What shapes the altitudinal range of a high mountain Mediterranean plant? Recruitment probabilities from ovule to seedling stage

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Recruitment is a complex process consisting of sequential stages affected by biotic interactions and abiotic factors. Assessment of these sequential stages and corresponding subprocesses may be useful in identifying the most critical stages. Accordingly, to assess the factors that may determine the altitudinal range limits of the high mountain Mediterranean plant *Silene ciliata*, a set of demographic stages, from flower production to establishment of 2-yr-old plants, and their influence on recruitment probability were examined using a step-by-step approach. We integrated florivory, pollination and pre-dispersal seed predation as pre-dispersal factors, and seedling emergence and survival as post-dispersal determinants of recruitment. Three populations were monitored at the southernmost margin of the species along its local altitudinal range. Previous studies suggest that seediness is strongly limited by summer drought especially at the lower boundary of the species, a situation that may worsen under current global warming.

Our results showed that recruitment was mainly limited by low seed production in the pre-dispersal stage and low seedling emergence and survival in the post-dispersal stage, probably due to environmental harshness in summer. By contrast, biotic factors responsible for propagule loss, such as flower and fruit predation, had a minor effect on the probability of plant recruitment. Although the relative importance of transition probabilities was similar among populations along the altitudinal range, comparatively lower flower production significantly reduced the number of recruited plants at the lowest altitude population. This demographic bottleneck, together with increased competition with other species favoured by climate warming, might collapse population growth and limit persistence at the lower altitudinal range of the species, raising its low local altitudinal edge.

Plant species are not expected to occur outside their geographical range due to environmental and/or biotic constraints. Similarly, decreased recruitment is considered a primary mechanism forcing changes in distributonal ranges and driving plant populations to local extinction (Bond 1995). As seed recruitment is one of the most critical processes in the life-history of plants (Kitajima and Fenner 2000), knowledge of this process may help predict species range constraints and trends in range shifts. However, recruitment is an extremely complex process involving successive life stages and biological mechanisms (Herrera 2000a). Therefore, to fully understand the ecological limits of plant distributions, we need to examine the multiple subprocesses involved, such as flower production, pollination, fruit development, seed dispersal, germination and seedling survival. All of these subprocesses are affected primarily by the physiological status of the plant and also by a complex set of abiotic factors and biotic interactions, such as flower and seed predation, and pollinator and seed dispersal mutualisms. However, most studies have only focused on single effects of a small number of abiotic or biotic factors (Louda 1982, Horvitz and Schemske 1984, Galen 1990, Galen and Butchart 2003) which may lead to a biased perspective of this complex process. Few of them have provided an integrated, sequential view of recruitment probabilities from the ovule to the seedling stage (Herrera 2000a, Gulias et al. 2004). Comprehensive, sequential assessment of recruitment may be the best approach for addressing regeneration constraints, as harsh abiotic impacts and/or biotic stress acting in just one step can be responsible for hindering a species’ presence at a particular site. Furthermore, factors that are favourable for a certain recruitment stage may be unfavourable for another (e.g. discrepancies between factors affecting emergence versus those affecting seedling survival, de la Cruz et al. in press).

In this context, special interest has recently arisen on how plants respond to climate warming. Many studies have explored reproductive stages and processes along environmental gradients and their response to climate warming (Louda 1982, 1983, Scherff et al. 1994, Louda and Potvin 1995, Dunne et al. 2003, Kudo and Hirao 2006), but they lack a sequential, integrated approach.

High mountain habitats are especially useful for evaluating the impacts of climate change because they provide...
sharp altitudinal gradients for testing ecological responses of biota to geophysical influences (Körner 2007). Among them, Mediterranean mountain environments impose both harsh summer drought and cold winter conditions that severely limit plant reproduction and growth (Cavieres et al. 2005, 2007, Ramírez et al. 2006, Giménez-Benavides et al. 2007a). High mountain plants are also presumably less exposed to biotic pressures, like those exerted by herbivores or pathogens, as biological activity has been found to decrease with increasing altitude (Molau et al. 1989, Galen 1990, Kelly 1998, Scheidel et al. 2001).

The purpose of our study was to assess the factors that determine the altitudinal range limits of Silene ciliata, a long-lived high mountain Mediterranean plant, in its southernmost distribution limit, and to identify the most critical life stages in the recruitment process. Thus, we evaluated the factors affecting each stage of the regeneration process from the ovule to the established seedling, and calculated transition probabilities along the altitudinal range of the species in central Spain for two years. Comparison of marginal and central populations along an altitudinal gradient may help us to determine the factors that limit the altitudinal range of the species and predict trends in altitudinal shifts due to global warming. Specifically, we hypothesized that the biotic and abiotic factors affecting recruitment would vary along the altitudinal range of the species. We expected recruitment to be increasingly controlled by biotic factors and water deficit as we move down the mountain, reaching a maximum at the lower altitudinal edge, which would eventually force local upward displacement.

Methods

Study species and study sites

Silene ciliata (Caryophyllaceae) is a long-lived high mountain plant which occurs in the main mountain ranges in the northern half of the Mediterranean basin. This species forms cushions of up to 2 cm in height and 15 cm in diameter. It flowers in late summer, with a peak in early August (Giménez-Benavides et al. 2007a). Flowering stems have 1–5 flowers and fruit capsules have up to 100 seeds that are passively dispersed by stalk vibration in August–September. Flowers are mainly preyed by the blister beetle Mylabris sobrina (Coleoptera: Meloidae) which nibbles petals and carpels preventing pollination, and occasionally by nectar-thieving ants (Hymenoptera: Formicidae) (Giménez-Benavides unpubl.). Fruits are almost exclusively consumed by the larvae of Hadena conspAratoiDeS (Lepidoptera: Noctuidae) and, rarely, by weevils (Coleoptera: Curculionidae). Hadena conspAratoiDeS is an Iberian endemic moth that lays its eggs in S. ciliata flowers when pollinating (the so-called nursery pollination, Giménez-Benavides et al. 2007c). Therefore, this moth species can act as a mutualist or parasite depending on the trade-off between pollination and seed predation (Giménez-Benavides et al. 2007c). Silene ciliata plants are rarely grazed by domestic cows, and dispersed seeds may be preyed by harvester ants (Giménez-Benavides unpubl.).

We selected three populations at different altitudes in Sierra de Guadarrama (Peñalara Natural Park, 40°N, 3°W), a mountain range located 50 km north of the city of Madrid where the species reaches its southernmost limit. The lowest altitude population was located at 1976 m in a moraine at Hoya de Peñalara at the Pinus sylvestris treeline and constitutes the lower regional boundary of the species, where it occurs in very fragmented, small subpopulations. The medium altitude population was located at 2256 m on the Peñalara summit, the highest peak of Sierra de Guadarrama. Here, Cytisus-Juniperus clumps are relatively scarce and the Festuca fellfield with S. ciliata dominates the landscape. This altitudinal gradient covers the overall altitudinal range of S. ciliata in Sierra de Guadarrama. As these populations represent the southernmost limit of this species’ distribution, they may be especially sensitive to changes in climate and land use. Sierra de Guadarrama is characterized by a Mediterranean-type climate. Mean annual precipitation in the Navacerrada Pass (1890 m) is 1350 mm, concentrated from early October to late May followed by a pronounced drought season from June to September. Temperature and rainfall varied considerably in the two years of study. Temperatures and summer rainfall were average in 2002, while an extreme heatwave in 2003 caused a relevant increase in maximum temperatures throughout the summer (Giménez-Benavides et al. 2007a). In the past 45 yr average annual temperatures have increased by ca 1.8 °C, while annual precipitation has remained constant. However, snow cover duration during the growing season has decreased 19.7 d (Giménez-Benavides et al. 2007a). As a consequence, the distribution and fitness of animal and plant species in this area have been significantly affected (Sanz et al. 2003, Sanz-Elorza et al. 2003, Wilson et al. 2005).

Sampling design

Flower predation, pollination and fruit predation
We randomly established three study plots (10–12 m²) spaced 10–50 m in each population (altitude). In each plot, 55–60 adult individuals (>2 cm in diameter) were randomly tagged. Cushions located at a minimum distance of 2 cm from each other were considered different individuals. As the species does not propagate vegetatively, each labelled plant was considered a genet. Every week throughout two growing seasons (2002 and 2003) we recorded the number of open flowers and mature fruits on each plant, as well as the number of flowers and fruits with signs of predation. Predated flowers presented nibbled petals and styles, and the gynoecium was also occasionally affected. Predated fruits were in most cases apparently intact from the outside but had a larva developing inside when opened. Sometimes fruits presented a hole produced by the exit of the larva after eating the seeds. All mature fruits were collected from each plant before opening to avoid seed dispersal, and seed number, non-fertilized ovules and larval predation were recorded in the laboratory. Fruit set (proportion of total flowers setting fruit) and seed set (proportion of total ovules setting seed) were calculated for each plant.
To assess whether the reproductive output of *S. ciliata* was affected by pollen limitation, we conducted a hand-pollination experiment in the field. In August 2003 we randomly chose 90 plants from a single population located in the middle of the local altitudinal gradient (2100 m, 1 km away from the Dos Hermanas population). The following treatments were randomly assigned to each plant: 1) passive autogamy: complete flowers were bagged before anthesis, 2) geitonogamy: pollen collected from one flower was applied to other flowers of the same plant with a fine paintbrush, 3) intrapopulational xenogamy: flowers from one plant were pollinated with a pollen mixture from 10 plants of the same population, located at a minimum distance of 10 m, 4) interpopulational xenogamy: flowers from one plant were pollinated with a pollen mixture from 10 plants of the Dos Hermanas population within 2 h after pollen collection. Pollen mixtures were stored in 2 ml plastic vials until use. Non-manipulated flowers were used as free-pollinating controls. In the geitonogamy and xenogamy treatments, anthers were removed before the onset of flowering and flowers were bagged with small pieces of anti-pollen mesh (3M®, St. Paul, MN) to exclude floral visitors. Flowers were monitored until withering or fruit development. Fruit set and seed number per capsule were obtained for each treatment.

**Seedling emergence and survival**

In 2002 and 2003, we collected mature seeds from 20 to 25 randomly selected mother plants in each population. Each year, seeds from each population were pooled together and stored at room temperature until sowing experiments were carried out. Seeds from 2002 were sown in May 2003 (immediately after snowmelt), whereas seeds from 2003 were sown in September 2003 (before the winter season). On each sowing date, four 16 × 16 cm quadrats were randomly established in small bare ground areas in each population. Fifty full-sized seeds, randomly selected from the pooled seeds, were slightly buried at 3 cm intervals in each quadrat in their population of origin. Seedling emergence and survival were monitored every 10 d during the snow-free season in 2004, and at the beginning and end of the season in 2005. Summer drought was assigned as the cause of death to dried-out seedlings without any other visible damage, herbivore/pathogen attack to dead seedlings with external signs of predation such as cotyledon removal, and winter frost to those seedlings that disappeared during winter or appeared uprooted after snowmelt. Additional details on the sowing method are provided in Gimeñez-Benavides et al. (2007b).

**Calculation of transition probabilities (TPs)**

We evaluated the probability of an ovule becoming a 2-yr-old seedling by decomposing it into a series of sequential subprocesses and intermediate stages (Fig. 1) and estimating the transition probabilities of each subprocess. “Ovules” were considered those present in the flowers of a mature plant, and “non-predated ovules” those remaining after flower predation. Similarly, “seeds” were considered those found in full-sized fruits after successful pollination, while “non-predated seeds” were those remaining after pre-dispersal seed predation. Finally, seedlings were classified into three categories: “emerged seedlings”, “1st summer seedlings” and “2nd summer seedlings”, corresponding to seedlings initially emerging from the ground, and those surviving after the first summer and second summer, respectively.

Then, transition probabilities were defined as follows: 1) TP₁ was the probability of an ovule escaping flower predation. Since ovules are packed in flowers and predated flowers do not develop any seed, this probability is equivalent to the probability of a flower escaping predation. We calculated this probability assuming that the number of ovules did not differ between predated and non-predated flowers. 2) TP₂ was the probability of a non-predated ovule being successfully fertilized and developing into a seed, i.e. the proportion of non-predated ovules that set seed. It thus combines fruit set and seediness (Herrera 2000a). 3) TP₃ was the probability of a seed escaping pre-dispersal predation by *H. consparcatoides* larvae. As each larva needs more than a single fruit to complete its development, it consumes all the seeds contained in one fruit before moving to the next.
(Giménez-Benavides et al. 2007c). Therefore, the probability of a seed escaping predation was equivalent to the probability of a fruit escaping predation, assuming that seed number did not differ between predated and non-predated fruits. 4) TP4 was the probability of seedling emergence (i.e. the ratio of the number of emerged seedlings to the number of seeds sown in each population). 5) TP5 was the probability of a seedling surviving from emergence to the end of the first summer. 6) TP6 was the probability of a seedling surviving from the end of the first summer to the end of the second summer, thereby becoming a 2-yr-old plant.

The first three TPs were calculated at the plant level, and mean values per plot (three per population) were used in the statistical analysis. The last three TPs were estimated at the sowing quadrat level (four per population). The product of the stage-specific transition probabilities (TPs) from the ovule to a given stage at plot level provided the cumulative probability of recruitment (CP) whereas the overall probability of recruitment (OPR), that is, the probability of an ovule becoming a 2-yr-old plant was obtained by multiplying all the transition probabilities involved (TPs). It should be noted that TP4, TP5 and TP6 were estimated only from the cohort of seeds produced in 2003, as emergence was negligible in the sowing trials carried out in 2002 due to an inadequate sowing date.

*Silene ciliata* shows considerable interpopulational differences in the percentage of adult plants that flower each year, mainly due to environmental conditions (Giménez-Benavides et al. 2007a). This trait may be relevant in recruitment dynamics since the ratio of established seedlings per adult plant will be very low in years or populations with few flowering individuals. Therefore, mean recruitment per plant (MRP) at each plot and year was calculated as follows:

\[
\text{MRP} = \frac{F_p \times \text{Flowers} \times \text{Ovules} \times OPR}{C_{30}}
\]

where \(F_p\) is mean flowering probability per plot, Flowers is the mean number of flowers per plant (at the plot level), Ovules is the mean number of ovules per flower (at the plot level), and OPR is the overall probability of recruitment. To assess to what extent the estimated values of recruitment per plant fit real recruitment values at the field sites, we carried out an additional census. In September 2003 (at the end of the growing season), we established five 1-m² plots at each population and counted the number of adult plants and seedlings (isolated rosettes with cotyledons or less than five pairs of leaves). Recruitment values in the field were then calculated as the number of seedlings per adult plant.

**Data analysis**

Differences in transition probabilities and cumulative probability of recruitment between populations, considering three plots per population, were analyzed by non-parametric Kruskal-Wallis tests, followed by Nemenyi post-hoc tests to perform multiple comparisons among populations (Zar 1999). The Bonferroni method was used to adjust the significance level for multiple comparisons. Differences among treatments in the hand-crossing experiment were also analyzed by a Kruskal-Wallis test, followed by a Nemenyi post-hoc test. Interannual differences in transition probabilities with two years of data were analyzed by Mann-Whitney tests. Statistical analyses were performed using the SAS (SAS Inst., Cary, NC) and SPSS (SPSS, Chicago) statistical packages.

**Results**

We observed significant altitudinal differences in flowering probability, with high flowering rates at the medium and high-altitude sites and low flowering rates at the low site, especially in the second year (Fig. 2). Flower, fruit and seed production per plant varied significantly among sites and between years. In general, reproductive output was the greatest at the high-altitude population and the lowest at the population located at the treeline, especially in the second year of the study due to the severity of summer drought (Giménez-Benavides et al. 2007a). Statistical differences among populations for each transition probability and each stage are shown in Table 1, whereas post-hoc tests are depicted in Fig. 2 and 4.

Interannual differences (p < 0.05) were only found in the flowering probability at the lowest altitude population, the probability of an ovule escaping flower predation (TP1) at the highest altitude population, and the probability of a seed escaping pre-dispersal predation (TP3) at the intermediate population.

**Flower predation, pollination and fruit predation**

Of the 502 tagged plants, 17.1 and 9.2% were attacked by florivores in 2002 and 2003, respectively, with some differences among sites (Table 2). However, 94.8% of produced flowers escaped predation by insects (TP1 in Fig. 2). The proportion of non-predated ovules setting seed (TP2) ranged between 40 and 60%, and did not differ significantly among sites (Fig. 2). Regarding pre-dispersal seed predation, 93.5% of produced fruits escaped from *Hadena* larvae across populations (TP3). The incidence of both flower and fruit predation was always higher at the low-altitude population (Table 2), but significant differences were only detected between populations at the low and high-altitude sites in 2003 (Fig. 2).

Fruit set and seed set of the pollination experiment are shown in Table 3. Non-manipulated bagged flowers (passive autogamy) produced both the lowest fruit set and seed set values. Interpopulational xenogamy had significantly higher fruit set than the geitonogamy and control treatments, but intrapopulational xenogamy did not differ from control flowers. However, no significant differences in seed set were found among treatments.

**Seedling emergence and survival**

Results were obtained just for the year 2003. Seedling emergence (TP4) started soon after snowmelt (May–June) and lasted until early July. Emergence was significantly greater at the medium-altitude site than at the lower site, whereas intermediate values were obtained at the high site as revealed by the post-hoc test (Fig. 2). On average, only 26%
of emerged seedlings survived until the end of the first summer (TP5), and barely 10% reached the 2-yr-old stage (TP6). The causes of seedling mortality were summer drought (85.8% of dead plants), winter frost (10.7%) and herbivore/pathogen attack (3.5%). The low-altitude population recorded the lowest seedling survival both after the first and the second summer (Fig. 2).

**Overall probability of recruitment and seedling density in the field**

The cumulative probability of recruitment per plant is represented in Fig. 3. The Kruskal-Wallis test by ranks found significant differences among populations, mainly in the post-dispersal stages of the recruitment process corresponding to the sowing experiment performed the second year. The most significant differences in the probability of setting an established seedling were found between the low and the medium-altitude populations. However, the high and medium-altitude populations did not differ significantly at any stage. The cumulative probability of recruitment declined sharply during fruit set, emergence and first summer survival, denoting the most critical processes (Fig. 3). As a result, the overall probability of recruitment (OPR) showed that the ovules produced in the medium-altitude population had the highest chance of reaching the 2-yr-old seedling stage, followed by those produced in the high-altitude population. However, when we estimated mean recruitment per plant, the high and medium-altitude populations yielded similar numbers of seedlings (Fig. 3). Moreover, mean recruitment per plant estimated directly in the field plots showed the same altitudinal pattern of recruitment (Fig. 4).

Figure 2. Partial fitness values of all subprocesses involved in the regeneration from the ovule to the seedling stage at low, medium and high-altitude *S. ciliata* populations in Sierra de Guadarrama. Post-dispersal subprocesses were assessed for only one season (2003). Vertical lines denote standard errors. Different letters denote significant differences between populations within each year (p < 0.05).
Discussion

Our results show that the overall probability of recruitment in *S. ciliata* is determined by a series of factors acting at sequential stages, with effects on each of them varying from negative to positive. Although many previous studies have explored the importance of seedling establishment in alpine environments (Chambers 1995, Galen and Stanton 1999, Niederfriniger and Erschbamer 2000, Forbis 2003, Cavieres et al. 2007) none of them have assessed the ultimate causes of variation among populations by using a multiphase approach. For instance, the most critical subprocesses limiting recruitment mainly rely on abiotic factors affecting seedling emergence and survival (specially water availability). However, a slightly decreasing trend in flower and seed predation with increasing altitude might also condition recruitment (Fig. 2), as previous works along altitudinal gradients have suggested (Molau et al. 1989, Galen 1990, Kelly 1998, Scheidel et al. 2001). In our case, neither flower nor fruit predation significantly affected overall recruitment of the species.

Florivory (i.e. herbivory focused on floral tissues) can be especially harmful for plant reproductive success, because it combines the direct reduction of ovules and the indirect effect of reduced flower display for insect attraction (Schemske and Horvitz 1988, Herrera 2000b, McCall and Irwin 2006). In alpine environments, flower predation is common and sometimes causes significant reductions in fecundity (Norment 1988, Galen and Butchart 2003). However, our results showed that flower predation by the blister beetle *M. sobrina* only slightly reduced *S. ciliata* seed output and flower predation was not significantly different along the elevational gradient.

Only 50% of non-predated flowers across populations produced seeds, suggesting certain pollinator limitation on fruiting success. Our hand-pollination experiment revealed that pollen from distant plants yielded a higher number of fruits than control plants, whereas control plants did not differ from intrapopulational crosses, suggesting some degree of inbreeding depression rather than pollen limitation. Pollinator scarcity and the consequent pollen limitation have traditionally been considered for plant sexual reproduction in high mountain environments (Bliss 1971, Kevan and Baker 1983, Totland 1994). However, our results suggest that the probability of an ovule becoming a seed may be more dependent on factors other than pollinator effectiveness, such as resource limitation or environmental harshness. In a previous study, Giménez-Benavides et al. (2007a) found that low fruit production was due to the late-flowering pattern of *S. ciliata*, which limited the use of snowmelt moisture during reproduction, and the subsequent summer drought.

Pre-dispersal seed predation has also been identified as a detrimental subprocess of plant reproductive success (Louda 1982, Louda and Potvin 1995), even in mountain environments (Molau et al. 1989, Freeman et al. 2003, Weippl and Stöcklin 2006). Our results suggest that most *S. ciliata* seeds escape pre-dispersal seed predation, so this factor, at least for the two years of this study, does not actually represent a severe limitation for seedling recruitment. However, seed predation by *H. consparratoidea* may be underestimated in our study because *Hadena* larvae

<table>
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<th>Flow. prob.</th>
<th>OPR</th>
<th>MRP</th>
<th>Non-pred. ovule</th>
<th>Emerged seedling</th>
<th>1-summer seedling</th>
<th>2-summer seedling</th>
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typically move to other host fruits once they have eaten the contents of the fruit where the egg was laid (Giménez-Benavides et al. 2007c). Since we collected fruits before seed dispersal to calculate seediness, some moth larvae were removed early from plants preventing subsequent fruit predation. Therefore, pre-dispersal seed predation could actually be higher and present an important source of propagule loss in *S. ciliata*.

The subsequent stages of seedling emergence and survival had a significant impact on the cumulative probability of recruitment, and accounted for most of the differences observed among populations (Fig. 3). Although seedling emergence was only measured in the field in one year, the obtained values were well below the potential germination capacity of the species under laboratory conditions (low altitude: 68%, medium altitude: 78% and high altitude: 58%, Giménez-Benavides et al. 2005). This suggests that local environmental factors played a major role in emergence success. The medium-altitude population had greater seedling emergence and, subsequently, a greater overall probability of recruitment than the high-altitude population, even though their seedling survival probabilities were similar (Fig. 3). Abiotic factors, especially drought, were the major cause of low seedling survival probability, particularly in the low-altitude population. Several authors have recently pointed out the existence of a severe water shortage in high Mediterranean mountain environments and its negative effect on plant regeneration (Castro et al. 2004, 2005, Lloret et al. 2004, Cavieres et al. 2005). In our case, soil moisture measurements at the experimental sowing sites showed an extreme water shortage during mid-summer, which accounted for most of the *S. ciliata* seedling deaths (Giménez-Benavides et al. 2007b). This summer drought was especially intense at the low-altitude population.

Recruitment per adult plant was notably higher in the medium and high-altitude populations compared to the low-altitude population where recruitment values were around five-fold lower (Fig. 3). Although the medium-altitude population reached a higher probability of recruitment per ovule, the high-altitude population yielded a similar number of 2-yr-old seedlings per adult plant due to both higher flowering probability and higher flower production (Fig. 2). Recruitment per plant values estimated by means of transition probabilities were significantly different from direct censuses in the field plots, although the altitudinal gradient pattern was similar (Fig. 3, 4). This suggests that some steps of the recruitment process that were not directly considered in our study may also play a relevant role. For instance, post-dispersal seed fates (e.g. predation and seed arrival to safe sites) are decisive subprocesses of the regeneration cycle that may also cause significant reductions in the reproductive output of perennial plants. Some studies have assessed the importance of sequential post-dispersal stages on plant recruitment, sowing that seed predation is one of the most important bottlenecks (Rey and Alcántara 2000, Traveset et al. 2003, Gulias et al. 2004, Lázar et al. 2006, Rodríguez-Pérez and Traveset 2007). In fact, both subprocesses have been reported to be important sources of seed loss in alpine environments (Chambers 1995, Muñoz and Cavieres 2006).

This study illustrates how the combination of observational and experimental surveys may help detect the most critical stages in the seed regeneration process, how they change along the altitudinal range of a mountain plant and, more specifically, what factors determine the final output in each population. This approach provides a very useful, standardized measure (i.e. the probability of an ovule becoming an established seedling) for comparing regeneration patterns under contrasting ecological scenarios, like peripheral vs central populations, disturbance regimes, management practices, etc. Although the time-consuming nature of these surveys limited our study to three populations to describe the altitudinal pattern, results were robust and led to relevant conclusions on the limiting factors of recruitment in the studied system.

In summary, our results showed that the most critical subprocesses of recruitment in *S. ciliata* were flowering probability of adult plants (very poor at the lower boundary), seed production, seedling emergence and seedling

| Table 2. Incidence of flower and fruit predation on low, medium and high-altitude *S. ciliata* populations in Sierra de Guadarrama (Madrid, Spain). | 2002 | 2003 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Florivory | Low | Medium | High | Mean | Low | Medium | High | Mean |
| % plants attacked (n = 502) | 15.7 | 14.5 | 21.1 | 17.1 | 12.0 | 10.9 | 4.7 | 9.2 |
| % flowers predated (n = 5796) | 8.1 | 5.1 | 6.0 | 6.2 | 10.2 | 6.2 | 0.7 | 3.4 |
| Pre-dispersal seed predation | 16.3 | 7.3 | 9.4 | 11.0 | 5.4 | 4.8 | 5.8 | 5.4 |
| % plants attacked (n = 502) | 16.3 | 4.7 | 4.1 | 7.4 | 16.3 | 6.4 | 2.9 | 5.2 |

<p>| Table 3. Fruit set and number of seeds per fruit after different pollination treatments in <em>Silene ciliata</em>. Superscripts denote Neményi post-hoc test. Treatments with the same letter do not differ significantly (p &gt; 0.05). |
|---|---|---|---|---|</p>
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<th>Pollination treatment</th>
<th>Flowers (n)</th>
<th>Fruit set</th>
<th>Seed set</th>
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<tr>
<td>Passive autogamy</td>
<td>49</td>
<td>0.30 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.14 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Geitonogamy</td>
<td>33</td>
<td>0.39 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.30 ± 0.22&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Intrapopulation xenogamy</td>
<td>33</td>
<td>0.45 ± 0.16&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>0.27 ± 0.21&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td>Interpopulation xenogamy</td>
<td>30</td>
<td>0.64 ± 0.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.20 ± 0.16&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td>Control</td>
<td>44</td>
<td>0.41 ± 0.13&lt;sup&gt;bd&lt;/sup&gt;</td>
<td>0.23 ± 0.13&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
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survival. These processes were highly dependent on local environmental conditions, and especially on summer drought which is exacerbated as altitude decreases (Giménez-Benavides et al. 2007a, b). Except for pollination which was not specifically measured in this study, biotic interactions such as herbivory were less restrictive for sexual regeneration than abiotic factors. This is a common feature of declining plant populations in their southernmost range (Eriksson 1996, García et al. 1999, 2000, Hampe and Arroyo 2002). As a result, the small, isolated populations located at the lower-altitudinal edge of the species (those most affected by summer drought) showed extremely lower recruitment probabilities and may, therefore, be highly affected by a recruitment shortage. Since this species is unable to reproduce vegetatively, the long-term persistence at the lower-altitude edge will ultimately depend on the longevity of adult plants. Sanz-Elorza et al. (2003) reported increasing density and upward displacement of the Cytisus-Juniperus shrub community in this mountain range, as a result of climate warming and land-use change. This community typically occurs interspersed in the lower and intermediate populations of S. ciliata, which is unable to grow beneath these shrubs. Therefore, in addition to the direct effects of climate harshness on the recruitment of S. ciliata, competition with shrubs may also promote the exclusion of this species from its current lower limit.

Figure 3. Cumulative probability of recruitment of low, medium and high-altitude S. ciliata populations, calculated by multiplying all the transition probabilities at each site for each year of study. Small plot represents mean recruitment per plant (number of ovules becoming 2-yr-old seedlings per S. ciliata adult plant). Vertical lines denote standard errors. *Differences between low and high altitude populations at p < 0.05, **differences between low and medium-altitude populations at p < 0.025. Separate analyses for each year.

Figure 4. Direct field estimation of mean recruitment per plant (number of seedlings per adult plant) in low, medium and high-altitude S. ciliata populations. Different letters denote significant differences between populations (p < 0.05).

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