Edge populations are frequently small and subject to stressful conditions that may compromise their long-term viability. Inbreeding can play an important role in small populations by reducing genetic diversity, leading to the fixation of deleterious mutations and, finally, carrying populations to an extinction vortex through inbreeding depression. Although stressful conditions may enhance the intensity of inbreeding depression, evidence to date is inconclusive in marginal habitats. Local adaptation, promoting native genotypes, and gene flow, reducing allele fixation, are two factors that can have different effects on the intensity of inbreeding depression. Three populations of \textit{Silene ciliata} distributed across an elevation gradient at the southernmost edge of the species distribution were used for this study. Several fitness components – germination, survival, and growth rate – were compared between inbred seedlings and seedlings from within- and between-population outcrosses, both in the field and controlled conditions. Overall, inbred seedlings had lower fitness than outcrossed seedlings. For most of the variables analysed, similar inbreeding depression effects were found in all three populations, but, for seed weight and seedling survival curve, inbreeding depression was only found in the low altitude population. Similarly, inbreeding depression was more evident in the field than in controlled chamber conditions. Outcrosses between populations contributed to an increase in most fitness estimates and populations, suggesting that the benefits of reducing inbreeding depression overrode the potentially deleterious effects of disrupting local adaptation. Our results suggest that inbreeding depression plays an important role in the fitness of early life stages of \textit{Silene ciliata} at its southernmost distribution limit, but only provided partial support to the hypothesis that stressful conditions enhance the expression of inbreeding depression.

Marginal populations, both those located at the eroding edge (rear edge) and at the expanding edge (front edge), have become the focus of many current studies on climate change (Hampe and Petit 2005, Parmesan et al. 2005). Rapid warming is expected to have a great impact on marginal populations and change species range limits (Sexton et al. 2009) with contrasting responses among species (Jump and Peñuelas 2005). This makes edge populations well-suited for assessing the ability to respond to the new environments that emerge during rapid climate fluctuations (Ackerly and Monson 2003).

Marginal populations are often fragmented and prone to extinction due to environmental fluctuations, demographic stochasticity and edge effects (Kawecki 2008). Isolation, which is frequently exacerbated due to fragmentation and poor habitat quality, is recognized as one of the major threats for the long-term viability of marginal populations (Gibson et al. 2009). This, together with small population size, reduces the genetic diversity of edge populations and can significantly increase their extinction risk (Sagarin and Gaines 2002). This low demographic viability results from a decrease in the number of reproductive individuals and a parallel increase in mating between close relatives (Lande 1993). Processes such as genetic drift and inbreeding depression can act as catalysts for extinction (Ellstrand and Elam 1993).

Inbreeding depression is widely accepted as one of the main causes of fitness reduction in small populations (Charlesworth and Charlesworth 1999). This situation can become especially unfavourable for self-incompatible or partially self-incompatible species, as they are more vulnerable to genetic deterioration (losing specific mating type alleles, Byers 1995), the negative effects of allele loss (Ellstrand and Elam 1993), and particularly the early stages of inbreeding depression in the population (Husband and Schemske 1995).

On the other hand, edge populations can have enhanced potential to deal with extreme environmental conditions through adaptation (Kawecki 2008). From a genetic point of view, the appearance of new alleles or the fixation of some mutations that support an adaptive advantage may be especially advantageous in marginal populations (reviewed by Kawecki and Ebert 2004). However, local adaptation processes require edge populations to have a minimum population size and genetic diversity where adaptive alleles and natural selection can take place (Kawecki 2008, Leimu and Fischer 2008).

Although some studies have found that stressful conditions enhance inbreeding depression in plant populations (Frankham et al. 2002), there is no clear evidence and other studies in other organisms have reported lower inbreeding depression under stress (Henry et al. 2003). Thus, this hypothesis remains controversial (Armbruster and Reed 2005) and still requires detailed examination (Paschke et al. 2005). Several local factors, like lineage effect (inbreeding in past...
generations, Falconer and Mackay 1996), nature of pollen donor (Paschke et al. 2005), original population size (Paland and Schmid 2003), different direct phenotypic response and different adaptive values of stable, nonplastic phenotypes (Cheptou and Donohue 2011) may also affect inbreeding depression, and, in some instances, diminish the pernicious effects of stressful environments. In this context, we hypothesized that discrepancies in relation to this hypothesis may be related to some extent to different degrees of local adaptation success in marginal and stressful conditions.

Mountains are especially well suited for testing hypotheses related to the adaptation and evolution of plants by comparative observations and manipulative experiments with populations along local elevation gradients (Körner 2007). When properly chosen, elevation gradients modify environmental conditions in a very predictable fashion (Walther et al. 2005) and put populations under different selective pressures (Gonzalo-Turpin and Hazard 2009). These gradients include both optimal and marginal environments which can be associated to central population conditions and the species’ rear and front edge conditions, respectively. Furthermore, some mountains are isolated islands where the arrival of migrants or gene flow from other areas is almost impossible. Low and high altitude limits in such mountains are ideal areas for testing whether inbreeding depression is more intense in marginal populations and whether it can be masked or influenced by local adaptation.

Our model plant, Silene ciliata, a Mediterranean high mountain specialist, is an appropriate species for this study since it shows evidence of local adaptation (greater seed germination and seedling survival of native individuals than seeds from other populations) in the lower populations of its southernmost limit in central Spain (Giménez-Benavides et al. 2007a). In addition, the S. ciliata populations at low altitudes are partially isolated from the upper ones because their flowering periods are significantly asynchronous (Giménez-Benavides et al. 2007b).

The aim of this study was to compare the incidence of inbreeding depression on locally adapted populations of S. ciliata across an environmental (elevation) gradient, where the lowest population experiences the most stressful conditions due to the drier conditions that develop there during the summer (Giménez-Benavides et al. 2007a). We combined field and germination chamber experiments and sowed seeds obtained from controlled selfings and outcrossings within and between populations. Specifically, we asked: 1) is there inbreeding depression in S. ciliata populations distributed across the species’ altitudinal range at its southernmost limit? 2) If so, is inbreeding depression related to the intensity of environmental stress? 3) Does gene flow from other populations increase or reduce seedling fitness (reducing inbreeding or reducing local adaptation, respectively)? 4) Do all populations react similarly to gene flow from other populations?

Material and methods

Study species and population selection

Silene ciliata (Caryophyllaceae) is a dwarf cushion perennial plant that inhabits Mediterranean mountain ranges of the northern Mediterranean Basin from the Cordillera Central in the Iberian Peninsula to the Massif Central in France, the Apennines in Italy and the Balkan Mountains (Tutin et al. 1995). Silene ciliata reaches its southernmost margin in central Spain in the Sierra de Guadarrama where populations are considered remnants because they are isolated from northern populations. Silene ciliata presents variable levels of ploidy in natural populations (Blackburn 1933) but in Sierra de Guadarrama, individuals in all populations are diploid (2n = 24, García-Fernández et al. 2012). The species is gynomonoecious, but spontaneous autogamy is restricted by pronounced protandry (Giménez-Benavides et al. 2007b).

Three populations of S. ciliata were selected along the largest local elevation gradient in Sierra de Guadarrama which mimics a steep global range distribution gradient of the species (Giménez-Benavides et al. 2007a, 2008). The lowest population (1980 m, hereafter ‘Low’) was situated in the lateral moraine deposit of the glacial cirque of Laguna de Peñalara. The intermediate population (2250 m, hereafter ‘Intermediate’) was located on the Dos Hermanas summit approximately 3 km from the Low population. The highest population (2420 m, ‘High’) was situated on the summit of Peñalara peak, the highest peak of Sierra de Guadarrama, approximately 3 km from the Intermediate population. All three populations are located in the Peñalara Natural Park, 50 km northwest of the city of Madrid (Fig. 1). All populations occur on gentle south-facing slopes and had a relatively high number of individuals (more than 200). Vegetation composition of these systems is detailed in Escudero et al. (2005). Previous studies carried out on these populations (Giménez-Benavides et al. 2007a, b, 2008) have observed that the altitudinal gradient associates to a gradient of environmental stress for this species, with the lowest population experiencing the most stressful conditions. Specifically, this population had significantly lower water availability in soil during the summer months which is critical for seedling survival (Giménez-Benavides et al. 2007a, 2008). Furthermore, the lowest population has a lower proportion of flowering plants and produces fewer flowers and fruits per plant as a result of water stress (Giménez-Benavides et al. 2007b), resulting overall in a lower population growth rate (Giménez-Benavides et al. 2010). The harsh conditions imposed by summer drought in Mediterranean mountain environments have also been documented in other studies (Cavieres et al. 2005).

Seed collection, cultivation of plants and manual crossings

In autumn 2006, mature fruits were collected from 20–25 healthy plants distanced at least 5 m from each other in the three populations. Fruits were dissected in the laboratory, and seeds were immediately cleaned and placed in petri dishes with moist filter paper according to population of origin. Petri dishes were wrapped in aluminium foil and stored at 4°C for humid stratification for 6 months to break seed dormancy (details in Giménez-Benavides et al. 2005). In spring 2007, the seeds were sown in plastic pots with a commercial substrate enriched in NPK and watered every 48 h. Plants were grown in the phytotron of the Cultive Laboratory (Rey Juan Carlos Univ., Móstoles, Spain) until February 2008.
when they were moved outside the greenhouse with the same water regime described above.

Plants flowered in May and June. Manual pollinations were carried out using a fine paint brush. Before anthesis, flowers were emasculated to avoid spontaneous self-pollination and covered with a cotton net to avoid accidental pollination. When the flower was unable to receive new pollen because the female structures were fading off, the net was removed. Developed fruits were removed from plants immediately before dispersal. Seeds were collected and placed in paper bags with silica gel.

We selected four flowering individuals from each population (Low, Intermediate and High) grouped in two pairs. On each plant, one flower was self-pollinated (self-pollination treatment) and a second flower was pollinated with pollen from its paired neighbour of the same population (within-population cross pollination treatment). A third flower was pollinated from a randomly selected individual from a different population. Likewise, the third flower of its paired neighbour was crossed with a random individual from the remaining population. These crosses with the third flower were called the between-population cross pollination. Thus, we obtained three sets of seeds for each population: 1) inbred seeds (four different seed subsets from each population, one from each mother plant); 2) within-population outcrossed seeds (two seed subsets, one from each reciprocal cross between paired individuals); and 3) between-population outcrossed seeds. In case 3, we obtained two seed subsets in each population by crossing individuals from different populations (Low-Intermediate, Low-High and Intermediate-High crosses, as each pair of plants in each population received pollen from the other two populations). All plants contributed to each seed subset in the same way and with the same number of seeds. The experimental crossing scheme is shown in Fig. 2.

Figure 1. Location of Peñalara Natural Park in the Madrid province in central Spain (white star) and 3D model map with the position of the studied populations.

Figure 2. Diagram of the types of crossing performed with plants at the greenhouse. Continuous lines represent self-crossings, discontinuous lines represent within-population outcrossings and pointed lines between-population outcrossings.
Germination tests and seed weight

One hundred seeds were selected from each seed subset (900 seeds total). All seeds were weighed with a precision micro balance. They were then placed in petri dishes and subjected to a cold humid stratification treatment for three months as described above.

For germination tests, we prepared four replicates of 25 seeds per type of crossing and population origin. Seeds were placed in 8 cm petri dishes with two layers of filter paper which were kept moistened throughout the germination tests. Germination tests were conducted in a germination chamber equipped with six cool-white fluorescent light tubes. Germination conditions were set at alternating 25/15°C temperature with a 16 h light/8 h darkness photoperiod. Seeds were surveyed every 2–3 days and removed from the dish after radicle emergence. Germination tests were maintained until no seeds germinated for two consecutive surveys. We calculated the total number of germinated seeds.

In situ seed germination, seedling growth and survival in field populations

In September 2008, 12 sites were randomly selected in each of the three original populations (Low, Intermediate and High). Each site consisted of a bare soil area where small stones and wooden sticks had been previously removed. At each site, we established a plot with a transparent plastic quadrat of 16 × 16 cm. The quadrat contained a four row × five column grid of cells. Each cell had a 1 cm diameter hole in the centre. This plastic sheet was used for accurate seed placement and to facilitate further monitoring. Two seeds were sown in each hole to ensure seed germination. In each population four plots were established for each of the three types of crossings. For each crossing type, only the seeds of the plants native to each population were sown. In selfings, five holes were used per maternal plant. In within-population outcrossings, ten holes were allocated to each crossing. Finally, in between-population outcrossings, ten holes were assigned to each of the two seed subsets derived from the crossings where the maternal population had participated. Thus, a total of 1440 seeds were sown.

Emergence, survival and growth were checked regularly. The first survey was made on 15 May 2009 after snow melt. Three surveys per month were carried out in June, July and August 2009. A last survey was made on 22 September 2009 to census the number of surviving plants at the end of the growing season. In each census, seed germination, seedling survival and seedling size (maximum diameter measured with a digital calliper) were determined. When both seeds in a hole germinated, the last seedling to emerge was clipped to avoid growing interference. This only happened one or two times in each population. Each dead seedling was assigned a most-likely cause of death. Thus, summer drought was assigned to dried-out seedlings without any visible damage, while herbivore attack was assigned to dead seedlings with external signs of predation, cotyledon removal or whole removal. Climate conditions during the monitoring period were similar to average values recorded for the last 30 years. Although climatic data for the specific locations were unavailable, annual rainfall at the closest weather station (Navacerrada Pass, 1890 m) is about 1400 mm with a very pronounced summer drought when less than 10% of annual precipitation occurs. Summer drought is a well-known process in Mediterranean systems (Beniston et al. 2007). High mountains in Mediterranean areas are also influenced by this process and summer drought has been considered as an important stress factor (Castro et al. 2005). Giménez-Benavides et al. (2007a) measured the soil water content (SWC) at the study site during the summer of 2004, a mild and wet year. SWC descended to less than 5% even at the highest locality (Peñalara peak 2428 m). SWC was inversely proportional to elevation throughout the growth season (June–September).

Data analysis

Germination chamber test and seed weight

Differences in seed mass and total seed germination were analysed using general linear models (GLM, McCullagh and Nelder 1989). For seed germination, the response variables were assumed to follow a binomial distribution (0 for no germination and 1 for germination), and thus we used the logit as link-function. For seed mass, the response variable was assumed to follow a Gaussian distribution and the identity function was used. GLMs were implemented using the crossing types (selfing, within-population outcrossing and between-population outcrossing) and the maternal populations (Low, Intermediate and High) as fixed factors, and including the interaction between these two factors. GLMs were implemented using SAS 9.0, using the GLIMMIX module (SAS Inst.).

Germination 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 data sets based on the comparison of possible distributions with the likelihood ratio test (Fox 2001). A log-logistic distribution was therefore used for the germination rate data. Analyses were performed with SAS 9.0, following the LIFEREG procedure (SAS Inst.).

Field sowing experiments

Seed germination and seedling survival were analyzed with general linear mixed models (GLMM, Breslow and Clayton 1993). Population origin (Low, Intermediate and High), type of cross (selfing, within-population outcrossing and between-population outcrossing), and the interaction between maternal population and type of cross were included in the analysis as fixed factors, while plot was treated as a random factor. Seed germination and seedling survival at the end of the growing season were assumed to be binomial variables (0 for no germination or seed death and 1 for seed germination or seed survival) and the logit link-function was used. As summer drought in Mediterranean mountains causes very low seedling survival, another GLMM model was developed using seedling survival at mid-summer (second survey in July) as the response variable. In this GLMM,
seedling size in the previous census (first survey in July) was also included as a fixed factor. The response variable (seedling survival) was introduced as described above. Seedling size for the same date was also analyzed with GLMM, including type of cross and population as fixed factors. In this analysis, seedling size was included as a normal variable, using identity link-function. Analyses were performed with SAS 9.0, using the GLIMMIX module.

Germination and survival rates at mid-summer and at the end of the experiment were also analyzed using an accelerated failure-time model as described above. A log-logistic distribution was used for the seedling emergence data set and Weibull distribution for seedling survival.

When the interaction between factors was found to be significant, independent GLMMs were carried out for each population. In these cases, the analyses followed the same conditions as explained before. The same strategy was followed for the accelerated failure-time models for germination and survival rates.

Reference population and estimations of inbreeding depression and inbreeding coefficient

In addition to evaluating the change in deviance in each of our multinomial fixed variables, population and type of cross, we also calculated a coefficient that indicated the change and intensity of this shift in relation to the performance of the High population (fixed population variable) and the between-population outcrossing (type of cross variable). The High population treatment was selected as a reference treatment because it was considered to represent the least stressful conditions for the species. The species is able to grow at altitudes far above our upper limit in other mountains with higher altitudes such as the Pyrenees. This suggests that the species has the potential to extend its upper limit far above the present one in its southernmost limit, but is constrained by the altitude of these mountains (Talavera 1991). Similarly, the between-population outcresses were used as a reference treatment because they represent a random outcrossing scenario for the species from which to estimate the magnitude of inbreeding depression. For each type of cross, the estimated coefficient was used to estimate inbreeding depression in populations. Positive values of this coefficient for selfing indicate inbreeding depression as a result of the sum of within-population inbreeding and among-population inbreeding sensu (Keller and Waller 2002), i.e. inbreeding depression due to relatedness between parents of within-population outcrosses, while the values of this coefficient for within-population outcrossing measure among-population inbreeding depression. Differences in the expression of inbreeding depression between populations were estimated through the type of cross × population interaction term.

Since we were aware of the novel approach followed to assess inbreeding depression we also estimated it with a more standard approach comparing fitness measures for selfing with those expected under random mating (between-population outcrosses, Frankham et al. 2002). Inbreeding depression ($\delta$) was calculated according to the expressions $\delta = 1 - (w_i/w_o)$, if $w_i \leq w_o$ and $\delta = (w_i/w_o) - 1$, if $w_i > w_o$, were $w_i$ and $w_o$ are the fitness measures for selfing and between population outcrossing, respectively (Ågren and Schemske 1993). Inbreeding depression estimates were calculated for seed weight, seed germination in field conditions and seedling size and seedling survival at mid-summer census.

Results

In general terms, between-population outcrossed seeds were heavier, germinated faster and had greater final germination percentages than within-population outcrossed and inbred seeds. Seedling survival and seedling size, two important fitness components, were also greater in between-population outcrossed seeds.

Seed weight and germination in the growth chamber

Seed weight for the three populations and the three types of crossings was $0.38 \pm 0.09 \text{ mg (mean + SD)}$. Seed weight was significantly affected by the population of the parent plants, the type of cross and their interaction (Table 1). In the Low and the Intermediate populations, between-population outcrossed seeds were significantly heavier than either within-population outcrossed seeds or inbred seeds. In the High population, however, within-population outcrossed seeds were the heaviest. Inbreeding depression estimates supported these results (Table 2). Low and Intermediate populations showed positive inbreeding depression values (higher at the Low population) whereas the High population did not experience any inbreeding depression.

Mean germination percentage in the chamber was 67 ± 27%. Seed germination was affected by population origin, type of cross and their interaction (Supplementary material Appendix 1 Table A1). In the Low population, within-population outcrossed seeds had greater germination percentages than between-population outcrossed seeds, while in the High population, between-population outcrossed seeds had the greatest germination percentages. The results from accelerated failure-time models of germination rates (Supplementary material Appendix 1 Table A2) showed significant effects for both factors. Within-population outcrossed seeds germinated faster than between-population outcrossed seeds, which germinated faster than inbred seeds.

Field sowing experiments

Although around 50% of the sown seeds germinated under field conditions, final seedling survival was barely 3% at the end of the experiment. The highest seedling mortality peak was during the month of July. Drought was assigned as the cause of death for more than 90% of seedlings.

Seed germination in field conditions was significantly affected by population and type of crossing and marginally affected by sowing plot (Table 3). No population × cross type interactions were found indicating that inbreeding depression levels were similar in the three populations (Table 2, 3). Between-population outcrossed seeds had the highest germination percentages (Fig. 3). On the other hand, germination in the Intermediate population was higher than in the Low population. Germination percentages of inbred seeds in the Intermediate and the High populations had lower variance than those of other cross types and inbred seeds in the Low population.
Table 1. GLM results for the effects of population, cross type and their interaction (Population × Cross) on *Silene ciliata* seed weight. An independent GLM was performed for each population because the interaction term was significant. Seed weight was considered as normal variable, using identity as link function. Between-population outcrossed seeds were considered the reference treatment for comparison with the other cross types.

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Seeds in the Low and Intermediate populations germinated faster than seeds in the High population, as shown by the accelerated failure-model (Table 4). No significant effects of cross type were detected with respect to between-population outcrossed seeds.

The GLMMs showed that seedling survival at the end of the growing season was significantly affected by sowing plot (as a random factor, Z value = 2.1 p = 0.017), while seedling survival at mid-July (when drought stress was only at the beginning, Table 5) depended on seedling size at the previous census (size correlated positively with survival). Cross type had only a marginal effect on seedling survival at mid-July, but significantly affected seedling size. Population origin had a marginal effect on seedling size (Table 5) with larger seedlings in the Low population. Supplementary material Appendix 1 Fig. A1, shows average seedling sizes for each population and cross type. The main differences were found in the Low population where between-population outcrossed seedlings were larger and inbreeding seeds had greater variance. However, standard inbreeding depression estimates showed high levels of inbreeding depression both in seedling survival and seedling size with higher values in the Low population (Table 2).

The shape of the survival curves for seedlings until the mid-July census was not affected by the factors included in the analysis or their interaction (Table 6). On the other hand, the shape of the survival curves until the end of the growing season was significantly affected by population, cross type and their interaction (Fig. 4, Table 6). The Low and Intermediate populations had higher survival rates than the High population. In independent survival models for each population (Supplementary material Appendix 1 Table A1), Low population inbred seedlings had lower survival rates than between-population outcrossed seedlings, whereas in the Intermediate population, between-population outcrossed seedlings had the highest survival rates.

### Discussion

This study provided information on how inbreeding depression is affecting the rear edge populations of a high mountain Mediterranean specialist at the southernmost limit of its whole distribution range. We found that inbreeding depression is present along the elevation range and that with our approach no differences were found in the intensity of inbreeding depression between populations for most fitness surrogates. However, in a few fitness surrogates, inbreeding depression was greater in populations or situations considered to be under greater environmental stress which was also found when standard estimates of inbreeding depression were assessed. Inbreeding depression runs in parallel to evidence of local adaptation in these populations (Giménez-Benavides et al. 2007a). Local adaptation is also supported by the accelerated failure-model (Table 4).
by the extremely low seedling survival found in the three populations (3% at the end of the study) which provides the necessary selective ground for evolution to operate (Kawecki and Ebert 2004). Decoupling the processes of inbreeding depression and local adaption seems critical for achieving a good understanding of species’ responses to stressful conditions.

Inbreeding depression in *Silene ciliata* populations

The greatest values of several fitness traits considered in this work (i.e. seed weight, germination or survival) were found in between-population outcrossed seeds. Inbreeding depression was not only detected when inbreeding was maximized through selfing, but also when within-population outcrosses were considered. This suggests the presence of biparental inbreeding, or at least, some degree of kinship between the parents in the studied populations. Biparental inbreeding has been documented in populations with low genetic diversity, and has often been found in marginal populations when compared to central ones (Eckert et al. 2008). Preliminary results obtained with microsatellite markers (SSR) developed for *S. ciliata* (Garcia-Fernandez et al. unpubl.) and with EST-SSR markers transferred from *Silene latifolia* (Moccia et al. 2009) showed significant departure from Hardy-Weinberg equilibrium and similar values of $F_{IS}$ and $H_E$ coefficients in the Low ($F_{IS} = 0.35, H_E = 0.51$), Intermediate ($F_{IS} = 0.39, H_E = 0.55$) and High populations ($F_{IS} = 0.47, H_E = 0.5$). Inbreeding depression has also been detected in wild and cultivated populations of other species of the genus *Silene* (Bernasconi et al. 2009).

**Table 3. GLMM results for seed germination of *Silene ciliata* considering population (Pop L: Low population, Pop I: Intermediate population), cross type and the interaction between both factors (Pop × Cross). Seed germination was considered as binomial variable (0 for no germination and 1 for germination) using logit as link function. High population was considered the reference treatment for comparison with the other populations.**

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<td>Selling</td>
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<td>0.466</td>
<td>25.1</td>
<td>-3.04</td>
<td>&lt;0.01</td>
<td>16.34</td>
</tr>
<tr>
<td>Between-pop. outcross</td>
<td>-1.371</td>
<td>0.466</td>
<td>25.1</td>
<td>-2.94</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Pop. × Cross type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.24</td>
</tr>
</tbody>
</table>

*Sowing plot was included as random factor; Z = 1.72, p = 0.04, Estimator ± error = 0.21 ± 0.12.*

**Relationship between inbreeding depression and stressful conditions**

No interactions were found between population origin and type of cross in most of the studied fitness components, suggesting that the expression of inbreeding depression is not different between populations. However, both seed weight and seedling survival curves showed inbreeding depression only in the Low and Intermediate populations. Assuming that the High population has the least stressful environmental conditions, this is consistent with the hypothesis that inbreeding depression may be more intense in the more stressful habitats (Armbruster and Reed 2005). The germination experiments also yielded results consistent with this hypothesis if we assume that natural habitats are a more stressful environment for germination than the germination chamber (inbreeding depression was found in the germination of all populations in the natural habitats (Table 3), but only in the High population in the germination chamber experiments). The standard inbreeding depression estimates showed differences between populations for seed weight, seedling survival and seedling size (Table 2) with the Low population having the greatest values. However, we feel that the results of our comparative approach are more appropriate as they allowed for a sound statistical assessment and considered the effects of other factors that were found to be relevant for the surveyed fitness components. Therefore, our results only provided partial support (based in just a few fitness components) to the hypothesis that stressful conditions enhance the effects of inbreeding depression.

Taking into account that the fitness components studied are concentrated in the early life stages and

![Figure 3. Percentage germination of *Silene ciliata* in each sowing plot (y-axis, plot average ± SD) for the field experiment, grouped by population and cross type (circle: inbred seeds, square: within-population outcrossed seeds and triangle: between-population outcrossed seeds).](image)
may not necessarily correspond to individuals’ total fitness (Armbruster and Reed 2005), the levels of inbreeding depression for the early life stage traits studied here might actually underestimate the total lifetime consequences of inbreeding for individuals in these populations. On the other hand, the definition of stressful conditions merits careful consideration, because the stressfulness of a habitat may differ between life stages and/or fitness components (Körner 2007). The replication of a second set of populations in an independent altitudinal gradient would have provided more consistent results. However, this proved to be very difficult to implement in this particular setting.

Discrepancies among studies comparing inbreeding depression under different environmental conditions suggest that other factors, in addition to stress, may affect inbreeding depression (Armbruster and Reed 2005). Local adaptation might help explain some of the apparently contradictory findings, since it could mitigate the effect of inbreeding depression through the benefits of the locally adapted genotypes (Kawecki 2008) or, on the contrary, enhance the pernicious effects of inbreeding, reducing quantitative genetic variation (Lande 1993). The same loci associated with inbreeding depression in some environments may even contribute to adaptation in others (Cheptou and Donohue 2011). Furthermore, local adaptation is also associated with other genetic processes and factors, such as the intensity of gene flow or available genetic diversity, which further complicate the interpretation of results (Kawecki and Ebert 2004). In this context, the expression of inbreeding depression within populations may also depend on the genotype. In our analyses we did not find a significant effect of maternal genotype (unpubl.) but this may be due to the reduced number of plants used to produce the seed stock.

**Gene flow, inbreeding depression and local adaptation**

Several fitness components were higher in between-population outcrossed plants than in within-population outcrossed plants, suggesting that gene flow between populations contributes to reducing inbreeding depression. These results also imply that the possible negative effects of gene flow reducing local adaptation in some genes were overridden by the reduction of inbreeding depression. However, it is noteworthy that this did not occur in all fitness components or studied populations. Thus, in the High population, seeds were heavier in within-population outcrosses than in between-population outcrosses. These results might be interpreted as a case where the optimal local arrangement of gene complexes is broken by the arrival of foreign gene flow (i.e. outbreeding depression, Whitlock et al. 2000). Similarly, greater germination of within-population outcrossed seeds was detected in the Low

Table 4. Accelerated failure-time analyses (log-logistic distribution) of total seed germination of *Silene ciliata* seeds considering population (Pop L: Low population, Pop I: Intermediate population, Pop H: High population), cross type and the interaction between both factors (Pop × Cross type). High population and between-population outcrossed seeds were considered the reference treatments for comparison with the other populations and cross types, respectively.

<table>
<thead>
<tr>
<th>Variable</th>
<th>DF</th>
<th>χ²</th>
<th>p</th>
<th>Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td></td>
<td>447.69</td>
<td>&lt; 0.01</td>
<td>0.933 ± 0.044</td>
</tr>
<tr>
<td>Population</td>
<td>2</td>
<td>346.24</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>Pop L</td>
<td>1</td>
<td>180.11</td>
<td>&lt; 0.01</td>
<td>−0.832 ± 0.062</td>
</tr>
<tr>
<td>Pop I</td>
<td>1</td>
<td>170.85</td>
<td>&lt; 0.01</td>
<td>−0.791 ± 0.061</td>
</tr>
<tr>
<td>Cross type</td>
<td>2</td>
<td>7.01</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Selfing</td>
<td>1</td>
<td>0.45</td>
<td>0.50</td>
<td>0.052 ± 0.573</td>
</tr>
<tr>
<td>Within-pop. outcross</td>
<td>1</td>
<td>0.61</td>
<td>0.44</td>
<td>−0.058 ± 0.073</td>
</tr>
<tr>
<td>Pop. × Cross type</td>
<td>4</td>
<td>2.59</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>Scale</td>
<td>1</td>
<td></td>
<td></td>
<td>0.191 ± 0.008</td>
</tr>
</tbody>
</table>

Table 5. GLMM results of seedling survival and seedling size of *Silene ciliata* at mid-summer (July census) considering as predictor factors population (Pop L: Low population, Pop I: Intermediate population, and Pop H: High population), cross type and the interaction between both factors (Pop × Cross type). Seed survival was analyzed as binomial variable, using Logit as link function while seedling size was considered a gaussian variable (identity as link function). High population and between-population outcrossed seeds were considered the reference treatments for comparison with the other populations and cross types, respectively.

<table>
<thead>
<tr>
<th>GLMM seedling survival</th>
<th>Effect*</th>
<th>Coefficient</th>
<th>SD</th>
<th>DF</th>
<th>t</th>
<th>p</th>
<th>Deviance change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.88 ± 0.43</td>
</tr>
<tr>
<td>Cross type</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4.71 ± 0.02</td>
</tr>
<tr>
<td>Selfing</td>
<td>−1.559</td>
<td>0.921</td>
<td>23.1</td>
<td>−1.69</td>
<td>0.10</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Within-pop. outcross</td>
<td>−0.503</td>
<td>0.929</td>
<td>21.7</td>
<td>−0.54</td>
<td>0.59</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Size at previous census</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.18 ± 0.01</td>
</tr>
<tr>
<td>Pop. × Cross type</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.53 ± 0.71</td>
</tr>
</tbody>
</table>

| GLMM seedling size     | Population | 3.238 | 1.029 | 21.3 | 2.6 | 0.02 | 3.49 ± 0.04 |
|                        | Pop L      | 0.433 | 1.024 | 20.4 | 3.15| <0.01| –            |
|                        | Pop I      | −0.967 | 1.102 | 26.3 | −0.88| 0.39| 4.62 ± 0.02 |
|                        | Cross type | −0.353 | 1.149 | 29.4 | −0.31| 0.76| –            |
|                        | Selfing    | –       | –     | –    | –   | –   | 1.16 ± 0.35  |
|                        | Within-pop. outcross| −0.353 | 1.149 | 29.4 | −0.31| 0.76| –            |

*Sowing plot was included in both analyses as random factor (seedling survival: Z = 1.53, p = 0.06; seedling size: Z = 2.56, p < 0.01 Estimator ± error = 1.7 ± 0.66).
population and faster germination was obtained in the growth chamber experiments. However, between-population outcrossed seeds had greater germination and marginally faster germination in natural habitats. This contrasting response may indicate the interaction of two processes: the better germination results of within-population outcrossed seeds in the growth chamber may reflect the consequences of breaking the local arrangement of gene complexes by foreign gene flow (between-population crosses) in a favourable environment where the deleterious effects of inbreeding are not expressed, whereas the better germination results of between-population outcrossed seeds in the field may indicate the greater effect of inbreeding depression in the overall balance of this fitness component, when environmental stress unmasks the differential performance of normal and deleterious alleles (Keller and Waller 2002). Although between-population outcrossing can be positive in the short-term, reversing the effects of inbreeding depression by hybrid vigour (Frankham et al. 2002), this initial effect could be masked by late outbreeding depression in the descendants due to the recombination of co-adapted gene complexes or the dilution of adapted alleles (Lynch 1991).

Local adaptation is highly influenced by gene flow between core and peripheral populations (Kawecki 2008). In this sense, gene flow is considered the most limiting force against local adaptation in marginal populations because it can carry alleles that counteract the effect of selection on the local gene pool (Kawecki 2008). However, recent studies of gene flow in populations along a geographical gradient have shown that some marginal populations can become locally adapted despite substantial gene flow (Sexton et al. 2009). For example, in mountain populations of Festuca eskia gene flow was concurrent with the development of local adaptation (Gonzalo-Turpin and Hazard 2009). On the other hand, Byars et al. (2009) found low levels of gene flow between local adapted populations of Poa aniwata across an elevation gradient. These apparently contradictory results suggest that the effect of gene flow on local adaptation might depend on the steepness of the gradient (Sexton et al. 2009) or other uncontrolled factors. We currently do not know the amount of gene flow between the studied populations of S. ciliata at the rear edge. Although populations are separated by only a few kilometres, gene flow is limited by differences in flowering phenology between populations (Giménez-Benavides et al. 2007b) and the close flowering altitudinal tracking of the local pollinator assembly, which goes up in response to the flowering shift in the community (Wilson et al. 2005). This flowering asynchrony could limit pollen flow between populations along the elevation gradient (Körner 2007).
enabling local adaptation (Byars et al. 2007, 2009), as recently found for the Low population through reciprocal sowings experiments (Giménez-Benavides et al. 2007a). Yet our results suggest that between-population outcrossing gave seeds an important advantage, suggesting that local adaptation and inbreeding depression may be operating at the same time (Cheptou and Donohue 2011).

Our results highlight that although local adaptation could play an important role in marginal populations by increasing the fitness of native genotypes with respect to foreign ones (Bradshaw 1984), inbreeding depression may override the effects of local adaptation, making the population more prone to extinction. They also reinforce the idea that inbreeding depression can limit the impact of local adaptation when populations in marginal habitats lose their contact with the rest of populations, a typical scenario in rear edge conditions (Hereford 2010).

Acknowledgements – We thank José Luis Margalet and all the greenhouse staff for caring for the Silene ciliata plants and Lori J. De Hond for linguistic assistance. We also thank the staff of the Parque Natural de las Cumbres, Circo y Lagunas de Peñalara for permission to work in the field area. This work was supported by the projects ISLAS (CGL2009-13190-C03-02), LIMITES (CGL2009-07229), MONTES and REMEDINAL 2. AG held a FPI fellowship (CGL2006-09431/BOS).

References


Supplementary material (available as Appendix O20219 at <www.oikosoffice.lu.se/appendix>). Appendix 1.


