


Seed dormancy and longevity in subarctic and alpine populations of *Silene suecica*

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Abstract Despite the strong environmental control of seed dormancy and longevity, their changes along latitudes are poorly understood. The aim of this study was to assess seed dormancy and longevity in different populations across the distribution of the arctic–alpine plant *Silene suecica*. Seeds of seven populations collected from alpine (Spain, Italy, Scotland) and subarctic (Sweden, Norway) populations were incubated at four temperature regimes and five cold stratification intervals for germination and dormancy testing. Seed longevity was studied by exposing seeds to controlled ageing (45 °C, 60% RH) and regularly sampled for germination. Fresh seeds of *S. suecica* germinated at warm temperature (20/15 °C) and more in subarctic (80–100%) compared to alpine (20–50%) populations

showed a negative correlation with autumn temperature (i.e., post-dispersal period). Seed germination increased after cold stratification in all populations, with different percentages (30–100%). Similarly, there was a large variation of seed longevity (p_{50} = 12–32 days), with seeds from the wettest locations showing faster deterioration rate. Subarctic populations of *S. suecica* were less dormant, showing a warmer suitable temperature range for germination, and a higher germinability than alpine populations. Germination and dormancy were driven by an interplay of geographical and climatic factors, with alpine and warm versus subarctic and cool autumn conditions, eliciting a decrease and an increase of emergence, respectively. Germination and dormancy patterns typically found in alpine habitats may not be found in the arctic.

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Introduction

Seed dormancy and seed longevity are two fundamental traits that affect plant regeneration. The first one is the mean by which plants regulate the germination of seeds in the appropriate period of the year, when conditions remain favourable long enough for the seedling to establish (Finch-Savage and Leubner-Metzger 2006); the second one is a quantitative trait to estimate the seed's life span (Kochanek et al. 2010; Mondoni et al. 2014). Both traits contribute to the formation of soil seed banks, a stock of viable seeds that allow species persistence when conditions are not suitable for germination and growth—also referred to as the capacity of species to disperse in time.

In alpine plants, physiological dormancy (*sensu* Baskin and Baskin 2014) is the most common type (Schwienbacher et al. 2011), which evolved to prevent seed germination before and/or during the cold winter season when a high risk of frost would otherwise result in low seedling survival (Marcante et al. 2012). After exposure to winter, the temperature window for germination usually widens toward lower values because of the process of loss of physiological dormancy. Hence, seed germination and seedling establishment of alpine plants tend to occur in early summer (Körner 2003) and/or even rapidly after snowmelt (Mondoni et al. 2015; Niederfringer Schlag and Ershbamer 2000). Interestingly, the seed dormancy level is predicted to drive changes in the timing of germination of alpine plants from spring to autumn in the context of climate warming. In particular, a warmer autumn can enhance seed germination in weakly dormant species (Mondoni et al. 2012; Orsenigo et al. 2015), with uncertainties related to the fate of seedlings developed in autumn and subsequently exposed to frost (Marcante et al. 2012).

Seed dormancy can be related to the season of major risk for the seedling survival (Mattana et al. 2014; Carta et al. 2014), suggesting that its presence and intensity may vary across populations which experience different climates. However, early studies on the relation between germination patterns and geographical distribution found only small differences across populations (Thompson 1970a, b). Nevertheless, subsequent investigations highlighted that maturation and even the post-dispersal environments may strongly affect the expression of dormancy and germination (Donohue 2005; Wagmann et al. 2012; Fenner 1991; Bernareggi et al. 2016; Carta et al. 2016), with warmer temperatures resulting in reduced seed dormancy and increased germinability (Fernandez-Pascual et al. 2013; García-Fernández et al. 2015). In this regard, despite numerous common features, alpine environments from equatorial to polar vary latitudes with respect to their atmospheric conditions (Nagy and Grabherr 2009), the amount and quality of solar radiation, photoperiod and the regime of precipitation, both in quantity and seasonal distribution (Chabot and Mooney 1985). Spring starts earlier in alpine ecosystems than in the arctic due to higher solar radiation, and although freezing typically occurs earlier in alpine areas than in the arctic, soil temperatures during the growing season are generally lower in the latter (Ernakovich et al. 2014). However, site-specific characteristics (e.g., topography) may confound this general pattern. Consequently, the possibility that selective pressures arising from contrasted climatic differences between arctic and alpine regions may act on germination and dormancy behaviour and that ecotypes might thus develop cannot be ruled out. However, little is known about intra-specific variation of germination in widely distributed arctic and alpine species.

In addition to genetic factors, seed longevity is also affected by environmental conditions under which seeds develop (Gasque and Garcia-Fayos 2003; Probert et al. 2009). For instance, plants from cold–wet environments produce short-lived seeds, compared to species/populations from warmer and drier climates (Daws et al. 2007; Kochanek et al. 2010; Mondoni et al. 2011). This can be explained by the low selective pressures for high-resistant seeds to ageing in the cold and wet arctic and alpine habitats, where seed depauperation over time is naturally low (Schwienbacher et al. 2010). In fact, seed ageing is accelerated under warm and wet conditions, as seeds developed under these conditions possess more efficient DNA repair mechanisms and antioxidant metabolisms (Ventura et al. 2012). Moreover, recent studies have demonstrated genetically-based seed longevity can be further modulated by the environment through trans-generational parental effects (Kochanek et al. 2011) and that such changes may potentially allow progeny adaptation to habitat conditions experienced by the maternal parent (Mondoni et al. 2014; Bernareggi et al. 2015).

Considering the effect of climatic factors on seed dormancy and longevity, both traits are expected to vary in space (e.g., between different populations). However, only few studies comparing alpine and subarctic populations of a species in seed dormancy (Wagmann et al. 2012) and none on seed longevity are available (Abeli et al. 2014). We thus assessed the pattern of seed germination, dormancy and longevity across the European range of *Silene suecica* (= *Lychnis alpina*), with the aim to highlight variation in such traits linked to climatic patterns. *S. suecica* is of particular interest because its distribution was strongly affected by climatic changes during the Quaternary (Haraldsen and Wesenberg 1993), which may have promoted population differentiation and different adaptations across its distribution range. In general, we hypothesized that traits related to seed dormancy and longevity differ between populations of *S. suecica* in Europe, with seeds from warmer locations showing lower dormancy and higher germinability and seeds from warmer and dryer locations showing higher longevity.

Materials and methods

Species description

Silene suecica (Lodd.) Greuter and Burdet, is a perennial, short-lived arctic–alpine–amphi–atlantic species in the Caryophyllaceae family (Nagy 2013). The species has its main distribution range in the subarctic and arctic areas in Scandinavia, Iceland, Greenland and north-western Canada, but also occurs in southern mountain chains of Europe (Alps, Apennines and Pyrenees; Haraldsen and Wesenberg 1993;

Aeschmann et al. 2004). It shows considerable morphological variation, which is reflected by the recognition of several intraspecific taxa (Nagy 2013). From a reproductive point of view, *S. suecica* is a hermaphrodite, protandrous, mainly out-crossing species, which is insect pollinated (Carlsson-Graner 2006). Literature suggests that vegetative reproduction does not take place (Warming 1920), plants dug out in the field showed the production of short lateral ramet from the main rosette, therefore, vegetative reproduction can occur (Abeli T., pers. obs.). The seeds are dispersed by gravity and usually fall within 0.5 m from the mother plant (Lundqvist and Andersson 2001). *S. suecica* produces a soil seed bank, estimated by Nagy and Proctor (1996) to be between 300 (July and October) and 1000 (February) seeds per m² at a depth of 4.5 cm.

Collection sites

Seeds of *S. suecica* were collected at the time of natural seed dispersal in September 2012 from seven populations from across its European geographical range (Fig. 1): two populations from the southernmost site of the species' occurrence in the northern Apennines (Italy, APE) and the Cantabrian mountains (Spain, SPA), two populations from the Italian Alps (ALP1 and ALP2), one population from the Scottish Highlands (SCO) and two populations from the northern distribution limit in Scandinavia [southern Norway (NOR) and northern Sweden (SWE)]. Detailed information about the sampling sites are provided in Table 1. After collection, seeds were processed (cleaned) and stored at room temperature for a maximum of 2 weeks until the beginning of the experiment.

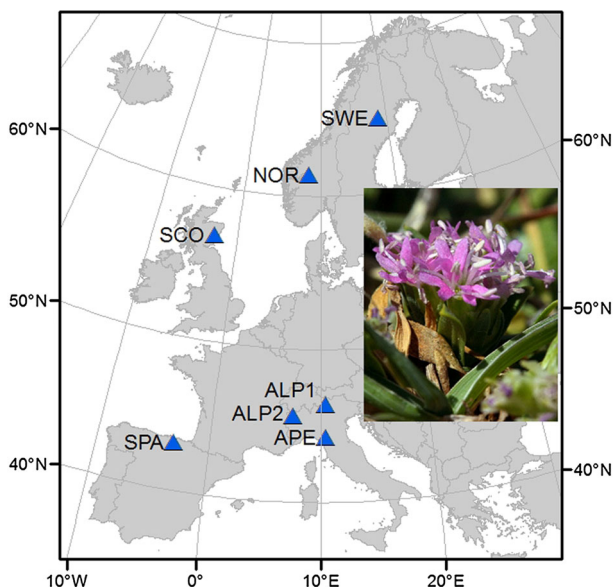


Fig. 1 Sampling locations of the seven populations of *Silene suecica* (colour figure online)

Laboratory germination

Experiments were conducted in temperature- and light-controlled incubators at 12 h daily photoperiods with a photosynthetically active radiation of 40–55 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (400–700 nm). All seeds were sown on 1% agar in distilled water, with three replicates of 30 seeds each per test (Baskin and Baskin 2014). Fresh seeds of all populations were exposed to 0 °C for 4, 12, 20 and 32 weeks to determine whether a period of cold stratification (CS) was required prior to germination. After each chilling period, 3 × 30 seeds were moved to each of the four alternating temperature regimes 5/0, 10/5, 15/10 and 20/15 °C (light 12 h/dark 12 h; light coinciding with the higher temperature, and dark coinciding with the lower temperature). Germination test on fresh seeds was also performed at all incubation temperatures described above, but without cold stratification. Germination tests were performed under alternating temperatures to simulate daily temperature fluctuations, as they occur in the natural environment, particularly in alpine/arctic habitats (Körner 2003). Furthermore, to evaluate whether ungerminated seeds after chilling were still dormant, a subsample of seeds which had been cold-stratified for 20 weeks was incubated with 250 mg/l of GA₃ incorporated into 1% agar at 15/10 °C (light conditions and methods of sowing as described above). This test was performed only with seeds from those populations where germination was < 80% (APE, ALP1, ALP2, SPA) after cold stratification. This test was not executed for SCO because of the limited number of available seeds. In each case, seeds were scored for germination at weekly intervals with germination defined as visible radicle emergence. At the completion of each germination test (5 weeks after sowing), non-germinated seeds were cut-tested to check their viability. Empty seeds or fungus infected with fleshy or dark embryo were considered non-viable, while those with a firm, fresh embryo were deemed to be still viable. Then, the final germination percentage was calculated excluding non-viable seeds.

Seed longevity

Seed longevity was determined using a standard rapid ageing protocol (Newton et al. 2009). For each population, 9 samples of 50 seeds were transferred to a sealed container over a non-saturated LiCl solution at 60% relative humidity (RH) and placed in a compact incubator (Binder FD53, Tuttlingen, Germany) in the dark at 45 ± 2 °C. The RH generated by the LiCl solution in the container was checked with a data logger (Tinytag View 2 Temperature/Relative Humidity Logger, Gemini Data Logger, Chichester, UK) placed inside the container. If necessary, the LiCl solution was adjusted by adding distilled water, stirring and allowing

Table 1 Location and climatic characteristics of the sampled populations

Pop ID	Location	Area (Country)	Latitude	Longitude	Altitude (m a.s.l.)	Growing season	No. of indiv. sampled	No. of flower. plants	Avg. annual T (°C)	Total annual prec. (mm)	Avg. August T (°C)	Total August prec. (mm)	Avg. autumn T (°C)	Total autumn prec. (mm)	Avg. growth season T (°C)	Total growth season prec. (mm)
WordClim																
Local																
APE	Mt. Prado	Apennines (Italy)	44°14'N	10°24'E	2040	5 months (Jun–Oct)	28	> 1000	3.32	853	8.58	67	– 0.05	277	9.11	384
ALP1	Mt. Sobretta	Alps (Italy)	46°23'N	10°28'E	2700	3 months (Jun–Aug)	24	198	– 1.73	1254	13.42	130	1.43	309	4.82	417
ALP2	Grauson Valley	Alps (Italy)	45°38'N	07°24'E	2500	6 months (May–Oct)	23	180	0.88	1083.40	9.61	131.8	1.26	357.8	8.21	451.6
SPA	Corisco	Cantabria (Spain)	43°05'N	04°47'W	2152	6 months (May–Oct)	20	250	5.58	555.00	15.3	46	5.9	169	11.22	326.2
NOR	Bygdin Lake	Scandinavia (Norway)	61°22'N	08°18'E	1070	3 months (Jun–Aug)	25	~ 100	– 0.40	787	7.22	66	1.43	242	8.58	239
SWE	Vormforsen	Scandinavia (Sweden)	64°51'N	18°42'E	230	5 months (May–Sep)	32	~ 170	– 0.69	603.80	9.2	98	– 0.03	147.7	8.43	208.9
SCO	Little Kinrannoch	British Isles (Scotland)	56°52'N	03°17'W	860	5 months (May–Sep)	29	> 1000	1.64	844.80	12.7	106.5	2.4	220.5	10.88	418.9
									3.61	1286	10.60	82	4.73	354	8.32	463
									6.53	812.10	13.1	66	6.13	167.8	10.58	307.5

the solution to equilibrate (Hay et al. 2008; Newton et al. 2009). One sample of 50 seeds for each seed population was removed after 2, 4, 9, 14, 19, 24, 29 and 39 days for germination testing, following the same methods as described above.

Climatic data

Long-term (~ 50 years) regional climatic data (ca. 1960–1990) at the sampling localities were extracted from WorldClim (Hijmans et al. 2005) in the highest possible resolution (i.e., 30 arc-seconds). Climatic data were annual temperatures and precipitation, mean temperature and total precipitation during seed development and maturation (August), post-dispersal (September–November, hereafter referred to as autumn) and growing season (i.e., months with average temperature > 5 °C; Table 1). The same climatic variables were also obtained from local meteorological stations, nearest to the sampling localities and for the year 2012 (when seeds were collected) to assess different effects of long-term regional data versus short-term local data on seed germination and longevity (Table 1).

Data analysis

Germination of fresh seeds

To test for differences between populations on the final germination of fresh seeds, we performed a Generalized Linear Model (GLM) with binomial error and logit link-function limited to germination data without CS. In this model, final germination was the response variable, while population was treated as the fixed effect. This analysis was performed separately for the two highest temperature regimes tested (20/15 and 15/10 °C), because germination of fresh seeds was extremely scarce at lower temperature regimes. In the case of significant differences between populations, least significant difference (LSD) pairwise comparisons were performed. Analysis was performed using SPSS 21 (IBM SPSS Statistics).

Germination after cold stratification

To test the effects of CS, temperature and population and their interactions on final seed germination, a GLM with binomial error and logit link-function was performed. Final germination was the response variable, while CS, temperature, population and their two-way interactions were treated as fixed effects. Finally, to detect whether ungerminated seeds at the end of the experiments were dead or still dormant, a separate GLM was performed to test the effect of gibberellic acid (GA₃) on the seed exposed to 20 weeks of cold stratification.

Seed longevity

Probit analysis was carried out on the data using GenStat Release 11.1 (VSN International Ltd, Oxford, UK) to estimate the time required for viability to fall to 50% (p50) by fitting the viability equation (Ellis and Roberts 1980):

$$v = Ki - (p/s) \quad (1)$$

where v is the viability (in normal equivalent deviates, NED) of the seed lot after p days in storage, Ki is the initial viability (NED) of the seed lot, and σ is the time (days, d) for viability to fall by 1 NED (i.e., the standard deviation of the normal distribution of seed deaths over time). Analysis of the residual deviance (variance ratio test following the F-distribution) was used to test for significance when constraining survival curve data for multiple seed lots to common estimates for Ki and/or σ .

Correlations with climatic data

Correlations between WorldClim data, local climate of 2012 from the nearby weather stations (see paragraph Climatic Data and Table 1), seed longevity and germination % were analyzed through Pearson's correlations. Data were checked for normality with the Kolmogorov–Smirnov normality test and transformed if necessary.

Results

Germination of fresh seeds

In most populations, fresh seeds germinated only at the warmest temperature regime tested (20/15 °C), but with significant differences between the populations (GLM $\text{Wald}\chi^2_{\text{Pop}} = 81.506$; $df = 6$; $P < 0.001$; Fig. 2). In particular, at 20/15 °C seed germination of the southern populations ALP1, ALP2, SPA and SCO showed the lowest germination (c. 20–40%), followed by APE (c. 50%). Conversely, seed germination of the Scandinavian populations, although statistically different between them, was significantly higher (c. 80–100%) than in the former group (Fig. 2). Moreover, SPA, NOR and SWE were able to germinate also at lower incubation temperature (15/10 °C), though to a lower extent (c. 10, 20 and 50%, respectively), and with significant differences between them (GLM $\text{Wald}\chi^2_{\text{Pop}} = 66.357$; $df = 2$; $P < 0.001$; Fig. 2).

Germination after cold stratification

The binary logistic model showed that seed germination differed across populations, stratification periods and incubation temperatures (Table 2). In general, germination

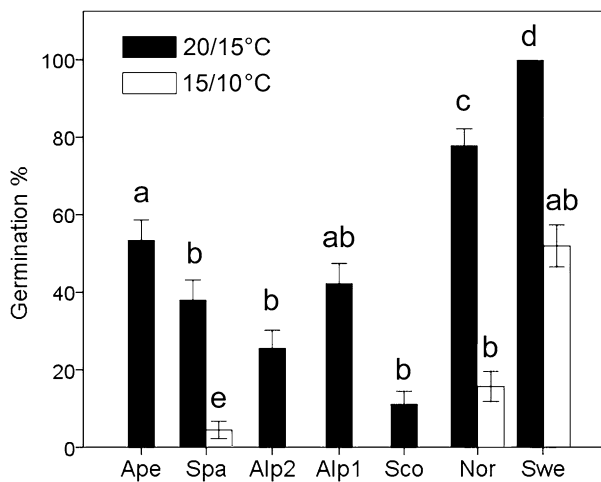


Fig. 2 Final germination percentage (\pm se) on fresh seeds (0 weeks cold stratification) of *Silene suecica* at two incubation temperature regimes (20/15 °C and 15/10 °C). Different letters indicate statistically significant differences in germination of fresh seeds (at $P < 0.05$)

Table 2 Results of binary logistic GLM on the effects of population, stratification period, incubation temperature, and their interactions on final seed germination of *Silene suecica*

Factor	Wald χ^2	df	P
Population	851.105	6	< 0.001
Stratification	363.415	4	< 0.001
Temperature	285.358	2	< 0.001
Pop*Strat	264.918	23	< 0.001
Pop*Temp	441.814	12	< 0.001
Strat*Temp	369.494	8	< 0.001

increased with increasing incubation temperatures and duration of cold stratification. Across seed lots, on average the Scandinavian populations (SWE and NOR) showed the highest germination, followed by ALP1, APE, ALP2, SPA and SCO (Fig. 3; Online Resource 2). However, because the effects of incubation temperature and duration of cold stratification were not consistent among the populations (i.e., significant interaction terms, Table 2), population-specific analyses were performed. These analyses revealed that cold stratification elicited a significant increase of seed germination at all temperatures only in seeds from the Alps (ALP 1 and 2). In the other populations, cold stratification significantly increased the germination at all temperatures except 5/0 °C in APE and NOR, at 15/10 and 20/15 °C in SCO and SPA and at 10/5 and 15/10 °C in SWE (Online Resource 1). Despite being statistically significant, the effect of cold stratification at the warmest temperature (20/15 °C)

was often lower (or null, in SWE) than that observed at the other temperatures, since germination was almost at maximum it could be at this temperature, even without the cold stratification treatment (i.e., on fresh seeds, Fig. 3). Finally, exposing ungerminated seeds to GA3 after 5 months of cold stratification promoted a significant increase of germination in all populations except for ALP1 (NOR and SWE were not tested as germination was almost complete following cold stratification treatments; Fig. 3, Table 3).

Seed longevity

In all populations, seed viability declined as the length of experimental ageing increased (Fig. 4), with a significant variation (12.1–32.2 days) in the time required for viability to fall to 50% (p_{50}) (Online Resource 3A). Variation in p_{50} between seed lots was explained mostly by differences in the initial seed viability (K_i), except for the Norwegian population (NOR) which showed a faster rate of deterioration (i.e., the slope term, σ^{-1}) (Online Resource 3B). Seeds collected from the Apennine population (APE, $p_{50} = 32.2$ days) and those from Scotland (SCO, $p_{50} = 12.1$ days) were the longest- and shortest-lived, respectively. Survival curves of seeds collected in Spain (SPA), the Italian Alps (ALP1 and ALP2) and Sweden (SWE) were similar (p_{50} on average = 24.05 days) and could be constrained to common values of both K_i and σ^{-1} without a significant increase of residual deviance (Fig. 4; Online Resource 3B). Furthermore, NOR had short-lived seeds compared to the other populations (except SCO), due to either a lower K_i and/or a faster σ^{-1} .

Correlations with climatic data

We found statistically significant correlations between seed traits and WorldClim as well as local (2012) climatic data. Because the overall germination percentage (mean germination percentage averaged across temperature and stratification intervals; Online Resource 2) was strongly related to percentage germinations at each stratification interval and temperature (data omitted), only correlations between the former variable and climatic data are shown. In particular, the overall germination percentage was negatively correlated to both local (Pearson's $r = -0.845$; $n = 7$; $P = 0.017$) and WorldClim (Pearson's $r = -0.807$; $n = 7$; $P = 0.028$) average autumn temperature. Moreover, deterioration rate (i.e., sigma) increased significantly, increasing the precipitation during growing season (Pearson's $r = 0.818$; $n = 7$; $P = 0.024$). We could

Fig. 3 Final germination percentages of seeds of *Silene suecica* after 0, 4, 12, 20 and 32 weeks of cold stratification (CS) at four incubation temperature regimes (5/0, 10/5, 15/10 and 20/15 °C). Also shown is the germination percentage with GA₃ (250 mg/l) after 20 weeks of CS (SCO not shown because of lack of seeds for additional tests)

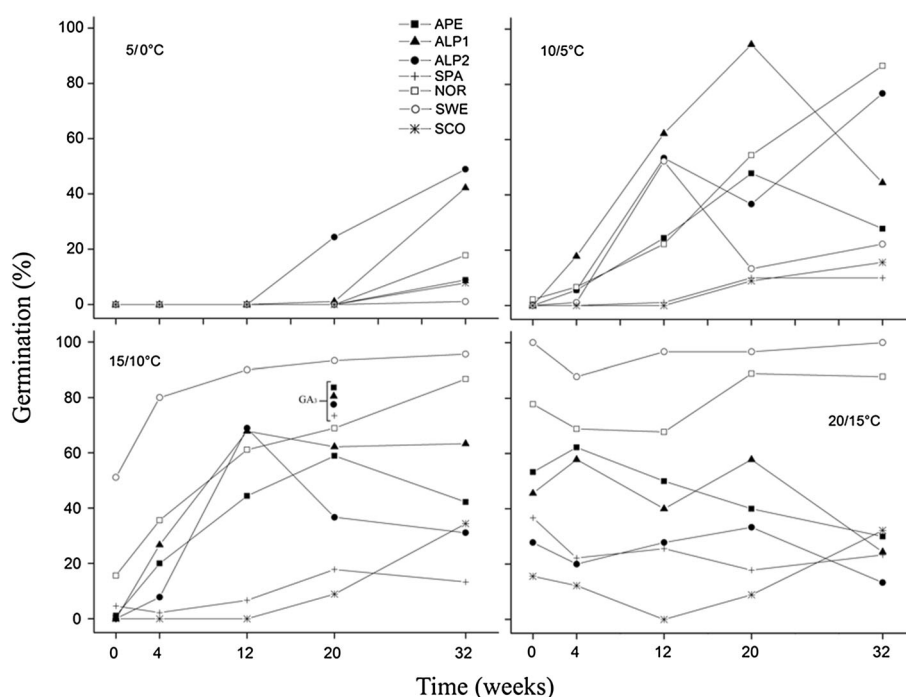


Table 3 Results of a GLM on the effects of population, GA₃ and their interactions on seed germination of 20 week cold-stratified seeds of four populations of *S. suecica* (APE, ALP1, ALP2, SPA)

Factor	Wald χ^2	df	P	Population	Wald χ^2	df	P
Population	64.035	3	< 0.001	APE	31.284	1	< 0.001
GA ₃	81.206	1	< 0.001	ALP1	0.578	1	0.447
Pop* GA ₃	21.897	3	< 0.001	ALP2	30.083	1	< 0.001

The effect of GA₃ on seed germination is also reported at population level. Models were performed with binomial error and logit link-function. Significant values are in bold

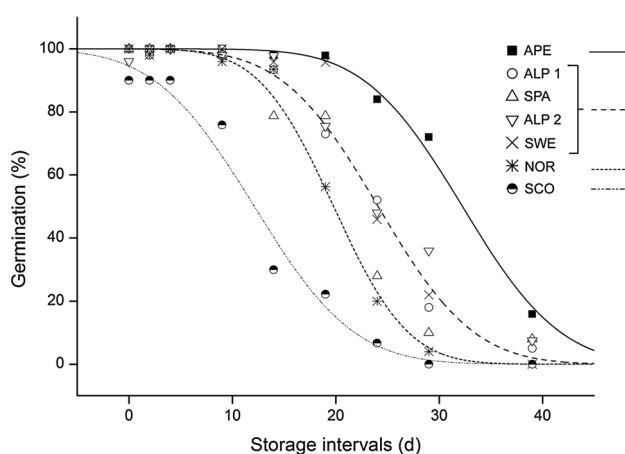


Fig. 4 Survival curves fitted by probit analysis for all seed lots. Analysis of residual deviance was not significantly different when constraining survival curves of ALP1, ALP2, SPA and SWE to common estimates for K_i and/or σ^{-1} (see Online Resource 3B); hence, seed longevity is shown as single curve for these populations

not find any correlation between p_{50} , germination percentage and other climatic data.

Discussion

This study shows that the fresh seeds of *S. suecica* germinated mostly under warm conditions (20/15 °C), but followed a different behaviour between subarctic (NOR and SWE) and alpine (APE, ALP1, ALP2, SPA and SCO) populations, germinating to high (80–100%; subarctic) and low (20–50%; alpine) percentages, respectively. Germination response at the stage of fresh seeds is an important trait, because it represents the potential seedling emergence after dispersal. In this regard, our results are consistent with the general assumption that fresh seeds of many arctic–alpine species germinate only at high temperatures, an adaptation to prevent autumn germination when a high likelihood of

frost events may result in a low success of seedling establishment (Cavieres and Arroyo 2000). Indeed, despite a substantial proportion of fresh seeds of arctic–alpine species being non-dormant or only conditionally dormant (sensu Baskin and Baskin 2014), the prevailing low temperatures in autumn are likely to prevent germination (Schwienbacher et al. 2011). Interestingly, the different germination response of fresh seeds of sub-arctic and alpine populations to incubation temperatures shown here suggest that alpine seeds are adapted to prevent autumn germination along a wider temperature range (i.e., towards warm conditions) than subarctic seeds, and therefore have a deeper dormancy state.

Any narrowing of the germination conditions is by definition an increase in the level of seed dormancy (Fernandez-Pascual et al. 2013), which is considered a bet-hedging strategy in unpredictable environments (Philippi 1993). In this regard, it has been suggested that dormancy is associated with environmental heterogeneity (Angevine and Chabot 1979) and that the weakest requirements for dormancy breaking should occur in populations that experience the longest and the most severe unfavourable period (Fenner and Thompson 2005). Following this view, the different levels of dormancy across populations of *S. suecica* indicate that autumn temperatures at the alpine sites per se might not always be low enough to prevent germination, as would conversely be the case for subarctic locations. Additionally, the significant decrease of germination percentage with increasing autumn temperature confirms that populations from warmer locations (e.g., SCO, SPA, see Table 1) consistently have a deeper dormancy state, as otherwise they might be triggered to germinate in autumn, with potentially lethal consequences for the seedlings.

In general, seeds of *S. suecica* showed a certain degree of non-deep physiological dormancy (sensu Baskin and Baskin 2014), which varied in its depth depending on the population. Indeed, seed germination increased significantly with an increase of length of cold stratification in all populations, but with a wide variation across them and incubation temperatures (Fig. 3; Table 2).

For example, seeds from the Alps significantly increased their germination with an increase of length of cold stratification at all temperatures (up to 80–100% in the best case, i.e., at 10/5 °C), thereby showing the widest temperature range for germination across the populations tested. Cold stratification played a lesser role in promoting seed germination in SWE, being consistently (almost) complete at 20/15 °C and null at 5/0 °C (Online Resource 1; Fig. 3). Incubation temperature was the main factor controlling the germination in this population, which was limited to warm conditions. The subtle influence of cold stratification in promoting seed germination and its high percentage (up to 100% in several tests) indicate a low dormancy state in the seeds of SWE. Conversely, seed germination in SPA and

SCO was low (max c. 20–30%), indicating a deep dormancy state in these populations.

The different patterns of dormancy loss and final germination observed across the populations tested (30–100%) may be either due to differences of seed viability, or germination behaviours across our populations. However, seeds of APE, ALP2, and SPA which had not germinated after 5 months of cold stratification germinated significantly better (though not to 100%) when exposed to GA₃, indicating that a proportion of such seeds were still dormant at that stage. This proportion of ungerminated seeds is unlikely to require longer periods of cold stratification to break their dormancy, while they may be programmed to form a soil seed bank. Many alpine plants are known to form a persistent soil seed bank (Schwienbacher et al. 2010), and physiological dormancy is one of the ways to ensure their persistence (in the soil seed bank) in often unpredictable environments (Arroyo et al. 2004; Leck et al. 1989). Following this view, the higher germination and lower dormancy of arctic populations of *S. suecica* compared to those showed by the alpine indicate that the former form smaller soil seed banks than the latter, though such possibility needs to be investigated.

The results presented here show that, for seeds placed under identical conditions (60% RH, 45 °C), seed longevity can significantly vary between populations. The estimates for p_{50} ranged between 12.1 days (SCO) and 32.2 d (APE). This confirms that seeds of *S. suecica* can be described as having medium longevity in air-dry storage, as shown for most alpine species (Mondoni et al. 2011). Whilst there is compelling evidence that seed longevity has a genetic basis, the possibility that the variation observed here is due to plastic adaptive responses to local environmental conditions cannot be ruled out (see e.g., Kochanek et al. 2011, Mondoni et al. 2014). In this regard, seed longevity increases with an increase in drought and temperature of the species growth site (Probert et al. 2009), our results show that seeds produced by plants in the Apennines (one of the drier and warmer location, Table 1) were long-lived compared to the other populations. Consistently, seed deterioration rate increased with increasing precipitation of the growing site. However, the (macro) climate at the sampling sites does not help explain the very low resistance to ageing of SCO, which is growing in one of the warmest places (though the second wettest). Similarly, seeds of SPA, which matured and developed in the warmest and driest place, had similar longevity to those of seeds of ALP1, which grow at the coldest and wettest site. Therefore, further studies should be made to define which factors might underlie the variation for seed longevity across populations.

The year of sampling tended to be warmer and drier than what was reported in WorldClim (Table 1), reflecting the

general warming trend happening in several alpine and arctic regions (Nogués-Bravo et al. 2007). Nevertheless, correlations between germination patterns and climate were consistent either using WorldClim, or local data, showing that both dataset can equally be used to predict germination pattern in response to climatic variations. However, the different seed dormancy, germination and longevity responses between alpine and subarctic populations of *S. suecica* suggest that further studies are needed to investigate the extent to which such variations are due to genetic differences and/or plastic responses to local environmental conditions. Indeed, seed germination is, despite its genetic base, a highly plastic trait, which means that its expression can be modified by maturation and the post-dispersal environment (Donohue 2005), as shown in several studies (Baskin and Baskin 1973; Carta et al. 2016; Mondoni et al. 2008; Mattana et al. 2014) and similar patterns have been found for the longevity of seeds (Mondoni et al. 2014; Kochanek et al. 2011). Common garden experiments are on the way at the Botanic Garden of Pavia (Italy) to understand the extent to which the variations observed here are due to genetic and/or maternal effects.

In conclusion, our results show that subarctic populations of *S. suecica* are less dormant, have a warmer suitable temperature range for germination and tend to germinate better (under optimal conditions), compared to alpine populations. Consequently, germination and dormancy patterns typically found in alpine populations may not be found in the arctic. Finally, despite any discussion about the possible effects of climate change on seedling survival being speculative, the marked differences in dormancy, temperature dependence for germination are worthy of comments. For example, considering the warming rate predicted for the next decades in the European mid-latitude mountains (c. + 0.3 °C/+ 0.5 °C per decade; Nogués-Bravo et al. 2007) and the arctic (c. + 5 °C/+ 9 °C within 2100; IPCC 2013), a significant increase of germination should be expected in *S. suecica*, either during warm spells at the end of summer or during winter. Such an increase would be higher in the subarctic regions, where the species' inherent germination constraints are lower.

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Declaration of authorship AM and TA conceived the idea; AM, TA and SO performed the experiments; JVM and UC-G provided the seeds and climatic data for the northern populations and contributed to the data interpretation. All the authors contributed to the writing of the text.

Compliance with ethical standards

Conflict of interest Andrea Mondoni, Simone Orsenigo, Jonas V. Müller, Ulla Carlsson-Graner, Borja Jiménez-Alfaro, Thomas Abeli declare they do not have conflict of interest.

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