

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

J. G. Segarra-Moragues · P. Catalán

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Abstract Taxa considered under low International Union for the Conservation of Nature categories of extinction risk often represent cases of concern to conservation biology. Their high relative abundance precludes management of the entire range due to limited economical resources. Therefore, they require a cost-effective management plan. *Borderea pyrenaica* (Dioscoreaceae), an endemic plant of the Central Pyrenees and pre-Pyrenees, reaches the French side of the Central Pyrenees on its narrow northernmost boundary at Gavarnie (Parc National des Pyrénées, PNP, France), where it is protected as Vulnerable and considered a priority species. We have used nuclear microsatellite population genetic data to design a management strategy for the 11 populations of *B. pyrenaica* present in this area and to identify Relevant Genetic Units for its Conservation. The 18 SSR loci analysed identified 56 alleles, 24 of which fulfilled the rarity criterion for this set of populations. Genetic structuring of populations and representativity values derived from regression analyses of probabilities of loss of rare alleles together support differentiation of the *B. pyrenaica* populations into different management units. Estimates derived from G_{ST} values indicate that five populations would adequately represent the 99.9% of the variation relative to most common alleles whereas calculations based on representativity values indicated that these five populations should equate the proportion

2:2:1 from the three different phylogeographical subdivisions of Gavarnie (Western, Eastern-1 and Eastern-2 ranges). This scheme would allow the preservation of 98.21% of the total *B. pyrenaica* alleles present in Gavarnie, according to the post glacial history of its populations. This conservation genetic approach could be applied to other low-extinction risk categories of extremely rare and subalpine plants in need of regulatory plans in European National Parks and Natural Reserves.

Keywords Allopolyploids · Genetic diversity · Microsatellites · Phylogeography · Population structure · RGUC

Introduction

Modern conservation schemes of endangered and threatened taxa usually involve several sources of data including demography, reproductive biology and genetics. Such designs are aimed at providing a comprehensive picture of population dynamics upon which solid management policies should be founded (López-Pujol et al. 2002; Oostermeijer et al. 2003; Rovira et al. 2002). Because the required degree of protection may depend on the species and on its category of extinction risk, a multidisciplinary approach will likely provide the most efficient conservation plans (Domínguez-Lozano et al. 2003; Heywood and Iriondo 2003). While a precautionary policy for Critically Endangered taxa encourages the protection and monitoring of the entire range of the species, this level of protection is usually either non-existent or limited for those taxa listed as Vulnerable or as a lower category of extinction risk. This is mainly because Vulnerable taxa have many populations and individuals. Generally, not all populations can be

J. G. Segarra-Moragues (✉)
ARAID-UNIZAR, Department of Agricultura y Economía Agraria, Escuela Politécnica Superior de Huesca, Universidad de Zaragoza, C/Carretera de Cuarte, Km1, 22071 Huesca, Spain
e-mail: jogasemo@unizar.es

P. Catalán
Department of Agricultura y Economía Agraria, Escuela Politécnica Superior de Huesca, Universidad de Zaragoza, C/Carretera de Cuarte, Km1, 22071 Huesca, Spain

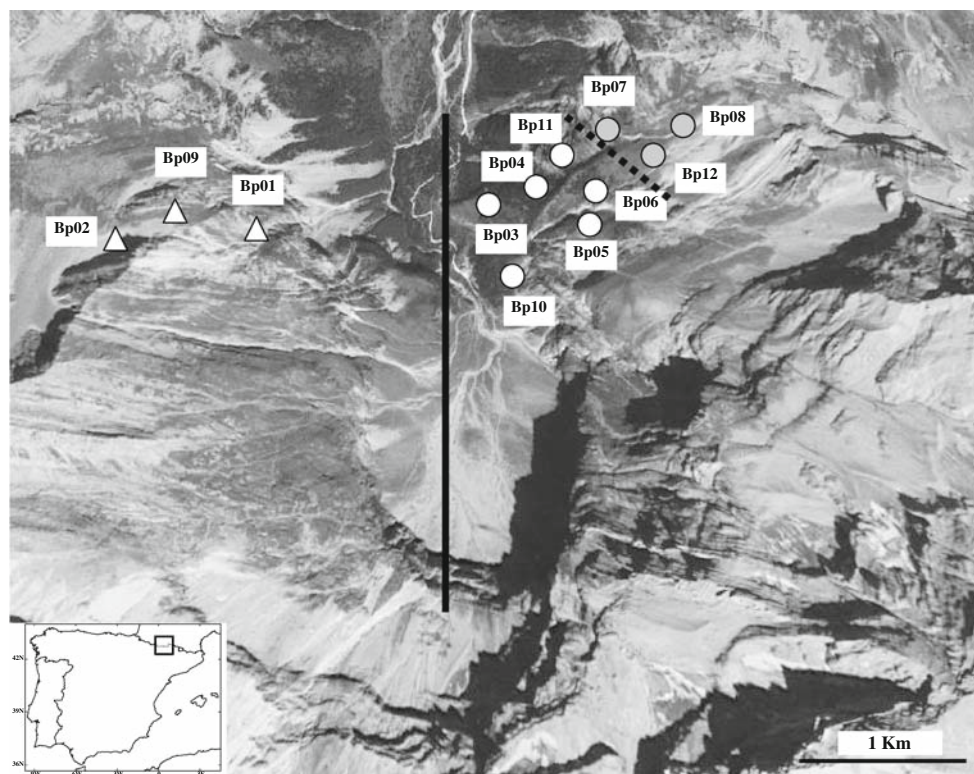


Fig. 1 Geographical location of the populations at Gavarnie. Codes of populations correspond to those indicated in Table 1. Triangles, western Gavarnie range; white circles, eastern Gavarnie range, EG1 and grey circles, eastern Gavarnie range, EG2. Bp09 is composed of a

single female individual and therefore was not included in this study. Solid line denotes the main geographical discontinuity between WG and EG groups and dotted line denotes the geographical separation between EG1 and EG2 groups, respectively

covered, so they have to be selected and prioritised for both in situ and ex situ conservation purposes in an objective manner. This is particularly true under limited availability of conservation resources, thus requiring the development of more elaborate genetic studies aimed at identifying relevant genetic units for conservation (RGUC) (Ciofi et al. 1999). Those plans should ensure the conservation of acceptable levels of genetic diversity with the minimum economic effort before a population decline occurs.

Borderea pyrenaica Miégeville is a dioecious strictly sexually-reproducing geophyte (García and Antor 1995a; Segarra and Catalán 2005) endemic to the Central Pyrenees and pre-Pyrenees (Spain–France). With some individuals living more than 300 years, *B. pyrenaica* has one of the longest life-spans reported for herbaceous plants (García and Antor 1995b). *Borderea pyrenaica* is regarded as a Tertiary relict of the pantropically distributed Dioscoreaceae (Burkill 1960) that adapted to high mountain habitats where it colonises mobile screes mostly above 1,800 m.a.s.l. Some of these adaptations to such unstable habitats deal with the shift from climbing to dwarf habit coupled with the development of creeping stems and inflorescences (Segarra-Moragues and Catalán 2006). *Borderea pyrenaica* shows a gemmate pollen ornamentation that is very restricted among the

Dioscoreaceae (Caddick et al. 1998; Schols et al. 2003, 2005) and is related to the mainly ant-pollination syndrome of the species (García et al. 1995).

Borderea pyrenaica is restricted to a narrow geographic area of about 160 km² in the Central Pyrenees and pre-Pyrenees. Most of its populations are located in Spain, where large populations cover wide expanses, whereas in France only 12 populations are known from the Gavarnie cirque (Segarra-Moragues et al. 2007). Historical records from the eastern neighbouring areas at the Troumouse and Estaube cirques (Dupias 1981) have not been confirmed after repeated inspections of potential sites by the Parc National des Pyrénées (PNP) guard service. Despite the short geographical distances between these populations (less than 4 km between the more distant ones; Fig. 1), some geographical discontinuities exist. Three of these populations (Bp01, Bp02 and Bp09) are located in the Western side of the Gavarnie cirque, referred hereafter as WG and are separated from the remaining ones by the central ravine waterfall (Fig. 1). Populations from the Eastern range referred hereafter as EG could be subdivided into two groups according to their separation by a high mountain crest (e.g., EG1: Bp03, Bp04, Bp05, Bp06, Bp10 and Bp11; EG2: Bp07, Bp08 and Bp12; Fig. 1). Population

Table 1 Populations sampled of *B. pyrenaica* from Gavarnie

	Range	Population name	Habitat	Size	<i>N</i>	N_A^a	A^a	H_O^a	H_E^a	F_{IS}^a
Bp01	WG	La Planette	Subalpine Pyrenean calcareous screes	>5,000	60	37	2.056	0.175	0.201	+0.130**
Bp02	WG	Crampettes	Subalpine Pyrenean calcareous screes	>100	60	44	2.444	0.221	0.235	+0.058 ^{ns}
Bp03	EG1	Chemin du Cirque	Subalpine Pyrenean calcareous screes	20	20	28	1.556	0.122	0.129	+0.056 ^{ns}
Bp04	EG1	Sentier Espugues	Subalpine Pyrenean calcareous screes	>100	60	29	1.661	0.129	0.146	+0.116*
Bp05	EG1	Rochers Blancs	Subalpine Pyrenean calcareous screes with <i>Festuca eskia</i> grasslands	>1,000	60	39	2.167	0.204	0.210	+0.032***
Bp06	EG1	Pailla Nord-Ouest	Central Pyrenean calcareous cliffs	>100	60	39	2.167	0.170	0.179	+0.049 ^{ns}
Bp07	EG2	Pailla Nord-Est	Subalpine Pyrenean calcareous screes + Central Pyrenean calcareous cliffs	<50	34	32	1.833	0.203	0.204	+0.007***
Bp08	EG2	Pailla bas	Subalpine Pyrenean calcareous screes	>1,000	60	28	1.778	0.188	0.200	+0.060*
Bp10	EG1	Hotel de Gavarnie	Subalpine Pyrenean calcareous screes	>100	60	33	1.833	0.141	0.168	+0.162***
Bp11	EG1	Hount Blanc	Central Pyrenean calcareous cliffs + mixed woodlands of <i>Abies</i> and <i>Fagus</i>	>1,000	60	33	2.111	0.164	0.170	+0.035 ^{ns}
Bp12	EG2	Pailla NE–Pailla bas	Subalpine Pyrenean calcareous screes	<50	30	28	1.556	0.183	0.172	−0.069*

For each population their geographical range, localities, habitat, estimated population size and number of sampled individuals and genetic diversity indices are given

N_A , total number of alleles; A , mean number of alleles per locus; H_O , H_E , observed and unbiased expected heterozygosities, respectively; F_{IS} ; fixation index. ^a Data from Segarra-Moragues et al. (2007); *ns*, non-significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

sizes vary according to habitat availability relative to the size of the scree, ranging from more than 5,000 individuals for the largest population of La Planette (Bp01) to only 20 individuals for the smallest population of Chemin du Cirque (Bp03, Table 1), apart from the single female individual found in Belleuve (Bp09). Rough estimates indicate the existence of more than 8,500 individuals in the Gavarnie (PNP) area.

Because of the narrow occurrence of *B. pyrenaica* in France, it has been classified under the International Union for the Conservation of Nature (IUCN 2008) category of Vulnerable and included in both the Annex I of the National List of Protected Species in France (Danton and Baffray 1995) and the Red Book of Endangered Flora of France (Olivier et al. 1995). As a consequence, *B. pyrenaica* has become a priority species within the PNP management plan of endangered Flora. Different studies have been conducted since 1999 in Gavarnie intending to improve the knowledge of *B. pyrenaica* spatial distribution, its ecology, and the size and fitness of its populations (Valadon and Fallour 2001). Nonetheless, a complementary set of detailed population genetic data are necessary to develop a successful management plan of the species (Valadon 2003). The purpose of our study was to establish the minimum number of conservation units representative of the total genetic variability, taking *B. pyrenaica* as a model in this microspatial scenario. In this respect, molecular markers have proved useful with respect to the evolutionary and biogeographical processes in plants (Avisé 1994; Taberlet et al. 1998; Caujapé-Castells and Jansen 2003), with special relevance at microgeographical

scales (Torres et al. 2003a, b; Segarra-Moragues et al. 2005a). Such information has become integral to conservation initiatives of endangered flora (Newton et al. 1999; Segarra-Moragues et al. 2005a, b) because it provides reliable data for comprehensive management and recovery plans without disrupting population relatedness that could have negative evolutionary implications (Prentice et al. 2003).

Earlier molecular studies demonstrated that *B. pyrenaica* populations were influenced by the oscillatory climatic changes of the Quaternary (Segarra-Moragues and Catalán 2002, 2003). Populations of this taxon were likely sheltered in warmer southern Pyrenean refugia during the cold periods, experiencing a rapid recolonisation after the ice retreat following different pathways to the northernmost areas of Gavarnie where it is currently distributed (Segarra-Moragues et al. 2007). The comparative evolutionary model analysis indicated that the infinite allele model (IAM, Kimura and Crow 1964) explained better than the stepwise mutation model (SMM, Kimura and Ohta 1978) the genotypic relationships among individuals and populations. This is because of the overwhelming influence of migration and genetic drift over mutation in the Quaternary history of the *B. pyrenaica* populations (Segarra-Moragues and Catalán 2008). The phylogeographical analysis of microsatellite patterns revealed that the populations of Gavarnie originated from two different migration routes, concordant with the West-East geographical separation, and showing significant overall population differentiation $F_{ST} = 0.194$ (Segarra-Moragues et al. 2007). The populations of the western range were closely related to the

western Spanish Pyrenean core of the Ordesa valley whereas those of the eastern range were closer to the eastern Spanish populations of the Pineta valley, showing a strong between-group differentiation $F_{CT} = 0.121$ (Segarra-Moragues et al. 2007). This indicates that there are at least two groups of *B. pyrenaica* populations with different origins and genetic composition in Gavarnie.

In this study we have used microsatellite markers to answer the following questions aimed at addressing the most adequate conservation genetic strategy for this taxon: (1) What is the distribution of the genetic diversity between the different geographical population cores of *B. pyrenaica* in Gavarnie? (2) What should be the number of populations that need to be preserved to represent adequately the genetic diversity present in this area? (3) Which populations should be selected to better represent the detected genetic diversity and therefore should be prioritised for in situ and ex situ conservation initiatives? The number of extant populations at Gavarnie, coupled with the relative inaccessibility of some of them impedes the continuous monitoring of the whole species range in this area thus suiting well the conservation premises stated for Vulnerable taxa.

Materials and methods

Population sampling, DNA extraction and PCR amplification

A total of 565 individuals collected from all 12 extant populations of *B. pyrenaica* at Gavarnie were sampled for this study (Table 1; Fig. 1). Population Bp09 was not included because it is composed of a single individual. However, we confirmed that it shared allelic relatedness with both populations Bp01 and Bp02 that correspond to the same geographical division (WG). Whenever possible, a ratio of male to female of 1:1 was kept in the sampling scheme and therefore sampling was restricted to flowering individuals. Fresh leaves from all sampled individuals were dried in silica gel and used for DNA isolation. DNA was extracted following the CTAB protocol of Doyle and Doyle (1990) adapted for miniprep extractions. DNA concentration was calculated by comparing to marker VII (Roche) concentration on gel; samples were diluted to a final concentration of ca. 5 ng/ μ l in Tris–EDTA 0.1 \times buffer.

Borderea pyrenaica has been reported to be an allotetraploid taxon based on molecular data (Segarra-Moragues et al. 2004; Catalán et al. 2006). Owing to its hybrid origin, most of the microsatellite primer-pairs amplified different alleles derived from one or the other parental genomes present in this species (Catalán et al. 2006). The duplicate

disomic allelic segregation permitted unambiguous encoding of genotypic data derived from 11 out of 17 simple sequence repeats (SSR) primer-pairs developed for the genus. The remaining 6 SSR primer-pairs amplified size-overlapping alleles derived from both parental genomes. Because reliable genotype scoring could not be performed in those 6 SSR regions they were not included in the study (Catalán et al. 2006). Amplification protocols for the different microsatellite regions were described elsewhere (Segarra-Moragues et al. 2003, 2004). Successful amplicons were obtained for all samples and their products were run on an ABI 310 automated sequencer (Applied Biosystems). Fragment lengths were assigned with GENESCAN and GENOTYPER software (Applied Biosystems) using ROX-500 as the internal lane standard.

Statistical analysis of microsatellite data

The 11 primer-pairs used in this study scored for 18 microsatellite loci, showing differential banding profiles. Four of the primer-pairs (Bc1169, Bp126, Bp1286 and Bp2214) amplified only one genetic dosage (from one of the putative parental genomes) encoding 4 SSR loci. The remaining seven primer-pairs (Bc1258, Bc1422, Bc1644, Bc166, Bp2256, Bp2290 and Bp2391) amplified both genomic dosages (from both putative parental genomes), encoding 14 SSR loci. Alleles of these loci could be assigned to their correspondent genomic complement and encoded as for conventional diploid taxa (Catalán et al. 2006). These loci were renamed as Bc1258a,b; Bc1422a,b, etc., respectively, to designate each of the two complements.

We estimated isolation by distance between populations of *B. pyrenaica* in Gavarnie as a factor likely to increase the effect of genetic drift producing differences in allelic frequencies (cf. Segarra-Moragues and Catalán 2008) and thus promoting local adaptation and an increase in number of different genetic units. *Borderea pyrenaica* is likely to be prone to this effect given the short dispersal distance of both seeds and pollen producing unique genotypic combinations within populations and geographical areas (Moritz 1994). For this purpose we calculated the correlation between log-transformed geographical distances and pairwise linearized F_{ST} values (i.e. $F_{ST}/(1 - F_{ST})$, Slatkin (1995)) between populations (Rousset 1997). Mantel tests using 1,000 permutations were conducted using NTSYSpc v. 2.11a (Rohlf 2002).

We used two different approaches to identify RGUCs among the *B. pyrenaica* populations from Gavarnie. One of these approaches entailed the estimators of population structure of Nei (1973). Total genetic diversity present in Gavarnie (H_T) was decomposed into the within-population (H_S) and between-population ($D_{ST} = H_T - H_S$) components, respectively, which were used to calculate the

coefficient of population differentiation ($G_{ST} = D_{ST}/H_T$). These values were calculated for each locus for the whole area of *B. pyrenaica* in Gavarnie and for each geographical subdivision (WG, EG, EG1 and EG2) within the Gavarnie cirque using GENETIX v. 4.04 (Belkhir et al. 2003). G_{ST} values were then used to estimate the number of populations that should be targeted to represent a given proportion of the genetic diversity according to the equation of Ceska et al. (1997), $P = 1 - G_{ST}^n$, where n is the number of populations to be sampled to represent a given proportion (P) of the among-population genetic diversity.

Because of the insensitivity of G_{ST} values to rare alleles (Neel and Cummings 2003), we used a second approach that dealt with the associated probabilities of loss of alleles present at low frequencies (rare alleles). We used the expression $L = (1 - p)^{2N}$ of Bengtsson et al. (1995) that estimates the probability that a sample size of (N) populations fails to include an allele with population frequency p (Caujapé-Castells and Pedrola-Monfort 2004). This analysis was carried out with alleles that fulfilled the following criteria of rarity: (1) had an overall frequency lower than 0.50 and (2) were present in less than 50% of the *B. pyrenaica* populations from Gavarnie. The observed (L_o) and the expected (L_e) values of the probabilities of loss were calculated. In both L_o and L_e p , corresponds to the average allele frequency over the populations in which each allele is present. However, for L_o , N in the exponential refers to the number of populations where the allele is actually present, whereas in L_e , N refers to the total number of populations considered. In other words, in L_e , an even average allele frequency is considered across all populations. $-\text{Log}L_o$ and $-\text{Log}L_e$ (y -axis) were plotted against the mean frequency of each allele (x -axis) and used for two linear regressions. The quotient between the slope of the expected regression line (based on L_e values) and the slope of the observed regression line (based on L_o values) estimates the representativity value (R) that indicates the proportion of rare alleles captured by sampling only one population (Bengtsson et al. 1995; Caujapé-Castells and Pedrola-Monfort 2004). Calculations of the probabilities of loss were carried out using TRANSFORMER-3 (Caujapé-Castells and Baccarani-Rosas 2005).

We based our conclusions on how many and which populations should be sampled through these two different approaches because they are based on different premises. First, G_{ST} is mostly influenced by between-population differences in frequencies of most common alleles (Culley et al. 2002) and second, rare alleles represent newly generated variants that may strengthen the species' capability to withstand environmental changes (Bengtsson et al. 1995). Therefore, a comprehensive estimate of RGUCs based on both complementary methods seems biologically more meaningful.

Results

The 18 SSR loci analysed detected a total of 56 alleles in the 11 *B. pyrenaica* populations in our study (Table 2). Seven alleles were exclusive to the western range and nine to the eastern range. Five out of the nine eastern alleles were restricted to single populations of EG1 and none was exclusive to EG2. The remaining 40 alleles were shared between different populations of the three geographical divisions (Table 2). Correlation between pairwise log-transformed geographical distances and linearized F_{ST} values ($r = 0.454$) revealed significant ($P = 0.010$) evidence of isolation by distance between populations. Population structure statistics of Nei (1973) detected a higher proportion of total genetic diversity within populations (>80%) than between populations (Table 3). This result was repeatedly recovered when calculations were conducted on the different geographical hierarchies. The highest value of apportionment of the between-population component was obtained for eastern Gavarnie (EG) ($D_{ST} = 0.060$, 18.75%), suggesting two geographically separated population groups. The highest value of the population differentiation coefficient (G_{ST}) resulted from the consideration of all populations into a single group (Gavarnie $G_{ST} = 0.196$). Further subdivision into different geographical hierarchies detected minimum G_{ST} values between populations of the western range ($G_{ST} = 0.059$) and higher among populations of the eastern range ($G_{ST} = 0.189$). Similar levels of population differentiation were detected among populations of the two eastern groups (EG1, $G_{ST} = 0.119$; EG2, $G_{ST} = 0.120$). Estimation of the number of populations to be monitored to represent a 99.9% of the total genetic diversity attributable to most common alleles resulted in 5 (4.24) populations among all populations considered as a single group. When this estimation was carried out separately for each geographical subdivisions, the estimated number of populations within each range were of 2 (2.44) for WG, 4 (4.15) for EG, 3 (3.25) for EG1 and 3 (3.26) for EG2.

Observed and expected probabilities of loss were calculated for 24 alleles that complied with the rarity premises explained in the methods (Tables 2, 4). The quotient between the slopes of the expected ($-\text{Log}L_e$) and the observed ($-\text{Log}L_o$) regression lines resulted in a representativity value of $R = 0.307$ for all Gavarnie populations (Fig. 3a), indicating that the sampling of a single population of Gavarnie would roughly account for the 30.7% of the 24 rare alleles present in this area. Independent estimations of representativities for the different groups of populations gave values of $R = 1.000$ for WG, $R = 0.263$ for EG, $R = 0.266$ for EG1 and $R = 0.314$ for EG2 (Fig. 3b–e). Based on the mean frequencies of rare alleles and their distribution between the different populations (Table 4), the area where each one of these rare alleles holds the lower

Table 2 Allele frequencies for 11 populations analysed of *B. pyrenaica*

Locus/allele (bp)	Population ranges										
	W Gavarnie		E Gavarnie 1 (EG1)					E Gavarnie 2 (EG2)			
	(WG)		Bp03	Bp04	Bp05	Bp06	Bp10	Bp11	Bp07	Bp08	Bp12
	Bp01	Bp02	(20)	(60)	(60)	(60)	(60)	(60)	(34)	(60)	(30)
Bc1169											
123	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
Bc1258a											
156	0.817	0.667	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
162*	0.183	0.333	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
Bc1258b											
145*	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
168	0.000	0.000	0.000	0.000	0.033	0.033	0.000	0.000	0.074	0.542	0.533
171	0.975	1.000	1.000	1.000	0.967	0.967	1.000	1.000	0.926	0.458	0.467
Bc1422a											
177*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.000
180	0.925	1.000	1.000	1.000	1.000	1.000	1.000	0.992	1.000	1.000	1.000
186*	0.075	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Bc1422b											
159	0.317	0.408	0.400	0.158	0.383	0.225	0.392	0.117	0.514	0.317	0.233
192*	0.000	0.017	0.000	0.000	0.008	0.000	0.133	0.017	0.000	0.000	0.267
195	0.258	0.342	0.050	0.058	0.050	0.100	0.133	0.183	0.000	0.358	0.000
198	0.350	0.200	0.550	0.784	0.442	0.633	0.292	0.641	0.368	0.325	0.500
201*	0.067	0.000	0.000	0.000	0.067	0.017	0.000	0.000	0.000	0.000	0.000
204*	0.000	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.118	0.000	0.000
210*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000
213*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000
216*	0.000	0.008	0.000	0.000	0.033	0.008	0.000	0.000	0.000	0.000	0.000
219*	0.008	0.017	0.000	0.000	0.017	0.017	0.050	0.000	0.000	0.000	0.000
Bc1644a											
160	0.867	0.550	0.950	0.700	0.717	0.858	0.883	0.933	0.985	0.758	1.000
163	0.133	0.450	0.050	0.300	0.283	0.142	0.117	0.067	0.015	0.242	0.000
Bc1644b											
169*	0.000	0.008	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
172	0.108	0.158	0.525	0.775	0.350	0.258	0.567	0.325	0.206	0.492	0.383
175	0.567	0.175	0.450	0.225	0.550	0.567	0.267	0.542	0.764	0.316	0.600
178	0.325	0.659	0.000	0.000	0.100	0.125	0.166	0.108	0.015	0.092	0.000
181*	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.025	0.015	0.100	0.017
Bc166a											
175	1.000	1.000	1.000	1.000	0.842	1.000	1.000	1.000	1.000	1.000	1.000
null*	0.000	0.000	0.000	0.000	0.158	0.000	0.000	0.000	0.000	0.000	0.000
Bc166b											
178	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Bp126											
232*	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.000
235	0.658	0.400	0.525	0.758	0.608	0.683	0.967	0.758	0.588	0.550	0.700
238	0.300	0.592	0.475	0.242	0.167	0.117	0.033	0.208	0.279	0.375	0.217
241	0.042	0.008	0.000	0.000	0.225	0.192	0.000	0.034	0.133	0.075	0.083
Bp1286											
123	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Bp2214											
204*	0.000	0.008	0.000	0.000	0.000	0.000	0.367	0.000	0.000	0.000	0.000
216	1.000	0.992	1.000	1.000	1.000	1.000	0.633	1.000	1.000	1.000	1.000
Bp2256a											
220	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Bp2256b											
223	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Bp2290a											
127	0.042	0.175	0.050	0.008	0.242	0.275	0.192	0.408	0.603	0.967	0.850
130	0.958	0.825	0.950	0.992	0.758	0.725	0.808	0.592	0.397	0.033	0.150
Bp2290b											
149	0.000	0.000	0.000	0.067	0.008	0.008	0.000	0.092	0.029	0.025	0.000
152	0.858	0.634	1.000	0.933	0.926	0.992	0.942	0.842	0.647	0.950	1.000
155*	0.000	0.008	0.000	0.000	0.058	0.000	0.025	0.066	0.324	0.000	0.000
158*	0.142	0.333	0.000	0.000	0.008	0.000	0.033	0.000	0.000	0.025	0.000
161*	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Bp2391a											
126	0.892	0.992	0.925	0.725	0.908	0.867	0.717	0.900	0.500	0.825	0.783
129	0.108	0.008	0.075	0.275	0.092	0.133	0.283	0.100	0.500	0.175	0.217
Bp2391b											
133	0.275	0.208	0.000	0.000	0.050	0.267	0.033	0.183	0.368	0.725	0.250
136	0.550	0.317	0.225	0.533	0.592	0.517	0.934	0.725	0.618	0.275	0.750
140	0.050	0.017	0.750	0.383	0.133	0.008	0.033	0.042	0.014	0.000	0.000
143*	0.000	0.000	0.000	0.025	0.225	0.183	0.000	0.008	0.000	0.000	0.000
146*	0.083	0.283	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
149*	0.000	0.092	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
153*	0.000	0.008	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.000
156	0.042	0.050	0.025	0.059	0.000	0.017	0.000	0.042	0.000	0.000	0.000
159*	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Asterisks indicate rare alleles used in calculations of probability of loss. Framed frequencies correspond to exclusive alleles of the western (blank filling) and eastern (grey filling) Gavarnie ranges, respectively. Bolded values are private alleles of a single population. Numbers in brackets indicate sample sizes

Table 3 Population structure statistics of Nei (1973) at the 13 polymorphic microsatellite loci scored in the 11 populations of *B. pyrenaica*

Range Subdivision	Gavarnie (total, 11)						Western Gavarnie (2)						Eastern Gavarnie (9)												
	WG			WG			WG			WG			WG			WG									
	H_T	H_S	D_{ST}	G_{ST}	H_T	H_S	D_{ST}	G_{ST}	H_T	H_S	D_{ST}	G_{ST}	H_T	H_S	D_{ST}	G_{ST}	H_T	H_S	D_{ST}	G_{ST}	H_T	H_S	D_{ST}	G_{ST}	
Bp126	0.492	0.443	0.049	0.100	0.521	0.482	0.039	0.073	0.473	0.435	0.038	0.081	0.438	0.391	0.047	0.108	0.531	0.522	0.009	0.017	0.438	0.391	0.047	0.108	0.531
Bp2214	0.066	0.044	0.022	0.336	0.008	0.008	0.000	0.004	0.078	0.052	0.026	0.340	0.115	0.077	0.038	0.325	0.000	0.000	0.000	–	0.115	0.077	0.038	0.325	0.000
Bp2290a	0.453	0.258	0.195	0.431	0.193	0.184	0.009	0.046	0.480	0.274	0.206	0.428	0.315	0.278	0.037	0.116	0.312	0.266	0.046	0.147	0.315	0.278	0.037	0.116	0.312
Bp2290b	0.214	0.180	0.034	0.162	0.387	0.365	0.022	0.057	0.160	0.138	0.022	0.137	0.117	0.112	0.005	0.042	0.239	0.191	0.048	0.201	0.117	0.112	0.005	0.042	0.239
Bp2391a	0.294	0.260	0.034	0.115	0.110	0.105	0.005	0.046	0.327	0.294	0.033	0.099	0.268	0.254	0.014	0.055	0.418	0.376	0.042	0.100	0.268	0.254	0.014	0.055	0.418
Bp2391b	0.633	0.487	0.146	0.231	0.715	0.687	0.028	0.038	0.601	0.442	0.159	0.265	0.590	0.454	0.136	0.232	0.500	0.419	0.081	0.162	0.590	0.454	0.136	0.232	0.500
Bc1258a	0.090	0.068	0.022	0.245	0.383	0.372	0.011	0.029	0.000	0.000	0.000	–	0.000	0.000	0.000	–	0.000	0.000	0.000	–	0.000	0.000	0.000	–	0.000
Bc1258b	0.201	0.119	0.082	0.407	0.025	0.024	0.001	0.013	0.234	0.140	0.094	0.401	0.022	0.021	0.001	0.023	0.473	0.377	0.096	0.203	0.022	0.021	0.001	0.023	0.473
Bc1422a	0.015	0.014	0.001	0.062	0.072	0.069	0.003	0.039	0.002	0.002	0.000	0.007	0.003	0.003	0.000	0.007	0.000	0.000	0.000	–	0.003	0.003	0.000	0.007	0.000
Bc1422b	0.666	0.600	0.066	0.098	0.702	0.691	0.011	0.015	0.640	0.580	0.060	0.094	0.602	0.557	0.045	0.074	0.692	0.625	0.067	0.097	0.602	0.557	0.045	0.074	0.692
Bc166a	0.028	0.024	0.004	0.146	0.000	0.000	0.000	–	0.035	0.030	0.005	0.143	0.051	0.044	0.007	0.136	0.000	0.000	0.000	–	0.051	0.044	0.007	0.136	0.000
Bc1644a	0.274	0.238	0.036	0.130	0.413	0.363	0.050	0.121	0.234	0.210	0.024	0.101	0.268	0.249	0.019	0.072	0.156	0.132	0.024	0.157	0.268	0.249	0.019	0.072	0.156
Bc1644b	0.628	0.525	0.103	0.164	0.603	0.536	0.067	0.111	0.583	0.523	0.060	0.103	0.587	0.533	0.054	0.093	0.553	0.502	0.051	0.093	0.587	0.533	0.054	0.093	0.553
Average	0.312	0.251	0.061	0.196	0.344	0.324	0.020	0.059	0.320	0.260	0.060	0.189	0.281	0.248	0.033	0.119	0.430	0.379	0.051	0.120	0.281	0.248	0.033	0.119	0.430

For each locus the total genetic diversity (H_T), the genetic diversity within (H_S) and between populations (D_{ST}), and the population differentiation coefficient (G_{ST}) are indicated, respectively, for the total range in Gavarnie, the western range (WG), the total eastern range (EG) and the subdivision of the eastern range into EG1 and EG2. Numbers in parenthesis indicate the number of populations included in each geographical subdivision

Table 4 Probabilities of loss of 24 rare alleles when populations of *B. pyrenaica* from Gavarnie are included as a single management unit

Locus	Allele	<i>N</i>				Average frequency	Probabilities of loss		Preferred sampling area
		Gavarnie	WG	EG1	EG2		L_o	L_e	
Bc1258a	162	2	2	0	0	0.258	0.303	0.001	WG
Bc1258b	145	1	1	0	0	0.025	0.951	0.573	WG
Bc1422a	177	1	0	1	0	0.008	0.983	0.832	EG1
	186	1	1	0	0	0.075	0.856	0.180	WG
Bc1422b	192	5	1	3	1	0.088	0.397	0.131	EG2
	201	3	1	2	0	0.050	0.735	0.324	WG
	204	2	1	0	1	0.063	0.771	0.239	EG2
	210	1	0	1	0	0.025	0.951	0.573	EG1
	213	1	0	1	0	0.017	0.967	0.690	EG1
	216	3	1	2	0	0.017	0.904	0.692	EG1
	219	5	2	3	0	0.022	0.803	0.617	EG1
Bc1644b	169	2	1	1	0	0.017	0.935	0.690	EG1
	181	5	0	2	3	0.041	0.656	0.395	EG2
Bc166a	Null	1	0	1	0	0.158	0.708	0.023	EG1
Bp126	232	1	0	1	0	0.008	0.983	0.832	EG1
Bp2214	204	2	1	1	0	0.188	0.436	0.010	EG1
Bp2290b	155	5	1	3	1	0.096	0.363	0.108	EG2
	158	5	2	2	1	0.108	0.318	0.080	WG
	161	1	1	0	0	0.025	0.951	0.573	WG
Bp2391b	143	4	0	4	0	0.110	0.392	0.076	EG1
	146	2	2	0	0	0.183	0.445	0.012	WG
	149	1	1	0	0	0.092	0.825	0.121	WG
	153	2	1	1	0	0.008	0.967	0.832	WG
	159	1	1	0	0	0.025	0.951	0.573	WG

N, number of populations where each allele was detected. L_o and L_e , observed and expected probabilities of loss. Preferred sampling area indicates the geographical range with higher probability to capture the corresponding allele. *WG* western Gavarnie; *EG1* eastern Gavarnie-1; *EG2* eastern Gavarnie-2 (upper crest)

probability of loss (i.e., the higher probability to be captured by sampling one population at random) was the western range in ten cases and the eastern range in 14 cases, the latter one being separated into EG1 in ten cases and EG2 in four cases. These values indicate that the proportion of populations to be sampled from each geographical subdivision can be expressed as 0.4:0.6 populations from the western-eastern ranges, respectively. The eastern range can be estimated according to the proportion 10:4, which gives a proportion of populations of 0.7:0.3 for EG1 and EG2, respectively. Considering the three geographical subdivisions independently for the relative proportion of populations to be monitored within each area, these values can approximately equal 0.4:0.4:0.2 for WG, EG1 and EG2, respectively.

Discussion

Genetic diversity may be associated with certain fitness traits, enabling a species to counteract environmental

changes. It supplies an increased adaptive capability to new habitats or competitive strength, leading ultimately to a higher biological success rate and to its maintenance in space and time (Gautschi et al. 2002; Paschke et al. 2002; Pluess and Stöcklin 2004). Most of the genetic studies addressing conservation issues have been conducted using molecular markers that are assumed to be selectively neutral (Avice 1994; Manel et al. 2005). This has raised the debate of whether such information may reveal the real evolutionary genetic potential of wild populations (Luikart et al. 2003; Holderegger et al. 2006), suggesting that population and landscape genetic studies should focus on genetic variation at quantitative and adaptive trait loci (Merilä and Crnokrak 2001; McKay and Latta 2002; Holderegger et al. 2008). Although this information would always be desirable, it is only currently available for few non-model organisms. It is not the case of many narrow endemics, such as *B. pyrenaica*, for which whole-genome knowledge is still scarce. Different studies have found a positive correlation between genetic diversity estimates using neutral markers and population

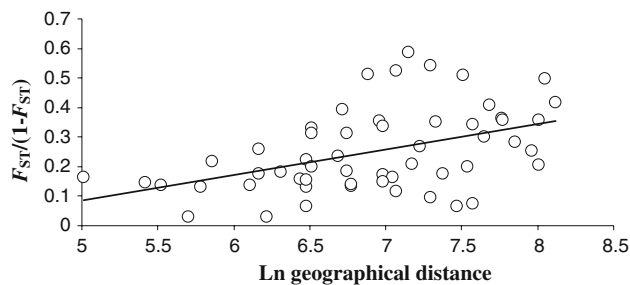


Fig. 2 Isolation by distance analysis. Correlation between pairwise log transformed geographical distances (x axis) and linearized F_{ST} values (Slatkin 1995; Rousset 1997) (y axis). Correlation between matrices was $r = 0.454$, $P = 0.010$

fitness (Gautschi et al. 2002; Paschke et al. 2002; Pluess and Stöcklin 2004). These results agree in that the larger the amount of neutral genetic diversity captured by appropriate management schemes, the higher the probability to retain the evolutionary potential of populations through indirect capturing of genetic variation at selectively non-neutral loci (Bataillon et al. 1996). This is especially true when allelic richness and genetic diversity is unevenly distributed among populations as a consequence of isolation by distance (Bataillon et al. 1996), like in *B. pyrenaica* (Tables 1, 2; Fig. 2). Accordingly, despite the inability of neutral markers to totally reflect the adaptive trends of the *B. pyrenaica* individuals, our analysis is an appropriate indirect approach to estimate the potential adaptive differences within the species.

Special emphasis has been traditionally devoted to those taxa regarded as Critically Endangered, which usually have low levels of genetic variation. Genetic data have been incorporated into their conservation plans or have been used to improve ongoing conservation initiatives (Petit et al. 1998; Torres et al. 2003a; Segarra-Moragues et al. 2005a, b; Orellana et al. 2009). Critically Endangered taxa often show extremely restricted ranges of distribution, sometimes restricted to a single population (Segarra-Moragues et al. 2005a). Habitat protection by various administrative means becomes a top priority for these taxa (Laguna et al. 2004). These populations are generally easy to handle, and genetic data can help in designing management strategies to avoid inbreeding depression and genetic erosion. Several studies have emphasized the failure of reintroduction programs due to the lack of adequate levels of genetic diversity in germplasm banks established before the availability of genetic data (Calero et al. 1999; Ibáñez et al. 1999; Maunder et al. 2001). Although some of these species may have been already depauperated at the time of collection, inappropriate collection schemes that ignored or did not have genetic data at hand cannot be discarded.

Conversely, Vulnerable taxa usually have wider distribution ranges and more populations and individuals. This

can make management of all their wild stocks difficult and expensive. Even when ex-situ strategies are considered these might fail to include a representative amount of the genetic variation (Maunder et al. 2001) or exceed the number of samples needed, resulting in a loss of efficiency and increased costs if genetic data are ignored (Neel and Cummings 2003). These taxa are ideally suited for genetic analyses. Genetic data can provide information on the levels and structure of genetic variation and can help to identify the population genetic relationships and to suggest the best sampling strategies to preserve adequate and representative levels of genetic diversity with minimum expense (Petit et al. 1998; Fraser and Bernatchez 2001; Fay 2003; Orellana et al. 2009). In this sense, population genetic studies of Vulnerable taxa are highly recommended to identify minimum relevant genetic units for conservation (RGUCs, Ciofi et al. 1999), which should be compatible with the maintenance of the evolutionary potential without interfering with the evolutionary history of the populations (Caujapé-Castells and Pedrola-Monfort 2004; Sánchez et al. 2004; Pérez-Collazos et al. 2008). This is particularly true for European taxa that inhabit high mountain areas and that have experienced different histories of population size contraction–expansion during the climatic changes of the Quaternary (Hewitt 1996, 2000; Taberlet et al. 1998; Stehlik 2000; Larena et al. 2002, 2006) that have conditioned their levels of genetic diversity and the phylogeographical relationships between populations (Vargas 2003). The knowledge of the relationships among populations is of primary interest because conservation genetics is biologically more meaningful if it accords with monophyletic groups (Moritz 1994; Soltis and Gitzendanner 1999).

Borderea pyrenaica fits well into this theoretical context. The restricted French range of this species promoted its classification as Vulnerable (IUCN 2008). In spite of the narrow range occupied in the northern Pyrenees (PNP area), the number of populations exceed human and economical resources for complete management. Furthermore, these populations are located in high, almost unaltered, mountain habitats that are unlikely to be threatened by extrinsic factors, making habitat protection a matter of secondary concern for this taxon. However, recent concerns about a rapid global climate warming in the Pyrenean alpine mountain belt could change its perspectives in the near future (Peñuelas and Boada 2003; Solomon et al. 2007). Therefore, genetic information was needed to devise a successful management plan for this species in this area (Valadon 2003).

How many and which populations?

By now a reliable answer to this question can be provided only in the light of genetic information derived from

molecular markers. Clustering analyses based on microsatellite data revealed that the populations of *B. pyrenaica* present in Gavarnie were derived from two different colonising routes followed during their postglacial immigration into this barren narrow geographical area in the Pleistocene (Segarra-Moragues et al. 2007). Accordingly, populations showed significant isolation by distance despite the short geographical distances separating populations (Fig. 2), strong overall population differentiation $F_{ST} = 0.194$ and strong between-group geographical differentiation $F_{CT} = 0.121$ (Segarra-Moragues et al. 2007), supporting the idea that they cannot be considered a single conservation unit. The G_{ST} values and the probabilities of loss of rare alleles both highlight the need to separate the *B. pyrenaica* area in Gavarnie into different management units. First we need to identify the minimum number of populations necessary to represent the genetic diversity of the area. G_{ST} values of Gavarnie suggest that the monitoring of five populations would represent the 99.9% of the genetic diversity attributable to most common alleles. This

figure exceeds by 9% previous suggestions for the successful accomplishment of conservation goals (Hamrick 1983; Caujapé-Castells and Pedrola-Monfort 2004) but agrees with the values recommended for narrow range endemics (López-Pujol et al. 2004; Sánchez et al. 2004; Pérez-Collazos et al. 2008). Further consideration of independent G_{ST} values for the three geographical areas of Gavarnie suggest an increase in the number of populations to be monitored to eight. This follows from the different levels of genetic diversity and the frequency variance of most common alleles, for which G_{ST} values are particularly sensitive (Caujapé-Castells and Pedrola-Monfort 2004), among populations. Since similar levels of variation would imply that both estimates should converge to a similar value, the estimate of Gavarnie as a whole, should equal the sum of the estimations for the three geographical divisions separately. Generally this concludes in the need to monitor a larger number of populations (Caujapé-Castells and Pedrola-Monfort 2004). In our case, the differences between both estimates are attributable to the

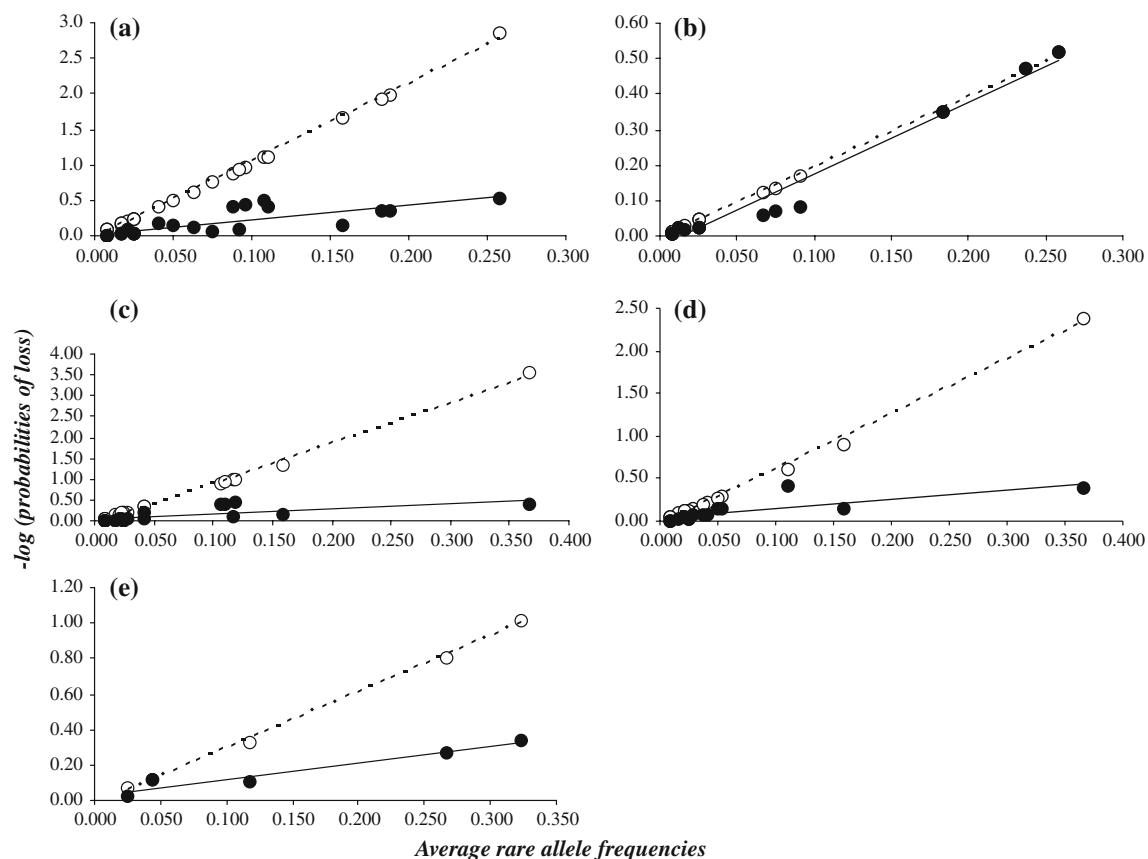


Fig. 3 Regression analyses of the average allele frequencies (x-axis) of alleles that fulfilled the criteria of rarity stated in the methods with $-\log$ probabilities of loss (y-axis). Solid lines with black dots and dashed lines with empty dots represent $-\log$ of the observed and expected probabilities of loss, respectively. The number of rare alleles considered (N_A) and the representativity values (R) for each

geographical subdivision were: **a** Gavarnie $N_A = 24$, $R = 0.3065$; **b** western Gavarnie (WG), $N_A = 17$, $R = 1.000$; **c** eastern Gavarnie (EG), $N_A = 17$, $R = 0.2626$, and EG hierarchy of two additional ranges; **d** EG1, $N_A = 16$, $R = 0.2663$ and **e** EG2, $N_A = 5$, $R = 0.3140$

variation in the allele frequencies that are shared among most of the populations. Therefore, there is no need to increase this figure by including more populations but to choose five populations among the more genetically diverse ones (Table 1).

The second question deals with which populations should be monitored to achieve an adequate representation of the genetic diversity of the area. Theoretical population genetics advises that populations should be selected in terms of both allelic richness and heterozygosity (Hamrick et al. 1991; Neel and Cummings 2003), because they represent complementary estimates of both the raw amount of genetic variation, as indicated by the number of alleles, and the heterogeneity of populations. Heterozygosity is also less biased by sampling errors and is correlated with general population fitness (Gautschi et al. 2002; Paschke et al. 2002; Pluess and Stöcklin 2004). An earlier population-genetic study conducted on Spanish and French *B. pyrenaica* populations detected similar levels of heterozygosity among all populations of this taxon from Gavarnie that had similar censuses and a higher allelic richness in those populations of the western range compared to those of the eastern area (Segarra-Moragues et al. 2007, Table 1). Therefore, larger populations should be prioritized for conservation (cf. Martínez-Palacios et al. 1999) in case some genetic diversity may have passed overlooked by small sample sizes. However, this seems unlikely within our broadly sampled study in which we should have a high probability to detect any allele with a frequency higher than 0.0009.

Representativity values derived from the analysis of the probabilities of loss of rare alleles (Table 4; Fig. 3) suggested that five populations should match the proportion of 2:2:1 each from WG, EG1 and EG2 geographical areas, respectively, implying the monitoring of the entire western range and three populations of the eastern range. We therefore recommend the following populations for conservation: Bp01 and Bp02 from the western range; Bp05 and Bp11 from EG1 and, Bp08 from EG2. A management strategy that guarantees the protection of these sets of populations would account for 23 (95.83%) of the 24 rare alleles in Gavarnie and 55 (98.21%) out of the 56 alleles detected in the French range of *B. pyrenaica*. Only one allele (Bp126-232), exclusive to Bp06 (Table 2), would be missed by this management scheme unless ex situ germplasm stocks are used as genetic reservoirs or it is eventually spread to other populations via gene flow. The populations selected here could serve for restoration or reinforcement if population decline occurs in any of the neighbouring populations of the same geographical division. Translocation of individuals or seeds between populations from either EG1 and EG2 seems feasible given the low genetic differentiation between these two groups.

However, this is not recommended between the two main geographical areas of Gavarnie (WG-EG) since they are derived from different Spanish southern Pyrenean ancestral populations. Although outbreeding depression is unlikely given the low genetic distances among all populations of *B. pyrenaica*, a degree of local adaptation and outbreeding depression can not be ruled out until experimental crosses between individuals of the two geographical ranges are performed and checked for fitness rates. Therefore, we recommend the management of these two areas separately.

Finally, if ex situ strategies are desirable for conservation of seeds in germplasm stocks from the targeted populations an additional advice can be provided in views of the available genetic data (Table 1). The populations of this rare species were determined not to be in Hardy–Weinberg equilibrium (Segarra-Moragues et al. 2007, Table 1), but deviating towards heterozygote deficiency. The significant positive F_{IS} values were explained as the consequence of the preferential mating between spatially close individuals, which favours inbreeding (Segarra-Moragues et al. 2007). This unexpected result for a dioecious taxon was related to the short dispersal of seeds and pollen that produce dense colonies of genetically related individuals and thus produce a strong within-population genetic structure. Accordingly, the genetic patches within sampling sites of *B. pyrenaica* are much smaller than the predefined populations. This same result was found for the sister taxon *B. chopardii* (Gaussen) Heslot, that shares similar reproductive traits with *B. pyrenaica* (Segarra-Moragues et al. 2005a), and is a likely example of a Wahlund effect. From a practical point of view, it would not be advisable to split geographically defined populations into many smaller genetic patches because this would further complicate their management. However, the strong within-population genetic structure cannot be ignored at the time of collection of seeds for ex situ storage, suggesting that this should be carried out from as many as possible spatially separated groups of individuals within populations to guarantee a good representation of all demes.

General conclusions

Narrow endemics that still have many populations are of concern because the protection of their entire range can be both expensive and politically challenging, so more restrictive conservation strategies are sometimes necessary. A balanced combination between the selection of the minimum number of populations needed to succeed in a conservation goal without compromising future genetic consequences by inadequate management is often desired. This study exploits information of microsatellite variation from a practical point of view and provides appropriate

recommendations for the adequate management of the populations of a Vulnerable taxon within a narrow geographical area by identifying RGUCs. Following these recommendations the proposed conservation strategy should minimize costs. This strategy should also guarantee the maintenance of representative amounts of genetic variation that can endow enough evolutionary potential for appropriately collected ex situ stocks and to in situ managed populations. This approach taken here should provide valuable theoretical basis for other candidate taxa needed of similar requirements.

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