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Conservation genetics of the rare Pyreneo-Cantabrian endemic *Aster pyrenaeus* (Asteraceae)

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Abstract

Background and aims *Aster pyrenaeus* (Asteraceae) is an endangered species, endemic to the Pyrenees and Cantabrian Mountain ranges (Spain). For its long-term persistence, this taxon needs an appropriate conservation strategy to be implemented. In this context, we studied the genetic structure over the entire geographical range of the species and then inferred the genetic relationships between populations.

Methodology Molecular diversity was analysed for 290 individuals from 12 populations in the Pyrenees and the Cantabrian Mountains using inter simple sequence repeats (ISSRs). Bayesian-based analysis was applied to examine population structure.

Principal results Analysis of genetic similarity and diversity, based on 87 polymorphic ISSR markers, suggests that despite being small and isolated, populations have an intermediate genetic diversity level (P % = 52.8 %, $H_E = 0.21 \pm 0.01$, genetic similarity between individuals = 49.6 %). Genetic variation was mainly found within populations (80–84 %), independently of mountain ranges, whereas 16–18 % was found between populations and <5 % between mountain ranges. Analyses of molecular variance indicated that population differentiation was highly significant. However, no significant correlation was found between the genetic and geographical distances among populations (Rs = 0.359, $P = 0.140$). Geographical structure based on assignment tests identified five different gene pools that were independent of any particular structure in the landscape.

Conclusions The results suggest that population isolation is probably relatively recent, and that the outbreeding behaviour of the species maintains a high within-population genetic diversity. We assume that some long-distance dispersal, even among topographically remote populations, may be determinant for the pattern of genetic variation found in populations. Based on these findings, strategies are proposed for genetic conservation and management of the species.

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Introduction

In alpine environments, the distribution of species is often fragmented due to pronounced mountainous topography and associated abiotic heterogeneity on small spatial scales (Kudo 1991; Molau 1993; Körner 2003). Alpine plant species usually form local populations of various sizes, exhibiting a marked ability for extended local persistence due to perenniality and/or clonality (Bliss 1971; Körner 2003). The characteristics of fragmented populations have profound consequences on the species genetic patterns, which are crucial to elucidate for adequate management of endangered populations and species. Genetic variation within plant species is determined by a number of different factors such as reproductive mode (sexual vs. clonal), breeding system (outcrossing vs. selfing), life-history traits, population history, geographical range or selective constraints (Loveless and Hamrick 1984). These factors are also mainly responsible for the way the total genetic variation of a species is partitioned between and within populations (Hamrick *et al.* 1992).

The spatial isolation that is often accompanied by a reduction in the levels of gene flow leads to isolation by distance and to a high genetic differentiation among populations. However, small-scale heterogeneity and spatially differentiated selective constraints can lead to high levels of diversity within populations (Gugerli *et al.* 1999; Till-Bottraud and Gaudeul 2002). For entomophilous plant species, small, isolated populations may provide too few mates and little attraction or reward for pollinators (Kunin 1997; Dauber *et al.* 2010), leading to a reduction in the quality and quantity of pollination services (Wilcock and Neiland 2002), particularly exacerbated when rare plants are surrounded by other flowering species (Duncan *et al.* 2004; Lazaro *et al.* 2009). This will reduce seed set and gene flow within and between populations. Such factors combine to erode genetic diversity within populations and enhance between-population differentiation (Rathcke and Jules 1993; Steffan-Dewenter and Tscharntke 1999). Moreover, species in small, isolated populations may lose genetic diversity through stochastic processes such as genetic drift and become less fit due to increased inbreeding (Ellstrand and Elam 1993; Byers and Waller 1999) and Allee effects, which can eventually lead to extinction (Groom 1998). Increasing population size and maximizing genetic diversity are among the primary goals of conservation management (Frankham *et al.* 2002; Van Dyke 2008).

The pattern of geographical variation in population genetic diversity and differentiation will be influenced by both historical and contemporary changes in population size and gene flow (Vucetich and Waite 2003). The effect of population history is especially significant for species that have survived the long glacial episodes of the Pleistocene because their current distribution and genetic pattern is the result of successive range shifts during glacial and interglacial cycles (Hewitt 2004).

Unlike plants from the Alps, very few studies have focused on the genetic diversity of plant populations in the Pyrenees (Segarra-Moragues and Catalán 2003, 2010; Segarra-Moragues *et al.* 2007; Lauga *et al.* 2009) and even less on those in the Cantabrian Mountains (Peredo *et al.* 2009); thus the present study provides new insights into genetic diversity patterns across the Pyrenees and the relationship between Pyrenean and Cantabrian Mountain ranges.

Aster pyrenaeus DC (Asteraceae) is a critically endangered perennial species, endemic to the French Pyrenees and Cantabrian Mountains (Cambecèdes and Largier 2003). The species was first identified and collected from an unknown Pyrenean population and planted in the Royal Gardens of the Kingdom of France around 1685. Native populations were extensively harvested by botanical collectors until the early 20th century (Cambecèdes and Largier 2003) and the species was thought to be nearly extinct in the early 1990s with only three known populations. However, because it prefers very steep mountain slopes, often with difficult access, its current distribution remained unknown. Today, 14 isolated populations (sometimes very small) are known in France and Spain (Cambecèdes and Largier 2009). The species has been protected since 1982 in France and 1990 in Spain. Recently, the main threat to the species has changed from collectors to the decrease in grazing animals, favouring the expansion of competitive species and habitat closure (Cambecèdes and Largier 2009). Thus, during the last decade, *A. pyrenaeus* has been a high priority for conservation efforts from both the French government (Directive Habitats 92/43/CEE) and the autonomous region of Asturias (Decreto 65/1995), increasing the urgency to document and understand the genetic structure of this endangered plant species. Previous field studies indicated that *A. pyrenaeus* is mainly an outcrossing species and produces wind-dispersed achenes with a pappus (Guzman *et al.* 2003; García 2004).

Given its biology, history and current distribution, we expect the populations of *A. pyrenaeus* to exhibit low levels of genetic diversity and high population differentiation and, consequently, smaller populations at greater risk of extinction. Indeed, many rare endemic species show low genetic diversity compared with widespread taxa [i.e. *Cycas guizhouensis* K.M. Lan & R.F. Zou (Xiao *et al.* 2004), *Chamaecrista semaphore* Moench (Da Silva *et al.* 2007)]. However, other rare species have been

shown to present rather high levels of genetic diversity [i.e. *Nouelia insignis* Franch. (Luan *et al.* 2006), *Physeria bellii* Mulligan (Kothera *et al.* 2007)]. Nevertheless, the current genetic pattern of *A. pyrenaeus* could mainly result from the response of the species to glacial/postglacial climatic changes. Basic knowledge on the past history and population dynamics of this species is indispensable to implement a preservation programme. The geographical structure of the genetic diversity still needs to be characterized in order to define appropriate sampling strategies for conservation purposes.

To characterize the genetic pattern of *A. pyrenaeus*, we used the inter simple sequence repeat (ISSR) technique, which has been widely applied in conservation genetics (Xiao *et al.* 2004; García-Gonzales *et al.* 2008; Crema *et al.* 2009; Su *et al.* 2009) and to resolve phylogeographical issues (Graves and Schrader 2008; Li *et al.* 2008). We first studied the within- and among-population genetic diversity in the Pyrenees and Cantabrian Mountains. We then inferred the genetic relationships between these populations with respect to their geographical locations. We used the results to establish recommendations for conservation, management and restoration of this endangered species. Finally, we propose a scenario describing the history of *A. pyrenaeus* populations during the last postglacial period.

Materials and methods

Studied species

Aster pyrenaeus ($2n = 18$) is a perennial herb 40–100 cm in height. It grows on calcareous rocky north- and east-facing slopes between 500 and 2400 m a.s.l. The flowering period extends from mid-July to mid-October (Guzman *et al.* 2003). It is a gynomonoecious species (the inflorescence has both hermaphroditic protandrous yellow disc florets and pistillate blue–lilac ray florets) mainly visited by *Cephus* sp. (Cephidae, Hymenoptera), *Neoascia podagrica* (Syrphidae, Diptera) and *Odontomya ornata* (Stratiomydae, Diptera) (Guzman *et al.* 2003). Autonomous self-pollination has occasionally been observed, suggesting that *A. pyrenaeus* cannot be considered fully self-incompatible (Guzman *et al.* 2003; García 2004). The species spreads vegetatively by short rhizomes and grows in clumps of numerous connected neighbouring shoots (Guzman *et al.* 2003).

Sampling procedure

We studied 10 populations in the French Pyrenees and two populations in the Cantabrian Mountain range in northern Spain (Fig. 1, Table 1). Population sizes are highly variable, ranging from 11 to ~2500 individuals (Table 1). Distances between populations varied from 1 km (PANL and PANH) to 400 km (CAU and DUJ; CAU and BUL). The number of individuals sampled in each population varied from 5 to 37, depending on population size and the difficulty of accessing the study site (Table 1). Sampling effort for populations TAC and CAU (i.e. five individuals sampled) was low due to the difficult access. Leaf material (one leaf per individual) was collected from a total of 290 individuals and stored in silica gel. DNA extraction was performed with the DNeasy Plant Mini Kit (Qiagen, Paris, France) according to the manufacturer's protocol, using 40 mg of dried leaf material. DNA concentration was determined by spectrophotometry with the NanoDrop™ ND-1000 (Thermo Scientific, Courtaboeuf, France).

ISSR procedure

Six of 49 ISSR primers produced clear and reproducible bands, and were hence selected for further study. Polymerase chain reaction (PCR) amplification was performed in a total volume of 25 μL, consisting of 20 ng of DNA template, 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 2 mM primer, 0.625 U *Go Taq* DNA (Promega, France) and purified water. Each PCR cycle consisted of the following steps: initial denaturation at 95 °C for 3 min, 38 cycles of 40 s denaturation at 95 °C, 40 s annealing at the primer's T_m (Table 2), 1 min extension at 72 °C and a final 5 min extension at 72 °C. For each primer, we determined the best annealing temperature by performing a gradient PCR. The PCR products were separated on 2 % agarose gels buffered with 1× TAE for 2 h 30 min at 100 V, detected by staining with ethidium bromide and photographed under ultraviolet light. Molecular weights were estimated using 50- and 100-bp DNA ladders (Promega). For all samples, PCR reactions were carried out using the same thermocycler. To assess the reproducibility of the ISSR patterns for each primer, PCR reactions were repeated twice for 50 samples. No band variation was detected when the two runs of a given DNA sample were compared. Positive controls were systematically included in each PCR run and in each electrophoresis gel to facilitate intergel comparisons, to check the efficiency of PCR, and to test for the reproducibility of ISSR patterns. Negative controls (without template DNA) were also included in every run to test for contamination in the reagents.

Data analysis

Inter simple sequence repeat bands were scored as present (1) or absent (0) and a binary matrix was manually constructed. Assuming that populations are in Hardy–Weinberg equilibrium ($F_{IS} = 0$), the software program AFLP-SURV v.1.0 (Vekemans *et al.* 2002) was used to estimate within-population genetic diversity

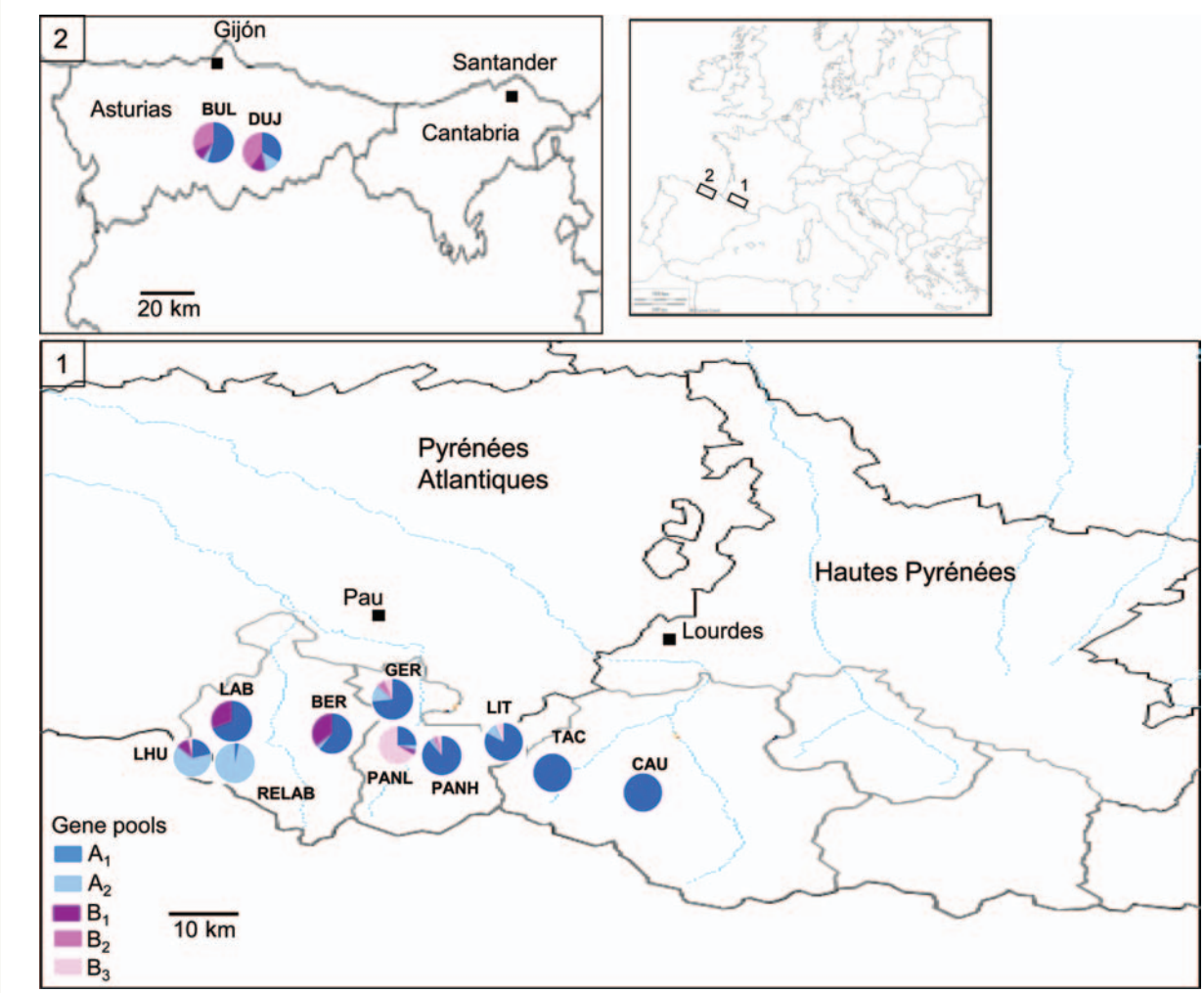

Fig. 1 Geographical structure of 12 populations of *A. pyrenaeus* in the Pyrenees (1) and Cantabrian Mountains (2) calculated using the STRUCTURE software v. 2.3.3. The pie charts give the proportions of the gene pools present in the population.

through the following parameters: percentage of polymorphic loci (*P* %), Nei's (1978) unbiased expected heterozygosity (*He*). Similarity of ISSR profiles between individuals was calculated using Nei and Li's (1979) similarity index ($S = 2n_{xy}/n_x + n_y$), where n_x and n_y refer to the number of ISSR bands in individuals *x* and *y*, respectively, and n_{xy} is the number of bands shared by both individuals *x* and *y*. Only polymorphic bands were considered in the index calculation. We checked the whole dataset for private fragments in populations.

To analyse the genetic differentiation and geographical structure of *A. pyrenaeus* populations, variation in ISSR patterning was examined with analysis of molecular variances (AMOVA) using ARLEQUIN v. 3.01 (Excoffier *et al.* 2005). Analysis of molecular variances was performed at different hierarchical levels: (i) between all the 12 populations included in a global analysis, (ii) between mountain ranges (Pyrenees vs. Cantabrians), and (iii) between populations in a given mountain range (either Pyrenees or Cantabrians). *F*-statistics were computed under the random mating hypothesis with ARLEQUIN v. 3.01 (Excoffier *et al.* 2005). This provided the unbiased F_{ST} estimator θ, following Weir and Cockerham (1984) for which 95 % confidence intervals were obtained by bootstrapping 1000 replicates over loci. Fisher's exact tests were performed, using Genepop v. 4 (Raymond and Rousset 1995; Rousset 2008), on marker frequencies at each locus between all pairs of populations to determine whether significant differences in marker frequencies existed between groups of individuals. To determine the genetic relationships among populations, the AFLP-SURV v. 1.0 (Vekemans *et al.* 2002) and the PHYLIP packages

Table 1 Name, location, altitude and size of the 12 studied *A. pyrenaeus* populations. The PAN site consists of two populations separated by ~1 km (PANL and PANH).

Population location	Population code	Elevation (m)	Longitude/latitude	Sample size	Population size
French Pyrenees					
Cauterets	CAU	1150	2°27′W/42°53′N	5	11
Cirque du Litor—Béost	LIT	1400	2°38′W/42°57′N	29	33
Vallon de Tachet—Arrens valley	TAC	1500	2°35′W/42°55′ N	5	20
Gerbe—Ossau valley	GER	1350	2°47′W/43°00′N	30	1000
Montagne de Pan (low part)—Ossau valley	PANL	600	2°46′W/42°58′N	26	50
Montagne de Pan (high part)—Ossau valley	PANH	900	2°46′W/42°58′N	28	1000
Pic de Bergon—Aspe valley	BER	1340	2°52′W/42°58′N	29	500
Laberouat—Aspe valley	LAB	1615	3°00′W/42°57′N	27	100
Refuge de Laberouat—Aspe valley	RELAB	1450	2°59′W/42°57′N	31	100
Piquet de Lhurs—Aspe valley	LHU	1480	3°01′W/42°55′N	28	50
Cantabrian Mounts (Spain)					
Bulne	BUL	800	4°50′W/43°14′N	22	2500
Val del Duje	DUJ	450	4°48′W/43°15′N	30	450

Table 2 Primers used in ISSR analyses of *A. pyrenaeus*, annealing temperature (T_m in °C) and number of reliable and polymorphic bands for each primer. B = (C, G or T), D = (A, G or T), R = (A or G), W = (A or T) and Y = (C or T).

ISSR sequence 5′ to 3′	T_m	No. of bands analysed	% of polymorphic bands
$BDB(ACA)_5$	50.6	20	90
$WB(GACA)_4$	46.0	10	70
$(GT)_8C$	46.0	9	78
$(AC)_8YG$	54.8	18	100
$(AG)_8C$	56.5	17	82
$(TG)_8RC$	46.0	28	96

(NEIGHBOR and CONSENSE; Felsenstein 1989) were used to calculate pairwise Nei's genetic distance (Nei 1978) between each population, and to construct a neighbour-joining (NJ) tree based on 10 000 permutated trees, bootstrapped across loci. Isolation by distance was tested by Mantel tests (10 000 permutations) performed between pairwise estimates of $F_{ST}(1-F_{ST})$ ratio and the logarithm of geographical distance (natural logarithm scale) for all samples (Rousset 1997) using ARLEQUIN v. 3.01 (Excoffier *et al.* 2005). We next applied a model-based clustering method to infer genetic structure and define the most adequate number of clusters in the whole dataset using the software STRUCTURE v. 2.3.3 (Pritchard *et al.* 2000). We set the number of clusters (K) from 1 to 14 and ran 20 independent runs for each K value. Each run consisted of a burn-in period of 10^5 steps followed by 10^6 Markov chain Monte Carlo repetitions, assuming an admixture model, a uniform prior for alpha and correlated allele frequencies with prior population information. We used the *ad hoc* statistic ΔK to identify the most likely number of clusters in the dataset (Evanno *et al.* 2005). Because independent runs can produce different permutations of the group labels, we used CLUMPP v. 1.1.1 (Jakobsson and Rosenberg 2007) to align the membership coefficient matrices from the 20 highest likelihood runs for each K_{max} (Full Search algorithm with random input order and 10^5 permutations to align the runs). The CLUMPP output consists of the same permuted matrices so that all replicates are as closely matched as possible. In order to detect substructure, we again applied the same Bayesian-based analysis within each predefined cluster. We then assigned each individual to a gene pool if the membership probability was >0.6 (Coulon *et al.* 2008).

Results

Within-population diversity

The six primer pairs used in the study generated a total of 102 reliable ISSR bands, of which 87 were polymorphic

Table 3 Genetic variability within the 12 *A. pyrenaeus* populations studied.

Population	*P* %	$H_E \pm$ SE	Genetic similarity (%)
CAU	33.3	0.13 ± 0.01	49.0
LIT	68.9	0.20 ± 0.02	50.3
TAC	33.3	0.13 ± 0.01	54.6
GER	67.6	0.23 ± 0.02	55.7
PANL	73.5	0.22 ± 0.02	50.3
PANH	70.1	0.21 ± 0.02	52.2
BER	67.8	0.20 ± 0.01	45.1
LAB	73.5	0.22 ± 0.02	44.0
RELAB	67.8	0.22 ± 0.02	50.1
LHU	75.8	0.23 ± 0.02	50.1
BUL	71.2	0.21 ± 0.01	50.4
DUJ	66.7	0.21 ± 0.02	45.5
Mean	63.2	0.21 ± 0.02	49.7
Species level	98.8	0.27 ± 0.01	
Mountain range level			
Pyrenees	97.7	0.27 ± 0.01	
Cantabrians	73.5	0.22 ± 0.01	

(85.29 %; Table 2). Within populations the mean percentage of polymorphic loci (*P* %) reached 63.2 %, ranging from 33.3 % (CAU and TAC) to 75.8 % (LHU; Table 3). The expected heterozygosity (H_E) reached on average 0.21 ± 0.02, and was between 0.13 ± 0.01 (CAU and TAC) and 0.23 ± 0.02 (GER and LHU). At the species and the Pyrenees mountain range levels, *P* % and H_E were very similar (Table 3) but were lower in the Cantabrians. The mean genetic similarity reached 49.7 % within populations. The LAB population had the lowest genetic similarity (44.0 %) while the GER population had the highest (55.7 %). No private fragments were found in any population. In each population, *P* % and H_E were significantly and positively correlated ($R^2 = 0.939$, $P < 0.001$). Furthermore, we detected no influence of population size on molecular diversity (*P* %: $R^2 = 0.072$, $P = 0.400$; H_E: $R^2 = 0.084$, $P = 0.360$).

Genetic differentiation and geographical structure

Whatever the hierarchical level considered, genetic variation was always much higher within populations (80.68–84.02 %, AMOVA; Table 4) than among populations (15.98–18.01 %). Φ_{ST} values were substantially similar (0.16–0.18 %), indicating a moderate among-population differentiation. Pairwise Φ_{ST} ranged from 0.075 to 0.351; all values differed from zero (Table 5), which was confirmed by global Fisher's exact tests ($\chi^2 = 1227.8$, d.f. = 174, $P < 0.001$). In contrast, genetic variation among mountain ranges was very low (~5 %) but still highly significant ($P < 0.001$). The result of the Mantel test indicated a limited isolation by distance, because the pairwise genetic distances measured as $F_{ST}/(1-F_{ST})$ and the logarithm of the distance between pairs of populations were not significant (Rs = 0.359, $P = 0.140$). Neighbour-joining analysis based on Nei's genetic distances failed to support geographical clustering. Only three groups were significantly identified, with moderate bootstrap support (>50 %; Fig. 2), i.e. Cantabrian populations, PANH and GER, and RELAB and LHU from Ossau and Aspe Valley, respectively. Geographical structure based on STRUCTURE revealed that two genetic clusters ($K = 2$) had the best *ad hoc* statistical fit (Fig. 3). A substructure within each previous cluster was found with $\Delta K = 2$ and 3 for the initial cluster A and B, respectively (Fig. 1). As a result, five different gene pools (A_1, A_2, B_1, B_2 and B_3) were identified. Eight individuals out of 290 were not assigned to a genetic group with a membership probability >0.6. For the smallest populations (CAU and TAC), the average membership coefficients (q_{mean}) were very high (0.99 and 0.98, respectively), indicating nearly perfect assignment of individuals. Only gene pool A_1 was detected in these small populations (Fig. 1). For larger populations, the pattern was not well structured since we detected two, three and four gene pools whatever the geographical distribution of the populations. In LHU and RELAB populations, A_2 was the dominant gene pool ($q_{mean} = 0.99$), and A_1 was the prevalent one in LIT, GER and PANH ($q_{mean} = 0.98$, 0.89 and 0.96, respectively). For LAB and BER populations, A_1 ($q_{mean} = 0.99$ and 0.95, respectively) and B_1 ($q_{mean} = 0.97$ and 0.90, respectively) were the dominant gene pools, while for PANL, population B_3 was the main gene pool ($q_{mean} = 0.95$). Interestingly, Cantabrian populations presented four gene pools in nearly equal proportions (Fig. 1).

Discussion

Within-population diversity

Endemic and narrowly distributed plants usually show lower levels of genetic diversity and higher levels of genetic structure compared with their relatives with wider distribution areas (Hamrick and Godt 1989; Nybom 2004). This is probably caused by the more accentuated effects of genetic drift and restricted gene flow in the rarer plants (Hamrick and Godt

Table 4 **Results of AMOVA based on 87 ISSR loci in two mountain ranges, Pyrenees and Cantabrians, at different hierarchical levels.**

Source of variation	d.f.	SS	Variance components	% of the total variance	Φ values	*P*
Global analysis						
Among populations	11	443.529	1.620	18.01	$\Phi_{ST} = 0.180$	<0.001
Within populations	278	2067.018	9.220	81.99		
Total	289	2510.547	10.850	100		
Pyrenees						
Among populations	9	360.209	1.504	15.98	$\Phi_{ST} = 0.159$	<0.001
Within populations	226	1859.866	9.253	84.02		
Total	235	2220.076	10.757	100		
Cantabrian Mountains						
Among populations	1	18.128	1.727	17.09	$\Phi_{ST} = 0.171$	<0.001
Within populations	53	207.152	9.006	82.91		
Total	54	225.280	10.734	100		
Pyrenees vs. Cantabrian Mountains						
Among mountain range	1	65.191	0.559	4.96	$\Phi_{CT} = 0.049$	<0.013
Within population among mountain range	10	378.338	1.510	14.37	$\Phi_{SC} = 0.140$	<0.001
Within populations	278	2067.018	9.227	80.67	$\Phi_{ST} = 0.183$	<0.001
Total	289	2510.547	11.298	100		

d.f., degree of freedom; SS, sum of squares.

Table 5 **Pairwise estimated Φ_{ST} values among 12 populations of *A. pyrenaeus*.** All values differed significantly from zero.

	CAU	LIT	TAC	GER	PANL	PANH	BER	LAB	RELAB	LHU	BUL	DUJ
CAU	—											
LIT	0.207	—										
TAC	0.351	0.213	—									
GER	0.170	0.095	0.210	—								
PANL	0.207	0.160	0.240	0.125	—							
PANH	0.175	0.080	0.135	0.147	0.136	—						
BER	0.200	0.129	0.208	0.140	0.124	0.103	—					
LAB	0.130	0.147	0.176	0.108	0.134	0.114	0.075	—				
RELAB	0.228	0.154	0.190	0.191	0.181	0.150	0.153	0.164	—			
LHU	0.203	0.126	0.160	0.149	0.139	0.130	0.145	0.118	0.089	—		
BUL	0.286	0.194	0.286	0.230	0.180	0.150	0.185	0.156	0.219	0.191	—	
DUJ	0.268	0.129	0.256	0.276	0.129	0.148	0.192	0.134	0.197	0.149	0.161	—

1989; Nybom 2004). For the endemic *A. pyrenaeus*, we revealed that whatever the mountain range or the population within a mountain range, most of the genetic diversity was found within populations. The same trend was commonly reported in outcrossing and/or perennial species (Hamrick *et al.* 1992). *Aster*

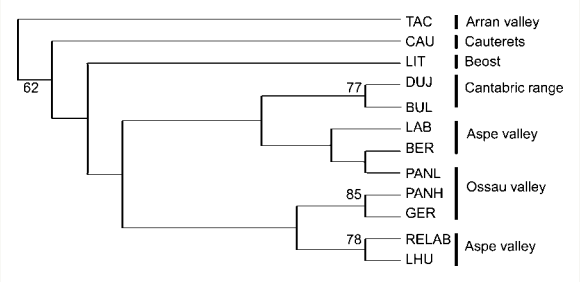

Fig. 2 Neighbour-joining phenogram based on Nei's unbiased genetic distance for the 12 studied populations. The corresponding valleys are indicated for the Pyrenean populations. Bootstrap values (>50) over loci (based on 1000 replicates) are indicated for each node.

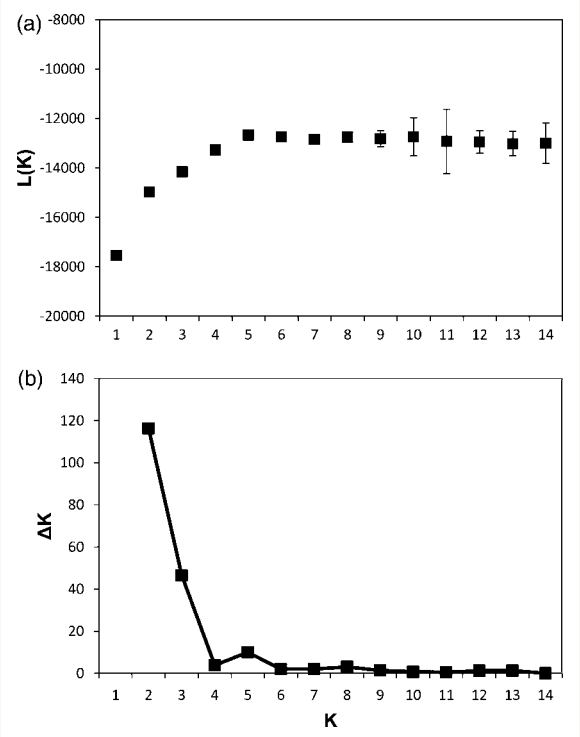

Fig. 3 The estimated mean logarithmic likelihood of *K* values (a) and Δ*K* values (b) ranging from 1 to 14 with 20 runs for each *K*.

pyrenaeus exhibits an intermediate level of mean intra-population genetic diversity (0.21 for Nei's expected heterozygozity H_E), which conforms to the value ($H_E = 0.20$) found by Nybom (2004) in a literature survey for endemic species using dominant markers. Comparisons with other studies are difficult since genetic diversity depends on numerous factors, such as life history, breeding system, growth life forms, geographical range and even the type of molecular method used (Powell *et al.* 1996; Nybom 2004). In spite of these complications, if we compare the results of studies using dominant markers it appears that the genetic diversity of *A. pyrenaeus* is similar to that of other alpine species: *Eryngium alpinum* L. ($H_E = 0.20$; Gaudeul *et al.* 2000), *Trollius europaeus* L. ($H_E = 0.22$ in the Alps and 0.197 in the Pyrenees; Despres *et al.* 2002), *Epilobium fleischeri* Hochst., *Geum reptans* L. and *Campanula thyrsoides* L. ($H_E =$ 0.19, 0.21 and 0.20, respectively; Kuss *et al.* 2008) and *Senecio boissieri* DC ($H_E = 0.19$ in the Cantabrian Mountains; Peredo *et al.* 2009). The values of H_E and *P* % suggest that even small populations can maintain a high level of genetic diversity. Thus, we suggest that no recent severe bottlenecks occurred or that genetic diversity may not respond immediately to reduction in population size (Young *et al.* 1996). However, these small populations could also result from recently dispersed individuals from different population sources. We did not find a significant correlation between population size and genetic diversity despite the theoretical prediction that small populations might lose genetic variation due to genetic drift, founder effects or population bottlenecks (Ellstrand and Elam 1993; Young *et al.* 1996). Therefore, we have no indication that natural fragmentation resulted in a pronounced loss of genetic diversity within *A. pyrenaeus* populations. Other studies on alpine plants showed an inconsistent pattern for genetic diversity and population size relationships. Some have detected a significant correlation (*E. alpinum*, Gaudeul *et al.* 2000; *T. europaeus*, Despres *et al.* 2002; *E. fleischeri*, Kuss *et al.* 2008) while others found no correlation (*Hypericum nummularium* L., Gaudeul 2006; *G. reptans*, Kuss *et al.* 2008; *C. thyrsoides*, Kuss *et al.* 2008; Ægisdóttir *et al.* 2009), depending on the sampling parameters used (i.e. sample size, breeding system, marker system; Nybom 2004). The within-population diversity is also likely to be influenced by some life-history traits of the species such as the type of breeding system. Outcrossing plant species tend to have higher genetic variation within populations, whereas populations of selfing species or species with a mixed mating system are often genetically less variable (Hamrick and Godt 1996; Till-Bottraud and Gaudeul 2002; Nybom 2004). In our study, genetic variability was mostly observed within population (80–84 %). On this basis, *A. pyrenaeus* can be considered as an outcrosser as previously found by García (2004), which contributes to maintaining within-population genetic variability.

Genetic and geographical structure

The genetic structure of plant populations reflects the interactions of various evolutionary processes including the long-term evolutionary history, such as shifts in distribution, habitat fragmentation, and population isolation, mutation, genetic drift, breeding system, gene flow and selection (Schaal *et al.* 1998). Factors such as isolation, small populations and gene flow may have a major influence on the levels of genetic diversity within and among populations (Hamrick *et al.* 1992). Continuous distribution of plants usually weakens the differentiation among populations (Wright 1951).

Our analyses of genetic structure revealed a moderate differentiation among populations ($\Phi_{ST} = 0.18$). The pattern of population differentiation is confirmed by Fisher's exact test, but the Mantel test revealed no correlation between geographical and genetic distances. In the two smallest populations, sampling effort was low; however, it represented 45.5 and 25 % of the entire population for CAU and TAC, respectively. Thus, it is possible that not all the genetic variability was sampled, but as the level of genetic diversity within these populations is similar to that of larger populations it could be assumed that the sampled individuals may reflect the variability of the population.

In a meta-analysis of RADP-based estimates of Φ_{ST} values, Nybom and Bartish (2000) and Nybom (2004) demonstrated that Φ_{ST} values for long-lived perennial, endemic and outcrossed species with wind-dispersed seeds have the lowest Φ_{ST} ($\sim$0.25). The values for Φ_{ST} found in *A. pyrenaeus* populations, although slightly lower, were similar to these. Moreover, compared with other alpine perennials, pairwise Φ_{ST} values among populations ranging from 0.07 to 0.35 (Table 5) are widely reported (Gugerli *et al.* 1999; Young *et al.* 2002; Pluess and Stöcklin 2004). This intermediate level of population differentiation, coupled with the fact that most of the genetic variance occurs within populations (>80 %), suggests that population isolation occurred recently (Gugerli *et al.* 1999; Segarra-Moragues and Catalán 2003; Pluess and Stöcklin 2004). This pattern of population differentiation is commonly described in other alpine plants and endemic species (Gugerli *et al.* 1999; Segarra-Moragues and Catalán 2003; Wesche *et al.* 2006). Genetic variation among all the populations or among the populations within a given mountain range was nevertheless highly significant ($P < 0.001$), which led us to conclude the occurrence of an impact of isolation. The Mantel test failed to reveal isolation by distance; therefore, geographical distance is not responsible for the reduction in gene flow between the different geographical locations. Although insect visitors to *A. pyrenaeus* flowers have been identified (Guzman *et al.* 2003), their effectiveness in pollination is unknown and there are no available data concerning pollen dispersal. However, given the remoteness of the populations, their isolation in valleys and the mountain barriers to pollinator displacements, pollen flow between populations appears unlikely. But given the inaccessibility of the *A. pyrenaeus* populations, currently sampled populations could be linked through isolated populations or individuals throughout the unexplored areas. The identification of new populations would tend to support this hypothesis. Between-population gene flow through seed dispersal is difficult to evaluate since no data on seed dispersal are available. Dispersal of achenes in the Asteraceae may be species dependent, and even in a single genus there may be variation in pappus length, weight and their associated dispersal capability (Andersen 1993). Thus, comparison with other species is problematic and more studies on seed dispersal in this species are needed. Yet, regarding the results obtained here, it may be considered that predominant wind flow in mountain areas could provide further opportunities for long-distance dispersal.

The Bayesian-based analysis performed by STRUCTURE allowed the detection of five different gene pools which did not reflect a particular structure in the landscape. Individuals were clustered in locations with variable estimated membership coefficients (0.60–0.99). The five genetic clusters included individuals belonging to different locations. Only small populations (CAU and TAC) were assigned to a single genetic group (A_1). This pattern is supported by the pattern of the NJ tree (Fig. 2), which reveals little congruence to the geographical distance between populations.

Other studies conducted in the Pyrenees on threatened endemic species showed the same genetic structure (*Borderea pyrenaica* Miègev. and *B. chouardii Gaussen* Heslot, Segarra-Moragues and Catalán 2003; *Delphinium montanum* DC, Simon *et al.* 2001). Past demographic events, and current gene flow, are likely to be responsible for this present-day structure of genetic variation.

A number of palaeoendemic taxa from the Pyrenees, like *A. pyrenaeus*, are the likely descendants of Tertiary ancestors (Gaussen and Lerede 1948). Ice sheets rarely reached altitudes <1000 m in the Pyrenees and Cantabrians (García-Ruiz and Marti-Bono 1994); thus, as there is no apparent isolation by distance, it is likely that the species became established in large populations at lower altitudes during Pleistocene glaciations. The large and perhaps continuous distribution of ancestral populations over lowland areas could have been favoured by both the calcareous habit of *A. pyrenaeus* and the

almost continuous presence of limestone in the Pyrenean piedmonts and Cantabrian Mountains with no significant geological barriers preventing gene flow through the population. Then the species successfully recolonized the open territories at higher altitudes with the retreat of the glaciers.

Implications for conservation

Within-population genetic diversity is usually needed to ensure population establishment and long-term persistence as well as long-term evolutionary potential of restored populations (McKay *et al.* 2005). Even small populations of *A. pyrenaeus* appear to maintain high genetic diversity. Thus, from a genetic point of view, they do not seem endangered. However, primarily allogamous species like this often experience chronic pollen limitation due to the scarcity of both pollinators and mates and heterospecific pollen deposition (Eckert *et al.* 2010), which, in turn, reduces seedling recruitment within populations. Moreover, the populations are geographically isolated with limited gene flow between locations. Thus, for such small allogamous populations, management decisions need to be taken to prevent population extinction. Besides close monitoring of the size and the changes in the genetic structure of all populations, two main management proposals for long-term conservation of *A. pyrenaeus* populations can be suggested.

The first should concentrate on small populations, which may suffer from pollen limitation due to scarcity of both pollinators and mates (further investigations are needed to confirm this hypothesis). In order to increase reproductive success of individuals in small populations and promote natural recruitment, it is possible to saturate stigmas with cross pollen from the same population. We also suggest that seeds be collected from each individual, to maintain growing seedlings offsite and use the *ex situ* plants materials for *in situ* reintroduction. This requires a good understanding of the regeneration niche of the species by a detailed study of sites where individuals are currently present (soil, exposure, etc.).

Closely related self-incompatible species may suffer biparental inbreeding depression. Although, in the studied *A. pyrenaeus* populations, individuals seem to be distantly related (mean genetic similarity = 49.6 %), we have no information on the genetic and spatial structures within populations (spatial autocorrelation). Therefore, pollen incompatibility between mates may occur between the more closely related individuals. The second management proposal could be the reinforcement of populations by seeds collected from surrounding populations with the same gene pool to avoid the possible negative effects of outbreeding depression. These include collecting plants or seeds locally, or from genetically close populations, matching climatic and environmental conditions between collection and restoration sites (McKay *et al.* 2005).

Aster pyrenaeus is located in areas where pastoral, forestry and hunting practices have long influenced the dynamics of the ecosystems. In the past, open areas were maintained within the range of *A. pyrenaeus* by livestock, agricultural burning and mowing practices. The abandonment of pastoralism, accentuated in recent years, leads to habitat closure due to forest propagation (and favours the expansion of the bracken, i.e. the rhizomatous fern *Pteridium aquilinum*, which could locally outcompete *A. pyrenaeus*) (Wencewiez 2002). A national action plan, drafted with scientists and managers, will lead to a better understanding of the threats, in particular, competition and the vegetation dynamics, and should lead to the proposal of management actions favourable to the species.

Conclusions and forward look

A high within-population genetic diversity is reported for the rare endemic *A. pyrenaeus* throughout its distribution range in the Pyrenees and Cantabrians, which can be explained by the outbreeding behaviour of the species. Despite the fact that it lives in isolated populations of different sizes, neither isolation of habitats nor population size affected genetic variability within the studied populations. Population differentiation was moderate, suggesting a restricted gene flow between populations and indicating that population isolation is probably relatively recent. Geographical distance was not found to be responsible for the reduction in gene flow between the different locations; in this context, we assume that some long-distance dispersal mechanism, even among topographically remote populations, may be the crucial determinant for the pattern of genetic variation found. Further research should focus on the pollen and seed dispersal strategies in the *A. pyrenaeus* populations, and studies based on other markers from cp DNA and/or nr DNA might help to elucidate the comprehensive phylogeography of the species.

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Contributions by the authors

All the authors contributed to a similar extent overall.

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Conflicts of interest statement

None declared.

References

Ægisdóttir HH, **Kuss P**, **Stöcklin J**. **2009**. Isolated populations of a rare alpine plant show high genetic diversity and considerable population differentiation. *Annals of Botany* **104**: 1313–1322.

Andersen MC. **1993**. Morphology and seed dispersal in several wind-dispersed Asteraceae. *American Journal of Botany* **80**: 487–492.

Bliss LC. **1971**. Arctic and alpine plant life cycles. *Annual Review of Ecology and Systematics* **2**: 405–438.

Byers DL, **Waller DM**. **1999**. Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. *Annual Review of Systematics and Evolution* **30**: 479–513.

Cambecèdes J, **Largier G**. **2003**. Projet de plan de restauration de l'Aster des Pyrénées (*Aster pyrenaeus* DC). *Acta Botanica Barcelonensis* **49**: 109–117.

Cambecèdes J, **Largier G**. **2009**. De l'analyse des causes de regression au projet de renforcement d'une population d'Aster des Pyrénées: l'apport des connaissances scientifiques. In: Llamas F, Acedo C. (coords.) *Botanic Pirenaico-Cantabrica en el siglo XXI*. Universidad de Leon, 345–359.

Coulon A, **Fitzpatrick JW**, **Bowman R**, **Stith BM**, **Makarewich CA**, **Stenzler LM**, **Lovette IJ**. **2008**. Congruent population structure inferred from dispersal behaviour and intensive genetic surveys of the threatened Florida scrub-jay (*Aphelocoma coerulescens*). *Molecular Ecology* **17**: 1685–1701.

Crema S, **Cristofolini G**, **Rossi M**, **Conte L**. **2009**. High genetic diversity detected in the endemic *Primula apennina* Widmer (Primulaceae) using ISSR fingerprinting. *Plant Systematics and Evolution* **280**: 29–36.

Da Silva RM, **Fernandes GW**, **Lovato MB**. **2007**. Genetic variation in two *Chamaecrista* species (Leguminosae) one endangered and narrowly distributed and another widespread in the Serra do Espinhaço, Brazil. *Canadian Journal of Botany* **85**: 629–635.

Dauber J, **Biesmeijer JC**, **Gabriel D**, **Kunin WE**, **Lamborn E**, **Meyer B**, **Nielsen A**, **Potts SG**, **Roberts SPM**, **Sõber V**, **Settele J**, **Steffan-Dewenter I**, **Stout JC**, **Teder T**, **Tscheulin T**, **Vivarelli D**, **Petanidou T**. **2010**. Effects of patch size and density on flower visitation and seed set of wild plants: a pan-European approach. *Journal of Ecology* **98**: 188–196.

Despres L, **Loriot S**, **Gaudeul M**. **2002**. Geographic pattern of genetic variation in the European globeflower *Trollius europaeus* L. (Ranunculaceae) inferred from amplified fragment length polymorphism markers. *Molecular Ecology* **11**: 2337–2347.

Duncan DH, **Nicotra AB**, **Wood JT**, **Cunningham SA**. **2004**. Plant isolation reduces outcross pollen receipt in a partially self-compatible herb. *Journal of Ecology* **92**: 977–985.

Eckert CG, **Kalisz S**, **Geber MA**, **Sargent R**, **Elle E**, **Cheptou P-O**, **Goodwillie C**, **Johnston MO**, **Kelly JK**, **Moeller DA**, **Porcher E**, **Ree RH**, **Vallejo-Marín M**, **Winn AA**. **2010**. Plant mating systems in a changing world. *Trends in Ecology and Evolution* **25**: 35–43.

Ellstrand NC, **Elam DR**. **1993**. Population genetic consequences of small population size: implications for plant conservation. *Annual Review of Ecology and Systematics* **24**: 217–242.

Evanno G, **Regnaut S**, **Goudet J**. **2005**. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14**: 2611–2620.

Excoffier L, **Laval G**, **Schneider S**. **2005**. Arlequin ver.3.1: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics* **1**: 47–50.

Felsenstein J. **1989**. PHYLIP—Phylogeny Inference Package (Version 3.2). *Cladistics* **5**: 164–166.

Frankham R, **Ballou JD**, **Briscoe DA**. **2002**. *Introduction to conservation genetics*. Cambridge: Cambridge University Press.

García J. **2004**. Contribution à l'étude de la biologie de la reproduction de l'Aster des Pyrénées (*Aster pyrenaeus* DC): pollinisations expérimentales au sein d'une population cultivée au Conservatoire botanique pyrénéen. *Rapport final du Conservatoire botanique pyrénéen*: 30–31.

García-Gonzales R, **Carrasco B**, **Peñailillo P**, **Letelier L**, **Herrera R**, **Lavandero B**, **Moya M**, **Caligari PDS**. **2008**. Genetic variability and structure of *Gomortega keule* (Molina) Baillon (Gomortegaceae) relict populations: geographical and genetic fragmentation and its implications for conservation. *Botany* **86**: 1299–1310.

García-Ruiz JM, **Marti-Bono C**. **1994**. Rasgos fundamentals del glaciarismo Cuaternario en el Pirineo Aragones. In: Marti-Bono C, García-Ruiz JM, eds. *El glaciarismo surpirenaico: Nuevas aportaciones*. Logroño: Geoforma Ediciones, 17–31.

Gaudeul M. 2006. Disjunct distribution of *Hypericum nummularium* L. (Hypericaceae): molecular data suggest bidirectional colonization from a single refugium rather than survival in distinct refugia. *Biological Journal of the Linnean Society* **87**: 437–447.

Gaudeul M, **Taberlet P**, **Till-Bottraud I**. **2000**. Genetic diversity in an endangered alpine plant, *Eryngium alpinum* L. (Apiaceae), inferred from amplified fragment length polymorphism markers. *Molecular Ecology* **9**: 1625–1637.

Gaussen H, **Lerede C**. **1948**. Les endémiques Pyrénéo-Cantabriques dans la région centrale des Pyrénées. *Bulletin de la Société Botanique de France* **96**: 55–83.

Graves WR, **Schrader JA**. **2008**. At the interface of phylogenetics and population genetics, the phylogeography of *Dirca occidentalis* (Thymelaeaceae). *American Journal of Botany* **95**: 1454–1465.

Groom MJ. **1998**. Allee effects limit population viability of an annual plant. *American Naturalist* **151**: 487–496.

Gugerli F, **Eichenberger K**, **Schneller JJ**. **1999**. Promiscuity in populations of the cushion plant *Saxifraga oppositifolia* in the Swiss

Alps as inferred from random amplified polymorphic DNA (RAPD). *Molecular Ecology* **8**: 453–461.

Guzman D, **Largier G**, **Villar L**, **Valadon A**. **2003**. Caractérisation écologique et étude préliminaire d'une population d'Aster des Pyrénées (*Aster pyrenaeus* DC) en vallée d'Ossau (Pyrénée, France). *Acta Botanica Barcinonensia* **49**: 95–107.

Hamrick JL, **Godt MJW**. **1989**. Allozyme diversity in plant species. In: Brown AHD, Clegg MT, Kahler AL, Weir BS, eds. *Plant population genetics, breeding and genetic resources*. Sunderland, MA: Sinauer Associates, 43–63.

Hamrick JL, **Godt MJW**. **1996**. Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences* **351**: 1291–1298.

Hamrick JL, **Godt MJW**, **Sherman-Broyles S**. **1992**. Factor influencing levels of genetic diversity in woody plant species. *New Forest* **6**: 95–124.

Hewitt GM. **2004**. Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences* **359**: 183–195.

Jakobsson M, **Rosenberg NA**. **2007**. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* **23**: 1801–1806.

Körner C. **2003**. *Alpine plant life: functional plant ecology of high mountain ecosystems*, 2nd edn. Berlin: Springer.

Kothera L, **Richards CM**, **Carney SE**. **2007**. Genetic diversity and structure in the rare Colorado endemic plant *Physaria bellii* Mulligan (Brassicaceae). *Conservation Biology* **8**: 1043–1050.

Kudo G. **1991**. Effects of snow-free period on the phenology of alpine plants inhabiting snow patches. *Arctic and Alpine Research* **23**: 436–443.

Kunin WE. **1997**. Population size and density effects in pollination: pollinator foraging and plant reproductive success in experimental arrays of *Brassica kaber*. *Journal of Ecology* **85**: 225–234.

Kuss P, **Pluess AR**, **Ægisdottir HH**, **Stöcklin J**. **2008**. Spatial isolation and genetic differentiation in naturally fragmented plant populations of the Swiss Alps. *Journal of Plant Ecology* **1**: 149–159.

Lauga B, **Malaval S**, **Largier G**, **Regnault-Roger C**. **2009**. Two lineages of *Trifolium alpinum* (Fabaceae) in the Pyrenees: evidence from random amplified polymorphic DNA (RAPD) markers. *Acta Botanica Gallica* **156**: 317–330.

Lazaro A, **Lundgren R**, **Totland O**. **2009**. Co-flowering neighbors influence the diversity and identity of pollinator groups visiting plant species. *Oikos* **118**: 691–702.

Li E-X, **Yi S**, **Qiu Y-X**, **Guo J-T**, **Comes HP**, **Fu C-X**. **2008**. Phylogeography of two East Asian species in *Croomia* (Stemonaceae) inferred from chloroplast DNA and ISSR fingerprinting variation. *Molecular Phylogenetics and Evolution* **49**: 702–714.

Loveless MD, **Hamrick JL**. **1984**. Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics* **15**: 65–95.

Luan SS, **Chiang TY**, **Gong X**. **2006**. High genetic diversity vs. low genetic differentiation in *Nouelia insignis* (Asteraceae), a narrowly distributed and endemic species in China, revealed by ISSR fingerprinting. *Annals of Botany* **98**: 583–589.

McKay JK, **Christian CE**, **Harrison S**, **Rice KJ**. **2005**. 'How local is local?' — A review of practical and conceptual issues in the genetics of restoration. *Restoration Ecology* **13**: 432–440.

Molau U. **1993**. Relationships between flowering phenology and life history strategies in tundra plants. *Arctic and Alpine Research* **25**: 391–402.

Nei M. **1978**. Estimation of average heterozygosity and genetic distance for a small number of individuals. *Genetics* **89**: 583–590.

Nei M, **Li WH**. **1979**. Mathematical model for studying genetical variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the USA* **76**: 5269–5273.

Nybom H. **2004**. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology* **13**: 1143–1155.

Nybom H, **Bartish IV**. **2000**. Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. *Perspectives in Plant Ecology, Evolution and Systematics* **3/2**: 93–114.

Peredo EL, **Revilla MA**, **Jimenez-Alfaro B**, **Bueno Á**, **Prieto JAF**, **Abbott RJ**. **2009**. Historical biogeography of a disjunctly distributed, Spanish alpine plant, *Senecio boissieri* (Asteraceae). *Taxon* **58**: 1–10.

Pluess AR, **Stöcklin J**. **2004**. Population genetic diversity of the clonal plant *Geum reptans* (Rosaceae) in the Swiss Alps. *American Journal of Botany* **91**: 2013–2021.

Powell W, **Morgante M**, **Andre C**, **Hanafey M**, **Vogel J**, **Tingey S**, **Rafalski A**. **1996**. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding* **2**: 225–238.

Pritchard JK, **Stephens M**, **Donnelly P**. **2000**. Inference of population structure using multilocus genotype data. *Genetics* **155**: 945–959.

Rathcke BJ, **Jules ES**. **1993**. Habitat fragmentation and plant-pollinator interactions. *Current Science* **65**: 273–277.

Raymond M, **Rousset F**. **1995**. An exact test for population differentiation. *Evolution* **49**: 1280–1283.

Rousset F. **1997**. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics* **145**: 1219–1228.

Rousset F. **2008**. GENEPOP'007: a complete re-implementation of the GENEPOP. *Molecular Ecology Resources* **8**: 103–106.

Schaal BA, **Hayworth DA**, **Olsen KM**, **Rauscher JT**, **Smith WA**. **1998**. Phylogeographic studies in plants: problems and prospects. *Molecular Ecology* **7**: 465–474.

Segarra-Moragues JG, **Catalán P**. **2003**. Life history variation between species of the relictual genus *Borderea* (Dioscoreaceae): phylogeography, genetic diversity, and population genetic structure assessed by RAPD markers. *Biological Journal of the Linnean Society* **80**: 483–498.

Segarra-Moragues JG, **Catalán P**. **2010**. The fewer and the better: prioritization of populations for conservation under limited resources, a genetic study with *Borderea pyrenaica* (Dioscoreaceae) in the Pyrenean National Park. *Genetica* **138**: 363–376.

Segarra-Moragues JG, **Palop-Esteban M**, **Gonzales-Candelas F**, **Catalán P**. **2007**. Nunatak survival *vs*. tabula rasa in the Central Pyrenees: a study on the endemic plant species *Borderea pyrenaica* (Dioscoreaceae). *Journal of Biogeography* **34**: 1893–1906.

Simon J, **Bosch M**, **Molero J**, **Blanche C**. **2001**. Conservation biology of the Pyrenean larkspur (*Delphinium montanum*): a case of

conflict of plant versus animal conservation? *Biological Conservation* **98**: 305–314.

Steffan-Dewenter I, **Tscharntke T**. **1999**. Effects of habitat isolation on pollinator communities and seed set. *Oecologia* **121**: 432–440.

Su Y, **Wang T**, **Sun Y**, **Ye H**. **2009**. High ISSR variation in 14 surviving individuals of *Euryodendron excelsum* (Ternstroemiaceae) endemic to China. *Biochemical Genetics* **47**: 56–65.

Till-Bottraud I, **Gaudeul M**. **2002**. Intraspecific genetic diversity in alpine plants. In: Körner C, Spehn EM, eds. *Mountain biodiversity. A global assessment*. Berlin: Parthenon Publishing, 23–34.

Van Dyke F. **2008**. *Conservation biology: foundations, concepts, applications*, 2nd edn. Berlin: Springer.

Vekemans X, **Beauwens T**, **Lemaire M**, **Roldan-Ruiz I**. **2002**. Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. *Molecular Ecology* **11**: 139–151.

Vucetich JA, **Waite TA**. **2003**. Spatial patterns of demography and genetic processes across the species range: null hypotheses for landscape conservation genetics. *Conservation Genetics* **4**: 639–645.

Weir BS, **Cockerham CC**. **1984**. Estimating *F*-statistics for the analysis of population structure. *Evolution* **38**: 1358–1370.

Wencewiez L. **2002**. *Analyse de la dynamique de la végétation sur les stations d'Aster des Pyrénées (Aster pyrenaeus DC) en relation avec les usages*. Mémoire de DESS Environnement en milieu rural, INP, ENSA Toulouse.

Wesche K, **Hensen I**, **Undrakh R**. **2006**. Genetic structure of *Galitzkya macrocarpa* and *G. potaninii*, two closely related endemics of Central Asian mountain ranges. *Annals of Botany* **98**: 1025–1034.

Wilcock C, **Neiland R**. **2002**. Pollination failure in plants: why it happens and when it matters. *Trends in Plant Science* **7**: 270–277.

Wright S. **1951**. The genetical structure of populations. *Annals of Eugenics* **15**: 323–354.

Xiao L-Q, **Ge X-J**, **Gong X**, **Hao G**, **Zheng S-X**. **2004**. ISSR variation in the endemic and endangered plant *Cycas guizhouensis* (Cycadaceae). *Annals of Botany* **94**: 133–138.

Young AG, **Boyle T**, **Brown T**. **1996**. The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology and Evolution* **11**: 413–418.

Young AG, **Hilla JH**, **Murray BG**, **Peakall R**. **2002**. Breeding system, genetic diversity and clonal structure in the subalpine forb *Rutidosis leiolepis* F. Muell. (Asteraceae). *Biological Conservation* **106**: 71–78.