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 (**???**). Such a global synthesis 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)). Therefore, how important cold stratification is for seed germination in alpine plant species remains unclear.

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. 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 (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 Apennines (Mondoni, unpublished), the Caucasus [Rosbakh, unpublished], the Qinghai-Tibet Plateau (Bu *et al.*, [2007](#ref-RN4728), [2008](#ref-RN4735); Liu *et al.*, [2013](#ref-RN3376)), the 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 using *The Plant List* (The Plant List, [2013](#ref-RN2321)) and the package *Taxonstand* (Cayuela *et al.*, [2019](#ref-RN4660)) in *R* (R Core Team, [2019](#ref-RN2315)). For each species, we checked its elevation range in 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 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: life form and life span 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/>); seed dormancy class (Baskin & Baskin, [2014](#ref-RN3214); Rosbakh *et al.*, [2020a](#ref-RN4743)); 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 embryo to endosperm ratio (Vandelook, unpublished). For the species missing seed mass or embryo values, we used genus or family averages.

## Description of the dataset

The final dataset used for 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 their dormancy classes did not differ between generalist and strict alpine species. Among 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 of 0.56. 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 (weighted average of the daily thermoperiod) ranged from 0 to 36 ºC, with 7,515 records of constant temperatures and 2,278 of alternating temperatures. Light was used in 8,927 records, and darkness in 866. The experiments were performed with unstratified seeds in 7,291 records, and of the rest, the majority (2,436) went through cold stratification. Scarification was applied in 119 records, and GA3 in 651. 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.

## Germination response variables

We used the final germination proportion as the main response variable for the germination treatments. We further calculated the mean time to germination and the germination uncertainty index with the package *GerminaR* (Lozano-Isla *et al.*, [2019](#ref-RN4752)). Mean germination time indicates the time it takes for half of the seed lot to germinate, while the germination uncertainty index estimates how scattered germination is through time. To calculate these indices, we used only records that had (i) more than 50% final germination; and (ii) less than 4 weeks of mean germination time; we established these limits to prevent the results being altered by a modification of seed dormancy status during the germination incubation.

## 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, 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 traits 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 (cue levels were the temperature treatments in the case of temperature; 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 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 only with 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, distribution (alpine vs. generalist), life form and life span.

# Results

## Germination temperature

Temperature had a positive effect on the final germination proportion (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 more intense in strict alpine than in generalist species. Seed mass and embryo:endosperm reduced the response to temperature: heavier and less endospermic seeds increased less their germination proportion and decreased less their germination time in warmer temperatures, although uncertainty did not show an interaction with mass or embryo:endosperm.

## Alternating temperatures

Alternating temperatures had a positive effect on the germination proportion (Fig. 3a), but not in germination time (Fig. 3b) or uncertainty (Fig. 3c). The positive effect on the germination proportion was slightly higher in the generalist species. Again, higher seed mass and embryo:endosperm reduced the positive effect of alternating temperatures.

## Light

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

## Scarification

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

## Stratification and GA3

In general, stratification increased the germination proportion (Fig. 3a). This positive effect occurred in both generalist and strict alpine species, although the effect was higher on the latter group. Stratification slightly reduced mean germination time (Fig. 3b), which was evident on the generalist species, but not on the strict alpines. Further, stratification reduced germination uncertainty as a general response (Fig. 3c) as well as on generalist and strict alpine species in particular. Seed mass did not interact with stratification, but species with smaller embryos had a smaller response to stratification in their germination proportion and reduced their germination time and uncertainty. GA3 had similar effects to stratification, with the main difference being that a higher seed mass generally reduced the response to GA3.

## Random effects and phylogenetic signal

Random factors influenced the germination response to the environment (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. Germination uncertainty 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 it was close to 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 plays an important role in plant recruitment in the harsh alpine environment. However, dormancy was similarly frequent in both strict alpine and generalist species, suggesting that this is a general strategy of temperate species but not restricted to alpine lineages. Seed dormancy is a common adaptation in seasonal climates where germination can be risky during certain seasons (Baskin & Baskin, [2014](#ref-RN3214)). The dominance of alpine species with dormant seeds suggests that germination after dispersal, usually in late summer or autumn, is delayed to favourable seasons for seedling establishment and survival such as spring and early summer (Hoyle *et al.*, [2015](#ref-RN3285)). There may also be a phylogenetic component to the frequency of some dormancy classes as, for example, morphological dormancy is common in the Campanulaceae, Gentianaceae and Ranunculaceae families (Baskin & Baskin, [2014](#ref-RN3214)), which are well represented in alpine floras.

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 confirm that cold stratification is important for promoting seed germination in species inhabiting alpine habitats, especially for strict alpine species. Our results also indicate that the hormone gibberellic acid (GA3) can be used as a substitute of cold stratification to overcome inherent physiological dormancy. Cold stratification decreases time to germination and increases overall percent germination. These results concur 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. Hence, the role of cold stratification on the different germination parameters assessed suggests that this allows seeds to sense the snow season, and thereby promotes germination to occur in the spring and summer snow-free season, 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)); @ 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.

Scarification removes mechanical barriers in species with physical dormancy, an alternative dormancy mechanism based on a water-impermeable seed coat (Baskin & Baskin, [2014](#ref-RN3214)). We found physical dormancy to be infrequent in alpine habitats, but when it does occur it could be broken by mechanical scarification via freezing-thawing cycles in spring and/or high diurnal temperature fluctuations in summer.

A small fraction of species in our dataset were classified as non-dormant. The response to high germination temperatures that we found suggests that, under a warming climate, 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. This raises a question on whether having a non-dormant or less dormant seed is 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 favoured.

## 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)). 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 results 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. 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)). Plants in high-elevation habitats generally do not experience drought limitation because precipitation increases while evapotranspiration decreases along elevational gradients (Schrier *et al.*, [2007](#ref-RN4914)).

Our results also show that germination is faster with increasing incubation temperature, especially in small seeded, endospermic species. This is not surprising, as alpine seed germination must be fast to match the short growing season. 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)). Accordingly, this pattern was stronger in strict alpine plants which, being restricted to higher elevations, presumably experience less unpredictable drought but more frost risk, compared with generalist species: precipitation and the probability of early or late season frosts generally increase with elevation (Körner, [2003](#ref-RN2392); 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 larger 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.

Finally, 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 later but can also take place in two seasons separated by a whole year (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 within individuals, across individuals within a population, or across populations; 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 snowmelt at the end of winter (Billings & Mooney, [1968](#ref-RN4712); Körner, [2003](#ref-RN2392); Mondoni *et al.*, [2012](#ref-RN3029)). Triggering seed germination at the very beginning of the spring season ensures that seedlings have time to establish during the short growing seasons (Körner, [2003](#ref-RN2392)) and grow to a critical biomass before the next winter (Schütz, [2002](#ref-RN2868)). 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.

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, it is not surprising that we detected a negative effect of seed mass on final germination values in the light and under alternating temperature regimes. The depth of seed burial in the soil is crucial for seedling emergence (Bond *et al.*, [1999](#ref-RN4898)), as seed mass - or better said the seed kernel, i.e. embryo and endosperm (Chen & Moles, [2018](#ref-RN4899)) - may represent a constraint for seedling emergence of small-seeded species. Therefore, small seeds are more likely to require light and alternating temperatures for germination, which ensures that germination does not occur too deep in the soil for seedling emergence (Pons, [2000](#ref-RN4904)).

## Seeds, embryos, and phylogenetic signal

Seed mass is a relatively constant trait in our data, with no differences between strict alpine and generalist species. This is in line with the general ambiguity of relationships between seed mass and elevation. In alpine species, the evidence of a relationship between seed mass and elevation is mixed, being either 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)) when looking at the species level. Similarly, at the population level, the relationship between seed mass and elevation is often absent (Mondoni *et al.*, [2011](#ref-RN3442); Bauk *et al.*, [2015](#ref-RN4896)). The length of the growing season might influence seed size. For instance, at higher elevations where snow duration is longer and thus the growing season is shorter, the production of smaller seeds might be favoured (Baker, [1972](#ref-RN4895); Wang *et al.*, [2014](#ref-RN4912)). On the other hand, natural selection may also favour the production of larger seeds in species at higher elevations because larger seeds enable plants to cope better with stressful environments (Westoby *et al.*, [1992](#ref-RN4913); Pluess *et al.*, [2005](#ref-RN4903)).

Much less information is available concerning a possible selective advantage of embryo:endosperm size in alpine species. However, 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 previously in Apiaceae species (Vandelook *et al.*, [2012](#ref-RN3685)). Although seed size and embryo:endosperm ratio are not different for generalist and strict alpine species in this study, these traits have been found to influence germination strategies in alpine species. For instance, 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)). Perhaps accumulation of reserves in cotyledons rather than in the endosperm may improve early growth rate and establishment in immediate germinating species (Hoyle *et al.*, [2015](#ref-RN3285)).

A general assumption of seed trait ecology is that seed mass and embryo:endosperm ratios are relatively homogeneous across related species (Moles *et al.*, [2005](#ref-RN4916); Vandelook *et al.*, [2012](#ref-RN3685)). In contrast, physiological responses to temperature and dormancy patterns are understood as more plastic traits that can quickly acclimatize in new situations (Fernández-Pascual *et al.*, [2019](#ref-RN2249)). Although our study is not focused on trait evolution, we detected close relationships between seed size and physiological responses of seeds, suggesting that both traits are connected and may be subjected to co-adaptation. While phylogenetic signal in seed germination has been found often, 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)) with 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 in the alpine environment. Further studies are required to assess whether the degree of the shared germination patterns among phylogenetically related species in the alpine environment is the result of trait conservatism, convergence, or both (Losos, [2008](#ref-RN4901); Revell *et al.*, [2008](#ref-RN3235)).

## 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.

## Conclusions

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:

* Physiological seed dormancy.
* A strong need for cold stratification to break dormancy, which can be substituted by GA3.
* A positive response to light.
* A positive response to alternating temperatures, although not so prominent as in generalist species.
* Slow and relatively synchronous germination.
* 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

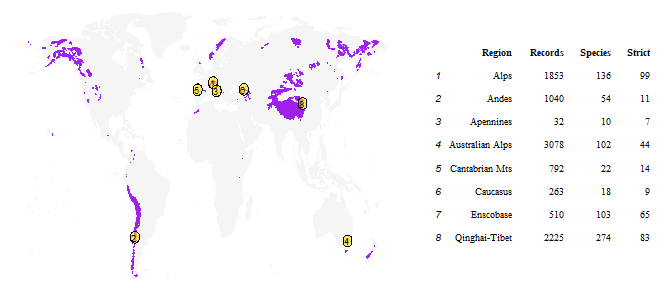


Figure 1: Geographical origin of the germination data with the number of records, species and strict alpine species per alpine region. Ensconet is not plotted because its records come from alpine regions throughout Europe. The purple areas correspond to the global extension of alpine environments according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

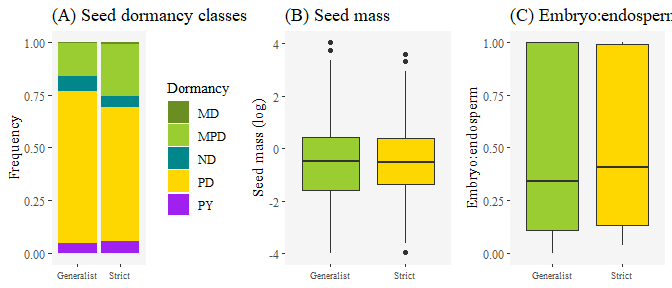


Figure 2: Seed dormancy classes, seed mass and embryo to endosperm ratio in the subsets of strict alpine and generalist species analysed in this study. Seed dormancy classes are abbreviated as follows: MD (morphological), MPD (morphophysiological), ND (non-dormant), PD (physiological), PY (physical).

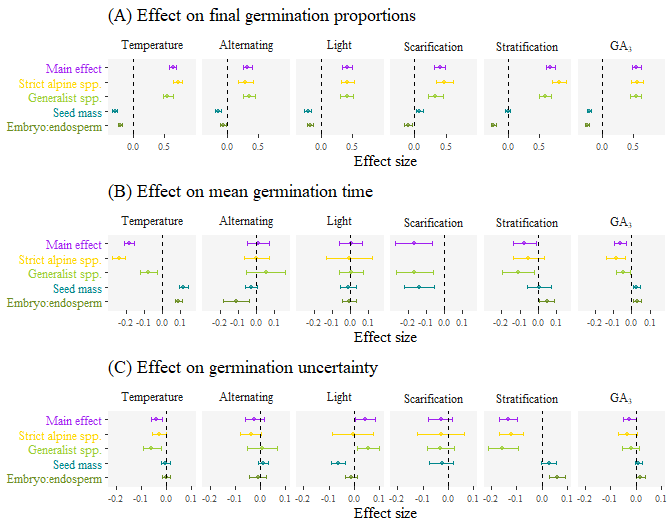


Figure 3: Effect of the germination environment on germination, according to the MCMC meta-analysis of the primary data. The dots indicate the posterior mean of the effect size, and the brackets its 95 % credible interval. The line of zero effect is shown. When the credible intervals overlap with the 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.

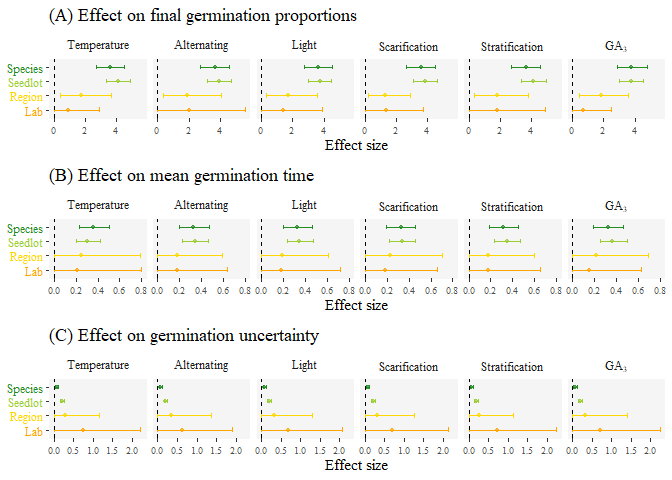


Figure 4: Effect of the random factors on germination, according to the MCMC meta-analysis of the primary data. The dots indicate the posterior mean of the effect size, and the brackets its 95 % credible interval. The line of zero effect is shown.

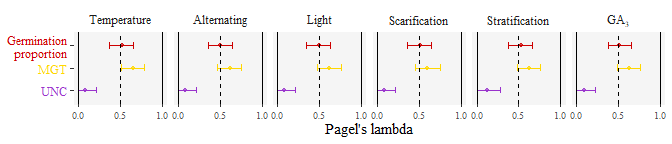


Figure 5: Phylogenetic signal in the response to the germination environment. When lambda = 0, related taxa are no more similar than expected by chance; when <U+03BB> = 1, the trait is evolving following a constant variance random walk or Brownian motion model

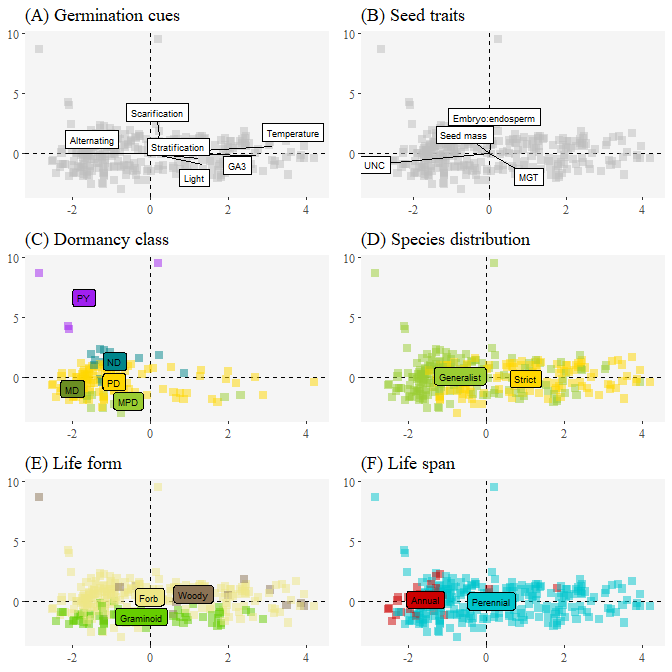


Figure 6: FADM ordination of the seed variables and the species distribution, life form and life span. Each square is a species. Labels indicate the contribution of the variables to the axes.