SILVA FENNICA

Silva Fennica 42(4) research articles

www.metla.fi/silvafennica · ISSN 0037-5330
The Finnish Society of Forest Science · The Finnish Forest Research Institute

Genetic Diversity and Structure of the Endangered *Betula pendula* subsp. *fontqueri* Populations in the South of Spain

Carmen Martín, Teresa Parra, Margarita Clemente-Muñoz and Esteban Hernandez-Bermejo

Martín, C., Parra, T., Clemente-Muñoz, M. & Hernandez-Bermejo, J. E. 2008. Genetic diversity and structure of the endangered *Betula pendula* subsp. *fontqueri* populations in the south of Spain. Silva Fennica 42(4): 00–00.

Betula pendula subsp. *fontqueri*, present in the south of Spain, has been considered in danger of extinction and, for this reason, some regional governments in Spain have included their populations in conservation programmes. In order to establish the genetic structure of the *Betula pendula* subsp. *fontqueri* populations, a random amplified polymorphic DNA (RAPD) analysis was carried out. Two *B. pubescens* populations were included in the study as taxonomic controls. *B. pendula* subsp. *fontqueri* populations were clearly differentiated through UPGMA, and showed significant pairwise genetic distance (Φ_{ST}) values between all pairs of populations obtained by AMOVA. Genetic diversity found between populations was not correlated to geographical distances. The significant differences among populations must be due to progressive isolation of *Betula* populations along their paleogeographical history, and more recently to the drastic fragmentation and reduction of some of these populations. The results obtained in this work show clear genetic differences which could be considered in the management of conservation strategies for *Betula pendula* subsp. *fontqueri* in its Iberian meridional distribution.

Keywords *Betula pendula, B. pendula* subsp. *fontqueri,* genetic diversity, population, RAPD
Addresses Universidad Politecnica de Madrid, Escuela Técnica Superior Ingenieros Agronomos, Ciudad Universitaria s/n, 28040 Madrid, Spain **E-mail** mariacarmen.martin@upm.es
Received 21 February 2008 **Revised** 11 April 2008 **Accepted** 14 April 2008
Available at http://www.metla.fi/silvafennica/full/sf42/sf424000.pdf

1 Introduction

The genus *Betula* is represented in Europe by four species, of which two are trees: *B. pubescens* Ehrh. (= *B. alba* L., according to Moreno & Peinado, 1990) and *B. pendula* Rothm. *Betula pubescens* is the most widely distributed taxon. It is found in the north of Europe and Asia. Its southernmost limit reaches some areas of the Iberian Peninsula. *B. pendula* is also distributed throughout most of Europe, being confined to mountains in the south. It also appears in the south of Asia (W Siberia, Iran, Anatolia) and north of Africa. A number of subspecies and varieties have also been recognised at different taxonomic levels in the past few decades.

In Spain, *Betula pubescens* is distributed throughout the northwest quarter of the Iberian Peninsula and the western half of the Pyrenees; while *B. pendula* populations occur along the eastern half of the Peninsula. *B. pendula* subspecies *fontqueri* is found mainly in scattered localities in the centre and SE mountains of Spain. It is also possible to find some relictic populations in mountains of Morocco (Moreno and Peinado 1990). Two varieties have been recognized within this subspecies (Moreno and Peinado 1990). The variety *fontqueri* may be found in the mountains of the 'Sistema Central' (in the center of the Peninsula) and in the mountains of the south of the country ('Sierra Nevada' and 'Sierras of Cazorla, Segura and Las Villas'). Its populations appear scattered and a significantly reduced number of individuals are found. The other variety of the subspecies *fontqueri*, var. *parvibracteata*, occurs in 'Montes of Toledo' and 'Sierra Morena' (in the centre of the country). In many cases, the distribution border between both varieties of the subspecies is not very clear.

Morphologically, *B. pubescens* differs from *B. pendula* mainly in the hairiness of young twigs and nutlets; which are glabrous in *B. pendula,* and often hairy in *B. pubescens.* Hence, the main difference that distinguishes the two *B. pendula* subspecies is: in subsp. *fontqueri* the fruit wings are overtopped by the styles; while in subsp. *pendula* wings are longer, or as long as the styles.

Betula pendula subsp. *fontqueri* reduced distribution (relictic populations, isolated in mountain areas) together with its low density, which has even been reduced in the last years, has resulted in its inclusion in the Red List of Plants of the IUCN, classified as endangered (IUCN 2001). The number of individuals in most of these populations reaches only a few hundred, although some populations can be made up of a few thousand. This situation reaches a critical level in 'Sierras of Cazorla, Segura and Las Villas' where all the populations amount to less than fifty trees. The localization of these populations in different Regional Government protected areas ('National Park of Sierra Nevada' and 'Natural Park of Sierras of Cazorla, Segura and Las Villas' in Andalusia – both of them are also biosphere reserves – and 'National Park of Cabañeros', in Castilla-La Mancha) has served to give a certain degree of protection to this subspecies. Yet, the lost of *B. pendula* subsp. *fontqueri* individuals urges for the development of more direct measures to preserve these populations. Some steps have already been taken; for instance, the Andalusia Regional Government has included the preservation of populations of this taxon as a priority in its conservation programs and recovery plans (Hernández Bermejo and Clemente Muñoz 1994, Blanca et al. 1999).

It is well known that it is essential to determine genetic diversity and structure of natural plant populations in order to assess conservation strategies (Holsinger and Gottlieb 1991). Not only for conservation purposes, but also to design rational ways of economic exploitation, knowledge of the extent and distribution of genetic variation may become a very helpful tool (Holsinger and Gottlieb 1991).

The study of *B. pendula* subsp. *fontqueri* populations in Spain can be of special interest to establish an adequate conservation strategy, which could match their potential use in the south of Spain. Due to its invasive nature in degraded habitats, and its ornamental value, its use for reforestation is increasing. Other traditional uses of the species are for timber production, and as a source of diverse substances with a wide range of applications, mainly in the pharmacological industry. The use of all these products, in a sustainable way, can contribute to the maintenance of the endangered populations of this taxon.

Different methods of DNA fingerprinting have proved to be useful, with a wide range of appli-

cations in plant population studies, such as the detection of genetic variation within and between populations, the characterisation of clones, the analysis of breeding systems, and the analysis of ecogeographical variation (Weising et al. 1995). One of the most commonly used methods is the PCR-derived RAPD (random amplified polymorphic DNA) (Williams et al. 1990), which has been employed in many plant population structure studies in recent years (reviews in Bartish et al. 1999, Bussell 1999, Nybom and Bartish 2000, Nybom 2004, Romeiras et al. 2007). Although RAPDs have been questioned, mainly due to their lack of reproducibility, several authors have shown that these problems may be solved provided that an appropriate amplification protocol is carefully followed (Parker et al. 1998). Besides, this technique offers two key advantages, firstly, it does not need large amounts of DNA for amplification reactions; and secondly, no previous knowledge of DNA sequences is required, which is quite interesting when working with wild plant species, for which this kind of information is unknown.

Therefore, the RAPD technique is widely employed in genetic structure population studies for many different plant species (Nybom 2004, Wesche et al. 2006), including some *Betula* species: *B. alnoides* (Zeng et al. 2003), and *B. maximowicziana* (Tsuda et al. 2004).

In the present study, a genetic analysis of individuals from different populations of *B. pendula* subsp. *fontqueri* from the Iberian Peninsula has been carried out. Some populations of a closely related taxon have been included (*B. pubescens* from the mountains in the centre of the Peninsula) to establish taxonomic differences and relatedness. The genetic study of the population structure of *B. pendula* subsp. *fontqueri* in Spain must be of great value for the establishment of a conservation strategy.

2 Materials and Methods

2.1 Plant Material

Ninety-four plants from six populations of *B. pendula* subsp. *fontqueri* were studied to assess their genetic variability. Five of them belonged to variety *fontqueri* and one population corresponded to variety *parvibracteata* (population from Riofrío). Twenty samples (ten per population) from two populations of the other Spanish *Betula* species, *B. pubescens,* were included in the study. These two populations were chosen due to their sharing the same distribution area as the *B. pendula* populations studied. Details of the populations are given in Table 1, and their geographical location is represented in Fig. 1.

Table 1. Taxonomic identity, location, estimated population size (number of individuals), number of samples and code used for each of the *Betula* studied populations. For geographic localization of populations see Fig. 1.

Population	Taxon	Region	Estimated population size	No. of samples	Code
Pontones	*Betula pendula* subsp. *fontqueri*	Sierras of Cazorla, Segura and Las Villas	20	11	P
Acebeas	*Betula pendula* subsp. *fontqueri*	Sierras of Cazorla, Segura and Las Villas	<10	2	A
Aguascebas	*Betula pendula* subsp. *fontqueri*	Sierras of Cazorla, Segura and Las Villas	<10	4	G
Riofrío	*Betula pendula* subsp. *fontqueri*	Montes of Toledo	20000	10	R
Somosierra	*Betula pendula* subsp. *fontqueri*	Sistema Central	≈300	17	S
Sierra Nevada	*Betula pendula* subsp. *fontqueri*	Sierra Nevada	≈200	50	N
Canencia	Betula pubescens	Sistema Central	≈1000	10	C
La Ventilla	Betula pubescens	Montes of Toledo	250	10	V

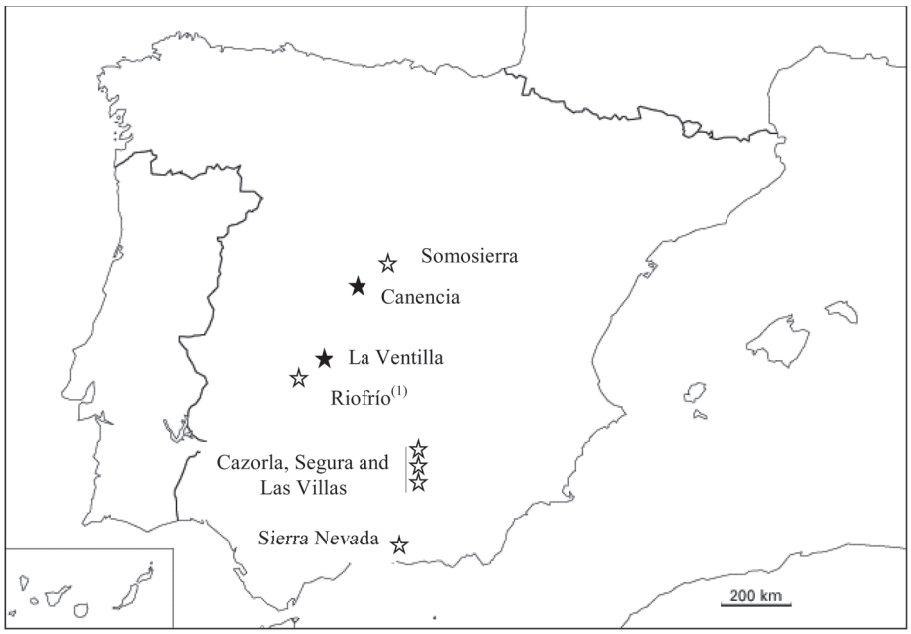

Fig. 1. Geographical location of the *Betula* populations studied in this work. ★ *B. pubescens,* ☆ *B. pendula* subsp. *fontqueri,* (1) the only var. *parvibracteata* population of *B. pendula* subsp. *fontqueri*, the other are var. *fontqueri.*

2.2 DNA Isolation

DNA was extracted from a small amount of tissue (20 mg approx.) of young leaves from single individuals using a modification of the cetyltrimethylammonium bromide (CTAB) protocol described by Gawel and Jarret (1991). The obtained DNA pellet was redissolved in 100 µL of sterile distilled water. DNA concentrations were estimated by a Hoefer TKO 100 DNA fluorometer.

2.3 PCR and Electrophoresis

Twenty decanucleotides of arbitrary sequence obtained from Operon Technologies Inc. (Alameda/CA, USA) were tested for PCR amplification. Six of them were chosen to assess the genetic variability of the samples: OPO-4, OPO-12, OPO-14, OPO-15, OPO-16 and OPO-20. DNA amplification reactions were performed in a volume of 25 µL containing approximately 10 ng of template DNA, 0.2 µM of a single decanucleotide, 200 µM of each dNTP and 1.2 units of *Taq* polymerase in the buffer provided by the manufacturer of the enzyme (*Biotaq of Bioprobe*). The reaction mixture was overlaid with a drop of mineral oil. Amplification was performed in a DNA Thermal Cycler PTC-100 ™ (MJ Research Inc.) programmed as follows: one cycle of 1 min at 94 °C, 35 cycles of 30 s at 92 °C, 1 min at 37 °C and 2 min at 72 °C, and finally one cycle of 3 min at 72 °C. Aliquots of 12 µL of amplification products were loaded on to 1.5% (w/v) agarose gels for electrophoresis in 1 × TBE buffer (Sambrook et al. 1989), followed by staining in ethidium bromide. The gels were visualised and photographed under UV light. Molecular weights were estimated by reference to a 100 Base-Pair Ladder (Pharmacia). All the amplifications were repeated at least twice, and only bands reproducible in several runs were considered for analysis.

2.4 Data Analysis

Specific amplification products were scored as present (1) or absent (0). The Jaccard coefficient

(Rohlf 1992) was employed to create the similarity matrix in order to construct a dendrogram by the UPGMA method (Rohlf 1992).

Genetic diversity was estimated using Shannon's information measure (Lewontin 1972) $H' = -\Sigma p_i \log_2 p_i$, where p_i is the frequency of a given RAPD fragment. Shannon index was calculated for two levels: the average diversity within populations (H'_{pop}), and the diversity within species (H'_{sp}). The proportion of diversity within populations can then be estimated as H'_{pop}/H'_{sp}, and the proportion of diversity among populations as $(H'_{sp}-H'_{pop})/H'_{sp}$.

In addition, the analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was implemented to estimate variance components for RAPD phenotypes, partitioning the variation among populations and among individuals within populations. The vector of marker presence/absence states for each individual was used to compute the distance metric $D = 100\,(1-F)$ for all pairs of individuals, where F is Nei and Li's (1979) estimator of similarity ($F = 2n_{xy}/n_x + n_y$), n_x and n_y being the total number of markers observed in individuals x and y, respectively, and n_{xy} the number of markers shared by the two individuals. The resulting distance matrix was subjected to AMOVA analysis. The significance level of variance component estimates was computed by non-parametric permutation procedures. Pairwise Φ_{ST} distances (analogous to F-statistics at the molecular level; Excoffier et al. 1992) were calculated among populations, and their level of significance were also tested by a permutation procedure. All analyses were undertaken with AMOVA version 1.55, provided by Laurent Excoffier (Genetics and Biometry Laboratory, University of Geneva, Switzerland). A Mantel matrix correspondence test was used to analyse correlation between genetic (Φ_{ST} values) and geographical distances among populations.

The use of Φ_{ST} values allowed for the estimation of the effective number of migrants (N_m) between populations [$N_m = ¼\,(1'/\Phi_{ST} - 1)$] as an estimator of gene flow (Freitas and Brehm 2001, Wright 1951).

3 Results

3.1 The RAPD Profile

A total of 101 markers (monomorphic as well as polymorphic) obtained with the six primers were used for the analysis of the 114 samples from the eight populations belonging to the two species studied. From them, 83 markers were found in *B. pendula* and 81 in *B. pubescens*. Sixty-two bands (61.2%) were shared by both species, seven of them being present in all the individuals.

Considering only the markers found in *B. pendula,* 10.89% (11 markers) were monomorphic, including the seven monomorphic for both species. Thirty one out of 83 markers present in *B. pendula* (37.35%) were shared by all the populations, while two markers (2.4%) were exclusive to Riofrío population, three (3.61%) were found only in Sierra Nevada population and other two (2.4%) in Somosierra; however, no exclusive markers were found in any of the populations from the protected area 'Sierras of Cazorla, Segura and Las Villas'. From the 81 markers found in *B. pubescens,* 24.69% (20 markers) were monomorphic and 49.38% (40) were shared by the two studied populations of this species. One of the monomorphic markers found in *B. pubescens* samples was exclusive for this species; however, no specific marker for *B. pendula* was found. Table 2 summarised the total number of markers

Table 2. Total number of markers observed for each *B. pendula* and *B. pubescens* population and number of polymorphic, monomorphic and population-specific markers in each case.

Population	Number of markers	Polymorphic markers	Monomorphic markers	Population-specific markers
Betula pendula subsp. *fontqueri*				
Pontones	55	11	44	-
Acebeas	47	6	41	-
Aguascebas	58	15	43	-
Riofrío	59	22	37	2
Sª Nevada	55	27	28	3
Somosierra	52	24	28	2
Betula pubescens				
La Ventilla	58	15	43	5
Canencia	63	39	24	11

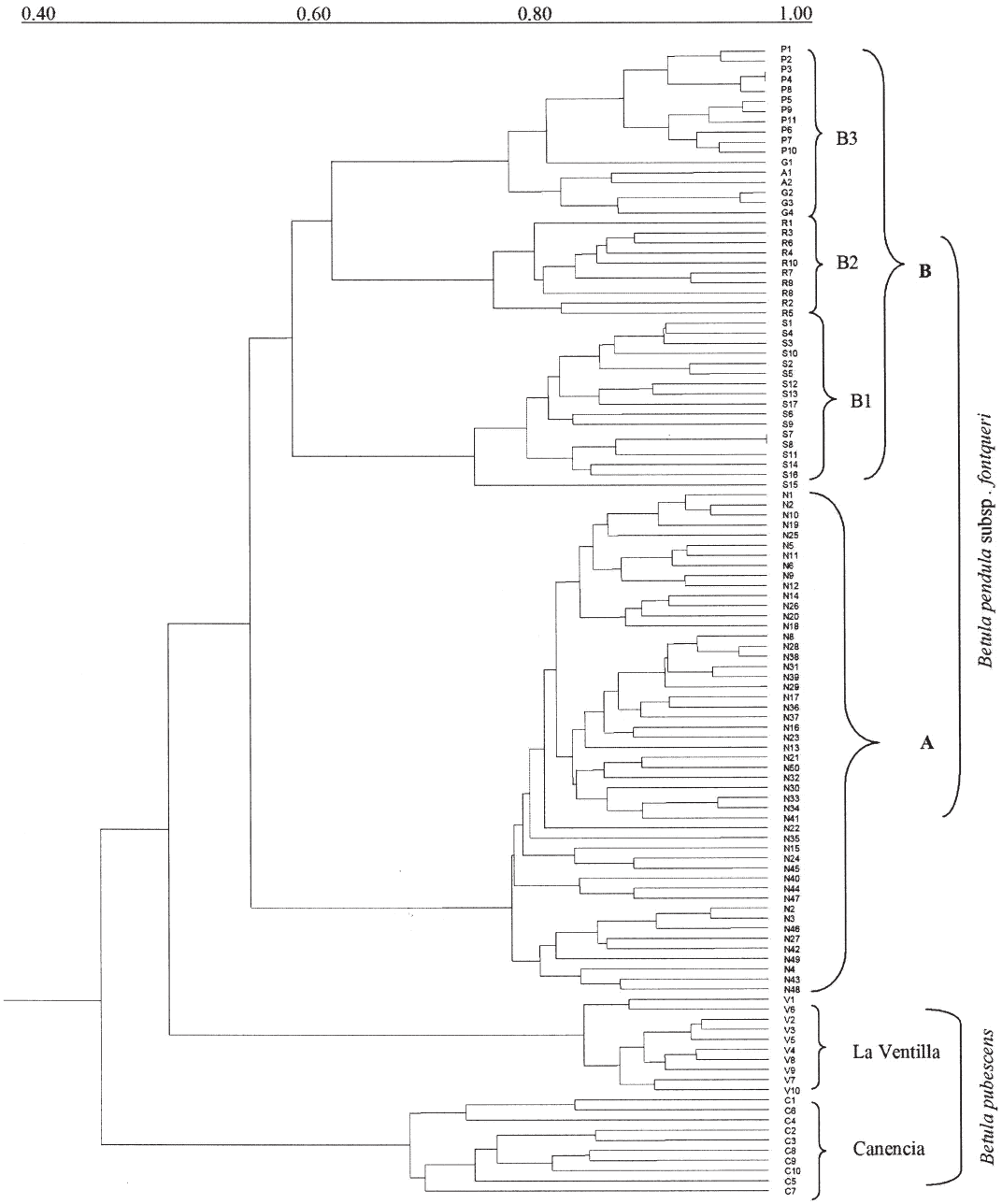

Fig. 2. Dendrogram of all the samples analysed from the *Betula* populations, using the UPGMA clustering method. Data derived from the RAPD analysis. (*B. pendula* subsp. *fontqueri* populations: P, Pontones; A, Acebeas; G, Aguascebas; R, Riofrío; S, Somosierra; N, Sierra Nevada).

observed for each population from each species, and the number of monomorphic and specific markers in each case.

3.2 Cluster Analysis

The dendrogram obtained by the UPGMA method from the 101 markers scored in the 114 samples of *B. pendula* and *B. pubescens* is shown in Fig. 2. Except in two cases, all the individuals showed a unique RAPD phenotype. The exceptions were found in two individuals from Pontones (P3 and P4) which had a 100% similarity, and other two individuals from Somosierra (S7 and S8).

Samples of the two *B. pubescens* populations included in the study were clearly separated from the *B. pendula* individuals. At the same time, each of these two populations presented clear differences one from the other, clustering in different groups. The highest homogeneity between individuals observed in all the populations studied was found in one of these *B. pubescens* populations, La Ventilla, with a level of similarity of 84%. In contrast, the other population of the same species, Canencia, showed the lowest similarity level within a given population.

The main cluster, which included all the *B. pendula* populations, was clearly subdivided into two groups, one containing all samples from the Sierra Nevada population (group A), and the other with the individuals from the other populations: Somosierra, Riofrío, Aguascebas, Acebeas and Pontones (group B). From all the *B. pendula* populations, Sierra Nevada showed the highest homogeneity between individuals (79% level of similarity).

Group B has three subgroups: B1, B2 and B3. Subgroup B1 corresponded to Somosierra individuals; subgroup B2 contained all the samples from Riofrío, which belongs to a different variety (var. *parvibracteata*) than the other *B. pendula* populations that correspond to var. *fontqueri.* Subgroup B3 clustered all the samples from Pontones, Acebeas and Aguascebas, and only Pontones samples were clearly separated in a cluster; individuals from the other two populations clustered together. The high similarity of these samples can be explained since the three populations belong to the same region (Sierras of Cazorla, Segura and Las Villas), included in a same protected area and very close each other.

3.3 Genetic Variation within and among Populations

Genetic variation in *B. pendula* subsp. *fontqueri* populations was estimated through Shannon's information measure and AMOVA. The two smallest populations considered in this work, Acebeas and Aguascebas, were excluded due to their extremely low number of individuals; their inclusion could result in a biased analysis. Therefore, the populations finally included in the analyses were Pontones, Riofrío, Somosierra and Sierra Nevada.

Estimates of Shannon's index of phenotypic diversity resulted in a mean diversity within the species (H'_{sp}) equal to 3.5780. The mean diversity within populations resulted in 1.1293, and the lowest level of within-population variability was found in Pontones population ($H'=0.7$). In addition, the highest diversity was found in Riofrío, the only population of var. *parvibracteata*, with $H'=1.5457$. Therefore, the proportion of the diversity within populations (H'_{pop}/H'_{sp}) was 36.38%, whereas the diversity among populations ($[H'_{sp}-H'_{pop}]/H'_{sp}$) resulted in 63.62% of the total diversity.

AMOVA obtained from the distance matrix (Table 3) showed highly significant ($P<0.001$) genetic differences among populations and among individuals. Estimation of genetic variation by AMOVA revealed that 64.22% of total variation was found among populations, and 35.78% within populations. These results are very similar to that obtained with Shannon´s measure.

3.4 Relationships between Populations

The genetic distances among populations obtained from the AMOVA (distances = Φ_{ST} between pairs of populations) showed large differences among populations, even when considering populations that clustered together. The level of genetic distance was in all cases very high and quite similar among all the different populations, ranging from 0.7106 (genetic distance between Sierra Nevada

Table 3. Analysis of molecular variance (AMOVA) for 84 individuals of *Betula pendula* subsp. *fontqueri* from the populations of Pontones, Riofrío, Sierra Nevada and Somosierra using 83 RAPDs markers. Statistics include sums of squared deviations (SSD), mean squared deviations (MSD), variance component estimates, the percentage of the total variance contributed by each component and the probability of obtaining a more extreme component estimate by chance alone.

Source of variation	d.f.	SSD	MSD	Variance component	% total variance	P
Among populations	3	476.69	158.90	8.59	64.22	<0.001
Within populations	84	402.20	4.79	4.79	35.78	<0.001

and Pontones) to 0.5927 (distance between Riofrío and Sierra Nevada) (Table 4). It is possible to consider every population clearly differentiated, since all distances between pairs of populations were significantly different from zero. Geographic distance did not explain the genetic distance among populations since the matrix of genetic distances among the four populations was not correlated with the corresponding matrix of geographic distances (Mantel Test: r=–0.431; p=0.18), a result that can be observed in the data of Table 4. Furthermore, the geographically closest populations (Pontones and Sierra Nevada) had the highest genetic distance.

Nm values are shown in Table 5. They were always lower than 1.0, suggesting a low level of gene flow between populations.

Table 4. Genetic distances, represented by the Φ_{ST} values (below diagonal), and geographical distances (above diagonal) in Km, between the four *B. pendula* subsp. *fontqueri* populations considered in the diversity partition analysis.

	Pontones	Riofrío	Somosierra	Sierra Nevada
Pontones	-	205	346	153
Riofrío	0.6639	-	236	261
Somosierra	0.6727	0.5956	-	515
Sierra Nevada	0.7106	0.5927	0.6066	-

Table 5. Effective number of migrants per generation (N_m) between the four populations of *Betula pendula* subsp. *fontqueri* considered in this work.

	Pontones	Riofrío	Somosierra	Sierra Nevada
Pontones	-			
Riofrío	0.1266	-		
Somosierra	0.1216	0.1697	-	
Sierra Nevada	0.1018	0.1718	0.1621	-

4 Discussion

The analyses of RAPD markers through different methods (cluster analysis, Shannon´s index and AMOVA) have revealed very similar interpretations of the genetic structure of the populations considered.

The populations of *Betula pubescens* included in this work were selected because of their geographical proximity to some of the *B. pendula* subsp. *fontqueri* studied. The results clearly separated *B. pubescens* and *B. pendula* populations, also showing a clear genetic differentiation between both *B. pubescens* populations.

The level of genetic diversity found in the *B. pendula* subsp. *fontqueri* populations from the Iberian Peninsula studied in this work might be considered high, since the 89.11% of RAPD´s markers obtained were polymorphic. This result is in accordance with the studies of other *Betula* species. Zeng et al. (2003), in their study of *Betula alnoides* populations, found that 64.1% of the RAPD markers were polymorphic. In this work, similar results from *Betula platyphylla* (Gao et al.

1999) are also cited. High levels of genetic diversity in *Betula* species are considered a natural effect of their life history and breeding systems (Zeng et al. 2003), since they are long-life perennial plants with outcrossing reproduction.

Populations of *B. pendula* subsp. *fontqueri* can be clearly distinguished from the dendrogram obtained. But only the three populations included in the protected area 'Sierras of Cazorla, Segura and Las Villas' clustered together. Within this cluster, it is possible to distinguish a well-defined group, containing all the samples from Pontones population; while samples from the other two populations of the area appear mixed. Another data obtained from the cluster, and corroborated by Shannon's index, is that Pontones shows the lowest within-population diversity. The high similarity found between individuals from this population could be due to its allegedly anthropic origin. In this case, the initial material could derive from one of the closest populations (probably Acebeas or Aguascebas), and thus explaining the close genetic relationship that is reflected in the dendrogram.

The observed difference between Sierra Nevada population and the other populations is very significant. Even the only var. *parvibracteata* population included in this work (Riofrío population) turned out to be more closely related to the other var. *fontqueri* populations than Sierra Nevada population. These unexpected results call for a special consideration of the Sierra Nevada population for conservation purposes. Likewise, a revision of the taxonomic consideration of Riofrío population would be helpful.

The study of the structure of the populations revealed an unusual distribution of the total diversity, within and among populations, for an outcrossing species. The level of diversity among populations, obtained from both Shannon's index and AMOVA, was near 60%. It is usually expected that long-lived outcrossing tree species retain most of their genetic variation within populations, but a wide and continuous range of distribution is necessary (Hamrick et al. 1992). Studying an outcrossing species (*Aconitum lycoctonum*), Utelli et al. (1999) found that the percentage of variation among populations did not completely correspond to the expected values for the breeding systems analysed. Not only the reproductive characteristics, but also data on eco-geographical differences and natural history of populations have to be considered, since they can affect genetic diversity (Utelli et al. 1999). In this sense, *B. pendula* populations considered in this work are geographically very distant from each other, and, in some cases, with important natural barriers separating them.

The history of the genus in the Iberian Peninsula could explain the high values of genetic distance between the *B. pendula* populations obtained in this work. During the Quaternary, the mountains of the Iberian Peninsula, and mountains of the south of Europe in general, were refugia for many species (not only plant but also animal species). The *Betula* populations in the Iberian Peninsula derived from those refugia are relictic populations. The isolation of these populations has contributed to the initiation of the process of taxonomic differentiation (Costa et al. 1997). In this case, the existence of a geographical barrier as is the wide depression (Baza depression) between the protected area of 'Sierras of Cazorla, Segura and Las Villas' and Sierra Nevada, which implies an interruption in the distribution of the species, could explain the differences found between these populations. Another historical factor that may help to explain this difference is the isolation of Sierra Nevada mountains during the last glacial period.

The pairwise genetic distances (Φ_{ST}) between populations obtained in the AMOVA analysis confirm the clear differentiation among populations of *B. pendula*. All Φ_{ST} values were significant and ranged from 0.5927 to 0.7106, with a mean value of 0.6403. This strong genetic differentiation among populations suggests that gene flow among the populations is very low. Genetic differentiation among populations has been reported in a number of other rare plant species and has been attributed to the absence of interpopulation gene flow (see Martin et al. 1999).

In similar genetic structure studies of other *Betula* species, the Φ_{ST} values were significant lower than the values that we have found in *B. pendula.* Tsuda et al. (2004) found a mean Φ_{ST} value of 0.156 in *Betula maximowicziana,* and even lower [Φ_{ST} ranging from 0.046 to 0.146 with a mean of 0.090] were the values obtained by Zeng et al. (2003) in *Betula alnoides.* RAPD-based esti-

mates of Φ_{ST} values are significantly correlated to live form (Nybom and Bartish 2000, Nybom 2004). According with this correlation, long-lived perennials are expected to show the lowest values (with a mean Φ_{ST} value of 0.25), and species with a mixed breeding present intermediate values (ranging from 0.25 to 0.4) (Nybom and Bartish 2000, Nybom 2004). Values obtained for *B. maximowicziana* and *B. alnoides* are in accordance with this explanation of Φ_{ST} values; however, the value obtained for *B. pendula* in this work is unexpectedly high. Similar unexpected high Φ_{ST} values were obtained in the Asian mountain endemic *Galitzkya macrocarpa* (Wesche et al. 2006), whose populations are strongly isolated. In this and other studies on alpine plants with high Φ_{ST} values, these results were interpreted as evidence of prolonged isolation, assumed to date back to the last glacial period (Schönswetter et al. 2002, 2004, Reisch et al. 2003, Wesche et al. 2006). The history of the *B. pendula* populations in the Iberian Peninsula is in accordance with this explanation.

Although the genetic distances between *B. pendula* subsp. *fontqueri* populations in Spain are significantly high, these differences did not correlate with the spatial distance. Loveless and Hamrick (1984) found just a weak correlation between geographical range and population differentiation; and it must be considered that geographical differentiation is frequently related to environmental differences across the range of a species. Differences among the populations studied in this work must be mainly due to the isolation originated by its life-history and, most recently, by habitat fragmentation.

5 Recommendations for Conservation

Our study on the genetic structure of *Betula pendula* subsp. *fontqueri* populations in the Iberian Peninsula can provide valuable information for the management and conservation of this endangered taxon. The genetic results obtained clearly showed a great differentiation between populations as a result of their historical isolation. Estimates of gene flow, based on Φ_{ST} values (number of migrants per generation, N_m, Table 5), give a mean value for *B. pendula* subsp. *fontqueri* of 0.1423 exchanged individuals per generation. This result is quite far from the minimum of one migrant per generation that is considered enough to maintain genetic exchange, and that indicates 'severe fragmentation' (IUCN 2001).

The diversity measured in this work is, however, considerably high. This can mean that a significant loss of diversity through genetic drift has not been detected, probably because the size of the populations is not critically low (Ellstrand and Elam 1993). Different considerations must be taken into account in populations from 'Sierras of Cazorla, Segura and Las Villas', where the limited population size could affect the diversity, and in consequence, their survival.

Although at this moment the size of some of the populations included in this work could be big enough to maintain a high level of diversity, the severe isolation suffered by this taxon makes it necessary to develop conservation strategies in order to avoid population decrease and, therefore genetic depauperation. Other factors such as environmental stochasticity and anthropic alterations of the habitat should be considered.

In conclusion, and taking into account the management of the endangered populations, we suggest the following guidelines:

The strong genetic differentiation found among populations of *Betula pendula* subsp. *fontqueri* allows us to suggest that each population should be considered as a distinct management unit. This implies that ex situ conservation strategies (seed collected, clonal multiplication, etc.) should be separately developed; and beside, distinct conservation programmes should be implemented for the in situ management of *Betula pendula* subsp. *fontqueri* populations, with the exception of the three populations located in the Natural Park of 'Sierras of Cazorla, Segura and Las Villas' (Pontones, Acebeas and Aguascebas), which could be considered as a same management unit.

The high genetic differences between Sierra Nevada and the other populations suggest the need to consider separately, and with special attention, the development of the management conservation programme for this population.

From a taxonomic point of view, the slight differences found between the Riofrío popula-

tion (which is classified as var. *parvibracteata* within this taxon) and the other populations from the var. *fontqueri,* should be considered. And then, its taxonomic status should be clarified in order to design a more convenient conservation programme for the species. Similarly, a taxonomic revision of Sierra Nevada population could explain the great differences found between this population and the others.

Finally, the use of RAPDs to determine the genetic structure of *Betula pendula* subsp. *fontqueri* populations has proved to be a useful tool. Besides, RAPDs should find a wider range of uses for plant population studies, notably genetics applications, in the field of genetic conservation, where molecular markers need to be developed at a reasonable cost (Hardy 2003).

Acknowledgements

This work was supported by the Spanish Government CICYT project no. AMB 96-C02-01. We thank the authorities of the Consejería de Medio Ambiente from the regional Government of Andalusia, and especially the curators of the Natural Parks of Sierra Nevada and Cazorla, Segura and Las Villas, as well as the managers of the National Park of Cabañeros. We also thank our colleagues Pilar Contreras, Josefa Prados, Alfonso Jimenez and José M. Iriondo for their collaboration with the field work. We are especially grateful to M. Elena González-Benito for his helpful comments and suggestions on this manuscript, and to Deborah McAllister for her English revision of the text.

References

Bartish, I.V., Jeppsson, N. & Nybom, H. 1999. Population genetic structure in the dioecious pioneer plant species Hippophae rhamnoides investigated by random amplified polymorphic DNA (RAPD) markers. Molecular Ecology 8: 791–802.

Blanca, G., Cabezudo, B., Hernandez-Bermejo, J.E., Herrera, C.M., Molero Mesa, J., Muñoz, J. & Valdés, B. (eds.). 1999. Libro rojo de la flora silvestre amenazada de Andalucía. Tomo I: Especies en peligro de extinción. Consejería de Medio Ambiente, Junta de Andalucía, Sevilla.

Bussell, J.D. 1999. The distribution of random amplified polymorphic DNA (RAPD) diversity amongst populations of Isotoma petraea (Lobeliaceae). Molecular Ecology 8: 775–789.

Costa, M., Morla, C. & Sainz, H. (eds.). 1997. Los bosques ibéricos. Ed. Planeta, Barcelona.

Ellstrand, N.C. & Elam, D.R. 1993. Population genetic consequences of small population size: implications for plant conservation. Annual Review of Ecology and Systematics 24: 217–242.

Excoffier, N.C., Smouse, P.E. & Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA restriction data. Genetics 131: 479–491.

Freitas, H. & Brehm, A. 2001. Genetic diversity of the Maqcaronesian leafy liverwort Porella canariensis inferred from RAPD markers. Journal of Heredity 92: 339–345.

Gao, Y.K., Nie, S.Q. & Zu, Y.G. 1999. Genetic structure analysis by RAPD in Betula platyphylla nature population in Northeast of China. In: Zu, Y.G., Sun, M. & Kang, L. (eds.). The application, method and theory of molecular ecology. China Higher Education Press Beijing & Springer-Verlag Heidelberg, Beijing, China. p. 196–205. (In Chinese).

Gawel, N.J. & Jarret, R.L. 1991. A modified CTAB DNA extraction procedure for Musa and Ipomoea. Plant Molecular Biology Reporter 9: 262–266.

Hamrick, J.L. 1990. Isozymes and the analysis of genetic structure in plant populations. In: Soltis, D.E. & Soltis, P.S. (eds.). Isozymes in plant biology. Chapman & Hall, London. p. 87–105.

— , Godt, M.J.W. & Sherman-Broyles, S.L. 1992. Factors influencing levels of genetic diversity in woody plant species. New Forests 6: 95–124.

Hardy, O.J. 2003. Estimation of pairwise relatedness between individuals and characterization of isolation-by-distance processes using dominant genetic markers. Molecular Ecology 12: 1577–1588.

Hernández Bermejo, J.E. & Clemente Muñoz, M. (eds.). 1994. Protección de la Flora en Andalucía. Consejería de Cultura y Medio Ambiente, Junta de Andalucía, Sevilla.

Holsinger, K.E. & Gottlieb, L.D. 1991. Conservation of rare and endangered plants: principles and prospects. In: Falk, D.A. & Holsinger, K.E. (eds.). Genetics and conservation of rare plants. Oxford

University Press, New York. p. 195–223.
I.U.C.N. 2001. IUCN Red list categories and criteria. IUCN, Gland, Switzerland.
Lewontin, R.C. 1972. The apportionment of human diversity. Evolutionary Biology 6: 381–394.
Loveless, M.D. & Hamrick, J.L. 1984. Ecological determinants of genetic structure in plant populations. Annual Review of Ecology and Systematics 15: 65–95.
Martín, C., González-Benito, M.E. & Iriondo, J.M. 1999. The use of genetic markers in the identification and characterization of three recently discovered populations of a threatened plant species. Molecular Ecology 8: S31–S40.
Moreno, G. & Peinado, M. 1990. Betulaceae. In: Castroviejo, S., Laínz, M., López González, G., Montserrat, P., Muñoz Garmendia, F., Paiva, J. & Villar, L. (eds.). Flora iberica, Vol. 2. Real Jardín Botánico, CSIC, Madrid. p. 38–43.
Nei, M. & Li, W.-H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences 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.
— & Bartish, I.V. 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.
Parker, P.G., Snow, A.A., Schug, M.D., Booton, G.C. & Fuerst, P.A. 1998. What molecules can tell us about populations: choosing a molecular marker. Ecology 79: 361–382.
Reisch, C., Poschlod, P. & Wingender, R. 2003. Genetic variation of Saxifraga paniculata Mill. (Saxifragaceae): molecular evidence for glacial relict endemism in central Europe. Biological Journal of the Linnean Society 80: 11–21.
Rohlf, F.J. 1992. NTSYS-PC: numerical taxonomy and multivariate analysis system. Exeter Software, New York.
Romeiras, M.M., Cotrim, H.C., Duarte, M.C. & Pais, M.S. 2007. Genetic diversity of three endangered species of Echium L. (Boraginaceae) endemic to Cape Verde Islands. Biodiversity and Conservation 16: 547–566.
Sambrook, J., Fritsch, E.F. & Maniatis, T. 1989. Molecular Cloning: a Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, New York.
Schönswetter, P., Tribsch, A., Barfuss, M. & Niklfeld, H. 2002. Several Pleistocene refugia detected in the high alpine plant Phyteuma globulariifolium Sternb & Hoppe (Campanulaceae) in the European Alps. Molecual Ecology 11: 2637–2647.
— , Tribsch, A., Stehlik, I. & Niklfeld, H. 2004. Glacial history of high alpine Ranunculus glacialis (Ranunculaceae) in the European Alps in a comparative phylogeographical context. Biological Journal of the Linnean Society 81: 183–195.
Tsuda, Y., Goto, S. & Ide, Y. 2004. RAPD analysis of genetic variation within and among four natural populations of Betula maximowicziana. Silvae Genetica 53: 234–239.
Utelli, A.-B., Roy, B.A. & Baltisberger, M. 1999. History can be more important than 'pollination syndrome' in determining the genetic structure of plant populations: the case of Aconitum lycoctonum (Ranunculaceae). Heredity 82: 574–584.
Weising, K., Nybom, H., Wolff, K. & Meyer, W. 1995. DNA fingerprinting in plants and fungi. CRC Press, Boca Raton.
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.
Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. & Tingey, S.V. 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18: 6531–6535.
Wright, S. 1951 The genetical structure of populations. Annals of Eugenetics 15: 323–354.
Zeng, J., Zou, Y., Bai, J. & Zheng, H. 2003. RAPD analysis of genetic variation in natural populations of Betula alnoides from Guangxi, China. Euphytica 134: 33–41.

Total of 36 references