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REPRODUCTIVE EFFICIENCY OF *PRIMULA ALLIONII*: IS THIS ENDEMIC PLANT REALLY THREATENED?

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ABSTRACT

Primula allionii is a primrose endemic to Maritime Alps. The plant was studied in three different plots representing the different structures of cliffs where the species usually lives. The reproductive success of the plant was analyzed in order to evaluate its conservation status. Mean plant dimensions and distribution within the plots were evaluated. The species resulted low morph biased (0.13), very fertile (83.53%) and with a quite good sexual organs efficiency of flowers (both in pin and thrum morphs) as demonstrated by the high rate of fruit (52.18%) and seed production (20-50% ovules developed in seeds). However, the populations remain in a demographic balance (Δ % = 0.38) and very few seedlings (0.21% of available seeds) were recorded in populations. The particular habitat of the plant does not favour a consistent population renewal. *Primula allionii* populations are not under a real threat at present: but the species and its loci deserve much more consideration in the future in order to preserve this possibly fragile habitat.

KEY WORDS: *Primula allionii*, demography, reproductive biology, morph bias, threat.

INTRODUCTION

Biological rarity is not easy to define, and it has, at least, three components: the total number of individuals of the species concerned, its geographical distribution and its habitat specificity (Zalba & Nebbia, 1999). In general terms, it is plausible that a species that is rare and restricted to a small region is exposed to extinction because of its possibly reduced genetic variation and greater sensitivity to natural or anthropogenic factors that could condition its survival (Wissel & Stöcker, 1991). Nevertheless, for a species to be rare it does not necessarily means to face a real risk of extinction (Gaston, 1994). Other data are required in order to assess the degree of threat faced by a given species.

Reproductive limitations in small plant populations (i.e. seedling recruitment) could be related to some of the following causes: autoincompatibility; deficiencies in pollination or seed dispersal; failure of germination or seedling establishment; seed predation (Zalba & Nebbia, 1999; Buza et al., 2000; Juenger & Bergelson, 2000; Tomimatsu & Ohara, 2002; Severns, 2003). In one of these particular conditions, morph frequencies of heterostylous plants with strict intramorph incompatibility deviate more strongly from equality with decreasing population size (Brys et al., 2003).

In conclusion, because the breeding system determines the quantitative (demographic) and qualitative (genetic) traits of a species, characterization of the population and morph ratio can detect if rare species are truly endangered (Zalba & Nebbia, 1999) and provides invaluable information to suggest appropriate conservation measures (Gaudeul & Till-Bottraud, 2004).

The aim of this work was to assess if *P. allionii* Loisel. besides being rare should be considered a threatened species. Several field observations were performed at two sites in the Roya Valley and over 3 consecutive years (2008-2010). More specifically, the main questions addressed were: (1) how is the consistence and demography of populations? (2) are the sexual organs efficient? (3) is the species able to cope with this particular habitat? (4) is it the species in danger?

MATERIALS AND METHODS

Species

P. allionii Loisel. is a small perennial rosette plant showing a specific affinity with carbonatic cliffs, absent on any other types of habitats. It mainly lives in mesoMediterranean and supraMediterranean bioclimates (between 500 and 1200 m a.s.l.), but locally may reach the subalpine belt (up to 2000 m at the Bec d'Orel Massif). This plant is dominant in the *Primuletum allionii* vegetational association together with *Asplenium trichomanes* L., *Campanula macrorhiza* J.Gay ex A.DC., *Hypericum coris* L., *Potentilla saxifraga* Ardoino ex De Not. and some other rare and endemic species like *Moehringia sedoides* (Pers.) Cumino ex Loisel. (endemic to SW Alps).

The species range is limited to a small area of the Maritime Alps. Populations (52) were recorded in the middle Roya Valley in France (N = 16) and in the Gesso of Trinità (N = 27), Roaschia (N = 8) and Vallegrande Valleys (N = 1) in Italy. In general, the populations are divided in two main groups: the first living into the North in Italy, the latter into the South in France, and they are probably the remains of a previous wider area of extension reduced and separated by the ice sheet during last glaciations. In fact, *P. allionii* is supposed to be a pre-glacial relict belonging to an ancient subtropical mountain climate (Martini, 1994).

Plants bloom from January to April according to geographical position and in general flowering period lasts three months (data in elaboration). As for other taxa of the genus, *P. allionii* is heterostylous and each specimen may bear long styled (pin – P – Figure 1A) or short styled (thrum - T - Figure 1B) flowers.

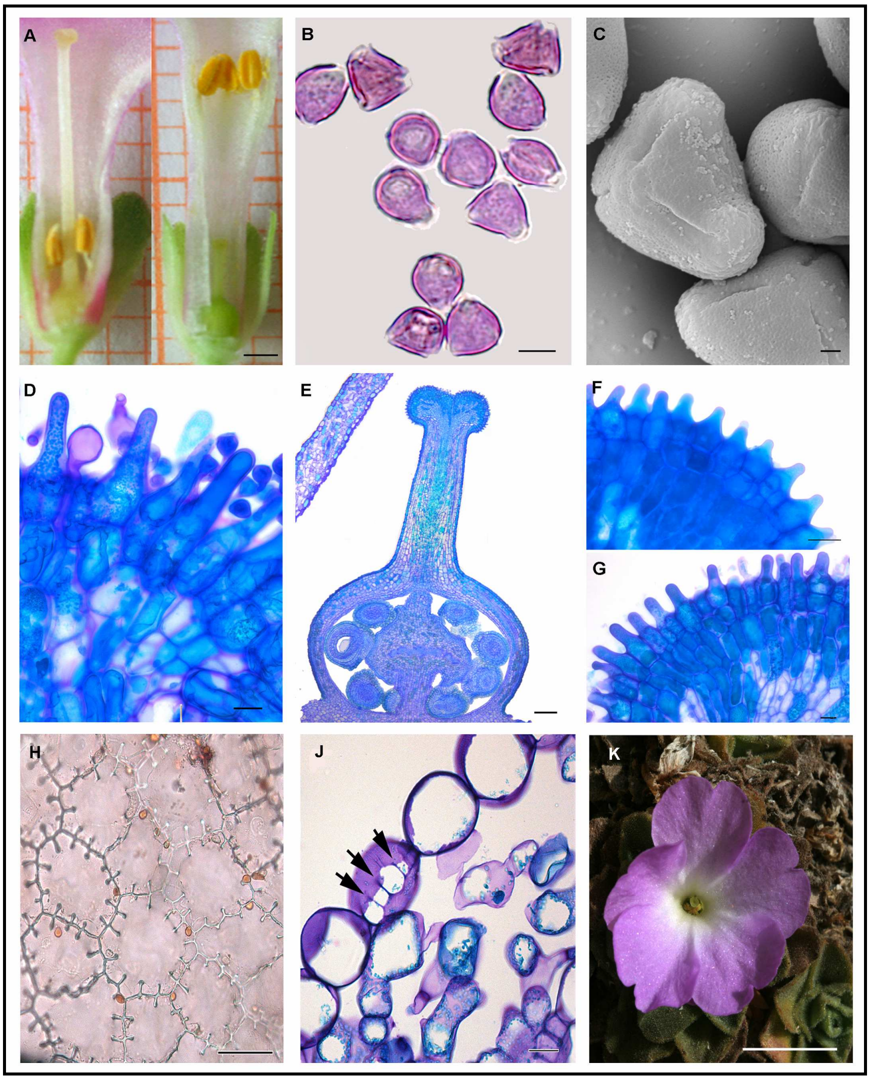

Fig. 1. **A**, mature flower in P and T morphs – bar = 1 mm; **B**, pollen grains, glycerol-gelatine and fuchsine staining – bar = 10 µm; C, SEM view of ellipsoidal 3(-4)-colpate pollen grains with small triangular polar area – bar = 1 µm; **D**, pollen grains germination on stigmatic papillae of hand-pollinated flowers, TBO staining – bar = 10 µm; **E**, longitudinal section of pistil in a mature flower at bloom, TBO staining – bar = 1 mm; **F**, longitudinal section of stigmatic papillae in T morph – bar = 10 µm; **G**, longitudinal section of

stigmatic papillae in P morph – bar = 10 μm; **H**, petal at optical microscope showing strengthened invaginations in epithelial cells - bar = 50 μm; **J**, petal longitudinal section at optical microscope showing strengthen columns in epithelial cells, TBO staining - bar = 20 μm; **K**, petal surface showing the "jewel-effect" in field - bar = 1cm.

Study sites and plots

Two natural populations from the southern group (Val Roya - France) were studied from November 2007 to November 2010 (three flowering seasons). The first population of 'Chiarin – Berghe Inferieur' [44°02'06"N 7°33'34"E; 1100 m a.s.l.; mean annual precipitation 1031.50 mm, mean annual temperature 11.5°C (http://junon.u-3mrs.fr/msc41www/poscli/B72.html), the second of 'Traya – Berghe Superieur' [44°01'44"N 7°34'07"E; 807 m a.s.l.; 1 km far from the previous]. They were selected for their easier accessibility during the flowering period, in winter.

The study was mainly conducted at the largest population of 'Chiarin – Berghe Superieur', harbouring thousand of individuals, where two representative plots (Pt1 – exp. S and Pt2 – exp. SW) were selected as the two typical population arrangements of *P. allionii*: the vertical and the overhanging cliffs respectively. A third plot (Pt3 – exp. NE) surrounding a cave was selected at 'Traya – Berghe Superieur'. Each plot was permanently marked with a plastic string delimiting the cliff at the mean height of 2 m from the ground and for a length of about 12 m. The three plots with a similar area (Pt1 – vertical cliff – 21.99 m^2; Pt2 – overhanging cliff – 24.52 m^2; Pt3 – cave margin – 23.28 m^2) harboured 120, 89 and 53 individuals, respectively.

Demography of populations

The plants were permanently listed, numbered and labelled on the cliff and then mapped in each plot. From November 2007 to November 2010 the demographic evolution of the plots was checked up and monitored by recording the eventual death of plant or the appearance of seedlings.

Flower description and sexual organs functionality

Anatomy of mature flowers was investigated in order to describe flower and sexual organs development. In March 2008 (at flowering peak) we collected 100 flowers (T = 50 and P = 50) from which anthers, pistils, petals and sepals were separately sampled. The samples were fixed in FAE (formalin-acetic acid-ethanol), alcohol dehydrated and embedded in 2-hydroxyethyl methacrylate resin (Technovit 7100, Heraeus Kulzer GmbH & Co.). From each flower organ longitudinal sections were cut at 8 μm intervals, mounted serially, and stained with toluidine blue (Sigma), pH 4.4 (O'Brien & McCully, 1981). Observations were accomplished with a Leica DM2000 optical microscope. Pollen grains (size of equatorial and polar axes) and stigmatic papillae dimensions (width, length and inter-papillar space) were observed and measured with Leica IM 4.0 software.

Pollen collected from dehiscent anthers and stigmata were sampled from 10 flowers belonging to P and T morphs, pollen grains and stigmatic papillae were analyzed as follow: a) fresh pollen grains were treated with glycerol-gelatine and fuchsine and observed with an optical microscope; b) fresh pollen samples (N = 100-150) and stigmata (N = 20), dehydrated or non dehydrated in ethanol series, were air-dried on SEM stubs, sputter-coated with gold, viewed with a Leo 1450 SEM at an acceleration voltage of 20 KV, and photographed with a Nikon Coolpix 5400 digital camera. We also verified the *in vivo* pollen germination on stigmata: open-pollinated flowers (N=50) from different individuals were sampled *in situ* and observed with a Leica DM2000 optical microscope and with a Leo 1450 SEM after pistils preparation as previously described for each technique.

Flower and fruit production

A complete survey of the plots was made each year and twice a month (from December to April) in order to verify the reproductive status of the plants. The sexual morph (P or T) of each specimen was detected during the flowering period, recording the number of flowers and fruits born. The correlation among individual dimension, flower and fruit production was calculated with Kendall Tau coefficient. The comparison among plots production was elaborated with ANOVA. Statistical analyses were performed with Statistica 8.0 (StatSoft, 2007). Morph/flower bias was calculated for each population as the difference between the total number of flowers produced by P an T morphs, respectively, and divided by the total number of flowers produced. It can vary from -1 (only T flowers present) to 1 (only P flowers present), with 0 indicating the equal frequency of both P and T flowers.

RESULTS

Demography of populations

The three plots harboured a different number of plants. In particular, individuals lived in holes and crevices and rarely formed clusters of individuals, distinguishable for their irregular shape and for the presence of flowers belonging to different morphs. The number of individuals seemed to decrease at increasing cliff steepness (Table 1).

	Cliff type	Area (m^2)	n	n/m^2	mA (cm^2)	Cov.%	dead	sdlg
Pt1	Vert.	21.99	120	5.46	33.46 (sd 35.26)	1.83	3	8
Pt2	Overh.	24.52	89	3.63	63.15 (sd 81.68)	2.29	8	9
Pt3	Cave	23.28	53	2.28	49.41 (sd 53.30)	1.13	9	2
mean		23.26	87.33	3.79	45.42	1.72	6.3	6.3

Table 1. Demographic data of the three study plots (Pt1-Pt3) and of the species (mean) during the whole study period. For each plot are reported: total surface in m^2 (area); number of specimens (n); plant density (n/ m^2); individual's mean area in cm^2 (mA); total coverage by all specimens (Cov.) in percentage; number of dead specimens recorded (dead); number of seedling observed (sdlg).

Each individual was formed by a clump of leafy rosettes originating from a unique rootstock. The typical size for a plant was about 7 x 8 cm (mean area 45.42 cm^2) and in general most of plants (88.28%) resulted small with a diameter lower than 10 cm. Few larger specimens (max. area 362.67 cm^2) were observed in the most protected and shadowy sites of the plot. Plant dimensions did not show any significant variation during the study period. Plant mean dimension varied from 33.46 (Pt1) and 63.15 (Pt1). Plant density varied from 2.28 (Pt3) and 5.46 (Pt1) individuals per square meter. The total plant coverage percentage in the plots varied from 1.13 (Pt3) and 2.29 (Pt2).

P. allionii showed a substantial demographic stability (Table 1) with a very low renewal of plant during the study period (Δ% = 0.38).

Flower description and sexual organs functionality

The androecium was fused with the perianth (Figure 1A) and consisted of a whorl of five stamens. Anthers measured 1.43 (sd 0.28) x 0.58 (sd 0.12) mm in P, while they resulted 1.60 (sd 0.36) x 0.65 (sd 0.12) mm in T.

Mature pollen grains (Figure 1B-C) were bi-nuclear, with a spermatic and a vegetative nucleus (Figure 1B arrows). Their shape was ellipsoidal, detecting a polar and a equatorial axis (always oblate), 3(-4)-colpate with small polar area. They had different dimensions in the two flower morphs: P grains [pol. 11.70 (sd 0.10) µm; eq. 15.60 (sd 0.20) µm] were bigger than T ones [pol. 16.70 (sd 0.10) µm; eq. 20.50 (sd 0.20) µm]. The exine [1.00 (sd 0.02) µm] rough in form was sculptured with reticulate ridges. Fractured pollen grains showed that short columella with thickened distal ends are connected to form the surface pattern. The nexine was irregular in thickness and shows a characteristic pattern of microperforations. Pollen grains were surrounded and hold together by pollenkitt. The germination test of pollen grains gave good results both *in vitro* [35.78 (sd 8.48) % in P; 60.45 (sd 8.42) % in T] and *in vivo* as observed in the histological sections (Figure 1D) and SEM images of hand-pollinated and open-pollinated flowers.

At anthesis the gynoecium was variable in length [8.25 (sd 1.16) mm in P; 3.17 (sd 0.56) mm in T] and it showed three functional parts (Figure 1E): a tricarpellar, unilocular glabrous ovary [∅ 1.53 (sd 0.24) mm] with a free-central placentation and many hemianatropous ovules [N = 30 (sd 8)], a style variable in length [0.60 (sd 0.13) mm in P; 0.45 (sd 0.11) mm in T] and a lobate stigma bearing numerous papillae [length 16.41 (sd 0.41) µm in T (Figure 1F); 21.03 (sd 0.40) µm in P (Figure 1G)].

Ovule and embryo degenerations were never observed. However, according to the gathered seed data, only 20-50% of ovules (calculated in 50 ovaries) were fecundated and developed in well formed seeds (percentage of seed-ovule ratio).

Anatomical sections of petals showed large and rounded epithelial cells refracting the light when the corolla is exposed to the sun (Fig 1K). These large epithelial cells were alternated with smaller ones. Their cell wall showed invaginations (Figure 1H) forming some (N = 5-10) strengthened column-like points (Figure 1J arrows).

Flower and fruit production

Overall flower and fruit productions of fertile plants (N= 213 corresponding to the 83.53%) during the three study years were reported in Table 2.

The plants flowered when they reached a diameter of 4-5 cm at least. The species (P= 57.28%, T= 42.72%, morph bias = 0.13) and the populations (Pt1: P= 52.73%, T= 47.27%, morph bias = 0.05; Pt2: P= 61.54%, T= 38.46%, morph bias = 0.23; Pt3: P= 57.89%, T= 42.11%, morph bias = 0.16) resulted constituted by a slightly greater number of individuals belonging to the P flower morph. The flower bias (0.206) and the fruit bias (0.209) were constant between P and T morphs.

The total productivity (Fr/Fl%) of *P. allionii* along the three years was estimated in 52.18% (Table 2) with weak variations among the plots and along the years. According to Kendall Tau coefficient plant dimensions had a low correlation with flower production (0.30 $p < 0.05$). On the contrary, a consistent relationship (0.82 $p < 0.05$) between the number of flowers and the number of ripe fruits per plant was present, as shown by the derived scattered plot (Figure 2).

			Fl	Fr	Fr/Fl	Pt	Y
2008	Pt1	P	65.63	69.43	51.42	48.61	50.78
		T	34.37	30.57	43.24		
	Pt2	P	53.33	57.89	49.11	45.24	
		T	46.67	42.11	40.82		
	Pt3	P	59.46	54.67	62.12	67.57	
		T	40.54	45.33	75.56		
2009	Pt1	P	60.54	61.38	52.51	51.79	50.98
		T	39.46	38.62	50.68		
	Pt2	P	54.87	55.79	49.53	48.72	
		T	45.13	44.21	47.73		
	Pt3	P	58.77	55.17	47.76	50.88	
		T	41.23	44.83	55.32		
2010	Pt1	P	64.17	62.64	47.19	48.33	53.93
		T	35.83	37.36	50.39		
	Pt2	P	58.82	59.44	53.13	52.57	
		T	41.18	40.56	51.79		
	Pt3	P	49.22	53.10	95.24	88.28	
		T	50.78	46.90	81.54		
TOT		P	60.31	60.48	52.33		**52.18**
		T	39.69	39.52	51.96		

Table 2. Flower (Fl) and fruit (Fr) production and reproductive success in percentage (Fr/Fl%) calculated on the total production (Fl and Fr) in the study plots (Pt1-Pt3), in the flower morphs (P and T) and in the species (TOT: P and T) during the study years (%Y) and in the whole period (bold).

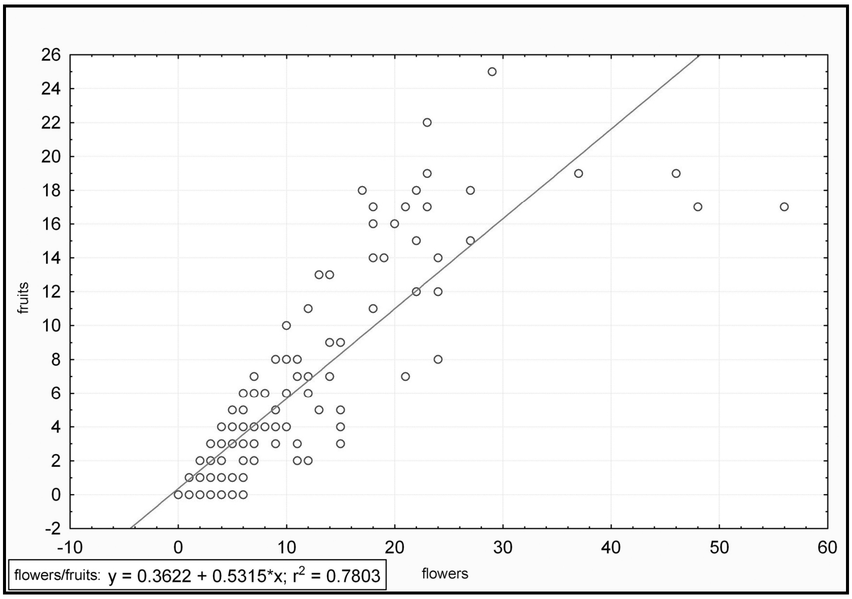

Fig. 2. Scattered plot derived from the comparison between flower bore by plants of *P. allionii* and the relative number of ripe fruits.

Reproductive efficiency data are reported in Table 3. *P. allionii* capsules contained on average 5.78 (sd 4.36) seeds [P= 5.35 (sd 3.77); T= 6.12 (sd 4.75)]. According to the mean fruits produced per year in the different plots (Table 3), a mean number of 650-1250 diaspores per plot per year was estimable. However, the real number of seedlings recorded was always very low (mean 0.21% of available seeds).

	2008				2009			
	Fr	seed	sdlg	%	Fr	seed	sdlg	%
Pt1	157	1140	3	0.26	290	2192	3	0.14
Pt2	95	528	4	0.76	95	657	3	0.46
Pt3	75	568	1	0.18	58	491	1	0.20
Tot	327	2031	8	0.39	443	3340	7	0.21
	2010				Total			
	Fr	seed	sdlg	%	Fr	seed	sdlg	%
Pt1	348	2428	2	0.08	795	5760	8	0.14
Pt2	143	603	2	0.33	333	1788	9	0.50
Pt3	113	757	0	0.00	246	1816	2	0.11
Tot	604	3788	4	0.11	1374	9159	19	**0.21**

Table 3. Reproductive efficiency observed in the three plots (Pt1-Pt3) and for the whole study (Tot): total productions of fruit (fruit) and seed (seed), seedlings (sdlg) and their percentage (%) related to the number of seed produced recorded in the three study seasons (2008-2009-2010).

DISCUSSION

Our results on the demography of populations showed a stability of the species despite the very low turnover of its specimens. The mean dimension of plants was in general quite small. As showed by Kendall Tau analysis, plant dimension had a low correlation with flower production and those specimens with a larger size did not show often a number of vegetative rosettes proportional to their global dimension. Some old plants showed many dead leafy rosettes in center of the clump and few number of living rosettes, generally along its margin. According to plants' dimension and growth rate recorded in *P. allionii*, longevity seems to be one of the main biological features of the species. No vegetative shoot proliferation was ever observed. Persistence by longevity has been rarely considered as an alternative to regeneration by seedling for plants showing multiple demographic strategies. Recently, a conceptual model of multiple demographic strategies for long-lived plants in stable habitats with high gradients of abiotic stress or interspecific competition was proposed for plants living on cliffs (Garcia & Zamora, 2003).

Among the three selected habitat types some differences were revealed in population structure: on vertical cliffs (Pt1) *P. allioni* seems to show a higher specimens density but a lower plant dimension average and a lower coverage percentage; on overhanging cliffs (Pt2) plant density decreases and mean dimension are larger so their coverage is sensibly higher. A different situation was described in the cave (Pt3) where the population density was very low as well as the coverage. On this base we can assume that vertical and overhanging cliffs are those habitats preferred by *P. allionii* as confirmed by the small number of dead plants and higher number of seedlings. However, the very low level of plant turnover observed in populations living on cliffs completely fits with the theory that regeneration by seedling would be mainly promoted under low abiotic stress or under low competition, whereas persistence by longevity and/or vegetative reproduction would predominate at high levels of abiotic stress or competition (Garcia & Zamora, 2003).

Even if microhabitat’s different levels of abiotic resources may act upon plant growth and dimensions and probably upon both pollen and seed production (Gaudeul & Till-Bottraud, 2004), the large number of fertile plants in the three study plots indicated no large differences among the populations.

The anatomic investigations on reproductive organs of flowers showed a good reproductive efficiency. Both male and female organs always showed a normal development and the final fruit and seed productivities demonstrated also an efficient reproductive biology.

In particular, pollen grain production was consistent in anthers with some difference in germination rate between the two flower morphs (two times higher in T). However, a substantial compensation might be supposed: P anthers contained a greater number of pollen grains than T ones due to their smaller dimensions. The final pollen availability acting in the breeding system might be almost equal between P and T plants.

The analysis of female flower organs in *P. allionii* confirmed its strict intramorph incompatibility typical of heterostylous plants. The correct combination of pollen/stigma between the two floral morphs is assured by the specific correlation between stigmatic papillae and pollen sizes of reciprocal floral morphs. The sectioned ovaries at different developmental stages showed always well formed ovules and embryos, even if only 20% of ovules was really fecundated and developed mature seeds. This limitation in seed development seems linked to a probably reduced pollen supply on stigmata by pollinators (data not shown).

This is in contrast with the particular arrangement of *P. allionii* petals that seem to be for a pollination improvement: the species is the only primrose species showing a special brightness of petal surface, defined as “jewel effect” by horticulturist (Richards, personal communication). Anatomical sections of petal highlighted an epidermal cell arrangements probably increasing light reflection but surely making petal more strength for a long time. The rounded adaxial cell walls of petal epithelial cells are probably responsible of the “jewel-effect” observed in the species (Richards, personal communication) and common in plants pollinated by bumblebees (Kay et al., 1981). This physical phenomenon makes the petal surface more shine and able to reflect the incident sun light (Dyer et al., 2007). On the contrary, the strengthened cell walls seem to be able to sustain big, heavy insects. These structures have never been described before in *Primula* but they are similar to those observed in *Petunia hybrida* (Zenoni et al., 2004) and comparable to the armed parenchyma of *Pinus*, whose cells are infolded with many cell wall invaginations (Wiebe & Al-Saadi, 1976).

P. allioni presented a large number of fertile plants (83.53%) and a consistent overall fruit production of 52.18% with weak variations among the plots (probably due to microhabitat features favouring pollen vectors presence) and along the years (weather's conditions during the flowering period). No correlation was evidenced between flower production and plant dimensions but fruit production of a plant was linked to the number of flowers borne each year. Those plants (even small) bearing many flower showed a high number of fruits in summer.

The lack of any consistent differences among productivities in the three study plots of *P. allionii* indicated that inter-morph pollen transfer by pollinators should be present and equal among populations in the whole distribution area, as already observed for the fertility of mainland and island populations of *P. mistassinica* (Larson & Barret, 1998).

On the other hands, population density were not good predictors of fertility within plots, suggesting that the cliff conformation (vertical, overhanging or cave) might influence local ecological factors resulting more important in governing reproductive success and population density. The best production was recorded in the plot with a vertical cliff and a higher sun exposition, probably enabling a better reproductive fitness. In these particular conditions, the presence of pollinators was more consistent being the flowering period in winter and early spring. These results, evidencing climate limitations, might be compared to those already reported for *P. veris* where a low habitat quality affected pollinator densities negatively (Brys et al., 2003).

The low biased morph frequency recorded was a further indication of the breeding system efficiency of *P. allionii*, in contrast to the results from other heterostylous species showing biased morph frequencies interacting with limited pollinator attraction in small populations (Byers, 1995; Kéry et al., 2002; Brys et al., 2003). For distylous and intramorph incompatible *Primula* species the probability of plants receiving suitable pollen increases when the different morphs are equally present in the population (Kéry et al., 2000). The equilibrium in the style-morph ratios consistently found in populations of *P. allionii* does not cast doubts on whether self-fertilization in *P. mistassinica* (Larson & Barret, 1998).

Because the breeding system determines both quantitatively (demographic) and qualitatively (genetic) crucial parameters, the characterization of the reproductive biology of endangered and locally distributed species provides invaluable information to understand if they are truly endangered (Zalba & Nebbia, 1999) and to suggest appropriate conservation measures (Gaudeul & Till-Bottraud, 2004). From this point of view *P. allioni* does not appear so much in threat.

Another parameter used by plant population biologists is population turnover. If a population has to survive over many generations, the offspring must replace the dying parent generation, so that population growth rates remain stable or positive (Oostermeijer, 2000). In general, the main problem of this event might be germination failure, seedling establishment or seed predation (Zalba & Nebbia, 1999).

In *P. allionii* the only potential limit on the stability of populations is the low number of seedlings recorded, which is likely to depend on high stress habitat where the plant lives. Its presence on cliff was probably induced in order to its low competitiveness against other plants: in any other rocky condition but not on vertical or overhanging cliffs, the species does not have any survival possibility for its very slow vegetative growth (personal data). *P. allionii* survival is mainly linked to plants longevity than to the fair probability that a seed of the thousands produced finds a small fissure or a hole on the cliff. Other investigations undertaken by us pointed out that the dissemination is mainly linked to gravity (unpublished data). Nevertheless, as a partial compensation, *P. allionii* shows a high percentage in germination (> 70% at 4-10°C – personal data), in contrast with data obtained on *P. veris* revealing a very low rate (0.01%) in seed viability (Brys et al., 2003; Jacquemyn & Brys, 2008).

Nowadays, *P. allionii* does not appear as a threatened plant even it lives in a specific habitat, naturally fragmented. Although its present demographic status seems quite strong, we can not exclude that future climate variations, in addition to slow renovation dynamics and habitat naturally fragmented, might change its reproductive biology and as a consequence its distribution range. Fragmented populations are often only insufficiently connected through seed/pollen dispersal and may form a non-equilibrium metapopulation with higher rates of local population extinction than of colonisation (Jacquemin et al., 2002; Lienert, 2004). Nevertheless, by taking in account the efficient pollination syndrome of *P. allionii* made by bumblebees (personal data) and the low morph bias recorded in the populations, the genetic exchange among the population seems to be assured despite seed dispersal might by difficult according to specific habitat. For this reason, the local population extinction cannot be considered a risk for the species.

CONCLUSIONS

The study highlights the usefulness of adopting several complementary approaches to understand the dynamic processes working in natural populations, and emphasized the conservative implications for *P. allionii*. Although the studied populations do not seem threatened in the near future, long-term monitoring appears necessary to assess the impact of future habitat modifications and to asses their genetic status.

Several plant species have evolved over long time ranges in naturally small, isolated populations and are well adapted to surviving under these special conditions. Several types of rarity are acknowledged in plant ecology: (1) rare species may be confined to a geographically narrow range, such as alpine plants, (2) they may occur only in a narrow and very specific habitat range, such as wetlands or (3) species may occur in small numbers and densities, which means that local population sizes are always small (Begon et al., 1990).

P. allionii can be classified into a combination of these categories. Whether a rare species also becomes endangered, depends on various attributes such as mode of reproduction, dispersal ability or habitat specificity and historical events acting upon them. Moreover, survival can also be largely dependent upon the structure of the intervening landscape or habitat. Like a rare orchid can quickly become extinct, when the habitat is increasingly destroyed (Lienert, 2004), *P. allionii* might be subdued to a similar event in the future.

Species conservation strategies must be oriented to reverse deterministic threats to maintain or increase population size as rapidly as possible, and they should include at least the following components:

– enlarge the adjacent natural reserves parks in order to protect not only the present range of the populations but also adjacent sites to allow recolonization;

– regulate the human activities in the cliff (climbing etc.);

– start *ex situ* conservation actions meanwhile assessing the genetic status of each population before proceeding with the aim of manipulating the extant population with releases from captivity.

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