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ORIGINAL ARTICLE

Population genetics of the endangered Canarian endemic *Atractylis arbuscula* (Asteraceae): implications for taxonomy and conservation

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Abstract We use the information provided by 36 RAPD loci and 15 morphological traits to describe and construe the population differentiation in *A. arbuscula*, a Canarian endemic Asteraceae threatened with extinction that exhibits a disjunct distribution in the islands of Gran Canaria (var. *schizogynophylla*) and Lanzarote (var. *arbuscula*). Our evaluation of morphological characters and the extent of RAPD differentiation found sets the stage for a taxonomic restructuring to hoist both var. *arbuscula* and var. *schizogynophylla* to subspecies category. Our genetic results suggest that fragmentation and generally low population sizes are jeopardising the survival of this species through a predominance of stochastic processes in microevolutionary dynamics, especially in Gran Canaria, where subpopulations exhibit the lowest levels of genetic variation and gene flow. If, as most Asteraceae, these plants have a sporophytic self-incompatibility system, the scarce reproductive turnover observed in the much smaller subpopulations from Gran Canaria (ssp. *schizogynophylla*) is possibly more influenced by a deficiency of S-alleles (that would be provoking the unavailability of compatible mating types, and a cascade of deleterious effects associated with the Allee effect) than by a reduced pollinator visitation frequency. Based on the depauperated values of genetic parameters for this subspecies, urgent mixed subpopulation reinforcements and reintroductions (with specimens belonging to the same subspecies) seem advisable on the grounds that they might allow the isolated habitat remnants to retrieve from inbreeding through the introduction of a new stock of S-alleles and the subsequent production of genotypes that may have a higher selective value.

Keywords *Atractylis arbuscula* · Canary Islands · Conservation genetics · RAPDs · Morphology · Taxonomy

Introduction

Atractylis arbuscula Svent. and Michaelis (Asteraceae) is a small Canarian endemic shrub (up to ca. 50 cm in height) with a disjunct distribution in the islands of Gran Canaria and Lanzarote that is considered as threatened with extinction by the Spanish National Catalogue of endangered species (Bañares et al. 2004, http://www.mma.es/portal/secciones/biodiversidad/inventarios/inb/flora_vascular/pdf/286.pdf). While the subpopulations from Lanzarote are within (or near) the Natural Preserve of the Archipiélago Chinijo (see Fig. 1), no protection is granted for the subpopulations of the Gran Canarian taxon, which are also of much smaller sizes (between 20 and 400 individuals). The very low number of plants counted in the last censuses (Rodríguez-Delgado et al. 2004), low seedling survival, substrate instability, and the proximity of populations to beaten trekking tracks make up direct threats to the plant's

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intrinsic survival capabilities. Furthermore, the habitat of most populations from Gran Canaria is undergoing a progressive and severe degradation given the large scale waste disposal associated with the many residential facilities in their surroundings.

Because of these direct threats and other potential ones, the Canarian Government suggested several specific conservation measures for this species (Rodríguez-Delgado et al. 2004), encompassing (i) the declaration of some subpopulations from Gran Canaria as "sites of scientific interest", (ii) a strategy to improve the management of the natural protected preserve in Lanzarote, (iii) seed collection, (iv) ex situ culture (both in vitro and in vivo), and (v) population enforcement. Since no thorough population studies for this species are available to date, there is an urgent need for ancillary population data to substantiate the aspects that entail in situ and ex situ conservation actions.

These conservation questions notwithstanding, *A. arbuscula* also poses an important taxonomic issue. At present, the subpopulations confined to Gran Canaria are considered as var. *schizogynophylla*, whereas those from Lanzarote are considered as var. *arbuscula*. However, the morphological differences between these varieties are very slight (Sventenius 1968), and have not been examined in depth. Thus, prior to any consideration concerning conservation strategies, it is necessary to clarify whether the infra-specific classification of *A. arbuscula* defines Evolutionarily Significant Units (ESUs, sensu Waples 1991) or merely represents a geographical subdivision with no further biological or taxonomic implications (Karl and Bowen 1999).

When their implementation fulfils stringent conditions in terms of consistency and reproducibility, Random Amplified Polymorphic DNA (RAPD) markers (Williams et al. 1990) have proven suitable to detect differences among closely related species (Elisiário et al. 1999; Sales et al. 2001; De Greef and Triest 1999; Koontz et al. 2001; Caraway et al. 2001; Bouza et al. 2002; González-Pérez et al. 2004). By using single, arbitrary 10-mers, this technique is capable of scanning multiple priming sites that are theoretically dispersed throughout the genome and close enough to allow efficient amplification. In general, RAPD amplification results in DNA fragments that are inherited as Mendelian dominant characters (Williams et al. 1990) and, most convenient for the case of the endangered *A. arbuscula*, it only requires small amounts of template DNA. Because of these characteristics and of their relative low cost as compared with other techniques, RAPDs are a proper first source of consistent molecular data to implement urgency in the conservation genetic assessments of *A. arbuscula*.

Our objectives in this paper are to use the information provided by RAPD markers and 15 morphological traits to (i) describe and construe the population genetic differentiation in *A. arbuscula*, (ii) assess the taxonomic implications of the detected genetic and morphological differences, and (iii) propose conservation guidelines cogent with the inferences made in (i) and (ii).

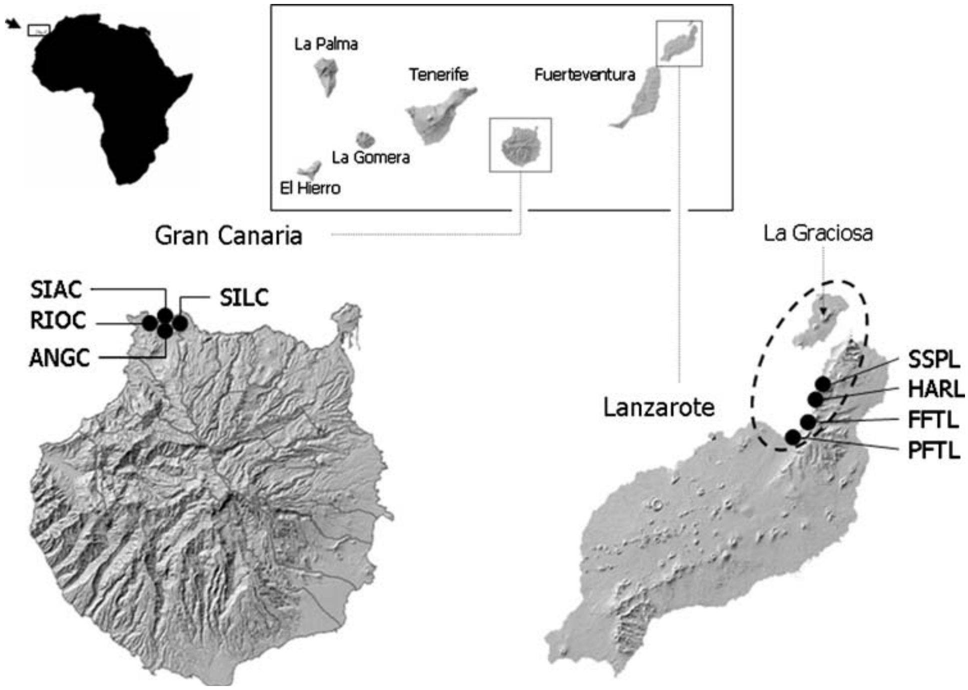

Fig. 1 Geographic map with the locations of the populations sampled for this investigation. Population codes correspond to Table 1. The *dashed ellipse* in Lanzarote circumscribes the area of the Archipielago Chinijo natural preserve

Table 1 Population designations and basic indicators of RAPD polymorphism in *Atractylis arbuscula* per population, island and primer

Island/population (code)	*N*	OPA-5		OPA-9		OPT-7		Across primers	
		P	*I*	*P*	*I*	*P*	*I*	*P*	*I*
Gran Canaria									
El Angosto (ANGC)	16	28.57	0.194	30.77	0.138	31.25	0.173	30.19	0.164
Acantilados de El Río (RIOC)	21	28.57	0.155	69.23	0.392	12.50	0.055	36.77	0.197
Cuesta de Silva-cliffs (SIAC)	10	0.00	0.000	69.23	0.403	6.25	0.020	25.16	0.155
Cuesta de Silva-litoral (SILC)	14	42.86	0.288	23.08	0.135	25.00	0.125	30.31	0.160
Lanzarote									
Salta Si Puedes (SSPL)	21	14.29	0.097	61.54	0.377	0.00	0.000	25.28	0.155
Haría (HARL)	5	42.86	0.241	46.15	0.270	0.00	0.000	29.67	0.144
Casas de Famara (FFTL)	32	71.43	0.337	76.92	0.474	75.00	0.381	74.45	0.406
Playa de Famara (PFTL)	26	71.43	0.418	76.92	0.396	62.50	0.293	70.28	0.355
Average *A. arbuscula* s. l.	18.1	37.50	0.216	56.73	0.323	26.56	0.131	40.26	0.217
Average Gran Canaria	15.3	25.00	0.159	48.08	0.267	18.75	0.093	30.61	0.169
Average Lanzarote	21.0	50.00	0.273	65.38	0.379	34.38	0.169	49.92	0.265

N sample size; *P* proportion of polymorphic loci; *I* Shannon's information index

Material and methods

Plant material

At present, *A. arbuscula* has a disjunct distribution in Lanzarote and Gran Canaria, which are separated by more than 250 km of sea. The distribution ranges of this taxon in each island are fragmented in discrete subpopulations that generally occur in very limited areas.

In Lanzarote, the species is found in stony and sandy soils, and in basaltic rocks only at the base of Famara's range (North of the island), and occurs in fragmented subpopulations distributing along barely 3 km of coast. In Gran Canaria, it grows in maritime phonolitic cliffs with steep slopes, forming small discontinuous subpopulations along less than 2 km of coast where halophytic vegetation prevails, occasionally containing xerophilous elements (Fig. 1).

We collected leaves of 145 individuals from eight subpopulations (four from Gran Canaria and four from Lanzarote) that thoroughly represent the known distribution areas of *A. arbuscula* (Fig. 1; Table 1). Leaf samples were put in zippered, unambiguously coded plastic bags containing silica gel, where they remained until further processed at the Laboratorio de Biodiversidad Molecular of the Jardín Botánico Canario "Viera y Clavijo". In all cases, sampling was carried out along transects representing the area of distribution of individuals.

Morphological study

We studied 15 morphological traits (five qualitative and ten quantitative), using plant material from both islands that corresponded to: ca. 25 specimens deposited at the herbarium LPA, between 50 and 80 achenes from three accessions kept at the seed bank of the JBCVC, and live plants cultured in this botanic garden. The floral and the capitulum bracts, which were not compared in Sventenius (1968) (Table 2), were examined thoroughly in this investigation (Tables 3, 4). Furthermore, the achenes and pappus of the Gran Canarian populations, which were not considered by Sventenius and Kahne (in Sventenius 1968), have been studied in this paper (Tables 3, 4). The observations and measurements of characters were carried out in a binocular lens Olympus-Tokio 259571. Quantitative characters were obtained from 15 to 25 measurements per sample (depending on availability), from which we calculated ranges of values. Extreme values for each character were also scored.

DNA processing

Leaves were crushed with liquid nitrogen using a sterile mortar and pestle until we obtained a fine-grained powder. DNA extractions were carried out with the Nucleon Phytopure kit (Amersham Pharmacia Biotech). The resulting DNAs were cleaned using the GFX PCR DNA and gel band purification kit columns (Amersham-Pharmacia Biotech) and then quantified in an Eppendorff® biophotometer, that gave us the necessary dilutions to bring the DNAs to a concentration of ca. 20 ng/µl, which was found to be optimal for RAPD amplification (Nereida Cabrera, unpublished data).

Amplification reactions were set in a 25 µl final volume of reaction mixture containing 23 µl of ABGENE

Table 2 Diagnostic characters examined for either variety of *Atractylis arbuscula* in Sventenius (1968)

Trait	*A. arbuscula* var. *arbuscula*	*A. arbuscula* var. *schizogynophylla*
Indument	Planta cortice ramoso-cinerea	Planta tota dense albido-pannosa
Leaves	Ramulis dense foliolosis	Ramis ramulis strictis, laxioris foliatis
Leaves/peduncles	Foliis 2–4 cm; pedunculis 1.2 cm	Foliis pedunculis conformis
Floral bracteae	Not studied	Bracteis floralis calathida non superantes remote spinosis vel inermis
Capitulum scales	Squamis omnibus apice spinescente vel longe aristatis	Not studied
Floscules	Floris centralibus 10- to 12-mm long, angustissimis longitudine pappi aequantibus	Flore discii 6-mm long, pappo corollae tubo aequanti
Corolla's lacinies	Corollae laciniis 2-mm long, acuminatis	Corollae lacinii brevioribus, breviter ovato-lanceolatis
Achenes	Achaenia 4-mm long, fusiformia-cylindrica, dense sericeo-villosa	Achaenia matura non visa
Pappus	Pappo setaceo, setis longe plumosis	Not studied

Note the gaps that prevent thorough comparison between the two taxa

Table 3 Qualitative data for the different characters studied in the populations of *A. arbuscula* in Lanzarote and Gran Canaria

Trait	*A. arbuscula* subsp. *arbuscula* (Lanzarote)	*A. arbuscula* subsp. *schizogynophylla* (Gran Canaria)
Leaf	Slightly silky-arachnoid or glabrescent with a long mucro	Woolly silky-arachnoid, with a short mucron
Capitulum scales	Ovate or angustiovate to angustiobovate or subspathulate, with wide scariose-hyaline margins, tip more or less rounded, slightly fimbriate, with a long mucro or edge brown-yellowish	Ovate or angustiovate to angustioblanceolate, with narrow scariose margins, sharp, atenuate in a more or less long mucron, purple or black
Capitulum	Proportionally narrower and slightly more elongated	Proportionally wider and slightly shorter
Ligulae	With lobules angustilanceolate, finished with a sharp peak	With lobules oblong or angustiovate, obtuse with an inconspicuous peak
Pappus	In general shorter than the floscules, plumose with short hairs up to one-fourth of its length	In general of the same length as the floscules, plumose with long hairs up to almost half of its length

Table 4 Ranges of values of the quantitative data for the different characters studied in the populations of *A. arbuscula* in Lanzarote and Gran Canaria (values between parentheses correspond to occasional extreme values that fall outside the range)

Trait	*A. arbuscula* subsp. *arbuscula* (Lanzarote)	*A. arbuscula* subsp. *schizogynophylla* (Gran Canaria)
Leaves (mucron)	(0.3) 0.6–1.0 (1.5)	(0.2) 0.3–0.6 (1.0)
Capitulum (length × width)	14.5–15.5 (17.0) × 5.5–6.5 (7.0)	(11) 13–16 (17) × (6.0) 7.0–8.0 (9.0)
Bracteae at the base of capitulum (spines)	(1.0) 1.2–1.8 (3.0)	(0.3) 0.5–1.0 (1.5)
Capitulum scales (spines)	(2.0) 2.5–3.5	1.5–2.0
Floscule length	8.5–9.2 (10.0)	(7.5) 8.5–9.0
Floscule lobule length	(1.3) 1.5–2.1	(1.0) 1.5–1.8 (2.0)
Ligulae length	(10.5) 11.5–14.0 (14.5) × 2.4–2.6	(11.0) 12.5–13.5 (14.5) × 2.5–2.8
Ligulae lobule length	(1.0) 1.5–2.2 (2.4)	1.0–1.8 (2.0)
Achene length	(2.6) 2.8–3.5 (4.0)	(2.5) 2.8–3.4 (3.8)
Pappus-hairs	Up to 2.5 (3.0)	Up to 3.8 (4.5)

Measurements are in mm

Mastermix (2.5 mM $MgCl_2$), 1 µl of genomic DNA and 1 µl of a 10 mM solution of the corresponding 10-mer primer used (we screened 14 primers from kits A and P of OPERON Technologies). The 96-well plates containing the amplification reactions were heat-sealed with an aluminum sheet and then loaded in an Eppendorf Mastercycler Gradient programmed for 45 cycles, each of which had the following temperature profile: 30 s at 94°C, 30 s at 36°C, and 1 min at 72°C. Before the first cycle, samples were subjected to a hot start (1.5 min at 94°C) and, after the last cycle, they underwent a prolonged extension at 72°C for 10 min. Amplification products were loaded in a 1.4% agarose gel that ran for 3 h at 90 mV. A 100 base-pair DNA ladder (Promega G695A) was added as a size gauge in all runs. Control samples containing all the reagents except for DNA were used to test that no self-amplification or DNA contamination occurred, and we randomly repeated some runs including individuals from all populations examined to confirm the consistency and reproducibility of the experiments. Markers that were inconsistently amplified in these repeated reactions were not included in the subsequent data analysis, where we only scored and used those RAPD fragments which were distinct and highly reproducible over the repeated multiple runs.

Gels were stained with ethidium bromide (0.5 µg/ml) and photographed under UV light using a Kodak DC 40 digital camera. These images were captured using the computer program PhotoCapt MW (Vilber-Lourmat), that estimated the band sizes and suggested prospective patterns of band presence/absence across individuals. Since the resolution of most gels only allowed us to distinguish the bands of the ladder up to 2,100 bp, the most reliable calibrations correspond to bands equal to or smaller than this size. Patterns of band presence/absence assigned by the program were manually checked and adjusted in all cases.

Genotype assignment for each amplified band was carried out conservatively, (i. e. only with amplification products that were clearly present or absent through all experiments for the data analysis), as this approach reduced the influence of non-reproducible, artifactual bands that might bias our analyses. The sizes of each scored fragment per primer were designed as loci, which were interpreted as diallelic characters; for each locus and individual, we assigned a "1" in case of band presence, a "0" in case of absence, and a "?" if the band resolution was not satisfactory. Bands appearing to have the same mobility were judged to represent the same locus.

Data analysis

The resulting data matrix was implemented in the computer program Transformer-3 (Caujapé-Castells and Baccarani-Rosas 2005) to generate the input files needed for the statistical analyses for all groupings of populations. Shannon's index (Lewontin 1972), F_{ST} values between pairwise combinations of populations, and Nei's (1973) population structure statistics per locus, per island and for *A. arbuscula* sensu lato (s. l.) were calculated using the program PopGene (1997). We ran AMOVAs within islands, between islands and for *A. arbuscula* s. l. using the computer program Arlequin (Schneider et al. 2000); significance of the values of F_{ST} was tested using the non-parametric approach described in Excoffier et al. (1992). To further substantiate the assessment of inter-population genetic differentiation, we carried out a principal components analysis (PCA).

Results

Morphological analysis

The comparative morphological study resulted in the following distinctive characters between the subpopulations from Gran Canaria and Lanzarote: indument and spininess of leaves and bracts; shape, margin and terminal tip of the capitular bracts; capitular length/width ratio; ligule teeth; and relative size and length of the pappus-hairs. The characteristics and value ranges of the qualitative and quantitative morphological variables used are given in Tables 2 and 3, respectively and shown in the drawing of Fig. 2.

Molecular analysis

Out of 12 ten-mer primers initially screened in *A. arbuscula*, only three (OPA-5, OPA-9 and OPT-7) produced bands that could be reliably scored (Table 1). Presumably, this low proportion of usable primers was due both to the highly stringent conditions of consistency and reproducibility that we implemented in order for a primer to be considered in the interpretations, and to the production of a considerable amount of secondary metabolites by these plants, that possibly undermined the DNA extraction yield and quality.

These three primers gave a total of 36 scorable loci with variable average levels of polymorphism for *A. arbuscula* s. l., that ranged from ($P = 56.73$, $I = 0.323$) in OPA-9 to ($P = 26.56$, $I = 0.131$) in OPT-7 (Table 1). Overall, the most polymorphic subpopulation was Casas de Famara (FFTL), from Lanzarote ($P = 74.45$, $I = 0.406$), and the more polymorphic one in Gran Canaria was Acantilados del Río (RIOC) ($P = 36.77$, $I = I = 0.197$). The less polymorphic population overall was SIAC, from Gran Canaria ($P = 25.16$, $I = 0.155$), and the less polymorphic one in Lanzarote was HARL ($P = 29.67$, $I = 0.144$). On

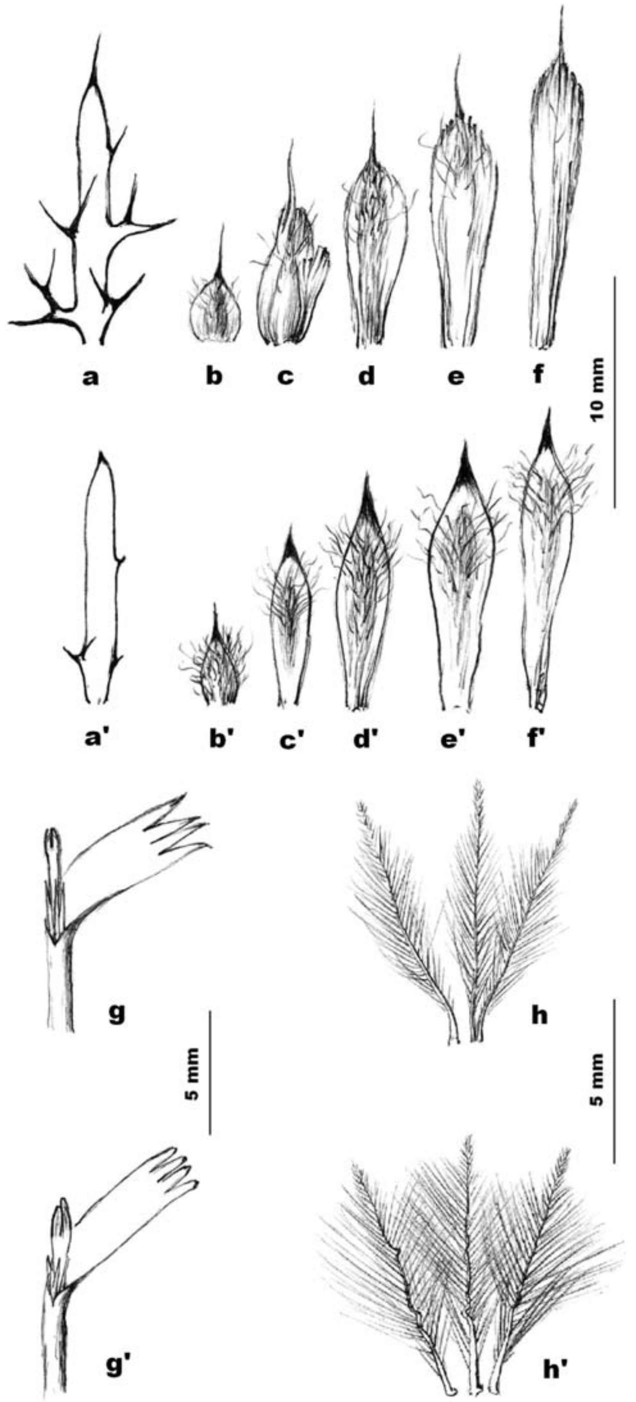

Fig. 2 Drawings of the main macro-morphological differences observed between *A. arbuscula* subsp. *arbuscula* (*a*–*h*) and *A. arbuscula* subsp. *schizogynophylla* (*a*′–*h*′). *a* and *a*′ bracts at the capitule's base; *b*–*f* and *b*′–*f*′ capitular scales; *g* and *g*′ ligule; *h* and *h*′ detail of the vilane. Drawings by A. Marrero-Rodríguez

average, the island of Lanzarote ($P = 49.92$, $I = 0.265$) was substantially more polymorphic than Gran Canaria ($P = 30.61$, $I = 0.169$) or even than *A. arbuscula* s. l. ($P = 40.26$, $I = 0.217$) (Table 1).

Table 5 Results of the AMOVA in *Atractylis arbuscula* s. l., between islands and within

Group and source of variation	SS	Variance	% Total
A. arbuscula s. l. ($F_{ST} = 0.330$***)			
Among populations	156.38	1.311	32.95
Within populations	309.39	2.667	67.05
Gran Canaria–Lanzarote (CL$F_{ST} = 0.294$***)			
Between islands	81.78	1.309	29.38
Within islands	383.99	3.148	70.62
Gran Canaria (C$F_{ST} = 0.308$***)			
Among populations	35.80	0.821	30.76
Within populations	86.83	1.847	69.24
Lanzarote (L$F_{ST} = 0.151$***)			
Among populations	38.81	0.574	15.12
Within populations	222.56	3.226	84.88

SS sum of squares; *% total* percentage of total variance contributed by each component

*** $P < 0.01$

The AMOVAs (Table 5) showed a sharp genetic differentiation for *A. arbuscula* s. l. ($F_{ST} = 0.330$***) and between islands (CL$F_{ST} = 0.294$***). Within islands, subpopulation differentiation in Gran Canaria (C$F_{ST} = 0.308$***) was closely similar to that calculated for *A. arbuscula* s. l., but more than twofold that for Lanzarote alone (L$F_{ST} = 0.151$***). Except for two values corresponding to Lanzarote, pairwise F_{ST}s were all significant (Table 6) and roughly threefold higher within Gran Canaria (average pairwise $F_{ST} = 0.324$) than within Lanzarote (average pairwise $F_{ST} = 0.136$), with the Gran Canarian subpopulation SIAC holding the largest values calculated. Nei's (1973) statistics of subpopulation subdivision

Table 6 F_{ST} values between all pair-wise combinations of populations in *Atractylis arbuscula* (lower diagonal) and indications of significance (+) or non-significance (−) of each value (upper diagonal)

	Gran Canaria				Lanzarote			
	ANGC	RIOC	SIAC	SILC	SSPL	HARL	FFTL	PFTL
ANGC		+	+	+	+	+	+	+
RIOC	0.392		+	+	+	+	+	+
SIAC	*0.537*	0.139		+	+	+	+	+
SILC	0.304	0.206	0.364		+	+	+	+
SSPL	0.313	0.466	0.529	0.507		−	+	+
HARL	0.281	0.451	0.511	0.527	**0.055**		−	+
FFTL	0.204	0.363	0.363	0.346	0.135	0.084		+
PFTL	0.320	0.429	0.427	0.423	0.230	0.195	0.116	

Italicised and bolded values signal, respectively, are the maximum and minimum values detected

Table 7 Nei's (1973) unmodified population structure statistics for the RAPD genetic variation detected in *Atractylis arbuscula*

Group	OPA-5			OPA-9OPA-9			OPT-7s primers			Across loci		
	Ht	Hs	Gst	Ht	Hs	Gst	Ht	Hs	Gst	Ht	Hs	Gst
A. arbuscula s. l.	0.296	0.148	0.500	0.302	0.219	0.275	0.183	0.087	0.525	0.260	0.151	0.433
Gran Canaria	0.104	0.090	0.138	0.206	0.160	0.218	0.107	0.055	0.482	0.139	0.102	0.279
Lanzarote	0.287	0.197	0.313	0.244	0.230	0.058	0.143	0.096	0.329	0.225	0.174	0.233

(Table 7) were substantially high ($G_{ST} = 0.433$ for *A. arbuscula* s. l.) and slightly higher in Gran Canaria ($G_{ST} = 0.279$) than in Lanzarote ($G_{ST} = 0.233$). The two first axes of the PCA (Fig. 3) explained 79.6% of the detected genetic variation and sharply separated the subpopulations from Lanzarote and Gran Canaria, save for six individuals from ANGC (Gran Canaria) and SSPL (Lanzarote) that intermingle between the regions of multivariate space corresponding to either island (see the discontinuous circle in Fig. 3).

The matrix of results, together with ancillary biological and abiotic information about *A. arbuscula*, will be deposited in a web database for public accessibility and dissemination of population genetic data of any kind that is presently being developed by an international team of scientists (http://www.exegen.org/sage).

Discussion

Morphology, RAPDS, and the taxonomy of *Atractylis arbuscula*

When Sventenius (1968) studied the specimens of this taxon, he found enough differences to consider the subpopulations in each island as belonging to a distinct variety, and he therefore described *Atractylis arbuscula* Svent. and Michaelis var. *arbuscula* for Lanzarote and *A. arbuscula* var. *schizogynophylla* Svent. and Kahne for Gran Canaria. However, the differences observed by this author referred mainly to general trends, as the indument type, leaf arrangement, leaf spininess, flower bracts or the size of floscules (i. e. disc flowers), and also to qualitative characters as the shape of the ligulae lobules (Table 2). Therefore, a first taxonomic question to ask in this investigation was whether those differences were reflected by other morphological characters and by neutral genetic markers (thereby accounting for heritable, taxonomically meaningful differences), or they could be considered just a result of environmentally-induced plasticity with no genetic basis, and thus not relevant for taxonomic purposes.

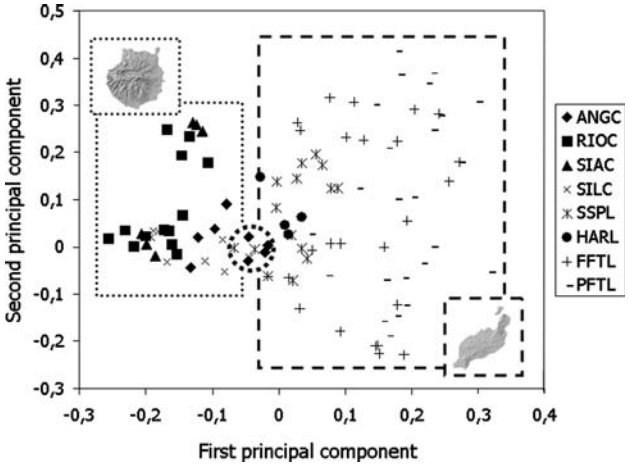

Fig. 3 Graphic representation of the two first PCA axes, which explained 59.6% of the detected genetic variation. Population codes correspond to Table 1. The circled individuals correspond to samples of either variety whose situation in the multivariate space does not permit unambiguous taxonomic ascription

Reproductive isolation underlies the existence of different taxa. Therefore, if the two islands of distribution of this Canarian endemic do indeed host different taxonomic entities, then we should expect them not to maintain a high genetic cohesion. In agreement with this prediction, our molecular population genetic data do indicate a distinct differentiation between islands as measured by the AMOVA (Table 5) and the G_{ST} value for *A. arbuscula* s. l. [which is roughly twice higher than those estimated for either island (Table 7)], and a sharp separation between the subpopulations of either variety in the multivariate space of the PCA (Fig. 3). Given the conservative nature of the RAPD study, the six ambiguous individuals halfway between the two islands in the PCA (circled in Fig. 3) probably represent the genetic similarity in the two closely related taxonomic entities examined, rather than a spurious result attributable to chance.

According to our molecular data, the two groups of subpopulations in Lanzarote and Gran Canaria can thus be considered as independent Evolutionarily Significant Units (ESUs, Waples 1991), as (i) they are reproductively isolated from each other, and (ii) each of them represents an important component of the evolutionary legacy of the species. As ESUs are widely equated to conservation units (Moritz 1994; Waples 1998; Karl and Bowen 1999), this result entails that any conservation strategy should consider the two islands of distribution separately.

What taxonomic rank should be given to these two distinct ESUs? In his morphological study, Sventenius (1968) did not take into consideration the shape of the basal capitular bracts or the scale shape in the involucral bracteae for his taxonomic descriptions. Despite he did study these characters, he described the former only for var. *schizogynophylla* and the latter only for var. *arbuscula*, without undertaking a comparison. Rather, he used heterogeneous sets of characters to describe each taxon independently (the characters used by Sventenius and the gaps that prevented a thorough morphological comparative treatment are accounted for in Table 2). Furthermore, the herbarium material collected by Dr. A. Kahne that was used by Sventenius (1968) to describe the taxon from Gran Canaria did not have fruits, so that this author could not observe the achenes of these plants.

The data generated by us on the variables already considered by Sventenius (1968) support the general morphological trends that this author highlighted, though our observations on the arrangement and clustering of leaves cannot be as conclusive as his. However, the morphological traits that are considered for the first time in this paper (Tables 3, 4; Fig. 2), together with the RAPD results discussed above, motivate the review of the taxonomy for this species and the establishment of the following infraspecific key

1. Leaves with a long mucro of ca. (0.3) 0.6–1.0 (1.5) mm. Bracts at the capitulum base pinnate-spiny, with spines long and simple or bifurcated with a long mucron. Scales of the capitulum narrowly obovate, with their extreme more or less obtuse and somewhat fimbriate, extensively mucronate-aristate, with wide scariose-hyaline margins. Lobules of the ligulae elongated, of ca. (1.0) 1.5–2.2 (2.4) mm, lanceolate, acute, mucronate. Pappus-hairs plumose, up to 2.5 (3.0) mm.

Atractylis arbuscula Svent. and *Michaelis* subsp. *arbuscula*

2. Leaves with a short mucro of ca. (0.2) 0.3–0.6 (1.0) mm or muticous. Bracts at the capitulum base entire or remotely spiny at the edges, with a very short mucro or muticous. Scales of the capitulum oblanceolate with a sharp tip, only slightly fimbriate (if at all) and with tip finished in a mucron, with narrow scariose margins. Lobules of the ligulae shorter, of 1.0–1.8 (2.0) mm, angustiovate-oblong, that end in a very short mucro. Pappus hairs plumose, up to 3.8 (4.5) mm.

Atractylis arbuscula Svent. and Michaelis subsp. *schizogynophylla* (Svent. and Kahne) Marrero Rodr. and Caujapé-Castells, *comb. & stat. nov*.

Basionym: *Atractylis arbuscula* Svent. and Michaelis var. *schizogynophylla* Svent. and Kahne in E. R. Sventenius, *Indices Seminum Horti Acclimat. Plantarum Arautapensi*, Pars IV: 43–60 (1968).

Levels of genetic divergence and conservation implications

Small population sizes and restricted distribution determine impoverished levels of genetic variation in *A. arbuscula*, and especially in subsp. *schizogynophylla* from Gran Canaria (Table 1). Within Asteraceae, our estimates rank similar to those for its congeneric Canarian endemic *Atractylis preauxiana* ($P = 49.3$, $I = 0.245$, Caujapé-Castells et al. 2008) and slightly higher than those reported by Friar et al. (1996) for the Hawaiian endemic *Argyroxiphium sandwicense* ($P = 12$–15). However, these indicators are very low as compared with RAPD data for other Canarian taxa. Bouza et al. (2002) report averages of respectively ($P = 91$, $I = 0.79$) and ($P = 68$, $I = 1.64$) for *Dorycnium spectabile* (Fabaceae) and *Isoplexis chalcantha* (Scrophulariaceae), two narrow Canarian endemics exclusive from the laurel forest in Tenerife and Gran Canaria (respectively), and González-Pérez (2001) also finds higher values in the Canarian endemic palm *Phoenix canariensis*. Thus, the results for *A. arbuscula* do not match the emerging picture of increased levels of genetic variation in Canarian endemics.

One distinct possibility to explain this result is that low population sizes are determining the genetic impoverishment detected in these taxa, as basic population genetic theory predicts a swift decrease of variation due to increased selfing, mating among related individuals and drift in small populations (Barrett and Kohn 1991), that make them more prone to extinction, independently of whether they belong to common or rare species (Honnay and Jacquemyn 2007).

The subpopulations from Lanzarote (which are bigger than those of the taxon from Gran Canaria) hold in general much higher levels of genetic variation (Table 1), and they are possibly not experiencing to such a large extent the deleterious genetic effects brought about by low sizes. In the absence of evidence for selection, the contrast between the distribution of genetic variation within these two taxa of *A. arbuscula* (Tables 5, 6 and 7) is best construed by the existence of a higher degree of genetic variation and interpopulation gene flow in Lanzarote, that allows for the introduction of a new stock of genetic variation in the subpopulations through pollen or seed flow.

The value of G_{ST} for the Gran Canarian subpopulations is fivefold higher than that in Lanzarote (Table 7), indicating a higher degreee of fragmentation in this island that possibly contributed to enhance the deleterious effects of low population sizes, as fragmented populations typically exhibit reduced gene flow (if at all) among remnant demes. Cheptou and Avendaño (2006) demonstrate a direct influence of fragmentation in the reduction of seed set in the allogamous Asteraceae *Crepis sancta*. The population size threshold beyond which these authors and earlier ones (e. g. Groom 1998, with *Clarkia concinna*) detected no clear influence of population size on seed set reduction is of about 50 individuals, which is clearly above the size of most of the isolates of *A. arbuscula* known in Gran Canaria. Therefore, these subpopulations might be additionally vulnerable to genetic erosion and loss of genetic diversity on account of the more severe fragmentation processes that they are undergoing. This also suggests that the habitat remnants of this taxon from Gran Canaria are too isolated to allow sufficient gene flow to enable replenishment of lost alleles, and have become unable to maintain a sufficient number of individuals to keep a mutation-drift balance.

Concommitant with these consequences, another of the main changes caused by fragmentation is the increased importance of stochastic processes resulting in a higher probability of a local population becoming extinct (Templeton et al. 1990, Young et al. 1996, Cheptou and Avendaño 2006). The small population sizes of the isolates of *A. arbuscula* in Gran Canaria not only determine a stochastic loss of alleles due to the action of drift, but they also probably help inducing a diminished reproductive efficiency, as pollination is a density-dependent process that requires a minimum number of individuals to proceed effectively (Richards 1986, 1997; Schemske et al. 1994; Young et al. 1996, 1999). Especially in insect-pollinated plants, pollen shortage may be caused by reduced pollinator visitation, since small populations have smaller, more diffuse, and therefore less attractive floral displays (Amarasekare 2004). The extremely low population sizes of the isolates of *A. arbuscula* (especially in Gran Canaria) may thus bring about a decreased interaction with vectors of animal pollination and seed dispersal leading to a substantial decrease of population fecundity (Heywood 1986), lower recruitment (Schemske et al. 1994; Courchamp et al. 1999), and inbreeding depression (Shafer 1990; Hawkes et al. 1997). According to both theoretical models (Charlesworth and Charlesworth 1987) and experimental work (Barrett and Charlesworth 1991; Pannell and Barrett 1998), this non-genetic reproductive syndrome, also known as the "Allee effect" (Allee et al. 1949; Lande 1988), may be of particular relevance for *A. arbuscula*, as outcrossing populations typically maintain more inbreeding depression and purge less deleterious recessives than selfing populations.

The generalised lack of population regeneration in subsp. *schizogynophylla* (Rodríguez-Delgado et al. 2004) and the extremely low seed production and seedling survival in its subpopulations indicate that the deleterious effects of the Allee effect are especially dramatic in this subspecies from Gran Canaria. If, as most Asteraceae studied to date, *A. arbuscula* possesses a self-incompatibility system, then the reduced reproductive turnover in these populations may be largely influenced by the stochastic loss of cross-compatible genotypes (Campbell and Husband 2007), irrespective of pollinator visits (no information is available on the pollination biology of *A. arbuscula*). In this context, one important factor (if not the only one) to explain the low seed set in the very small populations from Gran Canaria could be a deficiency of S-alleles, that would be provoking the unavailability of compatible mating types, as was shown recently for *Hymenoxys herbacea*, a very rare species of Asteraceae (Campbell and Husband 2007). Supportive indirect evidence for an influence of population size on seed set is given by the latest censuses in the much bigger subpopulations of subsp. *arbuscula*, that show that they are slowly expanding in Lanzarote, and display much higher levels of gene flow and connectivity (see Tables 5, 6, 7); probably they are also less prone to manifest the adverse consequences of inbreeding because of a higher number of compatible matings.

Overall, these features, together with population extinctions reported in recent times, substantiate the severe impact of low levels of genetic variation, genetic drift, scant population sizes, restricted gene flow and poor seed set and recruitment in the population dynamics and genetic makeup of subsp. *schizogynophylla*. The contrasting situation of *A. arbuscula* in its two islands of distribution is further aggravated by the fact that the Gran Canarian subpopulations are undergoing a relentless demographic and habitat degradation linked to the unsustainable development of the island. By contrast, populations of subsp. *arbuscula* are within (or in the surroundings of) the natural preserve of the Archipielago Chinijo (see Fig. 1), which warrants the protection of their habitats and, thus, provides a relative ecological stability. Hence, the implementation of conservation measures for the Gran Canarian subsp. *schizogynophylla* seems of utmost importance.

In order to retrieve the subpopulations of subsp. *schizogynophylla* from the sheer genetic depauperation they have undergone, in situ actions aimed at enhancing connectivity are essential. A sufficient level of connectivity among subpopulations would contribute to offset the adverse effects of genetic drift and habitat fragmentation through fostering re-colonisation, gene flow, and eventual expansion. With this purpose, reintroductions should be carried out in ecologically suitable locations, preferably undisturbed or within protected areas.

Parallel to these reintroductions, reinforcements are also necessary in all extant subpopulations to alleviate them from the deleterious effects of loss of genetic variation, drift and inbreeding. "Mixed" subpopulation reinforcements and reintroductions (i. e., with specimens from as many subpopulations as possible from the same island) seem preferable in this case on the grounds that they might allow the habitat isolates to retrieve from the negative effects of inbreeding and increase their diversity levels through the introduction of a new stock of genetic variation. The reinforced subpopulations will in general keep being small and, therefore, some of the new variation is liable to be lost by genetic drift. Nevertheless, the re-introductions would also facilitate an increase in the number of S-alleles, apparently much needed for more compatible matings to happen. Apart from inducing higher seed set levels, this expected upward surge of matings is bound to foster recombination and, thus, the production of new genotypes that may have a higher selective value (Templeton et al. 1990). Hence, this "mixed" strategy would probably ensure the survival of this subspecies through alleviating demographic stochasticity and increasing its global levels of genetic variation.

It is possible that a mixed reinforcement might eventually bring about outbreeding depression (OBD), so that it is in order to arrange a program of crosses to assess this possibility. However, as suggested with the populations of its congener *A. preauxiana* from Tenerife (Caujapé-Castells et al. 2008), it may be best to just make the mixed reinforcements as soon as possible, without waiting for the results of the crossing experiments. Since the eventual effects of OBD might not be detected readily for a variety of causes (see Caujapé-Castells et al. 2008), the smallest populations (which are on the verge of extinction) could go die off in the meantime.

Because of the poor seed set and seedling survival in the wild (Rodríguez-Delgado et al. 2004), it might be more sensible first to grow plants of subsp. *schizogynophylla in vitro* to have a sufficient amount of material to carry out the in situ conservation actions than to set up plant nurseries derived from seeds. By the same token, it does not seem urgent to collect seeds for ex situ banking until monitoring of the reinforced populations reveals an increased recruiting rate. For subsp. *arbuscula*, which is genetically much healthier than subsp. *schizogynophylla*, it seems sufficient at this stage to reinforce the smallest and genetically less variable subpopulations (HARL and SSPL) using a mixed strategy as explained above, considering that the Archipielago Chinijo preserve has a status of protected natural landscape.

The suggestions given might help overcome successfully the deleterious effects induced by inbreeding and lack of connectivity in the subpopulations of subsp. *schizogynophylla*. Yet, perhaps more important than heeding this basic genetic advice, any conservation strategy should warrant habitat protection measures aimed at halting the ongoing habitat degradation in the extant subpopulations of this subspecies. If these measures are not effectively implemented, then translocations of the reinforced subpopulations to the areas suggested for reintroductions should be urgently considered.

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