

Genetic differentiation in silicicolous *Echinospartum* (Leguminosae) indicated by allozyme variability

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Abstract. The genetic identities among several *Echinospartum* species and the genetic effects of isolation, small population size and decline in the restricted *Echinospartum algibicum* were studied both at adult and soil seed bank stages. The allozyme data support, to a large extent, population genetic predictions for genetic divergence. The genetic diversity parameters studied demonstrated that the aboveground population of *E. algibicum* is genetically less diverse than *E. ibericum* and *E. barnadesii*. Genetic identity between *E. ibericum* and *E. barnadesii* was very high, decreasing to levels considered typical for co-generic taxa between *E. ibericum* and *E. algibicum*. With regard to the soil seed bank of *E. algibicum*, this showed higher genetic variability than the adult population, greater extent of homozygosity, and significantly different allele frequencies at some loci. Despite the small population size, *E. algibicum* population maintains relatively high levels of genetic diversity both at adult and seed bank stages.

Key words: Allozymes, *Echinospartum*, endangered species, population differentiation, soil seed bank genetics, vicariant speciation.

It is generally accepted that the relative level of genetic variation can be a useful indicator of genetic history (Wolf et al. 1991), so one would expect the distribution of genetic variability to be similar in closely related taxa, where most

differences would mainly be the result of geographic range, demographic structure or reproductive system of the species (Olmstead 1990). In fact, many recent studies explore systematic, phylogenetic relatedness and ecological differentiation between populations, groups of populations and species by analysing genetic diversity based on allozyme variability (Gottlieb 1977, 1981; Olmstead 1990; Wolf et al. 1991; Chamberlain et al. 1996; Troia et al. 1997; Chamberlain 1998; Lee et al. 2000).

One of the reasons for plants becoming rare is due to natural or artificial habitat fragmentation and subsequent isolation. Moreover, persistent isolation can also lead to allopatric speciation by reducing or completely blocking gene flow between subpopulations which originally shared the same gene pool (Niklas 1997), but can also increase the rate at which genetic variation might be lost due to genetic drift (Amos and Hardwood 1998) and inbreeding depression (Hamrick and Godt 1989, Barrett and Kohn 1991, Dudash and Fenster 2000). Furthermore, rarity in plants has commonly been associated with a lack of polymorphism and higher risk of extinction (e.g. Godt and Hamrick 1998a). Nevertheless, Gitzendanner and Soltis (2000) have argued that this view of rare plants can be

an overgeneralization since a large body of experimental evidence has described highly polymorphic rare species (e.g. Young and Brown 1996). Analogously, Gitzendanner and Soltis (2000) have also stressed that studies comparing genetic diversity of threatened and rare plants should be done with co-generic or closely related taxa rather than with other unrelated threatened ones.

The existence of a persistent soil seed bank is common in a multitude of angiosperm taxa, playing an outstanding role in population ecology and evolutionary dynamics (Leck et al. 1989, Fenner 1992). From a genetic point of view, recruitment of seeds accumulated in a soil seed bank could theoretically mean a migration from the past and act as a genetic reservoir (Templeton and Levin 1979, Levin 1990). However, population genetic studies addressing questions such as the capability of seed banks to disperse genes through time, or to prevent homogenisation caused by random genetic drift, have scarcely been undertaken (Tonsor et al. 1993, Alvarez-Buylla et al. 1996, Cabin 1996, Cabin et al. 1998, McCue and Holstford 1998, Mahy et al. 1999). These could be specially interesting if dealing with taxa having small populations and facing risks for long-term survival (McCue and Holstford 1998).

The genus *Echinospartum* is considered to comprise five species forming two ecologically (silicolous vs calcicolous), anatomically, and karyologically unrelated groups (Talavera 1999). However, a taxonomic mess preceded this view and some other specific and subspecific taxa have been described (Rivas Martínez 1974, 1987; López 1982). Within the silicolous taxa, *E. algibicum* Talavera & Aparicio, related to *E. ibericum* Rivas Martínez, Sánchez Mata & Sancho and *E. barnadesii* (Graells) Rothm., was newly described showing a vicariant and restricted geographical distribution in high danger of extinction. This group of silicolous taxa seems to be a suitable case study, and here we present the results of a study using different allozymes for assessing the genetic identity at species

level along with the genetic effects of isolation, and small and declining population size in *E. algibicum*, both in the adult above-ground population and in the permanent soil seed bank.

Materials and methods

Plant taxa studied. *Echinospartum* is a genus in the family Leguminosae of long-lived spiny shrub mostly endemic to mountain areas (>1000 m above sea level) in the Iberian Peninsula and S France, and represented by either strictly calcicolous taxa [*E. boissieri* (Spach) Rothm. and *E. horridum* (Vahl.) Rothm.] or strictly silicolous ones (*E. barnadesii*, *E. ibericum* and *E. algibicum*). *Echinospartum barnadesii* is located in few sites within the geographical range of *E. ibericum*, which extends throughout central and north-western mountains of the Iberian Peninsula, with some isolated disjunct populations to the south (Fig. 1). Considered the southernmost population of this latter taxon (Aparicio and Silvestre 1987), *E. algibicum* was newly described based upon some morphological features, chromosome number and geographic isolation (Talavera and Aparicio 1995). In October 1994, the only known population of *E. algibicum* was found to be composed of 564 individuals, of which 89 were alive in a vegetative state (unable to set flowers) and only three, located at the top of rocky cliffs free from grazing, were able to flower and set fruit; the rest of the individuals were dead due mainly to intense and continuous grazing (Aparicio and Guisande 1995). In 1995 the site was fenced and protected from grazing and currently ca. 40 individual bushes have recovered blooming capacity. Later, Aparicio and Guisande (1997) showed the existence of a presumably ancient but viable (c. 80% germination rate) permanent soil seed bank of this species in a 0–15 cm soil depth layer with about 167 seeds m⁻². Geographical and genetic isolation of this species is stressed by pollination (predominantly performed by solitary-bees) and seed dispersal (small explosive legumes) (Ceballos, unpublished data) modes hindering the long range dispersal of genes.

Sampling. Five silicolous populations of *E. ibericum*, 1 of *E. barnadesii*, 1 of *E. algibicum* and 1 of the calcicolous *E. boissieri* for comparison, were sampled for this study (Fig. 1 and Table 1). Mature seed samples were collected from each

population (range 80–400 seeds) avoiding sampling more than 2–3 seeds from the same mother plant. Thirty randomly selected seeds from each population were then included in electrophoresis as this sample size should ensure a representation of alleles with moderate to high frequencies (Marshall and Brown 1975). The recovery of *E. algibicum* blooming capacity was a slow and cumulative process as recorded since 1997 to date. Therefore, due to the scarcity of seeds naturally produced in this species, allele frequencies in the population were calculated using a sample of flower buds collected from 30 adult plants. Previously, we checked that

E. algibicum flower buds showed identical isozyme activity (i.e. scoreable enzyme systems) and band intensity as a sample of fresh seeds (data not shown).

The genetic structure of the soil seed bank of *E. algibicum* was also studied by setting, in February 1997 and February 2000, eight 5 × 5 m plots across the population. In each plot, five randomly located 15 × 15 × 15 cm soil blocks averaging 3,75 kg per sample were dug out. These were left to dry and sieved selecting the fraction between 5 and 1 mm, where every *E. algibicum* seed could be carefully taken from the remaining material (Aparicio and

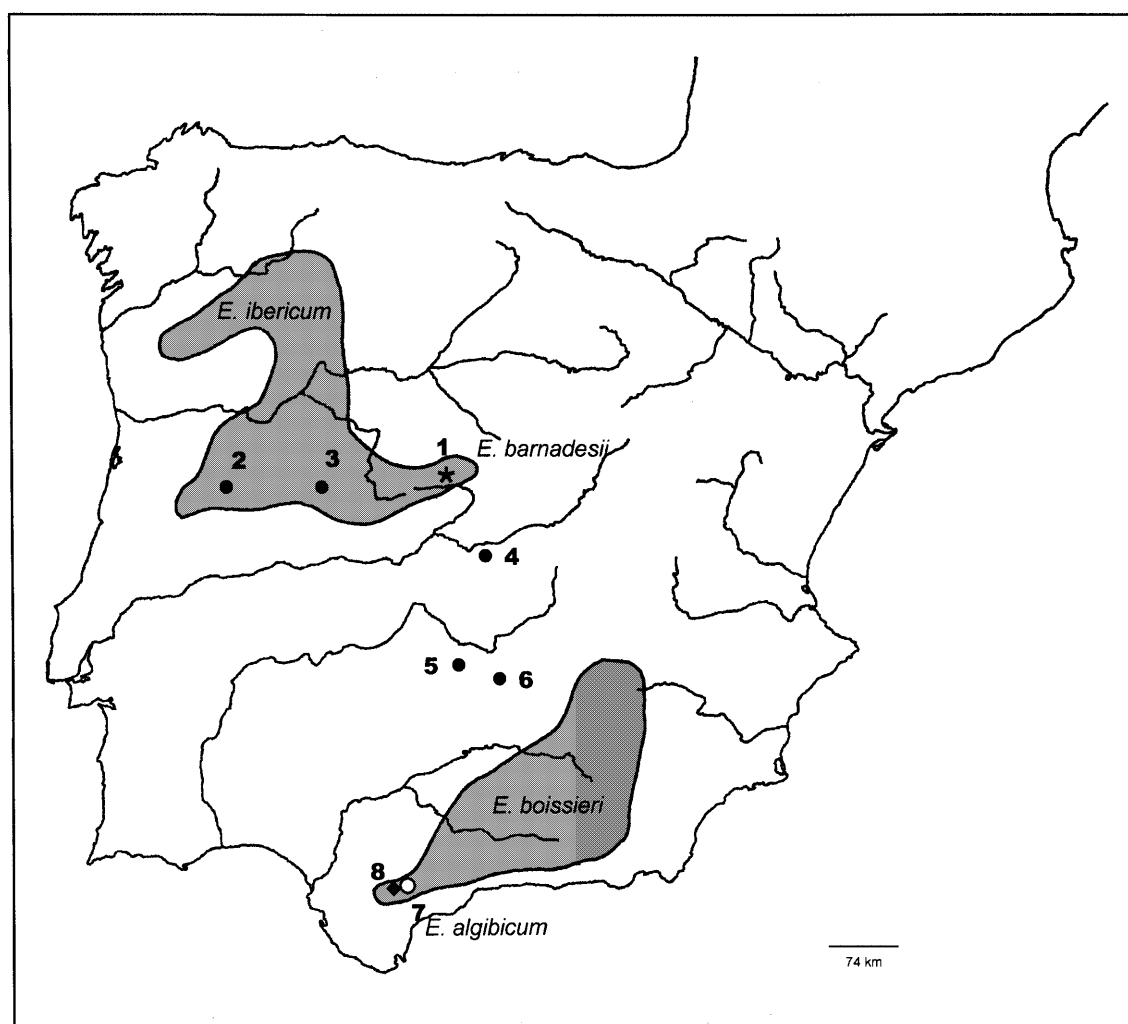


Fig. 1. Distribution area of the *Echinospartum* taxa and location of the studied populations. Shaded areas correspond to *E. ibericum* and *E. boissieri* distributions. Notice that populations 4, 5 and 6 of *E. ibericum* are isolated populations south of the main area. Distribution of *E. barnadesii* is within the *E. ibericum* area, both are silicolous. Despite the fact that the only population of *E. algibicum* is geographically within the distribution of *E. boissieri*, the former is strictly silicolous and the latter strictly calcicolous

Guisande 1997). A total of 106 seeds were recovered of which 77 were subject to electrophoresis (10 seeds from each sampled plot except for one in which only 7 were recovered). All the seeds in this sample were alive and gave as clearly scoreable bands after electrophoresis as contemporary seeds did.

Electrophoresis. All the seeds in this study were scarified, soaked in distilled water for two hours, and homogenised in three drops of DL-Dithiothreitol (0.065M) and Na₂HPO₄ (0.05 M) at pH = 7.00 buffer. The flower buds were collected in the field and kept refrigerated until protein extraction four hours later in the lab. These samples were homogenised in three drops of microbuffer containing 0.17% of β -Mercaptoethanol (Werth 1985). All the resulting extracts were immediately absorbed onto Whatman 3MM paper wicks of 3x15 mm, and kept at -80 °C until electrophoresis was performed less than a month later.

Electrophoresis was undertaken following the general protocols of Wendel and Weeden (1989) on a 12% starch, 2.5% sucrose gel. Nineteen enzymatic systems were tested, following the staining recipes of Soltis et al. (1983), although only five produced interpretable banding patterns. The enzyme systems isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH) and phosphoglucumutase (PGM) were resolved in histidine-citrate (pH 6.5/6.5) buffer (Cardy et al. 1983) and the leucine-amine peptidase (LAP) was resolved in the lithium hydroxide-boric acid/tris-citric acid (pH 8.1/ 8.0) (Ridgeway et al. 1970). Alcohol dehydrogenase (ADH) was resolved satisfactorily using both buffer systems. For those enzymes that presented more than one locus, the loci were numbered (and the alleles within them were labelled with letters) starting from the most anodally migrating form.

Data analyses. Genetic diversity was assessed by the proportion of polymorphic loci, at the 99% level (P_{99}), the mean number of alleles per locus (A) and per polymorphic locus (A_p), the effective number of alleles per locus (n_e) (Kimura and Crow 1964), the expected heterozygosity or gene diversity (H_e) (Nei 1978) and the observed heterozygosity (H_o). In order to compare the genetic structure between the soil seed bank and the adult population of *E. algibicum*, single locus differences in allele frequencies and inbreeding coefficient (F_{IS}) were calculated. Determination of statistical significance of allele frequencies between both stages was done by performing G -tests, where rare alleles with low frequencies were combined (see Tonsor et al. 1993). Estimated inbreeding coefficient confidence intervals were produced by bootstrapping over loci (2000 runs). Nei's genetic identity (I) values (Nei 1978) were calculated by pair-wise comparison of populations, and Nei's genetic distances among taxa were used to produce a dendrogram computed by UPGMA clustering (Sneath and Sokal 1973). All calculations, except G -tests, were made using the GDA (Genetic Data Analysis) software edited by Lewis and Zaykin (2000).

Results

Five enzyme systems were scored revealing eleven loci, two of them monomorphic (Table 2). Systems such as 6-phosphoglucuronate dehydrogenase (*6-PGD*) and phosphoglucosomerase (*PGI*) showed great activity but produced unscorable bands on the gels.

Several alleles were only present in *E. boissieri*, the species which possessed the highest number of private alleles (*Adh-1c*, *Adh-*

Table 1. Population location and size (number of adult individuals) of the four *Echinospartum* taxa studied

Species	Population number (#)	Location (UTM)	Altitude (m asl)	Population size	Soil type
<i>E. barnadesii</i>	1	Ávila: Hoyo del Espino (30S UK3315)	1520	> 1000	Siliceous
<i>E. ibericum</i>	2	Salamanca: Peña de Francia (29S QE1239)	1300	> 1000	Siliceous
	3	Cáceres: Candelario (29S TK2823)	1530	> 1000	Siliceous
	4	Toledo: El Rocigalgo (30S UJ6079)	1330	11	Siliceous
	5	Ciudad Real: Plaza del Judío (30S UH6671)	1100	~50	Siliceous
	6	Ciudad Real: Sierra Madrona (30S UH8658)	1300	~100	Siliceous
	<i>E. algibicum</i>	7	Málaga: Sierra de Grazalema (30S TF9772)	820	~100
<i>E. boissieri</i>	8	Cádiz: Sierra de Grazalema (30S TF8972)	1100	~100	Calcareous

Table 2 (continued)

Locus	<i>E. barnadesii</i>	<i>E. ibericum</i>					<i>E. algibicum</i>		<i>E. boissieri</i>
	#1	#2	#3	#4	#5	#6	#7 AAP	#7 SSB	#8
<i>d</i>	0.833	0.833	0.217	1.000	0.850	0.700	0.283	0.422	0.000
<i>e</i>	0.000	0.000	0.417	0.000	0.000	0.000	0.000	0.000	0.000
<i>f</i>	0.050	0.000	0.217	0.000	0.000	0.000	0.000	0.000	0.033
<i>Pgm-2</i>									
<i>a</i>	0.783	0.550	0.817	0.667	0.850	0.983	0.800	0.942	0.817
<i>b</i>	0.383	0.450	0.183	0.333	0.150	0.017	0.200	0.058	0.183

Table 3. Measurements of different genetic diversity parameters at population and species level in *Echinospartum*. N = Sample size, P_{99} = Percent of polymorphic loci (at 99% cutoff), A = Number of alleles per locus, A_p = Number of alleles per polymorphic loci, n_e = Effective number of alleles per locus, H_e = Expected heterozygosity (gene diversity), H_o = Observed heterozygosity. *Echinospartum ibericum* (overall) refers to values at species level

Species	Population (#)	N	P_{99}	A	A_p	n_e	H_e	H_o
<i>E. barnadesii</i>	1	30	0.727	2.364	2.875	1.541	0.283	0.152
<i>E. ibericum</i>	2	30	0.636	1.818	2.286	1.414	0.246	0.197
	3	30	0.727	2.182	2.625	1.571	0.278	0.185
	4	30	0.455	1.545	2.200	1.257	0.151	0.118
	5	30	0.545	1.818	2.500	1.399	0.201	0.112
	6	30	0.545	1.727	2.333	1.433	0.208	0.121
<i>E. ibericum</i> (overall)		150	0.727	2.455	3.000	1.470	0.253	0.147
<i>E. algibicum</i>	7 AAP	30	0.636	1.727	2.143	1.358	0.191	0.142
	7 SSB	77	0.727	2.000	2.375	1.408	0.217	0.094
<i>E. boissieri</i>	8	30	0.545	1.818	2.500	1.458	0.218	0.161

2*c*, *Idh-1c*, *Lap-3d* and *Pgm-1c*) either at high or low frequencies. With regard to the silicicolous taxa, three populations exhibited unique alleles: *Adh-1e* in *E. barnadesii* and *Pgm-1e* and *Adh-1f* in *E. ibericum* populations #3 and #4 respectively (Table 2).

Genetic diversity values, at both species and population level, were calculated and illustrated in Table 3. The *Echinospartum barnadesii* population showed the highest values for most parameters (P_{99} , A , A_p , H_e) followed by *E. ibericum* (#3) population which exhibited almost equal values. On the other hand, *E. ibericum* (#4) population presented the lowest values of these parameters with the exception of A_p . In all the studied populations the values of observed heterozygosity (H_o) were always lower than those of expected

heterozygosity (H_e), being the lowest H_o value in the seed bank *E. algibicum* population (0.094). Among the silicicolous species, the adult aboveground population of *E. algibicum* presented the lowest genetic variability values across all the calculated parameters.

The soil seed bank population of *E. algibicum* (#7 SSB) showed four distinctive alleles (*Adh-1d*, *Adh-2a*, *Lap-1a* and *Pgm-1a*) compared to the adult stage (Table 2) and higher genetic diversity values for all parameters except H_o (Table 3). Furthermore, *Adh-1d* and *Adh-2a* were not present in any other assayed population. On the other hand, the aboveground adult population (#7 AAP) possessed one allele that was absent in the seed bank (*Idh-1a*). However, this set of exclusive alleles was found at quite low frequencies

(Table 2). Across all loci, the two stages of the life cycle of *E. algibicum* showed statistical significant differences in allele frequencies at four of the loci: *Lap-1*, *Lap-2*, *Pgm-1* and *Pgm-2* (Table 4).

In *E. algibicum*, there was a difference of one order of magnitude in F_{IS} of the soil seed bank population compared to the above-ground adult stage, although confidence intervals bootstrapped over loci were still overlapping. However, homozygosity in both stages was significantly higher than expected in equilibrium (Fig. 2).

The pair-wise comparison of Nei's genetic identity values at population level, ranged between $I=0.983$ for the pair *E. barnadesii*–*E. ibericum* (#2) and $I=0.582$ for the pair *E. boissieri*–*E. ibericum* (#2) (Table 5). Within *E. ibericum* the different populations showed

an average genetic identity of $I=0.943$. At the species level, the highest identity values were found between *E. barnadesii* and *E. ibericum* ($I=0.987$) decreasing to $I=0.905$ for *E. ibericum*–*E. algibicum* (#7AAP). The lowest identity was found between *E. boissieri* and the rest of taxa, presenting an averaged value of $I=0.620$. The results of the pair-wise comparison of genetic distances at species level were used to plot a UPGMA phenogram (Fig. 3), which showed the existence of close genetic relationships between the silicicolous taxa in the genus, specially so between *E. ibericum* and *E. barnadesii*.

Discussion

Genetic relationships. The taxonomy in this group of silicicolous taxa is complex, and specific delimitation has traditionally been difficult (Rivas Martínez 1974, 1987; López 1982). Within this complex, *E. algibicum* has been newly described based upon the morphological and karyological variability exhibited by the single isolated population (Talavera and Aparicio 1995). We hypothesised that this species could be a result of vicariant speciation in a progenitor-derivative species system. From a genetic point of view and according to Gottlieb (1973) and Pleasants and Wendel (1989), a set of criteria should be fulfilled when seeking genetic divergence: (1) the set of *E. algibicum* alleles should be a subset of those of *E. ibericum* possessing no, or very rare, specific

Table 4. Single locus G -tests for differences in alleles frequencies between the soil seed bank and the adult stages of *Echinospartum algibicum*

Locus	G -value	P
<i>Adh-1</i>	0.01	< 0.890
<i>Adh-2</i>	0.01	< 0.890
<i>Idh-1</i>	1.95	< 0.162
<i>Idh-2</i>	0.0006	< 0.980
<i>Lap-1</i>	68.82	< 0.00001
<i>Lap-2</i>	23.67	< 0.000001
<i>Lap-3</i>	0.23	< 0.630
<i>Pgm-1</i>	4.64	< 0.030
<i>Pgm-2</i>	8.74	< 0.003

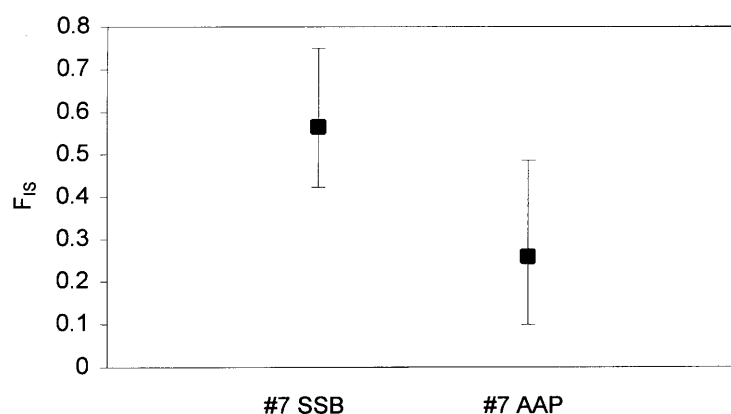


Fig. 2. Bootstrapped 95% confidence intervals of F_{IS} using nine polymorphic loci for the soil seed bank (SSB) and the aboveground adult population (AAP) of *Echinospartum algibicum*

Table 5. Nei's (1978) genetic identity (*I*) across the studied populations of *Echinospartum*. For *E. algibicum* the soil seed bank (SSB) and the adult surface plants (AAP) are treated as separated populations

	<i>E. barnadesii</i> #1	<i>E. ibericum</i> #2	<i>E. ibericum</i> #3	<i>E. ibericum</i> #4	<i>E. ibericum</i> #5	<i>E. ibericum</i> #6	<i>E. ibericum</i> #7 AAP	<i>E. algibicum</i> #7 SSB
<i>E. ibericum</i> #2	0.983	-						
<i>E. ibericum</i> #3	0.942	0.944	-					
<i>E. ibericum</i> #4	0.969	0.967	0.893	-				
<i>E. ibericum</i> #5	0.967	0.943	0.924	0.941	-			
<i>E. ibericum</i> #6	0.962	0.967	0.913	0.973	0.968	-		
<i>E. algibicum</i> #7 AAP	0.909	0.875	0.847	0.917	0.888	0.891	-	
<i>E. algibicum</i> #7 SSB	0.960	0.938	0.908	0.924	0.949	0.929	0.945	-
<i>E. boissieri</i> #8	0.631	0.582	0.592	0.620	0.596	0.603	0.667	0.640

alleles; (2) at the species level, the values of genetic variability should be lower in *E. algibicum* than in *E. ibericum*; and (3) at population level, the genetic identity should be slightly higher among the *E. ibericum* populations compared to the derivative species.

The allozyme data presented here seem to support to a large extent these premises. It is shown that in the 9 polymorphic loci we detected 25 alleles in *E. ibericum* and 24 in *E. barnadesii*, from which 17 were also present in the aboveground population of *E. algibicum*, whose *Lap-1* and *Pgm-1* frequencies appeared very different (Table 2). Regarding diversity, the allozyme data also demonstrated that at species level the aboveground populations of *E. algibicum* maintain lower degree of genetic variability than *E. ibericum* and *E. barnadesii* (Table 3). It was also found that genetic identity between *E. algibicum* (adult stage) and *E. ibericum* was mainly below the identity at population level within *E. ibericum*, fitting within the range of genetic identities calculated in other studies dealing with progenitor-derivative plant species in which speciation is supposed to be relatively recent (Pleasant and Wendel 1989).

On the other hand, the genetic identity between *E. barnadesii* and *E. ibericum* was higher than all the values found among the five *E. ibericum* studied populations falling within values corresponding to conspecific rather than congeneric populations. The levels of genetic diversity between *E. barnadesii* and *E. ibericum* were also very similar and they shared a gene pool basically integrated by the same alleles occurring at similar frequencies (Tables 2 and 3). These data apparently do not support the specific delimitation for *E. ibericum* and *E. barnadesii*; however, it must be stressed here that phenotypic divergence does not always correlate with genetic or allozyme divergence (Gottlieb 1973, 1974; Gottlieb and Pilz 1976; Wolf et al. 1991). The existence of reproductive barriers between these two species is still to be determined.

Overall, it is shown that *E. ibericum*, *E. barnadesii* and *E. algibicum* not only form

an ecological, karyological and morphological group (Talavera 1999), but also a genetic group of taxa probably interconnected during colder periods of the Quaternary. Interestingly, the soil seed bank of *E. algibicum* showed alleles actually present in *E. ibericum* and *E. barnadesii* (*Lap-1a* and *Pgm-1a*) but not detected in the adult stage, thus genetically linking the adult aboveground population with the other silicolous taxa in the supposedly progenitor–derivate species system. The calcicolous *E. boissieri* was, as expected, genetically unrelated to the set of silicolous species.

Genetics of the soil seed bank of *Echinospartum algibicum*. Many authors have demonstrated the importance of soil seed banks in plant ecology as a dormant population buried in the ground (Leck et al. 1989, Fenner 1992). Despite the strong demographic and evolutionary implications for demography and population biology, only a few studies have focused on the population genetics of soil seed banks. In most of these studies, clear spatial or temporal genetic differentiation have been found between seeds in the soil seed bank and other life cycle stages such as seedling and adult individuals. It has also been discussed that seed banks may function as a genetic record of the past, and have the potential to control, to some extent, the response of populations to new selective pressures by introducing genotypes from the past at several time scales (Templeton and Levin 1979, Ton-

sor et al. 1993, Cabin 1996, Cabin et al. 1998, McCue and Holsford 1998, Mahy et al. 1999).

Although most of these studies dealt with annual or short lived perennial plant species, and *E. algibicum* is a long lived perennial species with very low recruitment rates (Aparicio and Guisande 1997), our results are consistent with those reports cited above: the seed bank of *E. algibicum* showed (1) higher genetic variability, (2) significantly different allele frequencies at some loci and the existence of specific alleles, and (3) greater extent of homozygosity than the adult population.

Higher soil seed bank genetic variability is probably a consequence of multigenerational contributions of subsequent reproductive outputs which is consistent with the general view that seed banks may act as a genetic reservoir, even where novel genetic variation may accumulate with time (Levin 1990), and recruitment would mean a migration from the past.

We have also found that the allele frequencies differed significantly between soil seed bank and adult stages in four out of the nine polymorphic loci studied (*Lap-1*, *Lap-2*, *Pgm-1* and *Pgm-2*) (Table 4). At present, the role and the adaptative value, if any, of such differences and where selection in the life-cycle may have occurred is not clear, and only some population genetic studies have suggested the existence of a correlation between seed germination capability and differential isozyme genotypes (i.e. *Pgi* in *Plantago lanceolata* or

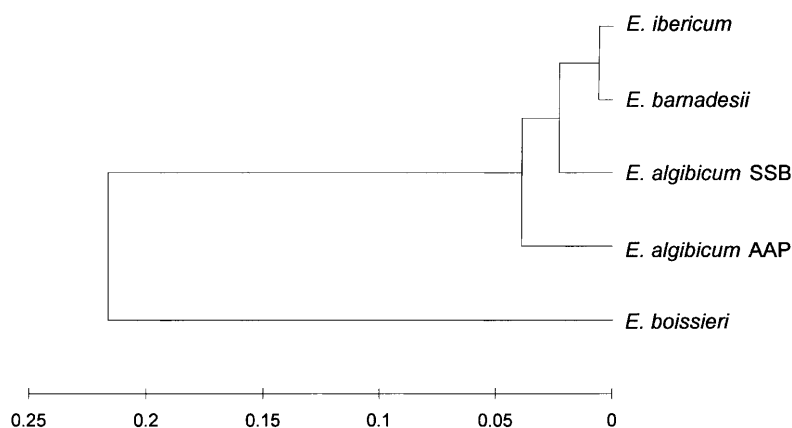


Fig. 3. UPGMA phenogram of the studied taxa of *Echinospartum* based on Nei's genetic distance (1978). For *E. algibicum* the soil seed bank (SSB) and the adult aboveground population (AAP) are treated as separate populations

Lesquerella fendleri) as a response to oxygen availability and soil moisture (Tonsor et al. 1993, Cabin et al. 1998). The observed significant differences in allele frequency of *Lap-1* and *Lap-2* remain to be investigated. The existence of apparently four stage-specific alleles in the soil seed bank, not present in the adult aboveground individuals, was also found. Similar results have been discussed by Cabin et al. (1998) and Tonsor et al. (1993) suggesting to be a possible consequence of natural selection, genetic drift, a combination of both, or completely due to chance. Our results showed that these 'unique' alleles occur at very low frequencies (below 2.6%) and appear to be a consequence of natural selection or a random effect of sampling.

Finally, the comparison between the soil seed bank and the aboveground adult plant population also showed higher homozygosity for the soil seed bank than in the adult population (Fig. 2). In order to explain this apparently generalised situation, three possibilities have been proposed in the literature (Tonsor et al. 1993): a higher proportion of inbreeding in past years, a Wahlund effect, and a lower capacity of homozygous genotypes to germinate. In fact, Cabin et al. (1997) found that seeds with more heterozygous genotypes in *Lesquerella fendleri* (Brassicaceae) had higher capacity of germination under different soil water regimes. Therefore, this raises methodological issues (Cabin et al. 1998) when homozygosity is calculated in samples coming from direct germination of viable seeds due to the genotypes with lower germination capacity -probably the most homozygous- can be undervalued. In our study, the direct method used for extracting seeds from the soil seed bank was free from substructuring and seems the most plausible cause for interpreting the unusually high values of homozygosity found in the soil seed bank of *E. algibicum*. Due to the fact that the population history of *E. algibicum* and how and when the incorporation of seeds to the permanent soil seed bank has taken place are unknown, inbreeding in past times and a Wahlund effect can not

currently account for such high levels of homozygosity in the soil seed bank of *E. algibicum*.

Conservation issues. Following theoretical predictions and empirical studies (e.g. van Treuren et al. 1991), small populations and geographically restricted taxa should exhibit low levels of polymorphism and genetic variability due to mainly genetic drift, inbreeding, and restricted gene flow. However, *E. algibicum* showed only relatively lower levels of genetic diversity than the widespread *E. ibericum*, and can not be considered to be genetically depauperate, coinciding with the results in many other studies dealing with genetic variability in endangered restricted species (Gottlieb 1974, Linhart and Premoli 1993, Lewis and Crawford 1995, Purdy and Bayer 1996, Shapcott and Playford 1996, Smith and Pham 1996, Godt and Hamrick 1998b, Neel and Ellstrand 2001). Factors such as hybridisation, multiple origins, the maintenance of genetic diversity in refuge populations, and recent speciation from a widespread species may influence genetic diversity in small populations (Karron et al. 1988).

The maintenance of genetic diversity is the basis for the survival of populations in the long term, since the loss of genetic variation can reduce the populations' capacity to adapt to environmental changes (Schonewald-Cox 1983, Richter et al. 1994) and may cause inbreeding depression (Arft and Ranker 1998, but see Schemske et al. 1994). As McCue and Holtsford (1998) have suggested, a soil seed bank can be partially responsible for the maintenance of the high diversity in populations, as it buffers the effects of small size or a genetic bottleneck. The loss, through extinction, of the genetic diversity of an adult population could be ameliorated by the soil seed bank, and local perturbations may be enough to reintroduce this diversity in new adult populations (Mahy et al. 1999). Therefore, in view of the data presented here, *E. algibicum* soil seed bank should be taken into account when decisions on the conservation of this species are discussed and implemented.

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