Seed germination ecology of alpine plants: a global meta-analysis of primary data

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# Summary (200 words)

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# Introduction

Alpine environments occur worldwide above the maximum elevation at which trees can grow (Testolin *et al.*, [2020](#ref-RN4750)). These treeless habitats are characterized by low temperatures, strong winds, unstable substrates, and short growing seasons (Körner, [2003](#ref-RN2392)). Although some alpine plants may reproduce clonally, sexual reproduction is the main strategy to maintain genetic diversity and to colonize suitable habitats in response to environmental changes. The short reproductive season of alpine habitats constrains the phenological timing of flowering, mating, seed development and seed dispersal. Further, dispersed seeds will need to germinate in the most appropriate period to ensure survival of seedlings under extreme environmental stress (Chambers *et al.*, [1990](#ref-RN4714); Schütz, [2002](#ref-RN2868); Forbis, [2003](#ref-RN4717)). 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](#ref-RN4691)). In alpine habitats, germination by seeds will be the final test for plant populations to cope with climate change and to determine whether they will persist or go into extinction.

Natural selection should favour seed germination requirements that reduce the probability of facing environmental conditions that are not appropriate for seedlings (Angevine & Chabot, [1979](#ref-RN3375)). The main physiological drivers of germination, water availability and temperature (Bewley *et al.*, [2013](#ref-RN3368)), are also the main ecological signals of climatic variation. Many plant species have also developed seed dormancy, by which germination is prevented during periods that are only ephemerally favourable, postponing germination to the right season (Baskin & Baskin, [2014](#ref-RN3214)). Different degrees of dormancy also ensure the distribution of offspring emergence across time, a bet-hedge effect against unpredictable environments (Venable, [2007](#ref-RN3065)). Other germination cues, such as the response to light (Carta *et al.*, [2017](#ref-RN4656)) and alternating temperatures (Thompson, [1977](#ref-RN1380)), allow for a fine-scale detection of germination micro-niches and safe sites (Jumpponen *et al.*, [1999](#ref-RN4719)). Given the heterogeneity of alpine climates and species lineages adapted to different regions, and the wealth of local studies on alpine seed ecology (reviewed in the following paragraphs), a current challenge is to synthesize, at the global level, the *seed germination spectrum* of alpine plants (**???**). Saatkamp et al. (**???**) define the seed ecological spectrum as the set of trait-based ecological responses of seeds that determines their ability to disperse, persist, germinate, and establish. A global synthesis of the alpine germination spectrum would allow us to (i) better understand how climatic changes will affect alpine plant diversity; and (ii) manage and eventually counteract regression of restricted alpine species.

Early studies on the germination ecology of alpine plants demonstrated that, in most species, recently dispersed seeds require relatively high temperatures for germination (Bliss, [1958](#ref-RN3258); Amen, [1966](#ref-RN3213); Billings & Mooney, [1968](#ref-RN4712)). Warm-cued germination has been considered as an adaptation to prevent seed germination at the time of seed dispersal (autumn) when temperatures are low and there is a high risk of frost (Cavieres & Arroyo, [2000](#ref-RN3393)). Indeed, germination of alpine seeds tends to occur after winter, mainly in early summer (Körner, [2003](#ref-RN2392); Mondoni *et al.*, [2015](#ref-RN2382)). In recent times, an increasing number of studies reported that fresh seeds of some alpine plants also germinate at cool incubation temperatures or during cold stratification treatments (Schwienbacher *et al.*, [2011](#ref-RN2943); Hoyle *et al.*, [2015](#ref-RN3285); Fernández-Pascual *et al.*, [2017a](#ref-RN2371); Cavieres & Sierra-Almeida, [2018](#ref-RN4713)). 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](#ref-RN4720)), or to attain an optimal size for overwintering (Billings & Mooney, [1968](#ref-RN4712)). The main question that remains to be answered is the generality of warm-cued germination across alpine micro-climatic niches.

Many alpine species have also been described as having seeds with deep physiological dormancy (Schwienbacher *et al.*, [2011](#ref-RN2943); Sommerville *et al.*, [2013](#ref-RN3703); Baskin & Baskin, [2014](#ref-RN3214)). Much like warm-cued germination, dormancy in recently dispersed seeds would prevent precocious germination under autumn cold conditions, when appropriate soil moisture and temperature are not likely to persist for more than a few weeks or days (Meyer & Monsen, [1991](#ref-RN3330)). A requirement for cold stratification to break dormancy would allow seeds to sense the snow season, thereby postponing germination to a better period for seedling survival and development. Thus, it can be expected that a cold stratification period is a common requirement for seed germination in alpine plants. Nonetheless, an early review (Amen, [1966](#ref-RN3213)) suggested that cold stratification was not a requirement for the seed germination of several alpine species from different mountains in the USA, an idea that was repeated in subsequent articles (Sayers, [1966](#ref-RN1484); Marchand & Roach, [1980](#ref-RN2995); Kaye *et al.*, [1997](#ref-RN4721)). 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](#ref-RN3703); Hoyle *et al.*, [2015](#ref-RN3285)). In contrast, 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](#ref-RN707)). 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 even after cold stratification (Cavieres & Sierra-Almeida, [2018](#ref-RN4713)). In a comparative study of species from different habitats of the Austrian Alps which assessed the germination of unstratified seeds, species from alpine grasslands had the lowest final germination proportion (**???**). Therefore, how important cold stratification is for seed germination in alpine plant species remains unclear.

The underlying question is whether seed dormancy plays a relevant role in postponing alpine germination during autumn and winter, or the warm-cued germination is enough to prevent germination under cool autumn temperatures. The response to this question becomes non-trivial under a warming climate, by which non-dormant seeds could germinate readily in autumn, and this would provide a longer time for seedlings to establish. Non-dormant seeds could possibly have an advantage as the growing seasons become longer, if seedlings become large enough to survive over winter. 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](#ref-RN3317); Mondoni *et al.*, [2015](#ref-RN2382)). However, we also might expect that seedlings from these species will be exposed to frost if snow starts to melt earlier and insulation during winter disappears, and therefore a mechanism that enables diverse germination timing (i.e. high germination uncertainty) should to be preferred.

Besides germination temperature and patterns of seed dormancy, the response of alpine seeds to light and alternating temperatures can provide further information on their capacity to detect fine-scale environmental cues. Solar irradiance in physiologically significant quantities penetrates only the first millimetres of the soil (TESTER & MORRIS, [1987](#ref-RN4909)) and fluctuations of diurnal temperature decrease with increasing burial depths: below 10 cm they might be too small to trigger germination of species requiring temperature variation (Van Assche & Vanlerberghe, [1989](#ref-RN4910)). Therefore, these cues can indicate whether seeds 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](#ref-RN4898)). The preference of alpine species to germinate in light (Densmore, [1997](#ref-RN4730); Mondoni *et al.*, [2009](#ref-RN4729)) could favour the creation of a persistent soil seed bank (Jaganathan *et al.*, [2015](#ref-RN4718)), which is advantageous in temporally and spatially unpredictable alpine environments (Kalin Arroyo *et al.*, [1999](#ref-RN4731); Cavieres & Arroyo, [2001](#ref-RN4732)). Indeed, alpine soil seed banks are more frequent than previously supposed, as it has been consistently reported in the last years (**???**; Jaganathan *et al.*, [2015](#ref-RN4718)). Nevertheless, other alpine species seem to prefer dark germination (Schwienbacher *et al.*, [2011](#ref-RN2943)), which may be interpreted as a strategy for detecting safe sites in rock crevices (Arslan *et al.*, [2011](#ref-RN3008)). Regarding alternating temperatures, 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](#ref-RN3376)). This is intriguing given that alpine habitats typically have a strong day-night climatic variation during the growing season. Although some germination studies report laboratory experiments conducted for constant and alternate temperatures, or light/dark conditions, they are very much case-dependent, therefore it is difficult to evaluate whether these factors show a consistent pattern across global alpine ecosystems.

Here, we collect primary germination data from eight alpine regions and four continents to investigate the germination response of alpine plant species to key environmental factors. We used raw data obtained from laboratory experiments to test the response of seed germination to temperature, cold stratification, light and alternating temperatures, and their relationships with dormancy classes, seed mass, and embryo:endosperm ratio obtained from bibliographic data. Our central question was to investigate the seed germination spectrum of alpine plants to test the consistency at the global scale of general assumptions about their seed germination ecology: in short, that alpine seeds (1) have physiological dormancy and (2) require cold stratification to break dormancy, (3) need relatively warm temperatures to germinate, (4) response positively to light and (5) do not require alternating temperatures for germination. To understand the ecological constraints of our meta-analysis on alpine species pools, we further analyse how the germination responses differ between strict alpine species which occur exclusively above the treeline, and generalist species that also occur at lower elevations.

# Materials and Methods

## Data collection

We conducted a meta-analysis of primary data (Mengersen *et al.*, [2013](#ref-RN4734)), which consists in pooling together original data from different local studies to synthetize their conclusions at the global level. The original data files, as well as R code for data cleaning, analysis and manuscript production are available at <https://github.com/efernandezpascual/alpineseeds>. To gather data, we contacted research groups who have performed experiments on seed germination of alpine plants, asking them to deliver 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 collected data from the Cantabrian Mountains of Spain (Fernández-Pascual *et al.*, [2017a](#ref-RN2371)), the European Alps [Mondoni *et al.* ([2009](#ref-RN4729)); Mondoni *et al.* ([2012](#ref-RN3029)); Tudela-Isanta *et al.* ([2018](#ref-RN4727)); Rosbakh, unpublished], the Italian Apennines (Mondoni, unpublished), the Russian Caucasus [Rosbakh, unpublished], the Qinghai-Tibet Plateau of China (Bu *et al.*, [2007](#ref-RN4728), [2008](#ref-RN4735); Liu *et al.*, [2013](#ref-RN3376)), the Chilean Andes [Cavieres & Arroyo ([2000](#ref-RN3393)); Cavieres & Sierra-Almeida ([2018](#ref-RN4713)); Briceño, unpublished] and the Australian Alps [Venn ([2007](#ref-RN4737)); Venn & Morgan ([2009](#ref-RN4736)); Sommerville *et al.* ([2013](#ref-RN3703)); Satyanti, unpublished]. We also collected data on European alpine species from *Enscobase*, the database of the European Native Seed Conservation Network (<http://enscobase.maich.gr/index.tml>).

Once received, we standardized the species names according to *The Plant List* (The Plant List, [2013](#ref-RN2321)) using the package *Taxonstand* (Cayuela *et al.*, [2019](#ref-RN4660)) in *R* (R Core Team, [2019](#ref-RN2315)). For each species, we recorded its elevation range from local floras (Hegi, [1906](#ref-RN4741); Pignatti, [1982](#ref-RN4739); Castroviejo, [1987](#ref-RN3243); Brach & Song, [2006](#ref-RN4742); Parolly & Rohwer, [2019](#ref-RN4740)), catalogues (Rodriguez *et al.*, [2018](#ref-RN4738)) and herbaria (*Australian National Herbarium*). 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 also compiled the following traits: (1) life form (forb, graminoid or woody) and (2) life span (annual or perennial) from the references used for the elevation, plus some extra references (Cabrera, [1982](#ref-RN4747); Oberdorfer, [2001](#ref-RN4744); Holubec & Krivka, [2006](#ref-RN4745); Shetekauri & Jacoby, [2009](#ref-RN4746); Zavala-Gallo *et al.*, [2010](#ref-RN4748); Al-Shehbaz, [2018](#ref-RN4749)) and online resources (<https://www.infoflora.ch/de/>; <http://plantnet.rbgsyd.nsw.gov.au/search/simple.htm>; <http://buscador.floraargentina.edu.ar/>; <https://patagoniawildflowers.org/>); (3) seed dormancy class following the classification system of Baskin and Baskin: physiological (germination prevented by the seed’s internal balance of hormones), morphological (germination prevented by an underdeveloped embryo at the time of dispersal), morphophysiological (both physiological and morphological dormancy present), physical (germination prevented by a water-impermeable seed coat) or non-dormant (Baskin & Baskin, [2014](#ref-RN3214); Rosbakh *et al.*, [2020a](#ref-RN4743)); (4) seed mass (Bu *et al.*, [2007](#ref-RN4728), [2008](#ref-RN4735); Liu *et al.*, [2013](#ref-RN3376); Royal Botanic Gardens, Kew, [2017](#ref-RN2987)) and (5) embryo to endosperm ratio (Vandelook, unpublished). For the species missing seed mass or embryo values, we calculated genus or family averages.

## Description of the dataset

The final dataset used in our meta-analysis contained 9,793 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 (291 strict alpine species and 370 generalists). Seed lots were originally sampled in 8 alpine regions of the world (Fig. 1), in both hemispheres.

Most species in the dataset (> 90 %) were perennial, and either graminoids or forbs. Most were reported to have dormant seeds (Fig. 2a), 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 morpho-physiological and physical. Both strict alpine and generalist species had a similar range of values for seed mass (Fig. 2b) and embryo to endosperm ratio (Fig. 2c). The values of seed mass ranged from 0.02 to 58 mg, with a median value of 0.56 mg. Embryo:endosperm ratio encompassed the full range of potential values, from endospermic species with very small embryos (0.001) to non-endospermic seeds (1). The median value was 0.34.

The total number of seeds used in the experiments was 365,508. 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 7,515 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 8,927 records, and kept in total darkness in 866 records. The experiments were performed with untreated (i.e. fresh) seeds in 7,291 records, and of the rest, the majority (2,436) went through cold stratification (< 5 ºC, in darkness). Scarification (i.e. an abrassion of the seed coat to allow water imbibition) was performed in 119 records, and GA3 (a plant hormone that promotes embryo growth and germination) in 651 records. Since germination incubations ran for different periods of time, we established a cut-off time of 8 weeks to calculate the final germination percentages, to have a homogeneous comparison across records.

## Germination response variables

We used the final germination proportion (i.e. the number of seeds germinated out of seeds sown) as the main response variable for the germination treatments. We further calculated the mean germination time and the germination uncertainty index with the package *GerminaR* (Lozano-Isla *et al.*, [2019](#ref-RN4752)). Mean germination time is the time that it takes for half of the seed lot to germinate, indicating the germination speed or rate in a given experimental condition. The germination uncertainty index estimates how scattered germination is through time, and gives 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). To calculate these indices, we used only records that met two conditions: (i) to have achieved more than 50% final germination percentage; and (ii) to have a mean germination time under 4 weeks. We established these limits to prevent the results being altered by a modification of seed dormancy status during the germination incubation (e.g. a fraction of the seed lot is non-dormant at the beginin 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).

## MCMCglmm models

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](#ref-RN4755)). To model final germination proportion, we used binomial MCMCglmms, while for mean germination time and germination uncertainty index we used gaussian MCMCglmms. Models had, as fixed effects, the experimental conditions (temperature, alternating temperature, light, scarification, stratification and GA3), plus their interaction with seed mass and embryo:endosperm, and the strict alpine or generalist character of the species. Random effects included a reconstructed phylogenetic tree for the 661, and also species identity, seed lot, lab, and alpine region. To create the phylogeny, we used the R package *V.PhyloMaker* (Jin & Qian, [2019](#ref-RN4753)). *V.PhyloMaker* contains a mega-tree which is an updated version of GBOTB for the seed plants (Smith & Brown, [2018](#ref-RN4754)). We placed taxa absent from the mega-tree at the genus-level basal node. In all models, all variables were scaled. 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 (Villemereuil & Nakagawa, [2014](#ref-RN4756)), 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 (λ) (Pagel, [1999](#ref-RN4757)), estimated simultaneously with the models by calculating the mean of the posterior distribution and the 95% CI of λ as indicated by ([2014](#ref-RN4756)). When λ = 0, related taxa are no more similar than expected by chance, while when λ = 1, the trait is evolving following a constant variance random walk or Brownian motion model; intermediate values of λ indicate a phylogenetic correlation in trait evolution that does not fully follow a Brownian motion model (Pagel, [1999](#ref-RN4757)).

## FAMD ordination

To visualize the alpine seed germination spectrum, we performed a Factorial Analysis of Mixed Data (FAMD) as implemented in the package *FactoMineR* (Le *et al.*, [2008](#ref-RN3166)). 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 created a continuous variable for each germination cue (i.e. temperature, alternating temperatures, light, scarification, stratification, and GA3). 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 hypothesis testing. For each species, we also calculated a single mean germination time (the minimum, i.e. the time taken at the most favourable treatment) and germination uncertainty index (the mean). We conducted the FAMD with only the 293 species for which we had mean germination time and germination uncertainty index values. We also included the continuous variables seed mass and embryo:endosperm, as well as the categorical variables dormancy class (physiological, morphophysiological, morphological, physical or non-dormant), distribution (strict alpine vs. generalist species), life form (forb, graminoid or woody) and life span (annual or perennial).

# Results

## Germination temperature

Temperature had a positive effect on the final germination proportion as shown by a credible interval not overlapping zero (Fig. 3a), indicating a preference for warmer temperatures. Increasing temperatures also decreased mean germination time (Fig. 3b) and the germination uncertainty index (Fig. 3c). The effect of warmer temperatures was slightly more intense in strict alpine than in generalist species, although the credible intervals of the two groups of species partially overlap. Seed mass and embryo:endosperm had a negative interaction with temperature, in other words, they reduced the response to temperature: heavier and less endospermic seeds increased less their germination proportion and decreased less their mean germination time in warmer temperatures. The germination uncertainty index did not show an interaction with seed mass or embryo:endosperm, as shown by the credible interval overlapping zero.

## Alternating temperatures

Alternating temperatures had a positive effect on the final germination proportion (Fig. 3a), but not in the mean germination time (Fig. 3b) or the germination uncertainty index (Fig. 3c). The positive effect on the germination proportion was marginally higher in the generalist species. As in the case of the mean germination temperature, higher seed mass and embryo:endosperm values reduced the positive effect of alternating temperatures on the final germination proportion. Alternating temperatures reduced the mean germination time more in seeds with low values of embryo:endosperm.

## Light

Light increased the final germination proportion (Fig. 3a), with no differences between strict alpine and generalist species. It did not affect the mean germination time (Fig. 3b), but it did increase the germination uncertainty index of generalist species (Fig. 3c). Heavier and less endospermic seeds responded less to light.

## Scarification

Scarification promoted the germination proportion (Fig. 3a) and reduced the mean germination time (Fig. 3b), but it did not affect the germination uncertainty index (Fig. 3c). Strict alpine species were slightly more responsive to scarification than generalist species. Heavier seeds were more responsive to scarification, while seeds with smaller embryos responded less.

## Stratification and GA3

In general, stratification increased the final germination proportion (Fig. 3a). This positive effect occurred in both generalist and strict alpine species, although the effect was higher in the strict alpine group. Stratification slightly reduced the mean germination time (Fig. 3b), 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. 3c), and slightly more so in generalist species. Seed mass did not interact with stratification, but species with smaller embryos had a smaller response to stratification in their final germination proportion and reduced less markedly their mean germination time and germination uncertainty index. GA3 had similar effects to stratification, with the main difference being that seed mass affected the response to GA3, whereas it did not interact with the response to stratification.

## Random effects and phylogenetic signal

Random factors had a significant effect on the MCMCglmm models (Fig. 4). The final germination proportion and the mean germination time were affected more strongly, on average, by the seed lot and the species identity. Alpine region and lab had a lower average effect, but 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. 5) was 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.

## FAMD ordination

The first FADM axis explained 15% of the variation. The quantitative variables with the largest contribution to this first axis were germination temperature, germination uncertainty, GA3 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 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 GA3 (Fig. 6). Axis 2 explained 11% of the variability. The main contributing variables were embryo:endosperm, mean germination time, seed mass and scarification; and the main categorical factors were physical dormancy, graminoid life form and morphophysiological dormancy. This axis separated (iii, bottom) morphophysiologically dormant species and graminoids with longer mean time to germination from physically dormant species that responded to scarification and had heavier, less endospermic seeds.

# Discussion

## Seed dormancy

Our dataset indicates that seed dormancy is frequent across many alpine regions of the world and postponing germination plays an important role in plant recruitment in the harsh alpine environment, although the frequency of dormant species is similar in strict alpine and generalist species. Seeds with the two most frequent dormancy classes, physiological and morphophysiological, require a stratification period during which they experience cold and wet conditions over a period of months (Baskin & Baskin, [2014](#ref-RN3214); Rosbakh *et al.*, [2020a](#ref-RN4743)). Our results show that cold stratification decreases time to germination and increases overall percent germination. Our results also indicate that the hormone gibberellic acid (GA3) can be used as a substitute of cold stratification to overcome inherent physiological dormancy. Physical dormancy, infrequent in our dataset, is broken by mechanical scarification, a process that could also occur naturally via freezing-thawing cycles in spring. Our results thus confirm that overwintering is essential for promoting seed germination in species inhabiting alpine habitats, especially for strict alpine species. This concurs with several studies that have shown that cold-stratification is important for seed germination in different alpine regions of the world (Cavieres & Arroyo, [2000](#ref-RN3393); Schütz, [2002](#ref-RN2868); Giménez-Benavides *et al.*, [2005](#ref-RN698); Shimono & Kudo, [2005](#ref-RN707); Sommerville *et al.*, [2013](#ref-RN3703); Garcia-Fernandez *et al.*, [2015](#ref-RN2355); Hoyle *et al.*, [2015](#ref-RN3285); Fernández-Pascual *et al.*, [2017a](#ref-RN2371); Cavieres & Sierra-Almeida, [2018](#ref-RN4713)). Under natural field conditions, cold stratification occurs over winter when seeds are covered by snow, allowing seeds to sense the snow season, and thereby promoting germination in the spring and summer, when conditions are more favourable for seedling survival and growth. Since global climate change is causing major changes in snow cover duration (Beniston, [2012](#ref-RN4917); Gobiet *et al.*, [2014](#ref-RN4918)), 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](#ref-RN3703)). This risk will be partly alleviated by the know plasticity of physiological seed dormancy in response to the seed maturation temperature, a mechanism of “seed memory” that facilitates acclimatisation to changing environments (Fernández-Pascual *et al.*, [2019](#ref-RN2249)), and which could reduce the stratification requirements of seeds produced in a warmer climate.

## Temperature regulation of germination

Our results show an increase of seed germination with incubation temperature, more pronounced in strict alpine compared to generalist species, confirming the findings of local studies (Walder & Erschbamer, [2015](#ref-RN3266); Fernández-Pascual *et al.*, [2017a](#ref-RN2371)). Our results also show that germination is faster with increasing incubation temperature, especially in small seeded, endospermic species. Small-seeded species have a lower rate of seeding survival under drought (Leishman & Westoby, [1994](#ref-RN4530)) but may require warmer germination temperatures than large-seeded species, especially in seasonal climates (Arène *et al.*, [2017](#ref-RN2399)). Considering that risk reduction is an important selective pressure for the evolution of germination traits (Venable & Brown, [1988](#ref-RN4915)), a plausible explanation for these patterns is that alpine plants received selection pressure from damaging spring frost and evolved an avoidance mechanism by germinating at high temperatures. Frost avoidance is an important survival strategy in alpine plants (TASCHLER & NEUNER, [2004](#ref-RN4908); Marcante *et al.*, [2012](#ref-RN4722); Rosbakh *et al.*, [2020b](#ref-RN4906)). Complementarily, strict alpine species might have been less subjected to drought damage during establishment compared to generalist species: as elevation increases, plants face less drought risk because precipitation increases and evapotranspiration decreases, but at the same there is a higher probability of early or late season frosts (Körner, [2003](#ref-RN2392); Schrier *et al.*, [2007](#ref-RN4914); Beniston, [2016](#ref-RN4897)). Taken together, our results indicate that germination patterns in alpine species are driven by an interplay of seed size and germination temperature to escape either drought or frost stress: small seeds selecting for fast germination at warm temperatures in order to escape unfavourable early-spring frost; and large seeds selecting for slower germination at cooler conditions, which should maximize emergence during snowmelt (when water availability is highest) and a well-developed root system to cope with desiccation risk in summer. Supporting this view, seedlings of alpine pioneer species have a low frost resistance (Marcante *et al.*, [2012](#ref-RN4722)), yet high heat tolerance up to 40–50°C (Marcante *et al.*, [2014](#ref-RN4902)).

Increasing temperatures also decreased germination uncertainty, showing that germination synchrony is a plastic trait driven by temperature, at least in alpine species. Indeed, while asynchronous germination is thought to be a form of adaptation to unpredictable alpine environments (Wagner & Simons, [2009](#ref-RN3690); Simons, [2011](#ref-RN3734)), our results indicate that staggered germination occurs when temperatures are still cool, likely as a bet-edging 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 summer drought. 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](#ref-RN2392); Hoyle *et al.*, [2015](#ref-RN3285); Satyanti *et al.*, [2019](#ref-RN4907)). Staggered germination is usually exhibited as a rapid first wave of germination followed by second wave several weeks or months later (Körner, [2003](#ref-RN2392)). If autumn seedlings grow faster than spring seedlings (Satyanti *et al.*, [2019](#ref-RN4907)), this will likely have cascading effects on individual fitness along the life of the plant (Donohue *et al.*, [2005](#ref-RN3036), [2010](#ref-RN2384)). Delving further into the staggering germination syndrome, such as examining whether the source of variation lies at the individual or population level; or whether such syndrome is conserved; will help us to understand further the ecology of the unique alpine flora and its fate under a changing climate.

## Alternating temperatures and light

We found a positive germination response to alternating temperatures and light, conditions that mimic the environment in the upper soil during the snow-free season (Billings & Mooney, [1968](#ref-RN4712); Körner, [2003](#ref-RN2392); Mondoni *et al.*, [2012](#ref-RN3029)). Alternating temperatures had a slightly lower effect on strict alpine species, compared with generalist species, but still had an effect. This partly contradicts the findings of Liu et al. ([2013](#ref-RN3376)), who reported a lack of a response to temperature fluctuation for the 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 in all months of the year and therefore fluctuating temperature alone may not be a reliable indicator of suitable conditions for seedling establishment and growth, while warm daily temperatures could be a cue that the short summer growing season has begun (Liu *et al.*, [2013](#ref-RN3376)). When analysed at the global level, alternating temperatures seem to increase germination of alpine plants, 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 under 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 (Pons, [2000](#ref-RN4904)).

## The alpine seed germination spectrum

The factorial analysis of mixed data (FAMD) separated generalist and strict alpine plants according to a “fast-slow” gradient of regeneration strategies. On one end of the gradient, strict alpine species tend to have slow and synchronous germination, require warm temperatures and light to germinate, and need stratification or GA3 to break physiological dormancy. On the other end, generalist species tend to have fast germination, show a bet-hedging strategy (high germination uncertainty), and show germination traits suggesting possible fast germination after specific cues (PY, alternating temperatures). The dormancy classes without a physiological component (non-dormant, physical, and morphological) appear associated with generalist species. While most species are perennial, the generalist group includes some annuals. Life forms are evenly distributed across regeneration strategies. This “fast-slow” gradient of regenerative strategies is similar to prominent gradients of fast or slow population dynamics (Silvertown *et al.*, [1992](#ref-RN4922); Nobis & Schweingruber, [2013](#ref-RN4921)), fast or slow developing leaf traits (Wright *et al.*, [2004](#ref-RN4923)), or large sets of plant ecological features (Grime, [1977](#ref-RN2279)). “Fast” regeneration can be understood as an opportunistic strategy to cope with frequently disturbed habitats such as avalanche ways, steep eroding slopes, regressing glaciers, and riverbeds (PIERCE *et al.*, [2007](#ref-RN4926); Gentili *et al.*, [2013](#ref-RN4925)). The “slow” regeneration of strict alpine species, on the other hand, seems to be a more specialized syndrome of those species that are truly restricted - and adapted - to the alpine belt.

A general assumption of seed trait ecology is that seed mass and embryo:endosperm ratios are relatively conserved across related species (Moles *et al.*, [2005](#ref-RN4916); Vandelook *et al.*, [2012](#ref-RN3685)). In our alpine dataset, both traits were relatively constant, with no differences between strict alpine and generalist species. This is in line with the general ambiguity of relationships between seed mass and elevation that have been found for alpine species, including examples of negative (Wang *et al.*, [2014](#ref-RN4912)), positive (Pluess *et al.*, [2005](#ref-RN4903)) or absent (Vandelook *et al.*, [2012](#ref-RN3685); Bauk *et al.*, [2015](#ref-RN4896)) relationships when looking at the species level. The similarity in embryo:endosperm size between generalist and strict alpine species confirms the absence of a correlation between embryo-seed size and elevation found in the Apiaceae (Vandelook *et al.*, [2012](#ref-RN3685)). In contrast with the supposed phylogenetic conservatism of these morphological and anatomical traits, physiological responses to temperature and dormancy patterns are understood as more plastic traits that can quickly acclimatize to new situations (Fernández-Pascual *et al.*, [2019](#ref-RN2249)). While phylogenetic signal in seed germination has been found, especially in highly selective environments (Carta *et al.*, [2016](#ref-RN3001); Arène *et al.*, [2017](#ref-RN2399); Fernández-Pascual *et al.*, [2017b](#ref-RN2865)), seed germination patterns can also be shared between phylogenetically distant species (Vandelook *et al.*, [2019](#ref-RN4911)) and have low or absent phylogenetic signal (Rosbakh & Poschlod, [2015](#ref-RN2365); Fernández-Pascual *et al.*, [2017a](#ref-RN2371); [2017](#ref-RN4900)). However, the phylogenetic signal found here indicates that evolutionary history cannot be neglected when studying seed germination patterns. Furthermore, we detected close relationships between seed-embryo size and physiological responses of seeds, suggesting that both sets of traits are connected and may be subjected to co-adaptation. 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](#ref-RN3703); Hoyle *et al.*, [2015](#ref-RN3285); Satyanti *et al.*, [2019](#ref-RN4907)). Taken together, these results indicate that seed morphology and physiology are connected aspects of the seed ecological spectrum and must be studied in the light of this connection and of their shared phylogenetic history.

In conclusion, our meta-analysis of primary data has shown that strict alpine species from different mountain regions of the world tend to show a specialized seed germination strategy which shows evidence of phylogenetic signal and is characterized by (1) physiological seed dormancy; (2) a strong need for cold stratification or GA3 to break dormancy; (3) a positive response to light; (4) a positive response to alternating temperatures, although not so prominent as in generalist species; (5) slow and relatively synchronous germination, and (3) an interplay with seed and embryo size, with smaller and more endospermic seeds being more responsive to warmth, light, and alternating temperatures.

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# Author Contribution

# 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>. Upon publication, a version of record of the repository will be deposited in Zenodo.

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# Figures

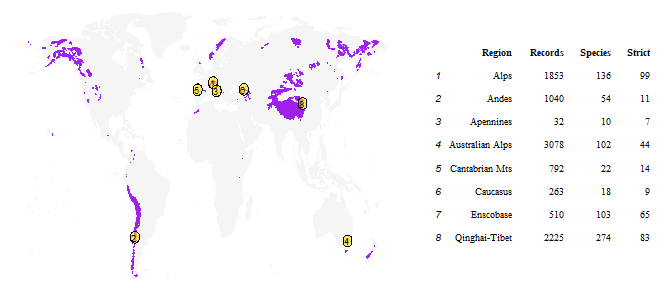


Figure 1: Geographical origin of the germination data with the number of records, species and strict alpine species per alpine region. Ensconet is not plotted because its records come from alpine regions throughout Europe. The purple areas correspond to the global extension of alpine environments according to Testolin *et al.* ([2020](#ref-RN4750)).

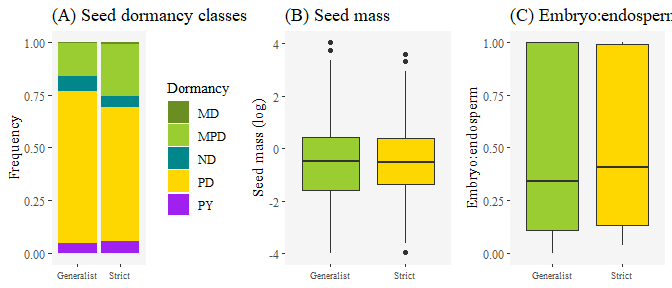


Figure 2: Seed dormancy classes, seed mass and embryo to endosperm ratio in the subsets of strict alpine and generalist species analysed in this study. Seed dormancy classes are abbreviated as follows: MD (morphological), MPD (morphophysiological), ND (non-dormant), PD (physiological), PY (physical).

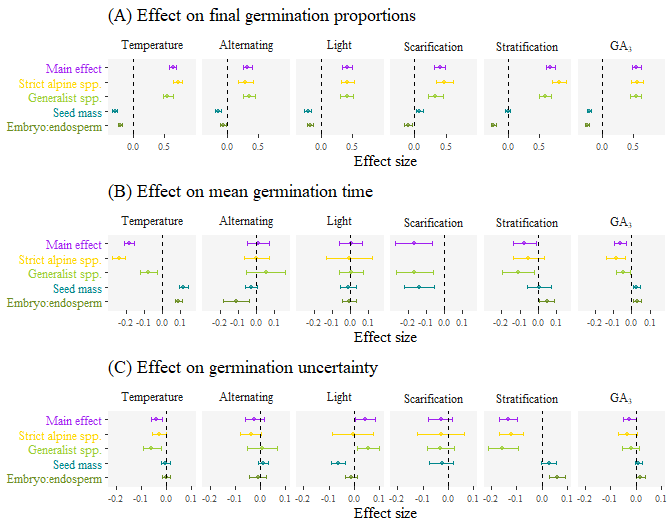


Figure 3: Effect of the germination environment on germination, according to the MCMC meta-analysis of the primary data. The dots indicate the posterior mean of the effect size, and the brackets its 95 % credible interval. The line of zero effect is shown. When the credible intervals overlap with the zero-effect line, the effect is not significant. 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:endosperm. 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.

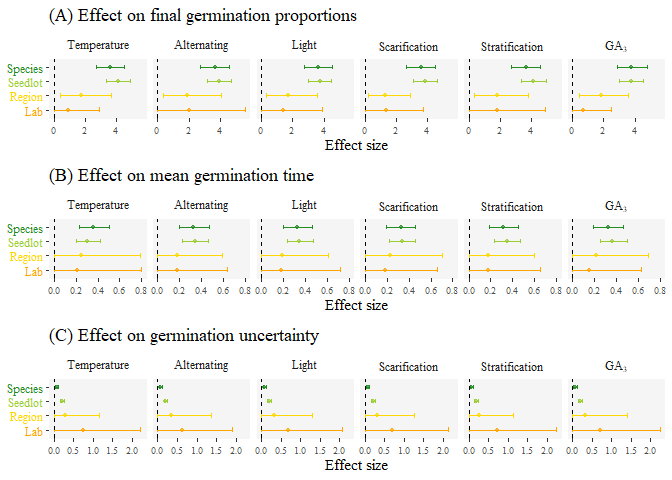


Figure 4: Effect of the random factors on germination, according to the MCMC meta-analysis of the primary data. The dots indicate the posterior mean of the effect size, and the brackets its 95 % credible interval. The line of zero effect is shown.

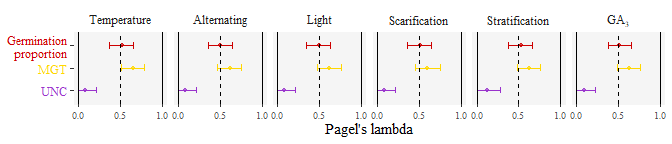


Figure 5: Phylogenetic signal in the response to the germination environment. When lambda = 0, related taxa are no more similar than expected by chance; when <U+03BB> = 1, the trait is evolving following a constant variance random walk or Brownian motion model.

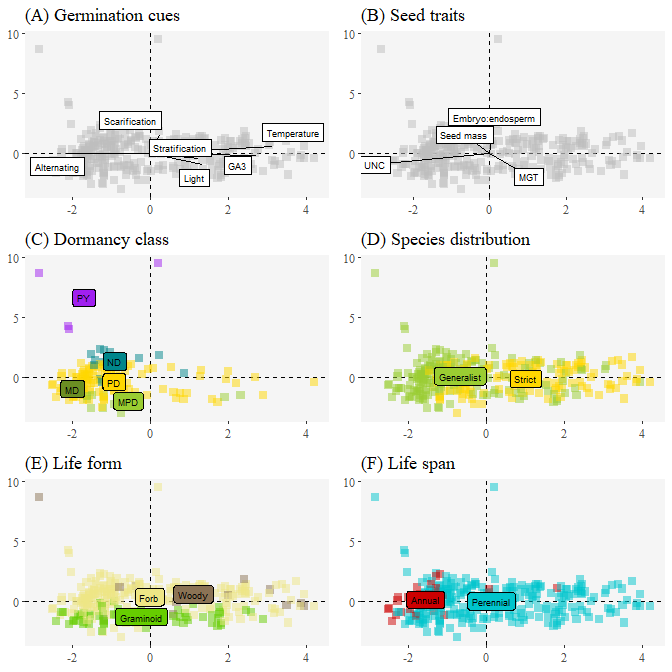


Figure 6: FADM ordination of the seed variables and the species distribution, life form and life span. Each square is a species. Labels indicate the contribution of the variables to the axes. Seed dormancy classes are abbreviated as follows: MD (morphological), MPD (morphophysiological), ND (non-dormant), PD (physiological), PY (physical).