A global meta-analysis of alpine seed germination ecology

# Introduction

Alpine environments occur worldwide above the maximum elevation at which trees can grow (Testolin et al. 2020). 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 appropiate period to ensure survival of seedlings under extreme environmental stress (Schütz, [2002](#ref-RN2868))(Chambers *et al.*, [1990](#ref-RN4714); 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 will go into extinction.

Natural selection should favor seed germination requirements reducing 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 regulated by climatic variation. Many plant species have further developed seed dormancy, by which germination is prevented during periods that are only ephemerally favorable (but with appropriate water and temperature conditions), ensuring germination in 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 niches and safe sites (Jumpponen *et al.*, [1999](#ref-RN4719)). Given the heterogeneity of alpine climates and species lineages adapted to different regions, a current challenge is to determine whether alpine plants have a common consisting of one or multiple responses that consistently respond to the same environmental conditions globally.

Early studies on the germination ecology of alpine plants demonstrated that, in most species, recently dispersed (fresh) seeds require relatively high temperatures for germination (Bliss, [1958](#ref-RN3258); Amen, [1966](#ref-RN3213); Billings & Mooney, [1968](#ref-RN4712)). High-temperature germination has been considered as an adaptation to prevent seed germination at the timing 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)), when the temperature window for germination usually widens to lower values (Nishitani, [1996](#ref-RN1021)). In recent times, an increasing number of studies highlighted that fresh seeds of 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.*, [2017](#ref-RN2371); Cavieres & Sierra-Almeida, [2018](#ref-RN4713)). This low-temperature 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 here is whether high- and low-temperature germination occur broadly in alpine environments as a set of possible plant strategies across micro-climatic niches.

Many alpine species have also been described as having deep physiological dormancy (Schwienbacher *et al.*, [2011](#ref-RN2943); Sommerville *et al.*, [2013](#ref-RN3703); Baskin & Baskin, [2014](#ref-RN3214)). Dormancy in fresh 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, in 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 (see also Sayers and Ward ([1966](#ref-RN1484)); Marchand and Roach ([1980](#ref-RN2995)); Kaye ([1997](#ref-RN4721))). More recently, Sommerville *et al.* ([2013](#ref-RN3703)) and Hoyle *et al.* ([2015](#ref-RN3285)) showed that in 19 and 54 Australian alpine plant species, respectively, a cold stratification period significantly increased seed germination only in half of the species tested. In contrast, Söyrinki (1938, cited in Körner ([2003](#ref-RN2392))), experimenting with 91 alpine species from the Alps, found that storage at winter temperatures (i.e. cold stratification) increased seed germination in the great majority of cases. Shimono and Kudo ([2005](#ref-RN707)) reported that cold stratification increased seed germination over a range of temperatures in most of the 27 plant species studied in the alpine zone of Japan. Cavieres and Sierra-Almeida ([2018](#ref-RN4713)) reported that, along an elevational gradient in the central Chilean Andes, cold stratification was an important requirement for seed germination in species from lower elevations, while species from higher sites did not germinate after cold stratification. Therefore, how important cold stratification is for seed germination in alpine plant species remains unclear.

Besides germination temperature and patterns of 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 (Arroyo *et al.*, [1999](#ref-RN4731); Cavieres & Arroyo, [2001](#ref-RN4732)). Indeed, alpine soil seed banks are more frequent that it was previously supposed, as it has been consistently reported in the last years (Venn & Morgan 2010; review). 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 Qinhai-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 have 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 alternate temperatures, and their relationships with seed mass, dormancy classes 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 of general assumptions about seed germination ecology at the global scale. To understand the ecological constrains of our meta-analysis on alpine species pools, we further analyze how the germination responses differ between strict alpine species which mostly occur above the treeline, and generalist species that also occur at lower altitudes.

# Materials and methods

## Data collection

We conducted a meta-analysis of primary data (Mengersen *et al.*, [2013](#ref-RN4734)) consisting of the analysis of ……. To gather data, we contacted research groups who have performed experiments on the 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.*, [2017](#ref-RN2371)), the European Alps [Mondoni *et al.* ([2009](#ref-RN4729)); Mondoni *et al.* ([2012](#ref-RN3029)); Tudela-Isanta *et al.* ([2018a](#ref-RN4727)); Rosbakh, unpublished], the Apennines (Mondoni, unpublished), the Caucasus [Rosbakh, unpublished], the Qinhai-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* (<http://enscobase.maich.gr/index.tml>). All species correspond to herbs or forbs, which are the most abundant life forms in alpine ecosystems.

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 subalpine 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); 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.*, [2020](#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 means.

## Description of the dataset

The final dataset contains 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 eight alpine regions of the world (**Table 1**), in both hemispheres. The total number of seeds used in the experiments was 365,508. The 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, in order to have an homogeneous comparison.

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## Germination response variables

We used the final germination proportion (GP) as the main response variable for the germination treatments. We further calculated the mean time to germination (MGT) and the germination uncertainty index (UNC) with the package *GerminaR* (Lozano-Isla *et al.*, [2019](#ref-RN4752)). MGT indicates the time it takes for half of the seed lot to germinate, while UNC 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 MGT; we established these limits to prevent the results being altered by a modification of seed dormancy status during the germination incubation.

## MCMC 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 MGT and UNC we used gaussian MCMCglmms. Models had, as fixed effects, the experimental conditions (temperature, alternating temperature, ligth, 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 relies on GBOTB for the seed plants (Smith & Brown, [2018](#ref-RN4754)), with updates, corrections and expansion. We binded taxa absent from the mega-tree to 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 Pagel’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 propotion 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 MGT (the min, i.e. the time taken at the most favourable treatment) and UNC (the mean). We conducted the FAMD only with the 293 species for which we had MGT and UNC 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

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## Germination temperature

Temperature had a positive effect on the final germination proportion (**Fig. 2**a), indicating a preference for warmer temperatures. Increasing temperatures also decreased mean germination time (**Fig. 2**b) and the germination uncertainty index (**Fig. 2**c). 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. 3**a), but not in germination time (**Fig. 3**b) or uncertainty (**Fig. 3**c). The posive 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. 3**a), with no differences between strict alpine and generalist species. It did not affect the germination time (**Fig. 3**b), but it did increase the germination uncertainty of generalist species (**Fig. 3**c). Heavier and less endospermic seeds responded less to light.

## Scarification

Scarification promoted germination (**Fig. 3**a) and reduced germination time (**Fig. 3**b), but it did not affect germination uncertainty (**Fig. 3**c). Strict alpine species responded slightly more to scarification. Heavier seeds were more responsive to stratification, while this treatment had a comparatively smaller effect on seeds with small embryos.

## Stratification and GA3

In general, stratification increased the germination proportion (**Fig. 3**a). isoccurred in both generalist and strict alpine species, although the effect latter speciesStratification slightly reduced mean germination time (**Fig. 3**b), which was evident on the generalist species, but not on the strict alpines. Further, stratification reduced germination uncertainty as a general response (**Fig. 3**c) 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 of 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, which might be representing different germination scoring schedules among research groups. 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 closer to zero, indicating a weak effect of phylogeny, which perhaps is also caused by a stronger effect of different scoring schedules.

## 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, strict, generalist, physiological dormancy and annual life span contributed to axis 1. In summary, the axis separated (i, left) generalist and annual species with physiological dormancy, a positive response to alternating temperatures and more germination uncertainty from (ii, right) strict alpine species with a preferences 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 and graminoid species with longer mean time to germination from physically dormant species that responded to scarification and had heavier, less endospermic seeds.

# Discussion

**Temperature regulation of germination**

Our results show an increase of seed germination with incubation temperature, more pronounced in strict alpine compared to generalist species. Alpine species showed higher optimal temperatures for germination than either subalpine species (i.e. species that live close to the treeline, Fernández-Pascual et al. (2017) or congeneric counterparts from below the treeline (Walder & Erschbamer, 2015). A plausible explanation for these results is that alpine species received selection pressures of damaging spring frost and evolved an avoidance mechanism by germinating at high temperatures; frost avoidance is an important survival strategy in alpine plants (Taschler and Neuner 2004; Rosbakh et al. 2020; Marcante et al., 2012). 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), yet high heat tolerance (up to 40–50°C; Marcante et al., 2014) and plants in high-elevation habitats generally do not experience drought limitation because precipitation increases and evapotranspiration decreases along elevational gradients (Van der Schrier et al., 2007).

Our results also show that germination is faster with increasing incubation temperature, especially in small seeded, endospermic species. This is not surprising, as seed germination must be fast to match the short (alpine) growing season. Small-seeded species have a lower rate of seeding survival under drought (Leishman and Westoby, 1994), but may require warmer germination temperature then larger seeded species, especially in seasonal climates (Arène et al. 2017). Accordingly, this pattern was stronger in strict alpine plants, which presumably experience less unpredictable drought compared with generalist species (i.e. being restricted to higher elevations). Taken together, our results indicate that germination patterns in alpine species is driven by an interplay of seed size and germination temperature, with 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 a well-developed root systems to cope with desiccation risk in summer. Indeed, despite the absence of significant differences in seed size between strict alpine and generalist species in our data, seed size correlates negatively with elevation at inter-species level (Qi et al. 2015), while precipitation and the probability of early or late season frosts generally increase with elevation (Korner 2003; Beniston 2000).

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 in unpredictable alpine environments (Simons 2011; Wagner & Simons 2009), our results indicate that staggered germination occur 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 condition (i.e. in late spring) may be another important strategy that increases seedling survival of alpine plants by avoiding summer drought.

**Dormancy and stratification**

Our study demonstrates 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 an important factor 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 and Baskin 2014). In the temperate montain flora, dormancy is achieved by different physiological mechanisms and seed anatomy (Amen, 1966; Schweinbacher et al. 2011; Baskin and Baskin, 2014). The dominance of alpine species with dormant seeds suggests that germination after dispersal, usually in late summer or autumn, is delayed to favorable seasons for seedling establishment and survival (spring – early summer; e.g. Hoyle et al. 2015). There may also be a phylogenetic component to the frequency of some dormancy classes as, for example, M(P)D is common in *Campanulaceae*, *Gentianaceaea* and *Ranunculaceae* families (Baskin and Baskin, 2014), species of which are common in alpine floras.

However, not all species from alpine habitats have dormant sees. We found that under higher temperatures, non-dormant seeds can germinate readily in autumn and would provide longer time for the seedlings to establish. Species with dormant seeds, on the other hand, would have a higher proportion of seeds that germinate earlier in spring as well as a higher frost risk and seedling mortality. It may seem that species with non-dormant seeds will likely have an advantage as the growing season becomes longer and seedlings would be 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; Mondoni *et al.* 2015). 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 enable diverse germination timing (high germination uncertainty) remains to be favored.

The extreme germination uncertainty syndrome, i.e. staggering germination so that both before and after winter seedlings occur, is known in alpine species (Körner 2003 based on Soyrinki (1938); Hoyle *et al.* 2015; Satyanti, Guja & Nicotra 2019). Seed dormancy is likely to play the major role in enabling this amplitude of temporal spreading of germination. Our results on germination uncertainty provide a confirmation that dormancy works as a spectrum and within a seed lot they can vary from low to deeply dormant. The divergence in germination timing is usually exhibited by a rapid first wave of germination followed by another wave of germination several weeks but can also takes place in two waves separated by a whole year (Körner 2003 based on Soyrinki (1938)). Germination timing has consequences on seedling establishment, e.g. autumn seedlings grow faster than spring seedlings (Satyanti, Guja & Nicotra 2019) and likely further along the ontogeny (Donohue *et al.* 2005; Donohue *et al.* 2010). Delving further into staggering germination syndrome, such as examining whether the source of variation lies either within individual or across individuals within a population or across populations and whether such syndrome is conserved will help us to understand further the ecology of unique alpine flora and their fate under changing climate.

Like cold stratification, the gibberalic acid (GA3) hormone can be used to help overcome inherent physiological dormancy. Our results showed that many researchers observed similar germination responses with the application of GA3 as with applying a cold stratification treatment. In some studies, GA3 is used to alleviate dormancy in seeds that received potentially inadequate germination cues (Hoyle et al. 2015; Baskin and Baskin 2014). In addition, using GA3 after, or in combination with other germination treatments, can alert researchers about the thresholds or strength of natural germination cues in some species. Deep physiological dormancy in alpine species is not uncommon (Schwienbacher et al. 2011), in which case, applying GA3 might improve germination rates when all other treatments fail to overcome this strategy. However, applying GA3 may be detrimental to germination in some instances (Hoyle et al. 2015), and result in negligible or no germination, whether or not seeds can return to a dormancy cycle or alter their germination requirements.

**Alternating temperatures and light**

Alternating temperatures and light regimes in alpine environments (together with a cold stratification requirement and a positive effect of increasing mean temperatures, as also detected this study) mimic the conditions that seeds experience in the upper soil, during snow-melt at the end of the winter (Billings and Mooney, 1968; Körner, 2003; Mondoni et al., 2012). Triggering seed germination at the very beginning of the spring season ensures that seedlings have the 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)). The slightly lower effect of alternating temperature detected for strict alpine respect to generalist species confirm the findings of Liu et al. (2013), who found a lack of a response to temperature fluctuation of the species distributed only at high elevations of the Tibet Plateau, arguing that in that area a high amplitude of temperature fluctuation occurs 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 maximum daily temperature could be a cue that the short summer growing season has begun (Liu et al., 2013), as confirmed by a higher effect of mean temperature for strict alpine respect to generalist species detected in this study. Solar irradiance penetrate to only the first millimeters into the soil in physiologically significant quantities (Tester and Morris,1987) and fluctuations of diurnal temperature decrease with increasing burial depts in the soil (at depts > 10 cm they might be too small to trigger germination of species requiring temperature variation; Assche and Vanlerberghe, 1989). 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), as seed mass (or better said the seed kernel, i.e. embryo and endosperm; Chen et Moles, 2018) may represent a constraint for seedling emergence of small-seeded species. Therefore, small seeds are more likely to require light for germination, which ensures that germination does not occur too deep in the soil for seedling emergence (Pons, 2000).

**Seed mass, embryo:seed ratio, 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 altitude. In alpine species, the relationship between seed mass with elevation is mixed, being either negative (Wang et al. 2014), positive (Pluess et al. 2005) or absent (Vandelook et al. 2012; Bauk et al. 2005) when looking at the species level. Similarly, at the population level, the relationship between seed mass and altitude is often absent (Mondoni et al. 2011; Bauk et al. 2015). 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 favour (Baker 1972; Wang et al. 2014). On the other hand, natural selection may also favour the production of larger seeds in species at higher altitudes because larger seeds enable plants to cope better with stressful environments (Westoby et al. 1992; Pluess et al. 2005).

Much less information is available concerning a possible selective advantage of embryo:seed size in alpine species. However, the similarity in embryo:seed size between generalist and strict alpine species confirms the absence of a correlation between embryo-seed size and altitude found previously in Apiaceae species (Vandelook et al. 2012). Although seed size and embryo:endosperm ratio are not different for generalist and strict alpine species in these 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 (Hoyle et al. 2015; Satyanti et al. 2019; Sommerville et al. 2013). 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).

A general assumption of seed trait ecology is that seed mass and embryo:seed ratios are relatively homogeneous across related species (refs). In contrast, physiological responses to temperature and dormancy patterns are more plastic traits that could be quickly adapted to new situations. 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 thus suggested to co-adaptation. Whilst the phylogenetic signal in seed germination has been often found, especially in highly selective environments (Carta et al., 2016; Arène et al., 2017), seed germination patterns can be also shared between phylogenetically distant species (Vandelook et al., 2019) with low or absent phylogenetic signal (Fang et al., 2017; Rosbakh and Poschlod, 2015). However, the phylogenetic signal found here indicate that the 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 results of trait conservatism, convergence or both (Losos 2008; Revell et al., 2008).

**Conclusions**

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