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High genetic diversity and population structure in the endangered Canarian endemic *Ruta oreojasme* (Rutaceae)

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Abstract Insular species are expected to have low genetic diversity, for their populations are often small and isolated, and characterized by restricted gene flow and increased incidence of inbreeding. However, empirical results do not always match this expectation. For example, population genetic analyses of several Canarian endemics, based mainly on allozymes, show levels of genetic diversity exceptionally high for insular species. To investigate whether genetic variation in rare species endemic to Canary Islands is low, as predicted by theoretical expectations, or high, as documented in some previous studies, we analysed genetic diversity of the endangered *Ruta oreojasme*, a rare endemic of the island of Gran Canaria, using microsatellite markers, which are more variable than allozymes. Our analyses identified very high levels of genetic diversity ($A = 7.625$, $P = 0.984$, $H_o = 0.558$, $H_e = 0.687$) for *R. oreojasme*. Even though the distribution of the species is restricted to the South of Gran Canaria, only one population shows low genetic diversity, isolation and signs of a recent bottleneck/founder event. Some intrinsic characteristics of *R. oreojasme* (hermaphroditism, proterandry and polyploidy), the relative climatic stability of the Canarian archipelago during Quaternary glacials/interglacials, the size of most populations (thousands of individuals), its age, and the relative proximity of the archipelago to the mainland might have contributed to the high diversity that characterises this endemic. As expected, given the marked topographic complexity of Gran Canaria, we found marked genetic structure in *R. oreojasme* populations. Our results support the observation that Canarian endemics are characterised by unexpectedly high genetic diversity and provides important insights for potential applications to the conservation of *R. oreojasme*.

Keywords Genetic diversity · Endangered · *Ruta oreojasme* · Microsatellite · Canary Islands

Introduction

Islands, with their high proportion of endemic forms, are 'hotspots' of biodiversity and considerably contribute to global diversity. The high biodiversity value of islands is well known, as are the threats to it. Islands, in fact, host many of the world's threatened species and are worthy of special attention in decisions on conservation prioritization (Whittaker and Fernandez-Palacios 2007). The Canary Islands (Spain) form a volcanic archipelago with seven main islands plus several minor islets located about 96 km from the north-western Atlantic coast of Africa (Fig. 1). These islands have a rich endemic flora, with around 680 endemic taxa constituting at least 50 % of their native plant species (Santos-Guerra 2001; Reyes-Betancort et al. 2008).

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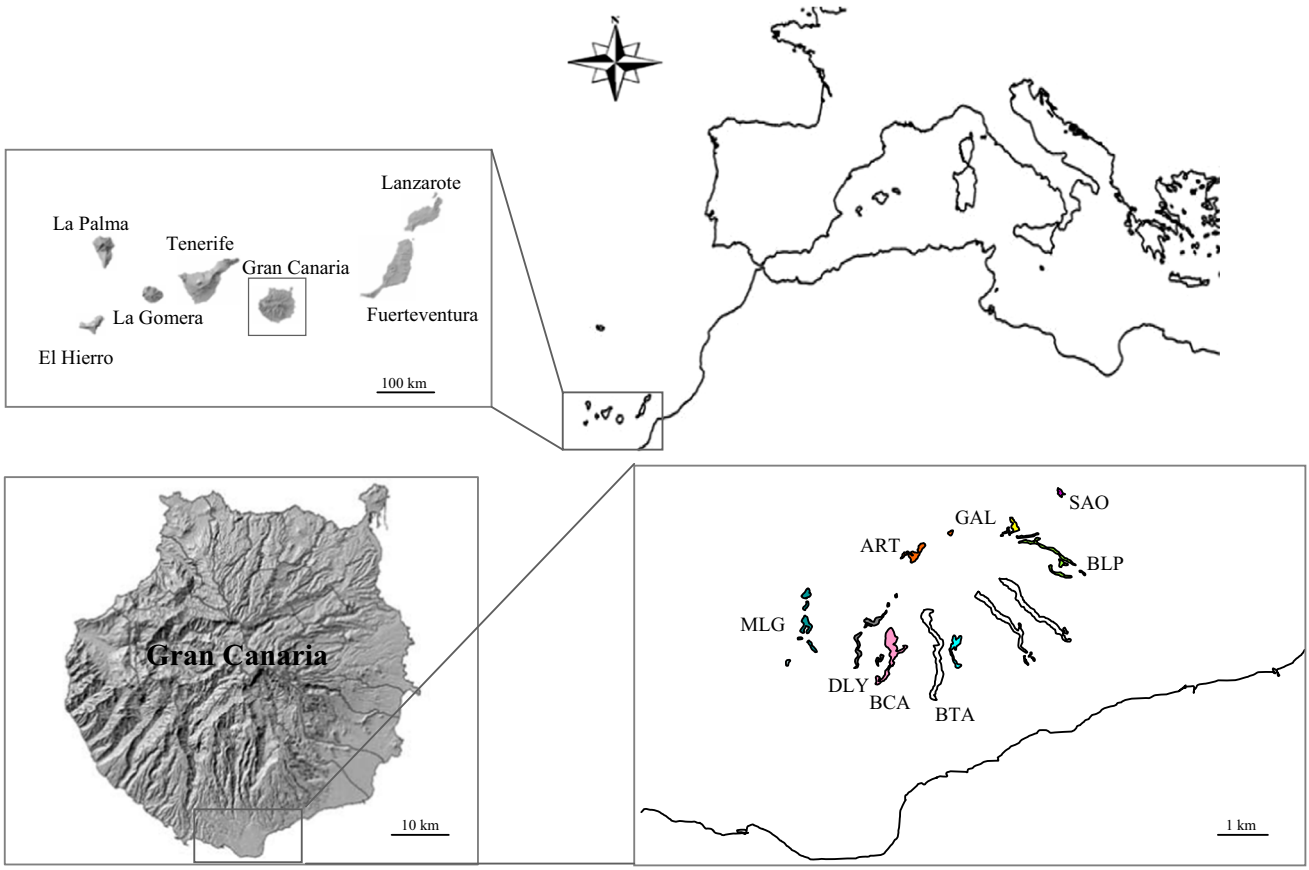

Fig. 1 Locations of known populations of *R. oreojasme*. Populations in *white* were not sampled because impossible to reach. Information on each sampled population is provided in Table 1

It is estimated that around 20 % of these endemics are endangered, with around 247 included in the Spanish Red Book of rare and endangered species (Bañares et al. 2004; Moreno 2008).

Insular species often consist of small and isolated populations characterized by restricted gene flow and increased incidence of inbreeding, hence experiencing a great impact of genetic drift. As a result, low genetic diversity is expected in island populations, with implications for their long-term potential for survival (Frankham 1998; Bouzat 2010). A loss in genetic diversity, in fact, is commonly associated with a reduction in fitness (Da Silva et al. 2006), and populations with low levels of genetic diversity are expected to be less able to adapt to environmental changes (Carrol and Fox 2008), thus imposing a strong priority on the conservation of island endemics.

However, empirical results on the genetic diversity of island populations do not always match the expectation of low genetic diversity. For example, Francisco-Ortega et al. (2000) reviewed allozyme data sets for 69 Canarian endemic species and found levels of genetic diversity exceptionally high for insular species, especially if compared to equivalent data for the flora of Pacific archipelagos (Crawford et al. 2001). More recently, Pérez de Paz and Caujapé-Castells (2013) updated the review by Francisco-Ortega et al. (2000) by adding 54 species (for a total of 123 taxa), and found that levels of genetic diversity are even higher than those reported before. The great geological age of the archipelago (ranging from 21 Ma in Fuerteventura to less than 1 Ma in El Hierro; Carracedo et al. 2002) and its proximity to the mainland (about 96 km from Fuerteventura to mainland Africa) were suggested as the main a-biotic explanations for the detected levels of genetic diversity (Francisco-Ortega et al. 2000; Pérez de Paz and Caujapé-Castells 2013). Pérez de Paz and Caujapé-Castells (2013) also found that some biotic traits (e.g., self-incompatibility or high chromosome number) were highly influential.

In the present study, we used highly polymorphic molecular markers (microsatellites) to study the amount and distribution of genetic diversity in a rare, endangered Canarian endemic. As recommended for genetic studies in endangered species (Pérez de Paz and Caujapé-Castells 2013), an extensive sampling was performed by collecting samples from all extant populations except for those that were physically impossible to reach.

Table 1 Description of *R. oreojasme* populations surveyed in this study

Population abbreviation	Location	Population size	Sample number	Coordinates	Altitude (m)	Area (Ha)
MLG	Montaña La Gorra	2350	29	27°49′10″N 15°36′11″O	350–475	75
DLY	Degollada de Las Yeguas	2880	30	27°48′57″N 15°34′57″O	225–550	50
ART	Arteara	5200	30	27°50′41″N 15°34′02″O	503–709	50
BCA	Barranco del Cañizo	3100	28	27°48′33″N 15°34′22″O	150–500	100
BTA	Barranco del Taliscal del Águila	1300	19	27°48′16″N 15°33′00″O	250–450	35
GAL	Gallegos	3300	25	27°51′20″N 15°31′40″O	622–761	35
BLP	Barranco de Las Palmas	2000	22	27°50′51″N 15°31′07″O	350–700	30
SAO	El Sao	185	22	27°52′00″N 15°30′46″O	375	8

Three more known populations were not sampled because impossible to reach

Ruta oreojasme Webb and Berth (Rutaceae) is a narrow endemic of the Canary Islands listed as endangered (EN) under the Spanish red list of vascular flora (Moreno 2008). A recent phylogenetic analysis of chloroplast DNA sequences revealed that the ancestor of this species and its two relatives in the archipelago (*R. pinnata* and *R. microcarpa*) colonised the Canary Islands from North Africa during the Miocene (Salvo et al. 2010). The study also supported the monophyly of *R. oreojasme* and that it split from its relatives in the archipelago about 7–8 Ma. The present distribution of *R. oreojasme* is restricted to the South of the island of Gran Canaria, where it occurs in 11 known populations (Fig. 1, Fig. S1). Ten of these populations are relatively abundant (thousands of individuals) and one is quite small (SAO, ~185 individuals; Table 1; Soto 2010). In the past, the occurrence of grazing in the distribution area of this species negatively affected the expansion and preservation of some populations, mainly due to trampling (Moreno 2008). The persisting impact of herbivores continues to limit seedling growth. Stochastic natural events, such as landslides, fires or droughts, represent other important threats for this species (Moreno 2008).

Given the endemicity of *R. oreojasme,* its conservation status and its rarity, this species represents an ideal case study to investigate whether rare species endemic to Canary Islands are characterized by low levels of genetic variation, as predicted by theoretical expectations and by empirical data from Pacific archipelagos, or by higher-than-expected levels of genetic variation, as found in other plants endemic to the Canarian archipelago.

The aim of this study is to assess the genetic variability of *R. oreojasme* populations in order to (1) determine whether *R. oreojasme* is characterized by high levels of genetic diversity, as found in other Canarian endemic plants and (2) use genetic information to design proper conservation strategies for this endangered species.

Materials and methods

Study species

Ruta oreojasme is a small, branched, procumbent to ascending shrub, up to 40 cm tall. It is characterised by pinnate leaves and light brown fruits with small black seeds (Bramwell and Bramwell 1994; Bañares et al. 2004). The yellow, tetramerous flowers are hermaphroditic and protandrous. Pollination is entomophilous and seed dispersal is barochorous (Bañares et al. 2004; Julia Pérez de Paz, pers. comm.). *R. oreojasme* blooms from April to June, fruiting in June–August. It occurs on rocky cliffs and escarpments at an altitude of 300–800 m (Fig. S1). As with most members of the genus *Ruta*, *R. oreojasme* is tetraploid, with $2n = 36$ (Stace et al. 1993).

Sample collection, DNA extraction and SSR amplification

In March–June of 2010 and 2011, plant material from 205 individuals was collected from eight of the 11 known

Table 2 Genetic diversity parameters inferred from microsatellite analysis

Population	N_A	P	A	A_P	$H_o \pm$ SD	$H_e \pm$ SD	$F_{IS} \pm$ SD
MLG	63	1	7.875	3	0.629 ± 0.054	0.723 ± 0.044	0.113 ± 0.056
DLY	72	1	9.000	3	0.671 ± 0.037	0.763 ± 0.038	0.106 ± 0.026
ART	68	1	8.500	5	0.592 ± 0.038	0.733 ± 0.033	0.178 ± 0.043
BCA	70	1	8.750	5	0.585 ± 0.056	0.706 ± 0.046	0.164 ± 0.049
BTA	50	1	6.250	3	0.540 ± 0.072	0.724 ± 0.041	0.240 ± 0.090
GAL	68	1	8.500	3	0.589 ± 0.104	0.725 ± 0.069	0.203 ± 0.108
BLP	64	1	8.000	6	0.494 ± 0.080	0.676 ± 0.076	0.265 ± 0.061
SAO	33	0.875	4.125	0	0.368 ± 0.072	0.446 ± 0.088	0.102 ± 0.087
Overall		0.984	7.625	3.625	0.558 ± 0.025	0.687 ± 0.023	0.172 ± 0.024

N_A total number of alleles across loci, P proportion of polymorphic loci, A allelic diversity, A_P number of private alleles, H_o observed heterozygosity, H_e expected heterozygosity, F_{IS} fixation index, *SD* standard deviation. For abbreviations of populations see Table 1

natural populations of *R. oreojasme* (19–30 individuals per population; see Fig. 1, Fig. S1 and Table 1 for details on each population). Leaf tissue samples were preserved in silica gel. Total genomic DNA was extracted using the QIAGEN® DNeasy plant mini kit following the manufacture's guidelines. Since the plants generated very viscous cell lysate, minor modifications were applied to the protocol to optimize genomic DNA quality and yield (see Meloni et al. 2013a).

Eight species-specific microsatellite (SSR) markers (RO57, RO59, RO62, RO70, RO71, RO72, RO77, RO79; Meloni et al. 2013a) were used for genetic analyses of *R. oreojasme* populations. Polymerase chain reaction (PCR) amplifications and genotyping were performed as in Meloni et al. (2013a).

Statistical analysis

Even though *R. oreojasme* is tetraploid (Stace et al. 1993), a maximum of two alleles per locus and per individual were detected in all populations. Because genetic analyses can be performed with standard population genetic tools developed for diploid organisms in tetraploid species that show disomic inheritance (Stift et al. 2008; Meloni et al. 2013b), our analyses were conducted assuming a diploid status for *R. oreojasme*.

We assessed genetic diversity by quantifying the number of alleles (N_A), proportion of polymorphic loci (P), allelic diversity (A), number of private alleles (A_P), observed (H_o) and expected (H_e) heterozygosity for each population across loci. Populations were tested for deviations from Hardy–Weinberg (HW) equilibrium using Fisher's exact test with the Markov chain algorithm (Guo and Thompson 1992). The fixation index, F_{IS}, was estimated in order to assess the departure from Hardy–Weinberg expectations due to non-random mating. Linkage disequilibrium (LD) between all different pairs of loci was tested within each population using Fisher's exact test. These analyses were performed with the web-based Genepop (Raymond and Rousset 1995; Rousset 2008) and GenAlEx v.6.5 (Peakall and Smouse 2012). The statistical methods implemented in BOTTLENECK (Piry et al. 1999) were used for detecting recent genetic bottlenecks in the study populations. Based on the observed number of alleles and the sample size of a population, the program computes the gene diversity expected under the assumption of mutation-drift equilibrium and compares it to the Hardy–Weinberg gene diversity (H_e; Nei 1987) to establish whether there is a significant deficit of gene diversity resulting from a recent bottleneck. As recommended by the authors of the program, a Wilcoxon sign-rank test (Luikart et al. 1998) was performed using 1000 bottleneck simulation replicates under the stepwise mutation model (SMM).

Genetic differentiation among populations was estimated by F_{ST} in FSTAT 2.9.3 (Goudet 1995) and by R_{ST}, an analogue of F_{ST} specific for microsatellite data, employing a stepwise mutation model (SMM, Slatkin 1995). R_{ST} was measured using the software SPAGeDi 1.4 (Hardy and Vekemans 2002). The same program was employed to determine if the values of F_{ST} and R_{ST} are significantly different by using an allele size permutation test (10,000 permutations): an observed R_{ST} significantly larger than the randomised R_{ST} indicates that the mutation process follows a stepwise mutation model and that mutations contributed to the observed genetic differentiation (Hardy et al. 2003). In addition, since F_{ST} can greatly underestimate differentiation when loci are highly variable (as it is commonly found with microsatellite markers; Hedrick 2005; Jost 2008; Meirmans and Hedrick 2011), we used SMOGD (Crawford 2010) to also calculate Jost's D_{est} using 1000 bootstrap replicates (Jost 2008).

In order to assess the hierarchical partitioning of genetic variation among and within populations, GenAlEx v.6.5 (Peakall and Smouse 2012) was used to conduct an

Analysis of Molecular Variance (AMOVA) following the procedure of Excoffier et al. (1992), Huff et al. (1993), Peakall et al. (1995), and Michalakis and Excoffier (1996) by estimating F_{ST} and using 999 random permutations of the data. With the same program, a Principal Coordinate Analysis (PCoA) based on a codominant genotypic distance matrix (Euclidean distances) was performed to visualise genetic relationships among individuals. Population structure was inferred using the Bayesian clustering method implemented by STRUCTURE (v. 2.3; Pritchard et al. 2000; Hubisz et al. 2009). The program uses a Markov Chain Monte Carlo (MCMC) procedure to estimate P($X|K$), the posterior probability that the data fit the hypothesis of K clusters, and assigns individual genotypes into clusters by estimating the membership coefficient Q for each individual based on allele frequencies at unlinked loci (independent of locality information). We tested all possible values of K from 1 to 8; for each K we ran an admixture model with correlated allele frequencies 20 times with a length of burnin period of 100,000 followed by 100,000 MCMC repetitions. We used two different methods to choose K. First, we employed STRUCTURE HARVESTER (Earl and vonHoldt 2012) to infer the statistically best-supported K by measuring ΔK (Evanno et al. 2005). This method provides the most accurate estimate of the number of clusters K (Evanno et al. 2005), but only for $K > 1$. Therefore, we also determined the best-fit value of K (including $K = 1$) using the criterion originally proposed by Pritchard et al. (2000) by calculating the average Ln probability of the data (Ln P($X|K$) for each value of K. After determining the most effective K for our data, we ran STRUCTURE with the admixture model and default parameter settings; the inferred ancestry of individuals was then determined using 100,000 iterations after a burnin period length of 100,000. Assignment was also carried out incorporating prior population information by using the option USEPOPINFO, a method that is considered useful to infer patterns of gene flow in recent generations by determining which individuals are immigrants to their sampled population or have recent immigrant ancestors (Pritchard et al. 2000; Falush et al. 2007). The program assumes an initially high probability that each individual is a resident of its sampling locality and calculates the posterior probabilities that individuals belong to their sampled locality/cluster. This method is sensitive to the migration rate (MIGRPRIOR, the probability that an individual is an immigrant to a given population) assigned as an initial condition (Pritchard and Wen 2004). Since we had no information on migration for our species, we conducted runs using a range of values for MIGRPRIOR (0.001–0.1), as suggested by Pritchard et al. (2000). We used the same burn-in and run length as in runs without prior population information.

To test for isolation by distance, a Mantel test (Mantel 1967) was applied to the matrices of pairwise population differentiation (calculated with both $F_{ST}/(1 - F_{ST})$ and D_{est}) and the matrices of geographical distance between populations with 999 random permutations using GenAlEx v. 6.5 (Peakall and Smouse 2012).

Results

Genetic diversity

With the exception of RO57 in population SAO, all loci were polymorphic (Table 2). The number of alleles identified at each population across all loci ranged from 33 to 72 (Table 2). Private alleles were found in all populations except SAO: three in MLG, DLY, BTA, GAL; five in ART, BCA and six in BLP (Table 2).

Based on the departure of F_{IS} from zero, 61 % of 64 tests showed significant HW equilibrium ($p > 0.05$). F_{IS} values were always positive (Table 2), meaning that the departure of genotype frequencies from Hardy–Weinberg expectations was always associated with a deficit of heterozygotes.

Significant linkage-disequilibrium (LD) at the 5 % level was detected at one pair of loci for populations MLG (RO57-RO71) and GAL (RO57-RO71), two for populations DLY (RO62-RO72, RO71-RO72), ART (RO59-RO62, RO57-RO70), BCA (RO57-RO72, RO77-RO79) and SAO (RO59-RO70, RO62-RO70), three for population BLP (RO62-RO72, RO57-RO77, RO72-RO77) and four for population BTA (RO57-RO62, RO57-RO70, RO59-RO70, RO59-RO71). Since locus RO57 was monomorphic in population SAO, it was impossible to perform the test for the seven pairwise combinations of loci that included this locus. Because we did not find any pair of loci in LD shared among all populations, we ruled out physical linkage as a possible reason for the detected LD.

Gene diversity, inferred from Nei's heterozygosity (H_e), was high ($0.676 < H_e < 0.763$) in all populations except for SAO where $H_e = 0.446$ (Table 2). Total gene diversity within the species was $H_e = 0.687$ (Table 2).

Inference of population demographic history

Under SMM, only population SAO showed significant signs of a recent bottleneck ($p = 0.039$), while all other populations were at mutation-drift equilibrium.

Population genetic structure

Genetic differentiation among populations was always statistically significant (F_{ST}, $p < 0.05$). F_{ST} values were

Table 3 Pairwise population estimates of F_{ST} (diagonal below) and D_{est} (above diagonal)

	MLG	DLY	ART	BCA	BTA	GAL	BLP	SAO
MLG		0.098	0.350	0.134	0.226	0.132	0.116	0.306
DLY	0.0428		0.283	0.055	0.178	0.148	0.084	0.232
ART	0.1288	0.1036		0.403	0.257	0.335	0.332	0.473
BCA	0.0594	0.0340	0.1470		0.159	0.197	0.081	0.254
BTA	0.0970	0.0710	0.1069	0.0714		0.185	0.211	0.228
GAL	0.0567	0.0567	0.1150	0.0872	0.0790		0.121	0.308
BLP	0.0581	0.0442	0.1387	0.0407	0.0866	0.0486		0.262
SAO	0.2315	0.1849	0.2665	0.2015	0.1945	0.2159	0.2235	

All values are statistically significant (95 % CI). For abbreviations of populations see Table 1

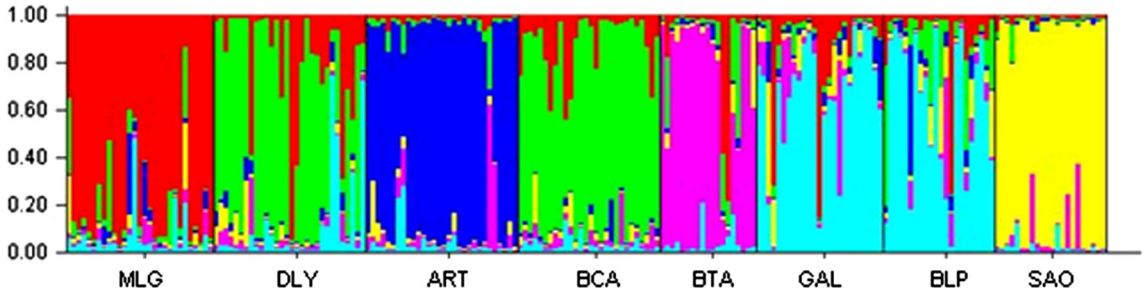

Fig. 2 Proportional membership (Q) of 205 individuals from the eight studied populations *of R. oreojasme* in the six clusters identified by STRUCTURE. Each individual is represented by a single *vertical bar*. The locality of origin for each individual is indicated below the figure

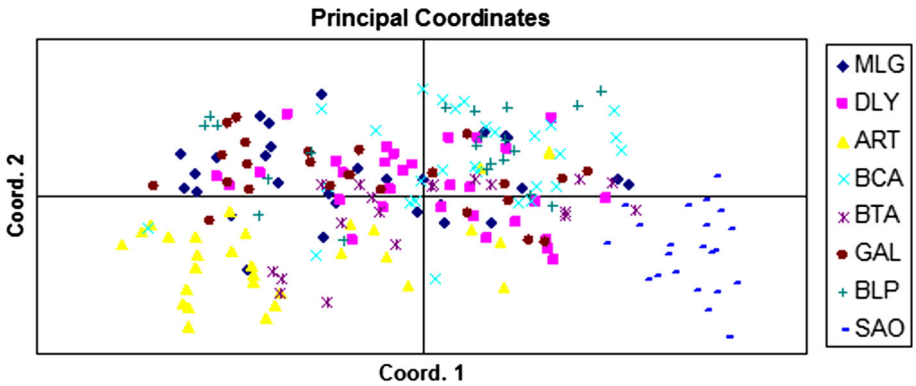

Fig. 3 Principal coordinate analysis (PCoA) based on the multilocus genotype of 205 *R. oreojasme* individuals. The percentage of the total variability explained by the first two components is 26.90 % (Coord. 1) and 19.84 % (Coord. 2). *Each symbol* represents a single plant from one of the eight studied populations. Information on each population is provided in Table 1

low, ranging between 0.0340 (BCA-DYL) and 0.2665 (SAO-ART); population SAO was the most differentiated, with $0.1849 < F_{ST} < 0.2665$ (Table 3). The overall genetic differentiation among populations was significant, with $F_{ST} = 0.097$ ($p = 0.01$). Observed R_{ST} values were not significantly higher than permuted R_{ST}, suggesting that stepwise mutations did not contribute to population differentiation and that statistics based on allele identity (F_{ST}) are likely to perform better than counterparts based on allele-size information (R_{ST}; Hardy et al. 2003). D_{est} values were much higher than the F_{ST} values, ranging between 0.055 (BCA-DYL) and 0.473 (SAO-ART; Table 3); across populations D_{est} was 0.275. According to the AMOVA, the within-population element explained most (87 %) of the total amount of genetic variation detected by our analyses.

Based on both the ΔK statistic and the Ln probability of the data (Ln ($X|K$)), the best-supported number of a posteriori genetic clusters was $K = 6$ (see Figs. S2 and S3 respectively). For $K = 6$, each cluster corresponds to one of the populations, except for populations DLY and BCA, which cluster together, as do populations GAL and BLP (Fig. 2). When we ran STRUCTURE with the USEPOPINFO option, the choice of MIGRPRIOR affected the program output. STRUCTURE identified no immigrants for MIGRPRIOR = 0.001, three immigrants (two in population DLY, one in population GAL) for MIGRPRIOR = 0.05, and five

immigrants for MIGRPRIOR = 0.1 (beyond those already identified, one in population ART and one in population BLP). Since we expect a low dispersal rate for *R. oreojasme* (it disperses its seeds by gravity; Bañares et al. 2004; Julia Pérez de Paz, pers. comm.) and we found differentiation among populations, we believe that the results based on the medium value of migration probability (MIGRPRIOR = 0.05) better describe the real situation. STRUCTURE also identified six individuals not readily classified as migrants, but not clearly assigned as residents either ($0.4 < Q < 0.8$; Bergl and Vigilant 2007), suggesting that these individuals are the products of admixture between populations.

Differentiation among populations is confirmed by PCoA, which shows separation among populations (Fig. 3). The first two components explain respectively 26.90 and 19.84 % of the total variability.

Mantel test for correlation between genetic differentiation and geographic distances among populations was not significant using both F_{ST} ($F_{ST}/(1 - F_{ST})$; $p = 0.139$, $R^2 = 0.046$) and D_{est} ($p = 0.398$, $R^2 = 0.001$) estimates.

Discussion

Genetic diversity

Our analyses revealed high levels of genetic diversity for the endangered Canarian endemic *R. oreojasme*. Even though the range of distribution of the species is restricted to the South of the island of Gran Canaria (Fig. 1), most *R. oreojasme* populations show fewer genetic signatures of isolation and small population size (i.e. low gene diversity, high homozygosity, high LD) than typically expected for insular endemics (Frankham 1998; Bouzat 2010).

In a review of allozyme variation in 449 plant species, Hamrick and Godt (1989) found that endemic species exhibited less diversity ($P = 0.40$, $A = 1.80$, $H_T = 0.096$) than the average measured across all taxa included in their analysis ($P = 0.50$, $A = 1.96$, $H_T = 0.149$). This study included relatively few island endemics. A study based exclusively on insular endemic plant species mostly from Pacific archipelagos showed that island endemics possess relatively limited amounts of allozyme variation, with mean estimates of genetic diversity that are approximately half of the average genetic diversity of plant species ($P = 0.25$, $A = 1.32$, $H_T = 0.064$; De Joode and Wendel 1992). More recent studies confirmed low genetic diversity for plants endemic to Pacific island archipelagos (Crawford et al. 2001). Allozyme diversity was also examined in Canarian endemic plants (Francisco-Ortega et al. 2000, Pérez de Paz and Caujapé-Castells 2013). These reviews confirmed generally low diversity in insular endemics, but revealed that species endemic to the Canary Islands harbour more genetic diversity than those endemic to the Pacific islands (Francisco-Ortega et al. 2000; Crawford et al. 2001).

The relatively high genetic diversity found in our study for *R. oreojasme* ($A = 7.625$, $P = 0.984$, $H_o = 0.558$, $H_e = 0.687$) supports the observation by Francisco-Ortega et al. (2000) and Pérez de Paz and Caujapé-Castells (2013) that endemic species from the Canarian archipelago are characterised by unexpectedly high genetic diversity. Several factors could provide an explanation for the high genetic diversity detected in *R. oreojasme*. Some are intrinsic characteristics of this species. Hermaphroditism, proterandry and polyploidy, which characterise *R. oreojasme*, are traits that increase genetic diversity and were found to be positively correlated to the level of genetic variation in other Canarian endemics (Pérez de Paz and Caujapé-Castells 2013). Historical factors could also explain the high genetic variation of *R. oreojasme* and other Canarian endemics with higher-than-expected genetic diversity. For example, the relative climatic stability of the Canarian archipelago during Quaternary glacial/interglacial periods (Francisco-Ortega et al. 2000; Pérez de Paz and Caujapé-Castells 2013; Rodríguez-Sánchez and Arroyo 2008) might have allowed populations of this species to persist through several climatic cycles and accumulate genetic differences by mutation and recombination. The size of *R. oreojasme* populations (thousands of individuals for all of them, except for SAO) might also have contributed to the development of genetic diversity, as big populations tend to accumulate more variation than small ones (Frankham 1996; Crawford et al. 2001). The low variation found in the only small population of the species (SAO; see Tables 1, 2) supports this observation. Finally, as for many Canarian endemics, the relative proximity of the archipelago to the mainland, and the old age of both island and species might have allowed more time for genetic diversity to accumulate in *R. oreojasme* populations (Francisco-Ortega et al. 2000; Crawford et al. 2001; Pérez de Paz and Caujapé-Castells 2013). The genus *Ruta* colonised the Canary Islands from North Africa in the Miocene (Salvo et al. 2010). The relatively short distance of the islands from the continent (about 96 km for Fuerteventura, the closest one, and 190 km for Gran Canaria, where *R. oreojasme* currently occurs) might have increased the probability of multiple immigration events into the archipelago by the common ancestor of the three Canarian *Ruta* species, thus alleviating the effects of founder events and increasing the genetic diversity of the ancestral genetic pool that gave origin to the extant species of *Ruta* in the archipelago (Salvo et al. 2010). Moreover, the relatively old (ca. 6–8 M yrs) age of *R. oreojasme*, which diverged first from the other two Canarian species

(Salvo et al. 2010) might have afforded for the accumulation of genetic variation through mutation, recombination, drift and selection.

Genetic diversity parameters measured for *R. oreojasme* (Table 2) are higher than those in the Canarian taxa included in Pérez de Paz and Caujapé-Castells's review ($A = 1.923$, $P = 0.502$, $H_o = 0.171$, $H_e = 0.185$; 2013). One possible explanation for this result is that the above-mentioned review is based on allozyme variation, while our analyses are based on microsatellite markers, which are more polymorphic and thus able to detect more variation than allozymes. Similarly to what we found for *R. oreojasme,* analyses based on microsatellite markers revealed very high genetic diversity in other Canarian endemics, including *Bencomia caudata* ($A = 5.13$, $H_o = 0.49$, $H_e = 0.62$; González-Pérez et al. 2009a), *Kleinia neriifolia* ($A = 12.5$, $H_o = 0.70$, $H_e = 0.85$; Chen et al. 2012) and *Myrica rivas-martinezii* ($A = 6.5$, $H_o = 0.49$, $H_e = 0.56$; González-Pérez et al. 2009b), with endangered species *B. caudata* and *M. rivas-martinezii* showing values of genetic diversity similar to that found in *R. oreojasme* and non-endangered Canarian species showing even greater diversity.

Population SAO is characterized by lower polymorphism, allelic diversity and heterozygosity than the other populations analysed in this study (Table 2). Its low genetic diversity, small size (Table 1), the presence of one fixed locus and the signs of a recent genetic bottleneck detected from our analyses might suggest that SAO (1) represents the last remnant of a larger population that has now partially disappeared, or (2) is a recently founded stand. The absence of private alleles in this population (Table 2) supports the latter possibility. The low genetic diversity of the small SAO population is however more likely the product of size reduction and isolation caused by intensive human activities (in particular grazing by goats) that affected the area surrounding this population in relatively recent times (M. Soto, pers. comm.). Small populations of other endangered, formerly more widespread species also occur in this region (i.e. *Solanum lidii, Convolvulus glandulosus, Juniperus turbinata spp. canariensis*; Soto 2010), suggesting that this area acts as a refuge where some species can survive human disturbance.

Population genetic structure

We found low values of F_{ST} for the populations of *R. oreojasme* we analysed (Table 3), especially if compared to other taxa endemic to the Canary Islands (Pérez de Paz and Caujapé-Castells 2013). These populations are located in the southern slope of Gran Canaria (Fig. 1, Fig. S1), which is affected by notable levels of erosion and is in the dismantling stage (Fernández-Palacios et al. 2011). Such populations are confined to a series of large and narrow concentric ravines (Fig. S1) that should promote geographic diversification among populations. Moreover, according to Bañares et al. (2004), *R. oreojasme* disperses its seeds by gravity (barochory). Thus, we expected low levels of gene flow and high differentiation among *R. oreojasme* populations on this southern slope of the island. Because F_{ST} is strongly influenced by levels of heterozygosity (Meirmans and Hedrick 2011), we cannot rule out the possibility that our low F_{ST} values may simply reflect the high values of heterozygosity that we found. We therefore also investigated the D statistic proposed by Jost (2008), as it is independent of levels of heterozygosity. D_{est} values were three times larger than the F_{ST} values ($F_{ST} = 0.097$, $D_{est} = 0.275$) and showed that *R. oreojasme* populations might be much more differentiated than would have been suggested by the F_{ST}. The differentiation among populations we found with D_{est} is supported by the evidence for population structure supported by STRUCTURE (Fig. 2) and PCoA (Fig. 3) analyses.

Population SAO, occupying a small area at the north-eastern extreme of the species range, is moderately differentiated from the other populations of *R. oreojasme* (Fig. 3; Table 3). A possible reason is that this population experienced a bottleneck caused by recent, intensive human activities. Moreover, the fact that SAO is located at the edge of the distribution of the species is another factor that could have reduced the exchange of genetic material and increased inbreeding in this population (Pearson et al. 2009).

Conservation implications

This study provides important insights into the genetic structure of *R. oreojasme* with potential applications to its effective conservation. The high genetic diversity that characterises this Canarian endemic suggests that the species is not at high risk of extinction due to genetic factors. Nevertheless, its very restricted distribution, the continued anthropogenic and environmental threats to its populations, and the genetic data obtained in this study (i.e., some loci deviating from HW equilibrium due to a deficit of heterozygotes and the presence of some loci in LD) highlight the necessity of developing measures for conservation. *In situ* conservation is essential and should aim to preserve all extant populations, for they all carry unique alleles. Because the main threats to *R. oreojasme* are habitat fragmentation and grazing, in situ conservation should be especially aimed at controlling grazing activities and at the general reduction of human impact on the populations. Special attention should be given to SAO, the only population characterised by low genetic diversity (Table 2): its small size (Table 1), genetic isolation

(Table 3) and the bottleneck/founder event recently experienced might make this population increasingly vulnerable through the effects of genetic drift. *Ex situ* conservation in botanical gardens or seed orchards is also advisable, to facilitate the eventual reintroduction of seedlings belonging to the same population whenever it seems necessary to restore genetic diversity and sustain fitness (Wilkinson 2001). Further research on the reproductive biology and dispersal ability of this species is fundamental for planning specific, and thus potentially successful, long-term conservation programs.

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