

Genetic structure and conservation priorities for *Silene sennenii* (Caryophyllaceae), a narrow endemic and critically endangered species of the Iberian Peninsula

J. LÓPEZ-PUJOL¹, J. FONT², J. SIMON¹ & C. BLANCHE¹

¹GREB, Laboratori de Botànica, Facultat de Farmàcia, Universitat de Barcelona, Avda. Joan XXIII s/n, E-08028 Barcelona, Catalonia, Spain. *E-mail: jlopezpu@ub.edu

²Grup de Recerca de Flora i Vegetació, Departament de Ciències Ambientals, Facultat de Ciències, Universitat de Girona. Campus Montilivi s/n, E-17071 Girona, Catalonia, Spain.

Summary

Allozyme electrophoresis was used to evaluate levels and distribution of genetic diversity in the critically endangered (CR) *Silene sennenii* (Caryophyllaceae), a narrow endemic plant species located in north-eastern Catalonia (Spain). At present, it only remains in five populations containing no more than 5,000 individuals, and subjected to different human pressures such as habitat fragmentation. From the 21 satisfactorily interpreted loci, low levels of genetic variation were detected ($P = 20.9$, $A = 1.31$ and $H_e = 0.071$), which may be related to small population size and isolation of populations. Moderate to high levels of inbreeding were also found, probably as consequence of the population's genetic structuring (biparental inbreeding). Conservation policies should be focused on maintaining population sizes in addition to preserving its habitat.

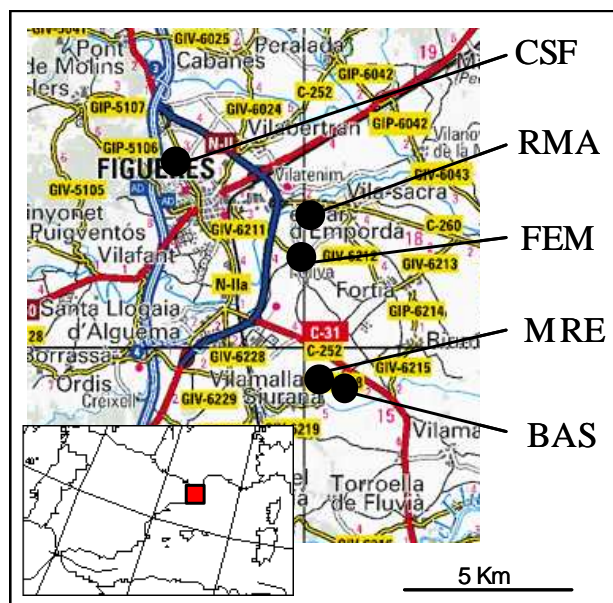
Introduction

Silene sennenii Pau (Caryophyllaceae) is a hemicryptophyte which annually produces abundant flower-bearing stems. Flowers are protandrous and arranged in dichasial inflorescences, with white or pink pale corolla. Petals withdraw during the hours of major sunstroke.

The flowering period, which takes place preferably between August and October, drives to a high production of fruits and seeds. Predation of flowers and fruits is low, although the observation of seedlings is difficult in the field.

However, germination shows high rates in experimental greenhouse conditions. It is a diploid species of $2n = 24$.

S. sennenii is a narrow endemic species geographically restricted to the Empordà plain (Catalonia, NE Iberian Peninsula), with its populations located below 100 m a.s.l.



It grows in dry perennial grasslands (Phoenician torgrass swards) installed on deep soils. In most cases this community is associated to margins of fields or slopes and, exceptionally, to old fields.

At present, only five populations containing no more than 5,000 individuals remain. They are highly fragmented, occupying together less than 0.1 km². Threats to this taxon are mainly anthropogenic (conversion of its habitat into irrigated croplands and expansion of urban and industrial areas). *S. sennenii* seems to be very sensitive to nitrogenous compounds in the soil since it is completely absent in the ruderal vegetation nearby. This species is listed as 'critically endangered' (CR) both in a study of threatened flora in Catalonia (SÁEZ, *et al*, 1998) and in the Red List of Vascular Spanish Flora (AIZPURU, *et al*, 2000).

Allozyme electrophoresis has been used to evaluate the levels of genetic diversity and its distribution within and among the five populations of *S. sennenii*. In addition, the conservation efforts are evaluated, and some strategies for its preservation both *in situ* and *ex situ* are suggested.

Material and methods

Young leaves were collected by a linear transect within populations, avoiding sampling ramets from the same genet. Enzymes were extracted with a cold buffer consisting of 0.05 M tris-citric acid, 0.1% cysteine-HCl, 0.1% ascorbic acid, 8% PVP-40, and 1mM 2-mercaptoethanol. Using horizontal 12.5% starch gels, 26 enzymes were tested, 11 of them were satisfactorily resolved (AAT, EC 2.6.1.1; ACO, EC 4.2.1.3; ACP, EC 3.1.3.2; ADH, EC 1.1.1.1; DIA, EC 1.6.99.-; IDH, EC 1.1.1.42; MDH, EC 1.1.1.37; 6PGD, EC 1.1.1.44; PGI, EC 5.3.1.9; PGM, EC 5.4.2.2; and PRX, EC 1.11.1.7) in four buffer systems (Tris-citrate/Lithium-borate pH 8.2, Tris-citrate pH 7.0, Morpholine-citrate pH 6.1, and Histidine-citrate pH 5.7), obtaining 21 interpretable loci: *Aat*, *Aco-1*, *Aco-2*, *Acp-1*, *Adh*, *Dia-1*, *Dia-2*, *Idh*, *Mdh-1*, *Mdh-2*, *6Pgd-1*, *6Pgd-2*, *Pgi-2*, *Pgm-1*, *Pgm-2*, *Prx-1*, *Prx-2*, *Prx-3*, *Prx-4*, *Prx-5*, and *Prx-6*.

Loci were numbered consecutively and alleles at each locus were labelled alphabetically, beginning from the most anodal form in both cases. The following statistics were computed: *P*, the percentage of polymorphic loci (0.95 criterion); *A*, the mean number of alleles per locus; *A_p*, the mean number of alleles per polymorphic locus; *H_o*, the observed heterozygosity; and *H_e*, the expected panmictic heterozygosity. A chi-square test (χ^2) was used to evaluate deviations of the *F* (fixation index) values of polymorphic loci from Hardy-Weinberg equilibrium. Population structure was analysed using Wright's *F*-statistics (*F_S*, *F_{ST}*, and *F_{IT}*), and gene flow was determined using Wright's equation: $Nm = (1 - F_{ST})/4 F_{ST}$. Nei's genetic identity (*I*) between pairs of populations was used to cluster those into a dendrogram following UPGMA. Finally, Mantel's test was conducted between genetic differentiation and geographical distances among pairs of populations. The software used was BIOSYS-1 and NTSYS.

Results and discussion

Genetic diversity

Among the 21 interpretable loci, we detected only 30 alleles. The two largest populations (BAS and CSF) displayed 30 alleles, whereas RMA and FEM 27 and 26 alleles, respectively, and the smallest one (MRE) harboured just 25 alleles. There is a positive correlation between the total number of alleles and population size ($r = 0.81$, $p = 0.097$), which may indicate that genetic drift could have been acting within populations, since the negative effects of drift are higher in small populations than in big ones. Genetic drift has probably removed rare alleles from the smallest populations (FEM and MRE; see Table 1). None of the populations harbour exclusive alleles.

Levels of genetic variation in *Silene sennenii* are low ($P = 20.9\%$, $A = 1.31$ and $H_e = 0.071$; see Table 1), as expected for endemic species ($P = 26.3\%$, $A = 1.39$ and $H_e = 0.063$; Hamrick & Godt, 1990). Isolation and fragmentation of populations, coupled with the high habitat specificity of this taxon, may explain these figures.

Table 1. Summary of genetic variation for 21 loci in the five populations of *Silene sennenii*. Rare alleles are those in frequencies < 0.05.

| Population | Population size | Sample size | Rare alleles | <i>P</i> | <i>A</i> | <i>H_o</i> | <i>H_e</i> |
|--------------------|-----------------|-------------|--------------|----------|----------|----------------------|----------------------|
| BAS | 1,084 | 70 | 1 | 28.6 | 1.43 | 0.050 | 0.081 |
| CSF | 3,209 | 70 | 4 | 23.8 | 1.43 | 0.052 | 0.074 |
| FEM | 49 | 24 | 1 | 19.0 | 1.24 | 0.055 | 0.050 |
| MRE | 4 | 4 | 0 | 19.0 | 1.19 | 0.060 | 0.109 |
| RMA | 172 | 32 | 3 | 14.3 | 1.28 | 0.030 | 0.041 |
| Mean | — | — | — | 20.9 | 1.31 | 0.049 | 0.071 |
| Standard deviation | — | — | — | 5.4 | 0.11 | 0.011 | 0.027 |

The significant deficiency of heterozygotes showed by the chi-square test [from 28 valid tests, 17 *F* values conformed to Hardy-Weinberg proportions ($p \geq 0.05$), while the remaining 11 were significantly different than zero ($p < 0.05$) and positive, indicating deficiency of heterozygotes] and the value of Wright's inbreeding coefficient ($F_S = 0.253$) can be explained by genetic substructuring of populations (Wahlund effect) achieved by biparental inbreeding. Most populations are composed by small units which contain only a few individuals. This structure may be caused by a limited pollinator activity or a restricted seed dispersal, or produced (or enhanced) by the current habitat fragmentation.

The value of genetic divergence among populations found ($F_{ST} = 0.271$) is above that obtained for several taxa subjected to fragmentation (about 10-20%). This, in addition to the inferred low value of gene flow ($Nm = 0.67$), might be interpreted as fragmentation has affected to populations. The significant heterozygosity deficit showed by the *AT* value (0.253) also gives support to a genetic substructuring of populations, probably enhanced by the fragmentation of populations.

There is a lack of significant correlation between geographic and genetic differentiation of populations (Mantel's test: $r = -0.078$, one-tailed $p = 0.505$), which shows that genetic differentiation has not occurred under the isolation-by-distance model. For instance, the closest genetic populations (CSF and BAS) are the most geographically distant (Fig. 1).

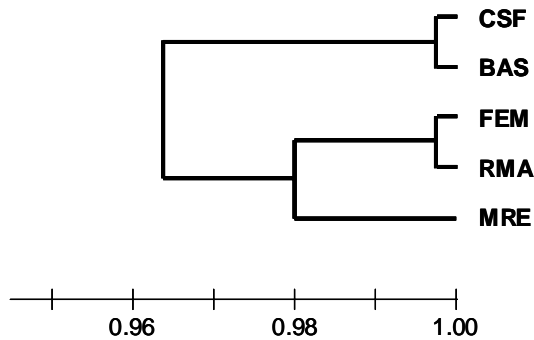


Fig. 1. Nei's genetic identity (*I*) among populations (UPGMA dendrogram)

Episodes of extinction by direct destruction of entire populations, in addition to bottlenecks derived from decrease in population sizes by habitat fragmentation, draw a more complex pattern of population differentiation such as found here. Foundation of new populations (e.g. CSF is located in the moat of Sant Ferran fortress, built in XVIII century onto an artificial hill) may also contribute to the pattern found.

Conservation

Currently there are no specific measures to protect *S. sennenii*. The extant populations are not included in any protected area, and its habitat (Phoenician torgrass swards; 6210), although listed in the Habitats Directive, is not considered priority (and therefore not included in Natura 2000 network). Given the extremely reduced occupation area of the populations, we recommend the creation of botanical microreserves for selected populations, because it is a figure directly focused to the management of the threatened species. Following the formula proposed by CESKA, *et al* (1997), the conservation of two populations may assure the preservation of 93% of the genetic diversity. Therefore, the selected populations should be the largest ones, i.e. CSF and BAS. The protection of a third population (RMA) would assure the conservation of 98% of genetic variation. A botanical microreserve should include these three populations.

Concerning *ex situ* protection measures, sampling for a germplasm bank should consist of about 50 individuals from the three same selected populations. Obtaining more information on breeding systems, pollination ecology, and the survival rates of seedlings, is essential to carry out an effective *ex situ* protection. The number of seeds per individual will depend on that data. Moreover, this species should be included in the Catálogo Nacional de Especies Amenazadas (State-level), or, alternatively, in the Autonomous-level legislation. Since in Catalonia there is not a catalogue of endangered flora, we suggest its urgent compilation.

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