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Assessment of the *ex situ* available genetic diversity of three species extinct in Nature using RAPDs.

Abstract

Sahuquillo, E., Aguinagalde, I. & Gómez-Campo, C.: Assessment of the *ex situ* available genetic diversity of three species extinct in Nature using RAPDs. – Bocconea 13: 599-603. 2001. – ISSN 1120-4060.

The *ex situ* available genetic diversity of three extremely rare Spanish endemic plants now considered extinct in Nature - *Lysimachia minoricensis* Rodr., *Diplotaxis siettiana* Maire and *Helianthemum cirae* Santos - has been studied on material preserved in a genebank using RAPDs markers. Either considering the number of amplification products obtained, the number of polymorphic bands, the similarity among individuals or the total variance detected, the maximum diversity always corresponded to *Diplotaxis siettiana* while intermediate values were obtained for *Helianthemum cirae* and the lowest for *Lysimachia minoricensis*. The correspondence of these results with the case histories of the three species is discussed.

Introduction

The study of population genetics has been identified as an important priority for species conservation. Knowledge of the amount and distribution of genetic diversity helps to establish proper conservation strategies and plans for further monitoring (Holsinger & Gottlieb 1991). Preservation of genetic diversity is essential for the conservation of endemic species (Hamrick & al. 1991) especially when population size is small (Lande 1988, Aguinagalde 1998, Lázaro & Aguinagalde 1998). Along this line of thought, we have analysed the genetic diversity of the available material of three Spanish species regarded as extinct in Nature. These are *Lysimachia minoricensis* Rodr., *Diplotaxis siettiana* Maire and *Helianthemum cirae* Santos. All are not only included as extinct in the red data books (Gómez-Campo 1987, 1997), but their case histories are particularly dramatic (see below). Random amplified polymorphic DNA markers (RAPDs) are specially suitable for studies of this type because it does neither need large amounts of plant material nor previous knowledge of DNA sequences (Williams & al. 1990).

Diplotaxis siettiana Maire (*Brassiceae*) is an annual species endemic to the Island of Alborán (S. Spain). In 1974 the last known natural population on the island presented 150-200 individuals distributed in less than one hectare around a helicopter landing platform.

Shortly after, this population disappeared due to several environmental changes in the island. Seeds collected in 1974 and subsequently replicated were conserved in the seed bank of the ETSIA/UPM in Madrid. Reintroductions were unsuccessful and the establishment of a protected area is judged necessary, even while the island is being used as a military base (Gómez-Campo 1987).

Helianthemum cirae Santos (*Cistaceae*) is a perennial species endemic to the Island of La Palma (Canary Islands) whose dotlike locality is within La Caldera de Taburiente National Park at an altitude over 1000 m. The only known individual disappeared in 1993 probably due to depredation by "arruis" (herbivores from Morocco introduced into La Caldera in the 60s). Two plants obtained from seeds of the original individual were grown in the Jardín de Aclimatación de La Orotava (Tenerife) and successful propagation of the existing material was carried out. At present, a population of twenty reintroduced individuals seems to grow healthily in the wild and proper monitoring is being made (Santos, pers. comm.).

Lysimachia minoricensis Rodr. (*Primulaceae*) is a perennial species endemic to a south-oriented ravine in Menorca (Balearic Islands). Since a long time it is considered to be extinct in the wild. The last collection of seeds was done in 1926. Fortunately, a colony of a few individuals was found in the Botanical Garden of Barcelona after the Spanish civil war (1939). This material was propagated and distributed to other institutions. Successive re-introductions have been carried out since 1959 with uncertain results (Gómez-Campo 1987).

Material and Methods

Plant material used was obtained from seeds stored in the ETSIA/UPM genebank. Special care was placed in the sampling to avoid a significant depletion of the scarce existing material.

DNA was analysed from 23 individuals (*Lysimachia minoricensis* 8, *Diplotaxis siettiana* 10 and *Helianthemum cirae* 5). Total genomic DNA was isolated from young leaves following modified CTAB method (Doyle & Doyle 1991). The DNA concentration was determined in a Beckman Spectrophotometer at 260 nm. Eight arbitrary 10-decamer primers (Operon Technologies, Ca, USA) were used for PCR amplification and reproducible polymorphic fragments were detected: Reaction mixtures were incubated in a DNA Thermal cycler (Perkin Elmer Cetus) using Amplitaq DNA Polymerase "Stoffel Fragment" (Perkin Elmer Cetus). Amplification products were analysed by electrophoresis in 1.5% agarose gels, detected by staining with ethidium bromide and photographed under UV light. DNA molecular size marker from Boehringer was used.

Amplification products were listed as discrete character status per strain (present/absent). These data were analysed using the NTSYS-pc package, version 1.5 (Rohlf 1992) to determine the similarity among the individuals for each species. Similarities were computed using the Jaccard coefficient. The individuals were clustered by UPGMA method in order to present the results in a dendrogram. In addition, analysis of molecular variance (AMOVA) obtained by using the distance matrix among haplotypes was employed to estimate the variance within each species (Excoffier & al. 1992).

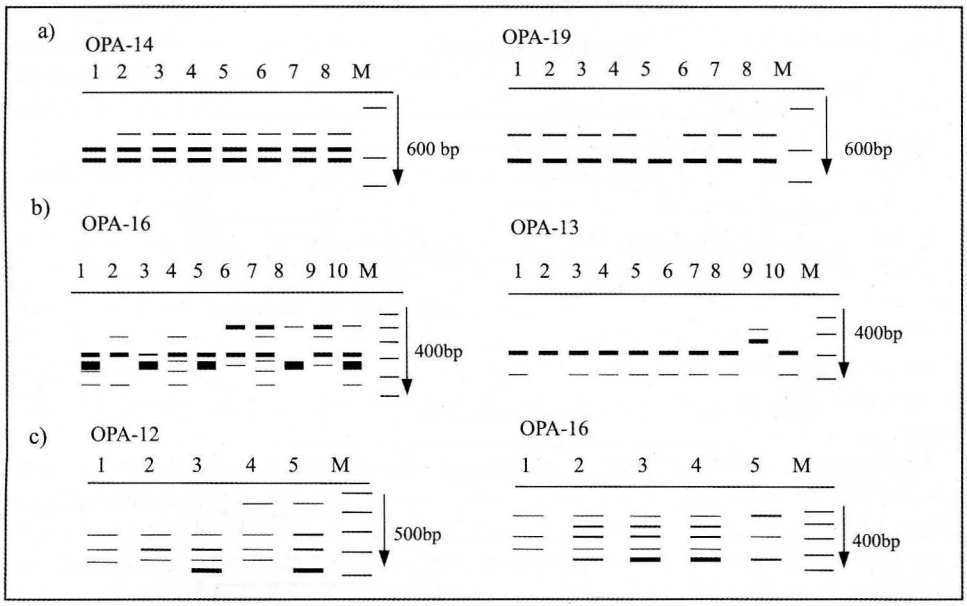

Fig. 1. RAPD profiles generated from individuals of: (a) *Lysimachia minoricensis*, (b) *Diplotaxis siettiana* and (c) *Helianthemum cirae* by five primers (OPA-12;13;14;16 and 19). M= molecular size markers.

Results and discussion

A total of 35 amplification products were generated from *Lysimachia minoricensis*, 48 from *Helianthemum cirae* and 66 from *Diplotaxis siettiana* and the percentage polymorphic bands for each species was 14%, 64% and 78%, respectively. The levels of polymorphism (Fig. 1) were very variable. For *H. cirae* it is similar to that found in other rare allogamous species such as *Erodium paularense* (44-51%) by Martin & al. (1997). This result is surprising if we take into account that the five analysed individuals belonged to the offspring of the unique plant found in Nature while *E. paularense* was sampled from a population with almost one thousand plants.

Cluster analysis within each species revealed different degrees of similarity among individuals. In *Lysimachia minoricensis*, 5 of 8 individuals showed 100% similarity and the rest exhibited over 90%. In *Helianthemum cirae* the levels of similarity ranged between 60-80% and in *Diplotaxis siettiana* between 45-75% (Fig. 2).

Total variance detected in each species as an estimate of the genetic diversity showed the lowest value in *Lysimachia minoricensis* (variance = 0.44) followed by *Helianthemum cirae* (variance = 4.33) while the highest value (variance = 23) was found in *Diplotaxis siettiana*.

Though little is known about their breeding system, everything seems to suggest that at least *Diplotaxis siettiana* and *Helianthemum cirae* (showy flowers, behaviour of their closest relatives, etc.) were allogamous in Nature. The higher genetic variability of *Diplotaxis siettiana* agrees with the generous sampling originally obtained from a - relatively! - large

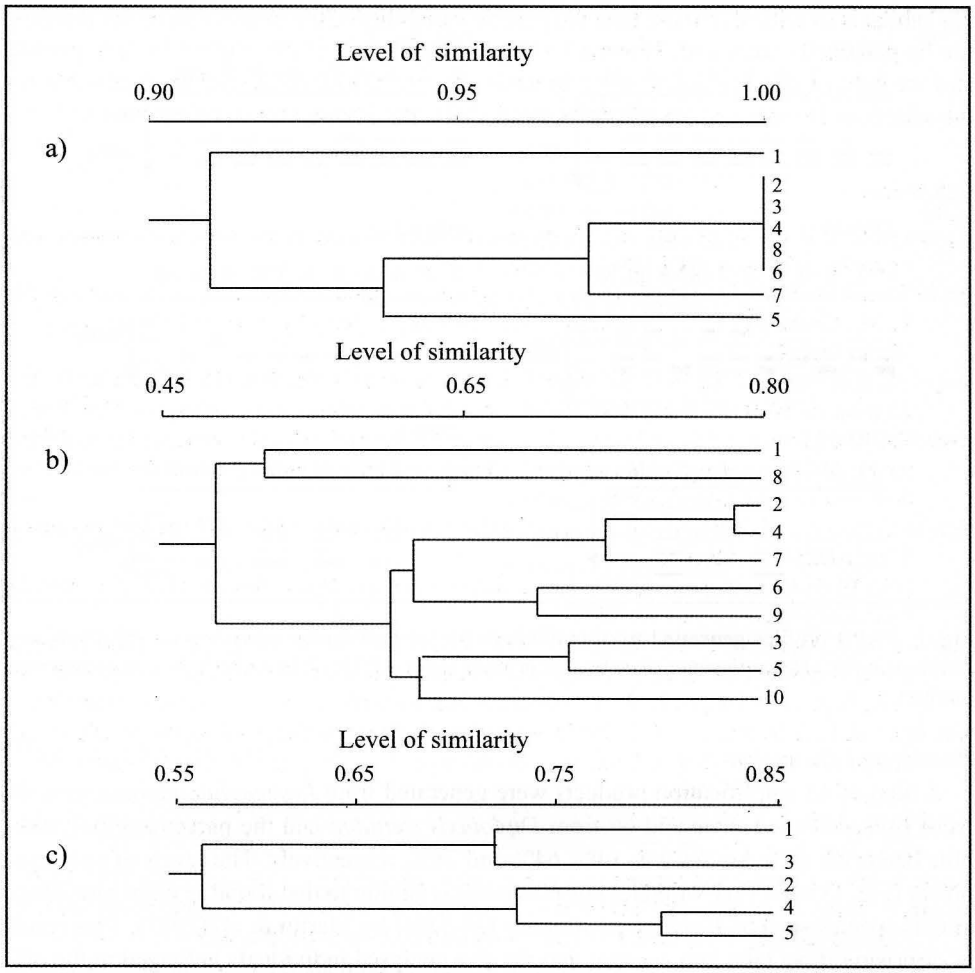

Fig. 2. Dendrograms indicating relationships between the studied individuals for each species analysed: (a) *Lysimachia minoricensis*, (b) *Diplotaxis siettiana*, (c) *Helianthemum cirae*.

population. The lower values for *Helianthemum cirae* and *Lysimachia minoricensis* also agree with their respective case histories and indicate that the unique known individual of *Helianthemum cirae* exhibited a considerable degree of heterozygosity. This in turn might be a hint for the existence of other unknown individuals in its rough mountainous surroundings.

Though endemic species are often genetically depauperate as it is, for instance, the case of *Brassica macrocarpa* (Lázaro & Aguinagalde 1998), it has been well documented that we cannot associate low genetic variation with endemism *per se* (Kruckeberg & Rabinowitz 1985). However, it is more than reasonable to associate low genetic diversity with danger of extinction (Beardmore 1983). All the material studied by us is already extinct, but our results could help to predict their chances of survival in the case they are successfully re-established in Nature. In such extreme cases, it is obvious that the persistence of the origi-

nal habitat is as critical or more than the genetic variability of the material since the last cannot be practically increased. However, a previous appraisal of this variability is important and we believe that the design of programmes for integrated conservation or possible reintroductions for these or other species should take into account this type of studies.

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