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POPULATION STRUCTURE AND GENETIC DIVERSITY OF TWO ENDANGERED ENDEMIC SPECIES OF THE CANARIAN LAUREL FOREST: *DORYCNIUM SPECTABILE* (FABACEAE) AND *ISOPLEXIS CHALCANTHA* (SCROPHULARIACEAE)

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We used random amplified polymorphic DNA (RAPD) markers to assess the levels and structuring of genetic variation in the Canarian laurel forest endangered endemics *Dorycnium spectabile* (Fabaceae) and *Isoplexis chalcantha* (Scrophulariaceae). Amplification of seven primers in the only two extant populations of each species resulted in 28 (*D. spectabile*) and 32 (*I. chalcantha*) markers that exhibited a polymorphism of 78% and 100%, respectively. The estimates of population subdivision show that ca. 75% and 25% of the total genetic variability of both species is explained by the within- and between-population components, respectively. Our favored hypothesis to explain the high levels of genetic variation detected in both endemics is that they originated from multiple introductions of continental ancestors. The recent fragmentation and degradation of the Canarian laurel forest probably brought about a severe reduction of interpopulation gene flow in both species that might have disrupted the genetic cohesion of once more widespread geographic ranges. Intrapopulation spatial autocorrelation analyses indicate that the genetic variability of *D. spectabile* and *I. chalcantha* is structured in family clumps whose maintenance and enhancement is best explained by assortive mating and short-range seed dispersal capabilities. Because of the extreme vulnerability of these two endemics and their high levels of interpopulation genetic differentiation, we recommend protecting all their natural areas of occurrence and avoiding mixing individuals from different populations. Patch size estimates derived from spatial autocorrelation were used to suggest seed collection strategies that minimize the probability of sampling genetically similar individuals.

Keywords: Canary Islands, conservation genetics, endangered species, gene flow, genetic variation, RAPDs.

Introduction

Dorycnium spectabile (Choisy ex Ser.) Webb and Berthel (Fabaceae) and *Isoplexis chalcantha* Svent. and O'Shanahan (Scrophulariaceae) are two endangered endemics from the Canarian laurel forest ecosystem. The genus *Dorycnium* contains ca. 15 species distributed in Macaronesia and the Mediterranean region and three species endemic to the Canary Islands. *Dorycnium spectabile* possesses hermaphroditic flowers, although it exhibits high levels of self-incompatibility (Calero and Santos 1988). Flowering occurs predominantly from May to July, pollination is by insects (Hymenoptera and Lepidoptera), and seeds disperse by gravity (M. Naranjo, personal communication). The only two known populations of this species (Teno and Güímar) occur in Tenerife and are separated by ca. 40 km (fig. 1). Only 24 individuals have been recorded in a recent census in Teno and 46 in Güímar (M. Naranjo, personal communication).

The genus *Isoplexis* (Scrophulariaceae) is considered to be a relict from the Tertiary (Bramwell 1972) and consists of four species strictly endemic to Macaronesia. *Isoplexis chalcantha* is a perennial monoecious protogynous shrub (M. Naranjo, personal communication) that follows allogamous behavior. It flowers predominantly from April to June, it is pollinated by birds and insects (Hymenoptera), and its seeds disperse by gravity. Until recently, *I. chalcantha* was known from five locations in the north of Gran Canaria (Sventenius 1968; Suárez 1980; Marrero 1989; Bramwell and Bramwell 1994). However, some of these populations have undergone a rapid decline in size that is attributable to the wholesale degradation of the Canarian laurel forest and to the use of the leaves of *I. chalcantha* for medicinal purposes. Currently, this endemic consists of two remnant populations (Los Tiles and La Virgen) separated by ca. 4 km (fig. 1). Only 19 individuals were recorded in a recent survey in Los Tiles, and 187 were found in La Virgen (M. Naranjo, personal communication).

As a result of the decrease in population number and size brought about by the destruction and fragmentation of the Canarian laurel forest and the ethnobotanical use of *I. chalcantha*, these two species have been classified as endangered according to the IUCN categories (Walter and Gillet 1997) and are listed in the *Red Book of the Canary Islands Flora* (Gómez-Campo 1996). Accordingly, they are protected by European and Spanish decrees: the European Directive of Habitats (Eu-

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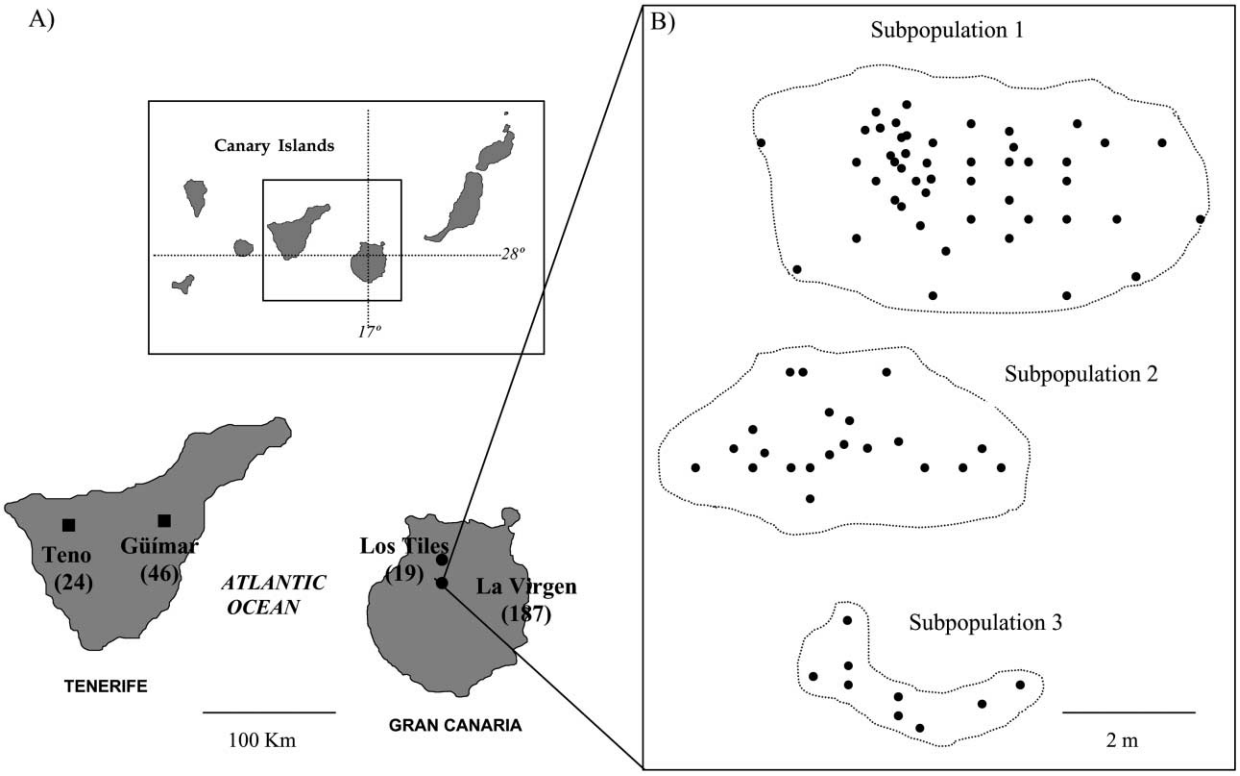


Fig. 1 *A*, Location of the populations of *Dorycnium spectabile* (filled squares) and *Isoplexis chalcantha* (filled circles) in the Canary Islands. Numbers in parentheses refer to the individuals censused in each population. *B*, Distribution of individuals in the three subpopulations from La Virgen population.

ropean Community 1992), Spanish law (BOE 1998), and Canarian regional law (BOC 1991).

Many conservation programs aim at maintaining existing levels of genetic variation in rare or threatened species (Frankel and Soulé 1981; Simberloff 1988; Barret and Kohn 1991) because this increases the chance of population long-term survival. Although detailed demographic data are known to be very important in designing conservation management programs aimed at the survival of endangered taxa (Lande 1988; Caro and Laurenson 1994; Schemske et al. 1994), genetic studies take a primary role in planning *in situ* and *ex situ* conservation efforts (Amos and Hoelzel 1992; Frankel et al. 1995; Gemmill et al. 1998; Francisco-Ortega et al. 2000).

The new methods of molecular biology have a wide range of applications in plant population studies. Among other important achievements, the characterization of DNA polymorphisms has facilitated the detection of genetic variation within and between populations, the identification of clones, the analysis of breeding systems, and the study of ecogeographical variation (Weising et al. 1995). The use of randomly amplified polymorphic DNA (RAPD) markers (Welsh and McClelland 1990; Williams et al. 1990; Martín et al. 1997) does not need either large amounts of DNA or previous knowledge of DNA sequence. It is, therefore, an ideal technique for conservation biology studies based on the assessment of genetic parameters in rare and endangered species, where plant material and background genetic information are often scarce. Moreover, RAPDs have been found to be particularly appropriate for studies involving small sample sizes, especially for outbreeders, because large numbers of polymorphic loci can be generated. RAPDs have permitted scientists to reach meaningful conclusions in studies aimed at characterizing and preserving the genetic diversity of threatened plant species (Rossetto et al. 1995; Sulaiman and Hasnain 1996; Ayres and Ryan 1997; James and Ashburner 1997; Martín et al. 1997; Palacios and González-Candelas 1997; Cardoso et al. 1998; Gillies et al. 1999; Morden and Loeffler 1999).

In this article, we use RAPD markers to estimate the amounts and structuring of genetic diversity in the remnant populations of *I. chalcantha* and *D. spectabile* in the Canarian laurel forest in an attempt to provide a background for the development of *in situ* and *ex situ* conservation programs for these endangered endemics.

Material and Methods

Field Collection

We analyzed 47 individuals of *Dorycnium spectabile* (13 from Teno and 34 from Güímar) and 58 individuals of *Isoplexis chalcantha* (four from Los Tiles and 54 from La Virgen) that represent 67% and 28% of the total known extant specimens of these taxa, respectively (fig. 1; table 1). The population of La Virgen was divided into three subpopulations on

Table 1

Summary of RAPD Results for *Dorycnium spectabile* and *Isoplexis chalcantha*

Primer and population	NF	PF	%P	Fragment sizes (bp)
Dorycnium spectabile:				
OPA 1:				
Teno	4	2	50.0	400–1050
Güímar	6	4	66.7	400–1100
OPA 4:				
Teno	3	3	100	500–750
Güímar	3	2	66.7	500–750
OPA 5:				
Teno	1	0	0.0	1250
Güímar	3	2	66.7	850–1250
OPA 6:				
Teno	6	4	66.7	800–1600
Güímar	6	6	100.0	800–1600
OPA 8:				
Teno	1	0	0.0	850
Güímar	1	0	0.0	850
OPA 9:				
Teno	4	4	100.0	650–1600
Güímar	4	3	80.0	500–1600
OPA 16:				
Teno	4	1	25.0	800–1500
Güímar	4	3	75.0	800–1500
Total:				
Teno	23	14	60.9	400–1600
Güímar	27	20	75.0	400–1600
Isoplexis chalcantha:				
OPA 5:				
Los Tiles	3	3	100	400–1100
La Virgen	4	4	100	400–1300
OPA 7:				
Los Tiles	1	0	0	550
La Virgen	1	1	100	550
OPA 8:				
Los Tiles	8	7	87.5	425–1650
La Virgen	9	9	100	325–1650
OPA 10:				
Los Tiles	0	…	…	…
La Virgen	1	1	100	1350
OPA 11:				
Los Tiles	6	5	83.3	500–1600
La Virgen	10	10	100	500–1750
OPA 16:				
Los Tiles	1	1	100	700
La Virgen	1	1	100	700
OPA 19:				
Los Tiles	4	3	75	650–1600
La Virgen	6	6	100	650–2000
Total:				
Los Tiles	23	19	82.6	400–1650
La Virgen	32	32	100	325–2000

Note. Number of total fragments obtained (NF), number of polymorphic fragments (PF), and percentage of polymorphic loci (%P).

the grounds of the conspicuous spatial separation among individuals in the field (fig. 1): La Virgen 1 ($n = 24$), La Virgen 2 ($n = 21$), and La Virgen 3 ($n = 9$). Individual plants were assigned a pair of Cartesian (x, y) coordinates representing their relative positions in populational space that were used for the spatial analyses. Two or three leaves were sampled from each individual, kept in a cooler, and transported to the lab, where they were stored at −80°C until DNA extraction was realized. Although DNA extractions were originally carried out for a higher number of individuals in all the populations surveyed, the amplifications of some samples (27% to *D. spectabile* and 16% to *I. chalcantha*) did not have enough resolution to warrant a reliable interpretation of their banding patterns, and they were excluded from the final analyses.

DNA Extraction

Ca. 1 cm^2 of a frozen leaf was ground in liquid nitrogen in a sterile mortar with a pestle, and the powder was transferred to a 1.5-mL tube. Total genomic DNA isolation followed that of Dellaporta et al. (1983), with the modifications suggested by Corniquel and Mercier (1994). The condition and concentration of the DNAs were tested by comparison with known quantities of prepurified calf thymus DNA (Amersham–Pharmacia Biotech) in a 1% agarose gel (Sambrook et al. 1989).

RAPD Amplification and Electrophoresis

Final reaction mixtures contained between 15 and 20 ng of genomic DNA and 2.5 μL 10× PCR Buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl); 160 μM each of dATP, dGTP, dCTP, dTTP; 1.5 mM $MgCl_2$; 1 unit AmpliTaq DNA polymerase (Perkin Elmer); and 0.4 μM of the primers. Amplifications were run in an Eppendorf Master gradient thermal cycler and gave optimal bands with an initial denaturation step of 94°C for 1.5 min followed by 45 cycles of 30 s at 94°C, 30 s at 36°C, 30 s at 72°C, and a final elongation cycle of 10 min at 72°C. Reaction mixes without DNA were run as negative controls in all RAPD amplifications. Twenty 10-mers of arbitrary sequence (Operon Technologies) were tested for PCR amplification. Seven of these (table 1) gave optimally reproducible polymorphic bands and were thereby selected for further analysis. Amplification products were resolved by 1.8% agarose gel electrophoresis in TBE buffer (45 mM Tris-Borate and 1 mM EDTA) and visualized under ultraviolet (UV) light after ethidium bromide staining. Gels were photographed using a Kodak DC40 digital camera. Scoring was carried out conservatively, excluding some markers that we considered unreliable. All polymorphisms whose intensities did not allow a straightforward interpretation were rechecked by subjecting them to amplification under the same conditions.

Data Analysis

The presence or absence of each fragment that could be scored was recorded in a binary data matrix from which the percentage of polymorphic bands per population was calculated. Average intrapopulational genetic diversity was estimated using Shannon's diversity index (Lewontin 1972). This index, that we designate as H_o, is suitable for RAPD data because of its insensitivity to the bias that can be introduced into the analysis by the inability to detect heterozygous individuals (Dawson et al. 1995). We calculated H_o at two levels: within populations (H_{pop}) and within species (H_{sp}). The proportion of diversity within and between populations was estimated by H_{pop}/H_{sp} and $(H_{sp} - H_{pop})/H_{sp}$, respectively.

Spatial autocorrelation (Sokal 1979; Griffith 1987) based on the vectors of presence or absence of each polymorphic marker was used to explore the within-population structuring of genetic variation as a function of the relative position of individuals in space. Moran's I (Moran 1948) was used as the coefficient of spatial autocorrelation. This index is based on the covariation of values at different spatial positions, and it varies from -1 to $+1$. Its expected value is $E(I) = -1/(n - 1)$, where n is the sample size (Oden 1984). When $I > 0$ for a given variable, it is said to exhibit positive spatial autocorrelation, meaning that points that are similar in terms of that variable tend to be juxtaposed in space. When $I < 0$, the variable is said to exhibit negative spatial autocorrelation, meaning that adjacent individuals are less similar than expected at random. The representation of Moran's I against distance classes is called "correlogram." When positive spatial autocorrelation is detected, the correlogram features a more or less monotonic decline of Moran's I as distance classes grow, with the higher value of I associated with the first distance class (which represents the individuals that are geographically closer). As a general rule, this pattern is expected when dispersal capabilities for a given species are low. When no spatial autocorrelation is detected, then the correlograms exhibit a more or less flat profile, with all I values around 0 for most distance classes, meaning that values of the surveyed variable are distributed randomly in the populational space. In the case of negative spatial autocorrelation, similar points tend to be separated in space, and the correlograms exhibit a peak of significant positive I values at high distance classes (that represent geographically distant individuals).

Matrices of geographical distance between pairs of individuals, departures from the expected value of Moran's I for each of five distance classes, and overall correlogram significances were calculated by the computer program SAAP (Wartenberg 1989) from Cartesian coordinates. Our use of spatial autocorrelation was addressed to find possible patterns of RAPD variability in space that could be informative of whether these populations are structured for this kind of genetic markers. This analysis was performed in *D. spectabile* populations and only in two out of the four sampled subpopulations of *I. chalcantha* (La Virgen 1 and La Virgen 2). The low numbers of individuals at La Virgen 3 and at Los Tiles in this analysis do not warrant meaningful conclusions (fig. 1).

Genetic distances between all possible pairwise combinations of individuals were computed from the presence or absence of markers using Dice's coefficient for the NTSYS-PC, version 2 (Rohlf 1993). The resulting genetic similarity matrix was input in NTSYS-PC (Rohlf 1993) and used to build a UPGMA similarity tree among the 47 and 58 individuals analyzed in *D. spectabile* and *I. chalcantha*, respectively. To further substantiate the estimates of genetic similarity, the individual vectors of presence or absence of RAPD polymorphisms were subjected to principal component analysis (PCA) using the software SPSS 6.1.3 (SPSS, Chicago).

The analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was implemented to estimate variance components for RAPD phenotypes and to test the significance of the partition of RAPD variation in both species at different hierarchical levels, i.e., among populations and among individuals within populations against the null hypothesis of no structure. We applied AMOVA using ARLEQUIN (Schneider et al. 2000). Genetic distances between pairwise combinations of populations were expressed as Φ_{ST} values, which are direct estimates of F_{ST} (Excoffier et al. 1992).

Results

Dorycnium spectabile

Of the seven primers assayed, OPA 8 was monomorphic in both populations and OPA 5 only in Teno. A total of 23 and 28 bands, varying in size from 400 to 1600 bp, were detected in Teno and Güímar, respectively (table 1). Of these, 14 were polymorphic in Teno (60.9%) and 21 in Güímar (75%). Although five exclusive bands from 500 to 1100 bp were found in Güímar, none of them was of any usefulness for labeling individuals from this population because they were not monomorphic.

Shannon's diversity index (H_o) was calculated for each polymorphic fragment (table 2) and was used as a measure of genetic diversity within populations. Güímar exhibited a higher level of within-genetic diversity ($H_o = 0.87$) than Teno ($H_o = 0.70$). The mean diversity within *Dorycnium spectabile* populations was $H_{pop} = 0.79$, and the mean diversity within the species was $H_{sp} = 0.96$. The proportions of diversity within and between populations were 85% and 15% to the total species diversity (table 2). Except for OPA 5, all polymorphic primers used detected more variability within populations than between populations.

A total of six (43%) and seven (32%) polymorphic bands showed significant correlograms ($P < 0.05$) in Teno and Güímar, respectively. The average correlograms of both populations showed a significant monotonic, clinal-like decline in the values of Moran's I as distance classes increased, indicating spatial structuring of overall RAPD variation (fig. 2). Plants in close spatial proximity (represented in the correlograms by the first distance classes) tend to be more similar genetically than nonadjacent plants. This general pattern was much more regular in *D. spectabile* than in *Isoplexis chalcantha*. The highest positive values of Moran's I were found among plants separated by <8 m in Teno and <16 m in Güímar (fig. 2).

The PCA (fig. 3) based on vectors of marker presence or absence summarized the interindividual relationship in terms of RAPD patterns in two multivariate components, which accounted for 54.4% of the total variation. All individuals from Teno were localized on the upper right of this representation and showed only a very slight overlapping with individuals from Güímar. No remarkable differences in the intrapopulation distributions of genotypes were observed.

The UPGMA tree displayed four subclusters (labeled I to IV in fig. 4) that showed a considerable degree of population intermixing, with only subcluster IV (that grouped 22 individuals from Güímar) exhibiting a homogeneous pattern. Three individuals from Güímar (G14, G15, G40) were not associated with any group in this cluster.

The genetic differentiation between both populations detected through the AMOVA (table 3) was considerable and highly significant ($P < 0.001$). Roughly 26% of the total genetic diversity was attributable to population differences ($F_{ST} = 0.264$) and 74% to within-population variability, which is con-

Table 2

Estimates of Genetic Diversity for the Six and Seven Primers That Produced Polymorphs in *Dorycnium spectabile* and *Isoplexis chalcantha*, Respectively

		OPA 1	OPA 4	OPA 5	OPA 6	OPA 9	OPA 16	Mean
Dorycnium spectabile:								
H_o:								
Teno		0.21	0.95	0.00	1.34	1.33	0.37	0.70
Güímar		1.54	0.39	0.80	0.95	1.11	0.46	0.87
H_{pop}		0.87	0.67	0.40	1.15	1.22	0.41	0.79
H_{sp}		1.38	0.59	0.89	1.13	1.35	0.44	0.96
H_{pop}/H_{sp}		0.63	1.14	0.45	1.01	0.90	0.94	0.85
$(H_{sp} - H_{pop})/H_{sp}$		0.37	−0.14	0.55	−0.01	0.10	0.06	0.15
	OPA 5	OPA 7	OPA 8	OPA 10	OPA 11	OPA 18	OPA 19	Mean
Isoplexis chalcantha:								
H_o:								
La Virgen	1.41	0.23	3.44	0.53	4.53	0.51	2.71	1.91
Los Tiles	1.31	0.00	3.31	…	1.75	0.50	1.31	1.36
H_{pop}	1.36	0.12	3.38	0.27	3.14	0.50	2.01	1.54
H_{sp}	1.44	0.37	3.53	0.52	4.55	0.51	2.80	1.96
H_{pop}/H_{sp}	0.94	0.31	0.96	0.51	0.69	0.99	0.72	0.73
$(H_{sp} - H_{pop})/H_{sp}$	0.06	0.69	0.04	0.49	0.31	0.01	0.28	0.27

Note. H_o, Shannon's Diversity Index per population; H_{pop}, average diversity within populations; H_{sp}, genetic diversity within species; H_{pop}/H_{sp}, proportion of genetic diversity within populations; $(H_{sp} - H_{pop})/H_{sp}$, proportion of genetic diversity between populations.

sistent with the lack of separation of the populations in either PCA or UPGMA analysis.

Isoplexis chalcantha

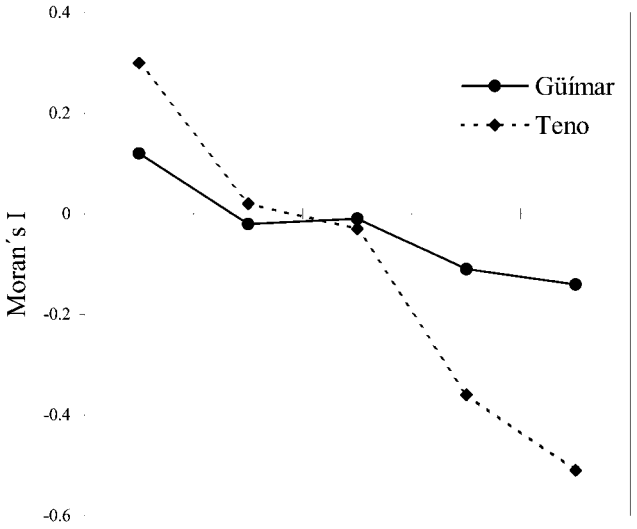
The seven primers assayed resolved a total of 23 and 32 bands (ranging from 325 to 2000 bp) in Los Tiles and La Virgen, respectively. Of these, 19 were polymorphic in Los Tiles (82.2%) and 32 in La Virgen (100%). None of the nine exclusive bands found in La Virgen (table 1) could be used as population markers because they exhibit intrapopulation variability.

Although La Virgen exhibited a higher level of genetic diversity ($H_o = 1.91$) than Los Tiles ($H_o = 1.36$), this may result from the very low number of individuals analyzed in the latter population (only four). The mean diversities within populations ($H_{pop} = 1.54$) and within the species ($H_{sp} = 1.96$) were higher than in *D. spectabile* (table 2).

Spatial autocorrelation analysis of subpopulations La Virgen 1 and La Virgen 2 resulted in significant ($P < 0.001$), moderate to high, positive values of Moran's I for the first distance classes (fig. 5). A total of five (18%) and seven (26%) loci showed significant correlograms ($P < 0.05$) in La Virgen 1 and La Virgen 2, respectively. The mean correlograms indicated that patch sizes are ca. 3 m for both subpopulations, as measured by the intersection of the correlogram with the abscissa.

The first two components of the PCA explained 68.1% of the total RAPD variability detected and separated Los Tiles and La Virgen sharply (fig. 6). However, this analysis did not have enough resolution to distinguish between subpopulations La Virgen 1 and La Virgen 2. Four main clusters (labeled I to IV in fig. 7) were observed in the UPGMA representation. All plants from Los Tiles clustered in group I. Groups II and III consisted of individuals from La Virgen 1 and La Virgen 2 (II) and of all individuals from La Virgen 3 with individuals from La Virgen 2 (III). Group IV contained exclusively individuals from La Virgen 1. Los Tiles cluster (group I) is more closely associated with some La Virgen clusters (groups II and III) than are other La Virgen plants (group IV). This is a further indication of the lack of separation of the two populations.

The proportion of diversity partitioned within and between populations was 73% and 27%, respectively, based on Shan-



Distance classes	1	2	3	4	5
Teno	0-3	3-4	4-6	6-9	9-17
Güímar	0-5	5-8	8-11	11-16	16-24

Fig. 2 Spatial autocorrelation analysis of *Dorycnium spectabile* populations. Distance classes are in meters.

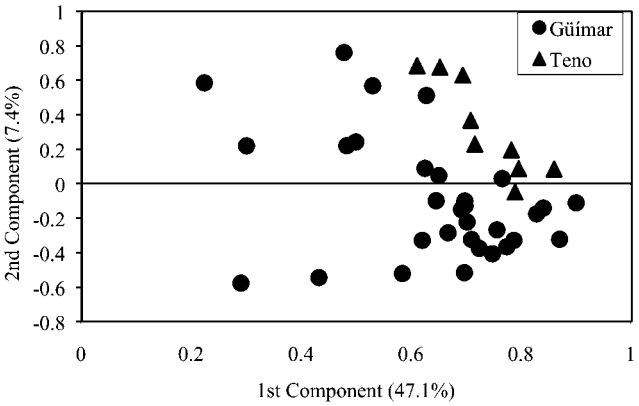


Fig. 3 Principal component analysis of *Dorycnium spectabile* populations. Percents in parentheses indicate the proportion of total variation explained by each component.

non's index (table 2). Quite similarly, the AMOVA showed that roughly 77% of the detected RAPD variation was found within populations and 23% between populations (table 3). The partition of the total variance associated with the subdivisions made in La Virgen showed that the RAPD variation within subpopulations was also higher than between them (83.9% vs. 16.1%, respectively; table 4). The genetic differentiation values (F_{ST}) between Los Tiles and the three La Virgen subpopulations ranged from 0.257 to 0.376 and were highly significant ($P < 0.001$) in all cases. The F_{ST} values between pairwise combinations of La Virgen subpopulations ranged from 0.117 to 0.251, with that between subpopulations 2 and 3 the lowest (table 5).

Discussion

An important objective of conservation genetics is to estimate the levels and apportionment of genetic variation in endangered species (Lacy 1988; Fritsch and Rieseberg 1996; Cardoso et al. 1998). Accurate estimates of genetic diversity are useful for optimizing sampling strategies driven at the conservation and management of genetic resources (Hamrick et al. 1991; Schaal et al. 1991; Chalmers et al. 1992; Cardoso et al. 1998). This bears special relevance in endangered species with a small distribution area and a reduced population size such as *Dorycnium spectabile* and *Isoplexis chalcantha*.

RAPD profiles are extremely sensitive to single-base changes in the target site (Williams et al. 1993), indicating that these molecular markers are especially suitable for genetic analyses at the intra- and interpopulation levels or for those aimed at comparing closely related species. Population genetics theory predicts that the subdivision of a population into small, isolated subpopulations brings about a loss of genetic diversity through drift (Templeton 1991). The severe fragmentation and degradation undergone by the remnants of their habitat in the

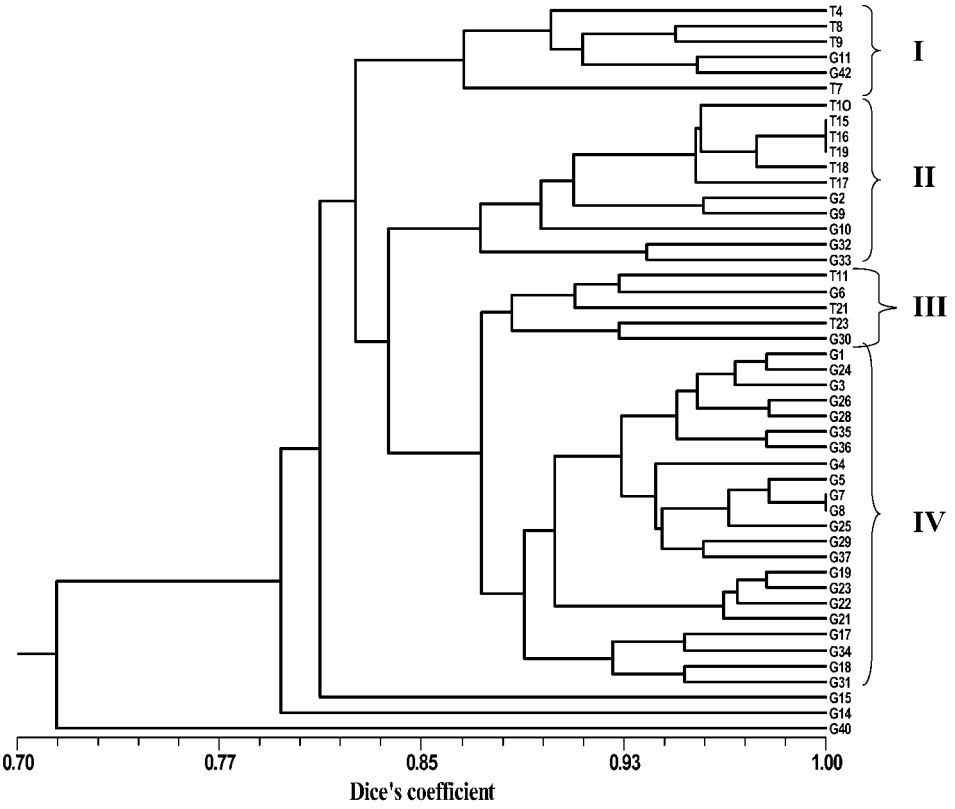


Fig. 4 UPGMA dendrogram of individuals of *Dorycnium spectabile* from Tenerife. Letters associated with the labels indicate the population of origin (*T*, Teno; *G*, Güímar).

Table 3

Analysis of Molecular Variance in *Dorycnium spectabile* and *Isoplexis chalcantha*

Species and source of variation	Sum of squares	Variance	% total
Dorycnium spectabile:			
Between populations	19.84	0.918	26.43***
Within populations	115.1	2.557	73.57
Coefficient of differentiation (F_{ST})	0.264***		
Isoplexis chalcantha:			
Between populations	16.63	1.54	23.04***
Within populations	288.33	5.14	76.96
Coefficient of differentiation (F_{ST})	0.230***		

Note. "% total" refers to the percentage of total variance contributed by each component.
*** $P < 0.001$.

laurel forest in past 200 yr has restricted the distribution and population sizes of both *I. chalcantha* and *D. spectabile*. So we would expect depauperated levels of genetic variation in the analyzed populations (Hamrick et al. 1991). This is especially true for Los Tiles (*I. chalcantha*), where only 19 extant individuals have been recorded. At odds with this expectation, levels of RAPD variation associated with either species are considerably high, regardless of the abrupt decrease in population sizes and swift habitat fragmentation (M. Naranjo, personal communication). Demographic structure of the populations presents a considerable number of individuals in the medium age classes, which could be a consequence of a great germinative and growth capacity *in situ* for both species. It bears mentioning that the number of individuals from Los Tiles was not enough to obtain statistically sound conclusions, and therefore our results for this population must be interpreted with extreme caution.

The proportion of polymorphic RAPD bands ranged from 61% to 75% in *D. spectabile* populations and from 82% to 100% in *I. chalcantha* populations (table 1). Similarly, total genetic diversity as measured by Shannon's index ranged from 0.70 to 0.87 in *D. spectabile* and from 1.36 to 1.91 in *I. chalcantha*. These estimates are clearly higher than those found in other narrowly distributed species—e.g., Smith and Pham (1996) found $P = 40\%–63\%$ in *Allium aaseae* (Alliaceae)—or in species with variable distribution patterns and population sizes such as *Hippophae rhamnoides* (Elaeagnaceae) (Bartish et al. 1999), *Astelia australiana* (Liliaceae) (James and Ashburner 1997) or *Haplostachys haplostachya* (Lamiaceae) (Morden and Loeffler 1999). The figures for *I. chalcantha* and *D. spectabile* also outnumber the levels of RAPD polymorphism detected in other endemics with more widespread distributions and much larger population sizes. Martín et al. (1997) report $P = 63\%$ and $H_{sp} = 1.31$ for *Erodium paularense* (Geraniaceae), an outbreeding endemic species of central Spain with population sizes of more than 1500 and 10,000 individuals; Friar et al. (1996) estimate $P = 12\%–15\%$ for *Argyroxiphium sandwicense* (Asteraceae), an endemic of the Hawaiian Islands. However, this last species has suffered an extensive bottleneck.

Several empirical works coincide in highlighting population sizes, breeding systems, phylogenetic relationships, mutation, and gene flow as the main factors that impact on levels of genetic diversity (Frankel et al. 1995; Weller et al. 1996; Francisco-Ortega et al. 2000). As discussed above, the rapid decrease in population size undergone by these two Canarian endemics does not manifest in depauperated genetic variation levels as estimated by RAPD markers. Population sizes not-

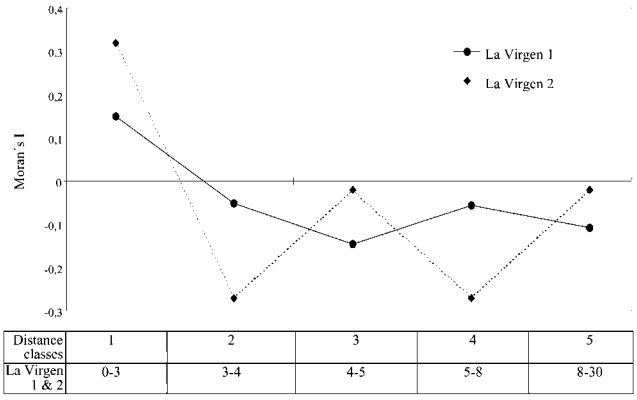


Fig. 5 Spatial autocorrelation analysis of *Isoplexis chalcantha* subpopulations 1 and 2 from La Virgen. Distance classes are in meters.

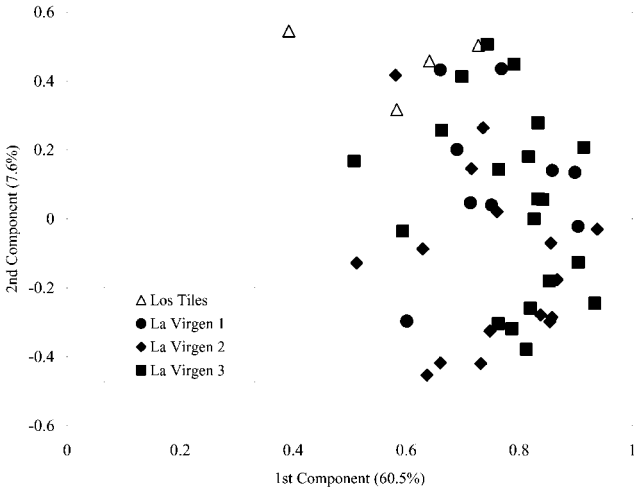


Fig. 6 Principal component analysis of *Isoplexis chalcantha* populations. Percents in parentheses indicate the proportion of total variation explained by each component.

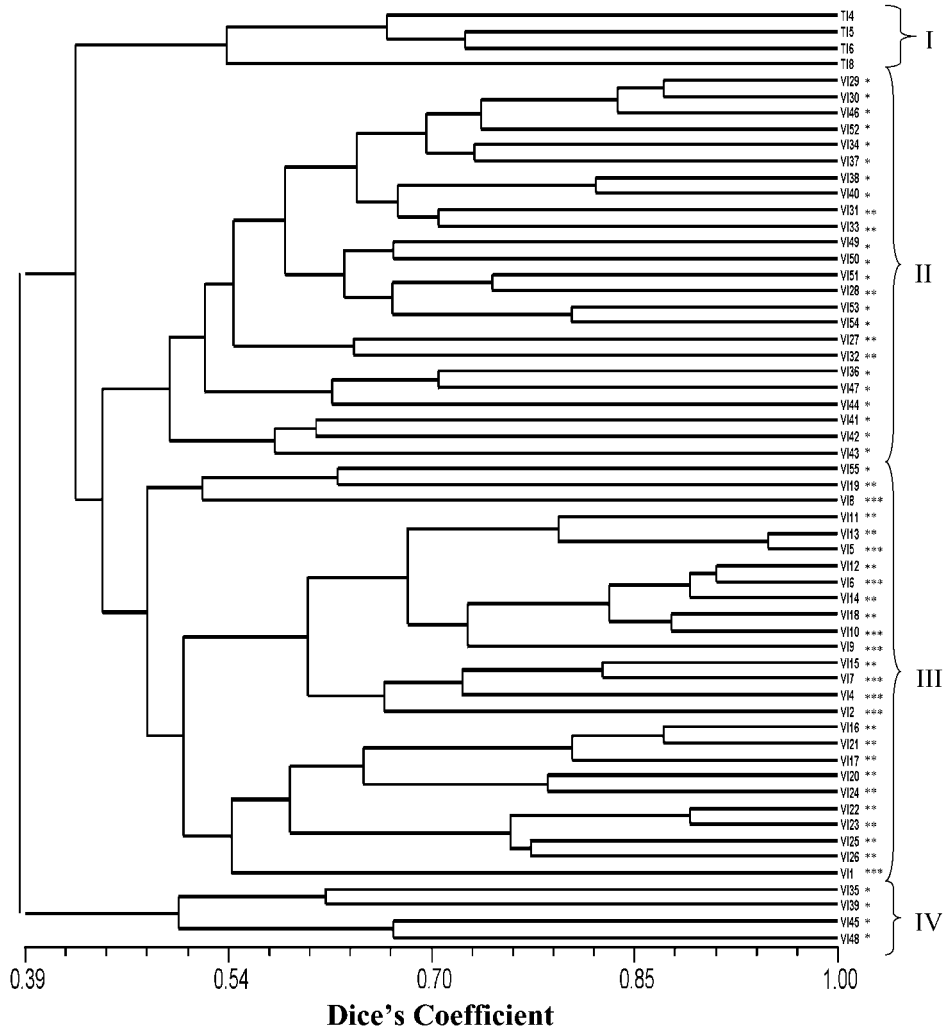


Fig. 7 UPGMA dendrogram of individuals of *Isoplexis chalcantha* individuals from Gran Canaria. Letters associated with the labels indicate the population of origin (*TI*, Los Tiles; *VI*, La Virgen). *, subpopulation 1; **, subpopulation 2; ***, subpopulation 3.

withstanding, the breeding systems of both species seem well suited to foster the maintenance and enhancement of genetic variation. *Isoplexis chalcantha* is allogamous (M. González, personal communication), whereas *D. spectabile* presents high levels of self-incompatibility (Calero and Santos 1988). Furthermore, the structure of the populations of both species present a considerable number of individuals in the medium age classes, which could be a consequence of an important germinative and growth capacity. Accordingly, the partitions of genetic variation within and between populations (ca. 73% vs. 27% and 85% vs. 15%, respectively) are in agreement with those expected for outbreeding species (Hamrick 1990; Huff et al. 1993; Nesbitt et al. 1995; Rossetto et al. 1995; Martin et al. 1997; Las Heras-Vazquez et al. 1999).

The hypothesis that some Canarian endemics represent old lineages that took refuge in the Macaronesian region during glaciations and desertification in Europe and northern Africa after the Miocene (Engler 1879; Bramwell 1976, 1990) has been recently bolstered empirically by Caujapé-Castells et al. (2001) through the estimation of divergence times associated with diversification events in *Androcymbium* (Colchicaceae). Under this "time" hypothesis, one explanation for the high levels of genetic variation in *I. chalcantha* and *D. spectabile* would be the progressive accumulation of mutations over time (Witter and Carr 1988; Francisco-Ortega et al. 2000). However, recent molecular phylogenies based on ITS markers for *I. chalcantha* (Carvalho and Culham 1998) place this species in a derived position with respect to its Mediterranean relatives. Although no time estimates are given for the age of the closer hypothetical progenitor of *I. chalcantha*, the finding of Carvalho and Culham (1998) rules out that this species represents an ancient diversification event within its lineage. Even if the origin of *I. chalcantha* dates back almost to the origin of the island of Gran Canaria ca. 14 million years ago (Carracedo 1996), our data indicate that the accumulation of mutations through time does not sufficiently explain the high levels of RAPD variation detected in this species, and other complementary factors need to be considered.

A more likely hypothesis to interpret the high levels of variation detected at least for *I. chalcantha* is that this endemic might

Table 4

Analysis of Molecular Variance of the Subpopulations from La Virgen (*Isoplexis chalcantha*)

Source of variation	Sum of squares	Variance	% total
Among subpopulations	39	0.88	16.09
Within subpopulations	235.32	4.61	83.91
Coefficient of differentiation (F_{ST})	0.161***		

Note. "% total" refers to the percentage of total variance contributed by each component.

*** $P < 0.001$.

have originated from multiple introductions of continental ancestors. In fact, there are no phylogenetic studies for Canarian species of *Dorycnium*; therefore, it is not certain whether age of this clade might have been an important factor in the high levels of genetic variation detected for this species. It is likely that multiple introductions of seeds from close mainland areas (mediated by birds, wind transportation, or a combination of both) influenced the high levels of variation detected in *I. chalcantha* and *D. spectabile*. This possibility is favored by the close proximity of the Canarian archipelago to Africa and by the emerging picture of higher genetic diversity in Canary Island endemics than in those from more remote archipelagos such as Hawaii (Francisco-Ortega et al. 2000). Under a multiple colonization scenario, the negative effects of genetic bottlenecks associated with founder events may have been less extreme, as has been argued for other Canarian endemics (Francisco-Ortega et al. 2000; Batista et al. 2001; Sosa 2001). In a similar way, Smith and Pham (1996) proposed multiple origins as a likely explanation for the high levels of genetic diversity in the narrow endemic *A. aaseae*. This alternative is favored by the close proximity of the Canarian archipelago to Africa and by the emerging picture of higher genetic diversity in Canary Island endemics than in those from more remote archipelagos such as the Hawaiian Islands (Francisco-Ortega et al. 2000).

The detection of patterns of significant spatial autocorrelation for the populations of *D. spectabile* and *I. chalcantha* indicates that they may be genetically structured, i.e., composed of subpopulations within which mating is approximately random but between which mating may be infrequent. The shortest length of any irregularly shaped patch size was estimated from the intersection of the correlograms with the abscissa (Sokal 1979). The distance at which the mean Moran's I values first intercept E(I) value may represent the shortest size of the true patches (Sokal 1979). Several factors have been reported to induce profiles of spatial genetic structure in plant populations, including limited dispersal (Slatkin 1993; Caujapé-Castells and Pedrola-Monfort 1997), clonal reproduction (Caujapé-Castells and Pedrola-Monfort 1997), selection at the microhabitat level (Wright 1946; Lande 1980), and assortive mating (Shapcott 1995; Caujapé-Castells et al. 1999). In both *I. chalcantha* and *D. spectabile*, gravity seed dispersal makes it likely that family clumps grow progressively as population recruitment proceeds, thereby fostering the reproduction among genetically similar individuals. The development of genetic neighborhoods and assortive mating might have been especially influenced in both species by the absence of adaptations for long-range dispersal and by the characteristics of capsule dehiscence, by virtue of which related seeds tend to set together. In *D. spectabile*, pods contain up to 10 seeds that are released only 2–3 m (at most) from the mother plant when valves open. In *I. chalcantha*, fruits are trilobular capsules that open progressively as the fruit ripens and release a high number of small seeds. The more regular decline in the values of Moran's I in *D. spectabile* (fig. 2) than in *I. chalcantha* (fig. 5) might be a reflection of a different impact of these similar dispersal capabilities on their genetic structures. Clonal reproduction and microhabitat selection are not feasible possibilities for explaining the observed patterns of spatial genetic structuring because vegetative propagation is not among the reproductive assets of either species, and we did not observe ecological heterogeneity within any of the sampled populations. Our results are in agreement with computer simulations that predict the rapid generation of large patches solely due to nearest-neighbor pollination and limited seed dispersal (Turner et al. 1982).

Although diversity between populations was lower than within populations (probably as a consequence of outbreeding), the AMOVA detected highly significant ($P < 0.001$) interpopulation genetic differentiation in both *D. spectabile* and *I. chalcantha* (F_{ST} values of 0.264 and 0.230, respectively). This indicates that these species may be genetically structured at spatial scales much larger than the intrapopulational. The PCA representation and the UPGMA tree (figs. 4, 6) substantiate this view by showing a general trend toward interpopulation differentiation. The F_{ST} values higher than 0.200 could be either the result of genetic drift or could reflect restricted levels of gene flow among populations. The high genetic diversity detected in both species makes it unlikely that the action of drift alone determined the degree of differentiation observed. Rather, it seems more feasible that the recent fragmentation of the Canarian laurel forest brought about a severe reduction of gene flow. Although the extant populations of either species are geographically adjacent as measured by linear distance on the map (roughly within 40 km for *D. spectabile* and 3 km for *I. chalcantha*), little interpopulation gene flow seems to be the rule for the two species. We must bear in mind that these populations are separated by abrupt geographical barriers (deep ravines and cliffs) that hinder genetic interchange. Sharp genetic differentiation has been also described in other endemic Canarian species (Kim et al. 1999; Francisco-Ortega et al. 2000). As discussed by Francisco-Ortega et al. (2000), high genetic differentiation values in Canarian endemics indicate that there must have been low levels of gene flow among populations despite their being predominantly outcrossing.

Because of a high degree of vulnerability of these two endemic species and the extremely low number of individuals in their extant populations, protection of their natural habitat,

Table 5

Pairwise F_{ST} Values between *Isoplexis chalcantha* Populations and Subpopulations

	Los Tiles	La Virgen 1	La Virgen 2
La Virgen 1	0.257		
La Virgen 2	0.296	0.130	
La Virgen 3	0.376	0.251	0.117

the Canarian laurel forest, is an obligate first step to help induce the recovery of populations. Elimination of many introduced animals (especially herbivores) and plants should have higher priority than transplanting endemic species, as suggested by Francisco-Ortega et al. (2000). Regulating the activities in the vicinities of the populations that may alter their fragile habitat and restricting public access to the area will probably help reduce the present state of degradation of the studied sites and facilitate the future monitoring of population dynamics.

However, this necessary habitat preservation might still prove insufficient if these populations represent genetically isolated "habitat islands" resulting from the fragmentation of a once widespread population whose genetic relatedness was maintained through moderate levels of gene flow. Templeton et al. (1990) showed that when there is no opportunity for recolonization, then each local extinction provoked by habitat fragmentation increases the probability of total extinction. If the severe and fast fragmentation of the Canarian laurel forest has disrupted the genetic cohesion of *I. chalcantha* and *D. spectabile*, then the survival of the extant populations of these endemics might be seriously threatened.

The genetic parameters estimated in this work present two important implications for eventual genetic recovery plans. First, the considerable genetic interpopulation differentiation found in both species suggests that each population should be considered as a distinct management unit in order not to break eventual coadapted gene complexes. Because of the inconspicuous genetic differences detected between subpopulations from La Virgen (*I. chalcantha*), they should be considered as a single collection area. Ideally, the germplasm sampling should be complemented with population reinforcement based on introductions of individuals from the same areas. Since the genetic makeup of individuals V35, V39, V45, and V48 from subpopulation 1 from La Virgen (*I. chalcantha*) and of individuals G14, G15, and G40 from Güímar are considerably different from the rest, as estimated by RAPD polymorphisms (figs. 4, 7), they must be given priority in the germplasm bank. More intensive sampling should be undertaken in the populations that appear to be more heterogeneous genetically (La Virgen for *Isoplexis* and Güímar for *Dorycnium*), with a view to obtaining a consistent representation of the species genetic variation. Second, the detection of significant profiles of intrapopulation spatial genetic structuring allows us to give advice on some aspects of fine-scale sampling strategies.

Sokal (1979) demonstrates that the first *X*-axis intercept is an operational estimate of the average length of the shortest side of true patches that are irregular in shape or variable in size. According to this interpretation, seed collection within populations of *D. spectabile* and *I. chalcantha* should be carried out at intervals of at least 3.5 m in *I. chalcantha* from La Virgen and 8 m in *D. spectabile* from Teno in order to minimize the probability of sampling genetically similar individuals.

Finally, although heeding the suggestions given above is likely to increase the populations' survival chances, we must bear in mind that these indications are based solely on an assessment of genetic variation through RAPDs. One possible shortcoming is that RAPDs might not be reflecting the differentiation in the traits that are critical for the survival and reproduction of the species (Olfelt et al. 2001). It is therefore necessary to investigate other important factors in the species biology that are crucial for population long-term survival. We hope that the implementation of the basic conservation measures proposed in this article will encourage the demographic and reproductive monitoring of these populations.

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