# # Discussion

**## Seed dormancy**

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 [@RN3214]. In the temperate mountain flora, dormancy is achieved by different physiological mechanisms together with seed anatomy [@RN3213; @RN2943; @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 [@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 [@RN3214], which are well represented in alpine floras.

However, not all species from alpine habitats have dormant sees. We found that, under high temperatures, non-dormant seeds can germinate readily in autumn, and this would provide a 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 seasons become longer, if 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 [@RN3317; @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.

The extreme germination uncertainty syndrome, i.e. staggering germination so that emergence occurs both before and after winter, is known in alpine species [@RN2392; @RN3285; @RN4907]. 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 later but can also take place in two waves separated by a whole year [@RN2392]. If autumn seedlings grow faster than spring seedlings [@RN4907], this will likely have further effects along the life of a plant [@RN3036; @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.

**## Dormancy break**

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 [@RN3214; @RN4919]. Our results confirm that cold stratification is important for promoting seed germination in species inhabiting alpine habitats, especially for strict alpine species. 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 [@RN3393; @RN2868; @RN707; @RN698; @RN3703; @RN2355; @RN3285; @RN2371; @RN4713]. Under natural conditions, a stable cold stratification treatment of the seeds occurs over winter when they are covered with 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 favorable for seedling survival and growth. Global climate change is causing large changes in the snow cover duration in several alpine areas around the world [@RN4917; @ RN4918]. In some areas, this is leading to shorter, or even the absence of, natural cold stratification periods under snow for some alpine species, and could suggest decreases in seed germination rates in the future, compromising their population viability and indirectly favoring species with no such requirement [@RN3703]. Thus, our global assessment suggests that major impacts on the natural regeneration process of alpine species may be expected according to the current trends in climate change.

The gibberellic acid hormone (GA~3~) can be used to overcome inherent physiological dormancy. Our results show that many researchers observed similar germination responses after the application of either GA~3~ or cold stratification. In some studies, GA~3~ is used to alleviate dormancy in seeds that received potentially inadequate germination cues [@RN3214; @RN3285]. In addition, using GA~3~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 [@RN2943], in which case, applying GA~3~ might improve germination rates when all other treatments fail to overcome this strategy. However, applying GA~3~ may be detrimental to germination in some instances [@RN3285], and result in negligible or no germination.

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

**## 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 that live close to the treeline [@RN2371], or congeneric counterparts from below the treeline [@RN3266]. Considering that risk reduction is an important selective pressure for the evolution of germination traits [@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 [@RN4908; @RN4906; @RN4722]. 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 [@RN4722], yet high heat tolerance up to 40–50°C [@RN4902]. Plants in high-elevation habitats generally do not experience drought limitation because precipitation increases while evapotranspiration decreases along elevational gradients [@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 [@RN4530] but may require warmer germination temperatures than large-seeded species, especially in seasonal climates [@RN2399]. Accordingly, this pattern was stronger in strict alpine plants which, being restricted to higher elevations, presumably experience less unpredictable drought compared with generalist species. Taken together, our results indicate that germination patterns in alpine species are 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 [@RN4905], while precipitation and the probability of early or late season frosts generally increase with elevation [@RN2392; @RN4897].

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 [@RN3734; @RN3690], 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.

**## 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 the winter [@RN4712; @RN2392; @RN3029]. Triggering seed germination at the very beginning of the spring season ensures that seedlings have time to establish during the short growing seasons [@RN2392] and grow to a critical biomass before the next winter [@RN2868]. The slightly lower effect of alternating temperature detected for strict alpine respect to generalist species confirm the findings of Liu et al. [-@RN3376], who found a lack of a response to temperature fluctuation of the species distributed only at high elevations of the 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 [@RN3376]. This is confirmed by the higher effect of mean temperature for strict alpine respect to generalist species detected in this study.

Solar irradiance in physiologically significant quantities penetrates only the first millimetres of the soil [@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 [@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 [@RN4898], as seed mass - or better said the seed kernel, i.e. embryo and endosperm [@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 [@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 [@RN4912], positive [@RN4903] or absent [@RN3685; @RN4896] when looking at the species level. Similarly, at the population level, the relationship between seed mass and elevation is often absent [@RN3442; @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 [@RN4895; @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 [@RN4913; @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 [@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 [@RN3703; @RN3285; @RN4907]. Perhaps accumulation of reserves in cotyledons rather than in the endosperm may improve early growth rate and establishment in immediate germinating species [@RN3285].

A general assumption of seed trait ecology is that seed mass and embryo:endosperm ratios are relatively homogeneous across related species [@RN4916; @RN3685]. In contrast, physiological responses to temperature and dormancy patterns are understood as more plastic traits that can quickly acclimatize in new situations [@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 thus subjected to co-adaptation. Whilst phylogenetic signal in seed germination has been often found, especially in highly selective environments [@RN3001; @RN2399; @RN2865], seed germination patterns can also be shared between phylogenetically distant species [@RN4911] with low or absent phylogenetic signal [@RN2365; @RN4900; @RN2371]. 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 [@RN4901; @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 GA~3~ 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 the majority of 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 [@RN4922; @RN4921], fast or slow developing leaf traits [@RN4923], or large sets of plant ecological features [@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 [@RN4926; @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 GA~3~.

\* A requirement for warm germination temperatures.

\* 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.