

RESEARCH PAPER

Comparative germination ecology of two altitudinal vicariant *Saxifraga* species endemic to the north of Spain

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Keywords

Adaptation; altitudinal gradient; germination; *Saxifraga canaliculata*; *Saxifraga trifurcata*; species replacement; stratification.

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ABSTRACT

Seeds of high-mountain species are thought to germinate rapidly, synchronously and at high percentages after a cold period, with limited dependence on the external environment; yet, empirical evidence only partially supports this behaviour. We performed a comparative study of the germination response of two closely related taxa along an altitude gradient in northern Spain. Seeds from several maternal families of six populations of *Saxifraga trifurcata* (lowland species) and *S. canaliculata* (highland species) were subjected to temperature and stratification treatments. Germination percentages and germination rates were analysed using generalised linear mixed modelling and accelerated failure-time modelling. We found that germination percentages and germination rates were high and dependent on incubation temperature in both species. Within species, seeds from higher altitudes had higher germination percentages under all conditions. Cold-wet stratification negatively affected germination success, particularly in the lowland species. Overall, the highland species was less responsive to the experimental treatments and showed more synchronous germination patterns. We conclude that seeds from these two *Saxifraga* species germinate as efficiently as species from other habitats, but have a narrower germination response, probably due to the stronger selective pressures in their harsh environments. Finally, a cold, wet stratification period is not a prerequisite for the germination of high-mountain *S. canaliculata*, and its strong negative effect on the germination of its lowland relative *S. trifurcata* may contribute to the altitudinal segregation of these two species.

INTRODUCTION

Germination and establishment are critical stages in the life cycle of plants and are therefore subject to Darwinian selection in the wild (Donohue *et al.* 2010). According to Baskin & Baskin (1998), natural selection favours germination patterns that reduce the probability of facing adverse environmental conditions for seedling establishment. In the case of high-mountain plants, the short growing season may be a major bottleneck for successful recruitment, because seedlings must reach a critical size to survive the long and harsh winter season (Maruta 1983, 1994; Chambers *et al.* 1990; Körner 1999; Schütz 2002; Forbis 2003; Shimono & Kudo 2005). Seeds must be adapted to germinate soon after winter, thus avoiding unfavourable seasons, and when temperature and soil moisture may be appropriate for germination, but not for persistence in the short term (Meyer & Monsen 1991). In this sense, several studies have reported that arctic and alpine species require higher temperatures to germinate than lowland plants. This can be interpreted as an adaptation to avoid germination in early spring or autumn, when a high probability of frost events may result in a low probability of seedling establishment (Billings & Mooney 1968; Chabot & Billings 1972; Cavieres & Arroyo 2000; Giménez-Benavides *et al.* 2005). With regard to germination timing, many high-

mountain plants produce seeds with different types of dormancy to avoid germination in the year of dispersal and favour rapid emergence after snowmelt (Billings & Mooney 1968; Baskin & Baskin 1998; Shimono & Kudo 2005). This ability to delay seedling emergence and build permanent and transient seed banks might be an adaptive response in alpine environments, as alpine soils are generally suitable for seed storage, post-dispersal predators and seed pathogens are scarce and cryoturbation and freeze-thaw cycles promote germination (Cavieres 1999). Furthermore, high inter-annual variability in the duration of the snow-free growing season makes some years more suitable for germination processes, further encouraging the establishment of seed banks (Cavieres 1999). Previous studies have found that the germination of many high-mountain plants is promoted by cycles of cold-wet stratification that release seed dormancy in transient and permanent seed banks (Baskin & Baskin 1998; Shimono & Kudo 2005; Giménez-Benavides *et al.* 2005).

Another important determinant of germination response is maternal effects. The mother plant's genotype may affect seed quality, including the level of seed dormancy and germination (Vange *et al.* 2004). We hypothesised that harsh environmental conditions may act as a strong environmental filter and allow little inter-individual variation in germination response, thereby constraining maternal effects more than in

milder environments. To our knowledge, this hypothesis has never been explicitly explored in germination studies on high-mountain plants.

The constraints imposed by cold and highly seasonal environments would presumably subject the germination of high-mountain and arctic plants to strong selective pressures and high canalization (Meyer & Monsen 1991; Cavieres & Arroyo 2000). If so, the germination ecology of high-mountain plants should take advantage of short temporal windows for establishment and show rapid and highly synchronous germination, low plasticity in germination response to contrasting conditions (like temperature or chilling) and low variability among individuals coexisting in a population. Empirical evidence, however, is inconclusive regarding this hypothesis, and a comparative method would be the most suitable approach for testing it (Baskin & Baskin 1998; Körner 1999).

Our study system consisted of two *Saxifraga* species endemic to N Spain: *S. trifurcata*, which inhabits lowland sites, and *S. canaliculata*, typical of high-mountain locations. This system is suitable for testing the intraspecific and interspecific determinants of germination response to altitude, as populations of the same species are located at different altitudes, and the species occur at different altitudinal ranges. Studies on the germination of single plant species along their altitudinal ranges are abundant in the literature (e.g. Dorne 1981; Cavieres & Arroyo 2000; Karlsson & Milberg 2007), but few studies compare closely related taxa replacing each other along an altitude gradient. This type of comparison offers an advantage in experimental design, because species-specific *versus* plastic germination responses can be tested separately. Our overall hypothesis was that the germination response of seeds of populations and species from different environments should differ in predictable ways, *i.e.* their germination percentage should be lower under laboratory conditions associated with higher risks in the field. We specifically expected higher-altitude populations to exhibit: (i) higher germination percentages and rates after a period of cold-wet stratification; (ii) faster germination rates than seeds from lowland origins; and (iii) lower variability in their germination response to contrasting ambient temperatures. We also expected them to be less affected by maternal effects, and their germination timing to be more synchronous in response to narrow selective pressures.

MATERIAL AND METHODS

The species

The two study species belong to the genus *Saxifraga* sect. *Saxifraga*, subsect. *Triplinervium* (Vargas 1993). *Saxifraga trifurcata*

urcata Schrad. is a cushion fissuricolous chamaephyte comprised of a large number of well-defined rosettes. Inflorescences (two to six per rosette) arise in the axils of the previous year's leaves. It is endemic to limestone rock fissures in the northern part of the Iberian Peninsula and occurs from sea level to ~1400 m a.s.l. *Saxifraga canaliculata* Boiss. & Reut. ex Engl. is morphologically similar to *S. trifurcata*, but has a more compact growth form with shorter and thicker leaves, and a single inflorescence arises from the centre of each reproductive rosette (Vargas 1991). It grows in limestone crevices from ~1300 to ~1900 m a.s.l. and is endemic to the Cantabric Range (Spain). Therefore, these two species are typical altitudinal vicariants, with a narrow contact zone of ~1300–1400 m where infrequent hybrids are occasionally sighted (*Saxifraga faucicola*; Diaz-González *et al.* 1990). Seasonality of reproduction in these species is similar, as *S. trifurcata* starts flowering in early spring and *S. canaliculata* in mid-spring (Milla *et al.* 2008).

Source populations and seed collection

Three populations per species were selected along a steep elevation gradient in Picos de Europa (Cantabric Mountain Range, Asturias province, Spain). Seeds of *S. trifurcata* and *S. canaliculata* were collected at 100, 1064 and 1360 m and at 1360, 1430 and 1872 m a.s.l., respectively. The highest population of *S. trifurcata* and the lowest population of *S. canaliculata* coexist. The length of the growing season along the altitudinal gradient (*i.e.* number of months with mean monthly temperature >5 °C) ranges from 12 months in the lowest population of *S. trifurcata*, to 4 months in the highest population of *S. canaliculata*. Furthermore, the difference in mean annual temperature from the lowest to the highest study populations was *ca.* 11 °C. All populations had a large number of individuals and inhabited ecologically similar sites (limestone rock outcrops). Detailed information on the sampled populations is provided in Table 1.

From July to August 2006, mature fruits were collected from four mother plants (*i.e.* seed families, herein) in each population of each species. Seeds were cleaned, placed in paper bags filled with silica gel and stored at room temperature in darkness for 4–5 months until trials started.

Germination tests

A total of 6450 seeds were planted in January 2007 under the following experimental design. Seed families (*i.e.* groups of seeds from each mother plant) were divided into batches of 25 full-sized seeds and placed in 258 Petri dishes on a double

Table 1. Location and climate of the study populations of *S. trifurcata* (lowland species) and *S. canaliculata* (highland species). All sites are located along an altitude gradient in north Spain. MAT stands for mean annual temperature. Growing season length is the number of months with mean monthly temperature >5 °C.

site (UTM)	species	altitude (m a.s.l.)	MAT (°C)	rainfall (mm-year ⁻¹)	growing season length (months)
30T 353 4807	<i>S. trifurcata</i>	100	14.7	1248	12
30T 337 4791	<i>S. trifurcata</i>	1064	8.2	1602	8
30T 338 4789	<i>S. trifurcata</i>	1360	6.9	1629	6
	<i>S. canaliculata</i>				
30T 338 4789	<i>S. canaliculata</i>	1430	6.7	1642	6
30T 339 4787	<i>S. canaliculata</i>	1872	3.9	1676	4

layer of moistened filter paper. Two Petri dishes per seed family and population were subjected to the following treatments: half of the seeds were subjected to a 8-h dark/16-h light photoperiod and three alternating temperature regimes (5/10 °C, 5/15 °C and 10/20 °C), simulating cold, temperate and warm environmental conditions after a snow-free winter period (typical of the lowland sites). Temperature ranges were based on mean temperatures of the study sites (Milla *et al.* 2008). The other half of the seeds was subjected to a cold–wet stratification treatment to simulate chilling conditions under snow-pack (typical of our highland sites). Petri dishes were covered with two more layers of filter paper and wetted with distilled water to ensure humid and dark conditions. Dishes were wrapped in aluminium foil and stored in a refrigerator at 2–4 °C for 2.5 months before germination tests.

The two sets of germination tests were carried out consecutively in three germination chambers (Selecta Hotcold GL, Barcelona, Spain) equipped with six cool-white fluorescent light tubes (Philips 18 W ‘TL’D standard type, wavelength 400–650 nm) providing a photon flux density of *ca.* 19 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and a relative humidity of about 70%. Filter papers were kept soaked throughout the experimental period. Every 5–7 days seeds showing radicle emergence were counted and removed. The arrangement of the dishes in the chambers was changed on each census date to avoid position effects. The experiments were considered to have finished when no radicle emergence was observed in a period of *ca.* 25 days. The remaining non-germinated seeds were then crushed, and seeds with a hard white embryo were considered viable. Final germination percentage was calculated excluding non-viable seeds.

Data analysis

Germination percentage

The effects of seed family, population of origin, stratification and chamber temperature on final germination percentage were analysed by fitting generalised linear mixed models (GLMMs). Since seed germination follows a binomial distribution (probability ranging from 0 to 1), we used a logit-link function and binomial error distribution (Venables & Ripley 1998; Schütz & Rave 1999). We performed a GLMM on the data for each species separately with experimental parameters (*altitude*, *stratification* and *temperature*) as fixed explanatory variables (stratification nested within temperature), and *seed family* as a random factor. In order to quantify the independent and shared relative importance of each factor in accounting for total variance in germination percentage, we carried out hierarchical partitioning of variance analyses (Chevan & Sutherland 1991; MacNally 2002). These procedures apply a hierarchical partitioning algorithm that implements independent and joint contributions of each explanatory variable to account for the total explained variance. This procedure is robust to deviations of the response variable from a normal distribution, allowing the explicit specification of binomial distributions, which are suitable for germination percentage data (Chevan & Sutherland 1991). GLMMs and variance partitioning analyses allowed us to test our first and third hypotheses on the effects of stratification

and altitude, and on the overall variability in response to the several experimental factors of the assay.

Additionally, we implemented a second model including *species*, *stratification* and *temperature* for the coexisting populations (1360 m) to compare species in a common environment. This latter model allowed us to test whether our first and third expectations, as outlined in the Introduction, hold when lowland and highland species, instead of lowland and highland populations, are considered. In all models, fixed factor effects were tested with *F*-tests, and the random factor was tested using the Wald *Z*-statistic test. The degrees of freedom at each level of variation were estimated using Satterthwaite’s method (Littell *et al.* 1996).

Germination rates

Germination rate was analysed using accelerated failure-time modelling. This method allows the use of right-censored data (*i.e.* experiments that end before all seeds germinate) to estimate parametric regression models. We used a maximum likelihood approach and the log-logistic distribution (Fox 2000). Since the germination curves of the stratified and non-stratified seed batches were visually very different, we implemented an independent model for each group with *species*, *altitude* and *temperature* as explanatory variables. *Post-hoc* pair-wise comparisons between groups were performed from the covariance matrix and statistics generated from tests of heterogeneity among groups (Fox 2000). GLMMs and failure-time modelling were performed using the GLIMMIX and LIFEREG procedures, respectively, of the SAS statistical package (SAS Institute, 1996). Analyses of hierarchical partitioning of variance were implemented using the hier.part package available for the R platform (R Core Development Team, 2008).

RESULTS

In general, all the seeds included in the experiment achieved high germination percentages, and mean germination percentage was 89% (Figs 1 and 2). The explanatory factors, *i.e.* altitude of seed origin, seed family, temperature and stratification, accounted for 24% and 18% of the total variance in germination percentages in *S. trifurcata* and *S. canaliculata*, respectively (Fig. 3).

Effects of cold–wet stratification on germination response of seeds from lowland and highland origin

Mean germination percentage tended to increase moderately with altitude in both species (see Fig. 2, and magnitude of altitude effects in Table 2). Cold–wet stratification did not affect the germination percentage of the highland species *S. canaliculata*, while it had a negative effect on germination of seeds of the lowland *S. trifurcata* (Table 2, Fig. 1). Stratification did not increase the germination percentage of seeds from higher altitudes in either species. Furthermore, GLMM models did not detect any significant differences in germination percentage between species at the coexisting altitude range, even though stratification negatively affected the germination percentage of *S. trifurcata* seeds at this site (Table 3).

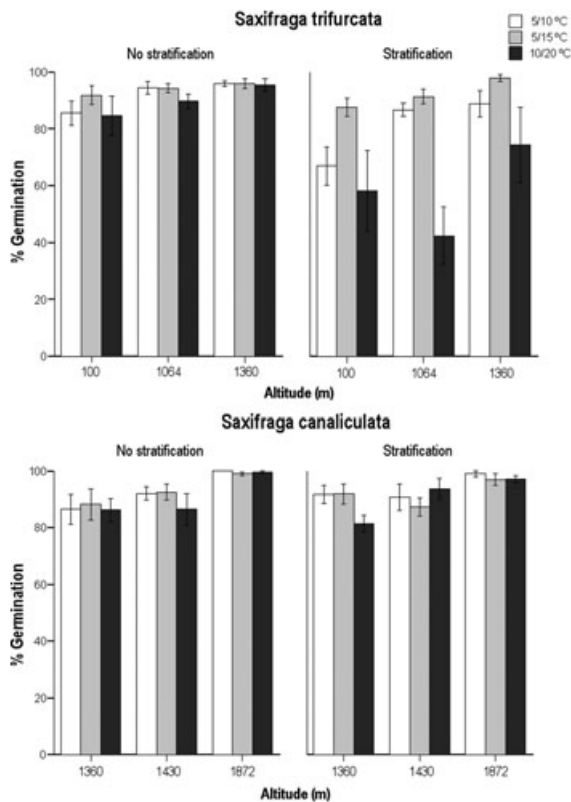


Fig. 1. Germination percentage (mean and SE) of *Saxifraga trifurcata* and *S. canaliculata* populations from the Cantabric Range (N Spain).

Results of accelerated failure-time analyses of germination rates are shown separately for stratified and non-stratified seeds. As stratification strongly affected germination rates by delaying seed germination (see Fig. 4), germination curves and the magnitude of other effects were easier to appreciate when analyses were carried out separately for each stratification treatment. Non-stratified seeds of both *S. canaliculata* and *S. trifurcata* generally germinated very rapidly under all treatments, irrespective of altitude of origin. In contrast, stratified seeds germinated more slowly (Fig. 4, Table 4).

Germination rates of seeds from contrasting altitudes

Similar germination rates were obtained for *S. canaliculata* and *S. trifurcata* seeds (Fig. 4, Table 4). Furthermore, seeds collected from higher-altitude sites tended to germinate faster, irrespective of stratification or temperature treatments (Fig. 4, Table 4). Non-stratified seeds germinated at a similar rate, irrespective of the ambient temperature in the growth chamber (Fig. 4, Table 4). However, once stratified, temperature was the factor that had the highest effect on germination rate in both species (see Table 4), with seeds at lower temperatures germinating faster (Fig. 4).

Variability and synchronicity of germination in response to temperature and seed family

In *S. canaliculata*, similar germination responses were obtained under the three assayed temperature regimes. In

contrast, *S. trifurcata* seeds germinated better at average temperatures, although this effect was only apparent after stratification (Table 2, Fig. 1). Seed family affected germination percentage in both species. Although the effect of seed family was of a similar magnitude and explained a similar fraction of total variance in both species, it had a significantly higher effect on germination percentage in *S. trifurcata* (Table 2, Fig. 3). Seed family also had a significant effect on germination rate (Table 4), with some family lines germinating faster than others in both species. In general, stratified seeds germinated more asynchronously than non-stratified seeds (Fig. 4).

DISCUSSION

The obtained results support our hypotheses that germination in the highland populations would take place faster, more synchronously and in a less variable manner. However, our expectation that germination performance would increase after a period of cold-wet stratification (chilling) was not supported by the obtained data: stratification reduced germination in all populations and species examined, although its negative effect decreased as altitude increased.

The germination percentages obtained in *S. trifurcata* and *S. canaliculata* were generally higher than those reported for other closely related *Saxifraga* spp. in the same Section (Webb 1950; Vargas 1994), as well as those reported for other angiosperms (see e.g. Grime *et al.* 1981). The two species showed similar germination percentages (at their coexisting altitude range) and overall germination rates, both of which were affected by seed family. However, other factors, such as cold-wet stratification, had a remarkably different effect on the germination percentages and germination rates of the two species. Lower variability and higher synchronicity were also found in the germination response of highland *S. canaliculata*. Within species, we found that seeds from highland populations germinated faster, more synchronously and at higher percentages than seeds from lowland populations.

Germination of high-mountain plants

Several germination traits have been claimed to be specific to high-altitude species. For instance, rapid onset of germination after snowmelt and high seed viability were considered adaptations of high-altitude species (see early screenings of Ludi 1933 or Söyrinki 1938). However, the high variability among alpine species casts doubt on these generalisations, and later comprehensive reviews have reported similar germination traits in alpine species and lowland or agricultural species (Baskin & Baskin 1998). In this respect, Körner (1999) concludes that, apart from a trend to increase germination after winter, alpine species do not show distinctive germination traits that could be considered an alpine germination syndrome.

When examined within species, *i.e.* comparable genetic entities, the germination performance of both saxifrage species increased with altitude. A number of studies have found germination performance decreases with altitude, while others have found no altitude effects, and yet others have obtained contrasting results depending on the species considered (Semwal & Purohit 1980; Dorne 1981; Holm 1994;

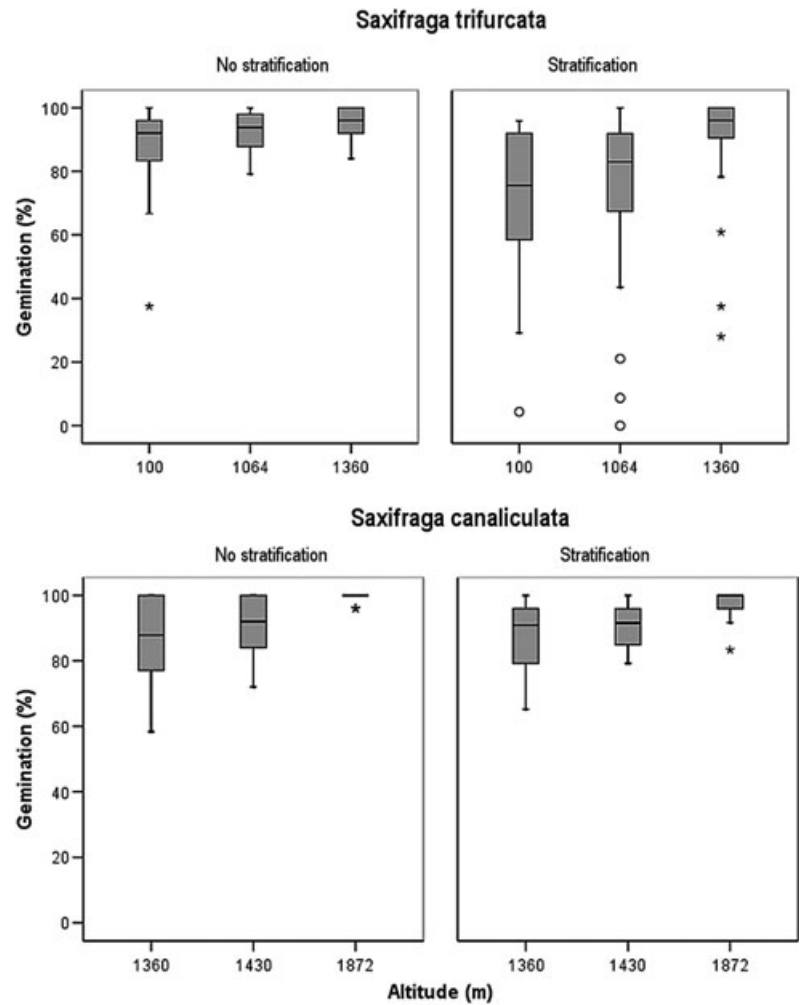


Fig. 2. Box plots summarising inter-population differences in germination response of non-stratified and stratified seeds of *S. trifurcata* and *S. canaliculata* (temperatures were pooled).

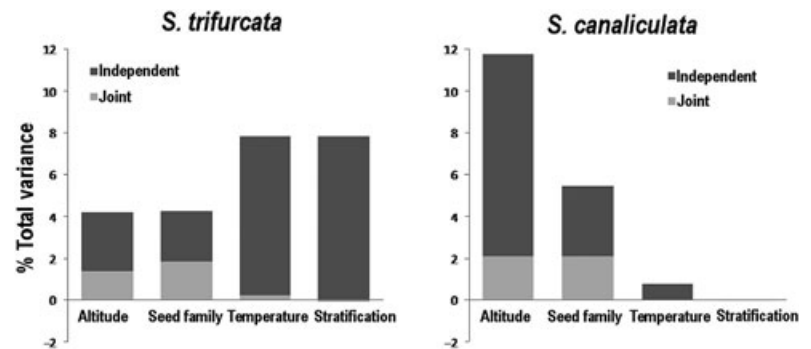


Fig. 3. Differences in variance partition analyses of germination response between *S. trifurcata* and *S. canaliculata*. Vertical bars represent the percentage of variance explained by each factor independently (dark grey), or jointly due to co-linearity with other factors (light grey), according to the analysis described in the Material and Methods. Total explained variance was 24% and 18% for *S. trifurcata* and *S. canaliculata*, respectively, according to the GLMMs shown in Table 2.

Nishitani & Masuzawa 1996; Vera 1997; Giménez-Benavides *et al.* 2005). In accordance with studies at the interspecific level, no general pattern can be established as to the intraspecific germination response of the studied species to altitude.

Germination response to environmental and maternal factors

In our *Saxifraga* species, higher incubation temperatures slowed down germination rates but did not consistently affect germination percentages. Only lowland *S. trifurcata* had

highly variable germination percentages after cold-wet stratification under the three assayed temperature regimes. These results do not agree with previous studies, which reported positive responses of germination percentages to increasing temperatures in high-mountain plants (see Giménez-Benavides *et al.* 2005; Graae *et al.* 2008; and refs. therein), suggesting an adaptation for seedling establishment in the very short growing season (Billings & Mooney 1968). With regard to germination rates, Lord (1994) found that low temperatures accelerated germination rates in *Festuca novae-zelandiae*

Table 2. Results of GLMMs for seed germination of *S. trifurcata* and *S. canaliculata*. *F*: Wald-type *F*-statistic used to evaluate if fixed factors explain a significant fraction of the deviance; Prob. (*F*): *P*-value of *F*-test; *Z*: Wald *Z*-statistic for random factors; Prob.(*Z*): *P*-value of *Z*-test.

<i>S. trifurcata</i>				<i>S. canaliculata</i>			
variable	df	<i>F</i>	Prob (<i>F</i>)	variable	df	<i>F</i>	Prob (<i>F</i>)
altitude	2	3.58	0.0449	altitude	2	32.83	<0.0001
temperature	2	21.39	<0.0001	temperature	2	3.42	0.0328
stratification	3	35.97	<0.0001	stratification	3	1.10	0.3461
		<i>Z</i>	Prob (<i>Z</i>)			<i>Z</i>	Prob (<i>Z</i>)
maternal plant (R)		2.87	0.0021	maternal plant (R)		1.87	0.0307

Table 3. Results of GLMMs for seed germination of *S. trifurcata* and *S. canaliculata* in the coexisting populations. *F*: Wald-type *F*-statistic used to evaluate if fixed factors explain a significant fraction of the deviance (with sign indicating the direction of the effects); Prob. (*F*): *P*-value of *F*-test; *Z*: Wald *Z*-statistic for random factors; Prob.(*Z*): *P*-value of *Z*-test.

variable	df	<i>F</i>	Prob (<i>F</i>)
species	1	0.39	0.5470
temperature	2	14.13	<0.0001
stratification	3	8.90	<0.0001
		<i>Z</i>	Prob (<i>Z</i>)
maternal plant (R)		2.02	0.0215

seeds from highlands but not in seeds from lowlands. Once again, this was not found our study, because low temperatures accelerated germination in all the seed batches tested. This could be interpreted as a trend for advanced seedling phenology as a strategy to compete for temporal regeneration niches. This hypothesis, however, requires further research.

Cold-wet stratification did not improve the germination response of the assayed *Saxifraga* species and, in fact, germination response was even lower compared to non-stratified seeds, especially in the lowland species *S. trifurcata*. Similarly, Giménez-Benavides *et al.* (2005) reported that the germination of high-mountain *S. pentadactylis* ssp. *wilkommiana* decreased after cold-wet stratification. However, the results obtained in these studies do not agree with most previous literature on alpine species (Chabot & Billings 1972; Bewley & Black 1982; Chambers *et al.* 1990; Baskin & Baskin 1998; Mondoni *et al.* 2009) and cast doubt on the generalisation that cold periods are required for effective germination in high-mountain environments. The germination percentage of non-stratified seeds was very high (80–100% across species and populations), suggesting low levels of physiological dormancy in these *Saxifraga* species. However, it should be taken into account that the seeds used in this study were stored in dry conditions at room temperature for 4–5 months before the experiments. It is known that many seeds require a period of dry storage to release primary dormancy and promote germination, a process called after-ripening (Finch-Savage & Leubner-Metzger 2006; Rubio de Casas *et al.* 2012). We therefore speculate that a fraction of the non-dormant seeds cycle back into a secondary dormant state when exposed to the chilling treatment, especially in the lowland species *S. trifurcata*. In many temperate species, prolonged periods of seed chilling can induce secondary dormancy (Baskin & Baskin 1998; Finch-Savage & Leubner-Metzger 2006; Rubio de Casas

et al. 2012). A cold period may trigger the perception of unfavourable conditions for germination, and a variable proportion of seeds may enter the permanent seed bank. Similar delayed and multimodal responses to germination have been documented for other mountain genera (Cavieres 1999), and in species of other habitats when germination patterns of single seed cohorts have been monitored for a long period (Karlsson & Milberg 2007).

Germination, like any other physiological process, is also dependent on the genetic and phenotypic factors of the seed and the mother plant. Several life-history traits, such as life form, seed set phenology, dispersal strategy and seed size, are known to affect seed germination (Rees 1996; Bu *et al.* 2008). We grouped the genetic determinants of these factors together through the inclusion of several seed families per seed origin in the experimental design. We found that seed family had a significant effect on germination percentages and germination rates, irrespective of species. This maternal effect on germination performance has also been reported for other species (Vange *et al.* 2004; Luzuriaga *et al.* 2006), but we are unaware of previous germination assays that used family lines in alpine species. These results highlight the importance of factors other than macroclimate (genealogical factors, microsite effects, *etc.*) in modulating physiological responses to altitude (Milla *et al.* 2009).

Segregation of species along altitude gradients and germination traits

Previous works have used germination response to determine how closely related species segregate across geographic space. For instance, Graves & Taylor (1988) analysed germination response in relation to the contrasting altitudinal distribution of *Geum rivale* (highland species) and *G. urbanum* (lowland species). They found that the lowland species tended to germinate at lower temperatures, which, when extrapolated to field conditions, could be risky in cold highland environments. This was interpreted following Grime *et al.* (1981) as a limitation for expanding geographic range towards colder regions. Our lowland and highland *Saxifraga* species, however, showed rather similar germination response. The most remarkable differences between these two species were higher synchronicity and lower responsiveness to cold-wet stratification and temperature in highland *S. canaliculata*. This supports our third hypothesis and highlights that species living in harsher habitats with shorter growing seasons should show less plasticity or more narrow germination traits (Debat & David 2001).

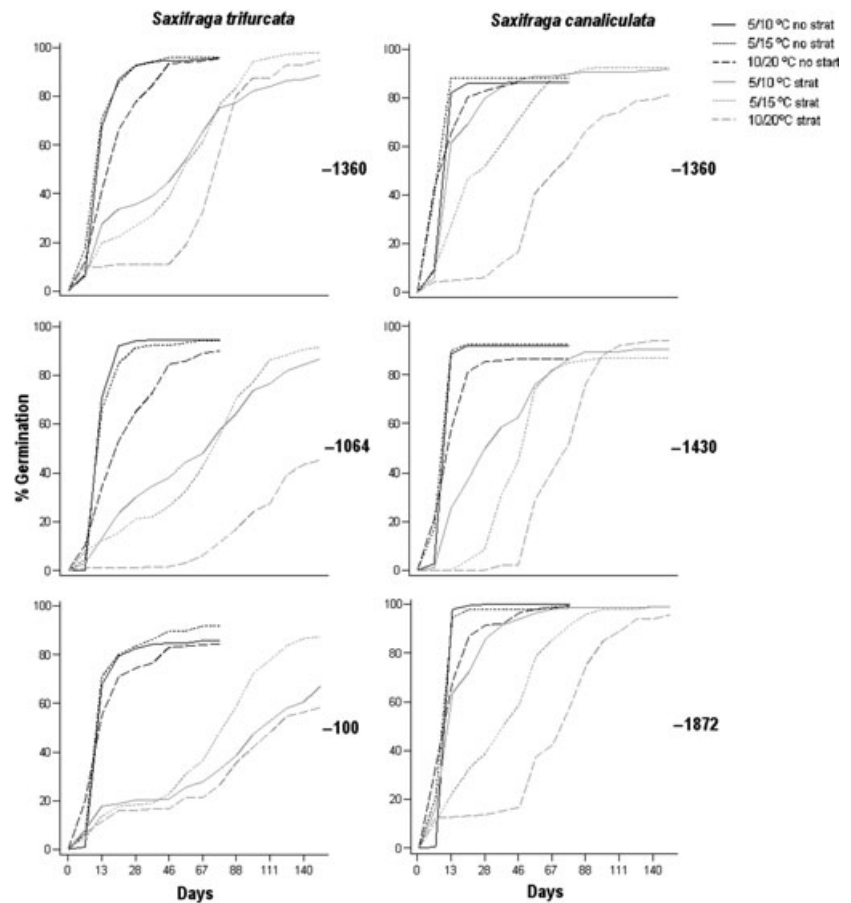


Fig. 4. Germination curves of *S. trifurcata* and *S. canaliculata*. Numbers on the right side of plots indicate the altitude of populations.

Table 4. Results of accelerated failure-time analyses (log-logistic distribution) for germination rate of *S. trifurcata* and *S. canaliculata*.

variable	df	chi-square	<i>P</i>
<i>no stratification</i>			
intercept		2646.26	<0.0001
species	1	4.43	0.0352
altitude	4	284.98	<0.0001
temperature	2	25.18	<0.0001
seed family	23	165.58	<0.0001
log likelihood = -2466.44			
<i>stratification</i>			
intercept		1594.99	<0.0001
species	1	1.79	0.1804
altitude	4	135.03	<0.0001
temperature	2	716.38	<0.0001
seed family	22	519.69	<0.0001
log likelihood = -2955.86			

The climate in the lowland study area is benign, with oceanic type regimes, mild winters, evenly distributed rainfall and monthly average temperatures over 5 °C throughout the year. This suggests that selection pressures towards fixed germination timing are low, since the risks to seedling establishment are low in all seasons. Thus, lowland *S. trifurcata* shows higher variability in response to temperature and stratification. Specifically, lower germination performance after cold

stratification could be an adaptive consequence of its rare exposure to cold winters in the populations of origin, and could contribute to constraining its distribution to mild, low-altitude regions. On the other hand, the extremely narrow germination response of *S. canaliculata* could make it a poorer competitor to exploit the diverse set of temporal regeneration niches in the lowlands. Further reciprocal transplant and demographic monitoring studies are needed to assess the relevance of the germination response reported here in accounting for the altitudinal segregation of vicariant species.

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