interspecific variability, respectively. Alpine region and lab had a lower average effect and more variability as shown by their credible intervals. The germination uncertainty index showed the opposite pattern, with a stronger effect of lab and alpine region. The phylogenetic signal in the response to all germination parameters (**Fig. 4**) was significantly positive for the final germination proportion and the mean germination time. For the germination uncertainty index, it overlapped with zero, indicating a weak effect of phylogeny. The results for the germination uncertainty index indicate that it might have been more sensitive to the frequency of germination scoring, which was different in different labs. Detailed values of phylogenetic signal for all model interactions are available in the results folder of the GitHub repository (see Data Availability Statement).

(A) Effect on final germination proportions



(B) Effect on mean germination time



(C) Effect on germination uncertainty

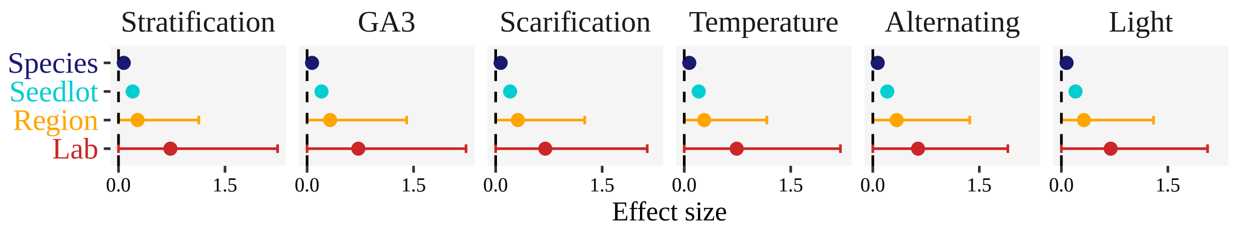


Figure 3: Effect of the random factors on germination, according to the MCMC meta-analysis of primary data. The dots indicate the posterior mean of the effect size, and the brackets its 95ç credible interval. The line of zero effect is shown.

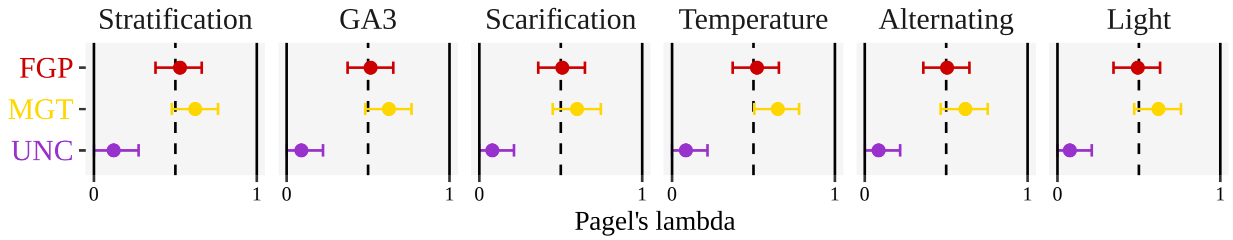


Figure 4: Phylogenetic signal in the response to the germination environment. When lambda = 0, related taxa are no more similar than expected by chance; when lambda = 1, the trait is evolving following a constant variance random walk or Brownian motion model. FGP = final germination proportion. MGT = mean germination time. UNC = germination uncertainty index.

FAMD ordination

The first FAMD axis explained 17% of the variation (**Fig. 5**). The quantitative variables with the largest contribution to this first axis were germination temperature, germination uncertainty, GA_3 and alternating temperatures. Among the qualitative factors, the strict alpine / generalist categories contributed to axis 1.

In summary, the horizontal axis separated (i, left) generalist species with a positive response to alternating temperatures and more germination uncertainty from (ii, right) strict alpine species with a preference for warmer temperatures and a positive response to GA_3. Axis 2 explained 12% of the variability. The main contributing variables were embryo:seed, mean germination time, seed mass and scarification; and the main categorical factors were physical dormancy and morphophysiological dormancy. This axis separated (iii, bottom) morphophysiologically dormant species with longer mean time to germination from (iv, top) physically dormant species that responded to scarification and had heavier, less endospermic seeds.

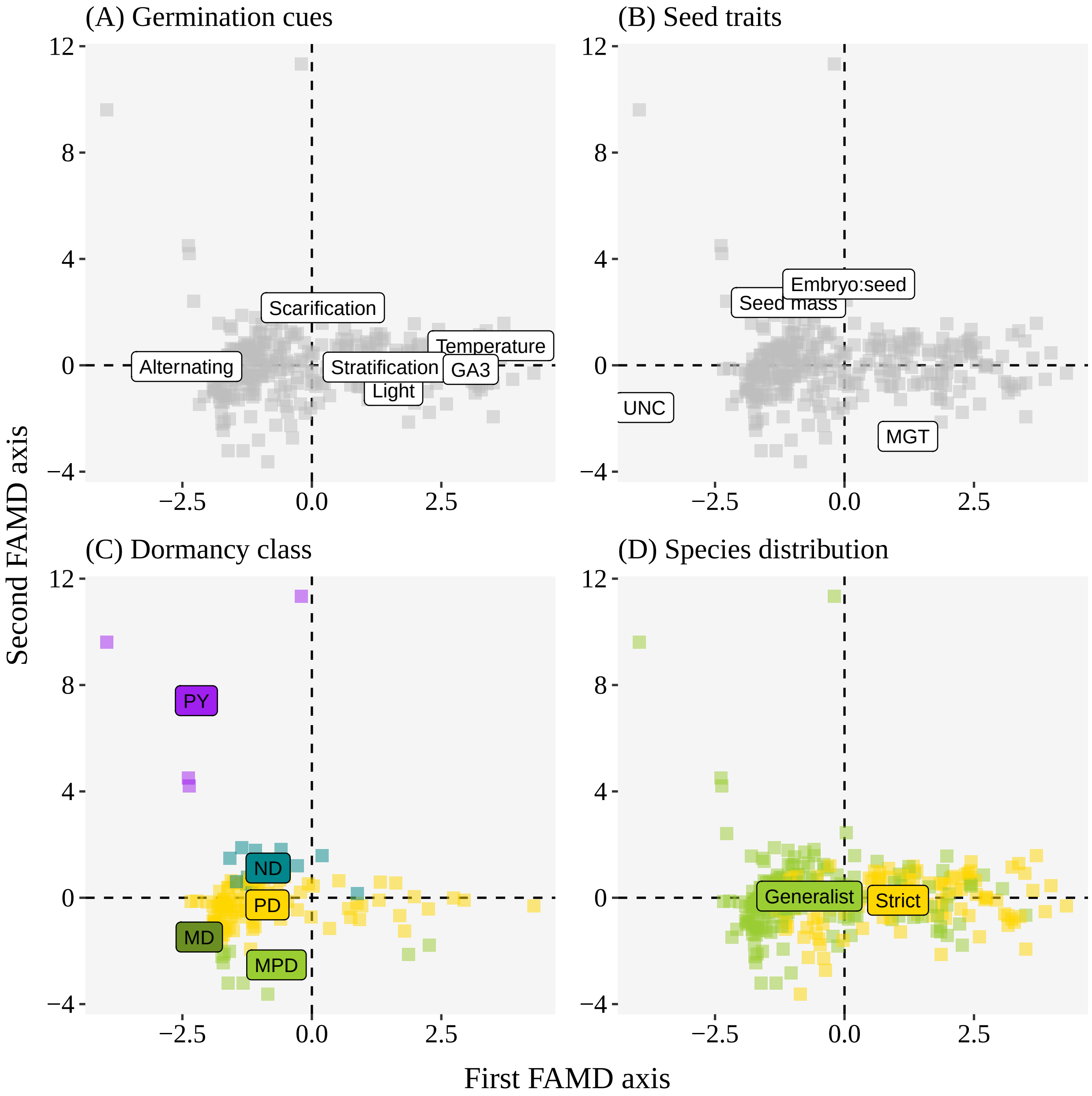


Figure 5: FAMD ordination of the alpine seed germination spectrum. Each square is a species. Labels indicate the contribution of the variables to the axes. Panels A and B correspond to quantitative traits; panel C and D to qualitative traits. MGT = mean germination time. UNC = germination uncertainty index. Seed dormancy classes are abbreviated as follows: MD (morphological), MPD (morphophysiological), ND (non-dormant), PD (physiological), PY (physical). Strict species are restricted to the alpine belt, generalist species can be found in the alpine belt but also below the treeline.

Discussion

Our meta-analysis of primary germination data shows that alpine seeds tend to germinate when, after weeks of exposure to conditions that mimic winter, they experience mid-summer temperatures. The germination ecology of strict alpine species is characterized by (1) physiological seed dormancy; (2) a strong need for cold stratification or GA_3 to break dormancy; (3) warm-cued germination; (4) a positive response to alternating temperatures, although not so prominent as in generalist species; (5) a positive response to light; (6) slow and relatively synchronous germination; and (7) an interplay with seed and embryo size, with smaller and more endospermic seeds being more responsive to stratification, warmth, alternating temperatures and light. Here we discuss the ecological significance of these responses and how they define the seed germination spectrum of alpine plants.

Seed dormancy

Our dataset shows that seed dormancy is frequent across alpine species and plays an important role in postponing plant recruitment to favourable times. The frequency of dormancy classes displayed by strict alpine and generalist species is similar, suggesting that this is a general trait of cold-adapted mountain species. Seeds with the two most frequent dormancy classes, i.e. physiological and morphophysiological, require a stratification period during which certain conditions (e.g. cold and wet, although other combinations are possible) are met over a period of months (Baskin & Baskin, 2014; Rosbakh *et al.*, 2020a). Indeed, our results show that cold stratification consistently promotes germination, while the phytohormone gibberellic acid (GA_3) works as a substitute of cold stratification to overcome physiological dormancy. Additionally, we found some species with physical dormancy. This dormancy type can be broken by mechanical scarification via freezing-thawing cycles in spring or extreme temperature fluctuations during summer, but it is also capable of responding to seasonal cycles of temperature and humidity (Van Assche *et al.*, 2003).

Together, these results confirm that overwintering is essential for promoting seed germination in alpine species, concurring with previous local studies (Cavieres & Arroyo, 2000; Schütz, 2002; Giménez-Benavides *et al.*, 2005; Shimono & Kudo, 2005; Sommerville *et al.*, 2013; García-Fernández *et al.*, 2015; Hoyle *et al.*, 2015; Fernández-Pascual *et al.*, 2017a; Cavieres & Sierra-Almeida, 2018). Since global climate change is causing major changes in snow cover duration (Beniston, 2012; Gobiet *et al.*, 2014), it could disrupt natural cold stratification cycles in alpine environments, compromising population viability and indirectly favouring species with no stratification requirement (Sommerville *et al.*, 2013). This risk will be partly alleviated by the plasticity of physiological seed dormancy in response to the seed maturation temperature (Fernández-Pascual *et al.*, 2013; Carta *et al.*, 2016b). 'Seed memory' via maternal effects facilitates acclimatisation to changing environments, and can reduce the stratification requirements of seeds produced in a warmer climate (Fernández-Pascual *et al.*, 2019).

Thermal regulation of germination

We found a general increase of seed germination with higher incubation temperatures. This effect was more pronounced in strict alpine compared to generalist species, confirming previous local studies (Walder & Erschbamer, 2015; Fernández-Pascual *et al.*, 2017a). It was also more pronounced in small seeded and endospermic species. Small-seeded species have a lower proportion of seedling survival under drought (Leishman & Westoby, 1994) but may require warmer germination temperatures than large-seeded species, especially in seasonal climates (Arène *et al.*, 2017). Considering that risk reduction is an important selective pressure for the evolution of germination traits (Venable & Brown, 1988), a plausible explanation for these patterns is that alpine plants received selection pressure from damaging spring frosts and evolved a frost avoidance mechanism by germinating at high temperatures (Taschler & Neuner, 2004; Marcante *et al.*, 2012; Rosbakh *et al.*, 2020b). Moreover, strict alpine species may be less subjected to drought damage during establishment compared to generalist species: as elevation increases, plants face less drought risk because precipitation increases (except in Mediterranean and semi-arid mountains), but at the same time there is a higher probability of early or late season frosts (Körner, 2003; Schrier *et al.*, 2007; Beniston, 2016). Together, these results indicate that germination patterns in alpine species are driven by an interplay of seed size and germination temperature to escape either frost or drought stress: small seeds are being selected for fast germination at warm temperatures, in order to escape unfavourable early-spring frost; while large seeds are being selected for slower germination under cooler conditions, which should maximize emergence during snowmelt (when water availability is highest) giving seedlings adequate time to develop a root system to cope with desiccation risks in summer. Supporting this view, seedlings of alpine pioneer species have a low frost resistance (Marcante *et al.*, 2012), yet tolerate heat up to 40–50°C (Marcante *et al.*, 2014).

In our analysis, increasing temperatures also decreased germination uncertainty, showing that germination synchrony is a plastic trait driven by temperature. While asynchronous germination is thought to be a form of adaptation to unpredictable environments (Wagner & Simons, 2009; Simons, 2011), our results indicate that asynchronous germination occurs when temperatures are relatively cool, likely as a bet-hedging strategy against the risk of early-spring frost. On the other hand, a fast and synchronised germination at warm conditions (i.e. in late spring) may be another important strategy that increases alpine seedling survival by avoiding drier conditions in summer. The extreme germination uncertainty syndrome, i.e. staggering germination so that emergence occurs both before and after winter, is known in alpine species (Körner, 2003; Hoyle *et al.*, 2015; Satyanti *et al.*, 2019). If autumn seedlings grow faster than spring seedlings (Satyanti *et al.*, 2019), this will likely have cascading effects on individual fitness along the life of the plant (Donohue *et al.*, 2005, 2010).

Alternating temperatures and light

We also found a positive germination response to alternating temperatures and light, conditions that mimic the environment in the upper soil and certain microsites during the snow-free season (Billings & Mooney, 1968; Körner, 2003; Mondoni *et al.*, 2012). Alternating temperatures had a slightly lower effect on strict alpine species, compared with generalist species, but still had some effect. This partly contradicts the findings of Liu et al. (2013), who found no response to temperature fluctuations for species distributed only at high elevations of the Qinghai-Tibet Plateau. Liu and co-workers argued that, in that area, high temperature fluctuations may occur throughout the year, and therefore fluctuating temperature alone may not be a reliable indicator of suitable conditions for seedling establishment and growth (Liu *et al.*, 2013). When analysed at the global level, alternating temperatures generally increase germination of alpine seeds, which corresponds well with the fact that most alpine regions have a strong day-night climatic variation during the growing season. It is also not surprising that we detected a negative interaction between seed mass and the germination response to light and alternating temperature regimes, since large seeds can afford to germinate at greater depths (where they are more protected from the alpine environment) and still reach the soil surface (Bond *et al.*, 1999; Pons, 2000).

The alpine seed germination spectrum

Using the whole set of traits compiled in this study we can visualize the seed germination spectrum of alpine plants. At one end of the spectrum, strict alpine species tend to germinate slowly and synchronously, require warm temperatures and light to germinate, and need stratification or GA_3 to break physiological dormancy. At the other end, generalist species tend to germinate fast, show a bet-hedging strategy (i.e. high germination uncertainty) and germination traits that suggest fast germination after specific cues (e.g. scarification or alternating temperatures). The dormancy classes without a physiological component (non-dormant, physical, and morphological) appear associated with generalist species. This gradient of regenerative strategies mirrors prominent gradients of fast or slow population dynamics (Silvertown *et al.*, 1992; Nobis & Schweingruber, 2013), fast or slow developing leaf traits (Wright *et al.*, 2004), or large sets of plant ecological features (Grime, 1977). 'Fast' regeneration can be understood as a plastic and opportunistic strategy to cope with frequently disturbed habitats such as avalanche ways, steep eroding slopes, regressing glaciers, and riverbeds (Pierce *et al.*, 2007; Gentili *et al.*, 2013). The 'slow' regeneration of strict alpine species, on the other hand, seems to be a more specialized syndrome and might be the result of convergent evolution acting on those species that are truly restricted to the alpine vegetation belt.

A general assumption of seed trait ecology is that seed mass and embryo:seed ratios are relatively conserved across related species (Moles *et al.*, 2005; Vandelook *et al.*, 2012). In our alpine dataset, both traits show no differences between strict alpine and generalist species. This is in line with the ambiguity of relationships between seed mass and elevation that have been found for alpine species, including examples of negative (Wang *et al.*, 2014), positive (Pluess *et al.*, 2005) or absent (Vandelook *et al.*, 2012; Bauk *et al.*, 2015) correlations. The similarity in embryo:seed ratio between generalist and strict alpine species also confirms the lack of correlation between embryo-seed ratio and elevation found in the Apiaceae (Vandelook *et al.*, 2012). In contrast with the supposed phylogenetic conservatism of these morphological traits, physiological germination responses are understood to be more plastic traits that can quickly respond to new situations (Fernández-Pascual *et al.*, 2019). While phylogenetic signal in seed germination is generally found, especially in highly selective environments (Carta *et al.*, 2016a; Arène *et al.*, 2017; Fernández-Pascual *et al.*, 2017b), germination patterns can also be shared between phylogenetically distant species (Vandelook *et al.*, 2019) and have low or absent phylogenetic signal (Rosbakh & Poschlod, 2015; Fang *et al.*, 2017; Fernández-Pascual *et al.*, 2017a). The phylogenetic signal in germination responses that we found here indicates that evolutionary history cannot be neglected when studying seed germination patterns. Furthermore, we detected that seed-embryo size influences germination responses to environmental cues, with smaller and more endospermic seeds being more responsive to warmth, light and alternating temperatures. Previous studies have shown that non-endospermic seeds are smaller and are likely to be non-dormant at the time of dispersal and thus germinate quicker than alpine endospermic seeds (Sommerville *et al.*, 2013; Hoyle *et al.*, 2015; Satyanti *et al.*, 2019). Together, these results indicate that seed morphology and physiology are connected and may be subjected to co-adaptation. This connection, intertwined with their shared phylogenetic history, may constrict the potential variability of the seed ecological spectrum.

Future directions

Our first global assessment of the seed germination spectrum in relation with seed morphology supports the existence of trade-offs between sets of regenerative traits (Saatkamp *et al.*, 2019), such as the relationship we found between seed/embryo size, germination speed and germination responses to temperature and light. Future studies will need to consider, as we did, evolutionary history and seed morphology as intrinsic constraints of the seed ecological spectrum; while the seed germination spectrum we described here will need to be explored at the community level (Jiménez-Alfaro *et al.*, 2016).

This study also highlights the general lack of seed germination studies on tropical alpine species. Although there is an increasing interest in the ecology of plants from tropical alpine regions, we could not find seed germination data to include in our meta-analysis. Compared with temperate alpine ecosystems, tropical alpine environments are relatively aseasonal and most plants have been reported as flowering throughout the year (Gehrke, 2018). Rather than being exposed to snow in winter and high temperatures in summer, tropical alpine plants face a year-round stress from night-time frost (Körner, 2003). These different selective pressures suggest that the cold-adapted germination syndrome of temperate alpine seeds may not be prevalent in the tropical alpine flora. That means that our study is valid for temperate alpine regions, where alpine habitats mainly occur (Testolin *et al.*, 2020), but not necessarily for tropical alpine systems. We thus recommend to focus future research on the seed ecology of high-mountain tropical areas.

Another aspect that calls for future efforts is a finer study of germination cues. To produce this meta-analysis, we had to assume some simplifications in order to merge disparate datasets: we considered coarse cues that are routinely recorded in germination tests, like the presence/absence of light, alternating temperatures or cold stratification. New research should aim to decompose these binary cues into finer quantitative variables which provide a more precise environmental signalling, such as the red:far red ratio (Jankowska-Blaszczuk & Daws, 2007), the length of the photoperiod (Black & Wareing, 1954), the amplitude of the diurnal thermal oscillations (Fernández-Pascual *et al.*, 2015) or the length and temperature of cold stratification (Pritchard *et al.*, 1996). In addition, new field research is necessary to assess germination phenology in response to temperature and water availability on the upper soil layer.

Conclusions

This study demonstrates how alpine plants from different regions of the world respond similarly to germination cues that support successful regeneration: cold stratification, warm and alternating temperatures, and light. These cues, particularly cold stratification and warm temperatures, are generally associated with cold-adapted herbaceous species in a broad sense (Fenner & Thompson, 2005; Baskin & Baskin, 2014; Rosbakh & Poschlod, 2015). Nonetheless, our results show that the response to stratification and temperature is more prominent in strict alpine species when comparing them with generalist plants that can grow opportunistically in the alpine belt. This suggests an evolutionary convergence of germination patterns in those species truly adapted to alpine ecosystems. We can conclude that, while alpine plants do not show a unique germination syndrome, they do show a more extreme or specialized version of the cold-adapted temperate germination syndrome.

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Author Contribution

BJA conceived the original idea. EFP, AC, AM, LC, SR, SV, AnS, LG, VB, FV, HB, KS, PP, KL, AN contributed data. EFP assembled and managed the database. AC and EFP analysed data. EFP led manuscript writing, with contributions from BJA, AC, AM, LC, SR, SV, AnS, LG, VB, FV, EM, ArS. All authors revised the manuscript and approved the final version.

Data Availability Statement

The original data, R code for the analysis and creation of the manuscript can be accessed at the GitHub repository https://github.com/efernandezpascual/alpineseeds. A version of record of the repository can be found at https://doi.org/10.5281/zenodo.4270344.

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