RESULTATS I DISCUSSIÓ

3. Resultats i discussió

3.1. Compendi de publicacions

3.1.1. **López-Pujol J**, Bosch M, Simon J & Blanché C. 2001. Allozyme diversity of the two endemic *Petrocoptis: P. montsicciana* and its close related *P. pardoi* (Caryophyllaceae). *Canadian Journal of Botany*, 79: 1379-1389.

3.1.2. **López-Pujol J**, Bosch M, Simon, J & Blanché C. 2002. Allozyme variation and population structure of the very narrow endemic *Seseli farrenyi* (Apiaceae). *Botanical Journal of the Linnean Society*, 138: 305-314.

3.1.3. **López-Pujol J**, Bosch M, Simon J & Blanché C. 2003. Population genetics and conservation priorities for the critically endangered island endemic *Delphinium pentagynum* subsp. *formenteranum* (Ranunculaceae). *Biodiversity and Conservation*, 12: 1937-1951.

3.1.4. **López-Pujol J**, Orellana MR, Bosch M, Simon J & Blanché C. 2003. Effects of habitat fragmentation on allozyme diversity and conservation status of the coastal sand dunes plant *Stachys maritima* (Lamiaceace) in the Iberian Peninsula. *Plant Biology*, 5: 504-512.

3.1.5. **López-Pujol J**, Bosch M, Simon J & Blanché C. 2004. Allozyme diversity in the tetraploid endemic *Thymus loscosii* (Lamiaceae). *Annals of Botany*, 93: 323-332.

3.1.6. **López-Pujol J**, Font J, Simon J & Blanché C. 2004. Genetic structure and conservation priorities for *Silene sennenii* (Caryophyllaceae), a narrow endemic and critically endangered species of the Iberian Peninsula. *Planta Europa Conference IV Proceedings*. Disponible a: http://www.nerium.net/plantaeuropa/Download/Procedings/Lopez_et_al.pdf

3.1.7. **López-Pujol J**, Álvarez N, Simon J & Blanché C. Allozyme variation and taxonomical implications of the endemic rocky plant *Erodium rupestre* (Geraniaceae). *Botanical Journal of Linnean Society*, en revisió.

3.1.8. **López-Pujol J**, Orellana MR, Bosch M, Simon J & Blanché C. Low genetic diversity in the tetraploid Pyrenean endemic larkspur *Delphinium montanum* (Ranunculaceae). Allozymic evidences for autopolyploidy and further diploidization. *International Journal of Plant Sciences*, en revisió.

3.1.1. **López-Pujol J**, Bosch M, Simon J & Blanché C. 2001. Allozyme diversity of the two endemic *Petrocoptis: P. montsicciana* and its close related *P. pardoi* (Caryophyllaceae). *Canadian Journal of Botany*, 79: 1379-1389.

Allozyme diversity of two endemic *Petrocoptis* species: *P. montsicciana* and its close relative *P. pardoi* (Caryophyllaceae)

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Abstract: Starch gel electrophoresis was used to evaluate the allozyme diversity, population structure, and taxonomic relationships of two closely related taxa endemic to the Iberian Peninsula: *Petrocoptis montsicciana* O. Bolòs & Rivas Martínez, restricted to the pre-Pyrenean belt of Catalonia and Aragon, and *Petrocoptis pardoi* Pau, endemic to the Bergantes River basin (Autonomous Community of Valencia). Seven populations of the two species were sampled, and 16 interpretable loci were found. Considerable polymorphism, more than expected in endemic species, was detected in both taxa. The percentage of polymorphic loci (P) when the most common allele had a frequency of <0.95, the mean number of alleles per locus (A), and the expected panmictic heterozygosity (H_e) were slightly higher in *P. montsicciana* (P = 70.3, A = 2.2 and H_e = 0.239) than in *P. pardoi* (P = 56.3, A = 1.9 and H_e = 0.192). Strong divergence between populations was found in both species (G_{ST} = 0.376 in *P. montsicciana* and G_{ST} = 0.354 in *P. pardoi*) because of the geographic isolation of populations and limited seed and pollen dispersal. Most loci showed deviations from the Hardy–Weinberg equilibrium, probably as a consequence of genetic substructuring of populations. Low germination and renewal rates, and some anthropogenic activities such as road work, climbing, and massive collection are the main threats to these species.

Key words: *Petrocoptis montsicciana*, *Petrocoptis pardoi*, Caryophyllaceae, allozyme electrophoresis, genetic diversity, conservation, endemic species.

Résumé : Nous avons utilisé l'électrophorèse sur gels d'amidon pour évaluer la diversité allozymique, la structure des populations et les rapports taxonomiques de deux taxons étroitement apparentés endémiques de la péninsule ibérique: le *Petrocoptis montsicciana* O. Bolòs & Rivas Martínez, limité à la ceinture pré-pyrénéenne de la Catalogne et l'Aragon, et le *Petrocoptis pardoi* Pau, endémique du bassin de la rivière Bergantes (Pays Valencien). Sept populations des deux espèces ont été échantillonnées et 16 loci interprétables ont été décelés. Un polymorphisme plus grand que celui attendu chez une espèce endémique a été trouvé dans les deux taxons. Le pourcentage de loci polymorphiques (P) lorsque l'allèle le plus commun a une fréquence < 0,95, le nombre moyen d'allèles par locus (A), et l'hétérozygocité panmictic attendue (H_e) sont un peu plus élevé chez le *P. montsicciana* (P = 70,3, A = 2,2 et H_e = 0,239) que chez le *P. pardoi* (P = 56,3, A = 1,9 and H_e = 0,192). Une forte divergence entre les populations a été trouvée chez les deux espèces (G_{ST} = 0,376 chez *P. montsicciana* et G_{ST} = 0,354 chez le *P. pardoi*), à cause de l'isolement géographique des populations et la limitation dans la dispersion des graines et du pollen. La plupart des loci montraient des écarts de l'équilibre de Hardy–Weinberg, probablement comme une conséquence de la sous-structuration génétique des populations. Les faibles taux de germination et de renouvellement, ainsi que des activités anthropiques, telles que les travaux routiers, l'escalade et la récolte massive sont les menaces principales pour ces espèces.

Mots clés : *Petrocoptis montsicciana*, *Petrocoptis pardoi*, Caryophyllaceae, électrophorèse d'alloenzymes, diversité génétique, conservation, espèces endémiques.

Introduction

In recent surveys of the genetic diversity of plant species using allozyme analysis, questions of evolution, population structure, taxonomic relationships, or conservation biology have been addressed (Hamrick and Godt 1996; Crawford 2000; Hogbin et al. 2000). Although some attempts to summarize the existing information on endemic species (Hamrick and Godt 1990, 1996; Gitzendanner and Soltis 2000) provide a consistent general background on allozyme diversity patterns, there is growing evidence that the many particular cases reported recently (Young and Brown 1996; Williamson and Werth 1999; Edwards and Sharitz 2000, among many others) suggest a far more complex picture. The richness of biodiversity demands further research into endemic species to develop models.

Mountain habitats in the Mediterranean region provide a number of clusters of related species or species groups with common patterns of geographic migration and evolutionary

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history resulting in similar patterns of phenotypic diversity. Particularly, cytobiogeographic approaches in recent decades (Küpfer 1974, 1981; Favarger 1974; Galland 1988, and references therein) developed models based on chromosome races as ploidy-multilevel complexes that generally fit with bioclimatic history and distribution areas of mountain endemic species (Küpfer 1981). A relatively common case is that of the vicariant species, restricted to narrow gorges or vertical walls, which have followed a generally north–south pattern of migration as a result of glaciation (Takhtajan 1986). They are a good model because of the mainly linear distribution of their populations along river valleys or mountain ranges and the distribution of individuals within populations that form a line along vertical walls. Their genetic and geographic (unidirectional) distances can be easily compared and their founder effects along migration paths can be measured. The allozyme diversity of some chasmophytes (rocky fissure plants) belonging to the genus *Antirrhinum* has been studied recently (Mateu-Andrés 1999; Torres 1999) and research into orophyte (mountain) endemic species of *Erodium* is currently in progress (J.M. Iriondo, personal communication; J. Simon, unpublished data).

Petrocoptis A. Braun ex Endl., traditionally regarded as a small genus restricted to the calcareous mountains of the northern Iberian Peninsula, comprises a set of perennial chasmophytic species, with narrow ranges and specializing in colonization of vertical rock walls and limestone caves (Montserrat and Fernández-Casas 1990). The main distinctive character of the genus is the conspicuous bearded hilum of the seeds, which is involved in seed dispersal and establishment (Montserrat and Fernández-Casas 1990; Mayol and Rosselló 1999).

Systematic reviews of this genus differ in the number of taxa recognized, ranging from 4 to 11 units. *Petrocoptis pardoi* Pau was originally described in 1898 (Pau 1898), and *Petrocoptis montsicciana* O. Bolòs & Rivas Martínez, in 1970 (Bolòs and Rivas-Martínez 1970). In subsequent treatments, *P. montsicciana* has been included as a subspecies of *Petrocoptis crassifolia* Rouy (Bolòs and Vigo 1974) and of *P. pardoi* (Montserrat 1976*a*; 1976*b*), but in 1990, it regained the status of species (Montserrat and Fernández-Casas 1990). The most recent taxonomic treatment by Mayol and Rosselló (1999) classified *Petrocoptis* as a subgenus of *Silene*, consisting of only four species. *Petrocoptis montsicciana* and *P. pardoi* were considered synonymous, under the name *Silene pardoi* (Pau) Mayol & Rosselló.

Both taxa have narrow geographic ranges and few individuals. *Petrocoptis montsicciana* is limited to the pre-Pyrenean area of Catalonia and Aragon, with an estimated population of around 11 000 individuals and extent of occurrence (IUCN 1994) of ca. 900 km^2. *Petrocoptis pardoi* is the southernmost species of the genus, located in the Bergantes River basin (Autonomous Community of Valencia and Aragon), with a similar number of individuals (around 10 000) but a very small geographic range (extent of occurrence: 27 km^2) (López-Pujol 2000). Both species are considered conservation priorities at regional, national, and European levels. *Petrocoptis montsicciana* is listed as "vulnerable" by Sáez et al. (1998) and Sainz et al. (1996) and is on the *Lista Roja de la Flora Vascular Española* (*Red List of Vascular Spanish Flora*) (Aizpuru et al. 2000). *Petrocoptis pardoi* is also listed as vulnerable both by Sainz et al. (1996) and Aizpuru et al. (2000). In addition, *P. montsicciana* is listed in Annex 2 of the "Habitats" directive of the European Union (EEC 92/43; Official Journal of the European Communities (OJEC) 1992) and in Annex 1 of the revision of the Bern Convention held in Stuttgart in 1992 (Council of Europe 1998).

The analysis of allozyme variation in closely related species enables inferences to be drawn about their phylogenetic relationships (Purdy et al. 1994; Runyeon and Prentice 1997; Kang and Chung 2000; Edwards and Sharitz 2000) and gives some insight into their management needs (Hamrick and Godt 1996). These taxa should have the same management needs, especially if they have similar habitats. *Petrocoptis montsicciana* and *P. pardoi* are closely related species that we can interpret either as a single taxon (Mayol and Rosselló 1999) or a progenitor–derivative species pair, with high genetic similarity (Gottlieb 1973; Crawford 1983).

Here we use allozyme electrophoresis to address the following questions: (*i*) what are the levels and distribution of genetic diversity in *P. montsicciana* and *P. pardoi*; (*ii*) what is the population structure of these chasmophytic plants; and (*iii*) what is the relationship between *P. montsicciana* and *P. pardoi* on the basis of their allozyme variation? We also make some comments on the conservation status of this species and suggest some strategies for its preservation.

Materials and methods

Plant material

Petrocoptis montsicciana and *P. pardoi* are small herbaceous perennials, morphologically indistinguishable, 10–40 cm tall with pink-purple flowers. Both species are $2n = 24$ diploid (Merxmüller and Grau 1968; Fernández-Casas and Ruiz-Rejón 1974; Fernández-Benito 1999). *Petrocoptis montsicciana* is outcrossed and pollinated mainly by long-tongued bees of the genus *Anthophora* (Hymenoptera) but also by some Diptera, such as *Bombylius* sp., and the Lepidoptera, *Macroglossum stellatarum*. Although the species is self-compatible, self-pollination rarely occurs because of protandry (M. Bosch, J. López-Pujol, J. Simon, and C. Blanché, unpublished data). Data on pollination of *P. pardoi* are very scarce in the literature; Sainz et al. (1996) reported *Apis mellifera* as the main pollinator. There are no data available on breeding systems. These species grow on limestone walls and caves at 250–1200 m altitude (Romo 1989; Sainz et al. 1996).

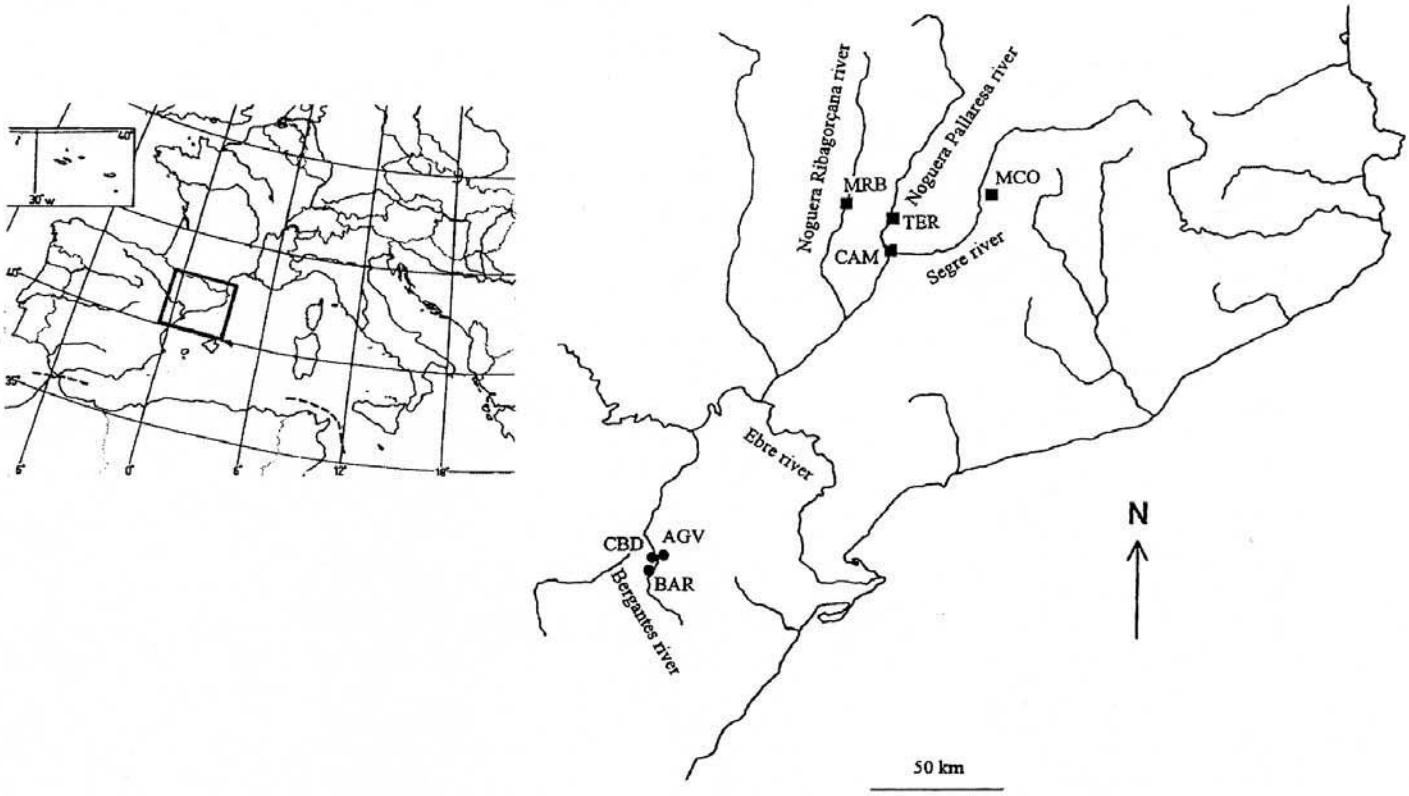
Sampling strategy

Genetic diversity was assessed through standard starch gel electrophoresis of allozymes (Soltis et al. 1983; Wendel and Weeden 1989). The sampled populations were selected using criteria of overall geographic range and accessibility (Table 1, Fig. 1). The populations of *P. montsicciana* at the Camarasa (CAM) and Terradets gorges (TER) were sampled from February to March 1999, whereas the other two populations, at Mont-Rebei pass (MRB) and La Móra Comdal rocks (MCO), were sampled from February to March 2000. The three populations of *P. pardoi*, at Aiguaviva (AGV), Cantal Badat (CBD), and Barranc de la Mare de Déu rocks (BAR), were sampled in February 2000. Sampling was mainly linear, since these species grow in vertical rocky walls, and comprised the range of populations when it was entirely accessible. Samples were collected about 50–100 cm apart, and consisted of

Table 1. Populations of *Petrocoptis montsicciana* and *Petrocoptis pardoi* studied.

Population code	Location (latitude, longitude)	Population size	Sample size
Petrocoptis montsicciana			
CAM	Camarasa gorges (41°53′N, 00°53′E)	>3200	31
MCO	La Móra Comdal rocks (42°07′N, 01°21′E)	~220	42
MRB	Mont-Rebei Pass (42°04′N, 00°40′E)	>2000	46
TER	Terradets gorges (42°02′N, 00°53′E)	~1750	21
Petrocoptis pardoi			
AGV	Aiguaviva rocks (40°47′N, 00°09′W)	~80	22
BAR	Barranc de la Mare de Déu rocks (40°44′N, 00°11′W)	~5000	30
CBD	Cantal Badat rocks (40°46′N, 00°10′W)	~600	31

Fig. 1. Sampled populations of *Petrocoptis montsicciana* (■) and *Petrocoptis pardoi* (●). CAM, Camarasa gorges; MCO, La Móra Comdal rocks; MRB, Mont-Rebei pass; TER, Terradets gorges; AGV, Aiguaviva rocks; BAR, Barranc de la Mare de Déu rocks; CBD, Cantal Badat rocks.



young leaves from hanging stems, which were placed into envelopes, transported to the laboratory, and stored at 4°C until extraction. Leaf samples were collected carefully to minimize the potential damage to populations.

Electrophoresis

Leaf fragments were homogenized in refrigerated porcelain plates using a cold extraction buffer consisting of 0.05 M Tris-citric acid, 0.1% cysteine-HCl, 0.1% ascorbic acid, 8% PVP-40, and 1 mM 2-mercaptoethanol. Extracts were absorbed onto 3 mm Whatman filter paper, either to be analyzed immediately or stored at –20°C until analysis 1 or 2 days later.

Using 12.5% starch gels, 21 enzymes were assayed, 10 of which were resolved in three buffer systems, obtaining 16 loci (*Aat*, *Aco-1*, *Aco-2*, *Adh*, *Dia-2*, *Dia-3*, *Mdh-1*, *Mdh-4*, *Me*, *6Pgd-1*, *6Pgd-2*, *Pgi-2*, *Pgm-1*, *Pgm-2*, *Prx-1*, and *Prx-2*). *Dia-1*, *Mdh-2*, *Mdh-3*, and *Pgi-1*, although variable, were not interpretable. Aspartate aminotransferase (AAT, EC 2.6.1.1), alcohol dehydrogenase (ADH, EC 1.1.1.1), and diaphorase (DIA, EC 1.6.99.–) were satisfactorily resolved on Tris-citrate – lithium-borate buffer (pH 8.2) (Scandalios 1969); aconitate hydratase (ACO, EC 4.2.1.3), malic enzyme (ME, EC 1.1.1.40), phosphoglucoisomerase (PGI, EC 5.3.1.9), and peroxidase (PRX, EC 1.11.1.7) were resolved on Tris-citrate buffer (pH 7.0); malate dehydrogenase (MDH, EC 1.1.1.37), phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44), and phosphoglucomutase (PGM, EC 5.4.2.2) were resolved on histidine–citrate buffer (pH 5.7) (Jeffries and Gottlieb 1982). Staining procedures for all enzymes followed the method described by Vallejos (1983), Shields et al. (1983), and Wendel and Weeden (1989), with small modifications (see Bosch 1999).

Genetic analyses

Loci were numbered consecutively, and alleles at each locus were labeled alphabetically, beginning from the most anodal form in both cases. Isozyme phenotypes were interpreted genetically ac-

cording to standard principles (Wendel and Weeden 1989). To calculate the levels of genetic diversity, the following statistics were computed: P, the percentage of polymorphic loci when the most common allele had a frequency of <0.95; 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. We also computed the mean fixation index, F, to compare genotype proportions with those expected under the Hardy–Weinberg equilibrium. The partitioning of genetic diversity within and between populations was analyzed using Nei's (1973) gene diversity statistics: total genetic diversity (H_T), genetic diversity within populations (H_S), genetic diversity between populations (D_{ST}), and proportion of total genetic diversity between populations (G_{ST}) were calculated for all populations. Gene flow (Nm) was determined using Wright's (1951) equation: $Nm = 1 - G_{ST}/4G_{ST}$. We also calculated Nei's (1972) genetic distance (D) and Nei's (1978) genetic identity (I). Using UPGMA (unweighted pair-group method with averaging), populations were clustered into a dendrogram on the basis of their pairwise values for I. The BIOSYS-1 version 1.7 software was used for most calculations and for dendrogram construction (Swofford and Selander 1989). Nei's (1973) H_T, H_S, D_{ST}, and G_{ST} diversity statistics were calculated using GeneStat version 3.31 (Whitkus 1988).

Results

Allelic richness and levels of genetic diversity

Among the 16 interpretable loci, we detected 53 alleles (49 in *P. montsicciana* and 39 in *P. pardoi*) from the two species (Table 2), 35 of which were common to both taxa. Fourteen alleles were unique to *P. montsicciana*, whereas only four were unique to *P. pardoi*. The richest population, with 39 alleles, was MCO, belonging to *P. montsicciana*; and the poorest populations, with 29 alleles, were both TER and BAR, which belonged to *P. montsicciana* and *P. pardoi*, respectively. Private alleles (found in only one population of one species) were found in four populations: *Aat-a*, *Aat-b*, *Adh-b*, and *Mdh-4c* in the CAM population; *Dia-2a*, *6Pgd-1d*, and *6Pgd-1e* at MRB; *Dia-3a* at BAR; and *6Pgd-2d* at AGV. Rare alleles (alleles with frequencies less than 0.05) were found in all populations of both taxa, totaling 17 alleles in *P. montsicciana* and 7 alleles in *P. pardoi*.

If loci are considered polymorphic when they have more than one allele regardless of frequency, then all loci in *P. montsicciana* were polymorphic. *Dia-2*, *Dia-3*, *Me*, *6Pgd-1*, *Pgm-1*, *Pgm-2*, *Prx-1*, and *Prx-2* were polymorphic in all populations, and *Adh* was monomorphic within each population. In *P. pardoi*, three loci (*Aat*, *Adh*, and *Pgi-2*) were monomorphic. The *Adh* locus showed the same allele in all populations studied (*Adh-a*) except CAM, which showed the *Adh-b* allele.

Petrocoptis montsicciana showed higher levels of genetic diversity than *P. pardoi* (Table 3): polymorphic loci (P) reached 70.3% in *P. montsicciana* and 56.3% in *P. pardoi*, and the number of alleles per locus and the number of alleles per polymorphic locus were higher in *P. montsicciana* (A = 2.2 ± 0.29 (mean ± SD) and A_p = 2.6 ± 0.33) than in *P. pardoi* (A = 1.9 ± 0.15 and A_p = 2.3 ± 0.11). Observed heterozygosity was lower than expected panmictic heterozygosity in both *P. montsicciana* (H_o = 0.121 ± 0.020 and H_e = 0.239 ± 0.038) and *P. pardoi* (H_o = 0.072 ± 0.006 and H_e = 0.192 ± 0.015). Taking both species together, the most variable population was MCO (H_e = 0.291, SE = 0.046), while the population with the least diversity was BAR (H_e = 0.175, SE = 0.047).

We used a χ^2 test to evaluate deviations of F from zero (Table 4). Nineteen of the 80 tests indicated Hardy–Weinberg proportions ($p \geq 0.05$), whereas 61 F values were significantly greater than zero ($p < 0.05$), indicating a deficiency of heterozygotes.

Partitioning of genetic diversity

Mean total gene diversity at the population level was slightly higher in *P. montsicciana* (H_T = 0.384) than in *P. pardoi* (H_T = 0.298) (Table 5). Genetic diversity within populations and genetic diversity between populations were also higher in *P. montsicciana* (H_S = 0.239 and D_{ST} = 0.144) than in *P. pardoi* (H_S = 0.192 and D_{ST} = 0.105). The proportion of total genetic diversity due to differences among populations (G_{ST}) was 0.376 in *P. montsicciana* and 0.354 in *P. pardoi*. Gene flow values were very low in both species (Nm = 0.415 for *P. montsicciana* and Nm = 0.456 for *P. pardoi*).

Both taxa exhibited low levels of genetic identity and high levels of genetic distance between pairs of the different populations sampled. Values for I were relatively low in two taxa, slightly higher in *P. pardoi* than in *P. montsicciana* (mean 0.870, range 0.805–0.963 for *P. pardoi*; mean 0.809, range 0.678–0.892 for *P. montsicciana*). In consequence, values for D among populations were high in both species (mean 0.142, range 0.038–0.217 for *P. pardoi*; mean 0.215, range 0.114–0.389 for *P. montsicciana*). The dendrogram from the UPGMA pairwise values for I analysis clustered the four populations of *P. montsicciana* and the three populations of *P. pardoi* (Fig. 2) as expected. Genetic identity among populations within the two taxa did not have a geographic pattern. Thus, the most distant populations in *P. montsicciana* (MCO and MRB; Table 6) were genetically closer than the nearest ones (CAM and TER). Between taxa, I was relatively high (mean 0.724, range 0.586–0.853) compared with identities between pairs of populations.

Discussion

Genetic diversity in *P. montsicciana* and *P. pardoi*

There is a general idea that rare or endemic species are genetically depauperate, suggested long ago by Stebbins (1942), who four decades later recognized that there was not a clear correlation between levels of genetic diversity and the rarity or commonness of plant species (Stebbins 1980). Although well-known compilations of plant allozyme data (Hamrick and Godt 1990, 1996) show a repetitive pattern of low genetic diversity in rare species, we should not assume this is a necessary characteristic of endemic species.

Low levels of genetic diversity found in endemic taxa may be due to the effects of small population size and (or) the taxa's isolation (Barrett and Kohn 1991) or to limited environmental ranges of habitat species (Babbel and Selander 1974). Since the habitats of *Petrocoptis* studied here are highly homogeneous and specialized (they consist of limestone exposed to the sun in gorges and narrow mountain passes), low levels of allozyme diversity in *P. montsicciana*

Table 2. Allele frequencies for 16 loci in four populations of *Petrocoptis montsicciana* (CAM, MCO, MRB, TER) and three populations of *Petrocoptis pardoi* (AGV, BAR, CBD).

Locus	Allele	CAM	MCO	MRB	TER	AGV	BAR	CBD
Aat	*a*	0.032	0.000	0.000	0.000	0.000	0.000	0.000
	b	0.548*	0.000	0.000	0.000	0.000	0.000	0.000
	c	0.419	1.000*	1.000*	1.000*	1.000*	1.000*	1.000*
Aco-1	*a*	0.935*	0.929*	0.848*	1.000*	0.955*	0.933*	0.903
	b	0.065	0.071	0.152	0.000	0.045	0.067	0.097
Aco-2	*a*	0.065	0.095	0.087	0.000	0.045	0.067	0.323
	b	0.935*	0.857*	0.891*	1.000*	0.955*	0.933*	0.645*
	c	0.000	0.048	0.022	0.000	0.000	0.000	0.032
Adh	*a*	0.000	1.000*	1.000*	1.000*	1.000*	1.000*	1.000*
	b	1.000*	0.000	0.000	0.000	0.000	0.000	0.000
Dia-2	*a*	0.000	0.000	0.022	0.000	0.000	0.000	0.000
	b	0.048	0.702*	0.043	0.024	0.000	0.000	0.000
	c	0.952*	0.298	0.913*	0.881*	1.000*	0.917*	0.871*
	d	0.000	0.000	0.022	0.095	0.000	0.083	0.129
Dia-3	*a*	0.000	0.000	0.000	0.000	0.000	0.167	0.000
	b	0.065	0.155	0.043	0.000	0.000	0.200	0.194
	c	0.935*	0.821*	0.924*	0.762*	0.864*	0.633*	0.806*
	d	0.000	0.024	0.033	0.238	0.136	0.000	0.000
Mdh-1	*a*	0.000	0.357	0.000	0.000	0.955*	0.050	1.000*
	b	1.000*	0.643*	1.000*	1.000*	0.045	0.950*	0.000
Mdh-4	*a*	0.000	0.000	0.000	0.000	0.955*	0.000	0.984*
	b	0.871*	1.000*	1.000*	1.000*	0.045	1.000*	0.016
	c	0.129	0.000	0.000	0.000	0.000	0.000	0.000
Me	*a*	0.177	0.024	0.076	0.095	0.068	0.000	0.065
	b	0.710*	0.679*	0.652*	0.833*	0.545*	0.800*	0.710*
	c	0.113	0.238	0.250	0.071	0.386	0.200	0.226
	d	0.000	0.060	0.022	0.000	0.000	0.000	0.000
6Pgd-1	*a*	0.839*	0.071	0.000	0.143	0.182	0.133	0.000
	b	0.161	0.095	0.043	0.857*	0.341	0.150	0.097
	c	0.000	0.833*	0.870*	0.000	0.477*	0.717*	0.903*
	d	0.000	0.000	0.076	0.000	0.000	0.000	0.000
	e	0.000	0.000	0.011	0.000	0.000	0.000	0.000
6Pgd-2	*a*	0.000	0.429	0.076	0.000	0.045	0.000	0.065
	b	1.000*	0.571*	0.685*	1.000*	0.182	0.050	0.726*
	c	0.000	0.000	0.239	0.000	0.523*	0.950*	0.210
	d	0.000	0.000	0.000	0.000	0.250	0.000	0.000
Pgi-2	*a*	0.548*	0.000	0.000	0.619*	1.000*	1.000*	1.000*
	b	0.435	0.107	1.000*	0.381	0.000	0.000	0.000
	c	0.016	0.893*	0.000	0.000	0.000	0.000	0.000
Pgm-1	*a*	0.000	0.000	0.000	0.000	0.364	0.583*	0.355
	b	0.000	0.262	0.467	0.357	0.636*	0.417	0.629*
	c	0.839*	0.726*	0.533*	0.500*	0.000	0.000	0.016
	d	0.161	0.012	0.000	0.143	0.000	0.000	0.000
Pgm-2	*a*	0.000	0.048	0.109	0.048	0.000	0.000	0.000
	b	0.758*	0.833*	0.891*	0.762*	0.932*	0.967*	0.903*
	c	0.226	0.071	0.000	0.190	0.068	0.000	0.097
	d	0.016	0.048	0.000	0.000	0.000	0.033	0.000
Prx-1	*a*	0.145	0.071	0.043	0.000	0.000	0.000	0.000
	b	0.855*	0.440	0.837*	0.643*	1.000*	1.000*	0.968*
	c	0.000	0.488*	0.120	0.357	0.000	0.000	0.032
Prx-2	*a*	0.565*	0.036	0.109	0.310	0.000	0.000	0.032
	b	0.435	0.810*	0.859*	0.690*	0.932*	0.800*	0.871*
	c	0.000	0.155	0.033	0.000	0.068	0.200	0.097

*Most frequent allele for each locus and population.

Table 3. Summary of allozyme diversity for 16 loci in four populations of *Petrocoptis montsicciana* and three populations of *Petrocoptis pardoi*.

Population	N	P	A	A_p	H_o	H_e
Petrocoptis montsicciana						
CAM	31	75.0	2.1 (0.2)	2.3	0.127 (0.043)	0.243 (0.047)
MCO	42	81.3	2.4 (0.2)	2.8	0.100 (0.039)	0.291 (0.046)
MRB	46	68.8	2.4 (0.3)	3.0	0.111 (0.059)	0.202 (0.045)
TER	21	56.3	1.8 (0.2)	2.4	0.146 (0.061)	0.221 (0.055)
Mean	35	70.3	2.2	2.6	0.121	0.239
SD		10.7	0.29	0.33	0.020	0.038
Petrocoptis pardoi						
AGV	22	43.8	1.9 (0.2)	2.4	0.068 (0.040)	0.198 (0.060)
BAR	30	62.5	1.8 (0.2)	2.2	0.069 (0.043)	0.175 (0.047)
CBD	31	62.5	2.1 (0.2)	2.4	0.079 (0.039)	0.204 (0.046)
Mean	27.7	56.3	1.9	2.3	0.072	0.192
SD		10.8	0.15	0.11	0.006	0.015
Two species mean	31.8	64.3	2.1	2.5	0.100	0.217
SD		12.3	0.26	0.29	0.030	0.038

Note: Values are means with SE given in parentheses. N, sample size; P, percentage of polymorphic loci; A, mean number of alleles per locus; A_p, mean number of alleles per polymorphic locus; H_o, observed heterozygosity; H_e, expected panmictic heterozygosity.

Table 4. Values of fixation index (F) for 16 loci in four populations of *Petrocoptis montsicciana* (CAM, MCO, MRB, TER) and three populations of *Petrocoptis pardoi* (AGV, BAR, CBD).

Locus	CAM	MCO	MRB	TER	AGV	BAR	CBD
Aat	0.382**	—	—	—	—	—	—
Aco-1	1.000***	1.000***	1.000***	—	1.000**	1.000***	1.000***
Aco-2	1.000***	1.000***	1.000***	—	1.000**	1.000***	1.000***
Adh	—	—	—	—	—	—	—
Dia-2	0.650ns	0.829***	1.000***	0.778**	—	0.782***	1.000***
Dia-3	1.000***	0.762***	0.545***	1.000***	1.000***	1.000***	1.000***
Mdh-1	—	1.000***	—	—	1.000**	0.649*	—
Mdh-4	–0.148ns	—	—	—	1.000**	—	–0.016ns
Me	0.429**	0.503***	0.484***	–0.144ns	0.834***	1.000***	1.000***
6Pgd-1	1.000***	1.000***	0.908***	1.000***	0.927***	0.925***	0.631**
6Pgd-2	—	0.708***	0.861***	—	0.928***	0.649*	0.621***
Pgi-2	–0.077ns	0.876***	—	–0.615*	—	—	—
Pgm-1	1.000***	–0.356ns	–0.878***	0.446ns	–0.375ns	–0.303ns	–0.282ns
Pgm-2	0.224ns	1.000***	1.000***	1.000***	–0.073ns	1.000***	0.262ns
Prx-1	0.870***	0.619***	0.309***	0.481ns	—	—	–0.033ns
Prx-2	0.410*	0.106ns	0.043ns	–0.448ns	–0.073ns	–0.042ns	0.162**

Note: Conformity with Hardy–Weinberg equilibrium was tested using χ^2 analysis: ns, $p \geq 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

and *P. pardoi* were expected. Surprisingly, these taxa had higher than average levels for endemic species (P = 26.3%, A = 1.39, and H_e = 0.063 at population level; Hamrick and Godt 1990), and for widespread species (P = 43.0%, A = 1.72, and H_e = 0.159; Hamrick and Godt 1990). Several studies of endemic and narrow species have demonstrated that geographic range is not always a good predictor of the genetic diversity of plant species. In the Mediterranean basin, which is considered today a biodiversity "hotspot" (Myers et al. 2000), we can find a large number of examples of endemic taxa with high levels of allozyme diversity: *Artemisia molinieri* (Torrell et al. 1999), *Seseli farrenyi* (J. Simon, M. Bosch, A. Rovira, J. Molero, and C. Blanché, unpublished data; López-Pujol 2000), *Delphinium bolosii* (Bosch et al. 1998), *Antirrhinum mollisimum*, and *Antirrhinum microphyllum* Rothm. (Mateu-Andrés 1999; Torres 1999). These last two species grow in the same type of habitat as *Petrocoptis*. Breeding systems also correlate with levels of genetic diversity, so that predominantly selfing species have less genetic diversity than outcrossing ones (Hamrick and Godt 1996). The two outcrossing and entomophilous taxa analyzed here showed much higher than average levels for outcrossing plants pollinated by animal species (P = 35.9%, A = 1.54, and H_e = 0.124; Hamrick and Godt 1990).

Large populations should maintain higher levels of genetic variability than small ones (Wright 1931; Kimura and Crow 1964; Barrett and Kohn 1991). Low levels of genetic diversity in small populations may be due to inbreeding, genetic drift, founder effects, genetic bottlenecks, or other his-

Table 5. Gene diversity statistics (Nei 1973) for 16 loci in four populations of *Petrocoptis montsicciana* and three populations of *Petrocoptis pardoi*.

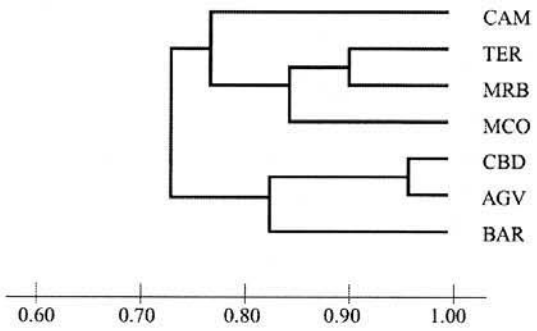
Locus	H_T	H_S	D_{ST}	G_{ST}
Petrocoptis montsicciana				
Aat	0.290	0.133	0.158	0.543
Aco-1	0.136	0.130	0.006	0.042
Aco-2	0.150	0.146	0.004	0.026
Adh	0.500	0.000	0.500	1.000
Dia-2	0.430	0.225	0.205	0.476
Dia-3	0.256	0.236	0.020	0.077
Mdh-1	0.178	0.117	0.062	0.347
Mdh-4	0.064	0.057	0.007	0.115
Me	0.451	0.439	0.012	0.027
6Pgd-1	0.800	0.265	0.536	0.669
6Pgd-2	0.344	0.243	0.101	0.293
Pgi-2	0.745	0.298	0.447	0.600
Pgm-1	0.516	0.451	0.066	0.127
Pgm-2	0.329	0.316	0.013	0.038
Prx-1	0.479	0.394	0.084	0.176
Prx-2	0.469	0.378	0.091	0.195
Mean	0.384	0.239	0.144	0.376
SE	0.052	0.034	—	—
Petrocoptis pardoi				
Aat	0.000	0.000	0.000	—
Aco-1	0.130	0.131	0.000	0.000
Aco-2	0.284	0.234	0.050	0.175
Adh	0.000	0.000	0.000	—
Dia-2	0.134	0.128	0.006	0.046
Dia-3	0.403	0.366	0.036	0.090
Mdh-1	0.635	0.061	0.573	0.903
Mdh-4	0.666	0.040	0.626	0.940
Me	0.465	0.445	0.020	0.043
6Pgd-1	0.486	0.422	0.063	0.130
6Pgd-2	0.671	0.390	0.281	0.419
Pgi-2	0.000	0.000	0.000	—
Pgm-1	0.508	0.485	0.023	0.046
Pgm-2	0.126	0.124	0.002	0.012
Prx-1	0.021	0.021	0.000	0.014
Prx-2	0.235	0.230	0.005	0.022
Mean	0.298	0.192	0.105	0.354
SE	0.062	0.044	—	—

Note: H_T, total genetic diversity; H_S, genetic diversity within populations; D_{ST}, genetic diversity between populations; G_{ST}, proportion of total genetic diversity among populations.

toric factors. Both *P. montsicciana* and *P. pardoi* show no correlation between population size and genetic variation, a phenomenon that has been reported in other studies (Maki et al. 1996; Williamson and Werth 1999). Among the seven populations sampled, the largest one (BAR with a population size of about 5000) had lower levels of allozyme diversity than smaller populations such as MCO (population size of about 200 individuals).

The values for H_o were lower than those for H_e in all populations of the two taxa. Deficiency of heterozygotes in plant species can be explained mainly in two ways: (*i*) inbreeding within populations and (*ii*) genetic substructuring of populations (Wahlund effect). Since both *P. montsicciana* and *P. pardoi* are outcrossing species that avoid self-pollination, inbreeding within populations is unlikely. However, limited seed and (or) pollen dispersal of these species could lead to a spatial structure in subpopulations. The analysis of *F* for all polymorphic loci in all populations revealed that 19 loci were in Hardy–Weinberg equilibrium, while 61 loci showed heterozygote deficiencies, probably because of local structuring of populations. Short flight distances performed by pollinators or repetitive visits to the same plant (M. Bosch, J. López-Pujol, J. Simon, and C. Blanché, unpublished data) may explain pollen dispersal between closely located and related individuals sharing very similar genotypes. Seed-dispersal mechanisms are not yet completely known but seem to be very limited. Seeds are dispersed by gravity, although spider webs, which are common on limestone walls, could act as seed receptacles (Sainz et al. 1996) and limit their dispersal. Another factor that could contribute to low seed dispersal is the promoting of seed deposition at the base of mother plants in *Petrocoptis* species, by the bending of pedicels (García 1993). Limited seed and pollen dispersal are thought to be the main reasons for the spatial substructure of populations in neighborhoods.

Fig. 2. Dendrogram resulting from UPGMA analysis of *Petrocoptis montsicciana* and *Petrocoptis pardoi* populations based on pairwise values of Nei's (1978) genetic identity (SD = 9.399%; cophenetic correlation = 0.731).



Distribution of genetic diversity

Although endemic species tend to show less genetic diversity (H_T) than more widespread species, such a pattern with respect to genetic divergence between populations (G_{ST}) is not found (Hamrick and Godt 1990, 1996). Outcrossing taxa have more genetic diversity than selfing species, with the former having more genetic diversity within species (H_S) and lower divergence among populations (G_{ST}) (Hamrick and Godt 1996). The H_T estimates in *P. montsicciana* and *P. pardoi* were higher than expected for endemic species (0.263) but similar to those expected for outcrossing animal-pollinated species (0.310). The H_S values were slightly higher in both taxa than expected for endemic species (0.163) and lower than expected for outcrossing animal species (0.243). The genetic divergence among populations found in *P. montsicciana* and in *P. pardoi*, which runs at near 40%, is much higher than the mean values for endemic and outcrossing animal species (0.248 and 0.197, respectively) (Hamrick and Godt 1996).

Table 6. Matrix of Nei's (1978) genetic distance (below diagonal) and Nei's (1978) genetic identity (above diagonal) between populations of *Petrocoptis montsicciana* and *Petrocoptis pardoi*.

Population	CAM	MCO	MRB	TER	AGV	BAR	CBD
CAM	—	0.678	0.770	0.828	0.595	0.691	0.586
MCO	0.389	—	0.867	0.821	0.690	0.769	0.715
MRB	0.261	0.142	—	0.892	0.733	0.853	0.744
TER	0.188	0.197	0.114	—	0.745	0.834	0.729
AGV	0.519	0.371	0.310	0.295	—	0.843	0.963
BAR	0.370	0.263	0.159	0.182	0.171	—	0.805
CBD	0.535	0.335	0.295	0.316	0.038	0.217	—

Gene flow estimates, as calculated from G_{ST} values, are consequently very low in both taxa (Nm = 0.415 for *P. montsicciana* and Nm = 0.456 for *P. pardoi*). A Nm value of 1.0 is considered enough to prevent divergence attributable to genetic drift (Wright 1951); thus, gene flow appears not to be a strong enough force to deter the random loss of alleles in *P. montsicciana* and *P. pardoi*. Geographic isolation of populations and limited pollen and seed dispersal may explain the low values of Nm. In both taxa studied, flight distances of insect pollinators are probably much shorter than distances between populations (mean distance between populations of *P. montsicciana* was 33.2 km, range 14.7–55.5 km; mean distance between populations of *P. pardoi* was 4.1 km, range 2.5–6 km), so the contribution of pollen dispersal to gene flow among populations is minimal.

Low seed and pollen dispersal observed in these species give low values of gene flow, but also promote structuring of populations in neighborhoods, as also occurs in the a chasmophyte, *Antirrhinum microphyllum* (Torres 1999). Low germination rates and seedling establishment (Sainz et al. 1996), as observed in *P. crassifolia* (García 1993), magnify structure in subpopulations, since they limit introduction of new genetic material into other neighborhoods.

Relationships between taxa

The genus *Petrocoptis* is a relict that appeared in the Tertiary age and lost contact with central European flora after the Quaternary glaciations. Speciation within the genus was probably due to the fragmentation of its distribution area, because of climatic changes (Mayol 1998). The most recent taxonomic treatment by Mayol and Rosselló (1999) postulates one single taxon, grouping together *P. montsicciana* and *P. pardoi* as the species *S. pardoi*. Genetic identity between pairs of populations was relatively low in the two taxa (mean I 0.870, range 0.760–0.884 for *P. pardoi*; mean I 0.809, range 0.678–0.892 for *P. montsicciana*), quite a bit smaller than expected for conspecific populations (mean I 0.950, range 0.900–1.000; Gottlieb 1981; Crawford 1983). These low levels of identity can be explained by the isolation of populations; however, for interspecific comparisons the range of values between populations is wider (0.586–0.853). Genetic identity between taxa is not much lower (mean I 0.724) than identities between populations and slightly higher than expected for congeneric species (mean I 0.670; Gottlieb 1981).

Another plausible hypothesis is that the two taxa form a pair of recently derived progenitor–derivative species for which: (*i*) there may be high genetic identities between both species; (*ii*) the progenitor should have greater genetic diversity than the derivative; (*iii*) the derivative should contain a subset of the alleles present in the progenitor; and (*iv*) there are few, if any, unique alleles to both species, because of inadequate time to accumulate new mutations (Gottlieb 1973; Crawford 1983). We assume that the derivative species is *P. pardoi*, since it has a much narrower geographic range than *P. montsicciana*. In addition, since *P. pardoi* is the only species of *Petrocoptis* distributed outside the Cantabrian–Pyrenean axis, it could be interpreted as a southern disjunction in the geographic range of *P. montsicciana*. *Petrocoptis pardoi* has less genetic diversity than *P. montsicciana* and exhibits a subset of the alleles present in *P. montsicciana*. Also, *P. pardoi* has only four unique alleles, whereas *P. montsicciana* has 14 unique alleles. The dendrogram from the UPGMA pairwise values for I analysis clearly showed two clusters: one for the populations of *P. montsicciana* and another for populations of *P. pardoi* (mean geographic distance between clusters is 169 km), although this does not necessarily support the progenitor–derivative hypothesis.

The north–south migration paths subsequent to glacial phenomena are well known in the Mediterranean flora (Stebbins 1971, Küpfer 1974, Galland 1988; Hewitt 1996; Cain et al. 2000). However, consequences of these migrations for the patterns of present-day diversity can vary greatly. Some species, such as *Abies alba*, have apparently given rise to an explosion of microspecies at their southernmost expansion because of their isolation from a former continuous area (*Abies nebrodensis*, *Abies pinsapo*, *Abies maroccana*, *Abies numidica*, etc.; see Greuter et al. 1989). The species of genus *Petrocoptis* are currently interpreted as Tertiary relicts and, as is the case with other Pyrenean endemics, a southwards migration under glacial conditions is assumed to have lead to (*i*) the isolation from central European stocks and (*ii*) the isolation of small units of narrow endemic species in distinct mountain ranges in southwestern Europe (Mayol 1998 and references therein). The *Petrocoptis* model suggests a difficult southwards expansion, which implies some genetic impoverishment, although it cannot be fully considered a case of genetic bottleneck, since so much genetic variation is still retained by *P. pardoi* populations. This limitation cannot be attributed to habitat restriction (there is a great diversity of similar habitats available south of the *P. pardoi* cluster) but rather to limitations in long-distance dispersal and the consequent restriction of gene flow between the newly established populations and the main gene pool of the ancestral species. A similar pat-

tern of impoverishment by loss of gene flow of extremely marginal populations with the main species gene pool has been reported in the ancestral-derivative pair *Delphinium fissum – Delphinium bolosii* group (Ranunculaceae) in the Mediterranean area during the Messinian period, in this case, mainly in an east–west direction (Bocquet et al. 1978, Bosch et al. 1998). Although genetic methods do not allow direct characterization of long dispersal events (Cain et al. 2000), the combinations of allozyme and biogeographic or geologic data provide useful ways of understanding speciation processes in pairs of derived progenitor species (Levin 2001, and references therein) at the appropriate geographic scale, particularly in endemic species.

Conservation implications

The knowledge of the genetic variation of a species is essential for managing a comprehensive conservation plan (Hamrick 1983; Falk and Holsinger 1991; Ellstrand and Elam 1993; Loeschcke et al. 1994). Maintenance of levels of genetic diversity is one of the main goals of conservation programs (Frankel and Soulé 1981; Simberloff 1988) and allows the preservation both of the potential for evolutionary change and of potential response to biotic and (or) abiotic environmental changes (Barrett and Kohn 1991).

Plant species survival can be affected by two kinds of threats: natural and anthropogenic. The natural risks to *P. montsicciana* and *P. pardoi* are related to their low germination and renewal rates, observed in the field by us and other researchers (Sainz et al. 1996; Mayol 1998). Preliminary recruitment rates data accumulated in the last 4 years (M. Bosch, J. López-Pujol, J. Simon, and C. Blanché, unpublished data) show that there were no newly established plants in the five monitoring plots designed in populations CAM and TER; populations maintained similarity in size, although some adult individuals were lost. However, their high levels of genetic diversity ensure their response to stochastic events of demographic, ecological, and (or) climatic origin. Several anthropogenic activities also threaten the survival of these species. Perhaps the most important one is the destruction of limestone by road construction or improvement. Other threats are climbing (a common threat to several chasmophytic species; Nuzzo 1995, 1996; Torres 1999), fires, and over-collection of individual plants.

Given the vulnerable status of these species, both in situ and ex situ conservation measures are needed. The clearest in situ measure is the protection of their habitat. At present, habitat protection is not the same for the two taxa. The largest population of *P. pardoi*, BAR (population size about 5000), constitutes a botanical microreserve (Diari Oficial de la Generalitat Valenciana 1994), but populations of *P. montsicciana* have no such specific protection. Rare alleles were found in all sampled populations but can easily be lost by processes of genetic drift, which are enhanced by reductions in population size (Barrett and Kohn 1991). Thus, a monitoring program for both taxa is required to ensure the maintenance of the present population sizes. Although seeds from both taxa are conserved in several seed collections of germplasm banks, this is not the best conservation policy because of their very low germination rates (5.5% success in *P. montsicciana*, see López-Pujol 2000).

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3.1.2. **López-Pujol J**, Bosch M, Simon, J & Blanché C. 2002. Allozyme variation and population structure of the very narrow endemic *Seseli farrenyi* (Apiaceae). *Botanical Journal of the Linnean Society*, 138: 305-314.

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Allozyme variation and population structure of the very narrow endemic *Seseli farrenyi* (Apiaceae)

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Analyses have been made of allozyme variation of the narrow endemic species *Seseli farrenyi* Molero & J. Pujadas (Apiaceae), which has only three known populations in Catalonia with a total of around 2000 individuals. All three populations were sampled and subjected to starch gel electrophoresis. Nine enzymes were resolved and 14 loci were interpreted. We detected high values of polymorphism ($P = 83.3\%$, $A = 3.0$, $H_e = 0.297$), far exceeding those expected for endemic species ($P = 26.3\%$, $A = 1.39$, $H_e = 0.063$). Genetic diversity was greater within populations than among populations, and the value of gene flow was very high ($Nm = 5.85$). Most loci showed deviations from Hardy–Weinberg equilibrium, possibly due to the presence of subpopulations. The main threats to this species are human activities (tourism, fires), while natural threats are minimal due to its high genetic diversity. Finally, we propose some conservation measures which include both *in situ* and *ex situ* strategies. *Botanical Journal of the Linnean Society*, 2002, **138**, 305–314.

ADDITIONAL KEYWORDS: Catalonia – conservation – genetic structure – isozyme electrophoresis – subpopulations.

INTRODUCTION

One characteristic of many rare species is their low levels of genetic variation. Limited genetic diversity has been reported for many rare plant species, and has been considered as either a cause or a consequence of rarity (Bosch *et al*., 1998; Gitzendanner & Soltis, 2000). However, the allozymic study of most rare species in recent years has demonstrated that there is no clear correlation between the amount of genetic variation and the rarity or commonness of plant species, as predicted by Stebbins 20 years ago (Stebbins, 1980). Compilations of isozyme data between rare and widespread congeners have shown that while some rare species show little genetic variation, others maintain levels of diversity equal to or exceeding widespread congeners (Gitzendanner & Soltis, 2000).

Seseli farrenyi Molero & J. Pujadas (Apiaceae) is a very narrow endemic whose range is restricted to the northern coast of Cape Creus, in north-eastern Catalonia (Spain). When originally described (Molero & Pujadas, 1979), only a single population of this species, located in Ses Estenedors (SES), was known. Later, Franquesa (1995) discovered two additional populations, in Es Camallerús (SCM) and Es Bol d'Es Prim (EBP). The three populations are geographically close (extent of occurrence is 0.47 km^2, according to the criteria of UICN 1994), and, together, have a total of *c*. 2000 individuals. The species is listed as 'endangered' (EN) both in a study of threatened flora in Catalonia (Sáez *et al.* 1998) and in a recently published Red List of Vascular Spanish Flora (Aizpuru *et al.*, 2000).

The systematic relationships of this species within the genus *Seseli* are not clear. Currently, it is included in section *Seseli*, which contains most species of this genus. *S. farrenyi* has been regarded as a subspecies of *Seseli elatum* L. (Bolòs & Vigo, 1990), but it is morphologically and ecologically closer to the *Seseli* species of Corsica and Sardinia [*S. praecox* (Gamisans) Gamisans and *S. bocconi* Guss.]. Thus, *S. farrenyi* could be considered a living relic, which probably reached the Iberian Peninsula during the Messinian period (Bocquet *et al.*, 1978; López-Pujol, 2000).

Here we used allozyme electrophoresis to address the following issues: (1) the genetic diversity within populations of *S. farrenyi*; (2) whether this species is genetically depauperate like many other rare plant species; (3) the levels of differentiation within and

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among populations; (4) whether there is genetic structure within populations, and whether this is correlated with spatial subpopulations. This information will be useful to inferring the conservation status of this species and for establishing priorities for its protection.

MATERIAL AND METHODS

Plant material

Seseli farrenyi is a small rosulate perennial herb (up to 20–30 cm) that produces abundant umbels, composed of (2) 4–8 (11) umbellar rays with small white flowers and ovoid seeds 2.2–3 × 2–2.5 mm (Molero & Pujadas, 1979). It is a diploid ($2n$ = 18; Fernández-Casas *et al.*, 1979), outcrossing and entomophylous species that is visited by a great variety of small insects (Rovira *et al.*, in prep.). It grows in fissures of schistous rocks in littoral scarps, on weakly acidic and sandy soils.

Sampling strategy

Genetic diversity was assessed using standard methods for starch gel electrophoresis of allozymes (e.g. Soltis *et al*., 1983; Soltis & Soltis, 1989). All three populations were sampled from October to November of 1999: 30 individuals from the smallest population and more than 100 individuals from the remaining two (Table 1; Fig. 1). To evaluate population substructure for the two largest populations, the collection of individuals was subdivided into spatially separated subpopulations within the populations that were observed in the field. All the samples collected were mapped and tagged, and accounted for about 13% of the total number of *S. farrenyi* plants. Young leaves from basal rosettes were collected, placed in envelopes and stored at 4 °C in the laboratory until extraction. Collection of leaf samples was done carefully to minimize damage to the populations.

Electrophoresis

Leaf fragments were homogenized in refrigerated porcelain plates using a cold extraction buffer consisting of 0.05 M tris-citric acid, 0.1% cysteine·HCl, 0.1% ascorbic acid, 8% PVP-40 and 1 mM 2-mercaptoethanol. Extracts were absorbed onto 3 mm Whatman filter paper, either to be analysed immediately or stored at <20 °C until analysis 1 or 2 days later.

Twenty-one enzymes were assayed, nine of which were resolved and interpreted using 12.5% starch gels and three buffer systems. Aspartate aminotransferase (AAT, E.C. [Enzyme Commission number] 2.6.1.1), alcohol dehydrogenase (ADH, E.C. 1.1.1.1), diaphorase (DIA, E.C. 1.6.99.), phosphoglucoisomerase (PGI, E.C. 5.3.1.9) and ribulose-bisphosphate carboxylase/oxygenase (RBC, E.C. 4.1.1.39) were satisfactorily resolved on tris-citrate/lithium borate buffer pH 8.2 (Scandalios, 1969); isocitrate dehydrogenase (IDH, E.C. 1.1.1.42) and malate dehydrogenase (MDH, E.C. 1.1.1.37) were resolved on morpholine buffer pH 6.1 (Odrzykoski & Gottlieb, 1984), while phosphogluconate dehydrogenase (6PGD, E.C. 1.1.1.44) and phosphoglucomutase (PGM, E.C. 5.4.2.2) were resolved on histidine-citrate buffer pH 5.7 (Jefries & Gottlieb, 1982). Staining procedures for all enzymes followed the method described by Vallejos (1983), Shields *et al.* (1983) and Wendel & Weeden (1989), with small modifications (see Bosch, 1999).

Table 1. Populations and subpopulations of *Seseli farrenyi* studied. Location of populations are detailed by UTM 1 × 1 km squares

Population code	Location	Population size	Subpopulation code	Sample size
SES	Ses Estenedors (31TEG2685)	90		30
SCM	Es Camallerús (31TEG2685)	716		
			SCM1	40
			SCM2	22
			SCM3	24
			SCM4	26
EBP	Es Bol d'Es Prim (31TEG2386)	1260		
			EBP1	26
			EBP2	22
			EBP3	20
			EBP4	25
			EBP5	21
			EBP6	21

Figure 1. Geographic distribution of *Seseli farrenyi*. • Known populations; SES Ses Estenedors; SCM Es Camallerús; EBP Es Bol d'Es Prim.

GENETIC ANALYSES

Loci were numbered consecutively and alleles at each locus were labelled alphabetically, beginning from the most anodal form in both cases. Isozyme phenotypes were interpreted genetically according to standard principles (Wendel & Weeden, 1989). To calculate the levels of genetic diversity, the following statistics were computed: P, the percentage of polymorphic loci when the most common allele had a frequency of <0.95; 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. We also computed the mean fixation index, F, to compare genotype proportions to those expected under Hardy–Weinberg equilibrium. The partitioning of genetic diversity within and among populations was analysed using Nei's (1973)

gene diversity statistics: total genetic diversity (H_T), genetic diversity within populations (H_S), genetic diversity between populations (D_{ST}), and proportion of total genetic diversity among populations (G_{ST}) were calculated for all populations. Gene flow (Nm) was determined using Wright's (1951) equation: $Nm = (1 - G_{ST})/4G_{ST}$. We also carried out the hierarchical F_{ST} analysis to evaluate the extent of genetic substructure. Genetic divergence among populations was estimated by calculating Nei's (1972) genetic distance (D), Nei's (1978) genetic identity (I) and Rogers's (1972) genetic similarity (S). Using UPGMA (unweighted pair group method with averaging), populations were clustered into a dendrogram on the basis of their pairwise values for I. Finally, Mantel's test (Mantel 1967) was conducted between values of Nei's genetic identity (I) and geographical interpopulation distances. Calculations of $P, A, A_p, H_o, H_e, F, F_{ST}$, D, I, S and dendrograms were done with BIOSYS-1 version 1.7 (Swofford & Selander 1989). Calculations of H_T, H_S, D_{ST} and G_{ST} were carried out using GeneStat version 3.31 (Whitkus 1988). We used the NTSYS package (Rohlf, 1994) to calculate Mantel's test.

RESULTS

Levels of genetic diversity

Among the 14 interpretable loci, we detected 49 alleles, and their frequencies were calculated (Table 2). If we consider that loci are polymorphic when they exhibit more than one allele, then 12 polymorphic loci were identified in all three populations (*Dia-1*, *Dia-2*, *Idh*, *Mdh-1*, *Mdh-3*, *Mdh-4*, *6Pgd-1*, *6Pgd-2*, *Pgi-1*, *Pgi-2*, *Pgm-2* and *Rbc*). There were no substantial differences in allele frequencies among the three populations: the same allele at 13 of the 14 loci showed the highest frequency. Four private alleles were found in the SCM population (*Mdh-1 a*, *Pgi-1 e*, *Pgi-2 a* and *Rbc a*), and two in the EBP population (*Aat a* and *Dia-2 d*), whereas none were found in the SES population. Rare alleles (alleles which are in proportion less than 0.05) were frequent in the three populations: 8, 17 and 15 in the SES, SCM and EBP populations, respectively.

Genetic diversity was quantified for each of the three populations, using the main indices of polymorphism (Table 3). *S. farrenyi* possesses high levels of genetic variability looking at their mean values: P was 83.3%, A was 3.0, A_p was 3.1, and H_o and H_e were 0.120 and 0.297, respectively.

Comparison to Hardy–Weinberg equilibrium

To compare genotype frequencies to those expected under Hardy–Weinberg equilibrium, the fixation

Table 2. Allele frequencies for 14 loci in three populations of *Seseli farrenyi*. The most frequent allele is underlined

		Populations		
Locus	Allele	SES	SCM	EBP
Aat	*a*	0.000	0.000	0.007
	b	1.000	1.000	0.993
Adh	*a*	0.833	0.946	1.000
	b	0.167	0.054	0.000
Dia-1	*a*	0.017	0.009	0.004
	b	0.833	0.813	0.948
	c	0.150	0.179	0.048
Dia-2	*a*	0.017	0.004	0.030
	b	0.133	0.138	0.289
	c	0.850	0.857	0.670
	d	0.000	0.000	0.011
Idh	*a*	0.017	0.018	0.059
	b	0.300	0.188	0.248
	c	0.683	0.786	0.663
	d	0.000	0.009	0.030
Mdh-1	*a*	0.000	0.013	0.000
	b	0.083	0.085	0.196
	c	0.917	0.902	0.804
Mdh-3	*a*	0.067	0.018	0.126
	b	0.933	0.982	0.874
Mdh-4	*a*	0.100	0.018	0.074
	b	0.900	0.938	0.896
	c	0.000	0.045	0.030
6Pgd-1	*a*	0.000	0.013	0.004
	b	0.967	0.915	0.937
	c	0.033	0.071	0.059
6Pgd-2	*a*	0.033	0.000	0.044
	b	0.233	0.098	0.304
	c	0.717	0.683	0.507
	d	0.017	0.214	0.141
	e	0.000	0.004	0.004
Pgi-1	*a*	0.017	0.027	0.026
	b	0.333	0.232	0.433
	c	0.383	0.496	0.422
	d	0.267	0.228	0.119
	e	0.000	0.018	0.000
Pgi-2	*a*	0.000	0.018	0.000
	b	0.117	0.277	0.015
	c	0.750	0.451	0.737
	d	0.133	0.219	0.230
	e	0.000	0.036	0.019
Pgm-2	*a*	0.000	0.125	0.004
	b	0.983	0.848	0.889
	c	0.017	0.027	0.107
Rbc	*a*	0.000	0.022	0.000
	b	0.167	0.281	0.107
	c	0.533	0.478	0.689
	d	0.300	0.174	0.159
	e	0.000	0.045	0.044

Table 3. Summary of genetic variation for 14 loci in three populations of *Seseli farrenyi*

Population	*N*	*P*	*A*	A_p	H_o	H_e
SES	30	78.6	2.6 (0.2)	2.6	0.124 (0.029)	0.285 (0.056)
SCM	112	85.7	3.3 (0.3)	3.5	0.137 (0.023)	0.302 (0.062)
EBP	135	85.7	3.1 (0.3)	3.3	0.100 (0.020)	0.305 (0.057)
Mean	92.3	83.3	3.0	3.1	0.120	0.297

N: sample size; *P*: percentage of polymorphic loci; *A*: mean number of alleles per locus; A_p: mean number of alleles per polymorphic locus; H_o: observed heterozygosity; H_e: expected panmictic heterozygosity. Standard error in parentheses.

Table 4. Values of fixation index (*F*) for 14 loci in three populations of *Seseli farrenyi*

Locus	SES	SCM	EBP
Aat	–	–	–0.007 ns
Adh	0.040 ns	0.119 ns	–
Dia-1	0.057 ns	0.188 ns	–0.051 ns
Dia-2	–0.028 ns	0.021 ns	0.698 ns
Idh	0.548 **	0.691 ***	0.715 ***
Mdh-1	–0.091 ns	0.154 ***	0.272 **
Mdh-3	1.000 ***	1.000 ***	1.000 ***
Mdh-4	1.000 ***	0.850 ***	1.000 ***
6Pgd-1	1.000 ***	0.261 ***	0.437 ***
6Pgd-2	0.458 ***	0.458 ***	0.682 ***
Pgi-1	0.901 ***	0.738 ***	0.844 ***
Pgi-2	0.754 ***	0.774 ***	0.780 ***
Pgm-2	–0.017 ns	0.358 ***	0.104 ***
Rbc	0.777 ***	0.702 ***	0.711 ***

Conformance to Hardy–Weinberg equilibrium was tested using chi-square analysis: ns $P \geq 0.05$; * $P < 0.05$; ** $P < 0.01$;*** $P < 0.001$.

Table 5. Gene diversity statistics (Nei 1973) for 14 loci in three populations of *Seseli farrenyi*

Locus	H_T	H_S	D_{ST}	G_{ST}
Aat	0.005	0.005	0.000	0.000
Adh	0.141	0.128	0.013	0.095
Dia-1	0.240	0.232	0.008	0.034
Dia-2	0.343	0.327	0.017	0.048
Idh	0.436	0.432	0.005	0.011
Mdh-1	0.223	0.217	0.006	0.028
Mdh-3	0.133	0.128	0.005	0.036
Mdh-4	0.166	0.164	0.001	0.009
6Pgd-1	0.114	0.114	0.000	0.001
6Pgd-2	0.546	0.517	0.030	0.055
Pgi-1	0.665	0.651	0.014	0.021
Pgi-2	0.542	0.497	0.045	0.083
Pgm-2	0.178	0.167	0.011	0.061
Rbc	0.608	0.586	0.022	0.036
Mean	0.310	0.297	0.013	0.041
Standard error	0.057	0.054	–	–

H_T: total genetic diversity; H_S: genetic diversity within populations; D_{ST}: genetic diversity between populations; G_{ST}: proportion of total genetic diversity among populations.

index (*F*) was calculated for all polymorphic loci in each population. We used the chi-square test (χ^2) to evaluate deviations of *F* from zero (Table 4). Twenty-eight *F*-values differed significantly from zero ($P < 0.05$) and all were positive, which indicates a deficiency of heterozygotes. Eleven *F*-values were consistent with Hardy–Weinberg expectations ($P \geq 0.05$).

Partitioning of genetic diversity

The mean total genetic diversity (H_T) was 0.310, and was distributed mainly within populations ($H_S = 0.297$) rather than among populations ($D_{ST} = 0.013$). Consequently, the value of G_{ST} was very low (0.041), indicating that only 4% of the total genetic variability was attributable to interpopulation differentiation (Table 5). The value of gene flow (*Nm*) was very high (5.85), indicating a substantial interchange of genes among populations. A *Nm* value of 1.0 is enough to prevent divergence by genetic drift (Wright 1951).

Genetic divergence among populations and subpopulations

Values for *I* (mean = 0.982, range: 0.973–0.987) and *S* (mean = 0.897, range: 0.879–0.901) were extremely high between pairs of populations (Table 6), as expected for conspecific populations (Gottlieb, 1981; Crawford, 1983). The values of genetic identities were in concordance with the proximity between populations (range of distances: 0.5–2.9 km). The UPGMA dendrogram based on pairwise *I*-values, placed the SES and SCM populations (only separated by 0.5 km) as most similar.

Table 6. Matrix of Rogers, (1972) genetic similarity (*S*, below diagonal) and Nei's (1978) genetic identity (*I*, above diagonal) between populations of *Seseli farrenyi*

Populations	SES	SCM	EBP
SES	–	0.987	0.986
SCM	0.912	–	0.973
EBP	0.901	0.879	–

Table 7. Hierarchical F_{ST} analysis in *Seseli farrenyi*

Comparison			Variance component	F_{XY}
X		Y		
Subpopulation	–	Population	0.19524	0.045
Subpopulation	–	Total	0.26205	0.060
Population	–	Total	0.06681	0.015

Table 8. Matrix of geographical distances* (below diagonal) and Nei's (1978) genetic identity (above diagonal) between subpopulations of *S. farrenyi*

Subpopulations	EBP1	EBP2	EBP3	EBP4	EBP5	EBP6	SES	SCM1	SCM2	SCM3	SCM4
EBP1	–	0.989	0.987	0.972	0.955	0.973	0.974	0.972	0.967	0.958	0.948
EBP2	0.050	–	0.988	0.979	0.967	0.984	0.987	0.975	0.973	0.956	0.947
EBP3	0.075	0.037	–	0.986	0.972	0.987	0.986	0.984	0.979	0.967	0.953
EBP4	0.135	0.090	0.055	–	0.989	0.983	0.975	0.963	0.961	0.948	0.936
EBP5	0.135	0.090	0.065	0.040	–	0.981	0.971	0.965	0.953	0.944	0.953
EBP6	0.100	0.060	0.045	0.050	0.037	–	0.973	0.963	0.960	0.937	0.937
SES	3.150	3.125	3.125	3.075	3.075	3.100	–	0.992	0.982	0.972	0.974
SCM1	2.850	2.825	2.800	2.775	2.775	2.800	0.425	–	0.989	0.994	0.992
SCM2	2.850	2.825	2.800	2.750	2.750	2.775	0.450	0.037	–	0.973	0.965
SCM3	2.800	2.775	2.775	2.750	2.700	2.750	0.525	0.100	0.075	–	0.991
SCM4	2.775	2.750	2.750	2.700	2.675	2.725	0.537	0.137	0.115	0.075	–

*Geographic distances are expressed in km.

To evaluate the extent of population substructure, we carried out the hierarchical F_{ST} analysis, as described by Williamson & Werth (1999). The designated hierarchy consisted of populations and micro-geographical subpopulations as observed in the field. Although divergence among subpopulations with respect to the total was low (F_{XY} = 0.060), the greatest amount was due to variation among subpopulations within populations (F_{XY} = 0.045) rather than to variation among populations (F_{XY} = 0.015) (Table 7).

We analysed genetic divergence among subpopulations, using the *I* and *D* parameters (Table 8). Values for *I* were high among pairs of subpopulations (mean = 0.970, range: 0.936–0.994), and values for *D* were, as a result, very low (mean = 0.030, range: 0.006–0.066). The dendrogram resulting from UPGMA (based on pairwise values for *I*) placed three subpopulations together from the EBP population (EBP1, EBP2 and EBP3), but it separated the other three subpopulations from these (EBP4, EBP5 and EBP6) and placed them in another cluster. The SCM2 subpopulation and the SES population were clustered together. Three subpopulations from the SCM population (SCM1, SCM3 and SCM4) were grouped together in a separate cluster (Fig. 2). Mantel's test showed a nonsignificant correlation between genetic variability and geographical distribution of subpopulations (r = –0.56).

DISCUSSION

GENETIC DIVERSITY IN *SESELI FARRENYI*

For many years, low levels of genetic diversity have been reported for rare and/or endemic species. This generalization has traditionally been related to the effects of small population size, the isolation of populations and adaptation to a uniform habitat (Barrett & Kohn, 1991; Ellstrand & Elam, 1993). The compilations of isozyme data show a pattern of genetic depauperation in rare species (Hamrick & Godt, 1990; Hamrick & Godt, 1996), but some rare species have high levels of genetic variability, such as *Layia discoidea* (Gottlieb *et al.*, 1985), *Adenophorus periens* (Ranker, 1994), *Daviesia suaveolens* (Young & Brown, 1996) and *Abronia macrocarpa* (Williamson & Werth, 1999).

Seseli farrenyi shows very high genetic polymorphism indices at the population level (see Table 3),

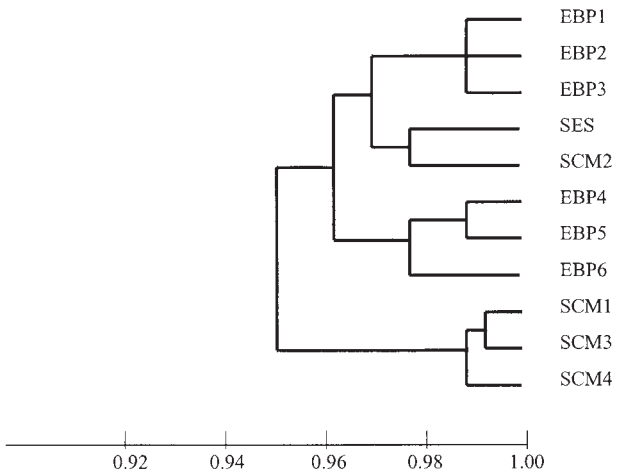

Figure 2. Dendrogram resulting from UPGMA analysis of *Seseli farrenyi* subpopulations based on pairwise values of Nei's (1978) genetic identity. Percentage standard deviation = 1.238, cophenetic correlation = 0.644.

much exceeding those expected for endemic species (P = 26.3%, A = 1.39, H_e = 0.063) and, surprisingly, those expected for widespread species (P = 43.0%, A = 1.72, H_e = 0.159) (Hamrick & Godt, 1990). Other endemic species of the western Mediterranean area, which have recently been studied and which are not reported in the papers cited by Hamrick & Godt, such as *Delphinium bolosii* (Bosch *et al*., 1998), *Petrocoptis montsicciana* (Simon *et al*., 1999), *Artemisia molinieri* (Torrell *et al*., 1999) and several species of *Antirrhinum* (Mateu-Andrés, 1999; Torres, 1999) show high levels of isozymic variability, suggesting both the conservative characteristics of endemism in that region and the need for further extensive genetic surveys of populations to draw a more comprehensive pattern of allozyme diversity in narrow endemic plants. Within the Apiaceae, *S. farrenyi* exhibits more genetic diversity than any other species of the family which have been studied isozymically: *Daucus carota* (P = 45.7%, A = 1.52, H_e = 0.151) (St. Pierre *et al.* 1990), *Aletes acaulis* and *Aletes humilis* (P = 58.4%, A = 2.45, H_e = 0.253 and P = 58.9%, A = 2.3, H_e = 0.235, respectively) (Linhart & Premoli 1993), *Ptilimnium nodosum* (P = 13.2%, A = 1.16, H_e = 0.052) (Kress *et al.* 1994), and several species of *Lomatium*: *L. rollinsii* (P = 18.4%, A = 1.22, H_e = 0.030), *L. serpentinum* (P = 2.8%, A = 1.03, H_e = 0.002), *L. laevigatum* (P = 2.6%, A = 1.02, H_e = 0.008), *L. dissectum* (P = 29%, A = 1.56, H_e = 0.120), *L. grayi* (P = 16.3%, A = 1.23, H_e = 0.056), and *L. triternatum* (P = 39.7%, A = 1.60, H_e = 0.106) (Soltis *et al.*, 1997).

The extent of variation in polymorphism indices among the three populations is very low (P = 78.6 – 85.7, A = 2.6 – 3.3, A_p = 2.6 – 3.5, H_o = 0.100 – 0.137, H_e = 0.285 – 0.305). The smallest population, SES, showed the lowest levels of diversity (P = 78.6%, A = 2.6, A_p = 2.6, H_o = 0.124 and H_e = 0.285), probably due to small population size (only 90 individuals recorded in 1999). One explanation for this is that this population is suffering from a relative bottleneck, although much genetic variation is still retained, because in 1979 at least 500 individuals were reported (Molero & Pujadas, 1979). Given their proximity (0.5 km), the SES population could be a remnant of the fragmentation of the SCM population. This hypothesis is supported by the ancient existence of some individuals between these two populations (Molero, pers. comm.), and their high genetic identity (I = 0.987). The dendrogram of subpopulations (Fig. 2) places the SCM2 subpopulation and the SES population in a cluster. The finding of private alleles in the SCM (four alleles) and EBP populations (two alleles) indicates a relatively ancient isolation between them.

The values of H_o are smaller than those of H_e for all populations, which suggests a certain degree of inbreeding. However, given that in *S. farrenyi* the prevalent mode of breeding is outcrossing (Rovira *et al.*, in prep.), we can attribute the observed levels of inbreeding to possible genetic substructuring of populations (Wahlund effect) and not to the mating system. An analysis of F-values reveals that 28 loci show heterozygote deficiencies while 11 are in Hardy–Weinberg equilibrium, which can explain a Wahlund effect in populations. The assumption of population substructuring is also supported by the hierarchical analysis of F_{ST}.

Distribution of genetic diversity

The mean value of H_T in *S. farrenyi* is relatively high compared with endemic species (0.310 vs. 0.263) (Hamrick & Godt 1990). The values of D_{ST} (0.013) and G_{ST} (0.041) indicate that most of the genetic diversity is distributed within populations and that there is very low divergence among populations, in contrast to expected values for endemic species composed of small, isolated populations (Barrett & Kohn, 1991). The value of Nm is very high (5.85), and is consistent with the low values of G_{ST}; consequently, the possibility of loss of alleles due to genetic drift is minimal. Gene flow in higher plants occurs via seed and pollen dispersal. The distribution of genetic variation results from this dispersal and varies according to the predominant mechanism. McCauley *et al*. (1996) classify plant species into four groups, depending on seed and pollen dispersal distances: (1) large distances in both seed and pollen, (2) small distances in both seed and pollen, (3) high pollen dispersal and low seed dispersal distances, and (4) low pollen dispersal and high seed dispersal distances. Although mechanisms of

seed dispersal are unknown in *S. farrenyi*, seed dispersal distance could be assumed to be high, given the light seed weight and both the high frequency and intensity of the wind in the Cape Creus region (Franquesa, 1995). Seeds of *S. farrenyi* do not have morphological adaptations for its dispersal by anemochory, although differences in seed dispersal distances between adapted and nonadapted seeds to wind are little in Apiaceae (Jongejans & Telenius, 2001). Pollination is provided by small insects such as wasps, solitary bees, ants, beetles and flies (Rovira *et al.*, in prep.) which travel short distances, thus pollen dispersal distances are short. Relatively large distances of seed dispersal and high proximity among populations can explain the high value of *Nm*. The mechanisms to prevent self-pollination (Rovira *et al.*, in prep.) could also contribute to a high *Nm* value. *S. farrenyi* seems to be in the fourth category, where low pollen dispersal implies high rates of selfing as a consequence of frequent crosses between genetically related neighbours, which promotes a deviation from Hardy–Weinberg equilibrium and a spatial aggregation of genetically similar genotypes ('spatial' subpopulations). However, high seed dispersal would reduce the probability of such crosses, thus attenuating the spatial substructure of populations.

We have evidence that only a small proportion of the individuals in populations of *S. farrenyi* (range 10–25%) blooms and sets fruits every year. Consequently, crossings are only possible between individuals with the same phenology, thereby producing 'temporal' neighbourhoods or subpopulations, a phenomenon which has been detected in other Apiaceae (Arús, pers. comm.). The combination of spatial and temporal neighbourhoods provides a complex structuring of subpopulations, which substantially reduces the effective population size of populations of *S. farrenyi* to below threshold levels of minimum viable population size (MVP < 250, see Iriondo, 1996). Surprisingly, despite this, levels of genetic diversity are high, indicating a lack of both genetic bottlenecking and inbreeding depression as a result of crosses between related individuals. To complete our knowledge of population substructuring in this species that results from the confluence of both spatial and temporal components, we must carry out both fine-scale sampling and electrophoretic analysis of the individuals with the same phenology.

Conservation implications

The small number of populations (three), and low values of occupation area (1 km^2) and extent of occurrence (0.47 km^2) have led to the classification of *S. farrenyi* as 'endangered' (EN) (Sáez *et al.*, 1998) according to the criteria of UICN (1994). The low population size (around 2000 individuals) confirms the endangered status of this species, despite its high levels of genetic diversity. Given its high levels of variability, and therefore its high capacity to respond to stochastic events (both ecological and climatic) (Torres, 1999), natural phenomena are not likely to threaten the survival of this plant. However, several anthropogenic threats, such as fires and tourism, can affect the survival of this species. Recently, a fire burnt around 70% of the total area of Cape Creus Natural Park (August, 2000), but it did not affect the distribution area of *S. farrenyi*.

Given the small number of individuals, measures of *in situ* conservation are needed. The three known populations of this species are included in the area of Cape Creus Natural Park (DOGC, 1998) and in a 'PEIN' area (DOGC, 1993), but the measures to ensure their protection are deficient. A more suitable measure would be the creation of a botanical micro-reserve, such as those in other autonomous territories of Spain, for example Valencian Country (DOGV, 1994) and Canary Islands (Francisco-Ortega *et al.*, 2000). Rare alleles are frequent in the three populations (see results), and can easily be lost by genetic drift (Barrett & Kohn, 1991). A reduction in population size leads to a rapid loss of these alleles (Torres, 1999). Thus, a monitoring program of *S. farrenyi* is required to ensure the maintenance of the present population sizes.

The main *ex situ* conservation policy is the maintenance of a seed bank. Seeds from the three populations are conserved in our laboratory at the Faculty of Pharmacy of the University of Barcelona. Two main questions must be addressed in the design of a seed collection strategy: the number of populations to be sampled, and number of seeds to be collected. According to Hamrick *et al.* (1991), two populations can be sampled to preserve 99% of the genetic diversity, while Lawrence *et al.* (1995) report that the effort required to conserve rare alleles is greater than its benefit to the evolutionary potential of the species, and consequently it is only necessary to preserve alleles with a frequency ≥0.05. It has also been reported that a sample of 67 seeds is enough to preserve alleles in frequency ≥0.05 if the germination rate is 100% (Torres, 1999). Our germination tests (56.2% success, cf. López-Pujol, 2000) show that a collection of 119 seeds is enough.

Speculating about causes which help to explain high levels of genetic variation found in *S. farrenyi* is difficult, given the current knowledge of the species. There are no records (from literature or herbarium specimens) suggesting that *S. farrenyi* was once more widespread, which could be an explanation to its great allozymic variation. Other reasons may be involved, such as the evolutionary history of this taxon. The relationship of *S. farrenyi* with other *Seseli* species

with similar morphology and ecology (*S. praecox* and *S. bocconi*), distributed in Mediterranean islands (Corsica, Sardinia), needs further investigation but molecular approaches to phylogeny and genetic diversity could be helpful to better understand the patterns of diversity of this complex of western Mediterranean forms. Finally, the study of intrinsic biological or genetic factors leading to high genetic diversity (mutation rates, crossing strategies, pre- or postzygotic selection, role of rosettes in conservation of genetic diversity with time, etc.) and additional molecular estimations of diversity also need to be developed in the future.

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Population genetics and conservation priorities for the critically endangered island endemic *Delphinium pentagynum* subsp. *formenteranum* (Ranunculaceae)

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Abstract. Isozyme electrophoresis was used to evaluate levels of genetic diversity and population genetic structure of the critically endangered (CR) perennial larkspur, *Delphinium pentagynum* subsp. *formenteranum* (Ranunculaceae), endemic to the island of Formentera (Balearic Islands, Spain). There is only one known population for this taxon, containing only 480 individuals. Moderate values of diversity were detected ($P = 40.7\%$, $A = 1.6$ and $H_e = 0.180$), within the range observed in other surveyed larkspurs, but higher than most island endemics. Moderate levels of inbreeding were detected, probably as a consequence of the population's genetic structuring (biparental inbreeding). Threats to this taxon are mainly anthropogenic (fires, grazing, pathway works, and building pressures), although given that only one population exists, stochastic risks cannot be ignored. Conservation of *D. pentagynum* subsp. *formenteranum* requires *in situ* strategies, such as implementing a monitoring program and establishing a 'botanical reserve', and *ex situ* strategies, such as collection and long-term storage of seeds.

Introduction

Endemic and rare taxa often show lower levels of genetic diversity than widespread species (Stebbins 1942; Falk and Holsinger 1991; Hamrick and Godt 1996), and this observation may be a consequence of their small population size and population isolation (Barrett and Kohn 1991; Ellstrand and Elam 1993; Neel and Ellstrand 2001). Such characteristics lead to the erosion of genetic variation and an increase in genetic divergence among populations, due to increased genetic drift, inbreeding, and reduced gene flow (Young et al. 1996). Today, it is assumed that island plants exhibit lower levels of genetic variation than mainland species. Likewise endemic island plants tend to show less genetic diversity than non-endemic island plants with mainland populations (Frankham 1997, 1998). There are several reasons for this genetic depauperation in endemic island species: (i) bottlenecks associated with colonization events and founder effects (either with or without speciation); (ii) species on islands often occur in very few and small populations, which leads to

inbreeding and drift; and (iii) adaptation to island environments, which includes loss of dispersal ability, limited capability to avoid predators or competitors, and lower disease resistance (Frankham 1998; Crawford et al. 2001). Although examples of clear depauperation in island endemic plants can be found in the literature (DeJoode and Wendel 1992; Crawford et al. 2001; Maki 2001), some studies have shown notable exceptions (Weller et al. 1996; Francisco-Ortega et al. 2000; Helenurm 2001; Hiramatsu et al. 2001).

There are very few studies on the genetic diversity of Mediterranean island endemic taxa, despite the Mediterranean being plentiful with them. The Mediterranean basin is currently considered to be one of the 25 world biodiversity 'hotspots' due to the exceptional concentrations of endemic species and the equally exceptional loss of habitat (Myers et al. 2000). For example, endemic species represent 7% of the Balearic Islands flora, 8% in Corsica and 8.6% in Crete (Affre et al. 1997). Allozyme surveys of endemic island species (or at least species which have island populations) are scarce and include those of *Cyclamen balearicum* (Affre and Thompson 1997), *Cyclamen creticum* (Affre et al. 1997), *Cytisus villosus* (Troìa et al. 1997), *Pinus brutia* (Panetsos et al. 1998), several taxa of the *Brassica oleracea* group (Lázaro and Aguinagalde 1998), *Lolium perenne* (Balfourier and Charmet 1994), *Lolium rigidum* (Bennett and Hayward 1999), *Lysimachia minoricensis* (Ibáñez et al. 1999), *Brassica insularis* (Petit et al. 2001) and some taxa of *Dactylorhiza* (Bullini et al. 2001).

Delphinium ser. *Pentagyna* B. Pawl. (Ranunculaceae) is a series restricted to the western Mediterranean basin that includes three species, *D. pentagynum* Lam., *D. emarginatum* C.B. Presl. and *D. sylvaticum* Pomel, as well as many lower taxa (Blanché 1991). The assumed speciation pattern of this group is based mainly on geographical and ecological isolation (Blanché et al. 1996). Puget et al. (1995) reported finding a single population of *D. pentagynum* on the island of Formentera (Balearic Islands, Spain), which was regarded some years later as a subspecies of the former, i.e. *D. pentagynum* subsp. *formenteranum* N. Torres, L. Sáez, Roselló and C. Blanché (Torres et al. 2000). This taxon differs from typical *D. pentagynum* in having narrower sepals and smaller corolla, spur, and follicles, characteristics which suggest an adaptation to a dry insular habitat (Torres et al. 2000).

This subspecies, the only known population at the time it was found, is located in Torrent de Cala Saona (UTM 31SCC68), in the southeast of Formentera (Figure 1). This island, about 85 km^2, belongs to the Pytiusic archipelago, the westernmost Balearic islands. However, in 2001 we located two additional close-by sites, which enlarged the population size to 480 individuals. The extent of occurrence is extremely low, about 0.10 km^2, according to IUCN criteria (IUCN 2001). This taxon has been listed as 'critically endangered' (CR) both in the Red List of Vascular Spanish Flora (Aizpuru et al. 2000) and in the Red Book of the Balearic Islands Vascular Flora (Sáez and Rosselló 2001).

Allozyme electrophoresis was used to evaluate levels of genetic diversity in this only known population of *D. pentagynum* subsp. *formenteranum*. Having observed its patchy distribution in the field, we also decided to analyse its genetic structure. The data obtained will provide important information about the taxon's state of

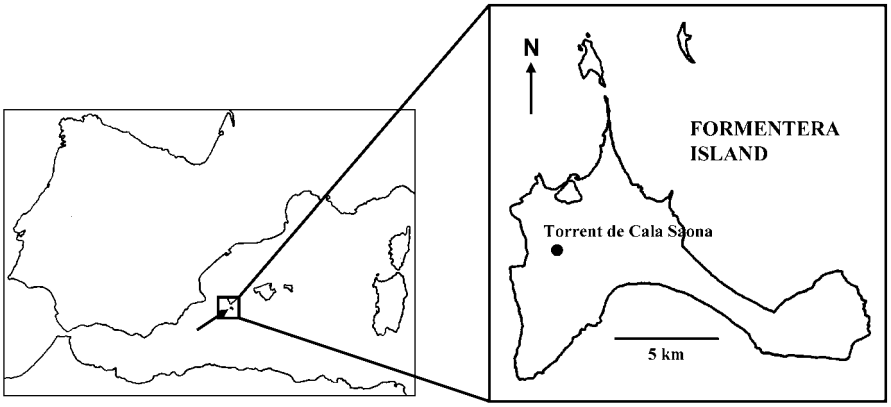

Figure 1. Location of the only known population of *Delphinium pentagynum* subsp. *formenteranum* in Formentera, Pytiusic archipelago (Balearic Islands, Spain).

conservation and a better understanding of its threats. Priorities and strategies for its preservation are also suggested.

Materials and methods

Plant material

Delphinium pentagynum subsp. *formenteranum* is a perennial herbaceous larkspur, (20) 40–70 (100) cm tall with palmatipartite leaves. It produces a basal rosette and (3) 4–6 (7) violet–blue flowers on a lax racemose inflorescence. Seeds are subpyramidal, 1.2–1.4 (1.5) mm long. It is a diploid species of $2n = 16$ (Torres et al. 2000) and little is known about its reproductive system. The taxonomically closest species are outcrossing (Bosch 1999). Pollination is entomophilous, as in other *Delphinium* species (Bosch et al. 1997; Bosch and Waser 1999; Williams et al. 2001). It grows in open dry scrub habitat (‘garriga’) on calcareous substrate.

Sampling design

The population of *D. pentagynum* subsp. *formenteranum* is subdivided into two main subpopulations separated by about 70 m, which we named ‘Central’ and ‘Eastern’ as well as a subpopulation termed ‘Western’, located about 600 m from the Central subpopulation. The Central subpopulation, the site originally found in 1995, contained 345 individuals, while Eastern had 132 and Western only had three individuals. As plants were distributed in patches within subpopulations, sampling of individuals was done in all the patches found (seven in the Central subpopulation, three in Eastern and only one in Western). Sample size varied according to patch size, although all individuals were sampled in small patches. In total, we sampled

119 mature (reproductive) individuals, which accounted for about 50% of the total number of adult reproductive plants. Sampling was performed in May 2001, and samples consisted of young leaves which were placed into envelopes, transported to the laboratory, and stored at 4 °C until extraction 1 or 2 days later. Collection of samples was done carefully to minimize damage to the population.

Electrophoresis

Genetic diversity was assessed using standard methods for starch gel electrophoresis of allozymes (Soltis et al. 1983; Soltis and Soltis 1989; Kephart 1990). Leaf fragments were homogenized on refrigerated porcelain plates using a cold extraction buffer consisting of 0.05 M tris-citric acid, 0.1% cysteine·HCl, 0.1% ascorbic acid, 8% PVP-40 and 1 mM 2-mercaptoethanol. Extracts were absorbed onto 3 mm Whatman filter paper, to be either analysed immediately or stored at −20 °C until analysis 1 or 2 days later. Using 12.5% starch gels, 17 enzymes were tested and six of these were satisfactorily resolved, obtaining nine interpretable loci: *Aat-1*, *Acp-3*, *Dia-1*, *Dia-2*, *Mdh-1*, *Mdh-3*, *6Pgd-1*, *6Pgd-2* and *Pgm-2*. *Aat-2* appeared highly variable, but was not interpretable. ACO, ME and PGI showed activity, but were not scorable due to poor or inconsistent resolution. An apparent, but uninterpretable gene duplication was detected in *Pgm-1*. Aspartate aminotransferase (AAT, EC 2.6.1.1) and diaphorase (DIA, EC 1.6.99.-) were satisfactorily resolved in tris-citrate/lithium-borate buffer pH 8.2 (Scandalios 1969); acid phosphatase (ACP, EC 3.1.3.2) and phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44) were resolved in morpholine buffer pH 6.1 (Odrzykoski and Gottlieb 1984); and malate dehydrogenase (MDH, EC 1.1.1.37) and phosphoglucomutase (PGM, EC 5.4.2.2) were resolved in histidine–citrate buffer pH 5.7 (Jefferies and Gottlieb 1982). Staining procedures for all enzymes followed the method described by Vallejos (1983), Shields et al. (1983), and Wendel and Weeden (1989), with slight modifications.

Genetic analyses

Loci were numbered consecutively and alleles at each locus were labelled alphabetically, beginning from the most anodal form in both cases. Isozyme phenotypes were interpreted genetically according to standard principles (Wendel and Weeden 1989). Allozyme frequencies at each locus were calculated for each subpopulation. To estimate the levels of genetic diversity, the following statistics were computed: P, the percentage of polymorphic loci when the most common allele had a frequency of <0.95; 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. The mean fixation index (F) for all polymorphic loci in each subpopulation was also computed to compare genotype proportions with those expected under Hardy–Weinberg equilibrium. The chi-square test (χ^2) was used to evaluate deviations of F from zero, with Levene's (1949) correction for small sample size. Population structure was analysed using Wright's (1965) F-statistics: F_{IS} measures levels of inbreeding within subpopulations, F_{ST} measures inbreeding

due to differentiation among subpopulations, and F_{IT} measures overall levels of inbreeding. Gene flow was determined using Wright's (1951) equation modified by Crow and Aoki (1984): $Nm = 1/4\alpha\ [(1/F_{ST}) - 1]$, where $\alpha = [n/(n-1)]^2$, n is the number of subpopulations and Nm is the average number of migrants exchanged per generation. A second estimate of gene flow was based on the frequency of private alleles (alleles found in a single population), using Slatkin's (1985) method, based on the following equation: $\ln(\bar{p}(1)) = a \ln(Nm) + b$, where $\bar{p}(1)$ is the mean frequency of private alleles, and a and b are constants determined by simulated data developed for a sample size of 25 (Slatkin 1985), equal to -0.505 and -2.440, respectively. The estimate of Nm was corrected for the *D. pentagynum* subsp. *formenteranum* mean sample size (39.7). Hierarchical F_{ST} analysis (Wright 1978) was also used to evaluate the pattern of genetic structure in patches and subpopulations. We also calculated Nei's (1978) genetic identity (I) between pairs of subpopulations and pairs of patches. Finally, Mantel's test (Mantel 1967) was applied to values of Nei's genetic identity (I) and geographical interpatch distances. Calculations of all parameters were done with BIOSYS-1 (Swofford and Selander 1989), and Mantel's test was calculated using the NTSYS package (Rohlf 1994).

Results

Sixteen alleles were detected among the nine interpretable loci, and their frequencies were calculated (Table 1). The richest subpopulation, where all 16 alleles were observed, was Central, while 15 were observed in Eastern and 13 were observed in Western. Only the Central subpopulation had a private allele, *Pgm-2a*. Rare alleles (those with frequencies <0.05) were found only in the Central subpopulation (*Dia-1a*, *Dia-2b*, and *Pgm-2a*), although two of them appeared in very low frequencies (<0.01). Four loci (*Acp-3*, *Mdh-1*, *6Pgd-1*, and *6Pgd-2*) were monomorphic across all subpopulations, while the other loci were polymorphic in at least one of the three subpopulations. *Pgm-2* was the only locus with four alleles; the remaining polymorphic loci only had two alleles. There were no substantial differences in allele frequencies among the three subpopulations, with the exception of *Pgm-2*.

Genetic variability was quantified for each of the three subpopulations using the main indices of polymorphism (Table 2). Mean values for *D. pentagynum* subsp. *formeneranum* at the population level were: $P = 40.7\%$, $A = 1.6$, $A_p = 2.4$, and $H_e = 0.180$. Values of P ranged from 33.3 to 55.6%, A ranged from 1.4 to 1.8, A_p ranged from 2.2 to 2.7, and H_e ranged from 0.152 to 0.222. At the species level, values of genetic variation were the following: $P = 55.5\%$, $A = 1.8$, $A_p = 2.4$, and $H_e = 0.172$.

Values of observed heterozygosity were lower than those of expected panmictic heterozygosity in all subpopulations (Table 2). Heterozygote deficiency was also detected from values of F for all polymorphic loci in each subpopulation (Table 3). The χ^2 test showed that 9 of the 13 F-values conformed to Hardy–Weinberg proportions ($P \geq 0.05$), while the remaining four were significantly greater than

Table 1. Allele frequencies for nine loci in the three subpopulations of *Delphinium pentagynum* subsp. *formenteranum*.

Locus	Allele	Subpopulations		
		Western	Central	Eastern
Aat-1	*a*	0.500	**0.714**	**0.575**
	b	0.500	0.286	0.425
Acp-3	*a*	1.000	1.000	1.000
Dia-1	*a*	0.000	0.005	0.132
	b	**1.000**	**0.995**	**0.868**
Dia-2	*a*	**0.750**	**0.994**	**0.941**
	b	0.250	0.006	0.059
Mdh-1	*a*	1.000	1.000	1.000
Mdh-3	*a*	0.000	0.149	0.053
	b	**1.000**	**0.851**	**0.947**
6Pgd-1	*a*	1.000	1.000	1.000
6Pgd-2	*a*	1.000	1.000	1.000
Pgm-2	*a*	0.000	0.038	0.000
	b	0.250	0.366	0.250
	c	**0.500**	0.231	0.125
	d	0.250	0.366	**0.625**

The most frequent allele for each locus and subpopulation is in boldface.

Table 2. Summary of genetic variation for nine loci in the three subpopulations of *Delphinium pentagynum* subsp. *formenteranum*.

Subpopulation	Size	*N*	*P*	*A*	A_p	H_o	H_e
Western	3	2.0	33.3	1.4	2.3	0.167	0.222
Central	345	95.0	33.3	1.8	2.7	0.094	0.152
Eastern	132	19.4	55.6	1.7	2.2	0.114	0.166
Mean		38.8	40.7	1.6	2.4	0.125	0.180
Standard deviation		49.4	12.9	0.2	0.3	0.038	0.037
Species level			55.5	1.8	2.4		0.172

N: sample size; *P*: percentage of polymorphic loci; *A*: mean number of alleles per locus; A_p: mean number of alleles per polymorphic locus; H_o: observed heterozygosity; H_e: expected panmictic heterozygosity.

zero ($P < 0.05$), indicating deficiency of heterozygotes at these loci, as all these values were positive. Values of F_{IS} (mean value of $F_{IS} = 0.358$) also indicated moderate levels of inbreeding within subpopulations. Heterozygote deficiencies found at some loci (4 of 13) cannot be explained by chance alone or by intensive inbreeding, as we would thus expect heterozygote deficiencies at all loci. Rather, they suggest that subpopulations of this taxon may be genetically structured, i.e. composed of minor units (Mayes et al. 1998; Williamson and Werth 1999; Batista et al. 2001). Nevertheless, some degree of selfing cannot be discarded to interpret the heterozygote deficiency. In order to evaluate the degree and pattern of genetic structure in the case of subpopulations into patches, we carried out a hierarchical F_{ST} analysis, with a designated hierarchy consisting of subpopulations and patches (Table 4). Although divergence among patches with respect to the total was low

Table 3. Values of fixation index (F) for all polymorphic loci in the three subpopulations of *Delphinium pentagynum* subsp. *formenteranum*.

Locus	Western	Central	Eastern
Aat-1	1.000 ns	0.669***	0.693**
Dia-1	–	−0.005 ns	0.309 ns
Dia-2	−0.333 ns	−0.006 ns	−0.062 ns
Mdh-3	–	1.000***	1.000***
Pgm-2	−0.600 ns	−0.015 ns	−0.129 ns

Conformance to Hardy–Weinberg equilibrium was tested using χ^2 analysis: ns: $P \geq 0.05$; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Table 4. Hierarchical F_{ST} analysis in *Delphinium pentagynum* subsp. *formenteranum*.

Comparison		F_{XY}					
X	Y	*Aat-1*	*Dia-1*	*Dia-2*	*Mdh-3*	*Pgm-2*	Across loci
Patch	Subpopulation	0.066	0.413	0.000	0.029	0.007	0.052
Patch	Total	0.044	0.410	0.110	0.044	0.042	0.068
Subpopulation	Total	−0.023	−0.007	0.110	0.015	0.035	0.016

($F_{XY} = 0.068$), the greatest amount was due to variance among patches within subpopulations ($F_{XY} = 0.052$) rather than to variance among subpopulations with respect to the total ($F_{XY} = 0.016$). These results indicate a slight genetic structuring of subpopulations into patches.

Genetic divergence among subpopulations was quantified by computing the F_{ST} parameter, an indicator of the genetic differentiation among subpopulations, and Nei's genetic identity (I). The mean value of F_{ST} was very low (0.023) with respect to the mean value of F_{IT} (0.373), indicating that only a small fraction of the taxon's total genetic variability is attributable to differentiation among the three subpopulations (Table 5). Wright's modified method (Crow and Aoki 1984) gives an estimate of Nm of 4.72, a value slightly higher than using Slatkin's (1985) method ($Nm = 3.26$). These values are really high, indicating a substantial interchange of genes among subpopulations. A Nm value of 1.0 is considered high enough to prevent divergence by genetic drift (Wright 1951). Values for I (mean = 0.994, range: 0.982–1.000) were extremely high between pairs of subpopulations, as expected for geographically close units without physical barriers (see Materials and methods). Western and Central subpopulations were genetically identical ($I = 1.000$). Values for I between patches were also high (mean = 0.986, range: 0.924–1.000). The Western patch was identical ($I = 1.000$) to some patches of the Central population, which would suggest, as pointed out above, that Western individuals come from the Central subpopulation (Table 6). Identities between patches of the Central subpopulation were very high, but they differed somewhat from Eastern patches. Mantel's test was applied for values of I and geographical interpatch distances, and showed a non-significant correlation between the two ($r = 0.20$; $P = 0.763$). Nor was a correlation found between the Central patches alone and their geographical distances ($r = -0.22$, $P = 0.176$). This indicates that there are no general restrictions

Table 5. Estimates of *F*-statistics for all polymorphic loci in the three subpopulations of *Delphinium pentagynum* subsp. *formenteranum*.

Locus	F_{IS}	F_{ST}	F_{IT}
Aat-1	0.680	0.015	0.685
Dia-1	0.250	0.087	0.316
Dia-2	−0.103	0.076	−0.019
Mdh-3	1.000	0.014	1.000
Pgm-2	−0.041	0.023	−0.017
Mean	0.358	0.023	0.373

F_{IS}: levels of inbreeding within subpopulations; F_{ST}: differentiation among subpopulations; F_{IT}: overall levels of inbreeding.

Table 6. Matrix of Nei's (1978) genetic identity between patches of *Delphinium pentagynum* subsp. *formenteranum*.

Patch	W	C1	C2	C3	C4	C5	C6	C7	E1	E2	E3
W											
C1	1.000	–									
C2	1.000	0.999	–								
C3	1.000	1.000	1.000	–							
C4	0.996	0.986	1.000	0.990	–						
C5	1.000	1.000	1.000	1.000	0.998	–					
C6	1.000	0.984	1.000	1.000	0.995	0.994	–				
C7	1.000	1.000	1.000	1.000	0.999	1.000	0.997	–			
E1	0.981	0.965	0.947	0.972	0.924	0.956	0.949	0.956	–		
E2	0.987	0.995	0.986	0.990	0.982	0.985	0.970	0.999	0.945	–	
E3	1.000	0.986	0.977	0.997	0.958	0.987	0.973	0.977	0.974	0.957	–

W: Western; C: Central; E: Eastern; Number: patch number.

to gene flow among patches, despite the slight genetic structuring detected within subpopulations (Table 4). Further research should be aimed at ascertaining pollen and seed dispersal patterns in order to provide a better understanding of how this structure could be created and its extent within subpopulations.

Discussion

Genetic diversity in Delphinium pentagynum *subsp.* formenteranum

Delphinium pentagynum subsp. *formenteranum* exhibits moderate to high levels of genetic diversity, higher than the average values at the population level reported for endemic species ($P = 26.3\%$, $A = 1.39$ and $H_e = 0.063$) and closer to those for widespread taxa ($P = 43\%$, $A = 1.72$ and $H_e = 0.159$) (Hamrick and Godt 1990, 1996). Island plants are expected to exhibit low levels of allozyme diversity (Frankham 1997). In a review of nearly 70 island endemic species, mainly from Pacific archipelagos, DeJoode and Wendel (1992) found reduced levels of genetic diversity ($P = 25\%$, $A = 1.32$ and $H_e = 0.064$). Low values were also found in a

Table 7. Genetic diversity in *Delphinium pentagynum* subsp. *formenteranum* and in other studied perennial larkspurs.

Taxa	*P*	*A*	H_e	Reference
D. bolosii	41.6	1.6	0.117	Bosch et al. (1998)
D. decorum	40.9	1.9	0.161	Koontz et al. (2001)
D. luteum[a]	69.2	1.8	0.211	Koontz et al. (2001)
D. montanum[b]	92.8	2.4	0.307	Simon et al. (2001)
D. nudicaule	65.0	2.0	0.295	Koontz et al. (2001)
D. variegatum subsp. *kinkiense* and subsp. *thornei*[c]	24.5	1.3	0.074	Dodd and Helenurm (2002)
D. variegatum subsp. *variegatum*	33.6	1.5	0.064	Dodd and Helenurm (2002)
D. viridescens	35.3	1.6	0.119	Richter et al. (1994)
D. pentagynum subsp. *formenteranum*	40.7	1.6	0.180	

P: percentage of polymorphic loci; *A*: mean number of alleles per locus; H_e: expected panmictic heterozygosity. All values given are population means. [a] Wild population only is considered; [b] this species is tetraploid; [c] the two subspecies have been combined in the analysis.

survey of 29 species endemic to the Juan Fernández Islands ($P = 21\%$ and $H_e = 0.044$; Crawford et al. 2001). In contrast, a survey of diversity of 69 endemic species from the Canary islands found very high levels of diversity ($H_e = 0.186$; Francisco-Ortega et al. 2000). Levels of genetic variation of *D. pentagynum* subsp. *formenteranum* are within the range of other perennial larkspurs (see Table 7), and this lends support to Gitzendanner and Soltis's (2000) argument that genetic diversity in plant congeners is often correlated.

One possible explanation for the relatively high levels of diversity found in *D. pentagynum* subsp. *formenteranum* could be the ability of individuals to remain dormant for many years (as seed or rootstock bank), as is the case for other *Delphinium* species (e.g. Lewis and Epling 1959; Koontz et al. 2001). This strategy allows genetically different individuals to be present at different years. What is clear is that *D. pentagynum* subsp. *formenteranum* is able to maintain some individuals (around 50%) at the rosette stage without blooming every year, as reported for *Delphinium bolosii* (Bosch 1999), and this may contribute to the strategy described above, having a positive effect on genetic diversity. This phenomenon, although reducing the number of mates in a population at a given year as a result of climatic constraints or other factors, may increase the overall effective population size (N_e) along time, because it allows crossings between different individuals at several years, maximizing the likelihood of crossings between all the individuals of the population.

Other explanations for the moderate to high diversity levels of this taxon are related to its historical traits. Island taxa in the Mediterranean have originated mainly via two well-known processes: (i) island isolation as a result of changes at sea level, and (ii) long-distance dispersal from the mainland (Thompson 1999). The population of Formentera is far (about 300 km) from the closest *D. pentagynum* s.l. populations recorded in the Iberian Peninsula and North Africa (Morocco and Algeria; Torres et al. 2000), lands to which Formentera could have been connected during the repeated drying in the Messinian salinity crisis (Bocquet et al. 1978), between 5.7 and 5.35 Mya (Gautier et al. 1994). At that time, land connections

between the Balearic islands were also present (Hsü et al. 1973), such that *D. pentagynum* could have reached other islands of the archipelago. However, *D. pentagynum* subsp. *formenteranum* has so far not been found in the other Balearic islands. The present geographic distribution of the *D. pentagynum* group does not, therefore, support the hypothesis that it is a remnant of a former continuous range for the Formentera population.

An old colonization by a single event of long-distance dispersal and subsequent island isolation is the most likely evolutionary origin of *D. pentagynum* subsp. *formenteranum*. Bottlenecks, and subsequent low population sizes, are associated with the establishment of colonizing ancestors on the island and the founding of new populations. As a consequence of these processes, inbreeding and genetic drift are expected to occur within populations (Frankham 1997, 1998; Crawford et al. 2001). In this study, we postulate an older origin of *D. pentagynum* subsp. *formenteranum* through a colonization event, which would have recovered genetic diversity following the founder effect by a process of speciation that seems to be in progress today. A peripatric speciation process may take place if the reduction of genetic diversity at foundation is followed by an increase in variability resulting from the appearance of new, locally evolved alleles (Mayr 1942). This type of speciation process is consistent with the moderate levels of diversity found in *D. pentagynum* subsp. *formenteranum*, in line with those of other larkspurs (Table 7), and the moderate levels of inbreeding detected, probably as a consequence of the slight genetic structuring found (biparental inbreeding) and some degree of selfing. Speciation based mainly on geographical and/or ecological isolation has already been suggested for series *Pentagyna* (Blanché et al. 1996), and in *D. pentagynum* subsp. *formenteranum* this could occur through the quantitative reduction of plant parts as an adaptive strategy to conditions of moderate to extreme drought, a phenomenon which has been previously described for other Mediterranean taxa of the tribe *Delphinieae* (Blanché et al. 1997; Bosch et al. 1997).

Conservation priorities

Island populations and insular endemics are more prone to extinction than are mainland populations or species, due mainly to genetic factors (inbreeding depression, loss of genetic variation, and genetic adaptation to island conditions) or to their interactions with demographic or environmental stochasticity, which are more severe than on the mainland (Frankham 1997, 1998). Furthermore, island populations are often small and few, as is the case for *D. pentagynum* subsp. *formenteranum*, and it is generally expected that small populations are more likely than larger ones to become extinct (Menges 1991). One of the major goals in the conservation of small and threatened populations is estimating the minimum population size necessary to have an acceptably low extinction probability, known as the minimum viable population (MVP) (Menges 1991). One MVP of the order of 10^3–10^6 individuals is enough to buffer environmental stochasticity and natural catastrophes, but this number falls to between 50 and 500 in terms of counteracting genetic stochasticity and to only 50 for preventing demographic stochasticity (Menges 1991). The size of the only known population of *D. pentagynum* subsp.

formenteranum is 480 individuals, but the effective population size (N_e) in terms of mature individuals is as low as 247, a number on the borderline of MVP estimates for countering genetic stochasticity, and which may be lower if there is differential reproductive success among the adults.

Plant species survival can also be affected by anthropogenic threats. The only population of *D. pentagynum* subsp. *formenteranum* is very close to a rural pathway which leads to a rubbish dump and some holiday homes. Work aimed at widening this pathway has increased the amount of earth near the plants, and machinery (bulldozers) has sometimes been parked beside the population. Evidence of grazing has also been observed inside the population, in the form of ovine excrement and bites on plants. Fires are a real risk to the survival of this taxon, and in 1996 a local fire affected a dry pasture only a few hundred metres from the population. Speculation on the land classification could also lead to the burning of this zone (for building purposes), an illegal practice that is very common on the Mediterranean Iberian coast and Balearic Islands (WWF 2002).

Given the status of this taxon, both *in situ* and *ex situ* measures are needed. The most obvious *in situ* measure is the protection of its habitat, maintaining levels of genetic diversity and the existing interactions between the species and its ecosystem without detaining evolutionary processes (Falk and Holsinger 1991). Genetic variation is the basis for potential evolutionary change in a taxon, enabling populations to adapt to changing environments and making them less susceptible to pest and disease pressures (Barrett and Kohn 1991). A more suitable measure would be the creation of a 'botanical reserve', such as those which already exist in other autonomous territories of Spain, for example, Valencian Country (Akeroyd 1998). This would enclose all subpopulations and patches, including the Western subpopulation (which contains only three individuals). The western subpopulation may have originated following dispersion of a genotype from the Central subpopulation (their genetic identity is 1.000), but at present there are no apparent trails that would aid in dispersal in that direction. Also, it may be a remnant of a fragmentation event (like the other two subpopulations) which affected an ancient larger population of *D. pentagynum* subsp. *formenteranum*. In any case a monitoring program is urgently required to ensure that the present population size is maintained.

In addition to genetic data, any plan to introduce or reintroduce *D. pentagynum* subsp. *formenteranum* will require data on the autoecology of the species, its demography, environmental requirements, pollination biology, reproductive system, and seed dispersal, among others (e.g. Koontz et al. 2001). The main *ex situ* conservation policy is maintenance of a germplasm bank. Given the limited area that this taxon occupies, the collection of seeds from all subpopulations is recommended in order to preserve all existing variability. Germination rates are still unknown at the moment, but are expected to be high, due to the good germination rates found in other species and subspecies of series *Pentagyna* (Bosch 1999).

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3.1.5. **López-Pujol J**, Bosch M, Simon J & Blanché C. 2004. Allozyme diversity in the tetraploid endemic *Thymus loscosii* (Lamiaceae). *Annals of Botany*, 93: 323-332.

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Allozyme Diversity in the Tetraploid Endemic *Thymus loscosii* (Lamiaceae)

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• *Background and Aims Thymus loscosii* (Lamiaceae) is a tetraploid perennial species endemic to the Ebro river basin (north-eastern Spain), which is included in the *National Catalogue of Endangered Species*. It is a tetraploid species ($2n = 54$), presumably an autotetraploid originated by the duplication of a $2n = 28$ genome and the subsequent loss of two chromosomes. Allozyme electrophoresis was conducted to survey the levels and distribution of genetic diversity and to test the previous autopolyploid hypothesis for its origin. In addition, both *in situ* and *ex situ* conservation measures are proposed.
• *Methods* Eight populations were sampled for analysis by standard methods of starch gel electrophoresis, and six putative enzymatic loci were resolved (five consistently and one only partially).
• *Key Results* Banding patterns exhibited no evidence of fixed heterozygosity and showed both balanced and unbalanced heterozygotes. In addition, most individuals showed a pattern consistent with the presence of three or four alleles at a single locus. High levels of genetic variability were found at population level (P = 85 %, A = 3·0, H_e = 0·422), in addition to a trend of an excess of heterozygotes.
• *Conclusions* Allozyme data support the hypothesis that *T. loscosii* is an autotetraploid, and the high number of alleles at some loci may be due to repeated polyploidization events. The high values of genetic variation found in this species agree with those expected for tetraploids. The excess of heterozygotes may be due to some barriers to inbreeding (e.g. occurrence of gynodioecy) and/or selection for heterozygosity.

Key words: Allozyme electrophoresis, genetic diversity, conservation, endemic species, tetraploidy, Lamiaceae, *Thymus loscosii*.

INTRODUCTION

Polyploidy is considered a significant mode of species formation and an important source of evolution in higher plants (Stebbins, 1980). In fact, estimates of the frequency of polyploidy in angiosperms vary from 30 % to 80 % (Otto and Whitton, 2000; Soltis and Soltis, 2000). The success of polyploidy can be explained by several factors such as increased heterozygosity and genetic diversity, enzyme multiplicity and increased biochemical diversity (Levin, 1983; Soltis and Soltis, 2000) and their establishment and commonness in nature may be favoured by vegetative (asexual) multiplication and a perennial habit (Otto and Whitton, 2000; Soltis and Soltis, 2000). Moreover, polyploids have a broader ecological amplitude than the parental diploids and a better colonizing ability, which allows for an increased availability of new ecological niches (Ramsey and Schemske, 1998; Soltis and Soltis, 2000).

Two major types of polyploidy are recognized in nature, allopolyploidy and autopolyploidy (Stebbins, 1947; Crawford, 1989; Soltis and Soltis, 2000), although these two terms represent the ends of a spectrum of variation. Allopolyploidy is thought to be the result of an interspecific hybridization process and subsequent chromosome duplication, while autopolyploidy is probably the consequence of polyploidization of conspecific individuals, generally by fusion of non-reduced gametes. Nevertheless, both allo- and autopolyploids may also be formed via a triploid bridge, in which there takes place a union of a reduced and an unreduced gamete (Ramsey and Schemske, 1998). Unfortunately, because allopolyploidy and autopolyploidy are often equated with interspecific and intraspecific polyploidy, respectively, classification of polyploids may also depend on species circumscription (Ramsey and Schemske, 1998).

Allopolyploids are characterized by disomic inheritance, bivalent formation at meiosis and fixed heterozygosity (non-segregating) due to the combination of two divergent parental genomes. In contrast, autopolyploids are expected to express polysomic inheritance (tetrasomic in autotetraploids) and may also show multivalent formation at meiosis. Two types of heterozygotes may be formed: balanced and unbalanced.

Thymus loscosii Willk. (Lamiaceae) is endemic to the Ebro river basin in north-eastern Spain. The classification of *Thymus* has long been considered difficult (Jalas, 1972; Morales, 1986), and *T. loscosii* has been included in three different sections, namely *Serpyllum* (Miller) Bentham, *Hyphodromi* (A. Kerner) Hálacsy and *Thymus*. The inclusion in the latter is the most widely recognized today (Greuter *et al*., 1986; Morales, 1986). *Thymus loscosii* is a tetraploid ($2n = 54$; Morales, 1986), presumably an autotetraploid, which probably originated by the duplication of a $2n = 28$ genome and the subsequent loss of two

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entomophilous, as in most *Thymus* species (Morales, 1986). The main pollinators are *Apis mellifera* and some species of *Bombylius*. It is gynodioecious, as for most species of *Thymus* (Morales, 1986; Manicacci *et al*., 1998). Despite being a self-compatible species, self-pollination is rare. The germination rate is low: 0 % reported by Morales (1986) and Sainz *et al*. (1996), 2 % by our group (Bosch *et al*., 2002). However, other researchers found higher rates under different temperature treatments, although with remarkable variation among populations (Albert *et al*., 2002). There is no active fruit dispersal; the nutlets are probably dispersed by gravity and/or surface water movements. *Thymus loscosii* might have a combined strategy of sexual and asexual reproduction, since vegetative propagation of stolons has been observed in the field. *Thymus loscosii* grows at 130–1010 m, in open sites in alkaline or saline loam substrates.

Populations of *T. loscosii* are located in several autonomous territories of Spain (Aragon, Castilla-León, Catalonia, Euskadi, La Rioja and Navarra), comprising 52 UTM 10 × 10 km squares (Blanché *et al*., 2000). The total number of individuals is probably greater than one million and the extent of occurrence is approx. 28 000 km^2. The species was included in the *Catálogo Nacional de Especies Amenazadas* (*National Catalogue of Endangered Species*) as endangered (Boletín Oficial del Estado, 1990), and at regional level is listed as rare in Aragon (Sainz *et al*., 1996), vulnerable in Navarra (Boletín Oficial de Navarra, 1997), and of special interest in Euskadi (Nekazal Ikerketa Eta Teknologia, 2002). However, the increasing number of new localities found in recent years has led to the exclusion of this species from the recent *Lista Roja de la Flora Vascular Española* (*Red List of the Vascular Spanish Flora*) (Aizpuru *et al*., 2000).

Allozyme electrophoresis was used to address the following issues: (*a*) whether there is evidence of autopolyploidy in *T. loscosii*, and (*b*) to describe the levels and distribution of genetic diversity. In addition, inferences are made about the status of conservation of this species together with some suggestions for strategies for its preservation.

MATERIALS AND METHODS

Sampling strategy

The study was focused in Catalonia (where six populations were sampled), but two additional populations were also sampled (one each from Aragon and Euskadi) to cover the entire geographic range of *Thymus loscosii* (Table 1 and Fig. 1). All populations were sampled in January–March 2001, and about 30 samples per population were collected. Sampling was conducted along a linear transect within each population and samples were collected about 50–100 cm apart to avoid collecting ramets from the same genet. Samples consisted of small fragments of branches which were placed into envelopes, transported to the laboratory, and stored at 4 °C until extraction 1 or 2 d later. Collection of samples was done carefully to minimize the potential damage to populations.

Electrophoresis

Genetic data were obtained through standard methods for starch gel electrophoresis of allozymes (Soltis *et al*., 1983; Wendel and Weeden, 1989). Leaves were detached from branches, and homogenized on refrigerated porcelain plates using a cold extraction buffer consisting of 0·011 M Tris–HCl, pH 7·6, 4 % sodium thioglycolate, 2 % polyethyleneglycol, and 8 % PVP-40 (polyvinyl-pyrrolidone). Analyses with an alternative extraction buffer (the composition is detailed in López-Pujol *et al*., 2001) were also performed but with poorer results. Extracts were absorbed onto 3 mm Whatman filter paper, and either analysed immediately or stored at –20 °C until analysis 1 or 2 d later. Using 12·5 % starch gels, 19 enzyme systems were assayed. Aspartate aminotransferase (AAT; EC 2.6.1.1), diaphorase (DIA; EC 1.6.99.) and phosphoglucoisomerase (PGI; EC 5.3.1.9) were satisfactorily resolved on a tris–citrate/lithium–borate buffer at pH 8·2; aconitate hydratase (ACO; EC 4.2.1.3) and phosphogluconate dehydrogenase (6-PGD; EC 1.1.1.44) were resolved on a morpholine buffer at pH 6·1 and phosphoglucomutase (PGM; EC 5.4.2.2) was resolved on histidine–citrate buffer pH 5·7.

Genetic analyses

Loci were numbered consecutively, and alleles at each locus were labelled alphabetically, beginning from the most anodal form. Isozyme phenotypes were interpreted genetically according to standard principles (Wendel and Weeden, 1989), but special attention was paid to phenotypes characteristic of tetraploids and to their interpretation (Gottlieb, 1981). To describe the levels of genetic diversity, the following statistics were calculated: P, the percentage of polymorphic loci at which the most common allele had a frequency of <0·95; *A*, the mean number of alleles per locus; H_o, the observed heterozygosity; and H_e, the expected panmictic heterozygosity. In autotetraploids, two types of H_e can be computed; H_e (Ce), the expected heterozygosity assuming random chromosomal segregation, and H_e (Cd), the expected heterozygosity assuming some level of chromatid segregation. Chromatid segregation is produced if 'double reduction' takes place, i.e sister chromatids segregate into the same gamete, a phenomenon specific to autopolyploids and which is dependent on the amount of tetravalent formation and the proximity of the locus to the centromere (see Bever and Felber, 1992; Ronfort *et al*., 1998). It is not known whether double reduction takes place in *T. loscosii*, and it was assumed that only chromosomal segregation occurs. This consideration allows a more conservative data processing method to be used regarding the type of polyploid origin for this species (auto- or allopolyploid), since double reduction increases the production of homozygous gametes as compared with what is expected under random chromosomal segregation in diploids and allopolyploids (Ronfort *et al*., 1998). The mean fixation index (*F*) was also calculated for all variable loci in each population, to compare genotype proportions with those expected under the Hardy–Weinberg equilibrium. We used a chi-square (χ^2) test to evaluate deviations

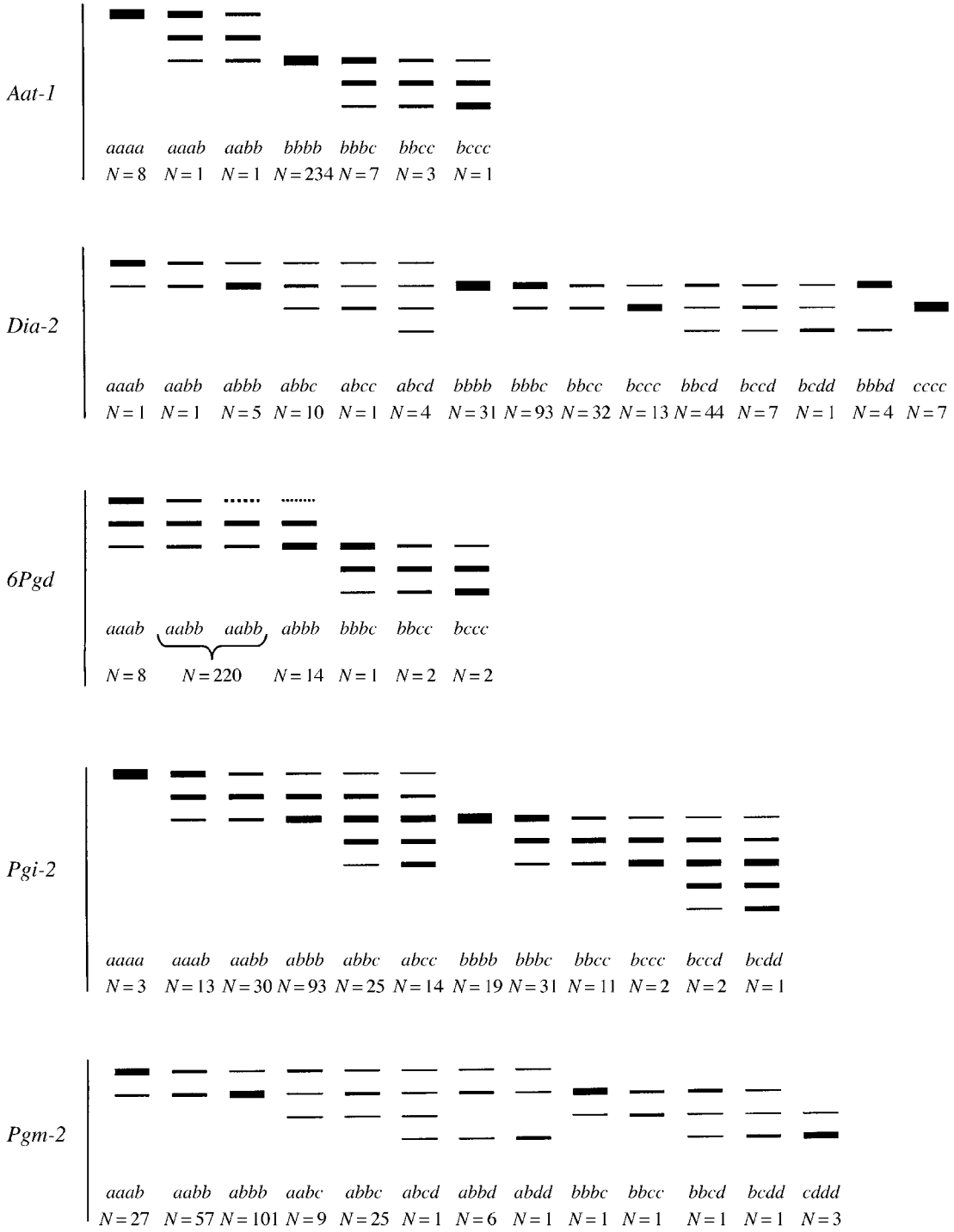

FIG. 2. Schematic banding patterns obtained for the five resolved loci in *Thymus loscosii*. All the inferred genotypes and the number of individuals showing each genotype are given below each phenotype.

of F from zero. The partitioning of genetic diversity within and between populations was analysed using Nei's gene diversity statistics (Nei, 1973): total genetic diversity (H_T), mean genetic diversity within populations (H_S), genetic diversity between populations (D_{ST}), and proportion of total genetic diversity between populations (G_{ST}) were calculated for all populations. Gene flow (Nm) was estimated by Wright's equation (Wright, 1951): $Nm = 1- G_{ST}/4\ G_{ST}$. Nei's genetic identity (I) (Nei, 1978) was also calculated between pairs of populations. A program for analysis of

autotetraploid genotypic data, AUTOTET (Thrall and Young, 2000), was used for calculations of H_o, H_e and F. Employing the appropriate options for tetraploids, BIOSYS-1 (Swofford and Selander, 1989) was used for the calculation of P, while GeneStat version 3·31 (Whitkus, 1988) was used to calculate H_T, H_S, D_{ST} and G_{ST}. The mean number of alleles per locus (A) was calculated by hand because data was included for the partially resolved locus *Aco-1*.

RESULTS

Interpretation of enzyme banding patterns

There were considerable difficulties in resolving enzymes in *T. loscosii*, due to interference during the extraction from polyphenolic substances and volatile oils. Up to 100 preliminary experiments were needed to obtain acceptable resolution. Of the 19 enzymes assayed, five were satisfactorily resolved, each with one interpretable locus: *Aat-1*, *Dia-2*, *6Pgd*, *Pgi-2* and *Pgm-2* (Fig. 2). *Aco-1* was poorly stained on gels and allelic dosage in heterozygote phenotypes could not be determined, but the locus was used for calculation of *A*.

Banding patterns in AAT, 6-PGD and PGI were consistent with dimeric enzymes, and in ACO, DIA and PGM with monomeric enzymes. AAT displayed two regions of activity in *T. loscosii*, encoded by two independent putative loci: *Aat-1* and *Aat-2*. *Aat-1* was highly monomorphic, with the majority of individuals being homozygous for *Aat-1b* allele. Alleles *Aat-1a* and *Aat-1c*, although less frequent, were also present. The locus *Aat-2* was too faint to be interpretable, but appeared to be variable.

ACO presented two zones of activity, encoded by two different putative loci (*Aco-1* and *Aco-2*). *Aco-1* was too poorly stained to interpret the allelic dosage, but four different alleles were detected at this locus, *Aco-1b* and *Aco-1c* being the most common. Some analysed individuals showed patterns of three and four bands, which could be interpreted as possessing three and four different alleles for this enzyme. The locus *Aco-2* could not be interpreted due to the poor staining of the bands.

Two different regions of activity were identified for DIA, from which only the most cathodal was consistently interpreted (*Dia-2*). Four alleles were detected, with *Dia-2b* displaying the highest frequency. Single-banded homozygotes and several types of heterozygotes were observed at this locus. Heterozygous phenotypes were distinguished by the number of bands and/or the relative intensity of band staining. Thus, it was possible to observe two-banded heterozygotes, with balanced and unbalanced staining activity, three-banded phenotypes with unbalanced staining activity, and four-banded phenotypes with equally strong bands.

6-PGD exhibited a single zone of activity, encoded by only one putative locus (*6Pgd*). All studied individuals showed either a two-banded or a three-banded phenotype, which is not consistent with the dimeric structure of this enzyme. This pattern can be attributed to the inactivity of the dimer *aa* in some populations, since this band appeared in other populations but was poorly stained. Three-banded patterns are consistent with both balanced phenotypes (*aabb*), which were the majority of observed genotypes, and unbalanced heterozygotes (*aaab*, *abbb*), which were less frequent. In the Vallclara (VAL) population we also observed heterozygous phenotypes with the *6Pgdc* allele.

PGI showed two different regions of activity, encoded by two putative independent loci (*Pgi-1* and *Pgi-2*), of which only *Pgi-2* could be interpreted. Four different alleles were detected, *Pgi-2b* being the most common in all populations. Complex banding patterns with up to five bands were observed in some individuals, which are consistent with the expression of three different alleles. Five (not six) bands were observed due to the co-migration of a homodimeric band encoded by the 'intermediate' allele with the heterodimeric band formed between the 'fastest' and the 'slowest' allele. Both balanced and unbalanced phenotypes with three or five bands were observed, but homozygous phenotypes were also present.

PGM presented two zones of activity. Only the most cathodal region was interpretable, controlled by the putative locus *Pgm-2*. Analysed individuals showed four different alleles at this locus, *Pgm-2a* and *Pgm-2b* being the most common. Some phenotypes of three and four bands were found, which correspond to individuals possessing three and four different alleles at this locus.

Levels and distribution of genetic diversity

Among the six interpretable loci (including *Aco-1*) 22 alleles were detected, the frequencies of which are given in Table 2. The richest populations were ALB, VAL and ELC (all displaying 16 alleles); the poorest populations were ULL, LGR and LPE (all with 13 alleles). Only one population-specific allele was found in *T. loscosii*, at VAL (*6Pgd-c*). In contrast, rare alleles (alleles with a frequency of <0·05) were common to all populations, representing 8 % (LGR) to 31 % (ALB) of the total number of alleles. It was found that 37·2 % of all plants possessed three or four alleles for at least one of the loci examined, with a maximum percentage of 58 % at *Pgm-2* in the MON population. Genetic diversity was high in *T. loscosii*, with the mean values over all eight populations of P = 85 %, A = 3·0, and H_e = 0·422 (Table 3). The most variable population was LGR (H_e = 0·479), while the least diversity was found in CHI (H_e = 0·399); however, values of the standard deviation (Table 3) showed that differences between populations were not statistically significant.

Values of observed heterozygosity were higher than those of expected heterozygosity in all populations except LGR, the only population with a positive value of F (fixation index) (Table 3). At loci level, 15 F-values were in accordance with Hardy–Weinberg expectations ($P \geqslant 0{\cdot}05$), while 21 F-values differed significantly from zero ($P < 0{\cdot}05$). Of these 21 values, four were positive and 17 were negative (Table 4). Positive values indicate deficiency of heterozygotes; negative values indicate excess.

Genetic diversity in *T. loscosii* was distributed mainly within populations (H_S = 0·429) rather than between them (D_{ST} = 0·015), i.e. genetic diversity attributable to

TABLE 2. *Allele frequencies for five loci in eight populations of* Thymus loscosii

Locus	Allele	ULL	ALB	LGR	VAL	MON	LPE	CHI	ELC
Aat-1	*a*	0·000	0·000	0·235	0·000	0·000	0·000	0·000	0·045
	b	**1·000**	**0·962**	**0·727**	**0·976**	**1·000**	**1·000**	**1·000**	**0·932**
	c	0·000	0·038	0·038	0·023	0·000	0·000	0·000	0·023
Dia-2	*a*	0·000	0·016	0·000	0·016	0·039	0·008	0·039	0·078
	b	**0·609**	**0·672**	**0·656**	**0·766**	0·297	**0·675**	**0·656**	**0·641**
	c	0·266	0·250	0·258	0·180	**0·609**	0·292	0·297	0·203
	d	0·125	0·062	0·086	0·039	0·055	0·025	0·008	0·078
6Pgd	*a*	**0·509**	**0·507**	0·485	0·427	0·492	0·475	0·500	0·476
	b	0·491	0·492	**0·515**	**0·484**	**0·508**	**0·525**	0·500	**0·523**
	c	0·000	0·000	0·000	0·089	0·000	0·000	0·000	0·000
Pgi-2	*a*	0·274	0·288	0·280	0·336	0·298	0·339	0·089	0·069
	b	**0·653**	**0·614**	**0·636**	**0·594**	**0·645**	**0·536**	**0·661**	**0·698**
	c	0·072	0·091	0·083	0·070	0·056	0·125	0·234	0·224
	d	0·000	0·007	0·000	0·000	0·000	0·000	0·016	0·009
Pgm-2	*a*	0·342	0·281	0·393	0·208	0·298	0·400	**0·577**	**0·537**
	b	**0·639**	**0·687**	**0·607**	**0·617**	**0·540**	**0·583**	0·388	0·361
	c	0·009	0·016	0·000	0·050	0·137	0·017	0·034	0·102
	d	0·009	0·016	0·000	0·125	0·024	0·000	0·000	0·000

The most frequent allele for each locus and population is indicated in bold.

TABLE 3. *Summary of genetic variation for five loci in eight populations of* Thymus loscosii

Population	*N*	*P*	*A**	H_o	H_e	*F*
ULL	30·2	80·0	2·83	0·491 (0·279)	0·402 (0·226)	–0·222
ALB	32·6	80·0	3·33	0·497 (0·249)	0·407 (0·189)	–0·221
LGR	29·0	100	2·83	0·469 (0·239)	0·479 (0·038)	0·022
VAL	31·4	80·0	3·17	0·439 (0·246)	0·418 (0·222)	–0·051
MON	31·4	80·0	3·00	0·470 (0·281)	0·425 (0·242)	–0·104
LPE	29·6	80·0	2·50	0·479 (0·270)	0·408 (0·232)	–0·175
CHI	31·2	80·0	3·00	0·452 (0·266)	0·399 (0·223)	–0·132
ELC	30·6	100	3·33	0·476 (0·246)	0·438 (0·178)	–0·086
Mean	30·7	85·0	3·00	0·472	0·422	–
s.d.		9·2	0·28	0·019	0·026	–

N, sample size; *P*, percentage of polymorphic loci; *A*, mean number of alleles per locus; H_o, observed heterozygosity; H_e, expected panmictic heterozygosity; *F*, mean fixation index.

Standard deviation in parentheses.

* Locus *Aco-1 is* included in its calculation.

interpopulation differentiation was low (G_{ST} = 0·033) (Table 5). Gene flow was high (*Nm* = 7·32), indicating a substantial interchange of genes between populations. Values for Nei's genetic identity (*I*) (Nei, 1978) were very high between pairs of populations (mean = 0·973, range 0·929–1·000; Table 6).

DISCUSSION

Autopolyploidy in Thymus loscosii

Morales (1986) hypothesized that *Thymus loscosii* is an autotetraploid. This hypothesis was based on chromosome numbers (as presented in the Introduction), and phylogenetic studies. Furthermore, according to R. Morales (pers. comm.) it may be inferred from its morphology that *T. loscosii* did not arise from hybridization of the potential diploid parentals, e.g. *T. zygis* or *T. vulgaris*.

To verify the hypothesis of an autopolyploid origin, extensive cytological (i.e. karyological meiotic analyses) and inheritance studies (allozyme progeny tests) would be needed. However, cytological analyses are technically extremely difficult in *Thymus*, due to the very small size of the chromosomes (cf. Morales, 1986), and inheritance studies would also be difficult due to low germination rates.

Allozyme data are extremely useful in distinguishing between autopolyploids and allopolyploids (Soltis and Rieseberg, 1986; Crawford, 1989). Tetrasomic inheritance in autotetraploids results in the formation of unbalanced as well as balanced heterozygotes in all possible combinations. In contrast, allotetraploids are expected to display fixed heterozygosity, at least at some loci at which loci on homoeologous chromosomes are fixed for different alleles. For example, when two alleles (*a* and *b*) are present at a locus in an autotetraploid, three types of heterozygotes can be produced, one balanced (*aabb*) and two unbalanced

TABLE 4. *Values of fixation index (*F*) for all polymorphic loci in eight populations of* Thymus loscosii

Locus	ULL	ALB	LGR	VAL	MON	LPE	CHI	ELC
Aat-1	–	0·238**	0·854***	0·204ns	–	–	–	0·492ns
Dia-2	–0·248**	–0·254***	–0·230**	0·013ns	0·057**	–0·199ns	0·077ns	–0·001ns
6Pgd	–0·299***	–0·324***	–0·314***	–0·084***	–0·301***	–0·303***	–0·303***	–0·305***
Pgi-2	–0·113*	–0·129*	0·078ns	–0·150ns	0·027ns	–0·043*	–0·065ns	0·007ns
Pgm-2	–0·223***	–0·256**	–0·148ns	0·015***	–0·192*	–0·179ns	–0·217ns	–0·179ns

Conformance to Hardy–Weinberg equilibrium was tested using chi-square analysis: ns, $P \geqslant 0{\cdot}05$; * $P < 0{\cdot}05$; **, $P < 0{\cdot}01$;***, $P < 0{\cdot}001$.

TABLE 5. *Gene diversity statistics (Nei, 1973) for five loci in eight populations of* Thymus loscosii

Locus	H_T	H_S	D_{ST}	G_{ST}
Aat-1	0·099	0·084	0·014	0·146
Dia-2	0·528	0·497	0·031	0·059
6Pgd	0·511	0·517	0·000	0·000
Pgi-2	0·531	0·520	0·010	0·019
Pgm-2	0·552	0·528	0·024	0·043
Mean	0·444	0·429	0·015	0·033
s.d.	0·193	0·193	0·012	0·056

H_T, Total genetic diversity; H_S, genetic diversity within populations; D_{ST}, genetic diversity between populations; G_{ST}, proportion of total genetic diversity between populations.

TABLE 6. *Matrix of Nei's genetic identity (Nei, 1978) between populations of* Thymus loscosii

Populations	ULL	ALB	LGR	VAL	MON	LPE	CHI	ELC
ULL	–							
ALB	1·000	–						
LGR	0·991	0·992	–					
VAL	0·994	1·000	0·976	–				
MON	0·968	0·957	0·938	0·932	–			
LPE	1·000	1·000	0·988	0·995	0·961	–		
CHI	0·978	0·971	0·967	0·953	0·941	0·985	–	
ELC	0·972	0·965	0·968	0·950	0·929	0·975	1·000	–

(*aaab* and *abbb*), which can be differentiated on zymograms by the intensity of band staining. Such a pattern would only be possible in an allopolyploid if the locus was heterozygous for *a* and *b* on both pairs of homoelogous chromosomes.

Allozyme data support the hypothesis that *T. loscosii* is an autotetraploid. No evidence of fixed heterozygosity was found in *T. loscosii*. Except for *6Pgd* and *Pgm-2*, all loci showed both homozygotes and heterozygotes (balanced and unbalanced). The *6Pgd* and the *Pgm-2* loci displayed only heterozygous phenotypes, but these were both balanced and unbalanced. Support for autopolyploidy was also given by genetic diversity data, as discussed below.

Levels of diversity in autopolyploids

The high levels of heterozygosity found in autotetraploids may also be a consequence of tetrasomic inheritance (Soltis and Soltis, 2000), because this mode of inheritance would reduce the effects of population bottlenecks and genetic drift. The literature provides us with several examples of higher levels of heterozygosity in autotetraploid plants than in their diploid counterparts: *Tolmiea menziesii* Torr. & Gray ($H_o = 0{\cdot}237$ in tetraploid populations and $H_o = 0{\cdot}070$ in diploid ones; Soltis and Soltis, 1989), *Heuchera micrantha* Dougl. ($H_o = 0{\cdot}150$ in tetraploid and $H_o = 0{\cdot}075$ in diploid; Ness *et al*., 1989), *Heuchera grossulariifolia* Rydb. ($H_o = 0{\cdot}159$ in tetraploid and $H_o = 0{\cdot}058$ in diploid; Wolf *et al*., 1990), *Dactylis glomerata* L. ($H_o = 0{\cdot}43$ in tetraploid and $H_o = 0{\cdot}17$ in diploid; Soltis and Soltis, 1993), *Rutidosis leptorrhinchoides* F. Muell. ($H_o = 0{\cdot}34$ in tetraploid and $H_o = 0{\cdot}22$ in diploid; Brown and Young, 2000), *Vaccinium oxycoccos* L. ($H_o = 0{\cdot}213$ in tetraploid and $H_o = 0{\cdot}067$ in diploid; Mahy *et al*., 2000) and *Centaurea jacea* L. ($H_o = 0{\cdot}54$ in tetraploid and $H_o = 0{\cdot}29$ in diploid; Hardy and Vekemans, 2001), among others. Judging from allozyme banding patterns, no population in the examined material of *T. loscosii* appeared to be diploid. Accordingly, only its levels of heterozygosity ($H_o = 0{\cdot}472$) can be compared, although cautiously, with the closely related diploid species *T. vulgaris* ($H_o = 0{\cdot}295$; Tarayre and Thompson, 1997).

The low number of loci resolved in *T. loscosii* illustrates the problems raised when allozyme studies are addressed to polyploid, woody and aromatic species, which are traditionally considered to be difficult (P. Arús, pers. comm.). To exemplify this, only four loci were interpretable in the analysis of the population structure of the closely related *Thymus vulgaris* L. (Tarayre and Thompson, 1997).

Genetic diversity in Thymus loscosii

Tetraploidy allows for the presence of three or four alleles at a single locus since loci are duplicated (Soltis and Rieseberg, 1986; Soltis and Soltis, 1989; Mahy *et al*., 2000). Compared with diploids, more enzyme variants and an increased biochemical diversity may contribute to the success of polyploids in nature (Levin, 1983). In *T. loscosii*, 37·2 % of all plants possessed three or four alleles for at least one of the examined loci, a value close to that obtained for an autotetraploid cytotype of *Tolmiea menziesii* (39 %; Soltis and Soltis, 1989), and higher than that reported for *Vaccinium oxycoccos* (12·1 %; Mahy *et al*., 2000). It is possible that the high number of alleles at some loci may be due to repeated polyploidization events, as discussed, e.g. for the grass *Dactylis glomerata* (Lumaret, 1988).

TABLE 7. *Genetic diversity in* Thymus loscosii *and in other autotetraploid species*

Taxa	P	*A*	H_o	H_e	Reference
Aster kantoensis Kitam.	36·9	1·53	–	0·142	Maki *et al.*, 1996
Centaurea jacea L.	–	3·54	0·54	0·38	Hardy and Vekemans, 2001
Dactylis glomerata L.	80·0	2·36	0·43	–	Soltis and Soltis, 1993
Heuchera grossulariifolia Rydb.	31·0	1·55	0·159	–	Wolf *et al.*, 1990
Heuchera micrantha Dougl.	38·33	1·64	0·150	–	Ness *et al.*, 1989
Rutidosis leptorrhynchoides F. Muell.	98·0	3·2	0·34	0·36	Brown and Young, 2000
Swainsona recta A.T. Lee	–	4·3	0·24	0·42	Buza *et al.*, 2000
Thymus loscosii Willk.	85·0	3·0	0·472	0·422	This study
Tolmiea menziesii Torr. & Gray	40·8	1·5	0·237	–	Soltis and Soltis, 1989
Turnera ulmifolia var. *elegans* Urb.	65·3	2·03	0·42	0·27	Shore, 1991
Turnera ulmifolia var. *Intermedia* Urb.	20·1	1·20	0·07	0·04	Shore, 1991
Vaccinium oxycoccos L.	38·9	1·66	0·213	–	Mahy *et al.*, 2000

If a species has both diploid and tetraploid populations, values of diversity given here are only for tetraploid populations.
All values given here are population means.
P, Percentage of polymorphic loci; *A*, mean number of alleles per locus; H_o, observed heterozygosity; H_e, expected panmictic heterozygosity.

The expected tetrasomic inheritance leads to high levels of genetic diversity in autotetraploids, as described by values of *A*, P and heterozygosity (Soltis and Soltis, 1989). High values of genetic variation in *T. loscosii* are within the range of other autotetraploids (Table 7). As observed in the field, *T. loscosii* can undergo vegetative propagation through stolons, which can maintain genetic variation, once produced, within populations. This phenomenon has already been reported in other species, such as *Populus tremuloides* Michx. (Cheliak and Dancik, 1982), *Erythronium albidum* Nutt. and *E. propullans* A. Gray (Pleasants and Wendel, 1989), *Iris cristata* Aiton (Hannan and Orick, 2000) and *Delphinium montanum* DC. (Simon *et al*., 2001).

In populations of autotetraploid species, equilibrium frequencies under random mating are reached after several generations, not in a single generation as for randomly mating diploid species (Bever and Felber, 1992). This can theoretically result in deviations from Hardy–Weinberg equilibrium (Mahy *et al*., 2000). Negative values for the fixation index were found at most loci and most populations of *T. loscosii*, indicating an excess of heterozygotes compared with Hardy–Weinberg expectations. The *6Pgd* and the *Pgm-2* loci displayed only heterozygous phenotypes in all examined populations. A negative fixation index could be explained by: (*a*) selection against homozygote survival promoting high heterozygosity; (*b*) random stochastic events; (*c*) barriers to inbreeding; and (*d*) sampling error. The excess of heterozygotes detected in *T. loscosii* might be explained by some barriers to inbreeding, e.g. ocurrence of gynodioecy, together with a marked protandry and low rates of selfing (Bosch *et al*., 2002). However, other reasons cannot be discarded, as for example selection for heterozygosity. Alternatively, vegetative propagation may have contributed to the maintenance of the heterozygote excess, but the extent to which vegetative reproduction occurs in *T. loscosii* needs further study.

Distribution patterns of genetic diversity within and among populations are primarily determined by genetic flow, genetic drift and natural selection. Only a minimal fraction of allozyme variation in *T. loscosii* (around 3 %) is due to differences between populations. This pattern is also expressed as a high value of gene flow (7·32), which is theoretically high enough to prevent divergence by genetic drift (Wright, 1951). However, judging from other species of *Thymus*, which are characterized by small pollen and seed dispersal distances (Tarayre and Thompson, 1997; Eriksson, 1998), gene flow should also be restricted in *T. loscosii*. Instead, the very high genetic identities found between populations and the high value of *Nm* may be explained by recent and rapid fragmentation from a wide, continuous area resulting in genetically similar populations. Chorological research in recent years has revealed the existence of many additional populations, which may indicate that *T. loscosii* formerly had a continuous distribution along the Ebro river basin. This area has been extensively replanted with forest during the last decades (Blanco *et al*., 1997), and open sites at which the species could grow have been lost. In addition, this area is currently experiencing a fragmentation process caused by change of land use, e.g. the extensive plantation of new vineyards in the westernmost part of the distribution area (Nekazal Ikerketa Eta Teknologia, 2002, and field observations by J. López-Pujol). Fragmentation may result in genetic impoverishment (Barrett and Kohn, 1991; Ellstrand and Elam, 1993), but polyploids may be less sensitive since genetic diversity is maintained by genome duplications.

Conservation

The optimal measures for species preservation are those *in situ*. The conservation of a species in its habitat allows for the maintenance of interactions between the species and its ecosystem without detaining evolutionary processes (Falk and Holsinger, 1991). Protection of habitat is generally limited to the protection of selected populations of species. Using the formula proposed by Ceska *et al*. (1997), $1 - (G_{ST})^n$ (where n is the number of populations), conservation of only two populations allows us to preserve 99·9 % of genetic diversity found in *T. loscosii*, due to the high genetic

similarity between populations. Nevertheless, given the variation of G_{ST} among loci, this may be an underestimate of genetic diversity detected in this species. Moreover, when choosing the populations to preserve, other considerations must also be taken into account, for instance large populations and populations with more intermediate allele frequencies must be favoured in order to reduce the probability of loss of alleles (Martínez-Palacios *et al*., 1999). For example, the combination of VAL and ALB populations contains 21 of the 22 detected alleles, and they are the largest sampled populations. However, if population LGR is also preserved (i.e. the three largest populations), then all alleles would be conserved. Collection of seeds for maintenance in germplasm banks as an *ex situ* strategy does not seem to be an appropriate alternative to conservation policy because of the low germination rates.

Apart from genetic criteria, two additional types of polymorphism may be considered: (1) chemical diversity and (2) the frequency of females within populations. Chemical diversity may play a role in adaptation to environment, as suggested in *T. vulgaris* (Thompson *et al*., 1998) and, if this also applies to *T. loscosii*, all chemotypes must be conserved. At present, only a single population of *T. loscosii* has been surveyed for phytochemical diversity (Molero and Rovira, 1983).

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TABLE 1. *Populations of* Thymus loscosii *studied*

Population code	Location	Population size	Sample size
ULL	Ulldemolins (Catalonia, Spain), 31TCF2075	~100	32
ALB	Albarca (Catalonia, Spain), 31TCF2075	300–500	33
LGR	La Granadella (Catalonia, Spain), 31TCF0380	~200	33
VAL	Vallclara (Catalonia, Spain), 31TCF3283	>500	32
MON	Montagut (Catalonia, Spain), 31TCF6885	~100	32
LPE	La Palma d'Ebre (Catalonia, Spain), 31TCF0172	50–100	30
CHI	Chiprana (Aragon, Spain), 30TYL3868	~100	32
ELC	Elciego (Basque Country, Spain), 30TWN3205	~100	33

Location of populations is detailed by UTM 1 × 1 km squares.

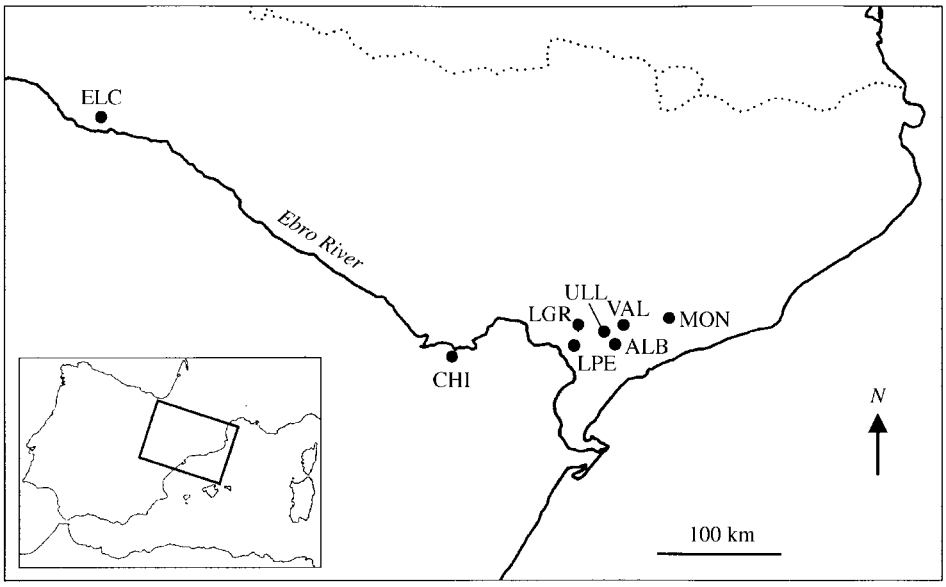

FIG. 1. Sampled populations of *Thymus loscosii*. ALB, Albarca, CHI, Chiprana, ELC, Elciego, LGR, La Granadella, LPE, La Palma d'Ebre, MON, Montagut, ULL, Ulldemolins, and VAL, Vallclara.

chromosomes, i.e. dysploidy ($2n = 4\times - 2$) (Morales, 1986). Morales (1986, 2002) has suggested a basic number in the genus *Thymus* of $x = 7$, which probably gave rise to the secondary basic numbers $x = 14$ and $x = 15$. Several studies report different ploidy levels in the same species, which indicates that polyploidization may occur frequently in this genus. Perhaps one of the most interesting cases is that of *Thymus herba-barona* Loisel., with $2n = 28$, 56 and 84 (Morales, 2002). In section *Thymus*, two ploidy levels (diploid and tetraploid) and five different numbers, $2n = 28$, 30, 54, 56 and 58, are found. According to Morales (1986), the tetraploid numbers (54, 56 and 58) have different origins: $2n = 56$ is probably derived from a duplication of a $2n = 28$ genome (i.e. autopolyploidy); $2n = 58$ may have originated from a hybridation of two taxa with $n = 14$ and $n = 15$ and a subsequent doubling of the chromosome number; and $2n = 54$ is probably derived from a $2n = 56$ plant which has lost two chromosomes (i.e. autopolyploidy followed by dysploidy). The taxonomically closest species to *T. loscosii* is *T. zygis* Loefl. ex L. (Morales, 1986), which has three subspecies in the Iberian peninsula, two diploid (subsp. *zygis* and subsp. *gracilis*, both with $2n = 28$) and one probably autotetraploid (subsp. *sylvestris*, with $2n = 56$). Morales (1986) studied the meiosis of *T. carnosus* Boiss. ($2n = 56$), from the same section, and reported some tetravalents, which supports the hypothesis that the number $2n = 56$ is derived by autopolyploidy. Judging from its morphology, it is difficult to believe that *Thymus loscosii* should have arisen by hybridization and, accordingly, an allopolyploid origin of the species is not supported by morphological data (R. Morales, pers. comm.).

Thymus loscosii is a perennial woody plant, 9–10 (15) cm tall, with abundant stoloniferous branches. The inflorescences are composed of whorls of small, whitish, zygomorphic flowers, with seeds disposed in up to four nutlets of about 0·4 × 1·1 mm. The pollination in *T. loscosii* is

3.1.6. **López-Pujol J**, Font J, Simon J & Blanché C. 2004. Genetic structure and conservation priorities for *Silene sennenii* (Caryophyllaceae), a narrow endemic and critically endangered species of the Iberian Peninsula. *Planta Europa Conference IV Proceedings*. Disponible a: http://www.nerium.net/plantaeuropa/Download/Proceedings/Lopez_et_al.pdf

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

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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 northeastern 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 *He* = 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.

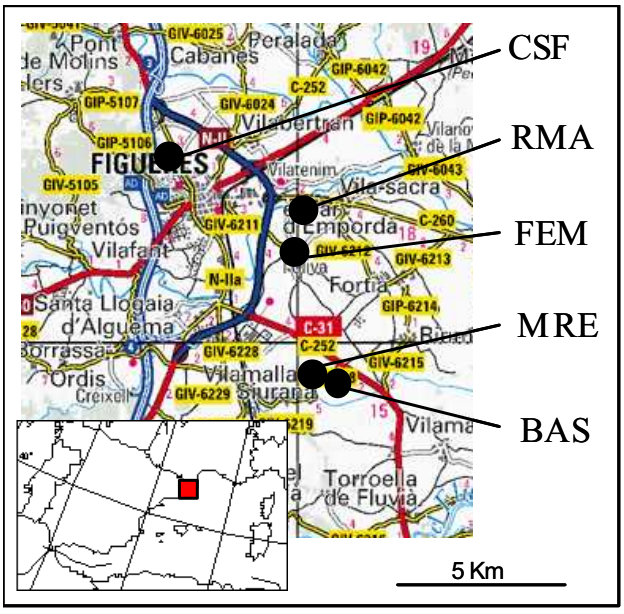
Introduction

Silene sennenii Pau (Caryophyllaceae) is a hemicryptophyte which anually 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 2*n* = 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 labeled 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; *Ap*, the mean number of alleles per polymorphic locus; *Ho*, the observed heterozygosity; and *He*, the expected panmictic heterozygosity. A chi-square test ($\chi 2$) 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 (*FIS*, *FST*, and *FIT*), and gene flow was determined using Wright's equation: $Nm = (1 - FST)/4\ FST$. 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 were 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) harbored 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 harbor exclusive alleles.

Levels of genetic variation in *Silene sennenii* are low (*P* = 20.9 %, *A* = 1.31 and *He* = 0.071; see Table 1), as expected for endemic species (*P* = 26.3 %, *A* = 1.39 and *He* = 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 aro those in frequencies < 0.05.

Population	Population size	Sample size	Rare alleles	*P*	*A*	*Ho*	*He*
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 (*FIS* = 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 (*FST* = 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 *FIT* value (0.253) also gives support to a genetic subtructuring 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).

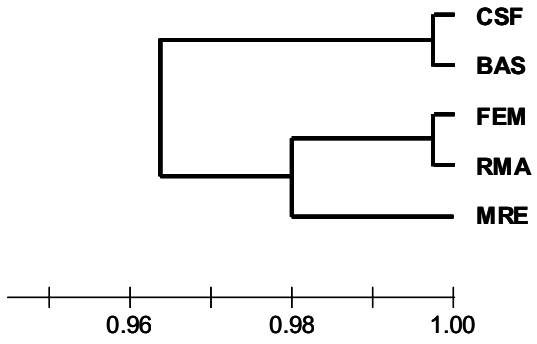


Fig. 1. Nei's genetic identity (*I*) among populations (UPGMA dendrogram)

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 prioritary (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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Allozyme variation and taxonomical implications of the endemic rocky plant *Erodium rupestre* (Geraniaceae)

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Running title: Allozyme variation of *Erodium rupestre*

Abstract

Erodium rupestre (Geraniaceae) is a taxon endemic to north-eastern Spain which has experienced significant changes in taxonomy and chorology. When first described, its distribution area was limited to Montserrat, a mountain near Barcelona, although later several populations from the pre-Pyrenean range were attributed to *E. rupestre*. However, some authors have assigned these as belonging to a distinct taxon. Starch gel electrophoresis has been used to resolve the taxonomic circumscription of the pre-Pyrenean populations and to evaluate the levels of genetic diversity and the population structure, taking into account all the populations studied (Pre-Pyrenean and Montserrat Mountain). Levels of genetic variation showed by *E. rupestre s.l.* are extremely scarce (P = 7.1%, A = 1.07, and H_e = 0.025), rather lower than those expected for endemic species. Moderate to high levels of inbreeding were detected, probably as a consequence of the population's genetic structuring (geitonogamy and biparental inbreeding). Genetic identity between the two presumable taxa is very high (I = 0.973), clearly suggesting that the two taxa form a single species. However, according to the allelic distributions among populations, the Montserrat population on the mountain itself may represent the first step of an allopatric speciation process.

Keywords: Electrophoresis – genetic diversity – inbreeding – allopatric speciation – Pre-Pyrenean plant – conservation.

1. Introduction

Estimation of genetic diversity and population structure give valuable insights into natural selection and gene flow forces shaping the evolutionary dynamics of natural populations (Tarayre and Thompson, 1997) and provide guidance for conservation and breeding programs (Falk and Holsinger, 1991; Hamrick and Godt, 1996). Species survival depends on the maintenance of enough genetic variability within and among populations to accommodate new selection pressures resulting from biotic and abiotic environmental changes (Barrett and Kohn, 1991; Lande and Shannon, 1996). Rare and endemic species are expected to show depleted levels of genetic variation due to genetic

drift and inbreeding in small populations, reduced gene flow among remnants (Young et al., 1996), and a high habitat specificity, all of which are often shown by these species (Barrett and Kohn, 1991; Ellstrand and Elam, 1993).

The Mediterranean Basin represents the species core of the genus *Erodium*, with approximately 60 species (Alarcón et al., 2003). The taxonomy of *Erodium* is extremely difficult and has suffered a good number of changes in the discrimination of taxa and assignment of taxonomic ranks. Most of the *Erodium* species are endemic since several isolated areas of the Mediterranean Basin (such as the Italian and Iberian Peninsulas, Greece and Turkey) have acted as secondary speciation centres, originating the current endemisms (Guittonneau, 1972). The subsection *Petraea* Brumh. constitutes a series of schizoendemic diploid taxa (2n = 20) generated by a slow and progressive geographic differentiation from a primitive ancestor, with reduced and disjunct distribution areas on the Western Mediterranean Basin (Guittonneau, 1972).

One of these endemics is *Erodium rupestre* (Pourr. ex Cav.) Cadevall (Geraniaceae), which was originally described as *E. supracanum* L´Hér. (L'Héritier, 1792-1802), but in 1963 was newly described as *E. rupestre* by Guittonneau (1963), and in a subsequent treatment was regarded as a subspecies, *E. foetidum* subsp. *rupestre* (Pourr. ex Cav.) O. Bolòs et J. Vigo (Bolòs and Vigo, 1990). Nevertheless, in the forthcoming revision of Geraniaceae for *Flora Iberica* (Aldasoro and Sáez, in press), this taxon will regain the status of species. In addition to the taxonomic changes, chorologic delimitation has also experienced significant changes. When described by Guittonneau (1963), its distribution area was delimited to Montserrat mountain, near Barcelona (Spain), in the same way as Bolòs and Vigo (1990). However, several field citations (cf. Romo, 1989) have allocated some populations located in the pre-Pyrenean ranges (see Fig. 1) to *E. rupestre* or to *E. foetidum* subsp. *rupestre* which, in contrast, were attributed by Bolòs and Vigo (1990) to other subspecies of *E. foetidum*, *E. foetidum* subsp. *glandulosum* (Cav. in Lamk.) O. Bolòs et J. Vigo (or *E. glandulosum* following other taxonomic treatments). Nevertheless, the leaves of the individuals of *E. foetidum* subsp. *glandulosum* from typical populations are very glandular, contrasting with the scarcity in glandular trichomes observed in the individuals of *E. rupestre* from Montserrat (L. Sáez, pers. comm.) and those in the pre-Pyrenean populations proposed to belong to *E. rupestre*. Aldasoro and Sáez (in press) consider these pre-Pyrenean populations as belonging to *E. rupestre* and consequently will enlarge its distribution area to the pre-Pyrenean belt of Catalonia and Aragon in Volume 9 of *Flora Iberica*.

Table 1
Studied populations of *Erodium rupestre s.l.* Location of populations is detailed by UTM 10 × 10 km squares

Population code	Location	Altitude	Population size	Sample size
MON	Montserrat (31TDG00)	1,017m	400	36
LAG	Laguarres (31TBG97)	1,151m	1,000-1,500	30
LOR	Mare de Déu del Lord (31TCG86)	1,122m	450	34
POB	La Pobla de Segur (31TCG37)	1,079m	500	32
ROM	Sant Romà de la Clusa (31TDG17)	1,417m	450	32

Allozymes are molecular markers that can be valuable in establishing relationships at low systematic levels (Crawford, 1983; Gottlieb, 1984); nevertheless, their limited resolution does not allow us to infer relationships at a higher level than

congeneric (Crawford et al., 2001). Allozymes have proved to be useful molecular markers in the discrimination of taxa in *Ulmus* (Machon et al., 1995), *Dactylorhiza* (Hedrén, 2001), *Lemna* (Crawford et al., 2001), *Aconitum* (Zhang et al., 2003) and *Antirrhinum* (Mateu-Andrés, 1999; Mateu-Andrés and Segarra-Moragues, 2003), among others. Indeed, allozyme markers have been used to establish new taxonomical syntheses and combinations (Mateu-Andrés and Segarra-Moragues, 2003). At present, no allozymic studies have appeared on Geraniaceae. Only RAPD markers were used to survey the genetic variation (Martín et al., 1997) and to identify and characterize newly discovered populations (Martín et al., 1999) of the Iberian endemic *Erodium paularense*, which also belongs to the same subsection as *E. rupestre*.

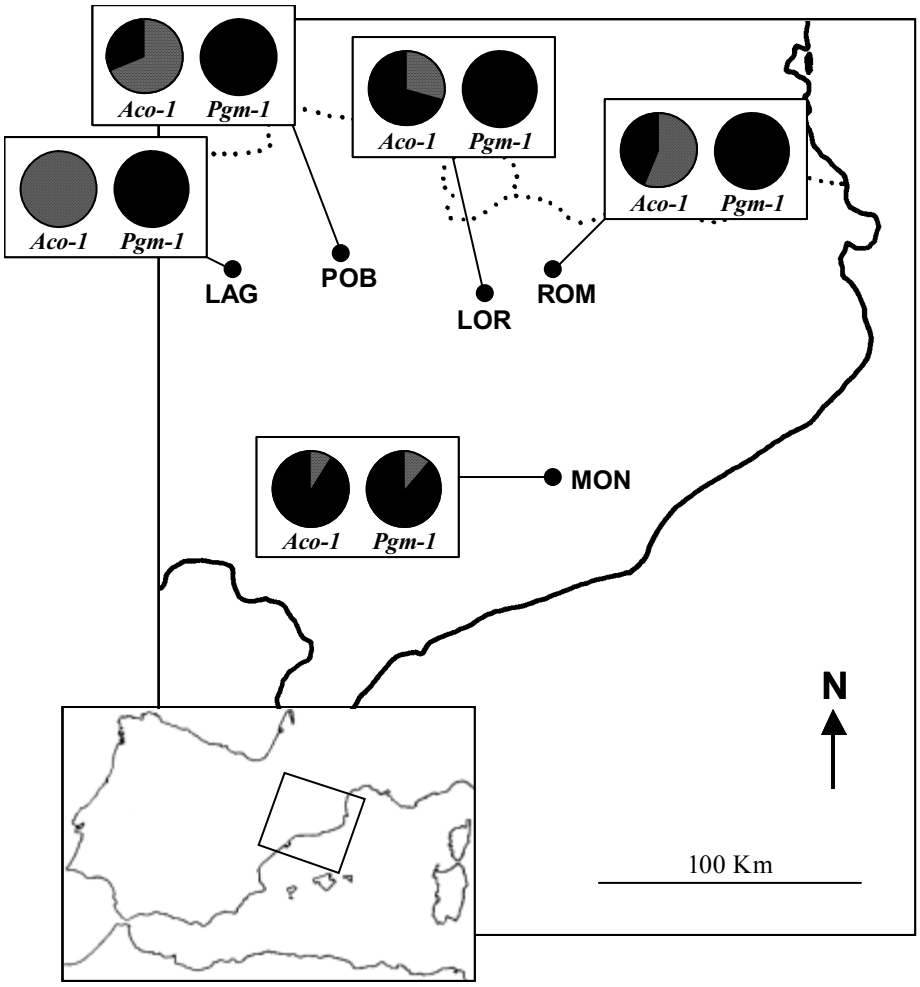

Fig. 1. Sampled populations of *Erodium rupestre s.l.* and their allelic distribution of polymorphic loci. POB: La Pobla de Segur; LAG: Laguarres; LOR: Mare de Déu del Lord; MON: Montserrat; ROM: Sant Romà de la Clusa. In black, fast allele; in grey, slow allele. The grey-shaded area represents the pre-Pyrenean.

On the other hand, allozyme electrophoresis is a powerful and cost-effective tool to assay genetic variation in plant species and which provides valuable guidance for conservation programs in rare and endangered species (Falk and Holsinger, 1991).

Herein we used allozymes to address two main issues concerning *E. rupestre*: (i) determination of the levels of genetic diversity and the population structure, taking into account all the populations studied (both those in the pre-Pyrenean area and on Montserrat); and (ii) insights concerning the taxonomic circumscription of the pre-Pyrenean populations described by Bolòs and Vigo (1990) as *E. foetidum* subsp. *glandulosum*, and the relationships between the latter and the 'original' population on Montserrat (*E. rupestre sensu stricto*). Finally, the state of conservation of *E. rupestre* is estimated, and some priorities and strategies for its conservation are suggested.

2. Materials and Methods

2.1. Plant material

Erodium rupestre is a small perennial chamaephyte (less than 20 cm) with leaves that are whitish-silvery on the upper surface with dense, straight, short and appressed hairs but green and subglabrous beneath. Flowers are pale pink, five-merous, and actinomorphic (sometimes slightly zygomorphic), often with spots only on the two upper petals. It is a diploid species with 2n = 20 (Guittonneau, 1965), and its pollination is entomophilous but not specialized, carried out by small insects. Although this species is self-compatible experimentally, the breeding system in nature is exclusively outcrossing (Álvarez, 2003). It grows in the cracks of limestone rocks but also in lithosol rocky beds on hilltops, at altitudes of 900-1600 m. According to the criteria of IUCN (2001), the extent of occurrence is around 7200 km;^{2} however, the occupation area is much lower (less than 500 km^2) due to its habitat specificity. This species was listed as 'vulnerable' (VU) both in a study of threatened flora of Catalonia (Sáez et al., 1998) and in the *Red List of Vascular Spanish Flora* (Aizpuru et al., 2000), although recently Aymerich and Sáez (2001) reduced its degree of threat to 'least concern' (LC).

2.2. Sampling design

A total of five populations were sampled from February to June 2003 (Fig. 1 and Table 1). Four of these corresponded to the pre-Pyrenean area, i.e. *E. foetidum* subsp. *glandulosum* according to Bolòs and Vigo (1990): Sant Romà de la Clusa (ROM) located in the easternmost distribution area, Laguarres (LAG), in the westernmost distribution area, and Mare de Déu del Lord (LOR) and La Pobla de Segur (POB), two intermediate populations. The remaining one was the population located at the historical site of Montserrat mountain (MON) where *E. rupestre* was originally described. Pre-Pyrenean populations were selected using criteria of overall geographic range and accessibility. Sampling was done by a linear transect within populations. A minimum of 30 individuals were selected from each population, usually collected about 50-100 cm apart to avoid ramet re-collection. Samples consisted of young leaves, which were placed into envelopes, transported to the laboratory and stored at 4°C until extraction one day later. Sampling was done carefully to minimize the potential damage to populations.

2.3. Electrophoresis

Genetic variability was assessed using standard methods for starch gel electrophoresis of allozymes (Soltis et al., 1983). Leaves were ground in refrigerated porcelain plates using three different extraction buffers due to the difficulties at the extraction step. One of the buffers (i) was a standard buffer consisting of 0.05 M tris-citric acid, 0.1% cysteine·HCl, 0.1% ascorbic acid, 8% PVP-40 (polyvinyl-pyrrolidone), and 1mM 2-mercaptoethanol. We also used two additional extraction buffers: one (ii) was specific for tissues with high levels of interfering substances (Wendel and Weeden, 1989), and the last one (iii) was specific for genus *Pinus* (Cheliak and Pitel, 1984), although with acceptable results for *E. rupestre*. The extracts were absorbed onto 2 × 6 mm paper wicks and stored at –80°C before being submitted to electrophoresis.

Twenty-seven enzymes were tested using horizontal 11% starch gels, 9 of which were satisfactorily resolved in morpholine gel buffer pH 6.1 (Odrzykoski and Gottlieb, 1984): Aspartate aminotransferase (AAT, EC 2.6.1.1), aconitate hydratase (ACO, EC 4.2.1.3), diaphorase (DIA, EC 1.6.99.-), isocitrate dehydrogenase (IDH, EC 1.1.1.42), malate dehydrogenase (MDH, EC 1.1.1.37), phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44), phosphoglucoisomerase (PGI, EC 5.3.1.9), phosphoglucomutase (PGM, EC 5.4.2.2), and shikimate dehydrogenase (SKD, EC 1.1.1.25). AAT and ACO were resolved using the extraction buffer (i), for IDH we used the extraction buffer (ii), and for SKD we employed the extraction buffer (iii). To resolve the remaining enzymes, we employed several combinations of extraction buffers: for DIA we used (i) and (iii); for MDH, 6PGD and PGM, (i) and (ii); finally, for PGI we employed a combination of (ii) and (iii). Up to 150 preliminary experiments were needed to obtain acceptable resolutions for the enzymes described. Fourteen putative loci were interpreted: *Aat-1*, *Aco-1*, *Dia-1*, *Dia-2*, *Dia-3*, *Idh*, *Mdh*, *6Pgd-1*, *6Pgd-2*, *Pgi-1*, *Pgi-2*, *Pgm-1*, *Pgm-2*, and *Skd*. Staining procedures followed the method described by Soltis et al. (1983) and Wendel and Weeden (1989), with slight modifications.

2.4. Genetic analyses

Loci were numbered consecutively and alleles at each locus were labeled alphabetically, beginning from the most anodal form in both cases. Interpretation of banding patterns was done on the basis of quaternary structure of isozymes, subcellular localization and number of loci usually expressed in diploid plants. Allozyme frequencies at each locus were calculated for each population. To estimate the levels of genetic diversity, the following statistics were computed: P, the percentage of polymorphic loci when the most common allele had a frequency of <0.95; 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. The mean fixation index (F) for all polymorphic loci in each population was also computed to compare genotype proportions with those expected under Hardy-Weinberg equilibrium. A chi-square test (χ^2) was used to evaluate deviations of F from zero, with Levene's (1949) correction for small sample size. Outcrossing rates (t) were estimated using the formula $t = (1 - F)/(1 + F)$ proposed by Weir (1990). Population structure was analysed using Wright's (1965) F-statistics: the inbreeding coefficient (F_{IS}), the fixation index (F_{ST}), and the overall inbreeding coefficient (F_{IT}). Gene flow was determined using Wright's (1951) equation modified by Crow and Aoki (1984): $Nm = 1/4\alpha\ [(1/F_{ST})-1]$, where $\alpha = [n/(n-1)]^2$, n is the number of subpopulations and Nm is the average number

of migrants exchanged per generation. A chi-square test was also performed to assess the statistical significance of F_{ST} values for each locus. On the other hand, we calculated Nei's (1978) genetic identity (*I*) between pairs of populations, which was used to cluster those into a dendrogram following UPGMA (unweighted pair group method with averaging). Finally, Mantel's (1967) test was conducted between genetic differentiation and geographical distances among pairs of populations to determine a possible 'isolation-by-distance' pattern of differentiation (Wright, 1943). BIOSYS-1 (Swofford and Selander 1989) was used for most calculations and for dendrogram construction, and NTSYS package (Rohlf, 1994) to conduct Mantel's test.

3. Results

Among the 14 interpretable loci, we only detected 16 alleles, due to the monomorphism of most loci (12 of 14). Only the MON population (which corresponds to *E. rupestre s.s.*) displayed all of them, whilst the remaining four populations, considered by Bolòs and Vigo (1990) as belonging to *E. glandulosum* subs. *rupestre*, exhibited 15 alleles, excepting LAG, which harboured 14 (Table 2). Therefore, the two presumable taxa shared 93.7% of the allelic richness. Nevertheless, the allele lacking in these populations (*Pgm-1a*) was found in MON only with a very low frequency (0.111). In the other polymorphic locus found (*Aco-1*), the faster allele, *Aco-1a*, was prevalent in LAG, POB and ROM populations, whilst the slower (*Aco-1b*) was most frequent in MON and LAG. No rare alleles (those with proportions less than 0.05) were found within populations.

Table 2
Allele frequencies for polymorphic loci in the five populations of *Erodium rupestre s.l.*

Locus	Allele	Populations				
		MON	LAG	LOR	POB	ROM
Aco-1	*a*	0.088	**1.000**	0.295	**0.683**	**0.556**
	b	**0.912**	0.000	**0.705**	0.317	0.444
Pgm-1	*a*	0.111	0.000	0.000	0.000	0.000
	b	**0.889**	**1.000**	**1.000**	**1.000**	**1.000**

The most frequent allele for each locus and population is in boldface.

Genetic variability was quantified for each of the five populations of *E. rupestre s.l.*, displaying the main indices extremely low values at population level (Table 3): P = 7.1%, A = 1.07, and H_e = 0.025. Values of P ranged from 0.0 (LAG) to 14.3% (MON), A ranged from 1.00 (LAG) to 1.14 (MON), and H_e ranged from 0.000 (LAG) to 0.037 (ROM). Therefore, LAG exhibited a total absence of polymorphism, whereas the most variable populations were MON and ROM. Values of observed heterozygosity were lower than those of expected panmictic heterozygosity in all populations with the exception of LOR (slightly higher) and LAG (both figures were zero), also reflected in the values of mean fixation index (Table 3). Outcrossing rates (t) were low and far from 1 (mean t = 0.427) in all populations excluding LOR (t = 1.500), which indicated a

general pattern of inbreeding within populations (Table 3). Significant deficiency of heterozygotes was also detected from values of F for all polymorphic loci in each population (data not shown). The chi-square test (χ^2) showed that 4 of the 5 F-values were significantly greater than zero ($p < 0.05$) and positive, indicating excess of homozygotes. The remaining value conformed to Hardy-Weinberg equilibrium ($p \geq 0.05$). High levels of inbreeding within populations were also supported by the mean value of F_{IS} (0.613; Table 4).

Genetic divergence among populations was quantified by computing the F_{ST} parameter, which measures differentiation among populations (Table 4). The mean value of F_{ST} was considerably high (0.372) with respect to the mean value of F_{IT} (0.757), indicating that a significative fraction of the genetic variability of *E. rupestre s.l.* is attributable to differences among populations because they are strongly divergent. The number of migrants per generation was consequently very low ($Nm = 0.27$), value from which can be inferred that the level of gene flow should be extremely limited among populations. The correlation between the matrix of the pairwise F_{ST} values and geographical distances (Table 5) gives support to a pattern of genetic differentiation of populations under the 'isolation-by-distance' model, since the value of Mantel statistical was significant and positive ($r = 0.733$, one-tailed $p = 0.038$ after 1000 permutations). This trend is also shown by the UPGMA dendrogram (Fig. 2), which widely separates the most distant pairwise of populations (MON-LAG). Values for Nei's (1978) genetic identity (I) were high between pairs of populations (mean = 0.982, range: 0.939-1.000 for *E. rupestre s.l.*; data not shown) since the monomorphic loci (12 of 14 in *E. rupestre s.l.*) are included in the calculation of this parameter. The genetic identity between the two presumable taxa (*E. foetidum* subsp. *glandulosum vs. E. rupestre s.s.*) was very high ($I = 0.973$), a value close to those of between pairs of populations considering all these as belonging to a single species. LOR was the most genetically close population with respect to MON (i.e. *E. rupestre s.s.*) from the group of populations presumably belonging to *E. foetidum* subsp. *glandulosum*.

4. Discussion

4.1. Lack of genetic diversity in Erodium rupestre s.l.

Levels of genetic variation showed by *E. rupestre s.l.* are extremely scarce ($P = 7.1\%$, $A = 1.07$, and $H_e = 0.025$), rather lower than those expected for endemic species according to the values given by Hamrick and Godt (1989), and similar to the lack of variability found in other narrow endemic species, such as *Pinus torreyana* (Ledig and Conkle, 1983), *Pedicularis furbishiae* (Waller et al., 1987), *Howellia aquatilis* (Lesica et al., 1988), *Bensoniella oregana* (Soltis et al., 1992), *Schwalbea americana* (Godt and Hamrick, 1998) and *Borderea chouardii* (Segarra-Moragues and Catalán, 2002). The well-known association between lack of genetic variation and endemism and/or rarity was established a long time ago (Stebbins, 1942) and most compilations of plant allozyme data (e.g. Hamrick and Godt, 1989; Hamrick et al., 1991; Karron, 1991, Gitzendanner and Soltis, 2000) show this repetitive pattern. The scarcity of genetic polymorphism associated with rare and endemic plants has been attributed to their small population sizes and isolation of populations, in addition to an adaptation to uniform habitat conditions (Barrett and Kohn, 1991; Ellstrand and Elam, 1993). Although size of

current populations of *E. rupestre* may reach high figures (from a few dozens to 1500-2000 individuals), these are often fragmented and scattered over large areas. However, habitat requirements for this species are highly specific (limestone rocks or lithosol rocky beds), which may be the determinant of the low levels of diversity detected. In that case, this species may present some degree of adaptation to the lack of genetic variability and could represent its "natural" situation. Nevertheless, literature is also replete with rare taxa which reveal unexpectedly high values of diversity (Gottlieb et al., 1985; Ranker, 1994; Young and Brown, 1996; Williamson and Werth, 1999; Neel and Ellstrand, 2001; López-Pujol et al., 2002; among others), which suggest that levels of genetic variation are greatly dependent, apart from the factors mentioned above, on several life-history traits (e.g. breeding system, seed dispersal mechanism, and life form), population history (e.g. occurrence of bottlenecks and founder events) and type of speciation (Hamrick and Godt, 1989; Booy et al., 2000; Dodd and Helenurm, 2002).

Table 3
Summary of genetic variation for 14 loci in the five populations of *Erodium rupestre s.l.*

Population	*P*	*A*	A_p	H_o	H_e	*F*	*t*
MON	14.3	1.14	2.00	0.004 (0.004)	0.026 (0.018)	0.846	0.083
LAG	0.0	1.00	1.00	0.000 (0.000)	0.000 (0.000)	—	—
LOR	7.1	1.07	1.50	0.036 (0.036)	0.030 (0.030)	-0.200	1.500
POB	7.1	1.07	1.50	0.007 (0.007)	0.031 (0.031)	0.774	0.127
ROM	7.1	1.07	1.50	0.000 (0.000)	0.037 (0.037)	1.000	0.000
Mean	7.1	1.07	1.50	0.009	0.025	0.605	0.427
Standard deviation	5.0	0.05	0.35	0.015	0.014	0.545	0.717

P: percentage of polymorphic loci; *A*: mean number of alleles per locus; A_p: mean number of alleles per polymorphic loci; H_o: observed heterozygosity; H_e: expected panmictic heterozygosity; *F*: mean fixation index; *t*: outcrossing rate. Standard error in parentheses.

4.2. Population structure

Two main potential causes explain the significant deficiency of heterozygotes found within populations: (i) the fact of the breeding system mainly consisting of selfing, and (ii) genetic substructuring of populations (Wahlund effect) achieved by biparental inbreeding (mating between close relatives). Although this species is self-compatible in experimental conditions, the breeding system in nature has been proved to be exclusively outcrossing due to the existence of marked protandry and herkogamy (Álvarez, 2003). Nevertheless, the asynchrony in flowering between flowers of the same individual may favor geitonogamy. Moreover, patterns of pollen and seed dispersal observed in *E. rupestre* may favor biparental inbreeding within populations. Pollination is highly unspecific, mainly due to disc-shaped flowers and visible nectar production, and carried out by a wide spectrum of small insects which are not capable of flying long distances (Álvarez, 2003). Bigger insects with high travelling capabilities, such as *Apidae* and Lepidoptera, are seldom observed in the field. Furthermore, only around 19% of the flower visits were performed by true pollinators; the remaining ones were thieves. On the other hand, seeds have no active dispersal mechanism; mericarps usually fall close to the plant and suffer a process of self-burying in the soil. Carrying of seeds by ants has been observed in the field (Álvarez, 2003), which may aid longer-

distance dispersal of fruits; nevertheless, this process has been associated with depredation by ants in *Erodium paularense* causing a depletion in the amount of seeds produced (Albert, 2003). These described mechanisms favor consanguinity within populations by means of geitonogamy and biparental inbreeding because pollen and seeds are shortly-dispersed. Moreover, seed production is very small, since the rate of viable seeds obtained per mericarp was low (c. 16.7%; Álvarez, 2003), representing a poor reproductive success and resembling that obtained in *E. paularense* (Albert, 2003). Individuals present a high longevity, probably up to 20 years, as found in *E. paularense* (at least 20 years; M.J. Albert, pers. comm.). Recruitment rates are probably low, based on our field observations; moreover seedling establishment is a limiting process in the viability of the related species *E. paularense* (Albert et al., 2001).

Table 4
Estimates of *F*-statistics for all polymorphic loci in the five populations of *Erodium rupestre s.l.*

Locus	F_{IS}	F_{IT}	F_{ST}
Aco-1	0.562	0.736	0.397 ***
Pgm-1	1.000	1.000	0.091 ***
Mean	0.613	0.757	0.372

F_{IS}: inbreeding coefficient , F_{ST}: fixation index, and F_{IT}: overall inbreeding coefficient. Statistical significance of F_{ST} values was tested using chi-square analysis: ns $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$,*** $p < 0.001$.

The life-history and autoecological characteristics observed in *E. rupestre* (long life-span, high habitat specificity, low recruitment levels and poor long-distance dispersal) are in agreement with those shared by high-persisting ancient species, taxa with a population dynamics of the remnant type (Eriksson, 1996). These species are characterized by having a strong population stability or very slow population growth rates (Picó and Riba, 2002; García, 2003). Their low mortality rates and long life-spans (in the case of the genus *Borderea* up to 300 years; García, 2003) make recruitment unnecessary for population persistence, this being sometimes very sporadic or even nonexistent due to the low availability of appropriate sites in their habitats. Such demographic patterns are exhibited by many relict species that occur in rocky habitats and are endemic to Mediterranean mountains in the Iberian Peninsula, Italy and the Balkans (Taberlet et al., 1998). When repeated cycles of glaciation occurred in the Quaternary, mountain areas in the Mediterranean basin acted as glacial refugia (Cardona and Contandriopoulos, 1979). Isolation of populations in those refugia and their survival to date, with or without speciation, has been discussed in depth but some questions still remain unclear (Thompson, 1999). Some examples of these rupicolous endemics in the Iberian Peninsula are the paleorelict and endemic genus *Borderea*, consisting of only two species, *B. chouardii* and *B. pyrenaica* (Segarra-Moragues and Catalán, 2002); the endemic genus *Petrocoptis*, which also originated in the Tertiary, containing a few species (López-Pujol et al., 2001); and *Ramonda myconi*, a narrow endemic species of this pre-glacial genus (Picó and Riba, 2002). All these taxa exhibit high longevity, habitat specificity, low recruitment rates and no long-distance dispersal capabilities, a common pattern shown by *Erodium rupestre s.l.* as well but also by other species of the similarly ancient subsection *Petraea* (Guittonneau, 1972), for example *E. paularense* (Albert, 2003) and *E. lucidum* (L. Sáez, pers. comm.).

Table 5
Matrix of geographic distances* (above diagonal) and Wright's (1965) genetic differentiation (F_{ST}, below diagonal) between populations of *Erodium rupestre s.l.*

Population	MON	LAG	LOR	POB	ROM
MON	—	131.7	63.7	102.1	66.2
LAG	0.702	—	92.9	42.0	116.9
LOR	0.067	0.544	—	52.2	28.5
POB	0.317	0.188	0.151	—	79.9
ROM	0.213	0.286	0.069	0.017	—

*Geographic distances are expressed in km.

On the other hand, all these life-history attributes are usually associated with low levels of intrapopulation genetic variation and high divergence among populations. Nevertheless, the population's history and type of speciation could have led to a different situation from the one theoretically predicted. This is the case in the two *Borderea* species, which show very low values of diversity within populations (P = 9.5%, A = 1.14 and H_e = 0.046 in *B. chouardii*, and P = 14.3%, A = 1.21 and H_e = 0.070 in *B. pyrenaica*) but also little interpopulation genetic divergence (G_{ST} = 0.035 in *B. pyrenaica*; *B. chouardii* has only one population; Segarra-Moragues and Catalán, 2002). These last figures have been attributed to severe genetic bottlenecks in the past (Segarra-Moragues and Catalán, 2002). In contrast, high intrapopulation genetic diversity but also significant interpopulation divergence were detected using RAPD markers in *E. paularense* [H_{pop} = 2.802 and $(H_{sp}-H_{pop})/H_{sp}$ = 31.75%; Martín et al., 1999] and allozymes in two taxa of *Petrocoptis*, *P. montsicciana* (P = 70.3%, A = 2.20, H_e = 0.239, and G_{ST} = 0.376) and *P. pardoi* (P = 56.3%, A = 1.90, H_e = 0.192, and G_{ST} = 0.354; López-Pujol et al., 2001). The schizoendemic nature of *E. rupestre*, in addition to the occurrence of repeated bottlenecks and extinction episodes due to several changes in their habitat conditions throughout history, may have contributed to its current extremely low levels of diversity.

4.3. Distribution of genetic diversity, biogeography and relationships between taxa

The erosion of genetic variation detected in *E. rupestre* may also be due to the genetic isolation of populations associated with the large geographical distances between them, and enhanced by the fact that populations are usually fragmented (J. López-Pujol, pers. obs.), consequently having low population sizes. The geographical pattern of population differentiation found in *E. rupestre* agrees with the 'isolation-by-distance' (IBD) model, which suggests that in a continuously distributed taxon with a dispersal capability less than its geographic range, there is less mixing among pairs of populations situated further apart from among those which are separated by shorter distances (Wright, 1943). This leads us to believe that genetic differences between populations should increase with distance separating those populations, contrasting with the randomized gene dispersal that occurs in the 'island' model (Wright, 1931). Nevertheless, the IBD of populations found in *E. rupestre* does not necessarily mean that populations are in equilibrium between genetic drift and gene exchange, because other processes can lead to apparent IBD (Slatkin, 1993). Without direct estimates of

gene flow, we cannot determine whether the indirect value of *Nm* obtained for *E. rupestre* reflect historical or current levels of gene flow. The large geographical distances (28.5 km - 131.7 km) and the relatively absence of suitable habitats among populations, in addition to the very limited dispersal capabilities of *E. rupestre*, give support to a lack of current effective gene exchange between populations. Rather, historical associations among populations and genetic drift have played a predominant role in shaping geographic patterns of genetic structure. The lack of rare alleles within populations, the maximum of two alleles shown by all polymorphic loci, and the vast majority of loci being monomorphic (12 of 14) may show that genetic drift has been acting within populations, removing alleles of low frequencies.

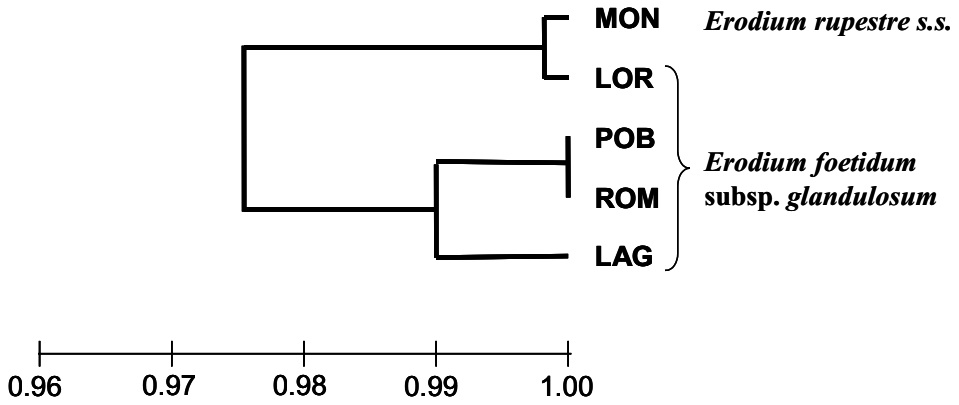

Fig. 2. Dendrogram resulting from UPGMA analysis of populations of *Erodium rupestre s.l.* based on pairwise values of Nei's (1978) genetic identity (SD = 1.616%; cophenetic correlation = 0.560).

The extinction of intervening populations in a continuous distribution could amplify the effects of IBD and allow genetic divergence to occur via an allopatric (geographic) pathway (Mayer et al., 1994). A geographic speciation (see Mayr, 1942) results from the gradual fragmentation of a wide-ranging species into geographic remnants, which evolve independently. The geographic subdivision decreases gene flow, and genetic drift and selection increase genetic divergence among populations; reproductive isolation is achieved gradually through spatial isolation. Most species groups undergoing gradual speciation include taxa in various stages of the process, as occurs in the *Streptanthus glandulosus* complex in California (Mayer et al., 1994). These different stages might be inferred using genetic identities of the compared taxa, since their values decrease inversely with phylogenetic distance. From other studies using allozymes, it may be inferred that mean genetic identities of conspecific populations are usually above 0.90 (mean = 0.95), whereas the genetic identities of congeneric taxa are usually below 0.70 (mean = 0.67) (Gottlieb, 1977; Crawford, 1983). One exception to this rule are the recently diverged progenitor-derivative species pairs, which show genetic identities typically more than 0.88 (Edwards and Sharitz, 2000).

In *E. rupestre s.l.*, the genetic identity between the two presumable taxa (*E. foetidum* subsp. *glandulosum vs. E. rupestre s.s.*) is very high (I = 0.973), clearly suggesting that the two taxa form a single species. Moreover, the two suspected taxa are morphologically indistinguishable, which gives support to the taxonomical treatment as a unique taxon in the forthcoming revision of Geraniaceae for *Flora Iberica* (Aldasoro

& Sáez, in press). Mediterranean flora is replete with examples of disjunct distributions of closely related species (Thompson, 1999), some in rupicolous endemic species. In the pair of *Borderea* species, *B. chouardii*, which has only one population near the edge of the distribution area of *B. pyrenaica*, is considered a diverged, although recently, species from *B. pyrenaica*, since both taxa have already reached a clear morphological distinction but genetic identity between both taxa is still high (I = 0.928; Segarra-Moragues and Catalán, 2002).

Nevertheless, as can be observed in Table 2 and Fig. 1, there is a trend in the allelic distributions which agrees with the IBD detected pattern. The prevalence of the faster allele detected in the locus *Aco-1* (*Aco-1a*) shows a decreasing gradient towards the east and the south, the two most distant populations being LAG and MON, those with the biggest differences in the *Aco-1a* frequency. This pattern may be explained by ancient gene interchanges among populations. The rocky hilltops, the current habitat of *E. rupestre*, could have been connected during the glaciations, thus allowing gene flow among populations. After the ice age, these connections would have been lost and other forces, such as genetic drift and selection, could have acted within the remnant populations. The MON population (which corresponds to *E. rupestre s.s.*) is the only one which exhibits the allele *Pgm-1a*, although with a low frequency (0.111). These new allele could be the result of a single mutation or, alternatively, the aforementioned drift has removed it from the northern populations but not yet from MON. In any event, the distinctiveness of MON population seems clear, and may represent the first step of an allopatric speciation process occurring in that disjunct population. Nevertheless, the origin of MON population by a long-distance dispersal southwards and further colonizing event from LOR population (I = 0.997 between the two populations) cannot be rejected.

4.4. Conservation implications

Inbreeding can lead to short-term decreases in individual fitness; the random loss of alleles in genetic drift reduces the ability to face habitat changes over the long-term (Young et al., 1996). These processes make populations more sensitive to local extinction, since they have reduced adaptability to biotic and abiotic environmental changes (Barrett and Kohn, 1991) and limited resistance to pest and diseases (Frankham, 1995). Despite the lack of allelic variation and the significant inbreeding detected, *E. rupestre* is a long-persisting species with a high population stability which has assured its survival up to the present. Only catastrophic natural events, or substantial human disturbances can cause the disappearance of populations. Anthropogenic threats are currently minor, since populations are usually isolated from urban areas and human activities. However, the destruction and/or fragmentation of natural habitats by road construction or their improvement, tourism pressure (e.g. on the LOR and MON populations, which are located beside crowded sanctuaries), some recreational activities (such as trekking and climbing), and fires are potential threats for this species.

Although this species is only moderately to slightly threatened, both *in-situ* and *ex-situ* conservation measures should be implemented. The preservation of the MON population must be prioritized, since it harbours a unique allele and may represent the initial step of an allopatric speciation process occurring in that population. Furthermore, MON is at present the most threatened population, due its proximity to Montserrat Abbey, one of the most visited Spanish tourist spots. The population is located beside one of the trekking trails of Montserrat mountain, and it may be easily collected by

visitors. Therefore, we recommend consideration of MON as a Conservation Unit (CU), which should be monitored and preserved through its delimitation as a botanical reserve. Despite the low levels of genetic variation of *E. rupestre*, to maintain a germplasm bank we recommend the collection of seeds from another source apart from MON to assure the conservation of the allele *Aco-1a*, e.g. ROM population, where both alleles of *Aco-1* exhibit intermediate frequencies.

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3.1.8. **López-Pujol J**, Orellana MR, Bosch M, Simon J & Blanché C. Low genetic diversity in the tetraploid Pyrenean endemic larkspur *Delphinium montanum* (Ranunculaceae). Allozymic evidences for autopolyploidy and further diploidization. *International Journal of Plant Sciences*, en revisió.

LOW GENETIC DIVERSITY IN THE TETRAPLOID PYRENEAN ENDEMIC LARKSPUR *DELPHINIUM MONTANUM* (RANUNCULACEAE): ALLOZYMIC EVIDENCES FOR AUTOPOLYPLOIDY AND FURTHER DIPLOIDIZATION

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Allozyme electrophoresis was conducted to survey the levels and distribution of genetic diversity of the tetraploid perennial larkspur *Delphinium montanum* DC. (Ranunculaceae). This species is endemic to the Eastern Pyrenees, both in the Spanish and French sides. It is considered an endangered plant, listed in the Spanish and the French red lists. Seven populations distributed within both countries were sampled, resolving 15 putative enzymatic loci belonging to eight enzymes. Banding patterns stained in gels revealed several enzymatic duplications which can be attributed to autotetraploidy, due to the presence of both balanced and unbalanced heterozygotes and the lack of fixed heterozygosity. However, values of variability in *D. montanum* (P = 23.8%, A = 1.48, and H_e = 0.082) are rather lower than the expected for autotetraploid species. This, in addition to the scarce presence of three or four different alleles at surveyed loci, could indicate that this species is currently suffering a process of diploidization. Cytological and morphological data also suggest diploidization of the genome of this larkspur. Some considerations about its conservation status are also provided.

Keywords: Allozyme electrophoresis, genetic diversity, tetraploidy, diploidization, Ranunculaceae, *Delphinium montanum*.

Introduction

Polyploidy is considered a significant mode of species formation and an important source of evolution in higher plants (Stebbins 1980); currently it is recognised that polyploidization may be the most common single mechanism of sympatric speciation in plants, and allow them to evolve faster or in novel directions than do related diploids, promoting therefore adaptative evolutionary change (Otto and Whitton 2000). Polyploidy is a widespread event in nature, but estimates of the frequency of polyploidy in angiosperms have widely varied along twentieth century: 30-35 % (Stebbins 1950), 47% (Grant 1981), and 70-80% (Lewis 1980; Masterson

1994). On the other hand, Otto and Whitton (2000) estimated that roughly 2-4% and 7% of all speciation events in angiosperms and ferns, respectively, involved polyploidy. Nevertheless, recent genomic studies have revealed that already all plants have undergone one of more episodes of polyploidization (Soltis et al. 2004). Repeated cicles of genome duplication, followed by extensive diploidization, have lead to many species to be functional diploids (Soltis et al. 2004).

The success of polyploidy can be explained by several genetic factors such as increased heterozygosity and genetic diversity, and enzyme multiplicity (i.e. the capacity to produce new heterodimeric proteins; Thompson and Lumaret 1992; Soltis and Soltis 2000; Soltis et al. 2004). These changes may lead to an increased biochemical diversity (Levin 1983), improving the ability of plants to respond to different environmental conditions (i.e. maintaining fitness; Thompson and Lumaret 1992), and allowing them to colonize new ecological niches (Ramsey and Schemske 1998). Moreover, inbreeding depression may be reduced in polyploids, because of the buffering effect of additional genomes masking deleterious alleles (Soltis and Soltis 2000). These, in addition to the possibility that genetic self-incompatibility may break down in polyploids (Otto and Whitton 2000), are reasons for the higher selfing rates observed in polyploids in comparison to the diploid progenitors. Self-compatibility, vegetative (asexual) multiplication and long life span (perenniality) may promote the successful colonization and establishment of polyploids, since these are adaptative strategies in situations of low availability of mating (Thompson and Lumaret 1992; Otto and Whitton 2000).

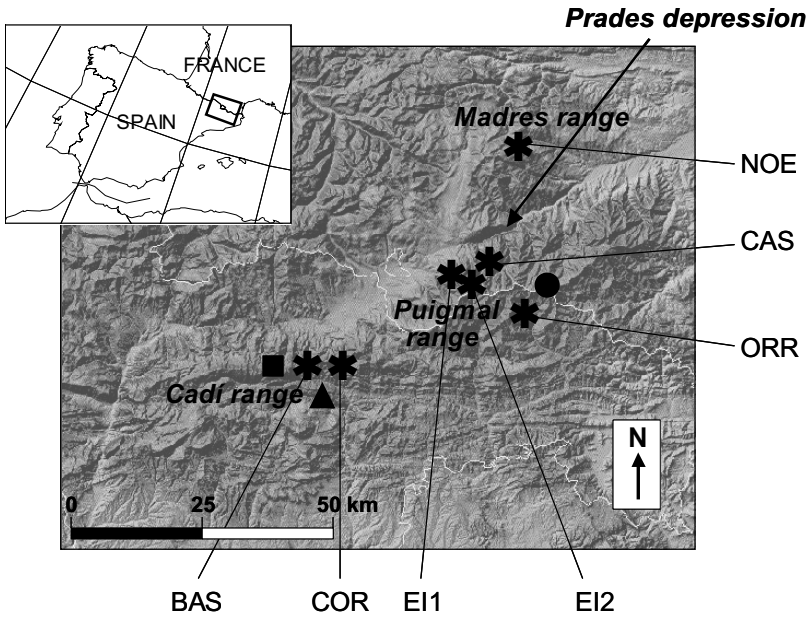

Fig. 1. Sampled populations of *Delphinium montanum*. BAS: Bastanist; COR: Els Cortils; ORR: Coma de l'Orri; EI1: Vall d'Eina (Lower); EI2: Vall d'Eina (Upper); CAS: Cambra d'Ase; NOE: Noedes. The triangle, the circle, and the square correspond to extinct populations (Pedraforca, Portella de Mantet, and Cava, respectively).

Two major types of polyploidy are recognized in nature, allopolyploidy and autopolyploidy (Stebbins 1947; 1950; Grant 1981; Crawford 1989; Soltis and Soltis 2000), although these two terms represent the ends of a spectrum of variation (Grant 1981, Thompson and Lumaret 1992; Soltis et al. 2004). Two major types of criteria

have been used to distinguish them: by cytologic and genetic criteria, and by their taxonomical rank (Soltis et al. 2004). Following Stebbins (1947; 1950) and Jackson (1982), allopolyploids are characterized by disomic inheritance, bivalent formation at meiosis and fixed heterozygosity (non-segregating) due to the combination of two divergent parental genomes. In contrast, autopolyploids are expected to express polysomic inheritance (tetrasomic in autotetraploids) and may also show multivalent formation at meiosis. However, these rules are often broken and, while autopolyploids may exhibit disomic inheritance, allopolyploids rarely exhibit multivalent formation (Otto and Whitton 2000; Soltis et al. 2004).

Alternatively, first Kihara and Ono (1926) and after Lewis (1980), identified allopolyploidy and autopolyploidy as interspecific polyploidy and intraspecific polyploidy, respectively. Allopolyploids are the result of a hybridization process between diferent species and subsequent chromosome duplication, while autopolyploidy is probably the consequence of crosses of conspecific individuals (within or between populations), generally by fusion of non-reduced gametes. Nevertheless, both allo- and autopolyploids may also be formed via a triploid bridge, in which takes place a union of a reduced and an unreduced gamete (Ramsey and Schemske 1998). This classification of polyploids, however, depends on species circumscription, a fact often subjected to a considerable variation on the criteria used (Ramsey and Schemske 1998).

It has been postulated in the past that allopolyploids were much more common than autopolyploids (Stebbins 1950; Grant 1981). However, it is currently recognised that autopolyploids are more frequent than traditionally mantained (Thompson and Lumaret 1992; Ramsey and Schemske 1998; Soltis et al. 2004). Autopolyploids were considered as maladaptative taxa (Soltis and Rieseberg 1986), because of their presumable slower development and reduced fertility due to meiotic irregularities (Ramsey and Schemske 1998). Permanent hybridity in allopolyploids due to fixed heterozygosity, which confers 'intrinsic advantages' (heterosis and homeostasis) and which lacks on autopolyploids, has been suggested as another reason to explain the incidence differences between the two types of polyploidy (Ramsey and Schemske 1998). Nevertheless, tetrasomic ratios provide autopolyploids with higher heterozygosity than their diploid parents and the possibility of three or four alleles at a single locus, which explain their success in nature as a rapid strategy of speciation and evolution.

Table 1

Populations of *Delphinium montanum* studied

Population code	Location	Elevation (a.s.l.)	Population size	Sample size
BAS	Bastanist (Spain), 31TCG98	2,200m	~2,500	72
COR	Els Cortils (Spain), 31TCG98	2,200m	~1,000	76
ORR	Coma de l'Orri (Spain), 31TDG39	1,850m	~500	74
EI1	Vall d'Eina (Lower) (France), 31TDG29	1,900m	~1,500	50
EI2	Vall d'Eina (Upper) (France), 31TDG29	2,100m	~80	24
CAS	Cambra d'Ase (France), 31DH20	2,400m	~200	70
NOE	Noedes (France), 31TDH31	1,750m	~700	72

Note. Location of populations is detailed by UTM 10 × 10 km squares.

Delphinium montanum DC. (Ranunculaceae) is a perennial larkspur endemic to the Eastern Pyrenees, on both the Spanish and French sides of Catalonia. Taxonomically it belongs to the series *Montana* B. Pawl., which includes two additional species: *D. dubium* (Rouy et Fouc.) B. Pawl., endemic to the Alps, and *D. oxysepalum* Borb. & Pax, restricted to the Carpathians. Pawlowsky (1970) suggests that the three taxa had a common ancestor before the last glaciation period, which probably suffered a fragmentation of its distribution area in the Quaternary. The high european ranges could have acted as refugia of those remnants, allowing an allopatric speciation process leading to the current three endemovicariants. *D. montanum* is a tetraploid with $2n = 4x = 32$, that is, the same ploidy level and chromosomic count than *D. dubium* and *D. oxysepalum* (Blanché 1991), which gives support to the suggested origin.

D. montanum is an herb of 15-50 (70) cm tall, with palmate and laciniate leaves. It produces a basal rosette and 15-20 flowers on a racemose inflorescence; each plant can produce up to 30 racemes. Flowers are blue, 23-33 mm long, and they have a spur of 11-15 mm which contains nectar. It exhibits a bee-flower syndrome due to the different color between floral pieces. Seeds are black and smooth, of 2.5-3.5 × 1.75-3.0 mm (Blanché 1991). Pollination is strictly dependent on pollinator activity (mainly by *Bombus hortorum*) to set seeds, and the mating system is basically outcrossing (Simon et al. 2001). Seed dormancy has been observed in the field (Bosch 1999). *D. montanum* grows in rocky places such as mobile or fixed screes, over calcareous substrates, at altitudes comprised between 1,600 and 2,200m. Aerial parts sprout in June, and flowering period is restricted to July and August, due to the cold and snowy conditions of its habitat during the rest of the year. Currently this species only remains in seven locations of the 10 described, four in the French side of the Pyrenees and three within the Spanish boundaries. The total population size is estimated in 6,280 mature individuals (Table 1). According to the IUCN recommendations to estimate the distribution area of the species (IUCN 2001), the extent of occurrence is about 827.5 km^2 and the occupancy area < 20 km^2. The species has been listed as VU ('vulnerable') in the *Red List of the Vascular Spanish Flora*; Aizpuru et al. 2000). *Delphinium montanum* will also be included in the second volume of the *Red Book of the French Endangered Flora* (Olivier et al. 1995).

Allozyme electrophoresis was used to address the following issues: (i) to obtain some insights about the polyploid origin for *D. montanum* (if autopolyploid or allopolyploid), and (ii) to describe levels and distribution of its genetic diversity. Also, we revise the status of conservation of this species and we suggest some strategies for its preservation.

Materials and Methods

Sampling strategy

All the seven extant populations of *D. montanum* were sampled (see Table 1 and Figure 1). Since the aerial part of the plant is only present during a short period of time (from June to September), and plants can be only surely distinguished from

Aconitum spp. during the flowering period (July-August), samples were collected in three different years: the two populations from Vall d'Eina (EI1 and EI2) and the population from Els Cortils (COR) were sampled in July 2002; populations from Bastanist (BAS) and Coma de l'Orri (ORR) were sampled in July 2003; finally, populations from Noedes (NOE) and Cambra d'Ase (CAS) were sampled in July 2004. Sampling was conducted along a linear transect within each population and samples were collected about 50-100 cm apart to avoid collecting ramets from the same genet. Samples consisted of small fragments of leaves which were placed into envelopes, transported to the laboratory, and stored at 4°C until extraction one or two days later. Given the threatened status of this species, collection of samples was done carefully to minimize the potential damage to populations.

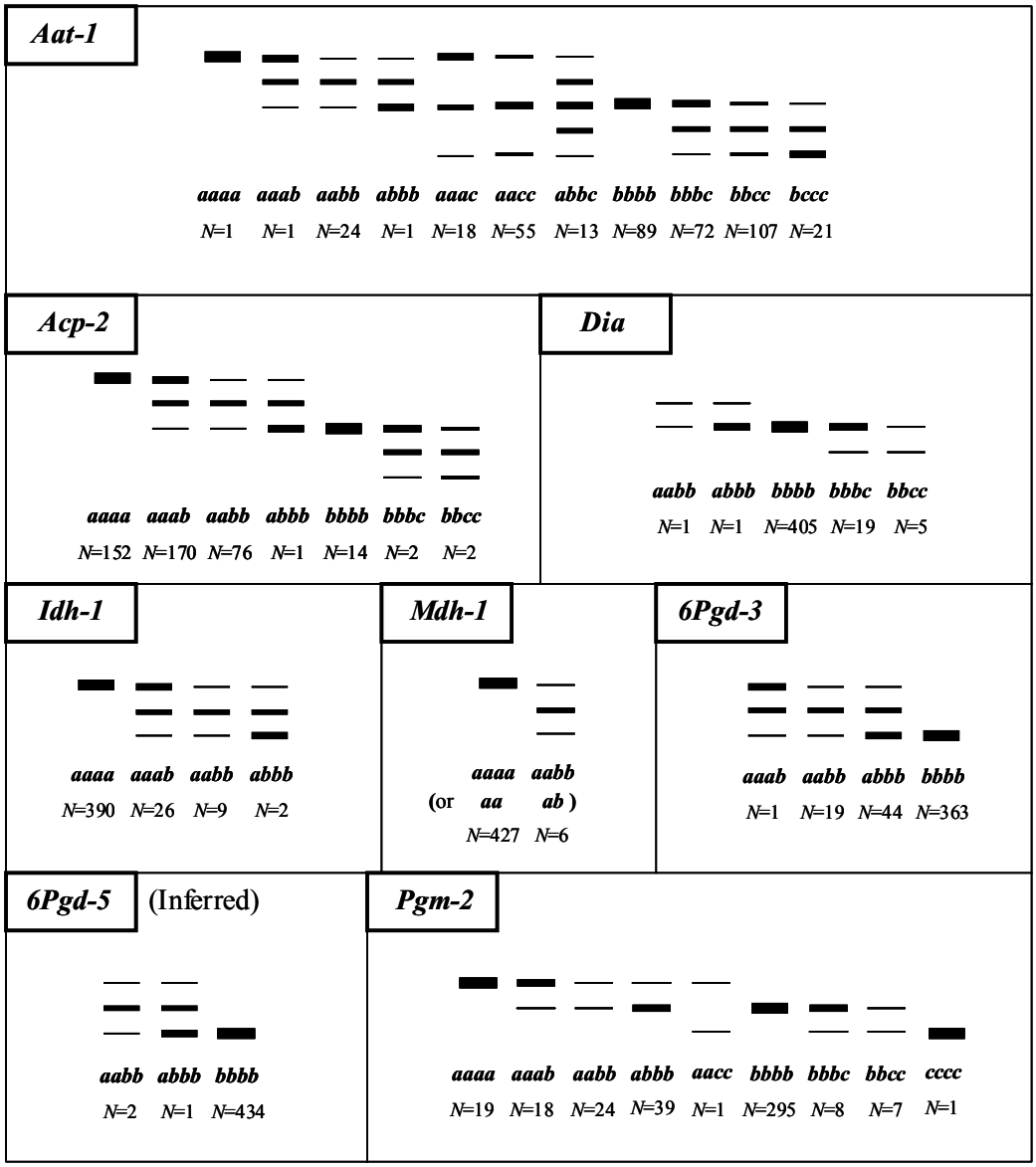

Fig. 2. Schematic banding patterns obtained for polymorphic loci in *Delphinium montanum*. All the inferred genotypes and the number of individuals showing each genotype are given below each genotype.

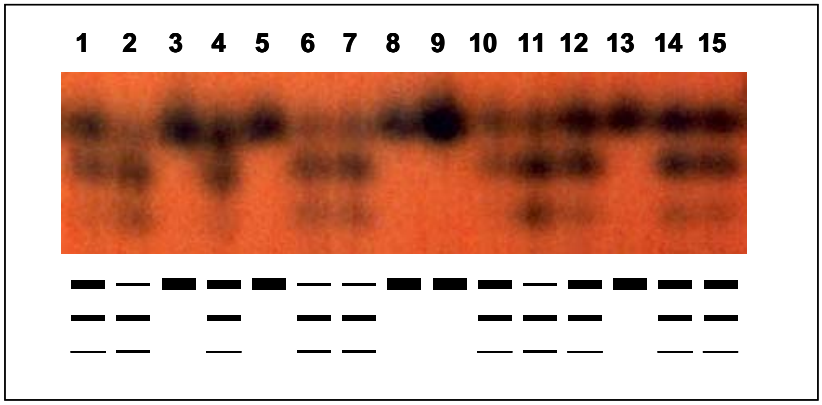

Fig. 3. Schematic banding patterns obtained for *Aat-1* in COR population of *Delphinium montanum*. Three types of genotypes may be inferred: homozygotes *aaaa* (individuals no. 3, 5, 8, 9 and 13) , balanced heterozygotes *bbcc* (individuals no. 2, 6, 7, and 11), and unbalanced heterozygotes *bbbc* (individuals no. 1, 4, 10, 12, 14 and 15).

Electrophoresis

Genetic data were obtained through standard methods for starch gel electrophoresis of allozymes (Soltis et al. 1983; Wendel and Weeden 1989). Leaves were homogenized on refrigerated porcelain plates using a cold extraction buffer consisting of 0.05 M tris-citric acid, 0.1% cysteine·HCl, 0.1% ascorbic acid, 8% PVP-40 (polyvinyl-pyrrolidone), and 1mM 2-mercaptoethanol. Extracts were absorbed onto 3 mm Whatman filter paper, either to be analysed immediately or stored at –80ºC for long-term conservation until electrophoresis. Using 11% starch gels, 8 enzyme systems were resolved. Aspartate aminotransferase (AAT, EC 2.6.1.1), diaphorase (DIA, EC 1.6.99.), and phosphoglucoisomerase (PGI, EC 5.3.1.9) were satisfactorily resolved on a tris-citrate/lithium-borate buffer at pH 8.2; acid phosphatase (ACP, EC 3.1.3.2), isocitrate dehydrogenase (IDH, EC 1.1.1.42), and phosphogluconate dehydrogenase (6-PGD, EC 1.1.1.44) were resolved on a morpholine buffer at pH 6.1; finally, malate dehydrogenase (MDH, EC 1.1.1.37), and phosphoglucomutase (PGM, EC 5.4.2.2) were resolved on histidine-citrate buffer pH 5.7.

Genetic analyses

Loci were numbered consecutively, and alleles at each locus were labeled alphabetically, beginning from the most anodal form. Isozyme phenotypes were interpreted genetically according to standard principles (Wendel and Weeden 1989), but special attention was paid to characteristic phenotypes of tetraploids and their interpretation (Gottlieb 1981). To describe the levels of genetic diversity, the following statistics were calculated: P, the percentage of polymorphic loci at which the most common allele had a frequency of <0.95; A, the mean number of alleles per locus; H_o, the observed heterozygosity; and H_e, the expected panmictic

heterozygosity. In autotetraploids, two types of H_e can be computed; H_e (*Ce*), the expected heterozygosity assuming random chromosomal segregation, and H_e (*Cd*), the expected heterozygosity assuming some level of chromatid segregation. Chromatid segregation is produced if 'double reduction' takes place, that is, sister chromatids segregate into the same gamete, a phenomenon specific of autopolyploids and which is dependent on the amount of tetravalent formation and the proximity of the locus to the centromere (see Bever and Felber 1992; Ronfort et al. 1998). The study of the meiotic behaviour suggested that double reduction does not take place in *D. montanum* (Blanché 1991), assuming therefore that only chromosomal segregation occurs. This consideration allows us to perform a more conservative data processing regarding the type of polyploid origin for this species (auto- or allopolyploid), since double reduction increases the production of homozygous gametes as compared to what is expected under random chromosomal segregation in diploids and allopolyploids (Ronfort et al. 1998). We also calculated the mean fixation index (*F*) for all variable loci in each population, to compare genotype proportions with those expected under the Hardy-Weinberg equilibrium. We used a chi-square (χ^2) test to evaluate deviations of *F* from zero. The partitioning of genetic diversity within and between populations was analysed using Nei's (1973) gene diversity statistics: total genetic diversity (H_T), mean genetic diversity within populations (H_S), genetic diversity between populations (D_{ST}), and proportion of total genetic diversity between populations (G_{ST}) were calculated for all populations. Gene flow (*Nm*) was estimated by Wright's (1951) equation: $Nm = 1- G_{ST} / 4 G_{ST}$. On the other hand, we calculated Nei's (1978) genetic identity (*I*) between pairs of populations, which was used to cluster those into a dendrogram following UPGMA (unweighted pair group method with averaging). A program for analysis of autotetraploid genotypic data, AUTOTET (Thrall and Young 2000), was used for calculations of *A*, H_o, H_e, and *F*. Choosing the appropriate options for tetraploids, BIOSYS-1 (Swofford and Selander 1989) was used for the calculation of *P*, while GeneStat version 3.31 (Whitkus 1988) was used to calculate H_T, H_S, D_{ST} and G_{ST}.

Results

Interpretation of enzyme banding patterns

The satisfactorily resolved enzymes displayed 15 putative loci: *Aat-1*, *Aat-2*, *Acp-2*, *Dia*, *Idh-1*, *Mdh-1*, *Mdh-2*, *6Pgd-1*, *6Pgd-2*, *6Pgd-3*, *6Pgd-4*, *6Pgd-5*, *Pgi-2*, *Pgm-1*, and *Pgm-2*. Seven of these loci (*Aat-2*, *Mdh-2*, *6Pgd-1*, *6Pgd-2*, *6Pgd-4*, *Pgi-2*, and *Pgm-1*) were monomorphic across populations, i.e. the phenotype of all examined individuals showed a single band (homozygote genotypes). By contrast, the eight remainder loci were polymorphic, and some of them exhibited a wide array of bands (see Figure 2). Banding patterns in AAT, ACP, IDH, MDH, and 6-PGD were consistent with dimeric enzymes, and in DIA and PGM, with monomeric enzymes. All banding patterns found for the interpretable loci are briefly described below.

Aspartate aminotransferase (AAT) displayed two regions of activity in *D. montanum*, encoded by two independent putative loci: *Aat-1* and *Aat-2*, being the last one monomorphic. *Aat-1* showed three diferent alleles. Whereas homozygotes corresponded only to the two fastest alleles (*Aat-1a* and *Aat-1b*), combinations of heterozygous phenotypes among all the alleles can be found. Several types of

heterozygotes were observed at this locus, which were distinguished by the number of bands and/or the relative intensity of band staining (Figure 3). Thus, we were able to observe three-banded heterozygotes (genotypes which are combinations of two alleles), either with balanced (same allelic dosage) or unbalanced staining activity (different allelic dosage). Although most of these heterozygotes are compounded by *Aat-1b and c* alleles, some consisted of combinations between *a* and *b* alleles or even *a* and *c*. Furthermore, some individuals showed a five-banded phenotype with balanced staining activity, which could be interpreted as having three different alleles for this enzyme (genotype *abbc*).

Table 2

Allele frequencies for polymorphic loci in seven populations of *Delphinium montanum*

Locus	Allele	BAS	COR	ORR	EI1	EI2	CAS	NOE
Aat-1	*a*	0.099	0.048	0.008	0.080	0.022	0.066	**0.564**
	b	**0.831**	**0.747**	0.444	**0.680**	**0.739**	**0.575**	0.000
	c	0.070	0.205	**0.548**	0.250	0.261	0.358	0.436
Acp-2	*a*	**0.875**	**0.586**	**0.761**	**0.890**	**0.833**	**0.631**	**0.868**
	b	0.125	0.386	0.239	0.110	0.167	0.369	0.132
	c	0.000	0.029	0.000	0.000	0.000	0.000	0.000
Dia	*a*	0.000	0.013	0.000	0.000	0.000	0.000	0.000
	b	**0.972**	**0.987**	**0.993**	**0.990**	**0.979**	**0.968**	**0.979**
	c	0.028	0.000	0.007	0.010	0.021	0.032	0.021
Idh-1	*a*	**1.000**	**0.967**	**0.864**	**1.000**	**1.000**	**0.996**	**1.000**
	b	0.000	0.033	0.136	0.000	0.000	0.004	0.000
Mdh-1	*a*	**1.000**	**1.000**	**1.000**	**1.000**	**0.875**	**1.000**	**1.000**
	b	0.000	0.000	0.000	0.000	0.125	0.000	0.000
6Pgd-3	*a*	0.000	0.039	0.101	0.052	0.043	0.007	0.103
	b	**1.000**	**0.961**	**0.899**	**0.948**	**0.957**	**0.993**	**0.897**
6Pgd-5	*a*	0.000	0.000	0.014	0.000	0.000	0.000	0.000
	b	**1.000**	**1.000**	**0.986**	**1.000**	**1.000**	**1.000**	**1.000**
Pgm-2	*a*	0.215	0.239	0.045	0.071	0.205	0.197	0.007
	b	**0.785**	**0.761**	**0.955**	**0.929**	**0.795**	**0.739**	**0.958**
	c	0.000	0.000	0.000	0.000	0.000	0.064	0.035

Note. The most frequent allele for each locus and population is in boldface.

Acid phosphatase (ACP) presented two zones of activity, encoded by two different putative loci (*Acp-1* and *Acp-2*). *Acp-1* was too poorly stained to be interpreted. *Acp-2* exhibited three different alleles, although the slowest one (*Acp-2c*) in a very low frequency (only four individuals from the 417 analysed; see Figure 2). Both single-banded homozygotes and several types of heterozygotes (both balanced and unbalanced) were observed, being the genotypes *aaaa*, *aaab* and *aabb* the most common.

A single zone of activity was detected for the diaphorase (DIA), encoded by one putative locus (*Dia*). Most of the individuals (*N*=405; see Figure 2) showed a

single-banded phenotype (interpreted as the *bbbb* genotype). Nevertheless, some analysed plants exibited two-banded phenotypes, either balanced heterozygotes and unbalanced ones, due to the monomeric structure of this enzyme. The presence of the fastest allele (*Dia-a*) is very rare (only showed by two individuals).

Two regions of activity were identified for isocitrate dehydrogenase (IDH), from which only the most anodal was consistently interpreted (*Idh-1*). Just two alleles were detected, but the slowest one (*Idh-1b*) appeared rarely (only shared by 37 individuals of the 427 analysed; see Figure 2). The most common inferred genotype was the homozygote *aaaa* (*N*=390). Although in low frequency, two kinds of three-banded heterozygotes were found, balanced (*aaab* and *abbb*) and unbalanced (*aabb*).

Table 3

Summary of genetic variation for 15 loci in seven populations of *Delphinium montanum*

Population	*P*	*A*	H_o	H_e	*F*
BAS	20.0	1.33	0.046 (0.090)	0.060 (0.119)	0.232 (0.075)
COR	20.0	1.60	0.072 (0.128)	0.096 (0.173)	0.250 (0.093)
ORR	26.7	1.53	0.098 (0.185)	0.094 (0.157)	-0.048 (0.058)
EI1	26.7	1.40	0.067 (0.136)	0.068 (0.139)	0.027 (0.013)
EI2	26.7	1.47	0.078 (0.137)	0.088 (0.141)	0.121 (0.074)
CAS	20.0	1.53	0.089 (0.196)	0.100 (0.194)	0.107 (0.135)
NOE	20.0	1.40	0.075 (0.170)	0.069 (0.138)	-0.087 (0.068)
Mean	**23.8**	**1.48**	**0.075**	**0.082**	**0.089**
Standard deviation	5.2	0.09	0.017	0.016	0.128

Note. *N*: sample size; *P*: percentage of polymorphic loci; *A*: mean number of alleles per locus; H_o: observed heterozygosity; H_e: expected panmictic heterozygosity; *F*: mean fixation index. Standard deviation in parentheses.

Malate dehydrogenase (MDH) showed at least three zones of activity, although only the most anodal was interpretable, controlled by the putative locus *Mdh-1*. Whereas most of the analysed individuals were homozygotes for the fastest allele (*Mdh-1a*), a few showed a three-banded balanced phenotype (interpreted either as *aabb* –i.e. with tetraploid load– or alternatively as *ab* –i.e. diploidized–).

Phosphogluconate dehydrogenase (6-PGD) exhibited up to five regions of activity, and all of them could be interpreted. The two most anodal loci (*6Pgd-1* and *6Pgd-2*) and the *6Pgd-4* locus showed a single-banded phenotype for all the analysed individuals, interpreted as the *aaaa* genotype. On the contrary, the *6Pgd-3* locus showed two different alleles, being the slowest one (*6Pgd-3b*) the most common (Figure 2). Both balanced (*aabb*) and unbalanced (*aaab* and *abbb*) heterozygous genotypes were inferred, but only homozygotes for the *6Pgd-3b* allele were found. The most cathodal isozyme (*6Pgd-5*) was too faint to be interpretable. Fortunately, an additional region of enzymatic activity appeared below the *6Pgd-4* locus, which was interpreted as intergenic heterodimers between *6Pgd-4* and *6Pgd-5*, since they consisted of one or two-banded (balanced or unbalanced) phenotypes. With the exception of three individuals, all plants exhibited a homozygous *bbbb* genotype.

Phosphoglucomutase (PGM) presented two zones of activity. Only the most cathodal region was interpretable, controlled by the putative locus *Pgm-2*. Analysed individuals showed three different alleles at this locus, *Pgm-2b* being the most common. Several types of two-banded homozygotes (since this enzyme behaves as monomeric), either balanced and unbalanced, were revealed in the starch gels, even between the fastest and the slowest allele. Although homozygotes with all the alleles were found, the most common was the genotype *bbbb* (*N*=295).

Levels and distribution of genetic diversity

Among the 15 interpretable loci we detected 27 alleles; the allelic frequencies of polymorphic loci are given in Table 2. The 'allelically' richest populations were CAS, COR, and ORR (all displaying 23 alleles); the poorest one was BAS, which scored 20 alleles. COR population showed two private (population-specific) alleles (*Acp-2c* and *Dia-a*), whereas EI2 and ORR harbored one private allele each one (*Mdh-1b* and *6Pgd-5a*, respectively). Rare alleles (those with a frequency of less than 0.050) were present in all populations, with a maximum of 5 in COR population. All the population-specific alleles were rare with the exception of *Mdh-1b* in EI2 population. Furthermore, some populations harbored extremely rare alleles, i.e. those with frequencies less than 0.010, such as ORR (*Aat-1a*, *Dia-c*), CAS (*Idh-1b*, *6Pgd-3a*), and NOE (*Pgm-2a*). All the populations shared the same allele as the most frequent across loci with the exception of *Aat-1*, where ORR and NOE exhibited a different allele (*Aat-1c* and *Aat-1a*, respectively). On the other hand, 13 of the 402 plants (3.2%) examined for the *Aat-1* locus had three alleles; no additional loci exhibited more than two alleles at individuals (see Figure 2).

Table 4

Values of fixation index (*F*) for all polymorphic loci in seven populations of *Delphinium montanum*

Locus	BAS	COR	ORR	EI1	EI2	CAS	NOE
Aat-1	0.225***	0.008*	-0.262***	0.031ns	-0.155ns	-0.021***	-0.269***
Acp-2	-0.076ns	0.462***	-0.083ns	-0.032ns	0.000ns	-0.244**	-0.117ns
Dia	0.143*	0.158***	-0.004ns	-0.010ns	-0.021ns	-0.033ns	0.319***
Idh-1	—	0.088ns	0.107ns	—	—	-0.004ns	—
Mdh-1	—	—	—	—	0.238***	—	—
6Pgd-3	—	0.132*	0.073ns	0.004ns	0.196ns	-0.007ns	0.124ns
6Pgd-5	—	—	0.254***	—	—	—	—
Pgm-2	0.454***	0.282*	0.535ns	0.158ns	0.488ns	0.699***	0.386***

Note. Conformance to Hardy-Weinberg equilibrium was tested using chi-square analysis: ns $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$,*** $p < 0.001$.

Genetic diversity was moderate to low in *D. montanum*, with mean values overall seven populations of $P = 23.8\%$, $A = 1.48$, and $H_e = 0.082$ (Table 3). The most variable population was CAS ($H_e = 0.100$), while the least diverse was BAS ($H_e =$

0.060); however, values of the standard deviation (Table 3) showed that differences between populations were not statistically significant. Values of observed heterozygosity were higher than those of expected heterozygosity in all populations except ORR and NOE, which showed a positive value of *F* (fixation index) (Table 3). At loci level, of the 39 valid tests the chi-square test (χ^2) showed that 22 *F*-values were in accordance with Hardy-Weinberg expectations ($p \geq 0.05$), while 17 *F*-values differed significantly from zero ($p < 0.05$). Of these 17 values, 13 were positive and 4 were negative (Table 4). Positive values indicate deficiency of heterozygotes; negative values indicate their excess.

Genetic diversity in *D. montanum* was distributed mainly within populations (H_S = 0.082) rather than between them (D_{ST} = 0.013); nevertheless, a significative fraction of the genetic variability is attributable to differences among populations (G_{ST} = 0.135; Table 5), i.e. populations are quite divergent. The number of migrants per generation was consequently low (*Nm* = 1.60), value from which can be inferred that the level of gene flow should be quite limited among populations. The UPGMA dendrogram (Figure 4), shows a clear separation of NOE populations from the remainder populations, which are clustered together. The most distant populations (NOE and BAS, which are separated 60 km) are also the most genetically distant ($I_{NOE\text{-}BAS}$ = 0.960), while the closest pair (EI1-EI2; 0.8 km) are also the genetically closest one ($I_{EI1\text{-}EI2}$ = 0.999; see Table 6).

Discussion

Autopolyploidy and diploidization in Delphinium montanum

Allozyme data are extremely useful in distinguishing between autopolyploids and allopolyploids (Soltis and Rieseberg 1986; Crawford 1989; López-Pujol et al. 2004). Tetrasomic inheritance in autotetraploids results in the formation of unbalanced as well as balanced heterozygotes in all possible combinations, because alleles at a given locus on the homologous chromosomes segregate at random. In contrast, allotetraploids are expected to display fixed heterozygosity since alleles at a given locus on homeologous chromosomes segregate independently (Ramsey and Schemske 2002). Therefore, if the loci on homoeologous chromosomes are fixed for different alleles, these loci will exhibit fixed heterozygosity. For example, when two alleles (*a* and *b*) are present at a locus in an autotetraploid, three types of heterozygotes can be produced, one balanced (*aabb*) and two unbalanced (*aaab* and *abbb*), which can be differentiated on zymograms by the intensity of band staining (the 'allelic dosage'). Such pattern would only be possible in an allopolyploid if the locus was heterozygous for *a* and *b* on both pairs of homoelogous chromosomes.

Allozyme data support an autotetraploid hypothesis for the origin of *D. montanum*. No evidence of fixed heterozygosity was found in none of the loci examined. In the eight polymorphic loci, all but one showed heterozygotes both balanced and unbalanced (see Figure 2). For instance, for the *Aat-1* locus, 113 of the 299 analysed heterozygote individuals (i.e. 37.8%) showed unbalanced banding patterns; for the *Acp-2*, 173 of the 251 (i.e. 68.9%) heterozygotes were unbalanced. The *Mdh-1* locus showed only balanced heterozygotes although these were only the

minority of the individuals analysed for this loci (6 of 433); the remainder exhibited a homozygous banding pattern (see Figure 2).

Table 5

Gene diversity statistics (Nei, 1973) for all polymorphic loci in seven populations of *Delphinium montanum*

Locus	H_T	H_S	D_{ST}	G_{ST}
Aat-1	0.583	0.444	0.139	0.238
Acp-2	0.351	0.325	0.026	0.074
Dia	0.036	0.036	0.000	0.000
Idh-1	0.050	0.045	0.005	0.093
Mdh-1	0.036	0.031	0.004	0.117
6Pgd-3	0.094	0.092	0.002	0.026
6Pgd-5	0.004	0.004	0.000	0.005
Pgm-2	0.266	0.250	0.016	0.062
Mean	**0.095**	**0.082**	**0.013**	**0.135**
Standard error	0.044	0.036	—	—

Note. H_T: total genetic diversity; H_S: genetic diversity within populations; D_{ST}: genetic diversity between populations; G_{ST}: proportion of total genetic diversity among populations.

Tetraploidy allows for the presence of three or four different alleles at a single locus since loci are duplicated (Soltis and Rieseberg 1986; Soltis and Soltis 1989; Mahy et al. 2000). In autopolyploids, segregation at a given locus can involve as many alleles as there are homologous chromosomes (Ramsey and Schemske 2002), i.e. up to four in autotetraploids. Compared to diploids, more enzyme variants and an increased biochemical diversity may contribute to the success of polyploids in nature (Levin 1983). For *D. montanum*, only thirteen (about 3%) of the electrophoretically analysed individuals possessed three alleles (all of those shared the genotype *abbc* at the *Aat-1* locus). This fact clearly gives support to the hypothesis that this species has an autopolyploid origin. Nevertheless, these figures are much lower than in other studied autotetraploid species. For example, in *Thymus loscosii*, 37.2% of all plants exhibited three or four alleles for at least one of the examined loci (López-Pujol et al. 2004); and the autotetraploid cytotypes of *Tolmiea menziesii* and *Vaccinium oxycoccos* had 39% (Soltis and Soltis 1989) and 12.1% (Mahy et al. 2000) of individuals with 3/4 alleles, respectively. The high number of alleles at some loci have been atributted to repeated polyploidization events, as discussed for, e.g., the grass *Dactylis glomerata* (Lumaret 1988).

Autotetraploids are usually characterized by their high levels of heterozygosity, which may also be a consequence of tetrasomic inheritance (Soltis and Soltis 2000), because this mode of inheritance would reduce the effects of population bottlenecks and genetic drift. The literature provides us with several examples of high levels of heterozygosity in autotetraploid plants (mean H_o = 0.279; see Table 7) and substantially greater than in their diploid counterparts, such as *Tolmiea menziesii* (H_o = 0.237 in tetraploid populations and H_o = 0.070 in diploid

ones; Soltis and Soltis 1989), *Vaccinium oxycoccos* (H_o = 0.213 in tetraploid and H_o = 0.067 in diploid; Mahy et al. 2000), or *Centaurea jacea* (H_o = 0.54 in tetraploid and H_o = 0.29 in diploid; Hardy and Vekemans 2001; see Table 7), among others. These figures are much higher than the value of heterozygosity for *D. montanum* (H_o = 0.075). Judging from allozyme banding patterns, none of the examined populations of Pyrenean larkspur appeared to be diploid, and all the reported chromosome counts are tetraploid (Blanché 1991).

Table 6

Matrix of geographic distances (above diagonal) and Nei's (1978) genetic identity between populations of *Delphinium montanum*

Populations	BAS	COR	ORR	EI1	EI2	CAS	NOE
BAS	—	5.4	49.0	39.8	40.0	42.2	60.4
COR	0.994	—	44.0	35.2	35.4	37.6	56.2
ORR	0.982	0.987	—	12.4	11.6	11.6	23.0
EI1	0.997	0.992	0.993	—	0.8	2.6	22.4
EI2	0.998	0.996	0.990	0.999	—	2.6	22.4
CAS	0.991	0.998	0.993	0.993	0.996	—	19.8
NOE	0.960	0.960	0.980	0.974	0.966	0.972	—

Tetrasomic inheritance leads to high levels of genetic diversity in autotetraploids, as described by values of *A*, *P* and heterozygosity (Soltis and Soltis 1989). Mean diversity values for autotetraploids are very high (*P* = 50.74%, *A* = 2.23, and H_e = 0.264; Table 7), significantly greater than reported values for diploid species (e.g. Hamrick and Godt 1989; Hamrick et al. 1991; Karron 1991, Gitzendanner and Soltis, 2000). Values of variability in *D. montanum* (*P* = 23.8%, *A* = 1.48, and H_e = 0.082) are rather lower than the expected for autotetraploid species, only comparable to those of *Turnera ulmifolia* var. *intermedia* (Shore 1991), and even lower to those found for the surveyed diploid perennial larkspurs (mean values: *P* = 41.4%, *A* = 1.6, and H_e = 0.147; see Table 8).

These relatively low levels of diversity and heterozygosity, coupled with the scarce presence of three or four different alleles at surveyed loci, could indicate that *D. montanum* is currently suffering a process of diploidization. This event consist of the gradual accumulation of structural differences in homologous chromosomes (producing a further divergence) and the evolution of genic factors that enforce preferential pairing in polyploids (Ramsey and Schemske 2002), which results in loss of duplicate expression in the case of autotetraploids (i.e. the switch from having four chromosomes that form a tetravalent at meosis –which leads to tetrasomic inheritance- to having two pairs of chromosomes each of which forms a bivalent, leading to disomic inheritance; Wolfe 2001). It is relatively common in both animals and plants that a single species can show a mixture of tetraploid and diploidized loci, because diploidization does not occur simultaneously for all chromosomes or even for all loci in a particular cromosome (Wolfe 2001). In that case, allozymes can be very useful in detecting loss of duplicate expression because the diploidized loci will not exhibit unbalanced banding patterns, which may be the case of *Mdh-1* in *D.*

montanum. The period of time spent from the formation of this 'paleotetraploid' in the Tertiary to nowadays may have contributted to the extensive ocurrence of genetic drift and allelic erosion within populations. These processes, coupled with diploidization of several segments of the genome along time, may explain the nearly absence of loci with 3/4 different alleles and the monomorphism of 7 of the 15 surveyed loci and the subsequent low values of genetic diversity when comparing to other autopolyploids (Table 7), most of them of recent origin.

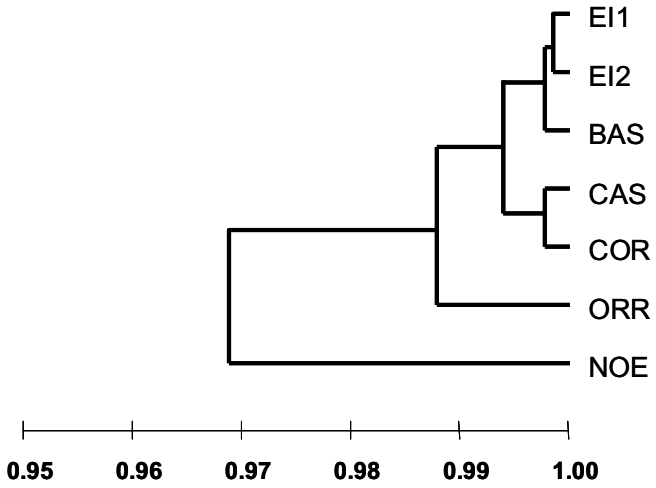

Fig. 4. Dendrogram resulting from UPGMA analysis of seven populations of *Delphinium montanum* based on pairwise values of Nei's (1978) genetic identity (SD = 0.474%; cophenetic correlation = 0.933).

In addition to banding patterns and data on genetic diversity, cytological and morphological data clearly suggest that the plant has suffered a significant process of diploidization in its genome. Morphological evidences on the karyotype (all chromosome pairs are quite distinct), the absence of the expected tetravalents at meiosis (only bivalents have been observed), and other anatomical characteristics (pollen size, stomata size and density, and stamen number are not higher than in some diploid counterparts; Blanché 1991; Bosch 1999). Nevertheless, to verify the hypothesis of an autopolyploid origin and further diploidization, extensive cytological (i.e. additional karyological meiotic analyses) and inheritance studies (allozyme progeny tests) would be needed.

Population genetic structure and conservation of Delphinium montanum

The moderate to low levels of inbreeding detected within populations (mean F = 0.089) gives us idea of a relative 'health' of populations at present. Most populations have a size (see Table 1) which seems enough to counteract the negative effects of genetic drift and inbreeding. Nevertheless, populations are rather divergent

(G_{ST} = 0.135) and both geographically (the mean distance among populations is about 27.3 km) and genetically isolated (Nm = 1.60, a value which may be insufficient to prevent divergence by genetic drift). However, indirect methods to estimate levels of gene flow (as done in this study) unlikely reveal current levels of interpopulation genetic exchange but historical ones, due to the unlikely interchange of genes among populations given the high interpopulation distances. Anyway, isolation of populations with the subsequent decrease in gene flow among them results in the loss of allele recovery capability by migration (Young et al. 1996).

Table 7

Genetic diversity in *Delphinium montanum* and in other autotetraploid species. If a species has both diploid and tetraploid populations, values of diversity given here are only for tetraploid populations

Taxa	*P*	*A*	H_o	H_e	Reference
Aster kantoensis Kitam.	36.9	1.53	—	0.142	Maki et al. (1996)
Centaurea jacea L.	—	3.54	0.54 (0.29*)	0.38	Hardy and Vekemans (2001)
Dactylis glomerata L.	80.0	2.36	0.43 (0.17*)	—	Soltis and Soltis (1993)
***Delphinium montanum* DC.**	**23.8**	**1.48**	**0.075**	**0.082**	**This study**
Heuchera grossulariifolia Rydb.	31.0	1.55	0.159 (0.058*)	—	Wolf et al. (1990)
Heuchera micrantha Dougl.	38.33	1.64	0.150 (0.075*)	—	Ness et al. (1989)
Rutidosis leptorrhynchoides F. Muell.	98.0	3.2	0.34 (0.22*)	0.36	Brown and Young (2000)
Swainsona recta A.T. Lee	—	4.3	0.24	0.42	Buza et al. (2000)
Thymus loscosii Willk.	85.0	3.0	0.472	0.422	López-Pujol et al. (2004)
Tolmiea menziesii Torr. & Gray	40.8	1.5	0.237 (0.070*)	—	Soltis and Soltis (1989)
Turnera ulmifolia var. *elegans* Urb.	65.3	2.03	0.42 (0.11)	0.27	Shore (1991)
Turnera ulmifolia var. *Intermedia* Urb.	20.1	1.20	0.07 (0.11)	0.04	Shore (1991)
Vaccinium oxycoccos L.	38.9	1.66	0.213 (0.067*)	—	Mahy et al. (2000)
Mean	**50.74**	**2.23**	**0.279**	**0.264**	

Note. *P*: percentage of polymorphic loci; *A*: mean number of alleles per locus; H_o: observed heterozygosity;
H_e: expected panmictic heterozygosity. All values given here are population means.
*Values of H_o in diploid populations.

Thereupon, population history may be another factor which could have contributed to the relatively low levels of genetic variability found in *D. montanum*, in addition to the hypothesized diploidization. There is a significant fraction of rare alleles and even extremely rare ones (see Results), which may be easily lost by genetic drift. Moreover, some populations show population-specific alleles, which also gives us idea of the very ancient population's establishment, probably before the Pleistocene glaciations. Genetic erosion of populations has probably lead to the decrease on the frequencies of some alleles up (or nearly up) to their elimination; population-specific alleles may come from mutations or massive loss of alleles in most populations. The long history of populations (from glaciations) has been demonstrated to be a crucial factor in modelling genetic structure of populations in the relative species *Aconitum lycoctonum* (Utelli et al. 1999), which may be the case for *D. montanum*.

The separation of NOE from the rest of populations showed by the UPGMA dendrogram may be due to a higher level of genetic isolation suffered by this population. If we observe the relief map (Figure 1), all the populations with the exception of NOE are located within the mountainous axis formed by the Cadí and Puigmal ranges. NOE population is located in the Madres range, manifestly segregated from the latter by the Prada-de-Conflent depression. The six southern populations are clustered together because they have had more likelihood of genetic interchange among them, whereas NOE remained isolated at least from the last glaciation allowing an independent evolution.

Table 8

Genetic diversity in the surveyed diploid perennial larkspurs

Taxa	*P*	*A*	H_e	Reference
D. bolosii	22.2	1.2	0.071	Orellana et al. (2004)
D. decorum	40.9	1.9	0.161	Koontz et al. (2001)
D. luteum[a]	69.2	1.8	0.211	Koontz et al. (2001)
D. nudicaule	65.0	2.0	0.295	Koontz et al. (2001)
D. pentagynum subsp. formenteranum	40.7	1.6	0.180	López-Pujol et al. (2003)
D. variegatum subsp. *kinkiense* and subsp. *thornei*[b]	24.5	1.3	0.074	Dodd and Helenurm (2002)
D. variegatum subsp. *variegatum*	33.6	1.5	0.064	Dodd and Helenurm (2002)
D. viridescens	35.3	1.6	0.119	Richter et al. (1994)
Mean	**41.4**	**1.6**	**0.147**	

Note. *P*, percentage of polymorphic loci; *A*, mean number of alleles per locus; H_e, expected panmictic heterozygosity. All values given are population means.
[a] Only the wild population considered.
[b] The two subspecies are combined in the analysis

Fortunately, all but one *D. montanum* populations are located within the boundaries of a legal figure of habitat protection, even those considered currently extinct (Table 9). Nevertheless, these belong to diferent administrations (French and Spanish) and the degree of protection is uneven. For example, the ORRI population is located into a Spot of Natural Interest (PEIN; see Table 9), which has a level of protection much lower than a Nature Reserve or a Natural Park. CAS population has not any degree of protection, and it is situated very close to a ski station. NOE population deserves a special protection due to its genetic singularity. This locality constitutes itself a Nature Reserve and a Natura 2000 site, and it can be considered a 'hotspot' of rare plants due to the presence of other endangered species in addition to the Pyrenean larkspur, such as *Hormathophylla pyrenaica*, *Ligularia sibirica*, and *Dracocephalum austriacum* (the three species are listed in the Annex I of Convention of Bern, in Annexes II and IV of Habitats Directive, and in Annex I of the French Red Book, where it also appears as VU), although the latter one may be currently extinct from the Pyrenees (Aymerich and Sáez 2001).

Although the size of extant populations seems enough to ensure an appropriate capability to respond to stochastic events, at least three classical localities might have been lost (one in the central scree of Pedraforca Mountain, another in Portella de Mantet, and the last one over Cava; see Table 9). Therefore, a close monitoring of extant populations is necessary to detect demographic fluctuations –widely reported

for this species (total population size reached almost 10.000 individuals in recent years; Aymerich and Sáez 2001)– or other incidences to ensure their long-term viability. One of these incidences may be the predation by several type of mountainous ungulates, such as *Rupricarpa pyrenaica* and *Ovis ammon* (Aymerich 2003). Predation by ungulates may represent a loss over 95% of seed set in some populations (Simon et al. 2001; Aymerich 2003). Recent field observations (July 2004) have revealed a massive predation of the reproductive structures of *D. montanum* in NOE population. Although polyploidy may buffer the animal grazing, long-term effects on conservation are expected. Therefore, monitoring of populations, regulation of ungulates density, and even the installation of physical barriers for the ungulates should be necessary for the plant survival.

Table 9

Known populations of *Delphinium montanum* and their habitat protection

Population	Country	Last record	Figure of habitat protection (and date of establishment)
Bastanist (BAS)	Spain	2003	Cadí-Moixeró Natural Park (1983)
Els Cortils (COR)	Spain	2002	Cadí-Moixeró Natural Park (1983)
Coma de l'Orri (ORR)	Spain	2003	PEIN* "Capçaleres del Ter i del Freser"
Vall d'Eina (Lower) (EI1)	France	2002	Vallée d'Eyne Nature Reserve (1993)
Vall d'Eina (Upper) (EI2)	France	2002	Vallée d'Eyne Nature Reserve (1993)
Cambra d'Ase (CAS)	France	2004	Unprotected
Noedes (NOE)	France	2004	Nohèdes Nature Reserve (1986)
Pedraforca	Spain	1984 (25 individuals); no individuals found in 2000 and 2002 surveys	Cadí-Moixeró Natural Park (1983)
Portella de Mantet	France	Not found since 1886	Mantet Nature Reserve (1984)
Cava	Spain	Not found since 1985 (200 individuals)	Cadí-Moixeró Natural Park (1983)

Note. *PEIN is an acronym which corresponds to *Pla d'Espais d'Interès Natural* (Spots of Natural Interest) created by the Autonomous Government of Catalonia (Generalitat de Catalunya).

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3.2. Discussió general

En els capítols anteriors (des del 3.1.1 al 3.1.8) s'estudien els nivells i la distribució de la variabilitat genètica en tot un seguit de tàxons escollits d'acord amb diferents criteris (endemicitat, grau d'amenaça, nivell de ploïdia, etc.; vegeu capítol 2.1 d'aquesta Memòria). Els isoenzims, però, atesa la seva versatilitat i baix cost, s'han emprat per a l'anàlisi de la variabilitat genètica en un gran nombre d'espècies vegetals des dels anys setanta arreu del món. Als Països Catalans, un nombre creixent d'espècies han estat estudiades amb l'ús d'aquests marcadors, especialment des de finals dels anys noranta. Per tant, creiem oportuna la realització d'una anàlisi conjunta de les dades derivades d'aquesta Tesi Doctoral amb les reportades a la bibliografia, amb el propòsit d'obtenir una visió global de quina és la quantitat de variabilitat genètica i com està repartida aquesta entre les poblacions, prestant especial atenció a les conseqüències conservacionistes que se'n puguin derivar.

3.2.1. *Patrons de diversitat genètica en relació a l'ecologia i als trets evolutius*

Botànics, ecòlegs i biòlegs de l'evolució estan d´acord que els vegetals no es distribueixen a l'atzar dins les comunitats. L'heterogeneïtat ambiental habitualment és citada com un factor important en aquest comportament, però també podrien intervenir-hi els patrons de colonització dels vegetals i factors estocàstics que afecten l'establiment i la mortalitat de les plantes (Hamrick, 1989). De la mateixa manera, la diversitat genètica de les poblacions vegetals tampoc es distribueix aleatòriament (Antonovics, 1971; Allard *et al.*, 1972; Hamrick & Allard, 1972; Turkington & Harper, 1979), fet al qual hom habitualment es refereix com "estructura genètica de les poblacions" (Loveless & Hamrick, 1984). L'estructura genètica és el resultat de l'acció conjunta d'una sèrie de forces evolutives, com ara les mutacions, la migració, la selecció natural i la deriva genètica, però també de tota una sèrie de factors biològics i ecològics, com ara el tipus de reproducció i de dispersió de les granes, les condicions de l'hàbitat o fins i tot l'acció de l'home (Huenneke, 1991; Heywood, 1991; Ellstrand & Elam, 1993; Frankham *et al.*, 2002). Aquesta suma de factors condueix a que les freqüències al·lèliques i/o genotípiques no estiguin distribuïdes a l'atzar en el sí de les poblacions, sinó que ho facin seguint un determinat esquema que pot ésser espacial o temporal (Loveless & Hamrick, 1984). L'estudi de l'estructura genètica de les poblacions naturals dins el camp de la biologia de la conservació és molt rellevant perquè la diversitat genètica es considera un indicador del potencial d'adaptació evolutiva de les espècies vegetals (Young *et al*., 1996; Booy *et al*., 2000).

En els últims 25 anys, han aparegut diversos estudis encaminats a determinar l'existència de correlacions entre la diversitat genètica de les espècies vegetals i les característiques biològiques, ecològiques i geogràfiques, amb l'objectiu d'explicar quins són els determinants dels nivells i de la distribució de la diversitat genètica de les poblacions. El motiu d'aquests estudis ha estat la cerca de característiques biològiques i ecològiques de les espècies –emprant la bibliografia disponible- que ens permetessin realitzar prediccions sobre els nivells i distribució de la variabilitat genètica, atesa la impossibilitat de dur a terme estudis genètics en totes les espècies amenaçades o almenys en una fracció significativa de totes elles (Hamrick & Godt, 1996a; Ellstrand & Elam, 1993). Per altra banda, l'actual crisi de biodiversitat recomana en moltes

ocasions la presa de decisions que assegurin la conservació de les espècies sense esperar a l'obtenció de dades genètiques (Spielman *et al.*, 2004).

El primer d'aquests treballs de síntesi és un recull de dades genètiques (nivells de diversitat a nivell poblacional) i ecològiques (12 característiques diferents) de 113 tàxons vegetals (Hamrick *et al.*, 1979). Posteriorment, Loveless & Hamrick (1984) examinaren l'existència de correlacions entre la distribució de la diversitat genètica entre poblacions i 14 característiques ecològiques diferents en 124 tàxons vegetals. El més cèlebre, però, és el recull de Hamrick & Godt (1990), a bastament citat en el camp de la biologia vegetal i la genètica de poblacions, on es recullen les dades genètiques de 449 tàxons a nivell d'espècie, a nivell de població i entre poblacions dins de la mateixa espècie, i es correlacionen amb 8 grans categories ecològiques: el gran grup filètic, el cicle vital, l'àmbit geogràfic, la distribució regional, el sistema reproductiu, el tipus de dispersió de les granes, el tipus de reproducció –sexual/asexual– i l'estatus successional. Amb posterioritat, els mateixos autors (Hamrick & Godt, 1996b) realitzaren una anàlisi on es comparaven les dades genètiques de gairebé 1.500 entrades (no necessàriament tàxons, atès que molts tàxons estaven representats amb més d'una entrada) amb diverses combinacions de dues característiques ecològiques.

D'aquests estudis, se'n desprenen tota una sèrie de conclusions amb importants implicacions de cara a la conservació de les espècies. En primer lloc, tot i que la variabilitat genètica depèn d'una teranyina de factors ecològics, biològics i també històrics, moltes vegades intímament lligats, hi ha dues característiques que sobresurten per damunt de la resta perquè proporcionen un grau més alt de variació de la diversitat genètica: l'àmbit geogràfic i el sistema reproductiu. Així, les espècies d'àmbit geogràfic restringit (les espècies endèmiques i les espècies rares) solen presentar un nivell de diversitat genètica menor, tant a nivell de població com d'espècie (Hamrick *et al.*, 1979; Hamrick & Godt, 1990); en canvi, no hi ha diferències significatives en la distribució d'aquesta diversitat entre poblacions (Loveless & Hamrick, 1984; Hamrick & Godt, 1990). Atenent al sistema reproductiu, les espècies al·lògames, especialment les anemòfiles, presenten més variabilitat genètica que les autògames o les de tipus mixt. Les autògames, en canvi, presenten un major nivell de diferenciació genètica entre poblacions (Loveless & Hamrick, 1984; Hamrick & Godt, 1990).

Aquests reculls s'han criticat, però, per mancar d'un plantejament estadístic prou congruent (Karron, 1987; Gitzendanner & Soltis, 2000; Cole, 2003). S'ha argumentat que els tàxons inclosos en aquests estudis s'han tractat com a mostres independents ignorant les seves relacions filogenètiques, i per tant violant les assumpcions dels mètodes estadístics emprats per a analitzar aquestes dades (*cf.* Gitzendanner & Soltis, 2000). Per a aquests autors (Karron, 1987; Gitzendanner & Soltis, 2000; però també vegeu Felsenstein, 1985; Silvertown & Dodd, 1996), la millor aproximació és limitar les comparacions a tàxons congenèrics en cas de no disposar d'aproximacions filogenètiques en un intent de controlar aquests efectes; les diferències entre parells d'espècies congenèriques són independents per a cada gènere, atès que els diferents tàxons que conformen un gènere tindran un avantpassat comú molt més recent que d'altres espècies que es puguin incloure en l'anàlisi (Gitzendanner & Soltis, 2000). Per contra, els reculls clàssics de diversitat genètica multi-espècie permeten la inclusió de totes les dades genètiques de la literatura disponibles, la qual cosa confereix una visió molt més global dels patrons de diversitat genètica de les espècies.

En el context de les comparacions entre tàxons congenèrics, Karron (1987) fou el primer en comparar 11 parells de congèneres rars i d'àmplia distribució pel que fa a la diversitat genètica (en termes de variabilitat intrapoblacional) i a la taxa d'al·logàmia. Les espècies rares es

caracteritzaven per uns nivells de variabilitat genètica més petits, però també per unes taxes d'al·logàmia menors. Més recentment, Gitzendanner & Soltis (2000) han realitzat aquest tipus d'anàlisi -entre espècies rares i d'àmplia distribució- amb més gèneres (34 generes representant 102 tàxons vegetals), comparant tant els nivells (específics i intrapoblacionals) com la distribució de la diversitat genètica, i reflectint una menor diversitat per a les espècies rares però gairebé idèntica distribució de la diversitat entre poblacions. Darrerament, Cole (2003) ha inclòs en la seva anàlisi un total de 247 espècies vegetals en 57 comparacions diferents de la diversitat genètica entre espècies congenèriques rares i comuns, amb idèntics resultats als reportats per Gitzendanner & Soltis (2000), amb la única novetat que es corroborava una menor variabilitat genètica per a les espècies autògames.

Una altra característica de les espècies que s'ha intentat correlacionar amb els seus nivells de diversitat genètica ha estat el grau d'amenaça. En el treball de Frankham (1995), es comparaven els nivells de variació genètica en 38 parells d'espècies (taxonòmicament properes) amenaçades-no amenaçades, i en 32 de les quals les espècies amenaçades mostraven una menor diversitat genètica. No obstant, només 6 dels 38 parells corresponien a espècies vegetals. En un treball molt més recent, Spielman *et al.* (2004) compara l'heterozigosi en 170 parells de tàxons (també taxonòmicament propers, encara que no necessàriament congenèrics) amenaçats-no amenaçats, dels quals 36 corresponen a plantes. D'aquestes, en 27 parells els tàxons amenaçats (dins d'alguna de les categories de la UICN: CR, EN o VU) presentaven menys heterozigosi que els tàxons no amenaçats. Una darrera característica per a la qual s'ha buscat correlació amb els nivells de diversitat genètica és el fet de tractar-se d'una espècie endèmica insular, cas en el qual les espècies vegetals mostren uns nivells de diversitat significativament més petits respecte als de les espècies continentals congenèriques (en 9 dels 10 parells analitzats; Frankham, 1997).

Finalment, són molt més nombrosos els estudis que correlacionen els nivells de diversitat a nivell de població (en comptes de a nivell d'espècie) amb diverses característiques de les poblacions, com ara la seva mida o bé si es tracta d'una població insular o continental. Tot i que aquestes correlacions es reporten en nombrosos estudis de diversitat en espècies vegetals, pocs són els treballs en que s'ha intentat trobar una pauta comuna per a les plantes i no només per a una espècie determinada. En aquest sentit, cal destacar dos treballs de Frankham. En el primer (Frankham, 1996), es reporta una correlació positiva entre els nivells de variació isoenzimàtica i el logaritme de la mida poblacional en 15 de les 16 espècies vegetals recollides en aquest estudi. Un segon treball (Frankham, 1997), que compara els nivells de variació genètica entre poblacions insulars i poblacions continentals, revela nivells significativament més alts per a les poblacions continentals en 8 de les 9 espècies recollides en aquesta anàlisi. En la revisió d'Ellstrand & Elam (1993) sobre les conseqüències genètiques de la petita mida poblacional, d'una llista de 10 espècies, en 7 existeix una correlació positiva entre la mida de les seves poblacions i els nivells de diversitat genètica.

3.2.2. *Els Països Catalans com a subconjunt del* hospot *Mediterrani*

La conca Mediterrània és considerada com un dels punts calents de biodiversitat mundial, atesa l'enorme riquesa florística que presenta (al voltant d'un 10% del total d'espècies que hi ha al planeta, concentrada en una àrea molt petita (només el 2% de la superfície terrestre; Médail & Quézel, 1999). A més, la flora de la regió Mediterrània es caracteritza per una elevadíssima taxa d'endemicitat (13.000 de les 25.000 espècies presents en aquesta àrea en són

endèmiques), fet que s'ha relacionat amb diversos factors com ara la història paleo-geològica i climàtica, la biogeografia, l'heterogeneïtat ecològica i geogràfica i la influència de l'home (Quézel, 1995; Médail & Quézel, 1997), tal i com es detalla en els capítols introductoris. Aquesta riquesa i heterogeneitat d'hàbitats, d'espècies i de poblacions, però també de camins i situacions evolutives diferents, converteix a la Mediterrània en un lloc on podem trobar una àmplia gamma de patrons de variabilitat genètica en els vegetals.

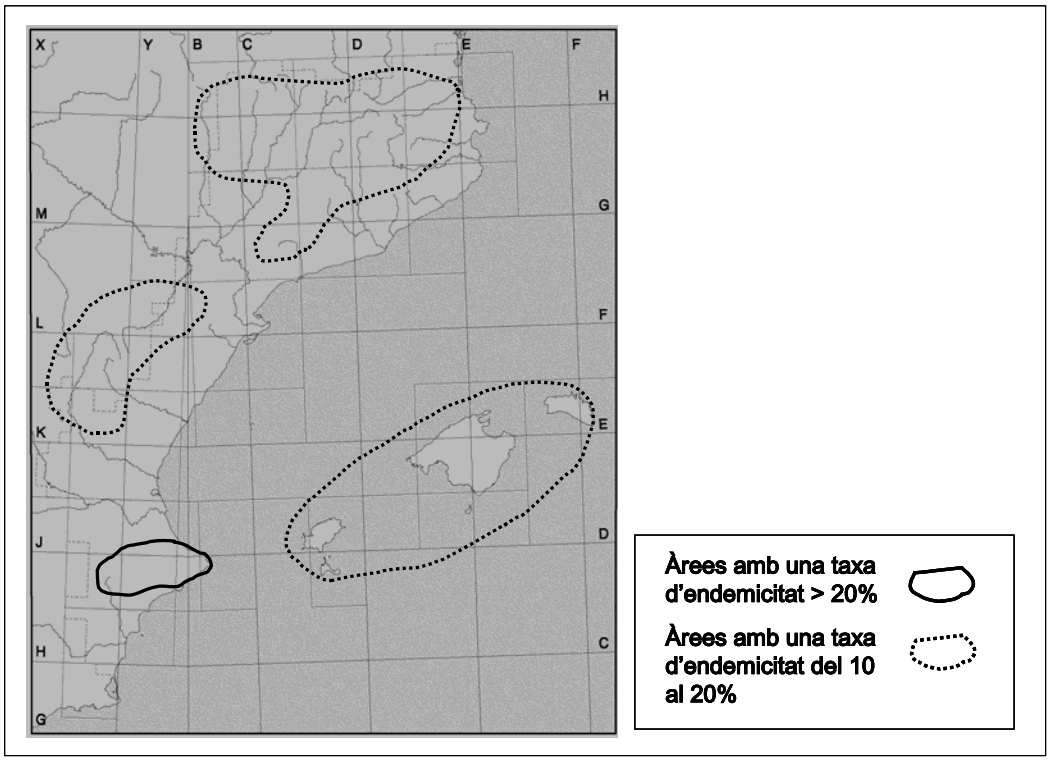

FIGURA 3.1. ÀREES D'ENDEMICITAT DELS PAÏSOS CATALANS, SEGONS DADES DE MÉDAIL & QUÉZEL (1997).

Hom anomena Països Catalans al conjunt de territoris de parla catalana més les terres de parla castellana de l'interior del País Valencià i la Vall d'Aran. Aquest territori, amb una extensió de prop de 70.000 km^2, engloba íntegrament tres Comunitats Autònomes de l'Estat Espanyol (Catalunya, el País Valencià i les Ilies Balears), part d'una altra comunitat també espanyola (la franja de Ponent, a l'Aragó), i la regió administrativament francesa de la Catalunya Nord (Departament dels Pirineus Orientals), a part d'un estat sobirà (Andorra). Els Països Catalans representen una de les àrees florísticament més riques de la conca Mediterrània, amb un total de 3.627 espècies i 4.605 tàxons incloent espècies, subespècies i varietats (Bolòs & Vigo, 1997). Amb una àrea que representa poc més del 2,9% de la superfície total de la conca Mediterrània, els Països Catalans contenen al voltant del 15% de tota la diversitat florística d'aquest *hotspot* mundial de biodiversitat en termes de nombre de tàxons (14,51% si tenim en compte només les espècies i 15,35% si també es prenen en consideració els tàxons a nivell subespecífic). Per tant, estem davant d'una de les àrees botànicament més riques de la Mediterrània occidental i, per extensió, de tota Europa. La taxa d'endemicitat és, però, en el seu conjunt, relativament pobre: segons les dades de Sáez *et al.* (1998), només 279 dels 4.605 tàxons que trobem als Països Catalans (o sigui, un 6,06%) hi són endèmics, degut a l'artificialitat d'aquest territori des del punt

de vista biogeogràfic. Tot i això, àrees significatives dels Països Catalans estàn incloses en alguns dels 10 *mini-hotspots* de biodiversitat mediterrània proposats per Médail & Quézel (1997), com ara les Illes Balears i el territori Diànic (País Valencià). Són també àrees florísticament molt riques (i amb taxes d'endemicitat superiors al 10%), les muntanyes catalanídiques (incloent terres ja situades a la província de Terol), i tots els Pre-Pirineus i els Pirineus orientals (vegeu Figura 3.1). Els Països Catalans poden ésser considerats, pert tant, com a una mostra representativa del *hotspot* Mediterrani.

L'objectiu d'aquesta part de la Memòria és múltiple. En primer lloc, criticar adequadament els resultats dels estudis realitzats als capítols anteriors, en el conjunt de les dades disponibles a la bibliografia. En segon lloc, intentarem esbrinar si la història paleo-geològica, climàtica i biogeogràfica dels Països Catalans (fenòmens d'orogènesi alpina, crisi del Messinià, glaciacions pleistocèniques) ha contribuït, almenys en part, als actuals patrons de diversitat genètica que mostren les espècies que habiten en aquesta regió. En tercer lloc, l'impacte continuat de les activitats humanes, significament agreujades durant l'últim segle, també pot haver contribuït a una modificació dels patrons de variació genètica en les espècies vegetals. A part de la història evolutiva de les poblacions (o "els processos dinàmics extrínsecs"; *cf.* Gray, 1996), els patrons de diversitat genètica de les espècies també estan influïts pels trets ecològics i biològics propis d'aquestes, les anomenades "propietats biològiques intrínseques" (Gray, 1996; Hamrick & Godt, 1996a; Booy *et al.*, 2000). Per aquesta raó, un dels objectius prioritaris d'aquesta secció és la prospecció de possibles correlacions entre les principals característiques ecològiques de les espècies (com ara tipus de reproducció, l'estratègia vital i l'hàbitat) i els nivells i la distribució de la seva diversitat genètica. Aquesta doble visió (històrica i actual), creiem que ens pot donar una perspectiva adequada i ens pot servir de model per intentar entendre els processos que han moldejat l'arquitectura genètica de les poblacions vegetals catalanes.

3.2.3. *Diversitat isoenzimàtica a la flora dels Països Catalans*

Per tal de valorar els resultats obtinguts a la Memòria i emmarcar-los adequadament, s'ha realitzat una cerca de la bibliografia disponible sobre diversitat isoenzimàtica en tàxons (espècies, subespècies, varietats o formes) dels Països Catalans o com a mínim amb algunes de les poblacions estudiades dins d'aquest territori, i s'ha construït una base de dades amb tots els tàxons dels quals hem pogut obtenir dades genètiques. Algun dels tàxons recollits són objecte d'estudi en més d'un treball, com ara *Quercus suber*; en aquests casos, i tal i com aconsellen Hamrick & Godt (1996b), convé tractar-los com a entrades independents i en cap cas calcular la mitjana dels paràmetres genètics, atès que els plantejaments, el material i els mètodes de cada treball solen ser molt diferents: la mida, el nombre de poblacions i el nombre de *loci* assajats són característiques que solen variar molt depenent de l'equip que realitza la recerca, i de vegades, cada treball se centra en una secció diferent de l'area de distribució del tàxon en qüestió. Per tant, en aquests casos cal considerar que cada entrada aporta una informació única i, donada la mida de la base de dades multi-tàxon (1 cas sobre 31 entrades en total), la redundància conseqüència de múltiples entrades per a determinats tàxons té un petit efecte en els valors mitjans dels diferents paràmetres genètics (*cf.* Hamrick & Godt, 1996b). Només hem recollit dades d'espècies de flora silvestre; aquest fet explica la no inclusió, per exemple, de *Lysimachia minoricensis*, atès que tots els individus analitzats electroforèticament estan cultivats en jardins botànics (Ibáñez *et al.*, 1999).

TAULA 3.1. NIVELLS I DISTRIBUCIÓ DE LA VARIABILITAT GENÈTICA PER A CADASCUNA DE LES 9 CARACTERÍSTIQUES ECOLÒGIQUES.

Tàxon	Nº de poblacions ***	Nº de *loci*	% d'al·lels rars per població**	*P*	*A*	H_o	H_e	F_{IS}	% d'al·lels privats per població	F_{ST}[a] o G_{ST}[b]	1	2	3	4	5	6	7	8	9	Referència
TÀXONS DIPLOIDES																				
1. *Aconitum lycoctonum*	19 (1)	10	—	29,0	1,50	0,093	0,100	0,066	—	—	D	AD	C	PC	S	AL	G	B	NA	Utelli *et al.* (1999)
2. *Antirrhinum pertegasii*	4 (4)	14	—	17,3	1,21	0,050	0,080	—	—	0,060[b]	D	E	C	PC	S	AL	G	R	A (VU)*	Mateu-Andrés (2004)
3. *Antirrhinum valentinum*	5 (5)	21	—	23,8	1,33	0,184	0,075	—	—	0,480[b]	D	E	C	PC	S	AL	G	R	A (VU)*	Mateu-Andrés & Segarra-Moragues (2000)
4. *Borderea chouardii*	1 (1)	21	4,17	9,5	1,14	0,078	0,046	-0,461	—	—	D	E	C	PL	S	AL	G	R	A (CR)	Segarra-Moragues & Catalán (2002)
5. *Borderea pyrenaica*	6 (1)	21	0	19,0	1,24	0,135	0,085	-0,500	—	—	D	E	C	PL	S	AL	G	R	NA	Segarra-Moragues & Catalán (2002)
6. *Brassica montana*	5 (3)	11	6,71	36,4	1,88	—	0,184	—	6,41	0,181[b]	D	AD	C	PC	—	M	G	R	NA	Lázaro & Aguinagalde (1998)
7. *Cheirolopus intybaceus*	5 (4)	10	1,92	15,8	1,18	—	0,247	—	5,05	0,293[b]	D	AD	IC	PC	—	AL	—	BE	NA	Garnatje *et al.* (1998)
8. *Cyclamen balearicum*	28 (19)	9	0,87	24,2	1,30	0,006	0,062	0,948	1,01	0,112[a]	D	AD	I	PL	S	M	LD	B	NA	Affre *et al.* (1997)
9. *Delphinium bolosii*	3 (3)	15	1,82	28,9	1,40	0,107	0,104	0,019	7,73	0,252[a]	D	E	C	PC	AS	M	G	BE	A (EN)	Orellana *et al.* (2004)
10. *Delphinium gracile*	3 (3)	11	10,02	45,5	1,77	0,198	0,159	0,039	18,46	0,073[a]	D	AD	C	A	S	M	G	PH	NA	Bosch, 1999
11. *Delphinium pentagynum* subsp. *formenteranum*	1 (1)	9	6,25	40,7	1,60	0,125	0,180	0,358	—	—	D	E	I	PC	AS	AL	G	BE	A (CR)	López-Pujol *et al.* (2003a)
12. *Delphinium verdunense*	2 (2)	11	16,11	40,9	2,15	0,154	0,205	0,451	17,31	0,028[a]	D	AD	C	A	—	M	G	AL	NA	Bosch, 1999
13. *Erodium rupestre*	5 (5)	14	0	7,1	1,07	0,009	0,025	0,613	1,25	0,372[a]	D	E	C	PL	S	AL	G	R	NA	López-Pujol *et al.* (inèd.)
14. *Hippocrepis balearica*	1 (1)	15	6,25	6,7	1,10	0,011	0,010	—	—	—	D	E	I	PL	S	AL	G	R	NA	González-Candelas & Montolío (2000)
15. *Hippocrepis grosii*	1 (1)	15	6,25	20,0	1,30	0,122	0,101	—	—	—	D	E	I	PL	S	AL	G	PS	A (EN)	González-Candelas & Montolío (2000)
16. *Hippocrepis valentina*	12 (12)	15	5,78	24,4	1,27	0,099	0,085	-0,179	3,35	0,171[a]	D	E	C	PL	S	AL	G	R	NA	González-Candelas & Montolío (2000)
17. *Lolium*	10 (2)	5	—	—	3,90	0,408	0,514	—	—	—	M	AD	I	A	—	AL	G	AL	NA	Oliveira & López (1999)

rigidum																			
18. *Petrocoptis montsicciana*	4 (4)	16	15,06	70,3	2,20	0,121	0,239	0,486	6,30	0,376[b]	D	E	C	PL	S	AL	G	R	NA	López-Pujol *et al.* (2001)
19. *Petrocoptis pardoi*	3 (3)	16	11,76	56,3	1,90	0,072	0,192	0,620	8,56	0,354[b]	D	E	C	PL	S	AL	G	R	A (VU)	López-Pujol *et al.* (2001)
20. *Pinus halepensis*	15 (6)	5	—	—	—	0,262	0,228	0,128	—	—	G	AD	IC	PL	S	AL	LD	B	NA	Agúndez *et al.* (1999)
21. *Pinus pinaster*	12 (2)	18	—	41,7	2,00	—	0,159	—	—	—	G	AD	IC	PL	S	AL	LD	B	NA	Salvador *et al.* (2000)
22. *Quercus suber*	18 (2)	14	—	32,1	—	0,135	0,145	0,123	—	—	D	AD	IC	PL	S	AL	LD	B	NA	Jiménez *et al.* (1999)
23. *Quercus suber*	7 (1)	13	8,33	61,5	1,85	0,189	0,229	—	—	—	D	AD	IC	PL	S	AL	LD	B	NA	Elena-Rosselló & Cabrera (1996)
24. *Seseli farrenyi*	3 (3)	14	31,09	83,3	3,00	0,120	0,297	0,592	4,41	0,041[b]	D	E	C	PC	S	AL	LD	PS	A (EN)	López-Pujol *et al.* (2002)
25. *Silene diclinis*	2 (2)	26	3,45	5,8	1,11	—	0,231	—	3,33	0,048[b]	D	E	C	PC	S	AL	G	PH	A (EN)	Prentice (1984)
26. *Silene hifacensis*	8 (8)	12	0,89	7,3	1,08	—	0,085	—	0,96	0,575[b]	D	E	IC	PC	S	AL	—	PS	A (VU)*	Prentice *et al.* (2003)
27. *Silene sennenii*	5 (5)	21	7,90	20,9	1,31	0,049	0,063	0,253	0	0,271[a]	D	E	C	PC	AS	—	LD	PH	A (EN)	López-Pujol *et al.* (2004b)
28. *Stachys maritima*	5 (5)	20	2,72	14,0	1,16	0,065	0,066	-0,102	0,95	0,316[b]	D	AD	C	PC	AS	AL	G	AL	A (CR)	López-Pujol *et al.* (2003b)
TÀXONS POLIPLOIDES																			
29. *Delphinium montanum*	7 (7)	15	12,78	23,8	1,48	0,075	0,082	0,089	2,51	0,135[b]	D	E	C	PC	S	AL	G	AL	A (VU)	López-Pujol *et al.* (inèd.)
30. *Medicago sativa* (silvestre)	9 (2)	5	—	90,0	3,10	—	0,255	—	—	—	D	AD	C	PC	—	AL	—	PH	NA	Jenczewski *et al.* (1999)
31. *Thymus loscosii*	8 (6)	5	18,40	83,3	2,94	0,474	0,423	-0,125	1,04	0,025[b]	D	AD	C	PC	AS	AL	G	BE	NA	López-Pujol *et al.* (2004a)

1. **Grup filogenètic**: G: gimnospermes; D: dicotiledònies; M: monocotiledònies.
2. **Àmbit geogràfic**: E: endèmica (present en menys de 50 localitats o bé en una àrea inferior a 10.000 km^2); AD: d'àmplia distribució (present de forma àmplia als Països Catalans i fins i tot extenent-se a territoris propers).
3. **Insularitat**: I: distribució (de les poblacions analitzades) només insular; C: distribució només continental; IC: distribució insular + continental.
4. **Estratègia vital**: A: plantes anuals (s'inclouen les bianuals); PC: perennes de vida curta; PL: perennes de vida llarga.
5. **Tipus de reproducció**: AS: estratègia mixta (asexual + sexual); S: sexual.
6. **Sistema d'encreuament**: AU: autògames; AL: al·lògames; M: estratègia mixta.
7. **Tipus de dispersió de les granes**: G: per gravetat (curta distància); LD: Estratègies de llarga distància (epizoocòria, endozoocòria, anemocòria, hidrocòria i altres).
8. **Hàbitat**: PS: penya-segats marítims; BE: brolla escleròfila; PH: prats i herbassars; R: afloraments rocosos; B: boscos; AL: altres.
9. **Grau d'amenaça**: A: amenaçada (llistada en alguna de les categories d'amenaça de la UICN o bé que s'ha constatat que el tàxon sofreix alguna amenaça real que posi en perill la seva supervivència); NA: no amenaçada. L'assignació a alguna de les categories d'amenaça s'ha realitzat emprant els criteris més recents de la UICN (2001).

*Categories UICN 1994.

** Per al seu càlcul només s'han considerat les poblacions amb N>10, donat que les de mida inferior no poden contenir al·lels amb una freqüència inferior a 0,05.

*** Entre parèntesi, nombre de poblacions localitzades als Països Catalans.

Només hem tingut en consideració, per altra banda, els treballs que contenien una interpretació genètica dels patrons de bandes electroforètics. En els tàxons on s'han assajat tant els *loci* monomòrfics com els polimòrfics (o bé quan tots resultaven ésser polimòrfics sense un coneixement previ), s'han extret o calculat (en aquells casos en el quals només es faciliten les dades genètiques en brut, sense treballar) els paràmetres descriptors dels nivells de diversitat genètica intrapoblacional: *A* (nombre mitjà d'al·lels per *locus*), *P* (percentatge de *loci* polimòrfics quan l'al·lel més comú es presenta en una freqüència inferior a 0,95), H_o (heterozigosi observada), H_e (heterozigosi esperada) i F_{IT} (coeficient d'endogàmia). Quan han estat disponibles les freqüències al·lèliques, també s'ha calculat el percentatge d'al·lels rars (en proporció inferior a 0,05) per població i el percentatge d'al·lels privats (exclusius) per població. També hem extret (o calculat) un indicador de la distribució de la variabilitat genètica entre poblacions: F_{ST} (índex de fixació) o, alternativament, G_{ST} (coeficient de diferenciació gènica), considerant ambdós equivalents (de fet, tenen el mateix valor quan només hi ha dos al·lels per *locus*; Hartl & Clark, 1989) per tal d'augmentar la mida mostral.

Basant-nos en les revisions publicades fins a la data (Hamrick *et al.*, 1979; Loveless & Hamrick, 1984; Hamrick & Godt, 1990), hem escollit una sèrie de característiques ecològiques dels tàxons amb una influència significativa sobre en els nivells i distribució de la diversitat genètica. Tots els tàxons de la base de dades multi-tàxon s'han classificat en una de les següents categories per a cada tret ecològic: (1) el gran grup filogenètic (gimnospermes, dicotiledònies o monocotiledònies); (2) l'àmbit geogràfic (endèmica restringida, endèmica, sub-endèmica o d'àmplia distribució); (3) la insularitat (distribució només insular, distribució només continental o distribució insular i continental); (4) l'estratègia vital (anuals, perennes de vida curta o bé perennes de vida llarga); (5) el tipus de reproducció (estratègia asexual + sexual o bé sexual); (6) el sistema d'encreuament (al·lògames o bé d'estratègia mixta), (7) el tipus de dispersió de les granes (per gravetat o bé amb una estratègia de llarga distància), (8) l'hàbitat (penya-segats marítims, dunes, brolla escleròfila, prats i herbassars, afloramentrs rocosos, boscos i altres); i (9) el grau d'amenaça (amenaçada o no amenaçada); i la categoria UICN d'amenaça (CR, EN o VU). S'ha afegit una darrera característica ecològica, el nivell de ploïdia, per a permetre la comparació respecte a la variabilitat genètica entre espècies diploides i poliploides. Els criteris per a la inclusió d'un tàxon en cada una de les categories de cada característica ecològica i les abreviatures emprades en aquest capítol estan detallades en la nota a peu de la Taula 3.1. La informació necessària per a classificar els tàxons en cada una de les categories s'ha obtingut en primer lloc de les publicacions originals; quan la informació no era disponible, s'ha recorregut a d'altres treballs que tracten altres aspectes dels tàxons en qüestió (com ara els sistemes reproductius), a diverses flores locals (com ara *Flora dels Països Catalans*; Bolòs & Vigo, 1984-2001), llibres vermells o publicacions d'altra mena. Per a tots els tàxons recollits a la base de dades multi-tàxon, hem calculat les mitjanes (ponderades pel producte del nombre de *loci* analitzats i el nombre de poblacions estudiades) dels diferents paràmetres genètics (*A*, *P*, H_e, F_{IT}, F_{ST} o G_{ST}, el % d'al·lels rars/població i el % d'al·lels privats/població) i els seus errors estàndard, per a cada categoria dels diferents trets ecològics recollits en l'apartat anterior. Les diferencies entre les diferents categories de cada tret ecològic s'han analitzat emprant un test d'ANOVA d'un sol factor.

En segon lloc, i sempre que ens ha estat posible, hem abordat l'anàlisi des del punt de vista de les comparacions entre tàxons congenèrics, tal i com suggereixen Karron (1987), Gitzendanner & Soltis (2000) i Cole (2003). No obstant, atesa la petita mida mostral, no s'ha pogut emprar cap dels tests estadístics apropiats per aquest tipus d'anàlisi (tests de dades aparellades), i

les comparacions que fem són només qualitatives. Només hem realitzat comparacions amb una selecció de trets ecològics (àmbit geogràfic, insularitat, grau d'amenaça i nivell de ploïdia) ja que els tàxons congenèrics solen pertànyer a la mateixa categoria per a la resta de característiques. Finalment, hem complementat el nostre estudi amb la comparació entre poblacions dins d'un mateix tàxon. D'aquesta manera, dins de la nostra base de dades multi-tàxon, hem seleccionat aquells tàxons que contenen poblacions que pertanyen a diferents categories per a determinats trets (insularitat i grau d'amenaça) dels que disposem de prou informació. Tots els càlculs estadístics s'han realitzat amb el programa STATGRAPHICS Plus 5.0.

3.2.4. *Anàlisi dels patrons de diversitat genètica*

NIVELLS I DISTRIBUCIÓ DE LA DIVERSITAT GENÈTICA

A la Taula 3.1 es recullen les dades isoenzimàtiques conegudes en el moment actual sobre la flora dels Països Catalans, que, incorporades les dades aportades en aquesta Memòria, ens donen un total de 124 poblacions, pertanyents a 31 tàxons. Com a resultat global de la nostra anàlisi, se'n pot derivar la conclusió que els valors mitjans de diversitat genètica per a espècies vegetals diploides dels Països Catalans són relativament pobres a nivell poblacional (P = 26,70%, A = 1,44, H_o = 0,090, H_e = 0,118), especialment si els comparem amb els derivats d'altres reculls de diversitat. Per exemple, en el primer recull de dades de variabilitat genètica de Hamrick *et al.* (1979) es reporten uns valors de diversitat superiors (P = 36,8%, A = 1,69 , H_e = 0,156) sobre un *pool* de 113 tàxons. No obstant, en un recull posterior amb una mida mostral molt més gran (653 entrades representant 449 espècies), Hamrick & Godt (1990) dónen uns valors lleugerament inferiors (P = 34,2%, A = 1,53, H_e = 0,113), més propers als obtinguts en aquest treball. De fet, l'heterozigosi observada, el paràmetre que millor descriu la variabilitat genètica (Berg & Hamrick, 1997), és lleugerament superior en la nostra anàlisi que en la de Hamrick & Godt (1990). A més, cal tenir en compte una sèrie de consideracions que expliquen aquests resultats: (i) els tàxons recollits a la nostra anàlisi representen una àrea geogràficament molt limitada –els Països Catalans- en comparació amb els treballs anteriorment esmentats, on s'inclouen tàxons de totes les latituds; (ii) una part important de les espècies estudiades isoenzimàticament ho és, precisament, pel seu caràcter d'endèmica i/o amenaçada i hom disposa de poques dades sobre espècies d'àmplia distribució. A més, alguns dels tàxons inclosos en la nostra anàlisi només disposen d'una única població, casos no inclosos en els reculls generals, però que hem tingut en compte aquí per tal d'augmentar la mida mostral; (iii) només s'han seleccionat les poblacions de les espècies situades geogràficament dins dels límits dels Països Catalans; i (iv) el nombre tant de gimnospermes com de monocotiledònies en el nostre estudi és anecdòtic, grups taxonòmics que solen presentar alts nivells de diversitat (vegeu Hamrick & Godt, 1990). A més, la major part dels tàxons estudiats electroforèticament a les nostres contrades són de distribució molt restringida, fet que sol venir acompanyat de manca de variabilitat genètica en aquests (Gitzendanner & Soltis, 2000; Cole, 2003). D'aquesta manera, es difícil parlar d'una situació de depauperació genètica en les espècies vegetals dels Països Catalans, sinó més aviat tot el contrari, tal i com s'espera en una regió que va actuar com a reservori de diversitat biològica (tant pel que fa a diversitat d'espècies com genètica) durant els períodes glacials del Quaternari (Hewitt, 1996; 1999), però que hauria

sofert una dràstica transformació dels seus ecosistemes originals i una pèrdua considerable tant de superfície com de qualitat dels hàbitats naturals, fenòmen especialment palpable en el darrer segle.

Els nivells de diversitat genètica per als nou tàxons estudiats en el marc d'aquesta Memòria són força variables: des de valors elevadíssims, com és el cas de *Seseli farrenyi* (P = 83,30%, A = 3,00, H_e = 0,297) i *Thymus loscosii* (P = 85,00%, A = 3,00, H_e = 0,422), -tot i que aquest últim és tracta d'un tetraploide-, fins a nivells extremadament petits, com ara els obtinguts per *Stachys maritima* (P = 14,00%, A = 1,16, H_e = 0,066) i per *Erodium rupestre* (P = 7,10%, A = 1,07, H_e = 0,025). Tot i això, es tracta de valors dins l'interval dels reportats a la bibliografia per d'altres espècies dels Països Catalans. Si repassem la Taula 3.1, trobem una gran disparitat de valors per als diferents paràmetres bàsics de diversitat, fet esperable donada l'enorme variabilitat en les característiques autoecològiques dels diferents tàxons analitzats electroforèticament: P varia des del 90,0% de *Medicago sativa* fins al 5,8% de *Silene diclinis*; l'interval de A va des de 3,90 per *Lolium rigidum* a només 1,08 per a una altra espècie de *Silene*, *S. hifacensis*; en darrer lloc, l'interval de l'heterozigosi esperada (H_e) és també força ampli: entre 0,514 altre cop per *Lolium rigidum* i 0,010 per *Hippocrepis balearica*.

TAULA 3.2. DIVERSITAT GENÈTICA PER A LES DIFERENTS CATEGORIES DE CADA TRET ECOLÒGIC DELS DIFERENTS TÀXONS RECOLLITS A LA BASE DE DADES MULTI-TÀXON (TAULA 3.1). L'EXPLICACIÓ DE LES DIFERENTS CATEGORIES DE CADA TRET ECOLÒGIC ESTAN DETALLATS A LA TAULA 3.1.

Categoria	Nombre de tàxons	% d'al·lels rars per població[1]	P	A	H_o	H_e	F_{IS}	% d'al·lels privats per població	F_{ST}[a] o G_{ST}[b]
(1) GRUP FILOGENÈTIC									
		—	—	—	—	NS	—	—	—
Gimnospermes	2	—	41,70	2,00	0,262	0,190 (0,049)	0,128	—	—
Monocotiledònies	1	—	—	3,90	0,408	0,514	—	—	—
Dicotiledònies	25	5,77 (7,18)	26,31 (20,69)	1,44 (0,47)	0,082 (0,057)	0,114 (0,154)	0,283 (0,406)	3,61 (5,63)	0,255 (0,165)
(2) ÀMBIT GEOGRÀFIC									
		NS	NS	NS	*	NS	NS	NS	NS
Endèmica	16	6,87 (8,11)	26,40 (23,52)	1,41 (0,52)	0,090 (0,049)	0,111 (0,083)	0,212 (0,389)	3,28 (3,03)	0,291 (0,179)
Àmplia distribució	12	3,67 (5,40)	27,29 (14,36)	1,50 (0,79)	0,088 (0,118)	0,132 (0,119)	0,434 (0,356)	4,29 (7,82)	0,179 (0,118)
(3) INSULARIRAT									
		NS	NS	NS	NS	NS	NS	NS	NS
Insular	5	1,87 (2,69)	23,36 (14,02)	1,42 (0,21)	0,037 (0,163)	0,086 (0,200)	0,918 (0,417)	1,01	0,112
Insular + continental	6	1,81 (4,03)	21,28 (21,43)	1,33 (0,46)	0,198 (0,064)	0,155 (0,063)	0,125 (0,003)	2,16 (2,89)	0,727 (0,199)
Continental	17	7,45 (8,50)	28,54 (22,26)	1,46 (0,52)	0,093 (0,053)	0,116 (0,080)	0,138 (0,402)	4,42 (5,97)	0,247 (0,154)
(4) ESTRATÈGIA VITAL									
		NS	NS	*	*	*	NS	***	NS
Anual	3	12,45 (4,31)	43,66 (3,25)	2,23 (1,14)	0,215 (0,136)	0,229 (0,193)	0,204 (0,291)	18,00 (0,81)	0,055 (0,032)
Perenne de vida curta	12	5,95 (9,37)	22,15 (20,73)	1,37 (0,53)	0,097 (0,045)	0,117 (0,081)	0,148 (0,254)	2,55 (2,80)	0,300 (0,180)
Perenne de vida llarga	13	3,50 (4,97)	29,95 (21,76)	1,44 (0,41)	0,072 (0,076)	0,108 (0,080)	0,349 (0,509)	3,15 (3,28)	0,219 (0,126)

(5) TIPUS DE REPRODUCCIÓ									
		NS	NS	NS	NS	NS	NS	NS	NS
Mixta (asexual + sexual)	4	2,56 (2,88)	21,70 (11,47)	1,28 (0,18)	0,068 (0,035)	0,075 (0,054)	0,079 (0,211)	1,77 (4,21)	0,285 (0,033)
Sexual	20	5,83 (8,44)	28,06 (22,92)	1,44 (0,52)	0,090 (0,071)	0,116 (0,080)	0,345 (0,454)	3,62 (5,89)	0,254 (0,193)
(6) SISTEMA D'ENCREUAMENT									
		NS	NS	NS	NS	NS	NS	*	NS
Al·lògama	22	6,27 (7,90)	26,39 (22,82)	1,44 (0,73)	0,104 (0,090)	0,128 (0,116)	0,124 (0,420)	3,25 (2,60)	0,298 (0,180)
Estratègia mixta	5	3,74 (6,25)	29,74 (8,67)	1,49 (0,35)	0,065 (0,082)	0,102 (0,059)	0,643 (0,437)	5,66 (7,48)	0,130 (0,089)
(7) TIPUS DE DISPERSIÓ DE LES GRANES									
		NS	NS	NS	NS	NS	NS	NS	NS
Gravetat (curta distància)	19	5,69 (4,94)	26,49 (17,99)	1,44 (0,66)	0,101 (0,091)	0,117 (0,113)	0,092 (0,390)	5,13 (6,11)	0,255 (0,154)
Altres mecanismes (llarga distància)	7	7,23 (13,15)	32,97 (24,18)	1,58 (0,69)	0,065 (0,093)	0,116 (0,088)	0,587 (0,357)	1,12 (2,31)	0,155 (0,118)
(8) HÀBITAT									
		NS	NS	NS	NS	NS	NS	NS	NS
Penya-segats marítims	3	9,75 (16,11)	29,41 (40,71)	1,63 (1,05)	0,120 (0,001)	0,145 (0,118)	0,592	2,01 (2,44)	0,412 (0,377)
Brolla escleròfila	3	2,29 (2,53)	22,10 (12,45)	1,20 (0,21)	0,110 (0,013)	0,155 (0,071)	0,075 (0,240)	5,79 (1,89)	0,243 (0,029)
Prats i herbassars	3	7,05 (3,35)	21,04 (20,04)	1,33 (0,34)	0,085 (0,105)	0,126 (0,084)	0,202 (0,151)	4,12 (9,84)	0,175 (0,122)
Afloraments rocosos	10	6,57 (5,22)	28,40 (21,37)	1,36 (0,40)	0,092 (0,057)	0,104 (0,077)	0,127 (0,536)	4,34 (2,87)	0,283 (0,149)
Boscos	6	1,40 (5,27)	29,56 (14,76)	1,45 (0,32)	0,064 (0,097)	0,108 (0,067)	0,711 (0,422)	1,01	0,112
Altres	4	5,13 (9,47)	18,85 (19,02)	1,42 (1,39)	0,106 (0,178)	0,123 (0,229)	-0,002 (0,391)	3,90 (11,57)	0,264 (0,204)
(9) GRAU D'AMENAÇA									
		NS	NS	NS	NS	NS	NS	NS	NS
No amenaçada	16	5,04 (8,84)	29,55 (22,81)	1,50 (0,54)	0,085 (0,042)	0,125 (0,079)	0,338 (0,391)	4,35 (3,40)	0,197 (0,191)
Amenaçada	12	6,67 (5,65)	23,68 (18,66)	1,49 (0,73)	0,095 (0,111)	0,110 (0,120)	0,177 (0,416)	2,67 (6,80)	0,310 (0,133)
(10) CATEGORIA UICN D'AMENAÇA									
		NS	NS	NS	NS	NS	NS	NS	NS
En perill crític (CR)	3	3,20 (1,73)	15,12 (16,86)	1,19 (0,26)	0,071 (0,031)	0,071 (0,072)	-0,108 (0,410)	0,95	0,316
En perill (EN)	5	9,61 (11,97)	29,32 (29,98)	1,56 (0,78)	0,081 (0,034)	0,144 (0,100)	0,272 (0,288)	2,89 (3,19)	0,180 (0,125)
Vulnerable (VU)	4	4,51 (7,69)	22,53 (21,20)	1,32 (0,36)	0,122 (0,072)	0,097 (0,056)	0,620	3,49 (5,37)	0,413 (0,224)
(11) NIVELL DE PLOÏDIA									
		NS	*	*	NS	NS	NS	NS	NS
Diploide	28	5,77 (7,18)	26,70 (20,41)	1,44 (0,65)	0,090 (0,089)	0,118 (0,104)	0,278 (0,394)	3,61 (5,63)	0,255 (0,157)
Tetraploide	3	13,63 (3.97)	40,67 (36,44)	1,89 (0,89)	0,164 (0,282)	0,164 (0,170)	0,041 (0,151)	2,18 (1,04)	0,110 (0,078)

[1]Per al seu càlcul només s'han considerat les poblacions amb N>10, donat que les de mida inferior no poden contenir al·lels amb una freqüència inferior a 0,05.

* $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$; NS: no significatiu.

Entre parèntesi, desviació estàndard.

TAULA 3.3. DIVERSITAT GENÈTICA PER A LES DUES CATEGORIES DE CADA TRET ECOLÒGIC DELS TÀXONS CONGENÈRICS. LES REFERÈNCIES PER A CADA UN DELS TÀXONS POT TROBAR-SE A LA TAULA 3.1.

Tàxon	Categoria	% d'al·lels rars per població*	*P*	*A*	H_o	H_e	F_{IS}	% d'al·lels privats per població	F_{ST}[a] o G_{ST}[b]
(1) ÀMBIT GEOGRÀFIC[1]									
Delphinium bolosii	Endèmic	1,82	28,9	1,40	0,107	0,104	0,019	7,73	0,252[a]
Delphinium pentagynum subsp. *formenteranum*	Endèmic	6,25	40,7	1,60	0,125	0,180	0,358	—	—
Mitjana (endèmics)		**2,71**	**30,9**	**1,43**	**0,110**	**0,117**	**0,075**	**7,73**	**0,252**
Delphinium gracile	Àmplia distribució	10,02	45,5	1,77	0,198	0,159	0,039	18,46	0,073[a]
Delphinium verdunense	Àmplia distribució	16,11	40,9	2,15	0,154	0,205	0,451	17,31	0,028[a]
Mitjana (àmplia distribució)		**12,46**	**43,7**	**1,92**	**0,180**	**0,177**	**0,204**	**18,00**	**0,055**
(2) INSULARIRAT									
Delphinium pentagynum subsp. *formenteranum*	Insular	**6,25**	**40,7**	**1,60**	**0,125**	**0,180**	**0,358**	**—**	**—**
Delphinium bolosii	Continental	1,82	28,9	1,40	0,107	0,104	0,019	7,73	0,252[a]
Delphinium gracile	Continental	10,02	45,5	1,77	0,198	0,159	0,039	18,46	0,073[a]
Delphinium verdunense	Continental	16,11	40,9	2,15	0,154	0,205	0,451	17,31	0,028[a]
Mitjana (continentals)		**7,67**	**37,0**	**1,69**	**0,147**	**0,144**	**0,121**	**13,38**	**0,144**
Hippocrepis balearica	Insular	6,25	6,7	1,10	0,011	0,010	—	—	—
Hippocrepis grosii	Insular	6,25	20,0	1,30	0,122	0,101	—	—	—
Mitjana (insulars)		**6,25**	**13,3**	**1,20**	**0,066**	**0,055**	—	—	—
Hippocrepis valentina	Continental	**5,78**	**24,4**	**1,27**	**0,099**	**0,085**	**-0,179**	**3,35**	**0,171[a]**
(3) GRAU D'AMENAÇA[2]									
Borderea chouardii	Amenaçada	**4,17**	**9,5**	**1,14**	**0,078**	**0,046**	**-0,461**	**—**	**—**
Borderea pyrenaica	No amenaçada	**0**	**19,05**	**1,24**	**0,135**	**0,085**	**-0,500**	**—**	**—**
Delphinium bolosii	Amenaçada	1,82	28,9	1,4	0,107	0,104	0,019	7,73	0,252[a]
Delphinium pentagynum subsp. *formenteranum*	Amenaçada	6,25	40,7	1,6	0,125	0,180	0,358	—	—
Mitjana (amenaçades)		**2,71**	**30,9**	**1,43**	**0,110**	**0,117**	**0,075**	**7,73**	**0,252**
Delphinium gracile	No amenaçada	10,02	45,5	1,77	0,198	0,159	0,039	18,46	0,073[a]
Delphinium verdunense	No amenaçada	16,11	40,9	2,15	0,154	0,205	0,451	17,31	0,028[a]
Mitjana (no amenaçades)		**12,46**	**43,7**	**1,92**	**0,180**	**0,177**	**0,204**	**18,00**	**0,055**
Hippocrepis grosii	Amenaçada	**6,25**	**20,0**	**1,30**	**0,122**	**0,101**	**—**	**—**	**—**
Hippocrepis balearica	No amenaçada	6,25	6,7	1,10	0,011	0,010	—	—	—
Hippocrepis valentina	No amenaçada	5,78	24,4	1,27	0,099	0,085	-0,179	3,35	0,171[a]
Mitjana (no amenaçades)		**5,82**	**23,0**	**1,26**	**0,092**	**0,079**	**-0,179**	**3,35**	**0,171[a]**
Petrocoptis pardoi	Amenaçada	**11,76**	**56,3**	**1,9**	**0,072**	**0,192**	**0,620**	**8,56**	**0,354[b]**
Petrocoptis montsicciana	No amenaçada	**15,06**	**70,3**	**2,2**	**0,121**	**0,239**	**0,486**	**6,30**	**0,376[b]**
(4) NIVELL DE PLOÏDIA									
Delphinium bolosii	Diploide	1,82	28,9	1,40	0,107	0,104	0,019	7,73	0,252[a]
Delphinium gracile	Diploide	10,02	45,5	1,77	0,198	0,159	0,039	18,46	0,073[a]
Delphinium pentagynum subsp. *formenteranum*	Diploide	6,25	40,7	1,60	0,125	0,180	0,358	—	—

Delphinium verdunense	Diploide	16,11	40,9	2,15	0,154	0,205	0,451	17,31	0,028[a]
Mitjana (diploides)		**7,55**	**37,3**	**1,68**	**0,145**	**0,147**	**0,134**	**13,38**	**0,144**
Delphinium montanum	Tetraploide	**12,78**	**23,8**	**1,48**	**0,075**	**0,082**	**0,089**	**2,51**	**0,135[b]**

*Per al seu càlcul només s'han considerat les poblacions amb N>10, donat que les de mida inferior no poden contenir al·lels amb una freqüència inferior a 0,05.

[1]Per a l'àmbit geogràfic, considerem endèmics els tàxons classificats en les següents categories de la Taula 3.1: ER i R. Considerem d'àmplia distribució la resta de categories: SE i AD.

[2]Per al grau d'amenaça, considerem que un tàxon està amenaçat quan està llistat en alguna de les categories d'amenaça de la UICN o bé que s'ha constatat que el tàxon sofreix alguna amenaça real que posi en perill la seva supervivència.

Un dels paràmetres que s'han considerat escassament en els diversos reculls de diversitat genètica però de gran importància, sobretot de cara a la gestió tant *in-situ* com *ex-situ* de les espècies, és el percentatge d'al·lels rars (aquells que estan en proporcions molt petites, per sota del 0,05%) per població (Caujapé, 1995). És un paràmetre que ens pot donar una idea de l'efecte de la deriva genètica sobre les poblacions (per exemple, una població que no contingui al·lels rars probablement pot haver patit els efectes erosius de la deriva genètica), i podria emprar-se com un indicador de perill real al que està sotmesa una població (per exemple, un elevat percentatge d'al·lels rars significa que una porció de la variabilitat genètica d'una població pot perdre's en unes poques generacions si aquesta es veu sotmesa a reduccions de la mida poblacional, fragmentació o fluctuacions demogràfiques importants). A les poblacions de les espècies vegetals diploides dels Països Catalans, prop del 6% del nombre total d'al·lels són rars, el que indica que hi ha una fracció significativa de la variació genètica que es troba en risc de perdre's en les properes generacions, sobre la qual convindria que les administracions gestores de la biodiversitat tinguessin una particular cura (constituïrien unitats de conservació, UC, amb una necessitat de seguiment específic). És notable el cas d'algunes espècies, com ara *Seseli farrenyi*, on gairebé la tercera part dels tots els al·lels estàn en freqüències inferiors a 0,05 (vegeu Figura 3.2). Un cop més, el caràcter d'endemisme, la mida poblacional i la història biològica de les espècies recollides, contribueix a explicar el perfil de les dades analitzades, en aquest cas, de raresa al·lèlica.

El segon dels processos que porta a una erosió de la diversitat genètica, junt amb la deriva genètica, és l'augment de les taxes d'endogàmia o consanguinitat, fenomen que sol produir-se quan disminueix la mida de les poblacions. El paràmetre més emprat en genètica de poblacions per a mesurar aquest augment de les taxes de consanguinitat és el coeficient d'endogàmia (F_{IS}; Wright, 1951), que ens mesura la desviació de la llei de Hardy-Weinberg (l'efecte d'encreuaments no a l'atzar) dins les poblacions, comparant l'heterozigosi observada i l'esperada. Per al conjunt de les espècies vegetals catalanes de les quals disposem de dades, el valor d'aquest paràmetre és significativament diferent de zero i positiu ($F_{IS} = 0,278$), el que indica que les poblacions catalanes estudiades són relativament endogàmiques, fruit en part de la petita mida poblacional i dels encreuaments obligats d'autogàmia biparental i, probablement també, d'una degradació dels hàbitats, sigui en forma de fragmentació i reducció de la mida poblacional, sigui en forma de disminució tant de la qualitat com de la quantitat dels serveis dels pol·linitzadors, depenent del grup d'espècies analitzat.

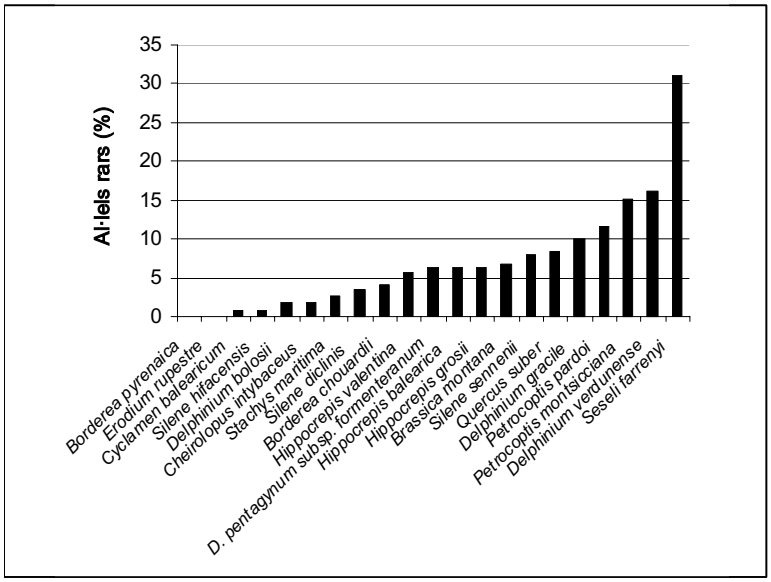

FIGURA 3.2. PERCENTATGE D'AL·LELS RARS PER POBLACIÓ EN LES ESPÈCIES RECOLLIDES EN EL NOSTRE ESTUDI DELS PAÏSOS CATALANS.

Una fracció prou significativa de la diversitat genètica dels tàxons estudiats dels Països Catalans és deu a diferències entre poblacions: la divergència genètica interpoblacional (donada pels paràmetres F_{ST} i G_{ST}, que considerem matemàticament com a equiparables) ens mostra un valor força elevat (0,255), sensiblement superior a l'obtingut per Hamrick & Godt (1990; G_{ST} = 0,224). Aquest diferenciació genètica tant marcada és un fet habitual a les poblacions de les espècies en els diferents refugis glacials del sud d'Europa (Hewitt, 1996; Thompson, 1999). Els grans fenòmens geològics com la orogènesi herciniana i alpina en primera instància, principals responsables de la topografia tan accidentada de les nostres contrades, i l'aïllament de nuclis poblacionals en diferents zones durant els períodes glacials que haurien actuat com a refugis, haurien conduït a un aïllament també genètic d'aquestes. Aquesta divergència entre poblacions s'hauria accentuat per l'enorme variabilitat microclimàtica que presenten les penínsules muntanyoses del sud d'Europa, com ara la península Ibèrica (Taberlet *et al.*, 1998; Tzedakis *et al.*, 2002). Dels 12 grans bioclimes reconeguts per Gaussen (1954), se'n presenten 4 als Països Catalans, els quals es subdivideixen en tota una munió de microclimes (hi ha més de 50 tipus diferents reconeguts; vegeu Bolòs & Vigo, 1984-2001). Un tercer factor, però, que pot haver contribuït a la remarcable diferenciació genètica de les poblacions catalanes és la destrucció i la fragmentació d'hàbitats soferta en aquesta regió, que haurien tingut tot un ventall de conseqüències genètiques sobre les poblacions vegetals: deriva genètica i consanguinitat, que comporten una disminució de la variació genètica intrapoblacional, i interrupció del flux de gens, que haurien conduït a un increment de la divergència entre poblacions. Tot i això, la manca de gimnospermes (un grup taxonòmic que sol presentar poca diferenciació interpoblacional, vegeu Hamrick & Godt, 1990) en la nostra mostra pot haver contribuït a obtenir un valor elevat de divergència genètica entre les poblacions catalanes.

TAULA 3.4. DIVERSITAT GENÈTICA PER A LES DUES CATEGORIES DE CADA TRET ECOLÒGIC DE LES DIFERENTS POBLACIONS D'UN TÀXON DETERMINAT. ELS VALORS DELS DIFERENTS PARÀMETRES SÓN MITJANES POBLACIONALS. LES REFERÈNCIES PER A CADA UN DELS TÀXONS POT TROBAR-SE A LA TAULA 3.1.

Tàxon	Categoria	Nombre de *loci*	Nombre de poblacions	% d'al·lels rars per població*	*P*	*A*	H_o	H_e	Referència
(1) INSULARIRAT									
Silene hifacensis	Poblacions insulars	12	6	1,19	9,70	1,11	—	—	Prentice *et al*. (2003)
	Poblacions continentals	12	2	0	0	1,00	—	—	Prentice *et al*. (2003)
Cheirolophus intybaceus	Poblacions insulars	12	1	0	10,00	1,10	—	0,346	Garnatje *et al.* (1998)
	Poblacions continentals	10	3	2,56	17,80	1,24	—	0,213	Garnatje *et al.* (1998)
Pinus halepensis	Poblacions insulars	5	1	—	—	—	0,196	0,199	Agúndez *et al*. (1999)
	Poblacions continentals	5	5	—	—	—	0,234	0,275	Agúndez *et al*. (1999)
(2) GRAU D'AMENAÇA[1]									
Delphinium bolosii	Poblacions amenaçades	15	2	4,54	26,7	1,40	0,095	0,097	Orellana *et al*. (2004)
	Poblacions no amenaçades	15	1	0	33,30	1,30	0,115	0,117	Orellana *et al*. (2004)
Delphinium montanum	Poblacions amenaçades	15	3	13,67	20,00	1,44	0,064	0,075	López-Pujol *et al*. (inèd.)
	Poblacions no amenaçades	15	4	12,21	25,02	1,48	0,083	0,087	López-Pujol *et al*. (inèd.)
Erodium rupestre	Poblacions amenaçades	14	3	0	7,10	1,07	0,013	0,019	López-Pujol *et al*. (inèd.)
	Poblacions no amenaçades	14	2	0	7,10	1,07	0,003	0,034	López-Pujol *et al*. (inèd.)
Petrocoptis montsicciana	Poblacions amenaçades	16	2	7,99	65,65	1,95	0,136	0,232	López-Pujol *et al*. (2001)
	Poblacions no amenaçades	16	2	22,13	75,05	2,40	0,105	0,246	López-Pujol *et al*. (2001)
Seseli farrenyii	Poblacions amenaçades	14	1	22,22	78,60	2,60	0,124	0,285	López-Pujol *et al*. (2002)
	Poblacions no amenaçades	14	2	35,52	85,70	3,20	0,118	0,303	López-Pujol *et al*. (2002)
Silene sennenii	Poblacions amenaçades	21	3	9,33	19,03	1,32	0,046	0,055	López-Pujol *et al*. (2004)
	Poblacions no amenaçades	21	2	3,33	23,80	1,31	0,055	0,095	López-Pujol *et al*. (2004)
Thymus loscosii	Poblacions amenaçades	5	3	19,23	93,33	3,16	0,481	0,441	López-Pujol *et al*. (2004)
	Poblacions no amenaçades	5	5	20,16	80,00	2,90	0,466	0,410	López-Pujol *et al*. (2004)

*Per al seu càlcul només s'han considerat les poblacions amb N>10, donat que les de mida inferior no poden contenir al·lels amb una freqüència inferior a 0,05.

[1]Per al grau d'amenaça, considerem que una població està amenaçada quan s'ha constatat que aquesta sofreix alguna amenaça real que posi en perill la seva viabilitat.

DIVERSITAT GENÈTICA I CARACTERÍSTIQUES ECOLÒGIQUES

1. Grup filogenètic: En el nostre recull, els paràmetres bàsics de diversitat ens donen valors més elevats per a les monocotiledònies, seguit de les gimnospermes i per últim les angiospermes. No obstant, en el nostre recull només disposem d'una monocotiledònia i dues gimnospermes, fet que resta validesa a aquestes comparacions. Les dicotiledònies dels Països Catalans (25 tàxons en total) sí que contenen però uns nivells de diversitat (P = 26,31%, A = 1,44, H_e = 0,114) molt similars i fins i tot superiors als reportats en l'estudi de Hamrick & Godt (1990; P = 29,0%, A = 1,44, H_e = 0,096), que, donades les característiques de la mostra –tal i com s'ha comentat anteriorment- denota una elevada riquesa genètica en aquesta regió. La divergència entre poblacions per a les dicotiledònies dels Països Catalans (l'únic grup taxonòmic del que disposem de dades genètiques) és també força important (25,5%), del mateix ordre que en el recull de Hamrick & Godt (1990; 27,3%).

2. Àmbit geogràfic: S'ha postulat que la variabilitat genètica és més gran en les espècies d'àmplia distribució que no pas en aquelles restringides a àrees geogràfiques petites. Diversos treballs que han comparat els valors de diversitat genètica entre espècies endèmiques i d'àmplia distribució han trobat diferencies significatives per als paràmetres A, P, H_o i H_e (Karron, 1987; Hamrick i Godt, 1990; Gitzendanner & Soltis, 2000; Cole, 2003). En canvi, sembla no haver-hi diferències entre espècies endèmiques i d'àmplia distribució pel que fa al coeficient d'endogàmia (Cole, 2003) o a la divergència genètica entre poblacions (Hamrick & Godt, 1990; Gitzendanner & Soltis, 2000). En el nostre cas, les espècies endèmiques presenten en general menors nivells de diversitat genètica, encara que les diferències no són significatives (vegeu Taula 3.2).

Convé destacar que les espècies endèmiques presenten més percentatge d'al·lels rars per població i una major divergència entre poblacions (0,297 *vs.* 0,179) respecte a les d'àmplia distribució, encara que en aquest cas les diferències tampoc són estadísticament significatives. Només en el cas de l'heterozigosi observada sí que hi ha diferències significatives entre els dos grups, encara que molt petites (p = 0,043). A nivell congenèric, l'únic gènere per al que podem comparar entre espècies endèmiques i d'àmplia distribució és *Delphinium* (Taula 3.3). Hom pot observar que les mitjanes reflecteixen una major variabilitat genètica per a les espècies d'àmplia distribució. Les poblacions de les espècies endèmiques presenten però una major divergència genètica que les de les d'àmplia distribució. Aquest fet pot deure's a la tipologia de l'endemicitat als Països Catalans (i per extensió a la conca Mediterrània). Segons Lavergne *et al*. (2004), les espècies endèmiques mediterrànies, a diferència de les d'àmplia distribució, produeixen menys flors i aquestes són més petites, presenten una separació estigma-antera menor, una relació P/O també més petita i també una menor producció de granes. Aquesta menor transferència de pol·len i granes limita les oportunitats de colonització de nous indrets i per tant afavoreix una més gran diferenciació genètica de les poblacions en les espècies endèmiques. El valor de divergència genètica entre poblacions de les espècies endèmiques dels Països Catalans (0,297) és més gran per a espècies rares/endèmiques d'altres reculls de diversitat genètica (0,206 a Gitzendanner & Soltis, 2000; 0,212 a Cole, 2003).

3. Insularitat: La insularitat és àmpliament considerada com un factor amb una enorme influència sobre els nivells i la distribució de la variabilitat genètica de les espècies vegetals, a causa de dues característiques biogeogràfiques que presenten les illes: una àrea geogràfica restringida i el seu aïllament (Rieseberg & Swensen, 1996). Les plantes que habiten en illes solen presentar una depauperació genètica bastant notable, provocada per diferents factors: (i) colls d'ampolla lligats a efectes fundadors, (ii) mida petita de les poblacions insulars, que afavoreix la

deriva genètica i la consanguinitat, i (iii) adaptació als ecosistemes insulars, que pot comportar una pèrdua de la capacitat de dispersió, una resistència limitada als depredadors i a les malalties (Frankham, 1998; Crawford *et al*., 2001). Una darrera generalització és la proporció significativa de diversitat genètica que està distribuïda entre les poblacions (Crawford *et al*., 2001).

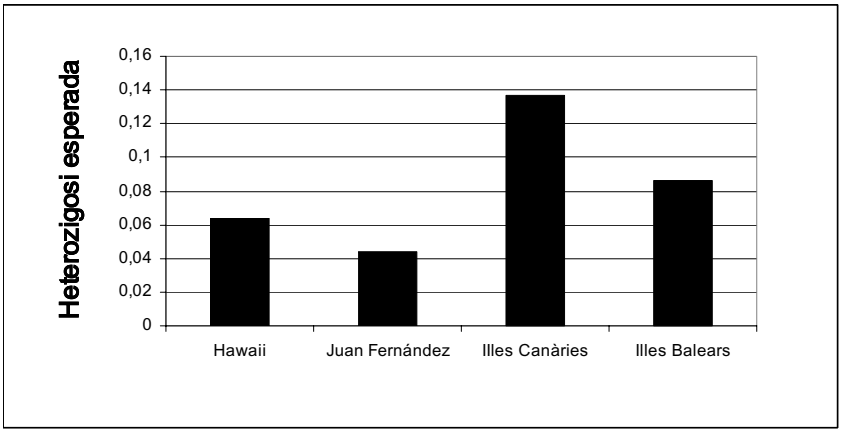

FIGURA 3.3. NIVELLS D'HETEROZIGOSI ESPERADA PER A LES ESPÈCIES INSULARS DE DIVERSOS ARXIPÈLAGS.

Els nivells de diversitat per als tàxons insulars dels Països Catalans, és a dir, baleàrics (P = 23,36%, A = 1,42, H_e = 0,086) són menors que per als tàxons continentals (P = 28,54%, A = 1,46, H_e = 0,116), encara que cap de les diferències és estadísticament significativa (Taula 3.2). Són valors lleugerament superiors als reportats per a illes oceàniques: Hawaii (P = 25,00% A = 1,32, H_e = 0,064; DeJoode & Wendel, 1992) i Juan Fernández (P = 21,00%, H_e = 0,044; Crawford *et al*., 2001), però no per a les Canàries (H_e = 0,137; Francisco-Ortega *et al*., 2000) (vegeu Figura 3.3). Destaca l'alt nivell d'endogàmia dels tàxons baleàrics (F_{IS} = 0,918), tal i com s'espera per a les espècies insulars; en canvi, la divergència interpoblacional és més aviat petita (G_{ST} = 0,112), a diferència dels tàxons canaris (G_{ST} = 0,281; Francisco-Ortega *et al*., 2000) i els de Juan Fernández (G_{ST} = 0,338; Crawford *et al*., 2001). Respecte a les comparacions congenèriques, mentre que per a *Hippocrepis* les espècies insulars presenten menys diversitat, per a *Delphinium* es dóna la situació contrària (Taula 3.3). A nivell poblacional, les poblacions insulars de *Cheirolophus intybaceus* i de *Pinus halepensis* contenen menys variabilitat genètica que les continentals; en canvi, al contrari del que s'espera, les poblacions insulars de *Silene hifacensis* presenten més diversitat que les continentals (Taula 3.4), degut però a la degradació severa del seu hàbitat al litoral alacantí i a la herborització massiva per part dels botànics (Prentice *et al*., 2003).

4. Estratègia vital: Els tàxons vegetals dels Països Catalans que mostren uns majors nivells de diversitat genètica són les plantes anuals, amb uns valors tant pel nombre mitjà d'al·lels per *locus* com per l'heterozigosi observada i esperada, significativament superiors als dels tàxons perennes, tant de vida curta com de vida llarga (vegeu Taula 3.2). Aquests resultats poden sorprendre si els comparem amb els obtinguts per per Hamrick *et al*. (1979) i, on són les plantes perennes de vida llarga les que presenten uns majors nivells de diversitat genètica. El mateix succeeix amb la distribució de la variació genètica entre poblacions, amb uns nivells de divergència de les plantes anuals catalanes inferiors als de les perennes, al contrari que l'obtingut per Hamrick i col·laboradors (Loveless & Hamrick, 1984; Hamrick & Godt, 1990). Per a aquests autors, les espècies perennes de vida llarga presenten uns nivells de diversitat intrapoblacional

més elevats degut a que tots ells solen presentar una combinació de característiques ecològiques que avaforeixen el manteniment d'alts nivells de variabilitat genètica: al·logàmia, alta fecunditat, i una major capacitat de dispersió tant del pol·len com de les granes, factors que alhora limiten la divergència genètica entre les poblacions (Hamrick *et al*., 1979; Loveless & Hamrick, 1984). La petita mida mostral per a les espècies anuals dels Països Catalans (només es recullen 3 tàxons) pot haver condicionat els resultats obtinguts en la nostra anàlisi, a part que, precisament aquestes 3 espècies són d'àmplia distribució, fet que pot esbiaixar l'anàlisi, en ser endèmiques moltes de les perennes recopilades.

5. Tipus de reproducció, sistema d'encreuament i tipus de dispersió de les granes: Els sistemes reproductius *sensu lato*, junt amb l'àmbit geogràfic, s'han postulat com un dels grans determinants tant dels nivells com de la distribució de la diversitat genètica en les espècies vegetals (Hamrick & Godt, 1990; Hamrick & Godt, 1996a). El tipus de reproducció (asexual, sexual o de tipus mixt), però, no sol tenir un efecte significatiu sobre els patrons de variació genètica de les plantes. En el cas de les espècies catalanes, els tàxons de reproducció mixta (que combinen l'estratègia asexual i la sexual) presenten uns nivells de diversitat lleugerament inferiors que els exclusivament sexuals; no obstant, la manca de significació estadística de les diferències i la petita mida mostral dels tàxons d'estratègia mixta (vegeu Taula 3.2), obliguen a interpretar amb precaució els resultats.

El sistema d'encreuament té un fort efecte sobre els nivells i la distribució de la diversitat genètica en els vegetals. Les plantes autògames es caracteritzen perquè exhibeixen una quantitat molt menor de diversitat genètica intrapoblacional i una divergència interpoblacional molt més acusada que les al·lògames (Hamrick & Godt, 1990; Hamrick & Godt, 1996a). En el nostre recull de diversitat genètica dels Països Catalans, no disposem de dades sobre espècies autògames, i la comparació entre espècies mixtes (que combinen taxes d'autogàmia i al·logàmia) i al·lògames, reflecteix uns nivells de diversitat intrapoblacional lleugerament superiors per a les al·lògames, encara que les diferències no són estadísticament significatives. Pel que fa a la divergència genètica entre poblacions, les al·lògames presenten uns nivells de divergència més alts que les mixtes –al contrari del que s'espera- encara que les diferències tampoc són significatives (vegeu Taula 3.2).

Pel que fa al sistema de dispersió de les granes, les espècies que dispersen les seves llavors per gravetat (és a dir, que les llavors cauen junt als parentals), solen exhibir menor diversitat intrapoblacional i una superior divergència interpoblacional que les plantes que dispersen les seves llavors pel vent, l'aigua o pels animals (Loveless & Hamrick, 1984; Hamrick & Godt, 1990). Per a les plantes dels Països Catalans, els tàxons que dispersen les seves llavors per gravetat presenten uns nivells de diversitat genètica intrapoblacionals menors i uns nivells de divergència interpoblacional més grans que les que dispersen les llavors a llarga distància, tot i que les diferències no són estadísticament significatives (Taula 3.2).

6. Hàbitat: Les espècies endèmiques mediterrànies es caracteritzen per habitar sovint llocs amb afloraments rocosos o en terrenys sobre llit rocós, amb pendents pronunciats i amb una vegetació de poca estatura, amb poques espècies acompanyants i amb una pobre cobertura d'espècies tant herbàcies com llenyoses (Lavergne *et al*., 2004). El 75% de les espècies endèmiques recollides en el nostre estudi (12 de 16; vegeu Taula 3.2) habiten en hàbitats rocosos: la majoria corresponen a afloraments rocosos de tipus calcari de mitjana i/o alta muntanya, mentre que alguns dels tàxons endèmics creixen en penya-segats litorals, com ara *Silene hifacensis* o *Seseli farrenyi*. Només una de les espècies que creix en hàbitats rocosos recollides en la nostra anàlisi és d'àmplia distribució (*Brassica montana*). Per tant, sembla haver-hi una clara

relació entre hàbitat rocós i endemicitat, fet ja posat de relleu en molts treballs anteriors (Stebbins, 1980; Major, 1988; Médail & Verlaque, 1997; Laguna *et al.*, 1998; Lavergne *et al.*, 2003). Si atenem a la Taula 3.2, podem comprovar que les dues tipologies d'hàbitat de naturalesa rocosa (penya-segats litorals i afloraments rocosos) es caracteritzen per uns alts nivells de diversitat genètica però alhora per una elevada divergència genètica entre poblacions. La flora que creix en prats i herbassars sembla la més pobre genèticament, mentre que els tàxons que habiten en boscos són els que presenten menys taxa de variabilitat distribuïda entre les poblacions. L'elevada divergència interpoblacional en les plantes d'hàbitats rocosos sembla tenir una base lògica atès el caracter discontinu d'aquest tipus d'hàbitat (en forma d'afloraments rocosos –habitualment de naturalesa calcària- aïllats). Els alts nivells de diversitat poden deure's al possible paper jugat pels massissos calcaris com a refugi de la flora Terciària (Küpfer, 1974; Chytrý *et al.*, 2003; Riba *et al.*, 2003). Molts dels endemismes rupícoles catalans i ibèrics corresponen a espècies amb una elevada capacitat de persistència, donades les seves característiques biològiques (elevada longevitat, escàs exit reproductiu) i de l'hàbitat en el qual creixen (reduïda competició per l'espai i la llum, substrat ric en nutrients), el que ha permès la seva supervivència fins als nostres dies. Aquestes característiques haurien permès també la conservació de com a mínim una part substancial de seva variabilitat genètica original, tot i les pèrdues provocades pels efectes de la deriva genètica i l'aïllament interpoblacional des de l'era glacial (Hewitt, 2004).

7. Grau d'amenaça: Teòricament, s'espera que les espècies amenaçades presentin nivells de diversitat menors que les no amenaçades, degut als efectes negatius que tenen les amenaces (sobretot de caire antropogènic) sobre les poblacions vegetals (disminució de la mida i fragmentació de poblacions, que condueix a una pèrdua de riquesa al·lèlica i a un augment de les taxes de consanguinitat). En el nostre estudi, tot i que les diferències no siguin estadísticament significatives (vegeu Taula 3.2), el valor mitjà dels paràmetres de variació genètica és més elevat per als tàxons no amenaçats que per als amenaçats (els que estàn llistats en alguna de les categories d'amenaça de la UICN). Si hom observa les dades de les comparacions congenèriques (per a tres dels quatre gèneres dels que disposem de dades, els tàxons amenaçats presenten menys variabilitat genètica que els no amenaçats; vegeu Taula 3.3). Pel que fa a la distribució de la diversitat genètica entre poblacions, també es detecten diferències: les espècies amenaçades presenten major divergència genètica interpoblacional que les no amenaçades, probablement degut a que la fragmentació de l'hàbitat (un dels principals efectes derivats de les activitats humanes als Països Catalans) té un clar efecte de disrupció del flux de gens entre poblacions. En canvi, si comparem els diferents paràmetres de variabilitat genètica entre les diferents categories UICN d'amenaça (VU, EN i CR), no s'aprecia un patró clar: els tàxons que estan en perill (EN) són els que mantenen majors nivells de variació intrapoblacional i menor divergència interproblacional; els tàxons classificats en perill crític sí que presenten –tal i com s'espera- els menors nivells de diversitat genètica (vegeu Taula 3.2). En tot cas, pot afirmar-se que, per a les espècies vegetals dels Països Catalans, les amenaces a les que estan sotmeses aquestes són responsables d'una pèrdua de variació genètica dins les seves poblacions naturals (en la mateixa línia que l'estudi de Spielman *et al*., 2004), però també d'un increment de la seva divergència genètica.

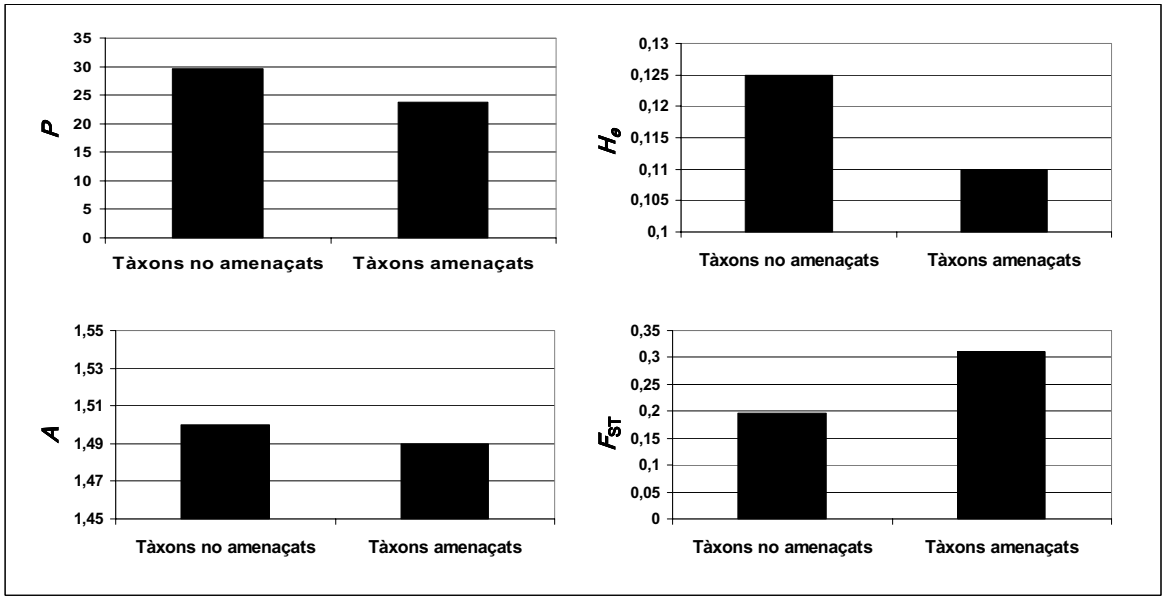

FIGURA 3.4. COMPARACIÓ ENTRE TÀXONS AMENAÇATS I NO AMENAÇATS PER A DIVERSOS PARÀMETRES DE DIVERSITAT GENÈTICA.

A nivell poblacional, de les 7 espècies per a les quals coneixem l'estat d'amenaça de cadascuna de les poblacions analitzades electroforèticament, en 6 d'elles les poblacions amenaçades presenten uns nivells de diversitat menor que les poblacions no amenaçades (vegeu Taula 3.4 i Figura 3.4). Només per a *Thymus loscosii*, una espècie poliploide, els nivells de diversitat per a les poblacions amenaçades són superiors que per a les no amenaçades.

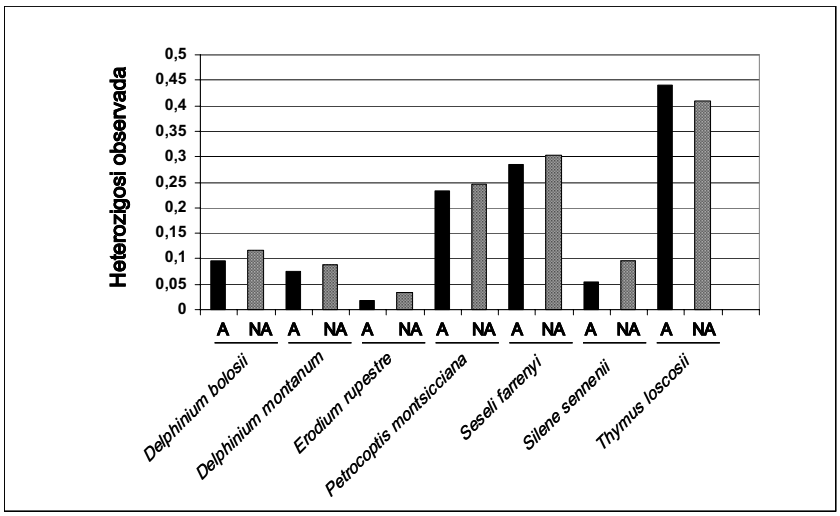

FIGURA 3.5. COMPARACIÓ ENTRE POBLACIONS AMENAÇADES I NO AMENAÇADES DE DIVERSOS TÀXONS PER ALS VALORS D'HETEROZIGOSI ESPERADA.

8. Nivell de ploïdia: L'última de les característiques biològiques que analitzem en el present treball, el nivell de ploïdia, té un efecte significatiu sobre la variabilitat genètica de les espècies vegetals. Els tàxons diploides dels Països Catalans, com es lògic, presenten menys diversitat que els tàxons poliploides: menys *loci* polimòrfics, menys al·lels per *locus*, menys heterozigosi (tant l'observada com l'esperada), però també un menor grau d'endogàmia i una menor divergència genètica entre poblacions (Taula 3.2). L'herència polisòmica dels poliploides té un efecte de reducció dels efectes negatius dels colls d'ampolla i la deriva genètica, i és alhora responsable dels alts nivells d'heterozigosi detectats habitualment en els poliploides (Soltis & Soltis, 2000), fet que tampona els efectes de la consanguinitat. Les diferències entre tàxons diploides i poliploides pel que fa a la distribució de la diversitat genètica entre poblacions tenen una explicació més incerta. L'hàbit perenne i una alta incidència de reproducció vegetativa, fenomens estretament lligats amb l'èxit ecològic dels poliploides, poden explicar en part aquest fet.

3.2.5. *Estat de conservació dels tàxons estudiats*

Els estudis de diversitat genètica de cada tàxon, atès que s'orienten cap a la seva aplicació en Biologia de la Conservació, s'han complementat amb un estudi detallat del seu estat de conservació, que ha comprès els següents punts, i que es recullen en una fitxa per a cada un dels tàxons:

1. Amenaces sobre els tàxons, tant si són d'origen natural (intrínseques del tàxon, dependents de la seva biologia) com d'origen antropogènic.
2. Grau d'amenaça segons els criteris de la IUCN, emprant la darrera versió de les categories d'amenaça (IUCN, 2001).
3. Proposta de mesures de conservació, tant *in situ* com *ex situ* (actuals i futures).

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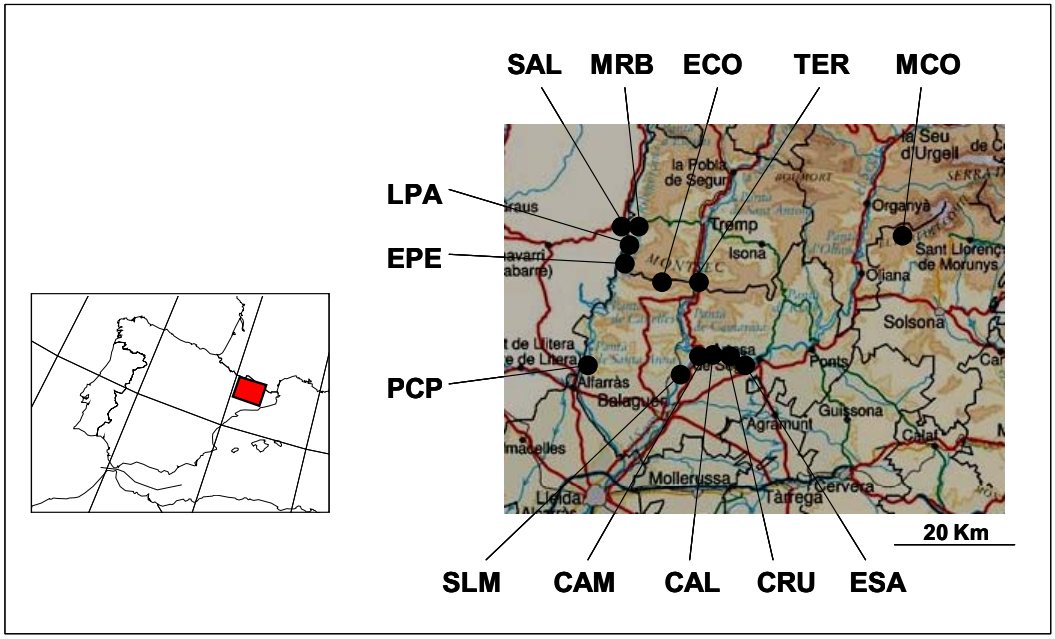
PROTECCIÓ LEGAL

- Directiva Hàbitats de la UE, Annex 2 (DOCE, 1992).
- Conveni de Berna, Annex 1 (Consell d'Europa, 1998).

COROLOGIA I DEMOGRAFIA

- Extensió de presència: 909,8 km^2 (2000).
- Àrea d'ocupació: 32 km^2 (2000).
- Mapa de localització de poblacions:



Codis de poblacions: PCP: Presa del Canal de Pinyana; EPE: Ermita de la Pertusa; LPA: La Pardina; SAL: Salteres; MRB: Congost de Mont-Rebei; ECO: Ermita del Colobor; SLM: Sant Llorenç de Montgai; CAM: Presa de Camarasa; CAL: Congost d'Alòs; CRU: Castell de Rubió; ESA: Ermita de Santa Maria de Salgar; TER: Congost de Terradets; MCO: La Móra Comdal.

A l'any 2000 hi havia 13 localitats conegudes de l'espècie (entre parèntesi, quadrícula UTM):

- PCP (31TBG93): 6 individus (2000).
- EPE (31TCG05): 209 individus (2000).
- LPA (31TCG05): 1.392 individus (2000).
- MRB (31TCG06): 2.089 individus (2000).
- SAL (31TCG06): 25 individus (Sainz *et al*., 1996).
- ECO (31TCG15): 83 individus (2000).
- SLM (31TCG23): 17 individus (2000).
- CAM (31TCG24): 3.258 individus (2000).
- CAL (31TCG24): 487 individus (2000).
- CRU (31TCG34): 6 individus (2000).
- ESA (31TCG34): 1.412 individus (2000).
- TER (31TCG25): 1.754 individus (2000).
- MCO (31TCG66): 223 individus (2000).

En total, hi havia 10.961 individus al 2000, 10.936 a Catalunya i només 25 a l'Aragó. Excepte en determinats casos, la majoria de vegades aquests censos s'han obtingut per estimació i no per recompte directe, a causa de la inaccessibilitat de les poblacions. A partir de recomptes amb l'ajut de prismàtics, s'ha corregit l'observació per un factor de 2,78, resultat de la mitjana d'individus totals per recompte directe en 5 parcel·les de 2 x 2 m.

AMENACES

Naturals:

■ Taxes de reclutament probablement molt baixes, observades tant pel nostre equip com per d'altres (Sainz *et al*., 1996; Mayol, 1998).

■ Petita mida poblacional en algunes localitats (PCP, SAL, ECO, SLM, CRU), que les fa altament susceptibles a factors estocàstics ambientals (sequera, incendis, etc.), demogràfics i genètics (*inbreeding*, deriva genètica), degut a que es troben sota de la seva MVP.

Antropogèniques:

■ Destrucció i/o fragmentació del seu hàbitat, bàsicament com a conseqüència de l'ampliació i millora de la xarxa viària (vegeu Figura 3.6, que correspon a la destrucció de paret de roca prop de l'Ermita de Santa Maria de Salgar, que ha provocat la desaparició d'alguns individus en eixamplar la carretera), i a la construcció d'embassaments i centrals hidroelèctriques. Un exemple és la destrucció de la localitat clàssica de la Font de les Bagasses, al congost de Terradets, com a conseqüència de l'eixamplament de la carretera. La millora de la via fèrria Lleida-La Pobla de Segur pot suposar una amenaça potencial.

■ Risc d'incendis forestals.

■ Activitats recreatives (escalada; s'han observat vies d'escalada en algunes de les poblacions).

■ Herboritzacions massives, sobretot a les localitats clàssiques (Camarasa, Terradets), constatable en *exsiccata* d'herbari diverses.

■ En el cas específic de la població de Camarasa, la instal·lació d'una passarel·la metàl·lica al marge esquerre del riu Segre ha deixat molts individus a l'abast de la mà, i de fet s'ha detectat l'arrencament d'alguns individus en visites successives a la població (vegeu Figura 3.7).

■ Possible ús en el futur de l'espècie en farmàcia, depenent de la recerca sobre proteïnes inactivadores de ribosomes (*RIPs*), amb potencial utilitat en les teràpies del càncer i de la SIDA (Arias *et al*., 1994).

Fig. 3.6. Destrucció de roca prop de Santa Maria de Salgar

Fig. 3.7. Passarel·la metàlica a la Presa de Camarasa

ESTIMACIÓ DE LA CATEGORIA D'AMENAÇA UICN

■ Categoria UICN mundial proposada (avaluació feta el 2004): **NT** (Quasi amenaçat), segons criteris UICN 2001. Amb les anteriors criteris de la UICN del 1994, l'espècie complia el criteri D2 (àrea d'ocupació menor de 100 km^2. Amb les noves categories aquest criteri s'ha rebaixat a 20 km^2; tot i això, la llarga llista d'amenaces que sofreix podria ocasionar la seva inclusió en alguna de les categories d'amenaça en un futur no massa llunyà.

Llistada també a:

■ *Catàleg de Plantes Vasculars endèmiques, rares o amenaçades de Catalunya (I. Tàxons endèmics*; Sáez *et al*., 1998): **VU** D2, segons criteris UICN 1994.

■ *Estrategias para la conservación de la Flora Amenazada de Aragón* (Sainz *et al*., 1996): **VU**.

■ *Lista Roja de la Flora Vascular Española* (Aizpuru *et al*., 2000): **VU** D2, segons criteris UICN 1994.

MESURES DE CONSERVACIÓ ACTUALS

■ Es conserven granes d'aquest tàxon al banc de germoplasma del Laboratori de Botànica de la Universitat de Barcelona.

■ Algunes de les poblacions estan situades dins d'àrees protegides: la població del Congost de Mont-Rebei forma part de la Reserva Natural Noguera Ribagorçana-Mont-Rebei (*Fundació Territori i Paisatge*) i del PEIN Serra del Montsec; diversos nuclis poblacionals també es troben dins aquest PEIN (Ermita de la Pertusa, La Pardina, Ermita del Colobor, Congost de Terradets). Per altra

banda, les poblacions de la presa de Camarasa, Sant Llorenç de Montgai i el Congost d'Alòs també es troben a l'interior d'un PEIN (Aiguabarreig Segre-Noguera Pallaresa).

MESURES DE CONSERVACIÓ PROPOSADES

***In-situ*:**

■ Segons la fórmula de Hamrick *et al.* (1991), conservant 4 poblacions es garantiria la preservació d'un 98,0% de la diversitat genètica detectada en *P. montsicciana*. Per tant, aconsellem la creació de micro-reserves per a les següents poblacions: CAM, MRB i TER, per ser les més grans, i MCO, per ser la que presenta més diversitat genètica: $H_e = 0{,}291$, sensiblement per sobre de la mitjana de l'espècie, però també la que conté un nombre superior d'al·lels; López-Pujol *et al.*, 2001). La conservació d'una cinquena població (LPA o ESA, ambdues amb mides superiors als 1.000 individus), permetria la preservació del 99,2% de la variabilitat genètica. L'elevat percentatge d'al·lels privats per població (6,30% del total) aconsella la conservació del màxim nombre de poblacions possible.

■ Vigilància de l'hàbitat per agents forestals.

■ Seguiment de les poblacions, sobretot aquelles sotmeses a majors pressions antròpiques, per tal d'observar la seva dinàmica demogràfica.

***Ex-situ*:**

■ Recol·lecció de granes per tenir duplicats en altres bancs de germoplasma. Atès l'elevat percentatge d'al·lers rars per població (15,06% del total), recomanem el mostratge d'almenys 5.436 granes, repartides en les 5 poblacions proposades per a la seva conservació *in situ*, atenent a la fórmula del CPC (1991): $C_p = 299 \times (1/0.055) = 5.436$; vegeu també Taula 2.6. Cal profunditzar en l'estudi de les condicions de germinació ateses les baixes taxes obtingudes pel nostre equip (5,5%; López-Pujol, 2000).

■ Cultiu i micropropagació en jardins botànics. Al Servei d'Hivernacles i Vivers de la Facultat de Farmàcia s'ha intentat el cultiu, però amb resultats negatius. Caldria desenvolupar la recerca en aquest aspecte.

Altres:

■ Coordinació entre les diferents administracions autonòmiques responsables de la conservació de l'espècie.

■ En principi no s'aconsella incloure aquest tàxon al *Catálogo Nacional de Especies Amenazadas*, atesa la seva classificació com NT segons els criteris de la UICN. En la mateixa línia, creiem que la seva inclusió dins la Directiva Hàbitats no està justificada, en detriment d'altres espècies recollides en aquesta Memòria en situació d'un risc molt superior (*Seseli farrenyi*, *Silene sennenii*). Tanmateix, el compliment de la legislació comunitària vigent fa esperar mesures de conservació en el marc de la Xarxa Natura 2000.

FITXA REALITZADA AMB LA COL·LABORACIÓ I DADES DE: Joan Devis, Ignasi Soriano, Josep Vicens, Anna Rovira, Julià Molero, Maria Bosch, Joan Simon, Cèsar Blanché.

BIBLIOGRAFIA: Mayol (1998); Mayol & Rosselló (1999); Mayol & Rosselló (2001).

Petrocoptis pardoi Pau

PROTECCIÓ LEGAL

■ *Catálogo de Especies Amenazadas de Aragón* (BOA, 1995).

■ *Ordre sobre Protecció d'espècies endèmiques o amenaçades de la Generalitat Valenciana* (DOGV, 1986).

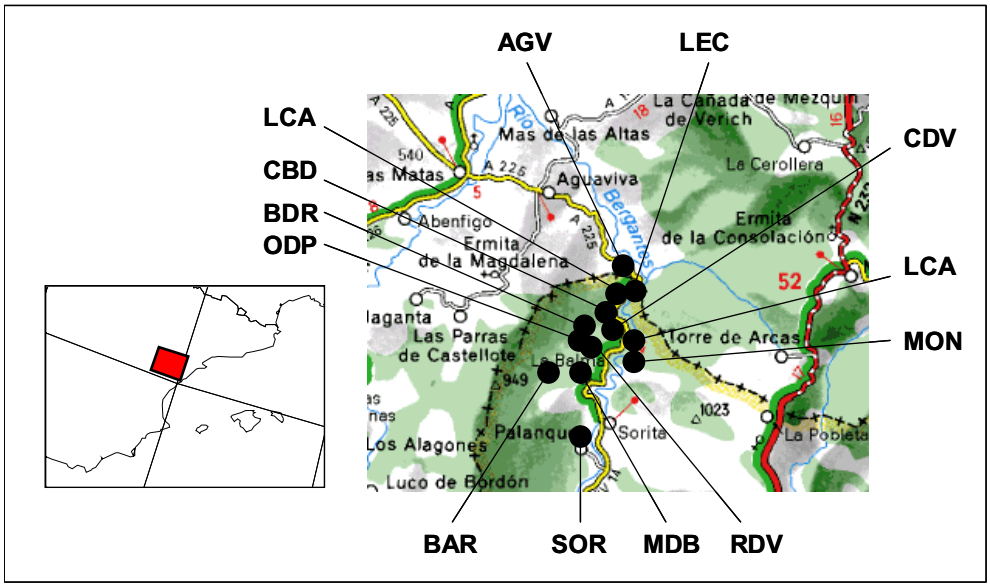
■ En el cas de considerar-la sinònim taxonòmic de *P. montsicciana*, quedaria inclosa dins la Directiva Hàbitats de la UE, Annex 2 (DOCE, 1992) i també dins el Conveni de Berna, Annex 1 (Consell d'Europa, 1998).

COROLOGIA I DEMOGRAFIA

■ Extensió de presència: 225,66 km^2 (2000).

■ Àrea d'ocupació: 27 km^2 (2000).

■ Mapa de localització de poblacions:



Codis de poblacions: AGV: Aiguaviva, BAR: Barranc de la Mare de Déu, BDR: Barranc de Rigores, CBD: Cantal Badat, CDV: Creu del Villar, LCA: Les Canalisses, LEC: Les Contiendes, LCO: Los Cabezos, MBD: Mare de Déu de la Balma, MON: Monagrell, ODP: Ombria de Pere, RDV: Racó d'En Vinadé, SOR: Sorita del Maestrat.

A la dècada dels 90 hi havia 14 localitats conegudes, segons Ibáñez *et al.* (1992), Sainz *et al.* (1996) i Mayol (s.d.):

- AGV (30TYL32): 80 individus (Sainz *et al.*, 1996).
- LEC (30TYL31, YL41): 50 individus (Mayol, s.d.).
- MBD (30TYL31): 190 individus (Ibáñez *et al.*, 1992).

- RDV (30TYL31): 500 individus (Mayol, s.d.).
- BAR (30TYL31): 5.000 individus (Mayol, s.d.).
- ODP (30TYL31): 2.000 individus (Mayol, s.d.).
- LCO (30TYL31): 12 individus (Ibáñez *et al.*, 1992).
- LCA (30TYL41): 250 individus (Mayol, s.d.).
- MON (30TYL41): 220 individus (Ibáñez *et al.*, 1992).
- CDV (30TYL31): 52 individus (Ibáñez *et al.*, 1992).
- CBD (30TYL31): 648 individus (Ibáñez *et al.*, 1992).
- SOR (30TYL31): 26 individus (Ibáñez *et al.*, 1992).
- BDR (30TYL31): 98 individus (Ibáñez *et al.*, 1992).

En total s'estima que hi ha 9.126 individus, la majoria al País Valencià.

AMENACES

Naturals:

■ Taxes de reclutament probablement molt baixes, observades tant pel nostre equip com per d'altres (Sainz *et al*., 1996; Mayol, 1998).

■ Petita mida poblacional en algunes localitats.

Antropogèniques:

■ Destrucció i/o fragmentació del seu hàbitat, bàsicament com a conseqüència de l'ampliació i millora de la xarxa viària; les obres de millora d'alguns trams de la carretera que uneix Aiguaviva amb Sorita del Maestrat han afectat a alguns dels nuclis poblacionals, fet que pot repetir-se amb noves obres que actualment estan en projecte.

■ Risc d'incendis forestals.

■ Herborització massiva, detectada a la localitat clàsica de l'ermita de la Mare de Déu de la Balma, on les herboritzacions continuades pels botànics (vegeu llista de plecs d'herbari a López-Pujol, 2000) des de finals del segle XIX han dut a la quasi completa desaparició de la població.

■ Activitats ramaderes: algunes de les balmes on hi ha individus de *P. pardoi* s'havien aprofitat per a la construcció de cabanes per a l'aixopluc del bestiar.

ESTIMACIÓ DE LA CATEGORIA D'AMENAÇA UICN

■ Categoria UICN mundial (avaluació feta el 2004): **VU** B1ab(iii)+2ab(iii), segons criteris UICN 2001.

Llistada també a:

■ *Estrategias para la conservación de la Flora Amenazada de Aragón* (Sainz *et al*., 1996): **VU**.

■ *Flora Endemica, Rara o Amenazada de la Comunidad Valenciana* (Laguna *et al.*, 1998): **VU**, segons criteris UICN 1994.

■ *Lista Roja de la Flora Vascular Española* (Aizpuru *et al.*, 2000): **VU** B1+2c; C2a, segons criteris UICN 1994.

MESURES DE CONSERVACIÓ ACTUALS

■ La població més gran (Barranc de la Mare de Déu, que compta amb uns 5.000 individus), constitueix una micro-reserva de flora de la Generalitat Valenciana (DOGV, 1994), el que li dóna un nivell òptim de protecció.

MESURES DE CONSERVACIÓ PROPOSADES

***In-situ*:**

■ Segons la fórmula de Hamrick *et al.* (1991), conservant 5 poblacions es garantiria la preservació d'un 99,4% de la diversitat genètica detectada en *P. pardoi*. La declaració de BAR com a micro-reserva permet la conservació de la majoria d'efectius de l'espècie, però no garanteix en absolut la preservació de la seva variabilitat genètica, atès que és la població que presenta menys al·lels i la menys diversa en termes de H_e (López-Pujol *et al.*, 2001). Per tant, es recomana la declaració d'almenys 4 micro-reserves més (CBD, ODP, RDV, LCA). L'elevat percentatge d'al·lels privats per població (8,56% del total) aconsella la conservació del màxim nombre de poblacions possible (un mínim de 5).

■ Vigilància de l'hàbitat per agents forestals.

■ Monitorització de les poblacions, sobretot aquelles sotmeses a majors pressions antròpiques, per tal d'observar la seva dinàmica demogràfica.

■ Limitar l'accés de persones i de ramat en les balmes amb presència de *P. pardoi*.

***Ex-situ*:**

■ Recol·lecció de granes per tenir duplicats en altres bancs de germoplasma. Atès l'elevat percentatge d'al·lers rars per població (11,76% del total), recomanem el mostratge d'almenys 5.436 granes, repartides en les 5 poblacions proposades per a la seva conservació *in situ*, atenent a la fórmula del CPC (1991): $C_p = 299 \times (1/0.055) = 5.436$; vegeu també Taula 2.6, i assumint una taxa de germinació similar a l'obtinguda per *P. montsicciana* (5,5%, López-Pujol, 2000).

■ Cultiu i micropropagació en jardins botànics. Mayol (1998) reporta però resultats molt deficients en el cultiu experimental de *P. pardoi*. Caldria desenvolupar la recerca en aquest aspecte.

Altres:

■ Incloure aquest tàxon al *Catálogo Nacional de Especies Amenazadas*.

■ Coordinació entre les diferents administracions autonòmiques responsables de la conservació de l'espècie.

FITXA REALITZADA AMB LA COL·LABORACIÓ I DADES DE: Emili Laguna, Patrícia Pérez, agents forestals de la Diputació de Castelló, Maria Bosch, Joan Simon & Cèsar Blanché.

BIBLIOGRAFIA: Mayol (s.d.); Ibáñez *et al.* (1992); Mayol (1998); Mayol & Rosselló (1999); Mayol & Rosselló (2001).

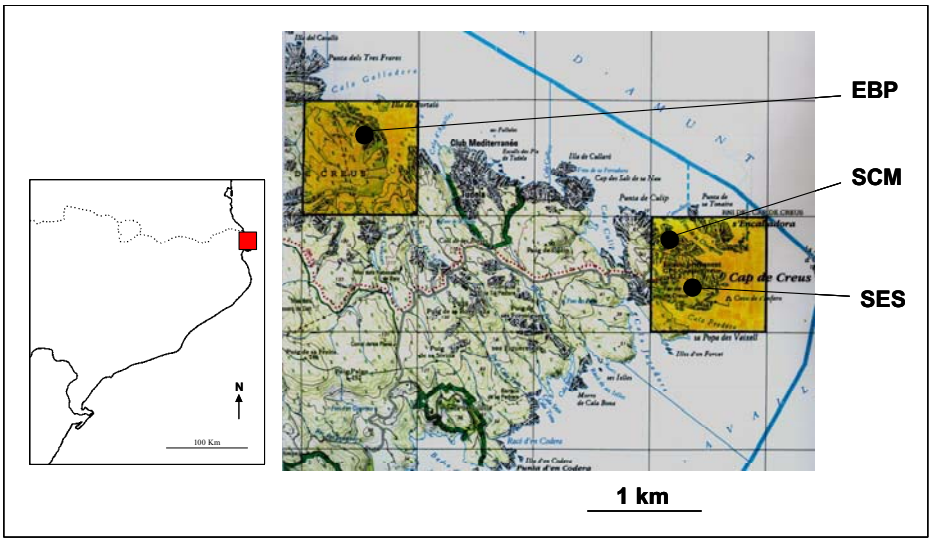
Seseli farrenyi Molero & J. Pujadas

PROTECCIÓ LEGAL

- Decret del Pla d'Espais d'Interès Natural (PEIN, Catalunya), Annex 3 (DOGC, 1993).

COROLOGIA I DEMOGRAFIA

- Extensió de presència: 0,47km^2 (2000); 0,27 km^2 (2004).
- Àrea d'ocupació: 1km^2 (2000).
- Mapa de localització de poblacions:



Codis de poblacions: EBP (Es Bol d'Es Prim); SCM (Es Camallerús); SES (Ses Estenedors).

Al 2000 hi havia 3 poblacions conegudes, a l'actualitat només en resten 2:

- SES (31TEG2685): 90 individus (2000); 0 individus (2004).
- SCM (31TEG2685): 716 individus (2000).
- EBP (31TEG2386): 1.260 individus (2000).

En total, hi havia 2.066 individus l'any 2000. Darrerament (2004) s'ha reportat la desaparició de la població de Ses Estenedors (J. Font, com. pers.), que a l'any 1979 comptava amb prop de 500 efectius (Molero & Pujadas, 1979), i que estava parcialment connectada amb SES a través d'alguns individus intermedis (J. Molero, com. pers.). Les dues poblacions grans estan molt fragmentades en veïnatges genètics (espacials i temporals), el que podria ocasionar davallades futures en el cens d'individus. Una roseta pot tardar entre 1 i 4 anys en emetre les inflorescències, i cada any floreix al voltant d'un 27% de les rosetes (Rovira *et al*., 2004).

AMENACES

Naturals:

■ Els episodis forts de tramuntana poden provocar l'assecament prematur de flors i fruits.

Antropogèniques:

■ Trànsit de persones (pescadors i banyistes bàsicament), detectat a les poblacions de Ses Estenedors i Es Camallerús. Aquest factor pot haver contribuït a la desaparició de la població de Ses Estenedors, puix que aquesta era travessada pel bell mig per un camí que condueix al paratge de S'Infern, molt visitat per la cova i la punta que porten el mateix nom. El trànsit de persones també pot potenciar la fragmentació de la població d'Es Camallerús, mentre que la població d'Es Bol d'Es Prim es troba allunyada de cap punt d'accés i no disposa de camí per arribar-hi.

■ Incendis forestals: la superfície del Parc Natural del Cap de Creus s'ha vist afectada per la successió de dos incendis forestals (estius del 2000 i 2001) en més del 50%, encara que no van arribar a la façana litoral, lloc on habita *S. farrenyi*.

ESTIMACIÓ DE LA CATEGORIA D'AMENAÇA UICN

■ Categoria UICN mundial proposada (avaluació feta el 2004): **EN** B1ab(ii,v)+2ab(ii,v), segons criteris UICN 2001.

Llistada també a:

■ *Catàleg de Plantes Vasculars endèmiques, rares o amenaçades de Catalunya (I. Tàxons endèmics*; Sáez *et al.*, 1998): **EN B1+2c**, segons criteris UICN 1994.

■ *Lista Roja de la Flora Vascular Española* (Aizpuru *et al.*, 2000): **EN** B1+2c, segons criteris UICN 1994.

■ *Atlas y Libro Rojo de la Flora Vascular Amenazada de España* (Bañares *et al.*, 2003): **EN** A2ac; B1ab(i,ii,v)+2 ab(i,ii,v), segons criteris UICN 2001.

MESURES DE CONSERVACIÓ ACTUALS

■ Es conserven granes d'aquest tàxon al banc de germoplasma del Laboratori de Botànica de la Universitat de Barcelona.

■ Els tres nuclis poblacionals estan situats dins una Reserva Natural Integral (RNI) del Parc Natural del Cap de Creus.

MESURES DE CONSERVACIÓ PROPOSADES

In-situ:

■ Creació de micro-reserves de flora per a les 2 localitats actuals, amb el que conservaríem tota la diversitat genètica present a l'espècie.

■ Seguiment poblacional per tal d'observar la dinàmica demogràfica de les poblacions. Tot i els alts nivells de diversitat genètica que presenta l'espècie, la forta presència d'al·lels rars fa preveure la pèrdua de variabilitat si es produeixen disminucions de la mida poblacional.

■ Definició de nous camins d'accés a les cales suficientment separats de les poblacions (especialment important per al cas d'Es Camallerús).

■ Vigilància de les poblacions per agents rurals.

***Ex-situ*:**

■ Recol·lecció de granes per tenir duplicats en altres bancs de germoplasma. Atès l'elevadíssim percentatge d'al·lers rars per població (31,09% del total; López-Pujol *et al.*, 2002), recomanem el mostratge d'almenys 2.990 granes, repartides en les 2 poblacions existents, atenent a la fórmula del CPC (1991) i al menor percentatge de germinació obtingut per l'equip de Biologia de Conservació del nostre laboratori (valors resportats d'entre 0 i 90% segons les condicions experimentals; Cerrillo, 2002): $C_p = 299 \times (1/0.10) = 2.990$; vegeu també Taula 2.6.

■ Cultiu i micropropagació en jardins botànics

Altres:

■ Incloure aquest tàxon al *Catálogo Nacional de Especies Amenazadas*. Atès que a Catalunya manca un catàleg de flora amenaçada, s'aconsella la seva redacció i la inclusió d'aquest tàxon.

■ Inclusió a la Directiva Hàbitats de la UE.

FITXA REALITZADA AMB LA COL·LABORACIÓ I DADES DE: Teresa Franquesa, Joan Font, Julià Molero, Anna Rovira, Maria Bosch, Joan Simon, Cèsar Blanché.

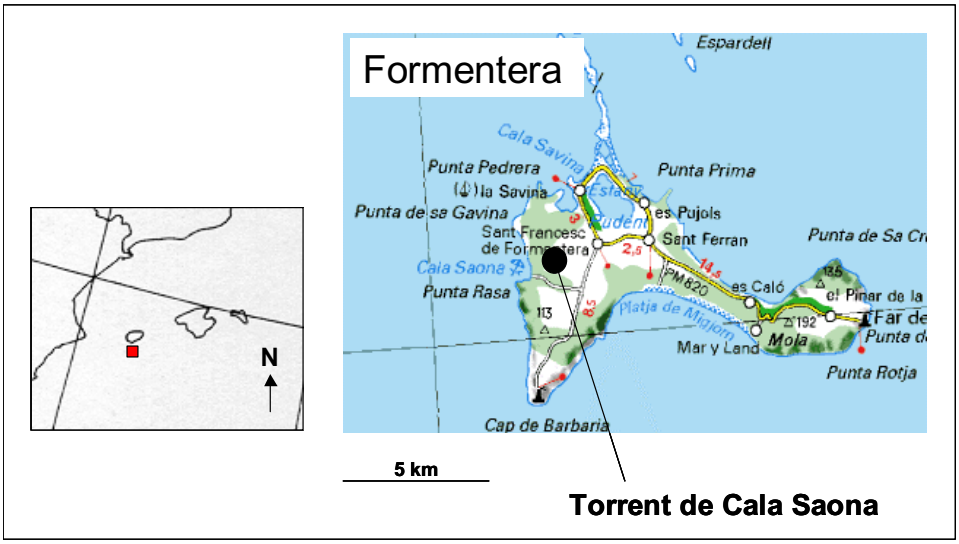
BIBLIOGRAFIA: Molero & Pujadas (1979); Rovira et al. (2004).

Delphinium pentagynum subsp. *formenteranum* N. Torres, L. Sáez, Rosselló & C. Blanché

PROTECCIÓ LEGAL
Inexistent.

COROLOGIA I DEMOGRAFIA

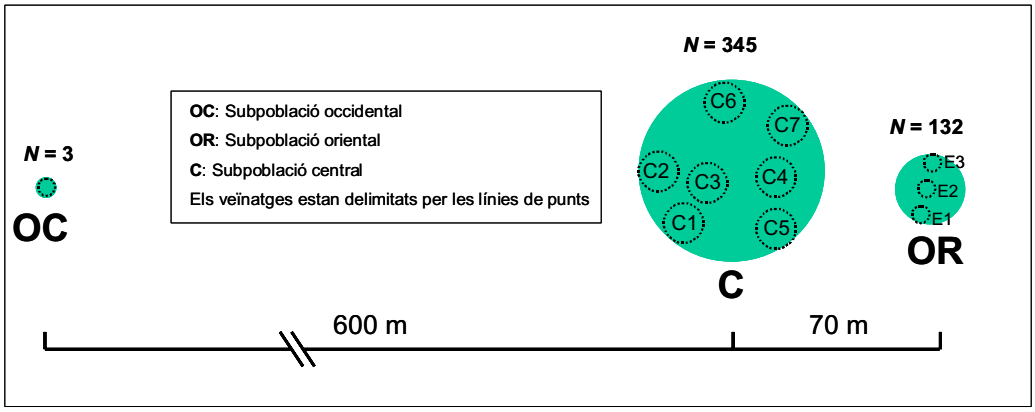
- Extensió de presència: 0.10 km^2 (2001).
- Àrea d'ocupació: 1 km^2 (2001).
- Mapa de localització de poblacions:



Hi ha una única població que conté 3 subpoblacions (totes localitzades dins la quadrícula UTM 31SCC68):

- Subpoblació occidental (OC): 3 individus.
- Subpoblació central (C): 345 individus.
- Subpoblació oriental (OR): 132 individus.

En total, hi ha 480 individus, però el nombre d'efectius madurs (que al 2001 desenvoluparen tija florífera) és només de 247, la resta van quedar en estat vegetatiu de roseta. Es pot, per tant, considerar que cada any floreixen entre un 40 i un 50% dels individus. Els individus de les dues subpoblacions grans estan alhora agrupats en veïnatges, tal com es mostra en el següent gràfic:



AMENACES

Naturals:

■ Petita mida poblacional (sobretot la el nucli occidental), susceptible a factors estocàstics ambientals (sequera, incendis, etc.), demogràfics i genètics (*inbreeding*, deriva genètica), propera al llindar de la MVP.

Antropogèniques:

■ Proximitat a un camí veïnal (que condueix a un abocador i a alguns xalets); l'ampliació del camí pot suposar (i de fet ha suposat) moviments de terra i aparcament de maquinària (excavadores) vora la població (vegeu Figura 3.8).

■ Pastura (ovins).

■ Risc d'incendi per la presència de restes combustibles (brossa i restes de llenya) prop de la població; de fet, el 1996 es declarà un petit foc només a uns centenars de metres de la població

■ Rarificació de pol·linitzadors.

■ Urbanització potencial de la zona (l'especulació urbanística pot conduir a l'incendi provocat de la zona per obtenir la requalificació dels terrenys, pràctica lamentablement estesa a les nostres contrades).

■ Degradació de l'hàbitat: per la proximitat de l'abocador s'abandonen restes de runa d'obra, trastos vells, llaunes, plàstics i d'altres abocaments al voltant de la població.

Fig. 3.8. Camí veïnal que condueix a l'abocador d'Es Cap de Barbaria

■ Increment de la freqüentació: asfaltat del camí (2003-2004) que facilita l'accés.

ESTIMACIÓ DE LA CATEGORIA D'AMENAÇA UICN

■ Categoria UICN mundial proposada (avaluació feta el 2001): **CR** A3c; B1ab(i,ii,iii)+2ab(i,ii,iii), segons criteris UICN 2001.

Llistada també a:

■ *Llibre Vermell de la Flora Vascular de les Illes Balears* (Sáez & Rosselló, 2001): **CR**, segons criteris UICN 2001.

■ *Lista Roja de la Flora Vascular Española* (Aizpuru *et al.*, 2000): **CR** A2c; B1+2ce; C2b, segons criteris UICN 1994.

■ *Atlas y Libro Rojo de la Flora Vascular Amenazada de España* (Bañares *et al.*, 2003): **CR** A3c; B1ab(i,ii,iii)+2ab(i,ii,iii), segons criteris UICN 2001.

MESURES DE CONSERVACIÓ ACTUALS

■ Es conserven granes d'aquest tàxon al banc de germoplasma del Laboratori de Botànica de la Universitat de Barcelona.

MESURES DE CONSERVACIÓ PROPOSADES

***In-situ*:**

■ Creació d'una micro-reserva de flora que inclogui els 3 nuclis poblacionals.

■ Vigilància de l'hàbitat i del camí per agents forestals.

■ Protecció de la població amb troncs o roques de manera no evident.

■ Netejar la llenya i altres restes combustibles dels voltants de la població.

■ Assegurar el flux genètic entre els tres nuclis poblacionals i evitar fragmentacions addicionals (cal un seguiment relativament intensiu). Tot i que la divergència genètica entre els tres nuclis és encara molt petita (F_{ST} = 0,023), els nuclis petits tenen menys al·lels mentre que el nucli central manté un al·lel privat (López-Pujol *et al.*, 2003a), fet que pot indicar interrupció en la migració genètica entre subpoblacions.

■ Prospecció intensiva per a la recerca de nous nuclis poblacionals.

■ Realització d'estudis demogràfics per tal d'avaluar un hipotètic reforç o reintroducció.

***Ex-situ*:**

■ Recol·lecció de granes per tenir duplicats en altres bancs de germoplasma. Atesa l'estructuració de les subpoblacions en veïnatges, cal recol·lectar granes de tots els nuclis per tal de conservar tota la variabilitat genètica existent. Atès el relativament elevat percentatge d'al·lels rars per població (6,25% del total; López-Pujol *et al.*, 2003a), recomanem el mostratge d'almenys 2.990 granes, atenent a la fórmula del CPC (1991) i assumint un percentatge de germinació d'un 10%, que ens ofereix suficient marge de seguretat en cas de desconeixement de la taxa de germinació d'una espècie: C_p = 299 x (1/0.1) = 2.990; vegeu també Taula 2.6.

■ Cultiu i micropropagació en jardins botànics.

Altres:

■ Inclusió d'aquest tàxon al *Catálogo Nacional de Especies Amenazadas* i al *Catàleg Balear de Flora Amenaçada*.

FITXA REALITZADA AMB LA COL·LABORACIÓ I DADES DE: Regidoria de Medi Ambient de Sant Francesc de Formentera, Cèsar Blanché.

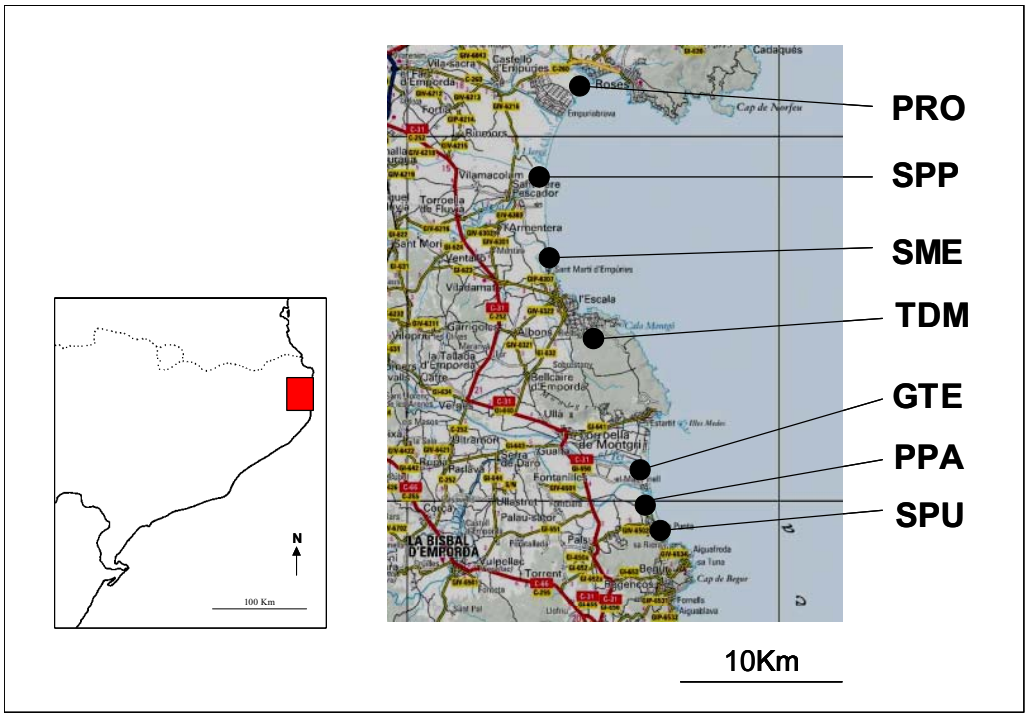
BIBLIOGRAFIA: Torres *et al.* (2000).

Stachys maritima Gouan

PROTECCIÓ LEGAL
Inexistent.

COROLOGIA I DEMOGRAFIA

- Extensió de presència: 35 km^2 (2003).
- Àrea d'ocupació: 8 km^2 (2004).
- Mapa de localització de poblacions:



Codis de poblacions: PRO: Platja de la Rovina; SPP: Sant Pere Pescador; SME: Sant Martí d'Empúries; GTE: Gola del Ter; PPA: Platja de Pals; SPU: Sa Punta; TDM: Torroella de Montgrí.

Al 2004 hi havia 7 localitats conegudes a la Península Ibèrica, dues de les quals pressumptament extingides:

- PRO (31TEG1278): 92 individus (2003).
- SPP (31TEG0968): 8 individus (2001); 0 individus (2002,2003).
- SME (31TEG0966): 1 individu (2003).
- GTE (31TEG1651, 31TEG1652): 9 individus (2003); 1 individu (2004).
- PPA (31TEG1649, 31TEG1650): 123 individus (2003).
- SPU (31TEG1747): 25 individus (2003); 0 individus (2004).
- TDM (31TEG1161): 25 individus (2004).

A l'actualitat, hi ha al voltant de 250 individus, encara que és una espècie sotmesa a unes importants fluctuacions demogràfiques (l'any 2001 es comptaren 177 individus, l'any 2002, 224 individus, el 2003 n'hi havia 250 i al 2004, 242) lligades a la desaparició de nuclis poblacionals i a subsegüents re-colonitzacions. Sembla que aquest fet pot constituir una estratègia que procura a l'espècie adaptabilitat a la mobilitat dels hàbitats de sorres dunars i a l'efecte dels temporals, relativament freqüents en aquesta regió (12 llevantades a l'hivern 2003-2004). S'ha detectat, però, una tendència a la fragmentació dels diferents nuclis poblacionals. La troballa d'una nova població a les dunes fòssils de Torroella de Montgrí, a 2 km de la línia de costa, aconsella noves prospeccions en aquest tipus d'hàbitat.

AMENACES

Naturals:

■ La petita mida de les diferents poblacions (algunes compten amb un sol individu), fa que siguin molt sensibles als factors de tipus estocàstic, ja que queden per sota de la seva MVP. Els temporals poden produir la desaparició –almenys temporal o permanent- de les poblacions per inundació o soterrament d'aquestes, tal com succeí amb els forts temporals registrats a la tardor-hivern del 2001, quan desaparegueren les localitats de SPP i SPA. Una ha reaparegut (SPA), mentre que l'altra (SPP) pot haver-se extingit definitivament.

Antropogèniques:

■ Fragmentació i destrucció directa del seu hàbitat, per la urbanització creixent del litoral gironí (construcció de nous edificis d'apartaments, passeigs marítims i altres infraestructures turístiques); remodelació de la zona de Ràdio Liberty.

■ Trànsit de persones (banyistes, campistes, *windsurfers*), motocicletes i de vehicles tot-terreny (vegeu Figura 3.9, Platja de Pals); construcció de carrils-bici.

■ Estacionament de remolcs d'embarcacions i embarcacions a la platja (vegeu Figura 3.10, Platja de Pals).

■ Competència i desplaçament per part d'espècies exòtiques (*Carpobrotus edulis*, vegeu Figura 3.11, Platja de Pals).

Fig. 3.9. Roderes de tot-terreny a la Platja de Pals

Fig. 3.10. Individu d'*Stachys maritima* la varador de Pals

Fig. 3.11. *Carpobrotus edulis* a la Platja de Pals

ESTIMACIÓ DE LA CATEGORIA D'AMENAÇA UICN

■ Categoria UICN regional (per als territoris de Catalunya, Espanya i la Península Ibèrica) proposada (avaluació feta el 2004): **CR** A1c+4c; B1ab(i,ii,iii,iv,v)+2ab(i,ii,iii,iv,v);C1, segons criteris UICN 2001.

Llistada també a:

■ *Catàleg de Plantes Vasculars endèmiques, rares o amenaçades de Catalunya (II. Tàxons no endèmics en situació de risc*; Sáez & Soriano, 2000): **VU** B1+2c; D2, segons criteris UICN 1994.

■ *Lista Roja de la Flora Vascular Española* (Aizpuru *et al.*, 2000): **VU** B1+2c; D2, segons criteris UICN 1994.

MESURES DE CONSERVACIÓ ACTUALS

■ Es conserven granes d'aquest tàxon al banc de germoplasma del Laboratori de Botànica de la Universitat de Barcelona

■ Dues de les localitats (SPP, SME) estan situades dins el perímetre del Parc Natural dels Aiguamolls de l'Empordà; i una part de PPA está localitzada dins de la Reserva "Basses d'en Coll", proposada com a Espai Natura 2000.

■ L'ens que fins al 2004 era responsable de la gestió privada de l'espai PEIN (Arenales del Mar, S.A.) s'ha fet càrrec de mesures de vigilància, limitació d'accés a l'embarcador de Pals, edició de materials informatius (tríptics, plafons) i realització d'activitats d'educació ambiental (visites guiades a escoles –2003-, itineraris naturalístics –ICHN, 2004-).

■ A principis de 2003, el Departament de Medi Ambient de la Generalitat de Catalunya promulgà un ordre (DOGC, 2003) en que és convocaven ajuts destinats a actuacions de conservació i recuperació de la fauna i la flora amenaçades, entre les que figurava *S. maritima*, tot i que finalment no es va dotar cap projecte.

MESURES DE CONSERVACIÓ PROPOSADES

In-situ:

■ Segons la fórmula de Hamrick *et al.* (1991), conservant 4 poblacions es garantiria la preservació d'un 99,0% de la diversitat genètica detectada en *Stachys maritima*. Per tant, aconsellem la creació de micro-reserves com a mínim per a les dues localitats més grans (PRO, PPA). Seria també convenient la preservació de la població de SME degut a la seva singularitat genètica (és l'única que conté 1 al·lel privat; i tot i que només disposi a l'actualitat d'un individu, no descartem un possible reclutament, atesa la dinàmica demogràfica d'aquesta espècie). Per últim, també creiem convenient la protecció de l'única població no litoral (TDM), atesa la seva singularitat d'hàbitat (dunes fòssils).

■ Seguiment intensiu de les poblacions per a detectar fluctuacions demogràfiques i amenaçes potencials.

■ Establiment de tot un seguit de mesures "toves", com ara el tancament de les dunes mitjançant cordes i estaques i la instal·lació de passarel·les alternatives d'accés a la platja.

■ Vigilància de les poblacions per agents rurals.

■ Reintroduccions en altres localitats de l'àrea de distribució històrica de l'espècie, i reforçaments de les poblacions existents. Hi ha un projecte preliminar de reintroducció d'*Stachys maritima* a les platges de Palamós (Girona).

***Ex-situ*:**

■ Recol·lecció de granes per tenir duplicats en altres bancs de germoplasma. Atès la manca gairebé total d'al·lers rars per població (2,72% del total; López-Pujol *et al.*, 2003b), recomanem el mostratge d'unes 1.720 granes, repartides en les 4 poblacions proposades per a la seva conservació *in situ*, atenent a la fórmula del CPC (1991) i al menor percentatge de germinació obtingut per l'equip de Biologia de Conservació del nostre laboratori (valors resportats d'entre 0 i 80% segons les condicions experimentals; Cerrillo, 2002): C_p = 172 x (1/0.10) = 1.720; vegeu també Taula 2.6.

■ Cultiu i micropropagació en jardins botànics

Altres:

■ Incloure aquest tàxon al *Catálogo Nacional de Especies Amenazadas*. Atès que a Catalunya manca un catàleg de flora amenaçada, s'aconsella la seva redacció i la inclusió d'aquest tàxon.

■ Realització de noves prospeccions en hàbitats de dunes fòssils.

FITXA REALITZADA AMB LA COL·LABORACIÓ I DADES DE: Carles Barriocanal, Joan Font, Maria Renée Orellana, Maria Bosch, Cèsar Blanché.

BIBLIOGRAFIA: Barriocanal & Blanché (2002); Barriocanal & Blanché (2003).

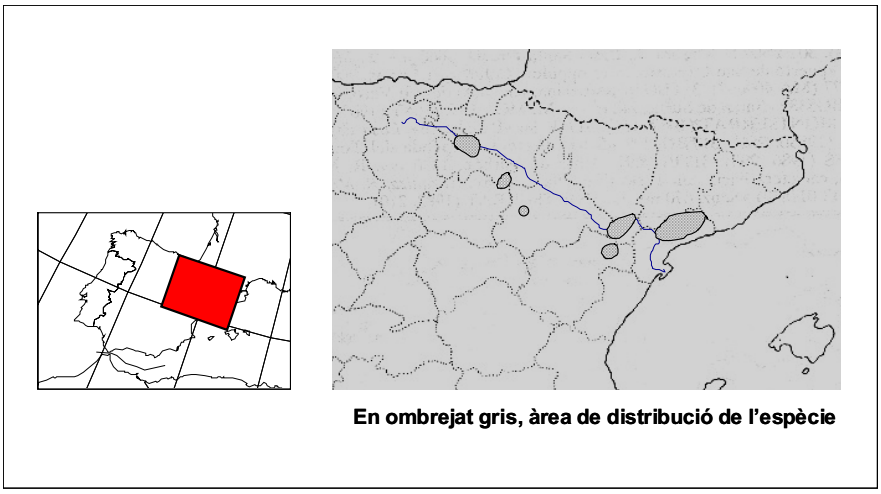
Thymus loscosii Willk.

PROTECCIÓ LEGAL

- Llistada al *Catálogo Nacional de Especies Amenazadas* (BOE, 1990) com "en perill".
- *Catálogo de la Flora Amenazada de Navarra* (BON, 1997), com a "vulnerable".
- *Catálogo de Especies Amenazadas de Aragón* (BOA, 1995), com a "en perill d'extinció".

COROLOGIA I DEMOGRAFIA

- Extensió de presència: 28.000 km^2 (2002).
- Àrea d'ocupació: 5.200 km^2 (2002).
- Mapa de localització de poblacions:



En ombrejat gris, àrea de distribució de l'espècie

És una espècie abundant; hi ha amb tota seguretat més de 100 poblacions, que probablement continguin en conjunt més d'1 milió d'individus. En total, es troba en 41 quadrícules UTM de 10×10 km, en 6 Comunitats Autònomes diferents, tot i que el nombre creixent de noves localitats trobades en anys recents fa preveure increments futurs en aquestes dades:

- Castella-Lleó: present en 2 quadrícules UTM 10×10 km (30TWM13, WM84), a la província de Sòria.
- País Basc: present en 4 quadrícules UTM 10×10 km (30TWN20, WN21, WN30, WN40), a la província d'Àlaba.
- La Rioja: present en 3 quadrícules UTM 10×10 km (30TWM84, WN20, WN40), a la província homònima.
- Navarra: present en 2 quadrícules UTM 10×10 km (30TWM95, WN50), a la província homònima.
- Aragó: present en 22 quadrícules UTM 10×10 km (30TXK65, YL33, YL34, YL35, YL43, YL44, WL77, XL14, XL16, XL23, XL29, XL38, XM01, XM10, XM70, YL36, YL46, YL47, BF46, BF56, BF57, BF68), a les províncies de Saragossa i Terol.

• Catalunya: present en 8 quadrícules UTM 10×10 km (31TCF07, CF08, CF17, CF27, CF28, CF38, CF49, CF68), a la províncies de Lleida i Tarragona.

AMENACES

Naturals:

■ No hi ha evidències de cap amenaça d'origen natural. En alguns punts, s'han reportat fenòmens d'hibridació amb *Thymus vulgaris*, *T. zygis* i *T. mastichina*; fins i tot, descrits com a *notho*-tàxons formals (*Thymus x rubioi*, *T. x aragonensis* i *T. x riojanus*, respectivament; Blanché *et al.*, 2002).

Antropogèniques:

■ Destrucció i/o fragmentació del seu hàbitat: la reforestació progressiva de la conca de l'Ebre en les darreres dècades pot fer desaparèixer part del seu hàbitat natural. Altres transformacions que poden afectar negativament l'espècie és la construcció de noves infraestructures, com ara el tren d'alta velocitat i l'ampliació de la xarxa de carreteres (pressions detectades sobre algunes poblacions catalanes, vegeu Figures 3.12 i 3.13: la primera correspon a les obres de l'AVE al seu pas per Tarrés, Lleida; la segona mostra l'ampliació d'un camí a La Granadella, Lleida; ambdues obres afectaven parcialment poblacions de *T. loscosii*), i el canvi d'ús del territori (la plantació de noves vinyes a la Rioja i País Basc ha produït la destrucció de poblacions senceres).

■ Pastura (en algunes poblacions aragoneses; Begoña García, com. pers.).

Fig. 3.12. Obres de l'AVE al seu pas per Tarrés

Fig. 3.13. Ampliació d'un camí a La Granadella

ESTIMACIÓ DE LA CATEGORIA D'AMENAÇA UICN

■ Categoria UICN mundial proposada (avaluació feta el 2004): **LC**, segons criteris UICN 2001; per a Catalunya, **NT**.

Llistada també a:

■ *Estrategias para la conservación de la Flora Amenazada de Aragón* (Sainz *et al.*, 1996): **R**.

■ *Especies Amenazadas de Flora en el País Vasco* (NIET, 2002): **"D'especial interès"**.

MESURES DE CONSERVACIÓ ACTUALS

■ Algunes de les poblacions catalanes estan situades dins dels límits PEIN ("Serra del Montsant", "Muntanyes de Prades", "Ancosa-Montagut"; cf. Blanché *et al.*, 2002).

MESURES DE CONSERVACIÓ PROPOSADES

***In-situ*:**

■ Vigilància de l'hàbitat i les localitats per agents forestals.

■ Prospecció intensiva per a la recerca de nous nuclis poblacionals.

■ Seguiment (i declaració de micro-reserves) de poblacions amb un interès especial (genotips o fenotips determinats): VAL, per ser la única població que presenta al·lels privats (López-Pujol *et al.*, 2004), MON, per presentar un fenotip morfològic particular (Blanché *et al.*, 2002), ELC, per ser la que presenta el percentatge més elevat d'invididus amb 3/4 al·lels per *locus* (21,7%; Blanché *et al.*, 2001), i ULL, per ser la que conté un percentatge més elevat de ginodioècia (18%; Blanché *et al.*, 2001). Atesa l'escassa diferenciació genètica entre poblacions (López-Pujol *et al.*, 2004), la preservació de només dues poblacions ja ens garantiria la conservació del 99,9% de la variabilitat genètica de *T. loscosii*. La preservació d'aquestes 4 poblacions proposades ens permetrà, per tant, la conservació del 100% de la diversitat genètica en termes tant de riquesa al·lèlica com de distribució de la diversitat, i també la conservació de morfologies i funcionalitats variades dins l'espècie.

***Ex-situ*:**

■ Recol·lecció de granes per tenir duplicats en altres bancs de germoplasma. Atès l'elevat percentatge d'al·lels rars per població (19,72% del total), recomanem el mostratge d'almenys 5.980 granes, repartides en les 4 poblacions proposades per a la seva conservació *in situ*, atenent a la fórmula del CPC (1991) i i al menor percentatge de germinació obtingut per l'equip de Biologia de Conservació del nostre laboratori (valors resportats d'entre 0 i 20% segons les condicions experimentals; *cf.* Cerrillo, 2002; encara que altres investigadors han assolit valors de fins al 96%; Albert *et al.* 2002): $C_p = 299 \times (1/0.05) = 5.980$; vegeu també Taula 2.6.

■ Cultiu (transplantament en testos) i micropropagació en jardins botànics.

Altres:

■ Atès que *T. loscosii* no està actualment en perill a l'Estat Espanyol, es podria excloure del *Catálogo Nacional de Especies Amenazadas*, encara que és una opció sobre la que cal reflexionar en profunditat ja que és perd un instrument legal que permet la protecció del territori si, al mateix temps, no s'inclouen noves espècies catalanes al *Catálogo*. Alternativament, sembla més adient la requalificació dins del mateix Catàleg, passant a la categoria "d'interès especial", que comporta legalment l'aplicació d'un pla de gestió en lloc d'un pla de recuperació.

■ A l'hora de seleccionar les poblacions o individus per a la seva conservació, tant *in-situ* com *ex-situ*, caldria tenir en compte la diversitat de quimiotipus i les taxes de ginodioència.

■ Coordinació entre les diferents administracions autonòmiques (l'espècie és present en 6 CC.AA. diferents).

FITXA REALITZADA AMB LA COL·LABORACIÓ I DADES DE: Llorenç Sáez, Julià Molero, Anna Rovira, Maria Renée Orellana, Maria Bosch, Joan Simon, Cèsar Blanché.

BIBLIOGRAFIA: Blanché *et al.*, 2000; Blanché *et al.*, 2001; Blanché *et al.*, 2002.

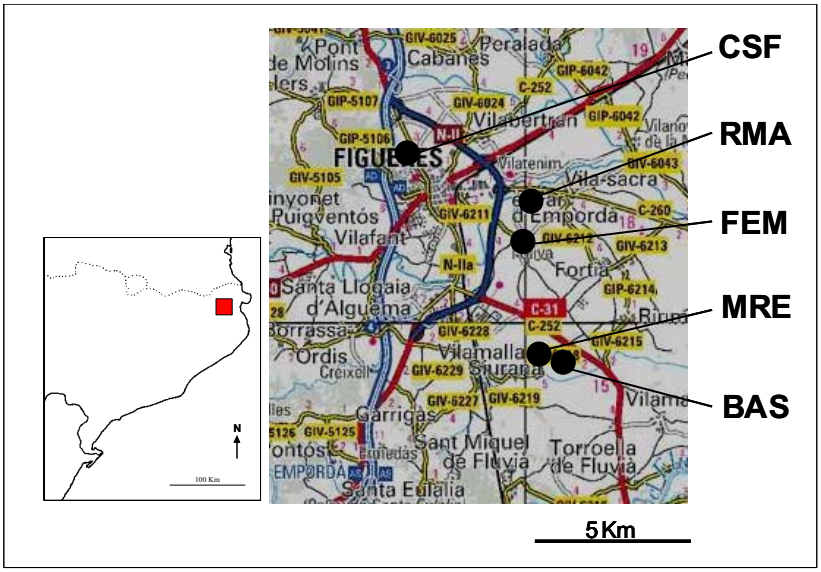
Silene sennenii Pau

PROTECCIÓ LEGAL

Inexistent.

COROLOGIA I DEMOGRAFIA

- Extensió de presència: 14,5 km^2 (2004).
- Àrea d'ocupació: < 3 km^2 (2004).
- Mapa de localització de poblacions:



Codis de poblacions: BAS (Baseia); CSF (Castell de Sant Ferran); FEM (El Far de l'Empordà); MRE (Mas Renart); RMA (Riu Manol).

Hi ha 5 poblacions conegudes:

- BAS (31TEG0073,EG0074, EG0172 i EG0173): 1.084 individus (2004).
- CSF (31TDG9579, DG9580, DG9581 i DG9680): 3.209 individus (2004).
- FEM (31TDG9978): 295 individus (2001); 49 individus (2004).
- MRE (31TDG9974): 4 individus (2004).
- RMA (31TDG9979): 172 individus (2004).

En total, hi ha 4.518 individus madurs (reproductius). Algunes poblacions, com Baseia o el Far de l'Empordà, estan molt fragmentades (atès que ocupen les vores dels camps de cultius i els marges de camins). La població més contínua és la del Castell de Sant Ferran, que ocupa el fossar en la majoria del perímetre del castell.

AMENACES

Naturals:

■ Petita mida poblacional en 3 de les 5 localitats on és present l'espècie (FEM, MRE, RMA), susceptibles a factors estocàstics ambientals (sequera, incendis, etc.), demogràfics i genètics (*inbreeding*, deriva genètica). En dues (MRE, FEM), la mida poblacional es inferior a la MVP, el que les posa en situació de risc extrem de desaparició.

■ Predació.

■ Competència vegetal natural.

Antropogèniques:

■ Fragmentació i destrucció directa del seu hàbitat, a causa de l'expansió dels polígons industrials i àrees de serveis, observat a la població RMA (vegeu Figura 3.14), a la construcció o ampliació de les vies de comunicació (l'ampliació d'un vial causà la desaparició de 246 individus de la població FEM l'any 2002, el que representava més d'un 80% dels seus efectius).

■ Conversió del seu hàbitat en terrenys de cultiu de regadiu.

■ Ús d'herbicides.

■ Ruderalització del seu hàbitat (*S. sennenii* sembla força sensible a la nitrificació dels sòls ja que és completament absent de la vegetació ruderal de les proximitats).

■ Projectes de remodelació del Castell de Sant Ferran (Figueres)

Fig. 3.14. Població del Riu Manol

ESTIMACIÓ DE LA CATEGORIA D'AMENAÇA UICN

■ Categoria UICN mundial proposada (avaluació feta el 2004): **EN** B1ab(i,ii,iii,iv,v)+2ab(i,ii,iii,iv,v), segons criteris UICN 2001.

Llistada també a:

■ *Catàleg de Plantes Vasculars endèmiques, rares o amenaçades de Catalunya (I. Tàxons endèmics*; Sáez *et al*., 1998): **CR** B2c,d+3c, segons criteris UICN 1994.

■ *Lista Roja de la Flora Vascular Española* (DD.AA., 2000): **CR** B2cd + 3c, segons criteris UICN 1994.

■ *Atlas y Libro Rojo de la Flora Vascular Amenazada de España* (Bañares *et al*., 2004): **EN** B1ab(i,ii,iii,iv,v)+2ab(i,ii,iii,iv,v), segons criteris UICN 2001.

MESURES DE CONSERVACIÓ ACTUALS

■ Inexistents per a l'espècie. El seu hàbitat (prats de *Brachypodium phoenicoides*; 6210) està llistat a la Directiva Hàbitats però no es considera prioritari (i per tant no inclòs dins la xarxa Natura 2000).

MESURES DE CONSERVACIÓ PROPOSADES

In-situ:

■ Segons la fórmula de Hamrick *et al.* (1991), la conservació de 3 poblacions asseguraria la preservació del 98,0% de la diversitat genètica, mentre que la conservació de 4 poblacions permetria la preservació de fins al 99,5% de la variabilitat. Atès que cap de les poblacions conté

al·lels privats (López-Pujol *et al.*, 2004), el disseny de micro-reserves per a les tres localitats més grans (CSF, BAS i RMA), que són les que també tenen més al·lels, sembla suficient.

- Seguiment poblacional per tal d'observar la dinàmica demogràfica de les poblacions.
- Adequació dels plans urbanístics locals per tal d'evitar noves destruccions de l'hàbitat.
- Vigilància de les poblacions per agents rurals.
- Limitació del trànsit de persones i vehicles als seus hàbitats.
- Restauració dels hàbitats o microambients (fenassars), mitjançant l'eliminació d'algunes espècies competidores.

***Ex-situ*:**

- Recol·lecció de granes per a conservar en bancs de germoplasma. Atès l'important percentatge d'al·lers rars per població (7,90% del total), recomanem el mostratge d'almenys 2.990 granes, repartides en les 3 poblacions proposades per a la seva conservació *in situ*, atenent a la fórmula del CPC (1991) i assumint un percentatge de germinació d'un 10%, que ens ofereix suficient marge de seguretat en cas de desconeixement de la taxa de germinació d'una espècie: $C_p = 299 \times (1/0.10) = 2.990$; vegeu també Taula 2.6.
- Cultiu i micropropagació en jardins botànics.

Altres:

- Incloure aquest tàxon al *Catálogo Nacional de Especies Amenazadas*. Atès que a Catalunya manca un catàleg de flora amenaçada, s'aconsella la seva redacció i la inclusió d'aquest tàxon.
- Realització d'estudis addicionals sobre l'autoecologia de l'espècie (demogràfics, de biologia reproductiva, etc.), necessaris per al correcte disseny d'estratègies de conservació tant *in-situ* com *ex-situ*.

FITXA REALITZADA AMB LA COL·LABORACIÓ I DADES DE: Joan Font, Cèsar Blanché.

BIBLIOGRAFIA: Font & Gesti (2002).

Erodium rupestre (Pourr. ex Cav.) Cadevall

PROTECCIÓ LEGAL

■ Decret del Pla d'Espais d'Interès Natural (PEIN, Catalunya), Annex III, per a l'espai Montserrat (DOGC, 1993).

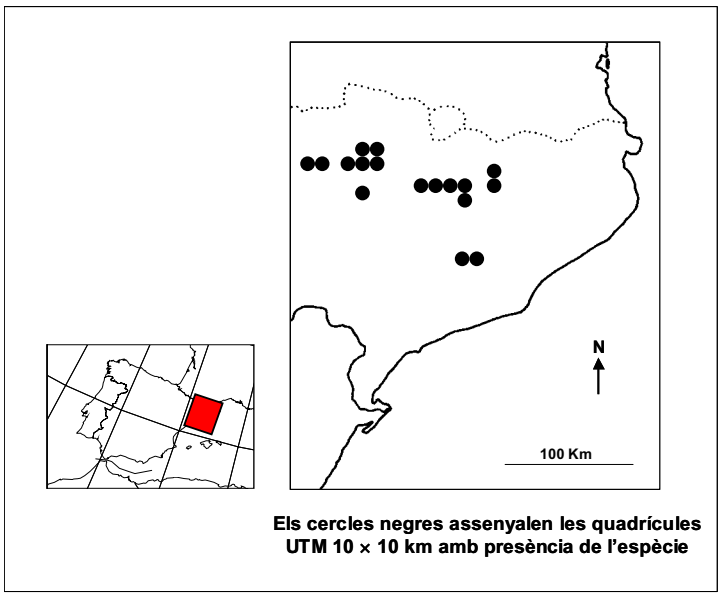
■ *Catálogo de Especies Amenazadas de Aragón* (BOA, 1995), "d'interès especial".

COROLOGIA I DEMOGRAFIA

■ Extensió de presència: 7.200 km^2 (2004).

■ Àrea d'ocupació: < 500 km^2 (2004).

■ Mapa de localització de poblacions:



Els cercles negres assenyalen les quadrícules UTM 10 × 10 km amb presència de l'espècie

És una espècie relativament abundant en el seu hàbitat; estimem a partir de visites al camp, i d'informacions d'altres investigadors, que hi ha una cinquantena de poblacions, que contenen en conjunt no menys de 20.000 individus. En total, es troba en 17 quadrícules UTM de 10×10 km, 15 a Catalunya i 2 a l'Aragó. Tretze de les quadrícules catalanes i les 2 aragoneses contenen les localitats pre-pirinenques, mentre que les dues quadrícules més meridionals de Catalunya corresponen a les poblacions de la Muntanya de Montserrat.

La llista de quadrícules UTM amb presència de l'espècie és la següent:

• Catalunya: 31TCG17, CG25, CG27, CG28, CG37, CG38, CG66, CG76, CG86, CG90, CG95, CG96, DG00, DG16, DG17.

• Aragó: 31TBG87, BG97.

AMENACES

Naturals:

■ Producció de llavors molt escassa (Álvarez, 2003).

■ Taxes de reclutament probablement molt baixes (observacions al camp).

■ Aquestes amenaces aparents poden realment no ésser-ho, a causa de l'elevada longevitat dels individus (vegeu imatge de la dreta, individu senescent), tret comú en les espècies que presenten una estratègia biològica d'alta persistència en hàbitats específics (Albert, 2003).

Fig. 3.15. Individu longeu d'*Erodium rupestre*

Antropogèniques:

■ Són poques a causa de la inaccessibilitat de la majoria de poblacions. Tot i això, algunes localitats estan situades en àrees força transitades, com ara les poblacions de la Muntanya de Montserrat (més de 5.000 visitants/any a través del funicular de Sant Joan), les de la Mare de Déu del Lord i Sant Romà de la Clusa. Una de les localitats clàssiques, la del castell de Laguarres, està sotmesa a una antropització creixent de l'hàbitat, puix que és un lloc on s'hi han instal·lat diferents antenes de telecomunicacions (telefonia mòbil).

■ Ús medicinal de l'espècie (Hernández *et al.*, 2003).

■ Risc d'incendis forestals.

■ Activitats recreatives (escalada, *trekking*).

■ Destrucció i/o fragmentació de l'hàbitat, per l'ampliació o la millora de la xarxa viària.

ESTIMACIÓ DE LA CATEGORIA D'AMENAÇA UICN

■ Categoria UICN mundial proposada (avaluació feta el 2004): **LC**, segons criteris UICN 2001.

Llistada també a:

■ *Catàleg de Plantes Vasculars endèmiques, rares o amenaçades de Catalunya (I. Tàxons endèmics*; Sáez *et al.*, 1998): **VU** D2, segons criteris UICN 1994.

■ *Dades sobre l'estatus d'algunes plantes endèmiques, amenaçades o rares a Catalunya (NE de la península Ibèrica)* (Aymerich & Sáez, 2001): **LC**, segons criteris UICN 2001.

■ *Lista Roja de la Flora Vascular Española* (Aizpuru *et al.*, 2000): **VU** D2, segons criteris UICN 1994.

MESURES DE CONSERVACIÓ ACTUALS

■ Es conserven granes d'aquest tàxon al banc de germoplasma del Laboratori de Botànica de la Universitat de Barcelona.

■ Algunes de les poblacions catalanes estan situades dins d'àrees protegides (les poblacions de Montserrat estan situades dins el Parc Natural de la Muntanya de Montserrat). Algunes de les poblacions pre-Pirinenques estan dins d'espais PEIN (Vall Alta de Serradell, Serres d'Odèn-Port del Compte, Serres de Busa-Els Bastets-Lord, Serra del Catllaràs).

MESURES DE CONSERVACIÓ PROPOSADES

***In-situ*:**

■ Segons la fórmula de Hamrick *et al.* (1991), seria necessària la conservació de 5 poblacions per a garantir la preservació d'un 99,3% de la diversitat genètica detectada en *E. rupestre*. L'estimació del coeficient de diferenciació gènica es basa en les diferències interpoblacionals en les freqüències al·lèliques. Atès que en l'estudi isoenzimàtic només apareixen 2 *loci* variables, si atenem a la taula de freqüències al·lèliques (López-Pujol *et al.*, en revisió), la conservació de dues poblacions (mitjançant una micro-reserva de flora) sembla del tot suficient: MON, atès que és la única població amb un al·lel privat, i ROM, que és la població en la qual els dos al·lels del *locus Aco-1* estan en freqüències molt properes a 0,5.

■ Limitació d'accés a les poblacions més transitades (Montserrat, Mare de Déu del Lord, Laguarres).

■ Vigilància de l'hàbitat per agents forestals.

■ Prospecció per a la recerca de noves poblacions.

***Ex-situ*:**

■ Recol·lecció de granes per tenir duplicats en altres bancs de germoplasma. Atesa l'absència d'al·lels rars, el mostratge de 1.720 granes, repartides en les 2 poblacions proposades per a la seva conservació *in situ*, atenent a la fórmula del CPC (1991) i assumint un percentatge de germinació d'un 10%, que ens ofereix suficient marge de seguretat en cas de desconeixement de la taxa de germinació d'una espècie: $C_p = 172 \times (1/0.10) = 1.720$; vegeu també Taula 2.6. Cal profunditzar en l'estudi de les condicions de germinació donats els resultats nuls obtinguts pel nostre equip (0%; Cerrillo, 2002) i les consideracions d'Albert (2003).

■ Cultiu i micropropagació en jardins botànics.

Altres:

■ Tot i que està llistada en el decret del PEIN, seria aconsellable incloure-la dins un catàleg de flora amenaçada de Catalunya que, donat que manca a la nostra Comunitat, s'aconsella la seva redacció urgent.

■ Coordinació entre les diferents administracions autonòmiques.

■ Realització d'estudis addicionals de biosistemàtica per a la detecció de possibles introgressions amb d'altres tàxons propers filogenèticament.

FITXA REALITZADA AMB LA COL·LABORACIÓ I DADES DE: Llorenç Sáez, David Guzmán, Pere Aymerich, Ignasi Soriano, Noemí Álvarez, Maria Bosch, Joan Simon, Cèsar Blanché.

BIBLIOGRAFIA: Estrada (1997); Álvarez (2003); Álvarez *et al*. (2004).

Delphinium montanum DC.

PROTECCIÓ LEGAL

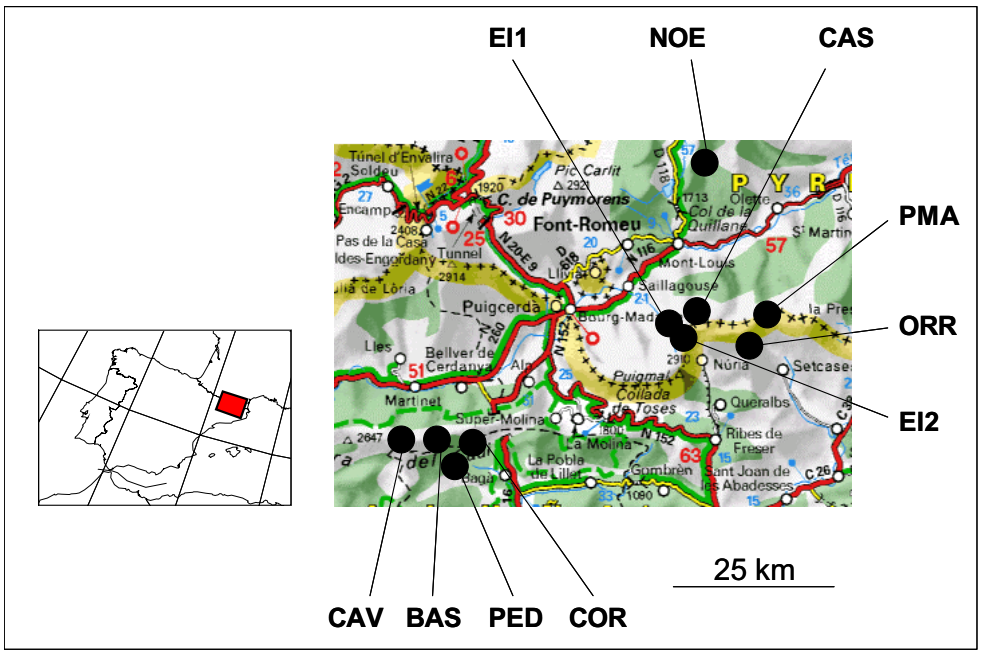
■ Decret del Pla d'Espais d'Interès Natural (PEIN, Catalunya), Annex III, a l'espai Capçaleres del Ter i del Freser (DOGC, 1993).

COROLOGIA I DEMOGRAFIA

■ Extensió de presència: 827,5 km^2 (2004).

■ Àrea d'ocupació: < 20 km^2 (2004).

■ Mapa de localització de poblacions:



Codis de poblacions: BAS: Bastanist; COR: Els Cortils; PED: Pedraforca; CAV: Cava; ORR: Coma de l'Orri; EI1: Vall d'Eina (Inferior); EI2: Vall d'Eina (Superior); CAS: Cambra d'Ase; NOE: Noedes; PMA: Portella de Mantet.

Al 2004 hi havia 10 localitats conegudes, tres de les quals pressumptament extingides:

- BAS (31TCG98): 2.000 individus (1997); 2.500 individus (2003).
- COR (31TCG98): 900 individus (1998); 1.000 individus (2002).
- PED (31TCG97): 25 individus (1984); 0 individus (2000, 2002).
- CAV (31TCG88): 200 individus (1985).
- ORR (31TDG39): 15 individus (1996); 500 individus (2003).
- EI1 (31TDG29): 700 individus (1997); 1.500 individus (2002).
- EI2 (31TDG29): 200 individus (1995); 80 individus (2002).

- CAS (31DH20): 200 individus (2004).
- NOE (31TDH31): 700 individus (2004).
- PMA (31TDG49): 0 individus (1994, 1995).

En total, hi ha 6,280 individus madurs (reproductius). És, però, una espècie que sofreix fortes oscil·lacions demogràfiques (la mida poblacional s'estimà en prop de 10.000 individus en anys recents; Aymerich & Sáez, 2001). La desaparició d'alguns nuclis poblacionals (Portella de Mantet, Pedraforca, i probablement, Cava) i les oscil·lacions demogràfiques en la resta podrien explicar-se en part per una dinàmica que seguiria el model de metapoblacions centre-perifèria (Gillman, 1997), i en part també per adaptació a un medi mòbil (tartera), amb la part subterrània capaç de rebrotar al cap d'un temps, a més de dinàmiques de reclutament potser irregulars.

AMENACES

Naturals:

■ Les tarteres inestables, un dels hàbitats potencials per a l'espècie, poden sofrir canvis importants o fins i tot desaparèixer com a conseqüència de despreniments de roques i allaus. L'espècie es troba adaptada a aquests canvis teòricament, però no són descartables extincions locals per causes naturals.

■ Predació per ungulats, bàsicament l'isard (*Rupricarpa* pyrenaica) i el mufló (*Ovis ammon*), que pot representar una pèrdua de més del 95% de la producció de granes en algunes poblacions, tal com s'ha demostrat a la població dels Cortils (Simon *et al.*, 2001) i a Bastanist (Aymerich, 2003), encara que s'ha suggerit que el reclutament no se'n veuria afectat (Aymerich, 2003). Al 2004 es va observar també una predació massiva d'òrgans reproductors a Noedes (vegeu Figura 3.16).

Fig. 3.16. Predació a la població de Noedes

■ Competència vegetal natural als marges de tartera, quan es produeix la fixació i el predomini subsegüent d'herbàcies cespitoses.

Antropogèniques:

■ Trànsit de persones, atrets per la bellesa dels paratges. La majoria de les poblacions estan localitzades dins de parcs naturals, àrees molt visitades pels turistes, sobretot a l'estiu, quan la planta és en flor. La població del Pedraforca estava situada a la tartera central de la muntanya, zona molt transitada; és aquest trànsit el que pot haver causat la seva desaparició per la degradació evident de l'hàbitat (nitrificació i alteració de la tartera). La població de Cambra d'Ase està situada a la vora d'una estació d'esquí i possibles ampliacions podrien afectar la planta.

ESTIMACIÓ DE LA CATEGORIA D'AMENAÇA UICN

■ Categoria UICN mundial proposada (avaluació feta el 2004): **VU** B1ac(iiii)+2ac(iii), segons criteris UICN 2001.

Llistada també a:

■ *Catàleg de Plantes Vasculars endèmiques, rares o amenaçades de Catalunya (I. Tàxons endèmics*; Sáez *et al.*, 1998): **VU** B1+2e; C1, segons criteris UICN 1994.

■ *Dades sobre l'estatus d'algunes plantes endèmiques, amenaçades o rares a Catalunya (NE de la península Ibèrica)* (Aymerich & Sáez, 2001): **VU** B2ac(iii); D2, segons criteris UICN 2001.

■ *Lista Roja de la Flora Vascular Española* (Aizpuru *et al.*, 2000): **VU** B1+2e; C1, segons criteris UICN 1994.

MESURES DE CONSERVACIÓ ACTUALS

■ De les 10 localitats conegudes de l'espècie, nou estan situades dins dels límits d'alguna zona protegida (BAS, CAV, PED i COR estan dins el Parc Natural del Cadí-Moixeró; ORR al PEIN "Capçaleres del Ter i del Freser"; EI1 i EI2 a la Reserva Natural de la Vall d'Eina, NOE a la Reserva Natural de Noedes, i PMA a la Reserva Natural de Mantet). La població de CAS no disposa de cap figura de protecció legal.

■ Existeix un pla de seguiment de l'activitat predadora d'isards sobre *D. montanum* al Parc Natural del Cadí (Aymerich, 2003).

MESURES DE CONSERVACIÓ PROPOSADES

***In-situ*:**

■ Seguiment de les diferents localitats amb presència de l'espècie, per tal d'observar: (i) la dinàmica demogràfica de les poblacions, i (ii) les possibles interaccions entre predadors i la planta.

■ Estudi de la conveniència i, si s'escau, col·locació de tanques a les localitats on pot produir-se predació per part d'ungulats.

■ Vigilància de les poblacions per agents forestals.

■ Limitació del trànsit de persones a les localitats més freqüentades.

■ Segons la fórmula de Hamrick *et al.* (1991), la conservació de només 3 poblacions garantiria la preservació d'un 99,7% de la diversitat genètica total detectada en *D. montanum*. La situació de 9 de 10 poblacions dins d'àrees protegides hauria d'assegurar la supervivència de l'espècie a llarg termini. L'única població sense instrument de protecció legal, CAS, és la que presenta més variabilitat genètica (H_e = 0,100), per la qual cosa creiem que és aconsellable l'adopció d'algun tipus de mesura conservacionista. La població de Noedes (NOE) pot representar una unitat evolutiva significativa (*ESU*) atesa la seva singularitat genètica (en el dendrograma de identitat genètica entre parells de poblacions, NOE se'ns separa clarament de la resta; López-Pujol *et al.*, en revisió), per tant cal donar la màxima prioritat a la seva conservació.

***Ex-situ*:**

■ Recol·lecció de granes per a conservar en bancs de germoplasma. Atès l'elevat percentatge d'al·lels rars per població (12,78% del total; López-Pujol *et al.*, dades inèdites), alguns dels quals es troben en freqüències extremadament petites (<0,01) recomanem el mostratge d'almenys

9.061 granes, com a mínim en les poblacions que contenen al·lels privats (COR, EI2, ORR) i aquelles evolutivament diferenciades (NOE), atenent a la fórmula del CPC (1991) i al menor dels percentatges de germinació trobat per Bosch (1999): $C_p = 299 \times (1/0.033) = 9.061$; vegeu també Taula 2.6.

■ Cultiu i micropropagació en jardins botànics de muntanya.

Altres:

■ Incloure aquest tàxon al *Catálogo Nacional de Especies Amenazadas*. Atès que a Catalunya manca un catàleg de flora amenaçada, s'aconsella la seva redacció i la inclusió d'aquest tàxon.

■ Coordinació entre les diferents administracions responsables de la conservació de l'espècie als dos costats dels Pirineus (estatals, autonòmiques i departamentals).

■ Realització de noves prospeccions en àrees properes tant a les localitats amb presència actual de l'espècie com a les localitats extingides.

FITXA REALITZADA AMB LA COL·LABORACIÓ DE: Josep Vigo, Pere Aymerich, Marcel Saule, Josep Vicens, Alain Mangeot, Maria Renée Orellana, Maria Bosch, Joan Simon, Cèsar Blanché.

BIBLIOGRAFIA: Blanché (1991); Bosch (1999); Simon *et al*. (1999); Aymerich & Sáez (2001); Bosch *et al.* (2001); Simon *et al.* (2001); Aymerich (2003).