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ORIGINAL ARTICLE

Population genetic suggestions to offset the extinction ratchet in the endangered Canarian endemic *Atractylis preauxiana* (Asteraceae)

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Abstract We examined the levels and apportionment of genetic variation of the 11 known subpopulations of *Atractylis preauxiana* at 95 RAPD loci to help streamline a conservation strategy for this Canarian endemic taxon, which is in a critical situation because of the constant exposure of plants to intensive, uncontrolled anthropic action in the last few decades. Our results revealed low genetic variation levels that match with the general picture of demographic and habitat degradation that this taxon is undergoing. Although geographic isolation between Tenerife and Gran Canaria is an effective barrier to gene flow, genetic heterogeneity within islands is also substantial, plausibly due to the negative impact of fragmentation on genetic variation. Our genetic results, together with declining population sizes, poor seedling survival, and recent population extinctions, compellingly indicate that *A. preauxiana* is undergoing an extinction ratchet, whereby every further local extinction will add up to the probability of total species' extinction. Our genetic results suggest that mitigating the deleterious consequences of this effect entails urgent mixed reinforcements of all sub-populations with sub-populations from the same island and urgent translocation of the two sub-populations from Tenerife that are doomed to extinction to ecologically suitable areas, together with seed collection and preservation in a convenient ex situ banking facility.

Keywords *Atractylis preauxiana* · Canary Islands · Endemic · RAPDs · Conservation · Fragmentation · Extinction

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Introduction

Atractylis preauxiana Sch. Bip. is a Canarian endemic Asteraceae exclusively known from south-eastern areas of the islands of Gran Canaria and Tenerife (Fig. 1) that is presently included in annex I of Bern's 1979 Covenant, and in the EU directive 92/43/CEE (May 1992), relative to the conservation of natural habitats and of the wild fauna and flora. It also appears in both the Catálogo Nacional de Especies Amenazadas and the Catálogo de Especies Amenazadas de Canarias and the Spanish and Canarian red lists of endangered plants (Bañares et al. 2004) and the Insular Biodiversity Plan of the island of Tenerife as "threatened with extinction".

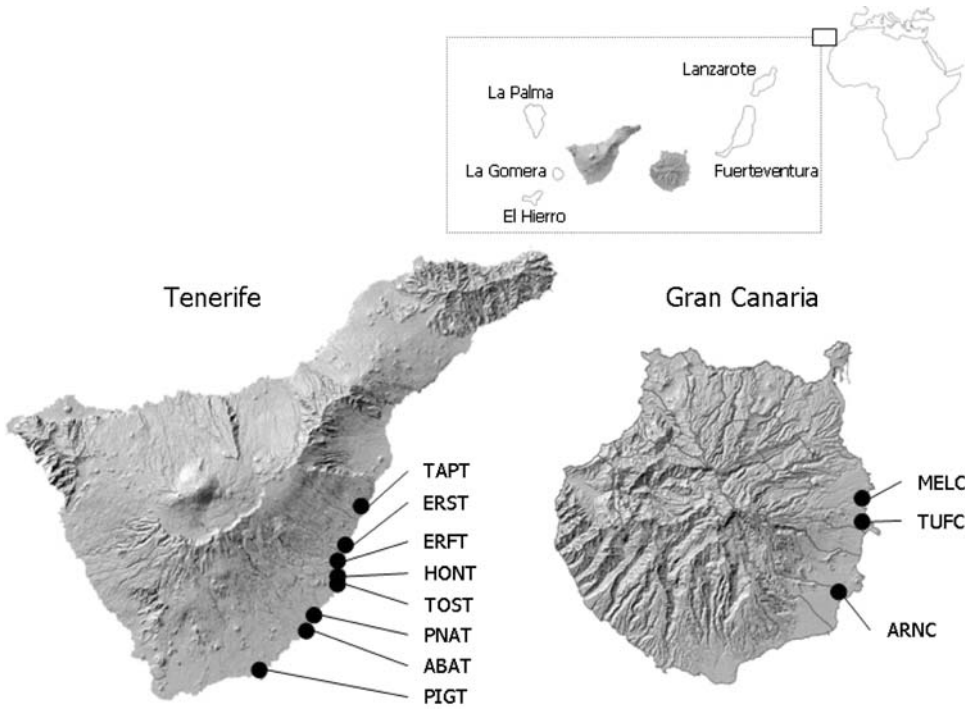

Fig. 1 Geographic map with the locations of the populations sampled for this investigation. Population codes correspond to Table 1

While in Gran Canaria the plant is only known from three subpopulations, in Tenerife it is found along a ca. 40 km coastal fringe from Tosca de Fasnia to San Miguel de Abona (see Fig. 1). In general, subpopulations are much bigger in Gran Canaria than in Tenerife (Table 1), and range in size from about 14,700 individuals in Arinaga (Gran Canaria) to only 10 in one of the stands in Tenerife, according to the latest censuses (Beltrán-Tejera et al. 1999; Rodríguez-Delgado et al. 2004; Mesa-Coello and Rodríguez-Núñez 2006).

The range of distribution of the species exhibits a severe fragmentation (especially in Tenerife), and subpopulations

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

Island/population (code)	*N*	*n*	OPA-4		OPA-5		OPA-8		OPA-9		OPT-7		Overall	
			P	*I*	*P*	*I*	*P*	*I*	*P*	*I*	*P*	*I*	*P*	*I*
Gran Canaria														
1. Arinaga (ARNC)	>2,500	20	48.00	0.216	66.67	0.352	63.64	0.278	45.45	0.155	68.18	0.335	67.37	0.258
2. Melenara (MELC)	<100	20	56.00	0.316	26.67	0.152	63.64	0.324	45.45	0.247	59.09	0.307	51.58	0.273
3. Tufia (TUFC)	<300	10	32.00	0.201	33.33	0.187	54.55	0.307	36.36	0.210	40.91	0.248	37.89	0.224
Tenerife														
4. La Tosca (TOST)	<50	21	72.00	0.353	73.33	0.365	81.82	0.390	68.18	0.341	77.27	0.368	73.68	0.360
5. La Hondura (HONT)	<300	22	36.00	0.208	33.33	0.213	36.36	0.229	27.27	0.152	36.36	0.206	33.68	0.198
6. Las Eras-1 (ERST)	<500	25	72.00	0.298	6.67	0.046	45.45	0.185	63.64	0.303	68.18	0.288	55.79	0.244
7. Las Eras-2 (ERFT)	<100	4	–	–	–	–	–	–	–	–	–	–	–	–
8. Tabaibal del Porís (TAPT)	<200	21	60.00	0.292	53.33	0.272	63.64	0.318	54.55	0.298	72.73	0.351	61.05	0.307
9. Punta de Abona (PNAT)	<100	36	68.00	0.312	13.33	0.081	45.45	0.192	36.36	0.183	45.45	0.251	44.21	0.218
10. Abades (ABAT)	<50	13	52.00	0.253	20.00	0.087	18.18	0.099	45.45	0.240	54.55	0.269	42.11	0.210
11. Polígono de Granadilla (PIGT)	<10	3	20.00	0.127	0.00	0.000	27.27	0.174	31.82	0.203	40.91	0.260	26.32	0.161
Average *A. preauxiana* s. l.			51.60	0.258	32.67	0.176	50.00	0.250	45.45	0.233	56.36	0.288	49.37	0.245
Average Gran Canaria			45.33	0.244	42.22	0.230	60.61	0.303	42.42	0.204	56.06	0.298	52.28	0.252
Average Tenerife			54.29	0.263	28.57	0.152	45.45	0.227	46.75	0.246	56.49	0.285	48.12	0.246

N Estimated population size according to Beltrán-Tejera et al. (1999) and Rodríguez-Delgado et al. (2004); *n* sample size; *P* proportion of polymorphic loci; *I* Shannon's information index

contain mostly adult, senescent or dead individuals; recent surveys in a locality near San Miguel de Abona (Tenerife) reported only dead individuals (Rodríguez-Delgado et al. 2004). Although a moderate to high number of seedlings can emerge (but only in rainy winters), most of these do not survive until the reproduction age (Rodríguez-Delgado et al. 2004). A small subpopulation in Granadilla de Abona (Tenerife) was discovered in 2002 by Otto and Barone Tosco (Otto, personal communication to the Gobierno de Canarias, http://www.atan.org/flora/atractylis/ doc101.pdf), but the approval of a project to build an industrial port in the area dooms it to destruction in the immediate future. Yet, the port authorities and the Viceconsejería de Medio Ambiente of the Gobierno de Canarias are streamlining the declaration of this area as a place of community interest (LIC). Furthermore, subpopulations of *A. preauxiana*, previously known in the coasts of Güímar (Tenerife), and Melenara (Gran Canaria) are already extinct (Rodríguez-Delgado et al. 2004). However, a new nucleus of some 150 individuals has been localized recently near the latter area.

Since no thorough population studies for this endemic species are available to date, the design of informed conservation strategies poses an urgent need for data. 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. preauxiana*, it only requires small amounts of template DNA. Two additional reasons support the use of RAPDs in the context of extremely endangered plant species like *A. preauxiana*. First, this technique makes it feasible to carry out a survey for molecular makers much faster than more detailed demographic or other studies to identify adaptive traits; this speed of analysis is a very convenient feature when the populations at issue require urgent data to base conservation decisions. Second, most usually RAPDs show differentiation between populations (e.g. Chong et al. 1994; Esselman et al. 1999; Fischer et al. 2000; Torres et al. 2003; Vilatersana et al. 2005), meaning that genetic divergence has had the opportunity to occur and that different populations might be worthy of detailed conservation consideration. In fewer occasions, low divergence also occurs (Black-Samuelsson and Andersson 1997; Darokar et al. 2004); in these cases, RAPDs are less informative, and they could indicate adaptation with strong selection driving divergence in certain characters faster than the divergence that occurs with the molecular markers. 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. preauxiana*.

Severe habitat fragmentation, declining population sizes, poor seedling survival and recent population extinctions indicate that *A. preauxiana* is possibly undergoing an extinction ratchet (*sensu* Templeton et al. 1990), by which the probability of species extinction irreversibly increases over time due to local population extinctions. Our objective in this paper is to help streamline a comprehensive conservation plan for the endangered variation of *A. preauxiana* using the information contained in RAPD markers and, more specifically, to set forth guidelines to halt and reverse the extinction ratchet in this Canarian endemic. Hopefully, our suggestions might be extrapolated to other plant taxa that also are in a similarly critical situation and demand urgent conservation strategies.

Materials and methods

Plant material and sampling

Atractylis preauxiana is a small (up to 25–30 cm) chamaephyte with: a thick woody stem ramified from the base; leaves up to 3 cm in length, whole, linear to oblanceolate and densely hirsute, whitish, green-grayish or silvery, with rigid spikes at the margins and on the apex; external involucral bracteae foliose, pinnatifid and spiny; internal involucral bracteae whole, brown-reddish at the edge, black at the tip and with a long apical spine; capitula solitary and terminal with white external ligulae, sometimes creamy or pinkish; cypselas with silvery hairs, vilane with feathery hairs joined at the base (Beltrán-Tejera et al. 1999; Bramwell and Bramwell 2001; Rodríguez-Delgado et al. 2004).

We collected leaves of 195 individuals from 11 localities (eight from Tenerife and three from Gran Canaria) that thoroughly represent the known distribution areas of *A. preauxiana* (Fig. 1; Table 1). Leaf samples were put in zipped, unambiguously coded plastic bags containing silica gel, where they remained until further processing 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.

DNA processing

The 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 (N. Cabrera, unpublished data). Amplification reactions were set in a 25 µl final volume of reaction mixture containing 23 µl of ABGENE 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 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 to confirm the consistency and reproducibility of the experiments. Markers that were inconsistently amplified in repeated RAPD reactions were not included in the subsequent data analysis.

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 the statistical 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. We assumed that bands with identical size represented the same locus.

Data analysis

The resulting binary 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 subpopulations. This matrix is soon to be included in a public database for molecular population genetic markers launched by the Cabildo de Gran Canaria that will be resident in the web site of this institution. Percentage of polymorphic loci (P), Shannon's information index (I, Lewontin 1972), F_{ST} values between pair-wise combinations of subpopulations, and Nei's (1973) population structure statistics per locus, per island and for *A. preauxiana* s. l. were calculated using the program Popgene (1997). AMOVAs within islands, between islands and for *A. arbuscula* sensu lato (s. l.) were carried out using the computer program Arlequin (Schneider et al. 2000); the 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 ran a principal components analysis (PCA) with the individual vectors of allele presence/absence.

Results

Out of 14 decamer primers initially screened in *A. preauxiana*, only 5 produced bands that could be reliably scored (Table 1). 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 two facts which influenced the yielding of DNA isolation: first, the production of a considerable amount of secondary metabolites by these plants, that possibly interfered with the extraction, and second, the physical condition of the leaves that was not healthy in most subpopulations. Since the results for the four individuals in ERFT were irregular for all screened primers, this subpopulation was excluded from the calculations.

These five primers gave a total of 95 scorable loci with variable average levels of polymorphism that ranged from ($P = 56.36$, $I = 0.288$) in OPT-7 to ($P = 32.00$, $I = 0.176$) in OPA-5 (Table 1). Overall, the most polymorphic subpopulation was TOST, from Tenerife ($P = 73.68$, $I = 0.360$), and the less polymorphic was

Table 2 Results of the AMOVA in *Atractylis preauxiana* s.l., between islands and within islands

Group and source of variation	SS	Variance	% Total
A. preauxiana s.l. ($F_{ST} = 0.295$***)			
Among populations	470.93	3.660	29.64
Within populations	981.56	8.690	70.36
Between islands (CT$F_{ST} = 0.163$***)			
Between islands	148.53	2.145	16.47
Within islands	1,303.96	10.87	13.02
Within Gran Canaria (C$F_{ST} = 0.331$***)			
Among populations	120.60	4.177	32.10
Within populations	347.36	8.838	67.90
Within Tenerife (T$F_{ST} = 0.226$***)			
Among populations	201.79	2.520	22.60
Within populations	618.21	8.630	77.40

SS Sum of Squares; % total refers to the percentage of total variance contributed by each component

*** $P < 0.001$

PIGT, also from Tenerife ($P = 26.32$, $I = 0.161$). On average, the island of Gran Canaria ($P = 52.28$, $I = 0.252$) was slightly more polymorphic than Tenerife ($P = 48.12$, $I = 0.246$; Table 1).

The AMOVAs (Table 2) showed a sharp genetic differentiation for *A. preauxiana* s. l. ($F_{ST} = 0.295$***). Within islands, subpopulation differentiation in Gran Canaria (C$F_{ST} = 0.331$***) was even higher than that calculated for *A. preauxiana* s. l. or for Tenerife alone (T$F_{ST} = 0.226$***). Pairwise values of F_{ST} (Table 3) were all significant and also higher within Gran Canaria (average pairwise $F_{ST} = 0.272$) than within Tenerife (average pairwise $F_{ST} = 0.247$), with the Gran Canarian subpopulation ARNC holding the largest values calculated. The two first axes of the PCA (Fig. 2) explained roughly 63% of the detected genetic variation and sharply separated ARNC

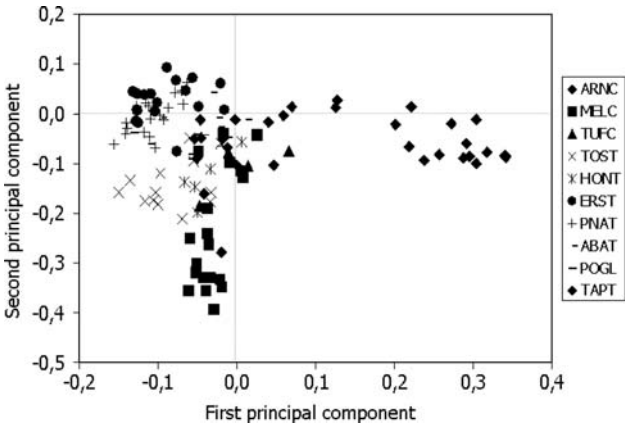

Fig. 2 Graphic representation of the two first PCA axes, which explained 63.6% of the detected genetic variation. Population codes correspond to Table 1

(Gran Canaria) and the rest of the subpopulations surveyed. Nei's (1973) statistics of subpopulation subdivision (Table 4) were high and slightly superior in Tenerife ($G_{ST} = 0.327$) than in Gran Canaria ($G_{ST} = 0.246$).

The matrix of results, together with ancillary biological and abiotic information about *A. preauxiana*, will be deposited in the web database SAGE (http://www.exegen.org/sage) an international initiative for public accessibility and dissemination of population genetic data of any kind.

Discussion

Small population sizes and restricted distribution are direct predictors of impoverished levels of genetic variation in *Atractylis preauxiana* (Table 1). Within Asteraceae, our estimates for this endemic rank similar to those reported by Friar et al. (1996) for the Hawaiian endemic *Argyroxiphium sandwicense* ($P = 12$–15). However, these indicators are low as compared with RAPD data for other Canarian taxa.

Table 3 F_{ST} values between all pair-wise combinations of populations in *Atractylis preauxiana* (lower diagonal) and indications of significance (+) or non-significance (−) of each value (upper diagonal)

	Gran Canaria			Tenerife						
	ARNC	MELC	TUFC	TOST	HONT	ERST	TBPT	PNAT	ABAT	PIGT
ARNC		+	+	+	+	+	+	+	+	+
MELC	0.355		+	+	+	+	+	+	+	+
TUFC	0.291	0.169		+	+	+	+	+	+	+
TOST	0.366	0.214	0.134		+	+	+	+	+	+
HONT	0.406	0.296	0.244	0.171		+	+	+	−	+
ERST	0.405	0.327	0.284	0.236	0.368		+	+	+	+
TBPT	0.435	0.331	0.304	0.215	0.385	0.148		+	+	+
PNAT	0.400	0.329	0.344	0.248	0.352	0.193	0.286		+	+
ABAT	0.440	0.324	0.292	0.201	0.368	0.277	0.357	0.180		+
PIGT	0.286	0.203	0.140	0.167	0.201	0.222	0.199	0.199	0.214	

Table 4 Nei's (1973) unmodified population structure statistics for the RAPD genetic variation detected in *Atractylis preauxiana*

Group	OPA-4			OPA-5			OPA-8			OPA-9			OPT-7			Across loci		
	Ht	Hs	Gst	Ht	Hs	Gst	Ht	Hs	Gst	Ht	Hs	Gst	Ht	Hs	Gst	Ht	Hs	Gst
A. preauxiana s. l.	0.251	0.169	0.326	0.208	0.118	0.432	0.260	0.164	0.368	0.236	0.155	0.343	0.288	0.191	0.336	0.250	0.162	0.351
Gran Canaria	0.164	0.138	0.162	0.239	0.156	0.348	0.250	0.197	0.212	0.202	0.134	0.336	0.238	0.197	0.173	0.219	0.164	0.246
Tenerife	0.247	0.171	0.308	0.170	0.102	0.399	0.225	0.150	0.334	0.224	0.164	0.268	0.280	0.188	0.327	0.229	0.155	0.327

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 islands of Tenerife and Gran Canaria (respectively), and González-Pérez (2001) also finds higher values than those of *A. preauxiana* in the Canarian endemic palm *Phoenix canariensis*. The results for *A. preauxiana* are at odds with the emerging picture of increased levels of genetic variation in Canarian endemics with respect to those in other geographic areas. One distinct possibility to explain this result is the influence of low population sizes, as basic population genetic theory predicts a swift decrease of variation due to inbreeding and drift in small populations (Barrett and Kohn 1991). Indeed, the biggest subpopulations (ARNC in Gran Canaria and TOST in Tenerife) are far more variable than the others (Table 1), and they are possibly buffering more efficiently the deleterious genetic effects brought about by low population sizes.

Although intra-population differentiation is low, the species as a whole holds a conspicuous and significant subpopulation differentiation ($F_{ST} = 0.295$***, Table 2). The AMOVAs also reveal a highly significant differentiation between islands ($F_{ST} = 0.163$***, Table 2), which is cogent with the absence of gene flow due to geographic isolation. As highlighted by the PCA representation, this difference is mostly attributable to the contribution of the Gran Canarian subpopulation ARNC that also holds the highest overall values of pair-wise F_{ST} values (Table 3).

It is not likely that this high level of subpopulation heterogeneity has been fostered by the action of qualitatively distinct selective forces in different areas, as superficial observations of the habitats revealed no obvious ecological differences, and Ewens–Watterson tests indicated that all loci surveyed can be considered neutral (data not shown). Furthermore, although geographic isolation between Tenerife and Gran Canaria is an effective barrier to gene flow, the detected levels of differentiation are not only due to this factor, as genetic heterogeneity within islands is also substantial, highly significant, and much higher in Gran Canaria ($F_{ST} = 0.331$***) than in Tenerife ($F_{ST} = 0.226$***). In the face of this evidence, our genetic results are best construed as indicative that habitat fragmentation has led to a critical situation where the influence of genetic drift associated with low population sizes and insufficient inter-population genetic interchange has overridden the reproductive capabilities of this species for survival. Examples where habitat fragmentation has acted to promote extinction are abundant and span a wide number of plant lineages (Niemelä and Baur 1998; Honnay et al. 2005). Though it would be possible that the higher number of subpopulations in Tenerife had fostered more opportunity for gene flow in this island (thereby inducing a slightly higher genetic cohesion), this is unlikely on the grounds of the high G_{ST} value in this island ($G_{ST} = 0.327$, Table 4), that is roughly one-third higher than that in Gran Canaria ($G_{ST} = 0.246$, Table 4). Thus, a higher number of subpopulations in Tenerife does not seem to relate to enhanced colonization success of *A. preauxiana* in this island, but to a much more radical influence of fragmentation in subpopulations that once exhibited a more continuous distribution.

Since most subpopulations hold very small sizes in both islands of distribution, and habitat fragmentation seems to have led to effectively diminished within-island inter-population gene flow, then *A. preauxiana*'s chances of extinction are bound to increase as a consequence of the cascade of effects related to genetic drift and inbreeding (Barrett and Kohn 1991; Templeton et al. 1990). According to Templeton et al. (1990), the disruption of genetic cohesion may entail an "extinction ratchet" in which each local extinction provoked by habitat fragmentation increases the probability of total species extinction. In fact, subpopulations of *A. preauxiana* previously known in the coasts of Güímar and Amarilla (San Miguel de Abona; Tenerife) and Melenara (Gran Canaria) are already considered extinct (Rodríguez-Delgado et al. 2004). This fact indicates a swift advance of the extinction ratchet in this Canarian endemic, which is further aggravated by the deleterious consequences of low subpopulation sizes and impoverished genetic variability levels in the extant subpopulations. In this context, we believe it highly likely that the imminent disappearance of the subpopulation at La Granadilla (PIGT) as a consequence of the construction of a port in that area will soon add up further to the extinction chances of the remaining isolates and, by extension, of the

species. The critical situation of *A. preauxiana* is probably the exclusive consequence of the constant exposure of plants to intensive, uncontrolled anthropic action for the last decades.

Conservation genetic insights

The genetic information that we obtained through RAPDs indicates that the low levels of intra-population genetic variation in *A. preauxiana* likely reflect several generations of inbreeding associated with low population sizes and restricted (if at all) levels of gene flow. Available demographic data bolster this interpretation by showing that seedlings are scarce and have in general a poor survival success (Rodríguez-Delgado et al. 2004). However, when we consider the subpopulations of *A. preauxiana* as an assemblage, it is evident that they have succeeded to retain a substantial amount of inter-population genetic heterogeneity, regardless of the forces that have acted to reduce variation. Therefore, a first urgent conservation measure that must be undertaken is to preserve ex situ the genetic variation of the species before it diminishes further, in order to warrant a source of germplasm for possible reintroductions or reinforcements. To this purpose, all extant subpopulations should be intensively sampled for seeds, which should be deposited in a proper germplasm banking facility. Since some of the subpopulations (HONT and TAPT in Tenerife, and ARNC and TUFC in Gran Canaria) are within the areas of protected natural landscapes, these collections might prioritize the known subpopulations that lack habitat protection.

In the face of the severe impact of habitat fragmentation on *A. preauxiana*, this ex situ conservation strategy should be paralleled by proper in situ actions encompassing at least (1) the reinforcing of all known subpopulations to compensate for demographic stochasticity and to maintain the existing genetic variation, and (2) the use of this genetic variation to protect the species from the advance of the extinction ratchet. These in situ actions seem essential because the higher the connectivity between habitat patches or subpopulations, the more potential for transfer of genetic material. A sufficient level of connectivity is thus of paramount importance for *A. preauxiana*'s survival, since it would contribute to offset the adverse effects of habitat fragmentation through (1) alleviating inbreeding and loss of genetic variation, and (2) fostering re-colonization of habitat patches in which subpopulations have gone extinct, colonization of new habitat patches, migration, and expansion.

At present, we do not have direct evidence of lowered fitness of inbred individuals in *A. preauxiana*, but poor seedling survival and diminished recruitment observed by the most recent monitoring of populations (Rodríguez-Delgado et al. 2004) suggests that they could be undergoing inbreeding depression. Nevertheless, absence of specific data about breeding systems or pollination biology in *A. preauxiana* imposes extreme caution in the interpretation of the possible causes of these symptoms. Although most Asteraceae are self-incompatible, important exceptions exist; for instance, Hiscock (2000) demonstrates a genetic flexibility in the sporophytic self-incompatibility system of *Senecio squalidus* that generates individuals with weakened self-incompatibilty, which could be crucial in explaining the success of this taxon as a colonizer in the UK. If *A. preauxiana* is self-incompatible, then the low seed set in the very small populations could be due to the low number of compatible matings. If it is self-compatible but needs an external pollinator, then lack of pollinators could limit seed set, and the selfed seeds should be about as fit as the outcrossed.

Several recent studies have shown that the deleterious effects induced by extreme inbreeding in small populations can be mitigated by the human-mediated introduction of even very small numbers of migrants (Tallmon et al. 2005; Edmands 2007). Carrying out "mixed" subpopulation reinforcements (i.e. with specimens from as many subpopulations as possible) seems preferable in the case of *A. preauxiana* on the grounds that they might allow the habitat isolates to retrieve from eventual inbreeding depression and increase their diversity levels through the introduction of a new stock of genetic variation. Although we would expect the artificially increased levels of intra-population variation to slump downwards in a few generations (i.e. the reinforced subpopulations will in general keep being small and, therefore, some of the new variation is liable to be lost by genetic drift), recombination is also bound to produce new genotypes that may have a higher selective value. Hence, a mixed reinforcement of the isolates of *A. preauxiana* would probably ensure the survival of the species through alleviating demographic stochasticity and increasing the global levels of genetic variation.

Since mixed reinforcements will usually give rise to interpopulation crosses, the assessment of the costs and benefits of intraspecific hybridization is a major concern. Admittedly, if the genetic differentiation detected in *A. preauxiana* was due to an underlying action of natural selection, a mixed reinforcement strategy might also bring about outbreeding depression (OBD, i.e. the decrease in the average fitness of the subpopulation as a consequence of the breakage of co-adapted gene complexes, Templeton 1986). Although our genetic and biological evidence does not suggest adaptation in the species, RAPDs are not known to be correlated with any fitness-related trait and it would be highly advisable to design a program of controlled crosses to assess whether OBD could pose a risk to the proposed mixed reintroductions previous to carrying

them out. Comparing the descendants of experimental crosses between the different population pairs with those of control crosses within each population could allow us to detect if there are inter-population crosses that induce a decline in fitness below the midparent [the most used comparative metric to gauge OBD, see Erickson and Fenster (2006) or Edmands (2007)]. If these cases were detected, then it would be advisable not to mix the corresponding populations. However, components of OBD can be expressed at late stages in F1 (e.g. upon outplanting, as shown in *Lotus scoparius* by Montalvo and Ellstrand 2001), appear several generations after F1 [as detected in *Chamaecrista fasciculata* by Fenster and Galloway (2000a, b)], or happen only in one of the habitats. Any of these factors might make it difficult to identify a problem readily, and the smallest subpopulations could go extinct in the meantime. Therefore, in the populations that are on the verge of extinction, it may be best to just make the mixed reinforcements as soon as possible, independent of carrying out a program of crosses.

Given that natural gene flow between subpopulations of *A. preauxiana* from Gran Canaria and Tenerife is highly unlikely, we consider it preferable to implement reinforcements and reintroductions with subpopulations from the same island, lest we might disrupt co-adapted gene complexes. Since some of the best known cases of OBD in plants are driven by disrupted interactions between genes and environment (e.g. Waser and Price 1994), we should also select the donor populations within areas environmentally similar to the reinforced ones, as indicated below.

It is worth stressing that precisely because every local extinction jeopardizes further the survival possibilities of *A. preauxiana*, all its subpopulations should be reinforced in order to prevent the advance of the extinction ratchet. Within this context of generalized urgency, in situ actions should preferably target the subpopulations that are the most ailing (and thus bound to become extinct in a shorter time) and in the areas of distribution that manifest more dramatically the consequences of habitat degradation. The subpopulations from Gran Canaria are in general bigger, exhibit a much lesser degree of fragmentation, and they occupy about 95% of the total species' distribution area. By contrast, subpopulations from Tenerife are more severely fragmented and contain a much higher proportion of adult, senescent or dead individuals (Rodríguez-Delgado et al. 2004).

Implementation of the suggested conservation strategy seems thus of utmost importance in Tenerife, and is additionally urged by the imminent construction of an industrial port in Granadilla de Abona and an urbanization in Las Heras, which doom the subpopulations from these areas (PIGT in Granadilla, and ERST and ERFT in Las Heras) to fast disappearance. In the context discussed, preventing the extinction of these subpopulations increases the chances to avoid other possible subpopulation extinctions of *A. preauxiana* in the near future. Thus, the individuals of these subpopulations should ideally be translocated to areas near their original locations. We believe that an ecologically suitable receptor site for ERFT and ERST would be the Tabaibal de Porís and, for PIGT, the area around Montaña Pelada, which neighbours a protected natural space. Parallel to these three necessary translocations and to the suggested mixed reinforcement strategy, it would also be advisable to make several reintroductions in order to enhance connectivity further and facilitate interpopulation gene flow in the future.

Heeding the genetic suggestions given in this paper might help overcome successfully the deleterious effects induced by the extinction ratchet that affects *A. preauxiana*. However, in order for these basic guidelines to be effective, they should be accompanied by legal measures aimed at halting and preventing further habitat degradation in the subpopulations that are not included in protected natural areas.

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