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DEMOGRAPHIC STRUCTURE AND REPRODUCTIVE SUCCESS OF *PRIMULA ALLIONII*, A PLANT ENDEMIC TO MARITIME ALPS

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ABSTRACT

Primula allionii is a primrose endemic to the Maritime Alps. In order to evaluate its conservation status, we investigated its demographic structure and reproductive success. We studied the plant in three different plots in Roya Valley (France), representing the different structure of cliffs where the plant usually lives. The mean dimensional attributes and the environmental characteristics of areas where plant occurred were evaluated and different size categories were defined. Plant occurrence appeared non-random, with relatively high density of individuals. We recorded a high rate of seed production and a low morph bias. However, the population remains in a demographic equilibrium and very few seedlings were recorded in the population. The habitat in which the plant lives does not allow a substantial population renewal because of its extreme ecological features (vertical cliff, low soil availability, etc). Although the species is not currently threatened, its slow renovation dynamics, together with the fragmentation of the existing populations, could expose a population to stochastic extinction or sudden declines. Future conservation strategies should aim to facilitate recovery if any such event would occur.

KEYWORDS

Primula allionii Loisel., endemic plant, reproductive success, morph bias, population structure.

INTRODUCTION

Biological rarity is a concept of difficult definition, and it is often driven by several factors such as the total number of individuals of the species of concern, its geographical distribution, its habitat specificity (Zalba & Nebbia, 1999), demographic stochasticity, stochastic deviations from sex ratio and subsequent reproductive failure. In general, a species with a geographically limited distribution is likely to be more exposed to extinction risk because of its reduced genetic variation and greater sensitivity to natural or anthropogenic factors that could condition its survival (Wissel & Stöcker, 1991). Nevertheless, being rare does not neccesarily mean that a species is facing a real risk of extinction (Gaston, 1994). Adequate information is required in order to assess the degree of threat faced by a given species: this includes its geographical distribution, total number of individuals and populations, population dynamics, habitat requirements, susceptibility to human activities and natural catastrophes, reproductive biology, habitat trends, etc. (Zalba & Nebbia, 1999; Tomimatsu & Ohara, 2002; Severns, 2003).

Reproductive limitations in small plant populations (i.e. seed reproduction) can depend on one or more factors such as self-incompatibility, deficiencies in pollination, failure of germination or seedling establishment, and seed predation (Zalba & Nebbia, 1999; Buza et al., 2000; Graur and Li, 2000; Juenger & Bergelson, 2000). In some plant species, higher geographic isolation among populations is correlated with an increased level of inbreeding and a decreased gene flow and population size (Richards et al., 1999; Richards, 2000). In small and isolated populations, morph frequencies of heterostylous plants with strict intramorph incompatibility deviate more markedly from equality with decreasing population size (Brys et al., 2003).

The breeding system of a species strongly influences parameters of great importance, both quantitatively (demographic) and qualitatively (genetic). Therefore, the characterization of the reproductive biology of endangered and narrowly distributed species is essential to determine which are truly endangered (Zalba & Nebbia, 1999) and provides valuable information to suggest appropriate conservation measures (Gaudeul & Till-Bottraud, 2004).

The aim of this work was to gather biological information to assess if the rare *P. allionii* should also be considered a threatened species, and hence worthy of conservation. We carried out field observations of the demographic and reproductive dynamics of populations in the Roya Valley (France). More specifically, the main questions addressed were: (1) what is the structure and the dynamics of the populations? (2) how many flowers and fruits are produced? (3) is the species able to preserve its status in a fragmented habitat? (4) is the species to be considered Critically Endangered, Endangered or Vulnerable according to IUCN criteria?

MATERIALS AND METHODS

Species

P. allionii is a small perennial rosette plant showing affinity with carbonatic cliffs. It mainly occurs in mesoMediterranean and supraMediterranean bioclimates (between 500 and 1200 m a.s.l.), but it may appear locally in the subalpine belt (up to 2000 m in the Bec d'Orel Massif). This plant is dominant in the *Primuletum allionii* plant community, often co-occurring with some other rare and endemic species like *Moehringia sedoides* (Pers.) Cumino ex Loisel. (endemic to SW Alps) and *Asplenium trichomanes* L (Martini, 1994; personal observation). The species distribution range is limited to a small area of the Maritime Alps: in particular, populations (total=52) live in the middle Roya Valley in France (n=16) and the Gesso of Trinità (n=27), Roaschia (n=8) and Vallegrande Valleys (n=1) in Italy. Populations are thus divided into two main groups in northern Italy and in southern France (30 km apart), and they are supposed to be the result of post-glacial colonization from South to North (Martini, 1982). *P. allionii* is believed to be a pre-glacial relict belonging to an ancient subtropical mountain climate (Martini, 1992, 1994).

Fig. 1. Specimens of *Primula allionii* from Berghe (Pt3) population (France). Pictures were taken in late February.

Each individual consists of a round and thick cluster of 1–15 ramets belonging to the same genet (Fig. 1). The ramets of a cluster derive from a single rhizome, whose growth rate does not exceed a few millimetres per year. Propagation is prevented by the rocky substrate where the species occurs (personal observation). The plants bear large flowers (4-5 cm in diameter) from January to April depending on the geographical position of specific populations: in general, the flowering period for each plant lasts three months (data in elaboration). Like many other taxa of the genus, *P. allionii* is heterostylous and each individual may bear long styled (pin - P) or short styled (thrum - T) flowers.

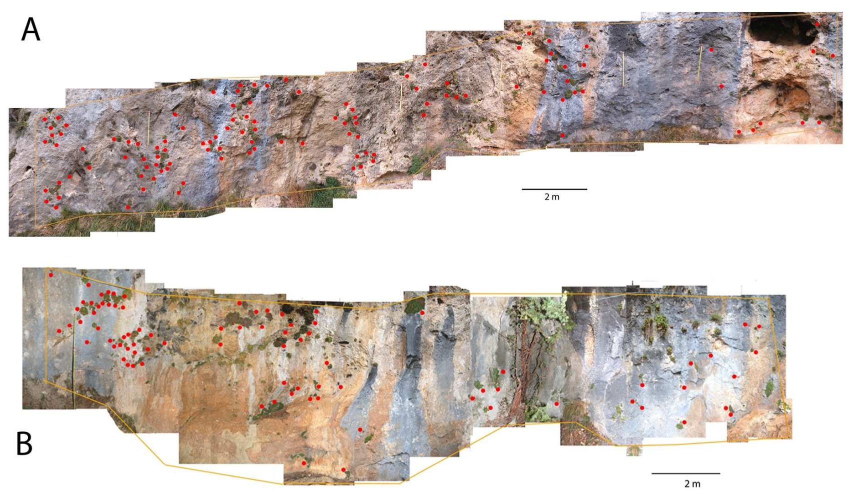

Fig. 2. Example of study plots: Pt1 (A) and Pt2 (B). Bar = 2 m.

Study sites and plots

Two natural populations from the southern group (Val Roya - France) were studied from November 2007 to November 2009 (two flowering seasons). The first population is located in 'Chiarin – Berghe Inferieur' [44°02'06"N 7°33'34"E; 1100 m a.s.l.; mean annual precipitation 1031.5 mm, mean annual temperature 11.5°C (http://junon.u-3mrs.fr/msc41www/poscli/B72.html)], the second in 'Traya – Berghe Superieur' [44°01'44"N 7°34'07"E; 807 m a.s.l.; 1 km distance from the other population]. They were selected only from the southern group for their easier accessibility during the flowering period in winter. No Italian populations were selected, because none of them were accessible during the flowering period due to the continuous snow cover.

The demographic study was mainly conducted at the largest population of ‘Chiarin – Berghe Inferieur’, consisting of some thousands of individuals, where two representative plots (Pt1 – exp. S and Pt2 – exp. SW; Fig. 2) were selected as the two typical arrangements of *P. allionii*: the vertical and the overhanging cliffs. A further third plot (Pt3 – exp. NE) was selected at ‘Traya – Berghe Superieur’. Each plot was permanently marked with a plastic string delimiting the cliff at a mean height of 2 m from the ground and for a length of about 12 m. The three plots had similar areas (Pt1 – vertical cliff – 21.99 m^2; Pt2 – overhanging cliff – 24.52 m^2; Pt3 – cave margin – 23.28 m^2).

Spatial structure and demography of populations

The position of each individual within the three study plots was mapped by a photographic scanning of each plot. Large series of photographs were produced with a camera (Nikon D50) at high resolution (8 Mb) maintaining a constant distance and angle from the cliff and visualizing a 2 m scale bar. Pictures were then joined together in one map (Fig. 2). This solution was adopted in order to translate the vertical structure of the plot, which could not be rendered using a GPS instrument, and to maintain the correct between-plant distances. The plants were permanently listed and numbered in the plots The final global map was elaborated using MapInfo Professional version 7.8 (MapInfo Corporation, Troy, New York) and overlapped with a grid (0.5-m side for each square). We reported on the grid the position of each plant, as well as the available micro-niches suitable for the species (holes, crevices, slits, pockets and hollows, typical of the Jurassic calcareous rock). The number of individuals per cell (each square within a plot) and the percentage of micro-niches were scored and their relationship was statistically analysed with Statistica 8.0 (StatSoft 2007) using Kendall Tau coefficient.

Between November 2007 and November 2009 the demographic dynamics of the plots were monitored, recording any deaths and seedling appearances. The sexual form (P or T) of each individual was recorded during the flowering period. To describe the demographic structure of each population, the individual size (obtained measuring the long and short diameters of a plant and by approximating the area as an ellipse) was recorded every year in order to detect any individual growth. Morph bias was calculated for each population as the value of the difference between the number of individuals of the two morphs, divided by the total number of flowering plants. It can vary from -1 (only T morph present) to 1 (only P morph present), with 0 indicating equal frequency.

Flower, fruit and seed sets

During the flowering season (from January to April) twice-weekly surveys were carried out in order to verify the reproductive status of each plant, recording the number of flowers and fruits. Between-plots flower and fruit productions were assessed using analysis of variance (ANOVA). All statistical analyses were performed with Statistica 8.0 (StatSoft, 2007). Any relationship among flower/fruit productions and individual size or floral morphs was investigated with a post hoc test unequal (HSD). In particular, the correlation between flower production and plant size for each plant was assessed graphically. During the last year all fruits produced in the plots were collected immediately before the opening of capsules. The seeds contained were observed under a Leica M205 C stereomicroscope and counted in order to estimate the mean production (n seed/plant).

RESULTS

Spatial structure and demography of populations

The study populations (Pt1-Pt3) numbered respectively 120, 89 and 53 individuals. They were constituted by an unequal number of individuals belonging to the two floral morphs (Table 1). In each plot the percentage of P was always a bit higher than T, as revealed also by the morph bias. *P. allionii* showed a substantial demographic stability with a low yearly recruitment of plants (Table 2). During the study period the number of dead plants (mean=2.67 , s.d.=1.63) was similar to the number of seedlings (mean=2.67, s.d.=1.06) appeared within each plot. Plant density (n/m^2) was relatively high (mean=3.79, s.d.=1.60). Some differences were evidenced in the three different plots (Pt1=5.46; Pt2=3.63; Pt3=2.28) with a higher density recorded on the vertical cliff. The density of plants per cell within each plot highlighted the existence of non-random distribution patterns (D=0.3122, p<0.01), showing a non-normal trend with many cells without any individual and very few ones with many plants (Fig. 3). The correlation between the number of plants per cell and the number of micro-niches per cell resulted 0.38 (p<0.001) for Pt1 and 0.35 (p<0.001) for Pt2.

	Pt1	**Pt2**	**Pt3**	**Tot**
P	51.89	62.50	60.61	56.41
T	48.11	37.50	39.39	43.59
P/T	0.03	0.25	0.21	0.12

Table 1. Percentage of the two floral morphs (P and T %), morph bias in the three plots (Pt1-Pt3) and in the whole study area (Tot).

		2007	2008	2009	TOT
Pt1	individuals	114	116	118	120
	dead		1	1	2
	seedlings	1	3	3	7
	Δ %		1.75	1.72	3.51
Pt2	individuals	82	84	83	89
	dead		2	4	6
	seedlings		4	3	7
	Δ %		2.44	-1.19	1.22
Pt3	individuals	50	48	45	53
	dead		3	5	8
	seedlings	2	1	2	5
	Δ %		-4.00	-6.25	-10.00
TOTAL	individuals	246	248	246	262
	dead		6	10	16
	seedlings	3	8	8	16
	Δ %		**0.81**	**-0.81**	**0.00**

Table 2. Demographic variation in the three plots (Pt1-Pt3) at the beginning of the study (2007) and after the two flowering seasons (2008-2009). For each plot the following data are reported: total number of living individuals (individuals); number of dead individuals recorded (dead); number of seedlings observed (seedlings); variation expressed as percentage (Δ %).

Individual plants, constituted by one or more ramets in a clump, showed a mean size of 46.77 cm^2 (about 7x8 cm in diameter; s.d.=59.85), ranging from a minimum of 2.36 cm^2 to a maximum of 362.67 cm^2. Few larger individuals deviate the mean value, but clumps resulted mostly small: 66.79% of them had an area lower than the mean value, as confirmed by the mode value of 15.70 cm^2 calculated on all individuals. Analysis of variance suggested there was a significant difference in size between the two populations [$F_{(2,\ 259)}=6.6275$, p=0.00156], as highlighted by the post hoc test unequal HSD (0.0040 $p \leq 0.01$).

No significant differences in size were found among plants belonging to the two floral morphs. P plants had a mean size of 48.03

cm^2 (s.d.=53.08) and a mode (15.70 cm^2) similar to that calculated for the species. T individuals had a mean size of 53.78 cm^2 (s.d.=69.49) but a lower mode (7.07 cm^2).

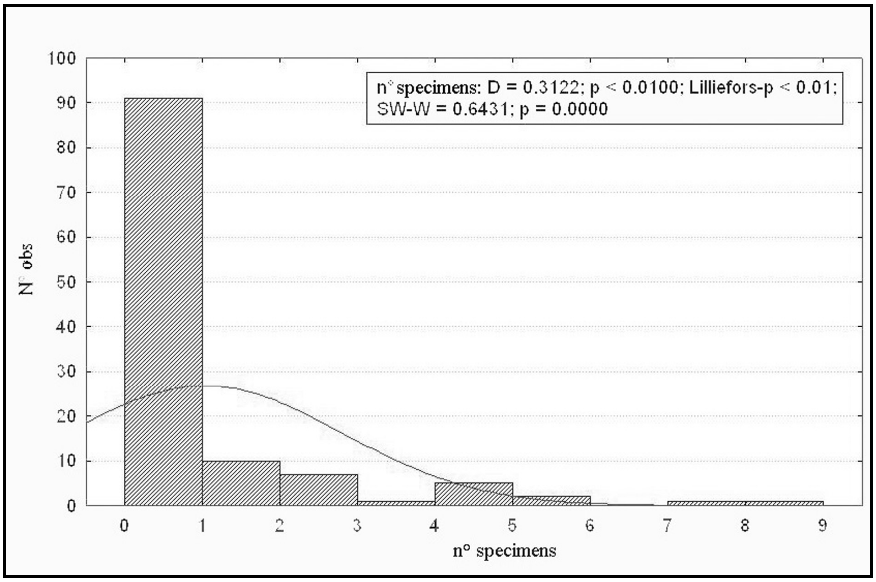

Fig. 3. Number of cells (N obs.) occupied by a progressive number of plants (n individual plants) in plots Pt1 and Pt2.

Flower, fruit and seed sets

P. allionii showed a high number of flowering plants (74.43%) and most of them produced ripe fruits (Table 3, columns 1-2). Fertile individuals in the populations ranged in percentage from 47.17% to 70.00%, bearing on average of 2.87 (s.d.=4.13) flowers per individual. The reproduction of *P. allionii* was not correlated to plant size: about a third of individuals was above the regression line and produced a higher quantity of flowers than the expected value related to its size, while other plants showed a lower production (Fig. 4A). Moreover, plant size had a low correlation with flower set (Kendall Tau coefficient=0.2963, $p<0.05$).

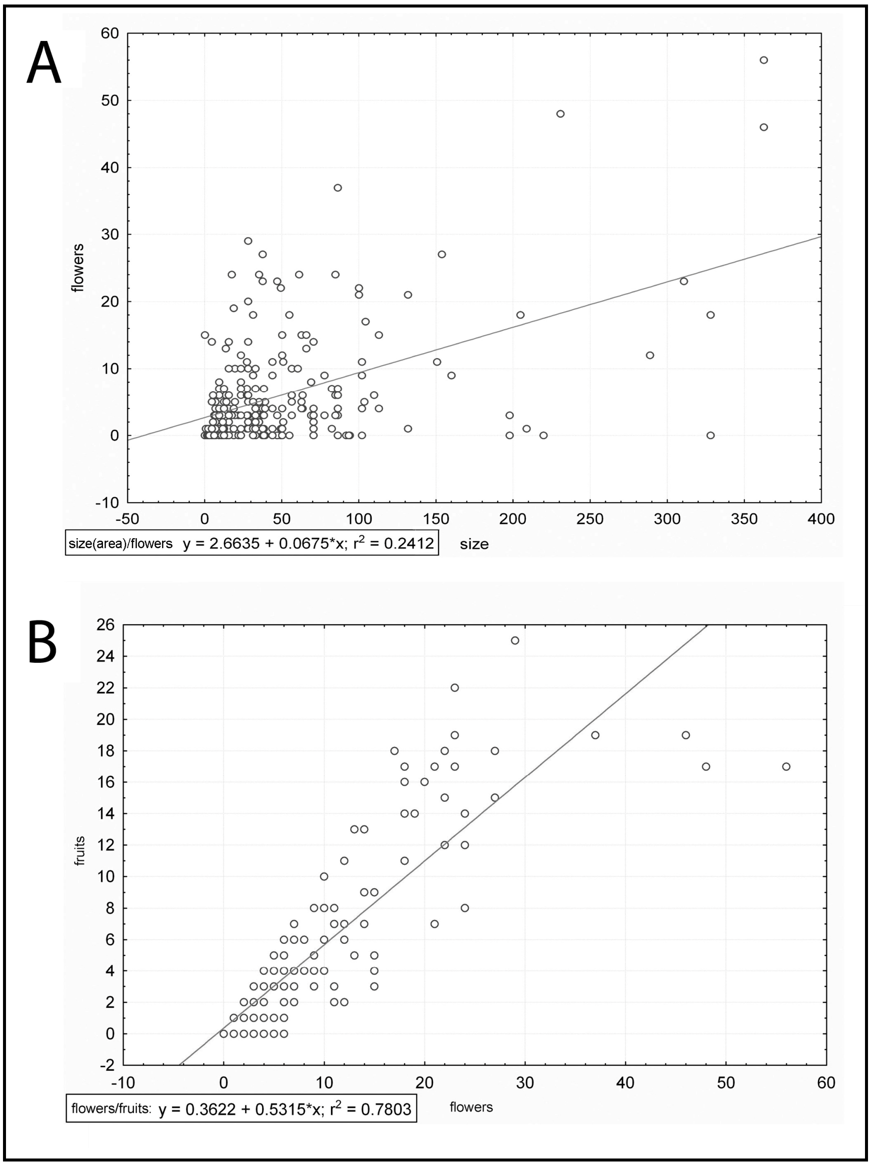

Fig. 4. Evaluation of population productivity (Pt1-Pt3). A) Flower production related with plant size. B) Correlation between flowers and number of ripened fruits for each plant. The regression lines and correlation coefficients (r^2) were reported in (A) and (B).

	Flow%	Frut%	Fl/pl	Fr/pl	Seed/pl
Pt1	87.50	70.00	3.64 sd 4.24	2.29 sd 2.91	3.84 sd 4.93
Pt2	62.92	49.44	2.28 sd 4.26	1.15 sd 1.86	1.38 sd 2.98
Pt3	62.26	47.17	2.12 sd 3.34	1.34 sd 2.20	1.89 sd 5.08
Tot	74.43	58.40	2.87 sd 4.13	1.71 sd 2.51	2.61 sd 4.22

Table 3. Flower and fruit productions in *P. allionii* within the three study plots (Pt1-Pt3). Percentage of flowering (Flow%) and fruiting (Fru%) plants, mean number of flowers (Fl/pl), fruits (Fr/pl) and seeds (Seed/pl) produced for each plant per year.

Weak variations in flower production were recorded among the plots (Table 3, column 3): ANOVA suggested some differences [$F_{(2, 259)}$=3.9310, p=0.02080] in flower production among the plots, whereas there were no significant differences in reproductive activities between the two floral morphs, with similar mean fruits and seeds production for T and P plants (Table 4). *P. allionii* completed the ripening process in six months during the summer. The total reproductive success was of 58.40%. Each plant produced a mean quantity 1.71 sd=2.51 of fruits per year. Most flowers yielded ripe fruits (Kendall Tau coefficient=0.8159, $p<0.05$; scatter plot: Fig. 4B).

Consistent variations were recorded among the plots (Table 3, column 4) also revealed by ANOVA, which highlighted differences in fruit production [$F_{(2, 259)}$=6.3082, p=0.00211]. In particular, the post hoc test unequal HSD pointed out a marked difference (0.00532 $p \leq 0.01$) in fruit production between Pt1 and Pt2. As previously reported for the flower production, there was no significant difference in fruit set between floral morphs (Table 4). The mean seed production per fruit was 2.61 (s.d.=4.22; Table 3). In the three plots a mean availability of

1,200 diaspores was recorded during the last year. However, the mean number of seedlings recorded was very low (n=7-8) corresponding only to a very low percentage of the seed set (mean 0.30%).

	n	Fl/pl	Fr/pl	Seed/pl
P	109	4.10 sd 4.56	2.50 sd 2.82	3.74 sd 5.08
T	85	3.59 sd 4.14	2.07 sd 2.45	3.25 sd 3.82

Table 4. Flower, fruit and seed productions in *P. allionii* within the two floral morphs (P and T). Mean number of flowers (Fl/pl), fruits (Fr/pl) and seeds (Seed/pl) produced for each plant per year.

We found a marked difference in seed production among the plots, confirmed by ANOVA [$F_{(2,\ 259)}$=10.2691, p=0.00005]; in particular, post hoc test unequal HSD pointed out a marked difference in fruit production between Pt1 and Pt2 (0.00019 $p\leq0.01$) and between Pt1 and Pt3 (0.03795 $p\leq0.05$). The seed set of the two morphs is reported in Table 5. P plant bore 3.39 (s.d.=4.97) seeds per fruit per year; T plant bore 2.83 sd=3.40 seeds per fruit per year. No significant differences were found.

DISCUSSION

In *P. allionii*, the distribution of individual plants within the plots appeared non-random and it was mainly explained by the presence of micro-niches suitable for seed germination and plant growth. This was probably the main determinant of the small number of seedlings recorded per year. However, the so specific habitat where *P. allionii* lives induces low competition and the demographic stability is maintained by a low mortality rate. Plant density appeared relatively high: however, to our knowledge no other studies exist on the demography of other cliff plants, preventing useful comparison of the data collected in this study.

Large population size and high density are good predictors for a good gene flow among individuals within a population (Lienert, 2004; Ishihama et al., 2006): both aspects were observed in this study for *P. allionii*. We observed a low bias in morph frequency, suggesting a good reproductive fitness for the species, in contrast with observations for other heterostylous species, where biased morph frequencies may interact with limited pollinator attraction in small populations (Byers, 1995; Brys et al., 2003; Kéry et al., 2003). In *Primula* species, which are distylous and intramorph-incompatible, the probability of plants receiving suitable pollen increases when the different morphs are equally present in the population (Kéry et al., 2000). The equilibrium of morph ratios we consistently found in populations of *P. allionii* cast no doubt on whether self-fertilization may occur as observed in *P. mistassinica* (Larson & Barrett, 1998).

In our study species, plant sizes were different among plots but not between the two floral morphs. This fact is in accordance with the evidence that microhabitat resources act upon plant growth and dimension (Gaudeul & Till-Bottraud, 2004). However, in our study statistical analyses suggested no influence of plant size on flower set, matching observations for *P. elatio*r (Jacquemyn et al., 2002). Conversely, differences among flower, fruit and seed sets were revealed in the three plots, but in all cases production values did not differ between different floral morphs. Particularly some differences were recorded in Pt1, where different levels of abiotic resources in different microhabitats might have influenced the reproductive efficiency of plant, acting upon pollen and seed production (Gaudeul & Till-Bottraud, 2004). The definition of these parameters was not an aim of this study: however, during our field surveys we noted differences in the frequency of pollinators in different plots, which might be influenced by abiotic patterns (light, hours of sun exposition, mean daily temperature) as already reported for *P. veris,* where a low habitat quality affected pollinator densities negatively (Brys et al., 2003). The lack of any consistent differences between the fertility in the two floral morphs of *P. allionii* indicated that inter-morph pollen transfer by pollinators should be effective in the whole area, as already observed for the fertility of mainland and island populations of *P. mistassinica* (Larson & Barrett, 1998).

Another parameter used by plant population biologists is population turnover. If a population is to survive over many generations, the offspring must replace the dying parent generation, so that population growth rates remain stable or positive (Oostermeijer, 2000). Such replacement might be disrupted by failure in germination or the seedling establishment and by high seed predation (Zalba & Nebbia, 1999). In *P. allionii* the main limit on populations stability (the low number of seedlings related to an equal number of dead plants recorded every year) is likely to depend on the particular habitat where the plant grows. Like many other endemic species of Alps (*Moehringia lebrunii*, *Moehringia sedoides* and *Potentilla saxifraga*), *P. allionii* is adapted to high stress habitats, such as vertical or overhanging cliffs (Casazza et al., 2005) where competition with other species is reduced. The strict correlation shown between micro-niches and plant presence suggests that seedling survival is mainly dependent on the probability of finding suitable habitat. Other personal investigations demonstrated that *P. allionii* seeds have a dissemination mainly based on gravity (Minuto, personal data) and the quite good viability of seed is accomplished by a germination in special conditions (Minuto, personal data).

P. allionii is really a rare endemic species with a restricted distribution range but, at present, it does not appear under threat. However, because of the naturally fragmented habitat, the slow renovation dynamics and the weak pollination biology (Minuto, personal data) we may assume that the plant lives in a dangerous equilibrium for the conservation of all its populations. For this reasons it become necessary to deeply investigate the reproductive biology of the plants because it seems to be the more fragile aspect of its survival.

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