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Article in *Plant Systematics and Evolution* · November 2008

DOI: 10.1007/s00606-008-0078-z

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Gnaphalium teydeum and *Gnaphalium luteo-album*: two taxa of the Canary Islands with different genetic histories

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Received: 20 February 2008 / Accepted: 21 July 2008
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Abstract *Gnaphalium teydeum* and *G. luteo-album* (Asteraceae) are two closely congeneric taxa native to the Canary Islands. While *G. luteo-album* is widespread in the Macaronesian Region, *G. teydeum* is endemic to the island of Tenerife and considered endangered by IUCN. Using the RAPD technique this study investigated the level and apportionment of genetic diversity of these taxa, trying to solve a taxonomic dispute related to *G. teydeum*. Based on the 102 DNA fragments generated by 11 primers, a high level of genetic differentiation was found between the taxa ($F_{ST} = 0.366$), with *G. luteo-album* showing levels of genetic variability ($P = 100\%$; $H = 0.246$) higher than those found in *G. teydeum* ($P = 75.5\%$; $H = 0.173$). UPGMA dendrogram and Bayesian cluster analysis clearly separated populations from both the species. Overall, results show that although morphological differentiation between *G. teydeum* and *G. luteo-album* is not strong, they show marked molecular divergence, supporting the current taxonomic status.

Keywords Canary Islands · Conservation genetics · Endangered species · Genetic diversity ·

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Gnaphalium luteo-album · *Gnaphalium teydeum* ·
RAPD

An increasing number of studies have demonstrated the value of genetic data in addressing issues of plant conservation biology, especially in identifying populations in which genetic factors are likely to affect their prospects of long-term survival, in developing approaches to reintroduction biology, or in resolving taxonomic uncertainties (Frankham et al. 2002; Ouborg et al. 2006).

One of these taxonomic uncertainties occurs in the Canary Islands, where only two taxa of *Gnaphalium* genera (Asteraceae) occur: *Gnaphalium teydeum* R. Knapp (= *Laphangium teydeum* Wildpret and Greuter) and *Gnaphalium luteo-album* (L.) (= *Laphangium luteo-album*), (Greuter 2003).

Gnaphalium teydeum is a small, herbaceous endemic plant, apparently perennial, which exclusively inhabits Tenerife, one of the Canary Islands. Its leaves are up to 12×5 mm in size, grouped in basal rosettes and whorls, tomentose, and with a whitish colour. This plant grows in locations of over 2,000 m in altitude and only two sites have been identified so far, approximately 8 km apart. In spite of its extreme rarity, the species has been used to characterize and designate a plant association, *Vulpio myuri-Gnaphalietum teydei* (Wildpret et al. 1997; Del-Arco et al. 2006). This association is exclusive to enclaves with notable humidity, such as wetlands with hot springs occurring around the fumaroles which are still active in the foothills of the volcano Teide at altitudes of ca 3,500 m (Greuter 2003; Bañares et al. 2004).

Very little is known about the reproductive biology of *G. teydeum*, but it has been described as perennial and

anemophilic (Greuter 2003; Bañares et al. 2004), and, similar to other Asteraceae, as having good powers of dispersal (Silvertown 2004). In addition, different studies have shown that most Asteraceae species have a sporophytic self-incompatibility (SSI) system (Burt 1977; Richard 1986). [Important exceptions exist in the recent literature where some deviation from this self-incompatibility has been reported (Grombone-Guaratini et al. 2004).] However, there are no data regarding this aspect of *Gnaphalium teydeum* or *G. luteo-album*. Knowledge on reproductive biology of the species is of great importance for the interpretation of genetic data and subsequent considerations regarding its conservation.

Gnaphalium luteo-album, by contrast, is a plant distributed worldwide, and native to the Macaronesian Islands (Greuter 2003; Izquierdo et al. 2004). It is an annual plant growing to a height of 60 cm, with alternate, simple, linear-lanceolate leaves, covered with whitish hairs.

Greuter (2003) defines *G. teydeum* as a diminutive version of *G. luteo-album*. According to this author, the two taxa differ mainly in the dimensions of their parts, all of which are smaller in *G. teydeum*. However, other authors have expressed doubts about the degree of separation between the taxa, and have raised the possibility that they are synonymous (Kunkel 1991; Bañares et al. 2004). No systematic study has yet been carried out to trying to clarify this position (Bañares et al. 2004).

However, due to its extreme rarity, *G. teydeum* has been ascribed the IUCN status of “endangered” (VVAA 2000). It is also protected by the Canarian Government, and is included in the Canarian Catalogue of Endangered Species (BOC 2001). The main problems affecting this species derive from a shortage of individuals and its low ecological plasticity, only developing in wet sites, which in the areas of its distribution, are rather scarce. To this should be added pressure from introduced herbivores (rabbits, goats, etc.), and from human activity in the area, especially for the population of Teide, where every year thousands of people visit the National Park, recently added to the UNESCO World Patrimony List. Nevertheless, stocks here appear to have some stability, and all the known populations are protected and included within Teide National Park (Bañares et al. 2004).

Genetic depletion, characteristic of species with a history of fragmented populations and very small population sizes, is believed to have a dramatic impact on the ability of the species to survive environmental changes (Segarra-Moragues et al. 2005; Ouborg et al. 2006), since these could be associated with certain fitness traits. In this respect neutral markers are useful in estimating the relative evolutionary importance of genetic factors such as gene flow and genetic drift, and in clarifying the

relationships between closely related taxa (Frankham et al. 2002).

During the last decade, several new DNA markers have emerged which have been rapidly integrated into the arsenal of commonly used routine laboratory tools available for genome analysis. Among these, Polymerase chain reaction (PCR)-derived markers, obtained with non-species-specific primers, have become exceedingly popular, since they do not require sequence information for the target species. The advantages and disadvantages of RAPD, AFLP and ISSR have been widely debated (Nybom 2004). Early RAPD analysis showed poor reproducibility; however, this technique has been enhanced through improved laboratory techniques and band scoring procedures (Skroch and Nienhuis 1995; Weising et al. 1995). Overall, direct comparisons between the three dominant markers show little difference between their performance (Nybom 2004). Its rapidity and simplicity, the absence of the need for any prior genetic information from the plant, and the fact that a large number of informative loci from across the entire genome can be assessed with RAPDs at relatively low cost, make this technique the most widely employed anonymous genetic marker in plant population studies (Nybom and Bartish 2000). Moreover, RAPD has been found to be particularly appropriate for studies involving small sample sizes, especially for outbreeders, because large numbers of polymorphic loci can be generated.

The general goals of this study are: (1) to use RAPD markers to obtain the information required to explore the relationship between *Gnaphalium teydeum* and *G. luteo-album* in the Canary Islands (our hypothesis being that genetic differences between two taxa should be higher than those within a species), and (2) to use this molecular information to help in assessing the protection status of *G. teydeum*, in order to formulate appropriate management and conservation strategies.

Materials and methods

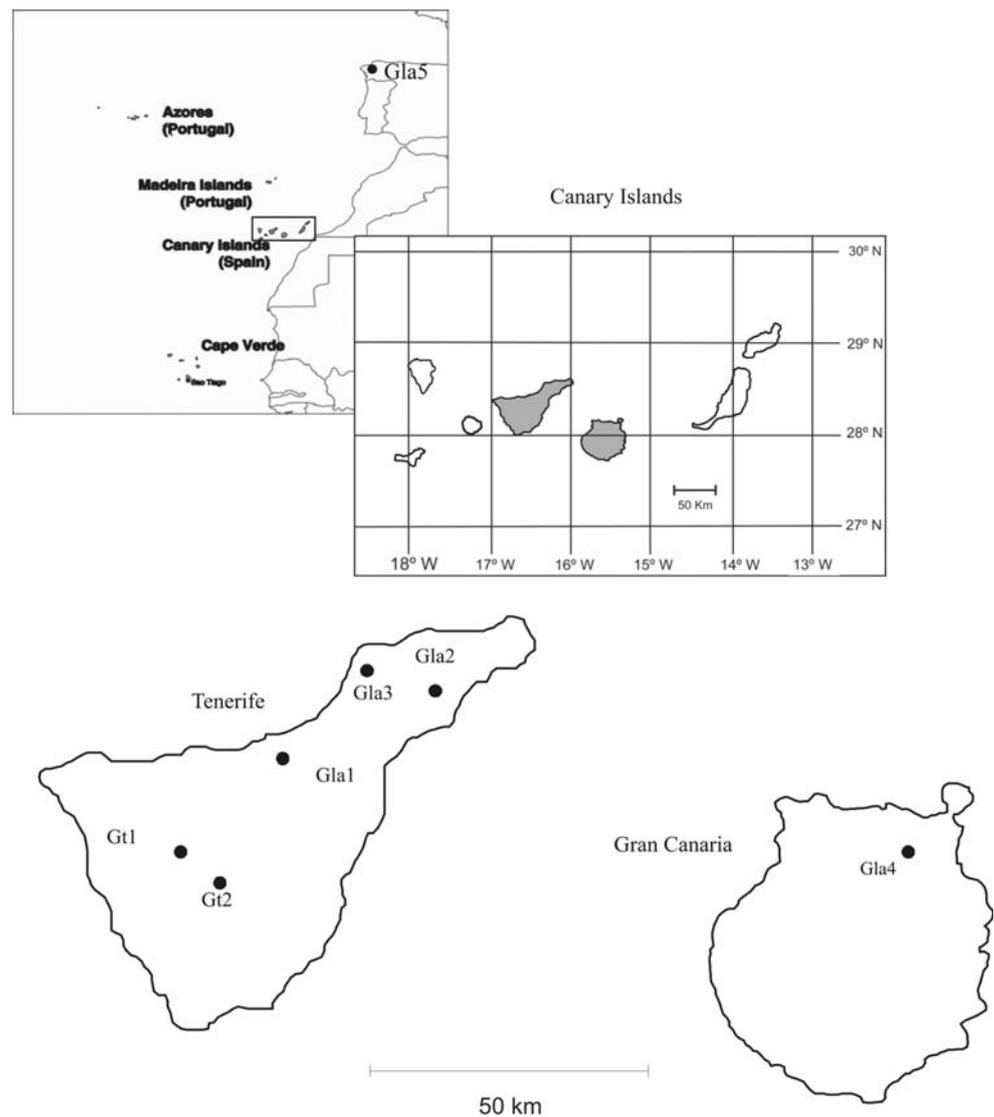
Plant sampling

Seventy-three individuals of *Gnaphalium teydeum* from the only two populations of the species known in Tenerife were sampled: 38 from Teide and 35 from Guajara. These sample sizes represent approximately 30 and 50%, respectively, of the total known individuals of these populations (Table 1; Fig. 1). Since one of the main goals of this study was to establish a genetic analysis comparing the natural populations of *G. teydeum* and *G. luteo-album*, samples from natural populations of *G. luteo-album* located in different geographical areas were collected. A hundred

Table 1 Sample size (*N*) and locality of *Gnaphalium teydeum* and *G. luteo-album* populations analyzed

Taxon	Code	Population	Location (Island)	<i>N</i>
<i>G. teydeum</i>	Gt1	Teide	Teide National Park (Tenerife)	38
	Gt2	Guajara	Teide National Park (Tenerife)	35
Total				73
<i>G. luteo-album</i>	Gla1	Valle Guerra	Tacoronte (Tenerife)	30
	Gla2	Agua García	Tacoronte (Tenerife)	31
	Gla3	Taco	La Laguna (Tenerife)	22
	Gla4	San José del Álamo	Las Palmas (Gran Canaria)	29
	Gla5	Sarela	A Coruña (Galicia); North of Iberian Peninsula	30
Total				142

Fig. 1 Locations of the populations sampled. Codes of populations correspond to those in Table 1



and forty-two individuals of *G. luteo-album* from five populations were collected: three populations were from the island of Tenerife—from Valle Guerra, Agua García and Taco—and the fourth population was from San José

del Álamo in Gran Canaria (another island in the Canaries). The final population from which samples were collected was in Galicia, at the River Sarela in the North West of the Iberian Peninsula (Fig. 1; Table 1).

DNA isolation

All samples were transported and conserved in zip-lock plastic bags with silica gel until their DNA extraction. About 1 cm² of dry leaf was macerated with a small amount of silica gel, with a sterile pestle and mortar, and the resulting powder transferred to a 1.5 ml tube. Total DNA was isolated following Doyle and Doyle (1987); 150 µl of total DNA samples were purified using QIAquick Nucleotide Removal Kit protocol (QIAGEN). DNA concentration and quality measures were performed by spectrophotometric measurements of absorption at 260 nm (Beckman Coulter DU 530).

RAPD amplification and electrophoresis

In the first assay, 48 primers from Operon Technologies (OPN 1-20, OPM 1-20 and OPL 1-8) were tested with ten randomly selected individuals from the Teide population (from Tenerife). Eleven RAPD primers (OPN4, OPN6, OPN9, OPN12, OPN13, OPN15, OPN16, OPN18, OPN19, OPN20 and OPM2) which produced clean and reproducible fragments were selected for further analysis.

DNA amplifications were carried out on 96-well Eppendorf PCR plates. In each well 20 ng of DNA, 23 µl of ReddyMix PCR Master Mix (AB-0619/LD, AB-gene), 0.25 µl BSA (0.4%) and 1 µl of oligonucleotide (OPERON) was placed. Amplifications were run in an Eppendorf Mastercycler Gradient, programmed for 45 cycles at the following thermal conditions: 30 s at 94°C, 30 s at 36°C, and 1 min at 72°C. The initial and final steps were at 94°C for 1.5 min, and 72°C for 10 s, respectively. RAPD products were resolved electrophoretically on 1.8% agarose gel, run at 200 V (60 mA) for 3 h in 1× TBE buffer. A 100 bp DNA ladder (100 Base-Pair Ladder, Amersham Pharmacia) was added as a molecular ruler. DNA was stained with ethidium bromide (0.5 µg/ml) and photographed under UV light ($\lambda = 302$ nm) using a Kodak DC 40 digital camera. The reproducibility of the PCR products was improved by maintaining standardized conditions with regard to all possible sources of variation. Thus, the same PCR machine and the same Taq polymerase were used throughout, and samples of each species were treated in separate amplification plates. In addition, RAPD reactions were repeated to confirm reproducibility, and markers that were inconsistently amplified in repeated RAPD reactions were not included in the subsequent data analysis. The concentration of DNA used was found to be optimal with respect to reproducibility and minimization of secondary ghost banding. Reaction mixes without DNA were run as blanks in all RAPD amplifications. Scoring was carried out conservatively, cross checked independently by at least two researchers, and any doubtful bands were excluded from the

analyses. Following referee recommendations, fragments larger than 2,000 bp were not considered because of the high probability of homoplasy. The presence or absence of each band was recorded for each individual and assigned a value of 1 or 0 depending on its presence or absence, respectively. The banding patterns were interpreted with the help of Kodak Digital Science program. Bands showing the same gel mobilities were assumed to be homologous, a rationale widely used in RAPD studies conducted in closely related taxa.

Data analysis

The presence/absence band data matrix was entered into TRANSFORMER 3b.01 software (Caujapé-Castells and Baccari-Rosas 2005) which allowed this data to be exported to different programs. Elementary genetic diversity statistics, percentage of polymorphic loci, and gene diversity (Nei 1973) were calculated using POPGENE 3.2 software (Yeh et al. 1997). Nei (1972) genetic distance matrix among populations was calculated using POPULATIONS 1.2.30beta software (Langella 2005). One thousand bootstrap replicates of the distance matrix were obtained, and UPGMA trees were generated and analyzed in MEGA version 3 (Kumar et al. 2004).

In order to identify the genetic differentiation between populations, pair-wise divergences were analyzed using the F_{ST} approach (Weir and Cockerham 1984) using AFLP-SURV 1.0 (Vekemans 2002).

To evaluate the genetic differentiation between *G. teydeum* and *G. luteo-album*, the population structure was also inferred using the Bayesian clustering analysis procedure implemented in STRUCTURE (Pritchard et al. 2000), designed to identify the K (unknown) populations of origin of the sampled individuals and assign simultaneously the individuals to the populations. The most likely value of K is assessed by comparing the likelihood of the data for different values of K . The admixture population model and independent allele frequencies were assumed following the STRUCTURE authors' advice. A series of independent runs were conducted for each value of K (the number of populations) between 1 and 13. Analyses consisted of a 10^5 burn-in period replicated, and a run length of 10^6 replicated. Populations or individuals were assigned to a cluster if their proportion of membership (q_i) to that cluster was equal to or larger than an arbitrary threshold of 0.800.

The genetic similarity matrix (Nei and Li 1985) between all possible pair-wise combinations of individuals was computed from the presence of bands using RAPDPLOT 2.3 (Black 1995). The reliability of the data matrix was evaluated using 1,000 bootstrap replicates. The resulting genetic similarity matrices were input to PHYLIP v3.5

(Felsenstein 1993) and used to build a consensus neighbour-joining similarity tree between the 73 individuals analyzed in *G. teydeum* and the 30 individuals from the Valle Guerra population of *G. luteo-album*.

Results

Genetic diversity levels

The 11 polymorphic RAPD primers assayed resolved a total of 102 bands (ranging from 335 to 1,950 bp) for all the individuals of both taxa analyzed. There were common RAPD fragments in *G. teydeum* but they were not exclusive to this species, since these bands were also recorded in *G. luteo-album*. However, 14 fragments exclusive to *G. luteo-album* were detected.

For *G. teydeum*, the Teide population (Gt1) showed, on the whole, higher levels of genetic variation than the Guajara population (Gt2) (Table 2). Genetic diversity values were $P = 67.6\%$, $NF = 69$, $H = 0.143$ and $P = 49.0\%$, $NF = 50$, $H = 0.140$ for the Teide and Guajara populations, respectively.

Table 2 Genetic diversity indexes per population and taxa

Taxon	Population	N	NF	P	H
<i>G. teydeum</i>	Teide	36	69	67.65	0.143
	Guajara	34	50	49.02	0.140
Overall		70	77	75.49	0.173
<i>G. luteo-album</i>	Valle Guerra	28	66	64.71	0.178
	Agua García	28	60	58.82	0.157
	Taco	19	41	40.42	0.113
	San José del Álamo	27	86	84.31	0.220
	Sarela	28	69	67.65	0.176
Overall		130	102	100	0.246

N sampled size, NF number of polymorphic fragments, P percentage of polymorphic loci, H gene diversity (Nei 1973)

In the case of *G. luteo-album*, the proportion of polymorphic loci among the five stocks spread over a wider range (40.4–84.3%), as did the rate of genetic diversity indexes (0.113–0.220). The population from San José del Álamo (Gran Canaria) showed the highest values of genetic diversity, while the population of Taco (Tenerife) showed the lowest genetic diversity, possibly due to the fact that fewer individuals were analyzed in this population.

At the species level, *G. luteo-album* clearly showed higher levels of genetic variability ($P = 100\%$, $NF = 102$, $H = 0.246$) than those found in *G. teydeum* ($P = 75.5\%$, $NF = 77$, $H = 0.173$) (Table 2), which accords with the wide spread of the former, and the endemic nature and low distribution of the latter.

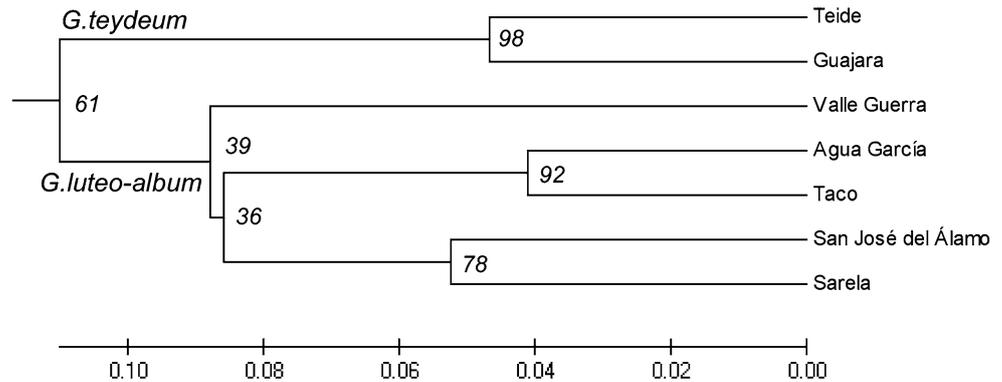
Genetic differentiation and genetic relationship between taxa

Overall, the analysis carried out on the RAPD matrix showed a clear genetic differentiation between the populations of the two taxa (Table 3). Genetic distance between the two populations of *G. teydeum* was 0.093, while genetic distance between populations of *G. luteo-album* ranged from 0.082 (between the Agua García and Taco populations) to 0.272 (between the Teide and Taco populations). Average genetic distance between *G. teydeum* and *G. luteo-album* populations (0.206) was always higher than those estimated between *G. luteo-album* populations (0.154) or between *G. teydeum* populations (0.093). In addition, the mean value of F_{ST} across all stocks of *G. luteo-album* (comparisons Gla-Gla) was 0.274, ranging from 0.160 between Sarela and San José del Álamo, to 0.370 between Taco and San José del Álamo. Only three of these values were higher than 0.300 (Table 3). However, the values of F_{ST} between populations of different taxa (Gt-Gla) were always higher than 0.300 (average $F_{ST} = 0.366$), ranging from 0.314 between Guajara and Valle Guerra to 0.479 between Teide and Taco (Table 3).

Table 3 Pairwise F_{ST} (below) and Nei (1972) genetic distance (above) between *G. teydeum* and *G. luteo-album* populations

Population	<i>G. teydeum</i>		<i>G. luteo-album</i>				
	Teide (Gt1)	Guajara (Gt2)	Valle Guerra (Gla1)	Agua García (Gla2)	Taco (Gla3)	San José del Álamo (Gla4)	Sarela (Gla5)
Teide (Gt1)		0.093	0.213	0.235	0.272	0.180	0.222
Guajara (Gt2)	0.236		0.168	0.184	0.199	0.169	0.217
Valle Guerra (Gla1)	0.371	0.314		0.205	0.152	0.203	0.143
Agua García (Gla2)	0.398	0.324	0.317		0.082	0.184	0.160
Taco (Gla3)	0.479	0.399	0.274	0.210		0.182	0.124
San José del Álamo (Gla4)	0.318	0.317	0.288	0.319	0.370		0.105
Sarela (Gla5)	0.371	0.371	0.222	0.299	0.284	0.160	

Fig. 2 UPGMA dendrogram, based on Nei (1972) genetic distance, including bootstrap support values in percentage, for populations of *G. teydeum* and *G. luteo-album* from the Canary Islands. Population codes follow Table 1



The UPGMA tree (Fig. 2) showed two main clusters. One of these included the two natural populations of *G. teydeum* (Teide and Guajara), with a high bootstrap support (98%). The second cluster grouped all the populations of *G. luteo-album*. Within this species cluster, the populations from San José del Álamo (Gran Canaria) and Sarela (on the north of the Iberian peninsula) were clustered together. The significant genetic relationship found between the populations of San José del Álamo and Sarela is surprising, since they are more than 4,000 km apart.

In the Bayesian analysis, using the total data set (142 individuals, 102 loci, 7 sampled populations) and $K = 2$ –13, the probability of the data was maximum, with $K = 12$, suggesting the presence of additional levels of structure in the total sample. Choosing a value of K that maximizes the posterior probability of the data (PPD) can be difficult to apply for complex data sets including many groups (Rosenberg et al. 2002). In the case of highly structured data, as K is increased the most divergent groups separate first into distinct clusters. Since the aim should be to find the smallest value of K that captures the major structure in the data, a second way to choose K is to consider the successive increase of the PPD for increasing values of K , which can be regarded as the increase in information at each addition of a set of allele frequencies.

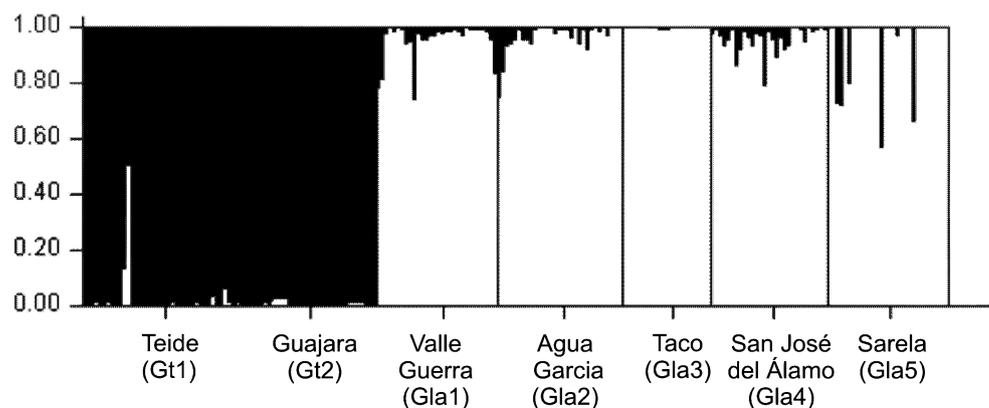
The PPD increased from $K = 2$ to $K = 12$, where it reached its maximum value and then reached a plateau. This result indicated that differentiation occurs between most of the sampling sites. Generally, each of the clusters of individuals obtained for a given value of K split into two clusters for $K + 1$.

The increase of the PPD was high for $K = 2$, but for $K > 2$ the increase in information became markedly less and showed gradually decreasing values. This result means that the information obtained by the three clusters (and the subsequent clusters) was much less important than the information obtained by the former two. Once two populations had been assigned to different clusters for $K = 2$, they never belonged to the same cluster for greater values of K . When two clusters ($K = 2$) were assumed the individuals were assigned asymmetrically to each group. Predefined populations assigned to the first group included all the *G. teydeum* populations, Teide and Guajara, while populations grouped in the second cluster included all the *G. luteo-album* populations (Fig. 3).

Conservation genetics of *G. teydeum*

The establishment of genetic differentiation between the taxa through phenotype RAPD, and the degree of threat to *G. teydeum*, enable the application of the results of genetic

Fig. 3 Bar plots for proportion of coancestry inferred from Bayesian cluster analysis. Each individual is represented by a thin vertical line, which is partitioned into K colored segments that represent the individual's estimated membership fractions in K clusters. Black lines separate individuals from different populations



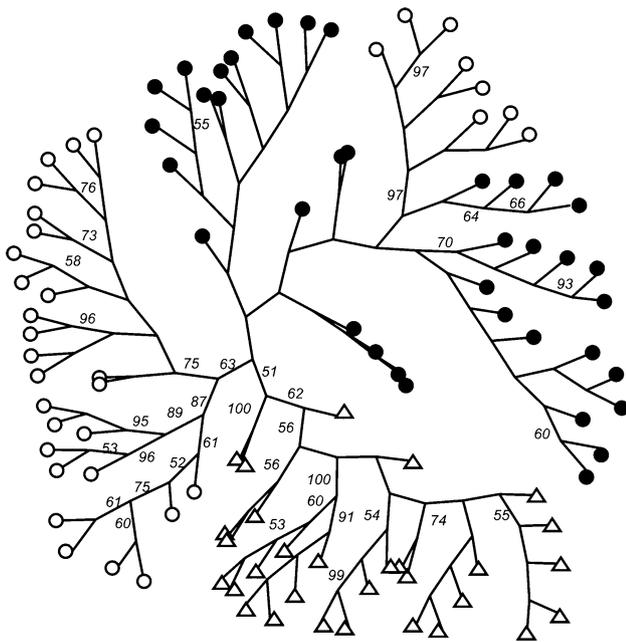


Fig. 4 Neighbour-joining (NJ) tree, based on Nei and Li (1985) genetic similarity, including bootstrap support values in percentage, showing the relationship among individuals of *G. teydeum* (Teide filled circle, and Guajara open circle) and individuals of *G. luteo-album* from Valle Guerra (open triangle), as outgroup

tests as a contribution to the conservation of this endangered species, thus fulfilling the second objective of this study.

A neighbour-joining (NJ) tree was produced, comparing the relationships between all the different RAPD phenotypes in the two populations of *G. teydeum*. In order to do this, a population of *G. luteo-album*, Valle Guerra (Gla1) from Tenerife, was used as the outgroup (Fig. 4). NJ clustering of phenotypes showed similar results to those from the UPGMA tree. The three major clades of this tree clearly separated the two natural populations of *G. teydeum* (Teide and Guajara), as well as the *G. luteo-album* population (Valle Guerra) (Fig. 4). Moreover, individuals from the two natural populations of *G. teydeum* were clustered separately according to their putative populations, except for a set of 8 individuals from Guajara, which were included in the Teide population subgroup. This constitutes a sign of the genetic relationships between the two populations of *G. teydeum* and proves that there is gene flow between them.

Discussion

Given their small population sizes and endemic character, as well as a very restricted geographic distribution, we expected *G. teydeum* populations to be genetically

impoverished, as has been shown to be the case for other limited and rare species (Ouborg et al. 2006).

At odds with this prediction, the levels of RAPD genetic variation of *G. teydeum* are surprisingly high. These are higher than most of those described by different authors using RAPDs in endangered and rare species with a reduced number of individuals in their populations, such as *Fitzroya cupressoides* (Allnut et al. 1999), *Goodyera procerca* (Wong and Sun 1999), *Antirrhinum microphyllum* (Torres et al. 2003), *Myrica rivas-martinezii* (Batista et al. 2004), and even higher than those found in *Isoplexis chalcantha* and *Dorycnium spectabile*, two other Canarian endangered species located in the Laurel forest (Bouza et al. 2002).

The precise historical distribution and population sizes of *G. teydeum* are very difficult to establish due to its rarity in the wild (Bañares et al. 2001). We cannot discount the possibility that the number of individuals of this species was larger in the past. As is the case with many other plant species from the Canary Islands, the relatively recent introduction of rabbits, goats and other herbivores has had a significant impact on the natural plant populations (García-Casanova et al. 2001) and this could explain the high genetic diversity detected in this endemic species. However, another explanation of the high genetic diversity of *G. teydeum* (and of *G. luteo-album*) may be the existence of outcrossing. Very little is known about the reproductive biology of *G. teydeum*, but it has been described as perennial and anemophilic (Greuter 2003; Bañares et al. 2004), and in common with other Asteraaceae, having good powers of dispersal (Silvertown 2004). Gene flow between populations seems to be occurring. Genetic distance between *G. teydeum* populations is only 0.093, and certain individuals of the Guajara population were grouped together with those of the Teide population (Fig. 4). These findings, together with the plant’s perennial character (Bañares et al. 2004), in which different generations overlap, could also be responsible for the high genetic variability detected.

According to Hamrick and Godt (1989), reproductive biology is the most important factor in determining the genetic structure of plant populations. They showed that outcrossing plant species tend to exhibit between 10 and 20% of the genetic variation among populations, while selfing species exhibit, on average, 50% of this variation between populations. In addition, RAPD analysis of outcrossing species has shown that levels of among population variation either approximate to or exceeds the average value of 14.8% (Huff et al. 1993; Huff 1997). Bouza et al. (2002), using RAPDs, found a coefficient of differentiation values (F_{ST}) of 0.264 and 0.230 among populations of two endangered endemic Canarian plant species; the self-incompatible *Dorycnium spectabile*, and the allogamous

Isoplexis chalcantha, respectively. In concordance, F_{ST} value between *G. teydeum* populations and *G. luteo-album* populations were 0.236 and 0.274, respectively (Table 3), as expected for a predominant outcrossing species. Contrary to this, *Gentianella germanica*, a rare self-compatible species showed higher variation among populations (0.51) (Fischer and Matthies 1998). Therefore, *G. teydeum* and *G. luteo-album* appear to behave as a self-incompatible species. This could indicate a sporophytic self-incompatible system, in common with most of the Asteraceae species (Burt 1977; Richard 1986). Therefore, taking into account the proportion of genetic diversity found among populations of outcrossing species as discussed above, it is suggested that *Gnaphalium teydeum* and *G. luteo-album* show self-incompatibility.

However, at the same time, we believe our results constitute a new example of the emerging picture of higher genetic diversity in Canary Island endemisms than in those from more remote archipelagos, such as the Hawaiian Islands (Francisco-Ortega et al. 2000; Sosa 2001; Sosa et al. 2002; Crawford et al. 2006), or the Juan Fernández Islands (Crawford et al. 2001). Different hypotheses have been proposed to explain these findings, including the suggestion that some Canarian endemisms represent old lineages that took refuge in the Macaronesian region during periods of glaciation and desertification in Europe and Northern Africa after the Miocene (Francisco-Ortega et al. 2000), and the progressive accumulation of mutations over time (Francisco-Ortega et al. 2000; Bouza et al. 2002). However, the factors responsible for this genetic diversity remain to be elucidated (Crawford et al. 2006). Supposing a monophyletic origin for both taxa, it would also be possible that the putative ancestor of *G. teydeum* was genetically diverse and that the current diversity reflects that history.

The natural tendency for colonization of the Canary Islands from the African continent, which seems to have occurred from east to west (Francisco-Ortega et al. 2000; Marrero and Francisco-Ortega 2001; Sosa 2001), could explain the pattern of genetic diversity and specific bands detected in samples from Tenerife in relation to those in Gran Canaria, since *G. luteo-album* in Tenerife (Valle Guerra, Agua García and Taco) has less genetic variation than in Gran Canaria (San José del Álamo). However, the make up of their bands is a subset of that found in Tenerife (15 exclusive alleles), with few specific markers (only five for Gran Canaria). Nevertheless, it should be borne in mind that only one population from Gran Canaria (27 individuals) is being compared with three populations from Tenerife (75 individuals). In fact, if the population from Gran Canaria is compared independently with each of the populations from Tenerife, the former shows a higher number of specific markers. This might suggest that

the few specific markers found in Gran Canaria are due to a stochastic event, and that an increase in sample size might show the natural and expected pattern. Therefore, we would expect high genetic diversity values in Gran Canaria in comparison to those in Tenerife, following a step-by-step colonization from the eastern to western islands.

Gnaphalium teydeum and *G. luteo-album* are closely related species, but while the former is exclusive to the archipelago—specifically to the island of Tenerife—and has a very small and restricted distribution, *G. luteo-album* is native and widespread, found throughout the Macaronesian islands, including the lower zones of Tenerife. Morphologically, the taxa are very similar (Greuter 2003) but our results show that *G. teydeum* is a species different from *G. luteo-album*. The various estimates of genetic differentiation analyzed show a clear separation of genetic pools between the species. Inter-species F_{ST} values were always found to be higher (average $F_{ST} > 0.300$) than those found intra-species (the average for *G. luteo-album* $F_{ST} = 0.274$), and similar values (F_{ST} ranging from 0.189 to 0.825) were found among *Antirrhinum* species by Jiménez et al. (2005), using RAPD. In accordance with the current taxonomic status of separate species, Bayesian clustering detected the uppermost level of structure at $K = 2$, corresponding to the expected difference between *Gnaphalium teydeum* and *G. luteo-album* (Fig. 3). Similarly, evidence from the UPGMA dendrogram and neighbour-joining tree reinforced the genetic differentiation, placing populations and individuals, respectively, in different non-overlapping groups (Figs. 2, 4), supporting the hypothesis that the two *Gnaphalium* species present in the Canary Islands are significantly differentiated. The use of all of these methods, and their congruent results, especially those obtained by the Bayesian methods approach, show that the two *Gnaphalium* taxa gene pools are differentiated.

G. luteo-album is considered as a species native to the Canary Islands (Izquierdo et al. 2004), and it would be feasible to consider *G. teydeum* as a derivative form of *G. luteo-album* which was limited to specialized habitats, reducing its size. Greuter (2003) used morphological characteristics to suggest that the endemic high mountain plant might be a specialised, recent derivative of the lowland species (*G. luteo-album*). In this sense, it is feasible that niche pre-emption by the early colonization of *G. luteo-album* (a very widespread species) may have inhibited the success of *G. teydeum*. Under this scenario the latter species was relegated to specialized habitats (hot and wet areas around the fumaroles). Similarly, a niche preemption is suggested by Carine et al. (2004) to account for the limited number of colonisations of *Convolvulus* into the region.

The high level of genetic similarity found between *G. teydeum* and *G. luteo-album* populations fits the values expected for closely related species. Considering the extensive distribution of *G. luteo-album* in the Macaronesian region, it is feasible that *G. teydeum* is recently derived from a common ancestor closely related to *G. luteo-album*. The hypothesis of a recent divergence from *G. luteo-album* gains additional support from the fact that *G. teydeum* has less genetic variation than *G. luteo-album*. Its allelic makeup is a subset of that found in *G. luteo-album*, with no unique alleles.

Molecular phylogenies for endemic plant groups of the Canary Islands (Francisco-Ortega et al. 2001) show that most of them are monophyletic and evolved from a single colonization of the archipelago (Crawford et al. 2006). This kind of case is common in different groups and species in the Canary Islands (Batista et al. 2004; González-Pérez et al. 2004; Olivia-Tejera et al. 2006).

We cannot exclude the hypothesis that morphological differences in *G. teydeum* might be just an adaptation; however, the results of this study suggest otherwise. Indeed, samples of both the taxa could be subject to common garden experiments to check the degree to which their differences in size are as a result of plasticity induced by the apparently extreme environment in which *G. teydeum* occurs.

Considerations for conservation genetics of *Gnaphalium teydeum*

RAPD analysis has provided useful information for conservation purposes of endangered species (Wong and Sun 1999; Sosa 2001; Bouza et al. 2002; Sosa et al. 2002; Batista et al. 2004; González-Pérez et al. 2004). These data can provide valuable information for conservation biologists because they allow the estimation of population genetic differentiation between and within populations and support the design of sampling strategies for *ex situ* collections (Sosa 2001; Frankham et al. 2002; Ouborg et al. 2006).

Due to the high degree of vulnerability of *G. teydeum*, and the extremely low number of individuals in their extant populations, the essential first steps in helping to initiate the recovery of these populations would be the protection of their natural habitat and the extension of the seed collection program. Measures have already been taken by the Teide National Park management to rescue this, and other native and/or endangered species, including the protection of both the locations of *G. teydeum* (Bañares et al. 2001).

Meta-analyses of the correlation between molecular and quantitative measures of genetic variation (Ouborg et al. 2006) conclude that molecular measures alone do not accurately reflect the evolutionary potential of populations.

However, the documentation of genetic diversity and differentiation between populations using molecular markers provides initial guidance for conservation and can contribute to setting conservation priorities among populations (Neel and Ellstrand 2003; Tallmon et al. 2004). Therefore, the outcomes of this study support a number of conservation approaches. In general, *in situ* reintroductions should be made periodically, with materials containing as much genetic diversity as possible, in an attempt to maximize the chances of there being genes suitable for survival and subsequent adaptation to a particular set of environmental conditions (Frankham et al. 2002; Sosa et al. 2002; Spielman et al. 2004). The high levels of genetic variation measured in both the populations of *G. teydeum*, despite their small population size (in terms of both number of plants and area occupied), support the proposal of different authors (Tansley and Brown 2000; Tschardt et al. 2002; Yip et al. 2006) that the use of small nature reserves could be a viable option for the conservation of this species in particular, and endangered Canarian endemisms, in general. Another view is that occasional reintroductions should be made from a donor population that matches the recipient population as closely as possible in its ecological and genetic makeup. Proponents of this strategy argue that this would increase the likelihood of both the survival and the integration of the immigrants, while contributing to the prevention of outbreeding depression, or the disruption of co-adapted gene complexes (Harrison and Patrick 2001). In the case of *G. teydeum* we consider that this approach is not necessary while the current high levels of genetic variation are maintained. However tracking these issues is highly recommended.

Finally, although acting on the above proposals is likely to increase the chances of survival of these populations, we must bear in mind that these indications are solely based on an assessment of genetic variation through RAPDs. We should consider the probability that there is genetic diversity and differentiation that could not be recorded by RAPDs due to technical restrictions (homoplasmy). Therefore, genetic differentiation between the species studied could be higher than those reported in this research. However, conservation targets and priorities which depend solely on markers must be applied cautiously, and should be interpreted as a low estimate of what needs to be conserved. It is therefore necessary to investigate other important factors in the species' biology which may be crucial for the long-term survival of the population. The data presented here provide guidance about which populations may be valuable from a genetic perspective, and could also serve as a valuable baseline for monitoring the effectiveness of establishing protected areas, and restoring and maintaining genetic diversity.

Acknowledgments This investigation was supported by the Ministerio de Educación y Ciencia, Dirección General de Investigación; Plan Nacional I+D+I 2004–2007; Ref: CGL 2004-03839. We are grateful to Dr. S. Ortiz (University of Santiago de Compostela), for his assistance in collecting samples. We also thank J. Sosa and A. Stephens for improving the English, and the two anonymous reviewers for providing a constructive criticism of the manuscript. Financial support has gratefully been received from the Ministerio de Asuntos Exteriores y Cooperación and the Agencia Española de Cooperación Internacional through the MAEC-AECI Fellowship for González-González PhD studies.

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