

RESEARCH PAPER

Effects of the duration of cold stratification on early life stages of the Mediterranean alpine plant *Silene ciliata*

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Keywords

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ABSTRACT

Cold stratification provided by snow cover is essential to break seed dormancy in many alpine plant species. The forecast reduction in snow precipitation and snow cover duration in most temperate mountains as a result of global warming could threaten alpine plant populations, especially those at the edge of their species distribution, by altering the dynamics of early life stages. We simulated some effects of a reduction in the snow cover period by manipulating the duration of cold stratification in seeds of *Silene ciliata*, a Mediterranean alpine specialist. Seeds from three populations distributed along an altitudinal gradient were exposed to different periods of cold stratification (2, 4 and 6 months) in the laboratory and then moved to common garden conditions in a greenhouse. The duration of the cold stratification treatment and population origin significantly affected seed emergence percentage, emergence rate and seedling size, but not the number of seedling leaves. The 6-month and 4-month cold stratification treatments produced higher emergence percentages and faster emergence rates than seeds without cold stratification treatment. No significant cold stratification duration \times seed population origin interactions were found, thus differential sensitivity to cold stratification along elevation is not supported.

INTRODUCTION

Mountains are considered one of the most vulnerable systems to global warming (Körner 2007). Model predictions forecast an increase in average temperatures of between 2.9 °C and 5.3 °C over the next 60 years in European temperate mountains, together with a decrease in precipitation, earlier snow melt at low altitudes and an increase in the frequency of extreme climate events, such as heat-waves or severe droughts (Beniston *et al.* 2007; Nogués-Bravo *et al.* 2007). The most pronounced temperature rise is projected to take place during the winter season (IPCC 2007). However, its consequences on ecological processes remain under-studied (Campbell *et al.* 2005). All these variations in the environment will most likely cause changes in current species distributions in these mountains (Thuiller *et al.* 2005).

Altitude acts as a surrogate for environmental change in a predictable way, with large variations at small spatial scales (Körner 2003). This strong variation along altitudinal gradients constitutes an exceptional tool for evaluating species responses to on-coming warming and for corroborating evolutionary hypotheses (Körner 2007). Organisms that inhabit alpine habitats are forced to react to these predictable changes along altitude (Jump & Peñuelas 2005). *In situ* responses (*e.g.* phenotypic plasticity, demographic changes or local adaptation) are cited as the most frequent reactions in mountain plants, as they may be unable to migrate to higher altitudes or latitudes due to limited dispersal capacities or a lack of available ground on colonised mountaintops (Engler *et al.* 2009). Moreover, mountain populations situated at the lowest eleva-

tion of altitudinal gradients have been the focus of studies on the effects of global change (*e.g.* biodiversity loss, Hampe & Petit 2005; microevolution, Sexton *et al.* 2009; extreme climate events, Herrero & Zamora 2014).

In arctic and alpine ecosystems, lower snow precipitation and early snowmelt are among the most important abiotic filters for plant life, because they profoundly affect early plant life stages (Walker *et al.* 1999; Körner 2003). However, little is known about the effects of climate change during winter conditions in temperate mountains and the consequences for organisms (Beniston 2003; Kreyling 2010). Snow cover has been described as essential for improving seed germination (Baskin & Baskin 1998), especially for alpine plants with small seeds (Schwienbacher & Erschbamer 2002). Seed dormancy prevents precocious seedling emergence after seed dispersal and avoids damage during winter conditions (Cavieres & Arroyo 2000; Körner 2003). This type of seed dormancy is naturally broken through exposure to low-temperature conditions characterised by the snow cover period and time of snowmelt and, experimentally, through cold stratification methods (Baskin & Baskin 1998).

Snow cover duration, which essentially depends on winter temperature and precipitation regime (Rutter *et al.* 2009), is decreasing as a result of increasing mean minimum winter temperatures. Variation in snow cover duration along altitudinal gradients has promoted different adaptations in many alpine plants, such as the generation of frost resistance (Venn *et al.* 2013) or the development of clonal traits (Evette *et al.* 2009). However, reduced snow cover duration may increase the frequency of topsoil freezing events in late spring (Kreyling

2010), damaging seedlings and adult plants (e.g. Schaerberg *et al.* 2008; Hennon *et al.* 2012). Nonetheless, snow cover effects are expected to vary between species, populations and regions (e.g. Cavieres & Arroyo 2000; Griffith & Loik 2010; Drescher & Thomas 2012). Finally, many other factors modulating seed germination and seedling emergence, such as light exposure, water availability, topsoil warming, alternating day–night temperature regime (*i.e.* Baskin & Baskin 1998; Körner 2003) or soil seed bank (e.g. Cavieres & Arroyo 2001) are also influenced by altitude.

Adaptation and responses to altitude in alpine plants have been described at different levels of fitness and life stages (Körner 2003). Since emergence and post-germination are crucial stages in plant development (Shimono & Kudo 2005), they seem ideal life stages for evaluating the existence of local adaptation in plant species (Donohue *et al.* 2010). The environmental conditions that stimulate seed germination also influence the expression of seedling and adult plant traits and act as selection pressures that promote local adaptation (Donohue *et al.* 2010). Evidence of local adaptation in seeds and seedlings can be obtained from reciprocal sowings in the field (e.g. Raabová *et al.* 2007; Leger *et al.* 2009). However, common garden experiments are also a valuable approach for testing local adaptation. They allow the control of all variables except the treatment factor to be evaluated (Kawecki & Ebert 2004) and help to determine if the differences found between considered populations have a genetic origin (Clausen *et al.* 1948; Parker *et al.* 2003).

Silene ciliata Pour. is a Mediterranean alpine plant that has shown local adaptation patterns in seed germination and seedling performance along altitudinal gradients (Giménez-Benavides *et al.* 2007a). Evidence of local adaptation in terms of differential drought tolerance has also been found in adults from the same elevation gradient (García-Fernández *et al.* 2013). Although previous studies have shown the importance of cold stratification in seed germination in *S. ciliata* (Giménez-Benavides *et al.* 2005), nothing is known about the possible variation of cold stratification requirements along an elevation gradient. Thus, our main objective was to study the effect of cold stratification period on seedling emergence and early seedling performance in *S. ciliata*, using seeds from different elevation populations grown under common environmental conditions. We assumed that differential response to the treatments among populations would imply genetic variation as a result of divergent selection pressures. Changes in stratification length are expected when duration of snow cover is reduced from higher to lower elevations. Similarly, these changes could be expected when snow cover is reduced due to climate warming. Specifically, we asked the following questions: (i) is seedling emergence dependent on the duration of the cold stratification period; (ii) is seedling growth affected by the duration of the cold stratification period; and (iii) are responses to cold stratification influenced by population origin along altitude?

MATERIAL AND METHODS

Silene ciliata Poir. (Caryophyllaceae) is a small cushion perennial plant that inhabits areas above the tree line in the Mediterranean mountain ranges of Southern Europe, from the Sistema Central in the centre of the Iberian Peninsula to the Alps and the Massif Central in France, the Apennines in Italy and the Balkan Peninsula (Tutin *et al.* 1995). Its southernmost distribution

limit is in the Sistema Central, central Spain, where populations are isolated from northern populations. Although the species is self-compatible, autogamy is restricted by pronounced protandry (García-Fernández *et al.* 2012b). All populations in the Sistema Central are diploid ($2n = 24$; García-Fernández *et al.* 2012a).

Three populations were selected along an altitudinal gradient on the Peñalara massif of Sierra de Guadarrama to be representative of the environmental conditions at which this species occurs (see Giménez-Benavides *et al.* 2007a, 2008). The population at the lowest altitude (1980 m, hereafter ‘Low’) is located on the west side of the moraine deposit of the glacial cirque of Laguna de Peñalara, coinciding with the tree line. The intermediate population (2250 m, hereafter ‘Intermediate’) is located on the Dos Hermanas summit, 3 km from the Low population. Finally, the population at the highest altitude (2420 m, hereafter ‘High’) is located on the summit of Peñalara peak, approximately 3 km from the Intermediate population. All three populations are located in the Parque Nacional del Guadarrama, 60 km northwest of the city of Madrid (Spain). All populations consist of over 250 healthy, reproductive adult individuals, and occur on south-facing slopes. Vegetation composition of these alpine systems is detailed in Escudero *et al.* (2005). Previous studies carried out on these populations (Giménez-Benavides *et al.* 2007a,b, 2008) found that the altitudinal gradient is associated with an environmental stress gradient, with the lowest population experiencing the most stressful conditions (*i.e.* scarce water availability in soil during the summer months, which is critical for seedling and plant survival; Giménez-Benavides *et al.* 2007a, 2008). As expected, snow cover duration also varies with altitude. From 2003 to 2005, snowmelt started in the Low population 1 week earlier than in the Intermediate population and 18 days earlier than in the High population (Giménez-Benavides *et al.* 2007a). During these years, the annual mean temperature of the Low population was 1.8 °C and 2.9 °C warmer than the Intermediate and High population, respectively (Giménez-Benavides *et al.* 2007b). In previous studies, we also found that the lowest population produced fewer flowers and fruits per plant, had lower population growth rates and a lower proportion of flowering plants (Giménez-Benavides *et al.* 2007b, 2010). Details of the environmental conditions at the study locations are found in Giménez-Benavides *et al.* (2007b). The harsh conditions imposed by summer drought in these mountains have also been documented in other Mediterranean-type mountains, like the Chilean Andes (e.g. Cavieres *et al.* 2005).

Seed collection and seedling recruitment experiments

In the second half of September 2009, mature seeds were collected at each population from 25–30 healthy adult individuals at least 5 m apart from each other. We collected a minimum of two fruits from each individual. In the laboratory, fruits were cleaned, immature seeds were discarded, and 400 seeds from each population were placed in Petri dishes (25 seeds per dish) with moist filter paper and wrapped in aluminium foil. Sets of 100 seeds from each population (four Petri dishes) were stored at 4 °C in a controlled environment chamber for cold stratification (Baskin & Baskin 1998) for different periods (6, 4 or 2 months) to break seed dormancy (see Giménez-Benavides *et al.* 2005 for details of seed stratification). Treatments were

started at 2-month intervals so that all treatments ended at the same time. The seeds for the 4- and 2-month stratification treatments were kept in dried silica gel at room temperature until the stratification treatment began. Another set of 100 seeds from each population was also stored in dried silica gel without cold stratification. A total of 1200 seeds were used for this experiment. The duration of the cold stratification treatments was established according to snow cover duration and altitudinal range in the Peñalara massif, which oscillates from 40 to over 220 days with relevant variation at small spatial scales (Palacios *et al.* 2003). Furthermore, climate models forecast a temperature increase of between 2.9 °C and 5.3 °C and a decline in precipitation of between 4.8 and 17% (Nogués-Bravo *et al.* 2008), which would imply a reduction in snow cover. Cold stratification treatments act as a proxy for snow cover, providing a constant low-temperature high-moisture environment that mimics the buffered low temperatures that occur under snow cover. In March 2010, the seeds were sown individually in rectangular plastic pots (*ca.* 5 cm³ volume) in a commercial substrate enriched with NPK. The pots were randomly distributed on a greenhouse bench of the CULTIVE Facility (<http://servicat.escet.urjc.es:8080>; Rey Juan Carlos University, Móstoles, Spain) and watered every 48 h. The light regime consisted of natural light with a 12-h photoperiod at the time of sowing and an average of 7.2 h of direct sunlight per day (AEMET, Agencia Estatal de Meteorología, Gobierno de España 2013). Temperatures in the greenhouse ranged from 25–30 °C (day) to 12–15 °C (night).

Monitoring of seedling emergence and seedling growth

Seedling emergence and seedling growth were surveyed twice a week during the first 2 months after sowing and then once a week thereafter. Emergence was recorded when cotyledons were visible in the substrate. Maximum diameter of each seedling was measured regularly using digital callipers, and the number of leaves was counted.

Data analysis: Seedling emergence and seedling growth

Differences in seedling emergence were analysed using generalised linear models (GLM). Emergence was considered a binomial response (0 for no emergence and 1 for emergence), and a logit link function was used. GLM were implemented using population origin (Low, Intermediate and High), duration of cold stratification (0, 2, 4 and 6 months) and the interaction of the two variables as fixed factors. GLM were implemented using the GLIMMIX procedure in SAS 9.0 (SAS Institute, Cary, NC, USA).

Emergence rate was also analysed using a right accelerated failure time model, *i.e.* a linear regression model in which the response variable is the logarithm or a known monotone transformation of a failure time (Fox 2001). This approach allows the use of censored data to estimate parametric regression models using a maximum likelihood approach. The best failure time distributions were chosen for the datasets based on comparison of possible distributions with the likelihood ratio test (Fox 2001). A log-logistic distribution was therefore used for the emergence rate data. Analyses were performed with SAS 9.0, using the LIFEREG procedure. Population origin, duration of cold stratification and the interaction between the two fac-

tors were considered in the analyses, and Tukey *post-hoc* comparisons were carried out when the factors showed significant effects.

Seedling size was also analysed using GLM. In this case, we considered seedling size at the end of the study as the dependent variable. Population origin, duration of cold stratification and the interaction between the two variables were considered fixed factors. Finally, another GLM was done including number of leaves at the end of the experiment as a response variable, as another estimate of plant performance and fitness, with the same fixed factors as described above. For these two GLM, the response variables (seedling size and number of leaves) follow a Gaussian distribution, and the identity link function was considered.

In spite of potential unwanted effects of pseudoreplication (Morrison & Morris 2000), the Petri dishes used to group seeds in the stratification treatments were not considered a random factor in these analyses because negligible effects of this factor have been observed in previous seed germination studies with this species (Giménez-Benavides *et al.* 2005; Lara-Romero & Iriondo, unpublished results).

RESULTS

Seedling emergence

A total of 254 seeds emerged (21%) in the greenhouse experiment. GLM results showed that population origin significantly affected seedling emergence ($P < 0.01$; Table 1). Seeds from the High population had lower emergence percentages than those from the other two populations (Fig. 1A, Table 1). Duration of cold stratification also had a significant effect on seedling emergence ($P < 0.01$; Table 1). The number of emerged seedlings in the treatment with no stratification (41 seeds) was significantly lower than in the 6-month stratification treatment (78 seeds; Table 1, Figs 1A, 2; Tukey *post-hoc* test $z = 5.45$, $P < 0.01$), but also than after the 2- or 4-month stratification treatments (Fig. 1A; 67 and 68 seeds, respectively; Tukey *post-hoc* $z = 4.79$, $P < 0.01$ and $z = 4.38$, $P < 0.01$, respectively). Differences in the

Table 1. Results of GLM for seedling emergence of *Silene ciliata* considering population origin (Low: Low population; Intermediate: Intermediate population), cold stratification period (0, 2 and 4 months) and the interaction between the two factors (Pop–Strat).

effect	solution for effects					deviance change	
	coefficient	SD	df	t	P	F	P
population	–	–	–	–	–	6.91	<0.01
low	0.17	0.33	1236	0.5	0.61		
intermediate	0.61	0.32	1236	1.88	0.06		
stratification	–	–	–	–	–	4.99	<0.01
0	–0.86	0.41	1236	–2.09	0.03		
2	–0.12	0.35	1236	–0.35	0.72		
4	–0.66	0.39	1236	–1.68	0.09		
pop–strat	–	–	–	–	–	1.89	0.07

Seedling emergence was considered a binomial variable (0 for no emergence and 1 for emergence) using logit link function. High population and a 6-month treatment were considered as reference for comparisons with other population and treatments.

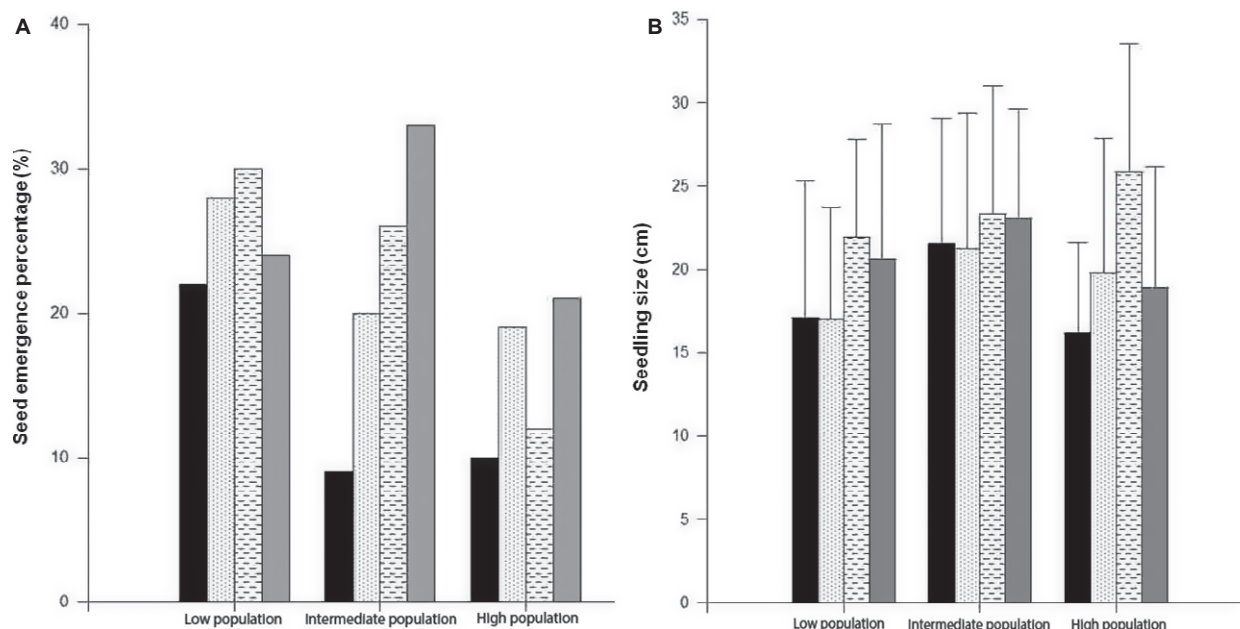


Fig. 1. A: Total seedling emergence percentage of *Silene ciliata* and B: seedling size at the end of the study (mean \pm SD, in cm), grouped by population (x-axis). Seed stratification treatments are depicted by bar patterns (black bars: no stratification; pointed bars: 2-month stratification; dashed bars: 4-month stratification; grey bars: 6-month stratification).

number of emerged seedlings between stratification treatments were not significant (Tukey *post-hoc* test $P > 0.05$; Table 1). No interaction was found between population origin and duration of cold stratification. The accelerated failure time model for emergence showed that duration of cold stratification had a significant effect on the seedling emergence curve. Seeds stratified for 6 months emerged significantly faster than seeds stratified for 2 months or non-stratified seeds. Significant differences according to population origin were also found. However, pair-wise differences between Low and Intermediate populations and the High population were not significant (Table 2). No significant population origin \times duration of cold stratification interactions were found (Table 2).

Seedling growth

Population of origin and duration of cold stratification also showed significant effects on seedling size (Fig. 1B, Table 3). Seeds from the 4-month cold stratification treatment reached the largest seedling size, whereas non-stratified seeds were smallest (Table 3, Fig. 1) although differences were not significant (Tukey *post-hoc* $z = 2.33$, $P = 0.09$). With regard to population origin, seedlings from the Intermediate population were the largest, independent of stratification treatment (Table 3). The GLM for number of leaves found no significant effects for both duration of cold stratification and population origin (data not shown).

DISCUSSION

Our results show that duration of cold stratification and population origin significantly affected seedling emergence, seedling emergence rate and seedling size in *S. ciliata*. Seeds from the intermediate population performed better than those from the

local distribution limits in our mountain system (largest seedling size and highest emergence percentage after 6-month stratification). This concurs with the accepted idea that populations in the centre of realized niches are the most optimally adapted and show traits associated with higher fitness values (Baker 1972; Herrera & Bazaga 2008). Contrary to our expectations, we found that the interaction between the two experimental factors was not significant, indicating similar effects of cold stratification on all populations, independent of origin.

Seedling emergence

Similar to our previous findings on emergence behaviour in *in situ* field sowing (Giménez-Benavides *et al.* 2007a; García-Fernández *et al.* 2012b) and in sowings under controlled conditions in Petri dishes (Giménez-Benavides *et al.* 2005, 2007a; García-Fernández *et al.* 2012b), *S. ciliata* seeds required a cold stratification treatment to break seed dormancy. However, *S. ciliata* seeds have not been found in the permanent seed banks (García-Camacho 2009), which suggests that seeds germinate during the next growing season after dispersal. All populations benefited from the cold stratification treatments in a similar way (Fig. 1A).

The effect of cold stratification in subarctic, alpine and Mediterranean mountain plants has been widely documented (e.g. Schwenbacher & Erschbamer 2002; Mondoni *et al.* 2012). Different germination responses of populations distributed along altitudinal gradients have also been described in other high-mountain specialists under a Mediterranean-type climate (Cavieres & Arroyo 2000). These authors found that seeds from high-altitude populations of *Phacelia secunda* in Chile needed longer periods of cold stratification to germinate. However, the altitudinal gradient was larger than that considered for *S. ciliata* populations (1800 m in *Phacelia secunda* versus 600 m in

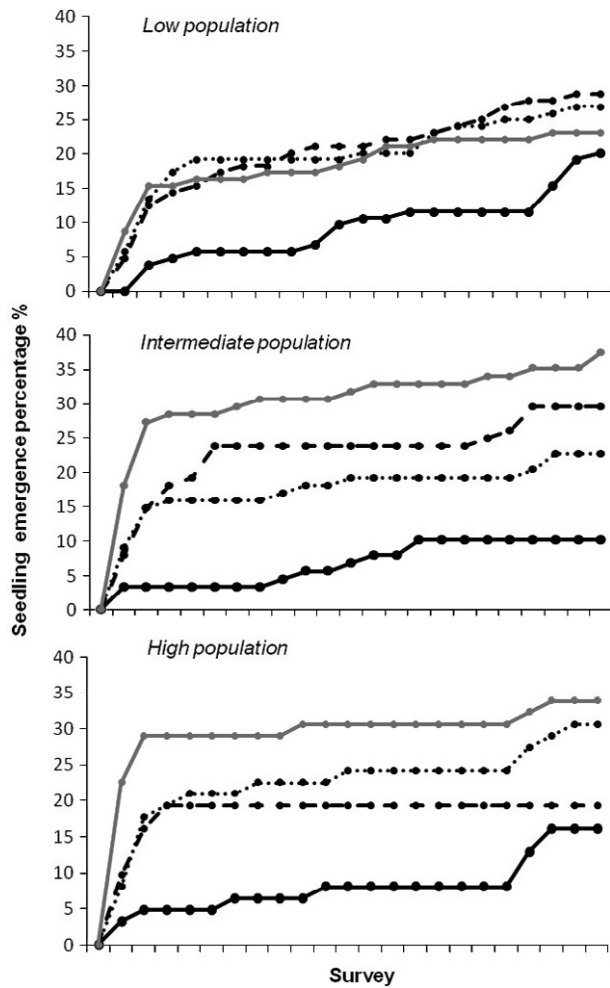


Fig. 2. Seedling emergence percentage (y-axis) grouped by duration of the cold stratification treatment (black line: no stratification; pointed line: 2-month stratification; discontinuous line: 4-month stratification; and grey line: 6-month stratification).

Table 2. Accelerated failure analyses time analyses (log-logistic distribution) of total seedling emergence of *Silene ciliata* considering population origin (Low: Low population; Intermediate: Intermediate population), cold stratification period (0, 2 and 4 months) and the interaction between the two factors (Pop–Strat).

variable	df	χ^2	P	estimate
intercept		1137.38	0.09	0.348 ± 0.204
population	2	7.86	0.02	–
low	1	2.87	0.09	0.51 ± 0.29
intermediate	1	1.19	0.27	0.29 ± 0.27
stratification	3	47.18	<0.01	–
0	1	20.02	<0.01	1.82 ± 0.41
2	1	5.03	0.03	0.72 ± 0.32
4	1	0.03	0.85	0.06 ± 0.33
pop–strat	4	6.09	0.41	–
scale				0.59 ± 0.03

High population and a 6-month treatment were considered as reference for comparisons with other population and treatments.

Table 3. Generalised linear model results for seedling size of *Silene ciliata* 1 month after germination, considering population (Low: Low population; Intermediate: Intermediate), cold stratification period (0, 2 and 4 months) and the interaction between the two factors (Pop–Strat).

effect	solution for effects					deviance change	
	coefficient	SD	df	t	P	F	P
population	–	–	–	–	–	3.81	0.02
low	1.69	2.18	241	0.77	0.44		
intermediate	4.14	2.05	241	2.01	0.04		
stratification	–	–	–	–	–	1.96	<0.01
0	–2.69	2.81	241	–0.96	0.33		
2	0.86	2.31	241	0.37	0.71		
4	6.93	2.64	241	2.62	0.01		
pop–Strat	–	–	–	–	–	1.09	0.37

Seedling size was considered a normal variable using identity as link function. High population and a 6-month treatment were considered as reference for comparisons with other population and treatments.

S. ciliata), which could have increased the differences in selection pressures in the early life stages. Although seeds always needed a cold stratification treatment for enhanced germination, the differences found in response to the duration of stratification among populations might suggest some adaptive differences along altitude. Thus, while the highest emergence percentage in the Low population was obtained when seeds were submitted to 4 months of cold stratification, seeds reached the highest emergence percentage in the Intermediate and the High populations after a 6-month cold stratification period (Fig. 1). This is supported by the results of the emergence rate models (Fig. 2, Table 2), which showed that seeds from the Intermediate and High populations emerged faster after 6 months of cold stratification, whereas seeds from the Low population emerged at similar percentages in all cold stratification treatments (Fig. 2). This suggests that certain environmental variables linked to altitudinal variation, such as biotic and abiotic factors (Rees 1996; Finkelstein *et al.* 2008) or microsite factors (Tripathi & Khan 1990), could be acting as selective forces on the mechanisms that regulate seed germination (Meyer *et al.* 1997). These results are supported by evidence of local adaptation recently documented in this Low population (*e.g.* Giménez-Benavides *et al.* 2007a; García-Fernández *et al.* 2012b,c, 2013). The lower requirement for a cold stratification period observed in the Low population may be of adaptive value under current warmer conditions, although several other factors (*e.g.* fluctuations in soil moisture and frost, seed or seedling predation; Wenny 2001; Moody & Meentemeyer 2001; Walck *et al.* 2011) involved in seedling emergence and seedling root development are being modified in all populations as a result of global warming.

Our common garden experiment showed a low seedling emergence percentage (average 21%). The obtained emergence rates were lower than those obtained in field sowings (*ca.* 50%; Giménez-Benavides *et al.* 2007a; García-Fernández *et al.* 2012b) with natural snow cover or under controlled conditions in a growth chamber in Petri dishes (*ca.* 70%, with cold stratification; Giménez-Benavides *et al.* 2005, 2007a; García-Fernández *et al.* 2012b). The low emergence percentage obtained in

the garden experiment might be due to insufficient variation in daily temperature conditions in the greenhouse (Billings & Mooney 1968), differences in the length of the after-ripening period or seed sensitivity to desiccation in silica gel. Nonetheless, the many other factors that influence seed viability and emergence percentages might also be altitude-dependent, such as inbreeding (e.g. increasing the number of aborted seeds and germination percentages; García-Fernández *et al.* 2012b), solar irradiation and light quality (i.e. Walker & Evenson 1985; Baskin & Baskin 1998), seed size (Stöcklin & Bäumler 1996; Körner 2003) and maternal factors (Giménez-Benavides & Milla 2013).

Seedling growth and development

Seedling growth and size were also dependent on population origin and duration of cold stratification. Because seedlings were grown under common environmental conditions, the differences found between populations must have a genetic basis or be caused by maternal effects. A previous study using manual pollination found that pollen origin (autogamy *versus* allogamy) affected seedling size in native populations (García-Fernández *et al.* 2012b). This suggests that different degrees of inbreeding in the populations may be responsible for the observed differences in seedling growth and size. However, García-Fernández *et al.* (2012c) found no significant differences in inbreeding between the studied populations. On the other hand, maternal effects are known to influence seed germination (e.g. Koornneef *et al.* 2002) as well as seedling growth (Antonovics & Schmitt 1986; Roach & Wulff 1987). It is noteworthy that seedlings from the Low population always had the smallest size, both in this study and in a previous study carried out in the natural populations (Giménez-Benavides *et al.* 2007a). This is congruent with the less favourable conditions for reproduction found at the Low population due to summer drought, which could result in seeds with fewer nutritional reserves (Giménez-Benavides *et al.* 2007b). Further studies,

considering F₁ and F₂ generations, are necessary to disentangle maternal and genetic effects (e.g. Fernández-Pascual *et al.* 2013) or genetic regulation (Meyer & Pendleton 2000).

Concluding remarks

The lack of significant differences in the seedling emergence percentage and emergence rate between the 4-month and the 6-month cold stratification treatments, and the differences between the latter and the 2-month and control treatments suggest that a certain level of stratification is needed to maintain current levels of seedling emergence. No significant population × stratification period interaction was found, showing no evidence of adaptation to local conditions. Further research, including additional populations, within-population response variation or a gradual gradient in the cold stratification process (i.e. additional duration treatments with smaller differences between them), is needed to explore more accurately if there is a threshold of snow cover duration at which *S. ciliata* seeds experience significant decreases in seedling emergence and seedling growth and to provide insight on the adaptive evolution implications of reduced snow cover duration as a result of climate warming.

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