

A review of the allozyme data set for the Canarian endemic flora: causes of the high genetic diversity levels and implications for conservation

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- **Background and Aims** Allozyme and reproductive data sets for the Canarian flora are updated in order to assess how the present levels and structuring of genetic variation have been influenced by the abiotic island traits and by phylogenetically determined biotic traits of the corresponding taxa; and in order to suggest conservation guidelines.
- **Methods** Kruskal–Wallis tests are conducted to assess the relationships of 27 variables with genetic diversity (estimated by A , P , H_o and H_e) and structuring (G_{ST}) of 123 taxa representing 309 populations and 16 families. Multiple linear regression analyses (MLRAs) are carried out to determine the relative influence of the less correlated significant abiotic and biotic factors on the genetic diversity levels.
- **Key Results and Conclusions** The interactions between biotic features of the colonizing taxa and the abiotic island features drive plant diversification in the Canarian flora. However, the lower weight of closeness to the mainland than of (respectively) high basic chromosome number, partial or total self-incompatibility and polyploidy in the MLRAs indicates substantial phylogenetic constraint; the importance of a high chromosome number is feasibly due to the generation of a larger number of linkage groups, which increase gametic and genotypic diversity. Genetic structure is also more influenced by biotic factors (long-range seed dispersal, basic chromosome number and partial or total self-incompatibility) than by distance to the mainland. Conservation-wise, genetic structure estimates (F_{ST}/G_{ST}) only reflect endangerment under intensive population sampling designs, and neutral genetic variation levels do not directly relate to threat status or to small population sizes. Habitat protection is emphasized, but the results suggest the need for urgent implementation of elementary reproductive studies in all cases, and for *ex situ* conservation measures for the most endangered taxa, even without prior studies. In non-endangered endemics, multidisciplinary research is needed before suggesting case-specific conservation strategies. The molecular information relevant for conservation should be conserved in a standardized format to facilitate further insight.

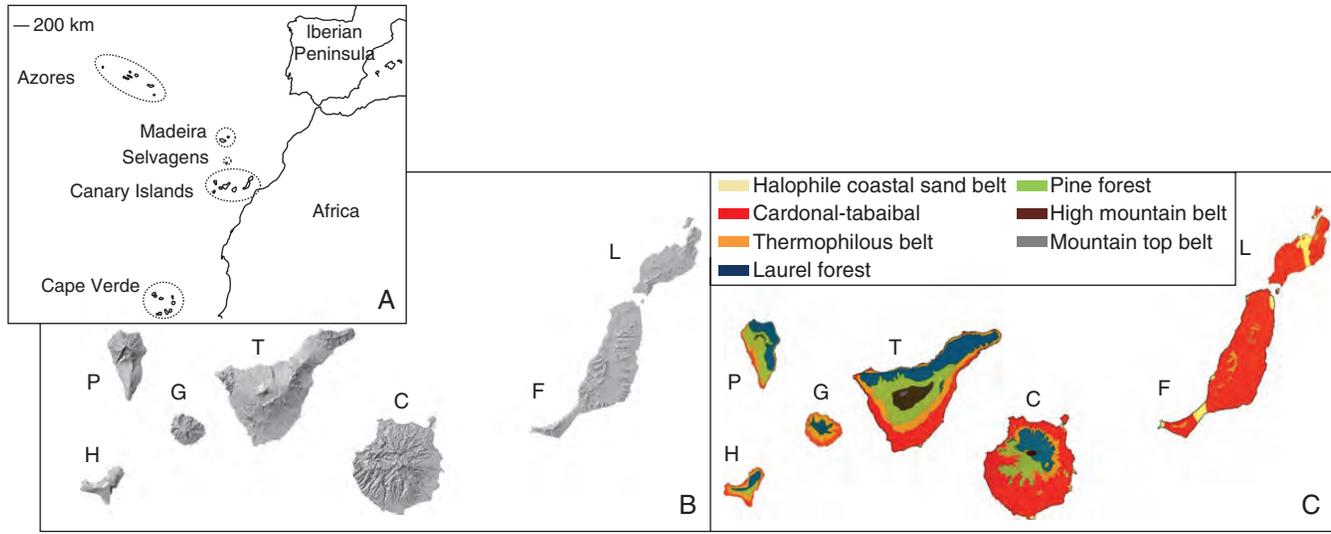
Key words: Canary Islands, allozymes, genetic diversity, conservation, meta-analysis, biotic features, abiotic factors.

INTRODUCTION

The Canarian archipelago is an oceanic insular hotspot situated west of the African continent, between 27–29 °N and 13–18 °W. Unlike many other such enclaves, it is close to the nearest mainland (Africa, see Fig. 1), it has a considerable geological antiquity (Carracedo *et al.*, 2002) and it was relatively stable climatically during Quaternary glacial/interglacial periods, according to palaeo-climatic data (e.g. Rodríguez-Sánchez and Arroyo, 2008). The prolonged interaction of these unique geo-climatic characteristics with the changes in the insular landscape through geological time (Whittaker *et al.*, 2008) has generated a highly diverse flora. Both relict taxa, the closest congeners of which are no longer extant in the mainland (Bramwell, 1976; Vargas, 2007), and more modern taxa generated by recent isolation in the different ecological and topographical zones shaped by the ruggedness of the terrain coexist in the current endemic flora, estimated to consist of approx. 610 species (Martín-Esquível *et al.*, 2001; A. Santos-Guerra, unpubl. data). The naturally and severely

fragmented geography of most islands has given rise to a rich island-exclusive diversity (an estimated 399 single-island endemics, Fig. 1), but it also impedes the thorough botanical exploration of many areas. Therefore, the currently recognized number of endemics in the Canarian terrestrial flora is probably an underestimate, and further exploration is still needed for an accurate census.

Similarly, the causes of the high genetic diversity in the endemic flora of the archipelago need to be better understood. Allozymes, intersimple sequence repeats (ISSRs), nuclear microsatellites, random amplified polymorphic DNAs (RAPDs) and amplified fragment length polymorphisms (AFLPs) have provided the most relevant insights until now (see fig. 12.3 in Caujapé-Castells, 2011). Allozymes are by far the most abundant source of data, and several investigations have used these markers to understand the population genetic variation levels and structuring of many endemic lineages and to propose informed guidelines to mitigate the main factors that threaten them (e.g. Francisco-Ortega *et al.*, 2000; Batista *et al.*, 2002; Fernández-Palacios *et al.*, 2006;



Island characteristics	ISLAND						
	H	P	G	T	C	F	L
Maximum elevation (m)	1503	2426	1485	3718	1949	807	670
Area (km ²)	287	708	370	2034	1560	1661	846
Complexity (number of conspicuous ravines)	Low	Medium	High	Medium	High	Low	Low
Island age (My)	1.12	2	12	7.5	14.5	20.6	15.5
Minimum distance to mainland (km)	375	350	310	275	190	90	100
Potential vegetation zones (see map in 'C')	4	6	5	7	6	3	3
Number of exclusive endemic plant species	14	39	42	122	81	12	14
Total number of bird species	47	44	50	60	61	49	41
Total number of insect species	1038	2072	1722	3928	2604	1316	1099

FIG. 1. Physical–biological maps of the Canary Islands, and values of the island-dependent variables tested. (A) Geographical location of the Canary Islands in the Macaronesian hotspot; (B) geographical map of the islands; and (C) main potential vegetation zones, modified from Del Arco-Aguilar and Rodríguez-Delgado (2000). Single-letter island abbreviations are used throughout the paper and correspond to: C, Gran Canaria; F, Fuerteventura; G, La Gomera; H, El Hierro; L, Lanzarote; P, La Palma; and T, Tenerife. Values of maximum height, island area and number of potential vegetation zones (slightly modified following Sunding, 1972) were taken from Del Arco-Aguilar and Rodríguez-Delgado (2000); maximum island age was taken from Carracedo *et al.* (2002); the number of exclusive plant endemics, total number of endemic species, total number of bird species and total number of insect species are from Martín *et al.* (2001).

Suárez-García *et al.*, 2009). However, only Francisco-Ortega *et al.* (2000) (FOEA hereafter) reviewed the allozyme genetic variation of Canarian plant endemics to propose hypotheses about the origins of the high genetic variation levels in this flora, and to provide conservation indications. According to FOEA, the high genetic diversity levels of the Canarian flora relative to most other oceanic islands could be explained by a combination of (1) the great geological age of the islands and (2) gene flow from nearby mainland areas. Based on FOEA and their own results, Crawford *et al.* (2001) contended that the close proximity of the Canaries to the mainland should determine a higher probability of multiple introductions, which would alleviate the genetic bottlenecks associated with founder events and result in higher overall genetic diversity; however, they also suggested that this hypothesis could be unlikely because molecular phylogenetic studies suggest a monophyletic origin of most Canarian endemics investigated (FOEA). Crawford *et al.* (2001, 2011) also

considered a higher incidence of interspecific hybridization and pseudo-self-incompatible colonizers with mixed mating systems as a potential source of genetic diversity in the Canarian flora. Nevertheless, these important suggestions could not be tested with the limited data set that FOEA used. Importantly, and owing to the much lower level of knowledge of some reproductive characteristics at that time, FOEA did not examine the influence of other abiotic and biotic factors on genetic diversity levels and structuring of the Canarian flora.

Some researchers (e.g. Reed and Frankham, 2001; Iriando *et al.*, 2008; Freeland *et al.*, 2010) contended that only markers subjected to selection should be used in evolutionary studies. However, Leinonen *et al.* (2008) convincingly showed that neutral markers may accurately predict quantitative genetic structuring (Q_{ST}), and therefore be useful for analysing differentiation processes. Assuming that the genetic parameters in the independent data sources are sufficiently

comparable to allow an objective appraisal of evidence, our main aim in this investigation is to use the updated allozyme data set for the Canarian endemic terrestrial flora to assess how the abiotic characteristics of the different Canary Islands (the island-dependent selective pressures) and the biotic traits (reproductive and other) of the plant endemics (determined by the phylogenetic history of the colonizing taxa) have interacted to generate the present levels and structuring of genetic variation in the current flora. As an important subsidiary milestone to achieve this main objective, we will update the estimates of levels and apportionment of genetic diversity in this flora. Because of the higher fragility and diversity inherent to the floras of oceanic insular hotspots like the Canaries (Caujapé-Castells *et al.*, 2010), we will also use our results to suggest conservation guidelines that address the urgent multidisciplinary targets set in the reformulated Global Strategy for Plant Conservation 2011–2020 (GSPC hereafter; see <http://www.cbd.int/gspc/gspcpreview/>).

For the sake of brevity, we refrain from comparing our results with those in other oceanic archipelagos, as this will be the focal subject of a subsequent collaborative paper.

MATERIALS AND METHODS

The Canary Islands

The Canarian archipelago consists of seven major oceanic islands that belong to Spain, though they are geographically situated near mainland Africa, with the easternmost and oldest island (Fuerteventura) some 96 km off the Atlantic coast of Morocco. Figure 1 synthesizes the most relevant physical–geographical traits of the Canary Islands, with some of their general biological features. All these were used as variables in our analyses.

The allozyme data set for the Canarian endemic flora. Barely a decade after the review by FOEA, the allozyme data set for the terrestrial flora of the archipelago consists of data for 623 populations that represent 123 taxa from 104 species (vs. 69 species considered in FOEA), 33 genera (vs. 18 in FOEA) and 16 families (vs. eight in FOEA). For all the tests concerning lineage-dependent biotic factors, we used the averages of genetic diversity indicators for the 123 taxa in the data set (Annex 1 in http://demiurge-project.org/matrix_digests/15). To test the island-dependent abiotic factors, we used a matrix with the averages per taxon and island of distribution (132 entries, see Annex 2 in http://demiurge-project.org/matrix_digests/15). Many of the genotype matrices generated at the Jardín Botánico Canario ‘Viera y Clavijo’ – Unidad Asociada CSIC (JBCVCSIC hereafter), and ancillary information associated with them, are permanently deposited as ‘genetic diversity digests’ in the free public information system Demiurge (search ‘allozymes’ in <http://demiurge-project.org/>) in the data standard provided by the software Transformer-4 (T4 hereafter).

The biotic features of the Canarian endemic flora

A variety of sources were used to associate the taxa included in this survey with relevant biotic data (see the document by Pérez de Paz *et al.*, in http://demiurge-project.org/matrix_digests/15). Since reproductive data are essential for understanding genetic

variation levels, the current knowledge of this aspect for the Canarian flora has been updated based both on (1) a thorough review of the available literature; and (2) empirical research conducted over the last decades by the ‘Departamento de Biología Reproductiva y Micromorfología’ at the JBCVCSIC of the Cabildo de Gran Canaria (most of it in the process of publication).

Overall, the biotic traits considered include those evaluated by Hamrick and Godt (1996), Weller *et al.* (1996), Gitzendanner and Soltis (2000) and Crawford *et al.* (2001). To minimize the use of redundant variables in assessing the effect of the breeding system (auto/xenogamy and mixed mating systems) on genetic diversity, we only tested its two main components: self-incompatibility (total or partial) and sexual system (hermaphroditism, sexual dimorphism and dioecy). Although these variables can be somewhat redundant with pollination and seed dispersal, they are complementarily related to gene flow and genetic isolation (G_{ST}/F_{ST}). Other variables considered relevant were chromosome numbers [see Hamrick *et al.* (1979) or Pérez de Paz *et al.* (2007) for the Canary Islands], dispersal vectors including diplochory (i.e. both long- and short-distance seed dispersal in the same plant due to the action of two or more dispersal vectors) or the different sampling strategies used (Caujapé-Castells, 2010).

Data analysis

Genetic diversity levels per taxon were estimated by the mean number of alleles per locus (A), the proportion of polymorphic loci (P), and expected and observed heterozygosity (H_e and H_o , respectively). The apportionment of genetic variation among populations was measured indistinctly by G_{ST} (Nei, 1973) or F_{ST} (Wright, 1951), depending on the parameter used in the corresponding data source. The differences in the values of genetic parameters among groups of biotic and abiotic variables were in all cases tested using non-parametric Kruskal–Wallis tests (Kruskal and Wallis, 1952).

Due to the incompleteness and the heterogeneous limitations of the different data sources, sample sizes vary within the same group depending on the genetic parameter tested. The structuring of genetic variation as measured by the values of G_{ST} or F_{ST} could only be tested for some biotic and abiotic variables.

We conducted multiple linear regression analyses (MLRAs) with the less correlated biotic and abiotic variables for which Kruskal–Wallis tests showed significant differences to (1) select the biotic and abiotic variables that best explain the levels of genetic variation and structure of the Canarian flora; and (2) determine the relative importance of each selected variable. The relative support for each regression model was estimated using Akaike’s information criterion (AIC; Akaike, 1974), the preferred model being the one with the minimum AIC value (Akaike, 1974). For the variable ‘distance to mainland’ in the MLRAs with G_{ST} , we used the values for taxa distributed in one or several islands from (a) only the easternmost group (Fuerteventura and Lanzarote); (b) only Gran Canaria; and (c) the westernmost group (Tenerife, La Gomera, La Palma and El Hierro). See the column ‘Islands’ in Annex 1 of http://demiurge-project.org/matrix_digests/15 for details.

RESULTS

The average genetic variation levels of the Canarian endemic flora ($A = 1.781$) were even higher than those in the taxa assessed by FOEA ($A = 1.659$), and the contribution of the taxa newly added to the allozyme data set was highly significant with respect to the data set used by FOEA ($A = 1.923$ vs. $A = 1.659$, P -value < 0.001 , respectively). Intensive sampling always resulted in significantly higher genetic diversity levels than extensive sampling (see ‘Miscellaneous variables’ in Table 1).

Closeness to the mainland and both a few and many altitudinal ecological zones were the island-dependent abiotic factors that most significantly influenced high population genetic variation levels in the plant groups examined (Table 1). The biotic factors that most significantly determined higher levels of genetic variation (see Table 1) were mostly intrinsic (absence of vegetative reproduction, total or partial self-incompatibility, xenogamy and mixed mating system), high basic chromosome number, polyploidy, and both intrinsic and extrinsic (diplochory with both abiotic and biotic seed dispersal vectors, and taxa on islands with a high number of bird species recorded). Taxa with generally large population sizes had significantly higher genetic diversity levels than taxa with lower population sizes (Table 1). Taxa of genera in which there have been no significant radiations on the Canaries (genera with ≤ 2 endemic taxa) tended to have higher genetic variation and less genetic isolation (G_{ST}/F_{ST}) than those that experienced radiation. DNA content was inversely related to the levels of population genetic variation. Taxa with some degree of threat (CR + VU + EN) were significantly less genetically variable than non-threatened taxa, but they also tended to be (non-significantly) less genetically isolated (Table 1).

The degree of genetic isolation as measured by G_{ST} or F_{ST} was non-significantly lower in the updated allozyme data set ($G_{ST} = 0.245$ overall, $G_{ST} = 0.231$ for the taxa reviewed here for the first time) than that given by FOEA ($G_{ST} = 0.281$). The value of G_{ST} was significantly higher just for taxa with (1) absence of vegetative reproduction; (2) long-distance seed dispersal; (3) generally small/intermediate population sizes; and (4) > 2 endemic taxa per genus (‘radiating genera’ in Table 1).

Based on Table 1 and on correlation analyses (not shown), the less correlated variables used for the MLRAs were the basic chromosome number, ploidy, self-incompatibility, seed dispersal distance, distance to the mainland and population size. Population size was excluded from the MLRAs because it may be influenced by combinations of other variables. According to this regression analysis (Table 2, Fig. 2), a high basic chromosome number, total or partial self-incompatibility, a shorter distance to the mainland and polyploidy were, respectively, the factors that most influence the generation of the high levels of allozyme genetic variation detected in the Canarian flora. The variables that most influenced genetic isolation as measured by G_{ST} were, respectively, long-distance seed dispersal, low base chromosome number, self-compatibility and a greater distance to the mainland (Tables 1 and 2, Fig. 2).

DISCUSSION

Abiotic factors and genetic variation

Abiotic factors are important to explain the levels and structuring of genetic diversity on islands, because some of them (e.g. a short distance to the mainland) may increase the probability and frequency of propagule arrival. After arrival, other abiotic factors (e.g. natural barriers and habitat diversity, or absence thereof) may also further facilitate or impede gene flow within the islands. The effects of all these factors on genetic diversity are also closely associated with the lineage-dependent biotic traits of the colonizing taxa (see below).

FOEA proposed that gene flow with the mainland and island age may be directly related to the high allozyme diversity levels detected in the Canarian endemic flora, a suggestion that remained untested because of various limitations of their data set. With the current data set, it is impossible to assess if the levels of gene flow with the mainland have been high, especially because only a few investigations provide comparisons with mainland congeners [e.g. *Argyranthemum* (Asteraceae), Francisco-Ortega *et al.* (1995); *Androcymbium* (Colchicaceae), Pedrola-Monfort and Caujapé-Castells (1996); *Olea guanchica* (Oleaceae), Lumaret *et al.* (2004)]. Nonetheless, our tests indirectly bolster this possibility by showing that closeness to the mainland does yield significantly higher genetic diversity levels in all the parameters tested (Tables 1 and 2, Fig. 2). The influence of this factor may be further accentuated by habitat complexity, as taxa on islands with many altitudinal vegetation zones also have higher diversity levels (Table 1).

The hybrid swarm theory predicts that multiple colonizations of closely related taxa will promote both hybridization and adaptive radiation on oceanic islands (Carlquist, 1966; Seehausen, 2004). Similarly, the main tenet of the ‘surfing syngameon’ hypothesis (Caujapé-Castells, 2011) is that multiple colonizations of phylogenetically close individuals to the older, easternmost Canaries (F, L, C in Fig. 1) created genetic diversity sinks on these islands that, in some lineages, may have facilitated subsequent colonization and diversification in the more topographically and ecologically complex westernmost islands (T, G, P, H in Fig. 1). Genetic admixture giving rise to high genetic variation levels in the easternmost islands is bound to have been additionally promoted by the relatively shallow relief of most areas in Fuerteventura, Lanzarote and the eastern side of Gran Canaria, by the ecological uniformity of these enclaves and by their similarity to the mainland [see, for example, Lambrinos (2001) or Seehausen (2004) for other contexts].

Although this hypothesis would conflict with estimates of low colonization rates from the mainland to the eastern islands by Sanmartín *et al.* (2008), these authors also conceded that extinction may have severely warped their results. Furthermore, the allozyme data bolster the surfing syngameon scenario by pinpointing significantly smaller genetic variation levels and higher genetic isolation in radiating plant genera, with most of their species concentrated in the westernmost islands (Table 1).

TABLE 1. *Kruskal–Wallis non-parametric test results for the biotic and abiotic variables considered, and categories tested (below the variables, when needed). Only taxon-level tests are shown for the basic genetic parameters (A, P, H_o, H_e, G_{ST}/F_{ST}) that have a value in Annex 1 of Caujapé-Castells and Pérez de Paz (2011)*

Variables and categories tested	A	P	H _o	H _e	G _{ST} /F _{ST}
REPRODUCTIVE BIOLOGY RELATED VARIABLES					
Vegetative reproduction	1-514, 1-845*	0-338, 0-474*	0-164, 0-143	0-134, 0-177*	0-106, 0-274**
Taxa with vegetative and sexual reproduction vs. only sexual	21, 89	21, 102	18, 73	19, 102	15, 68
Self-incompatibility [†]	1-306, 1-875***	0-164, 0-507***	0-091, 0-152	0-105, 0-183***	0-360, 0-230
Self-compatible vs. partially and totally self-incompatible taxa	18, 92	20, 103	7, 84	20, 101	9, 74
Sexual systems	1-804, 1-822, 1-351	0-447, 0-542, 0-287	0-153, 0-137, 0-109	0-170, 0-194, 0-113	0-259, 0-177, 0-250
Hermaphroditism vs. sexual dimorphism vs. dioecy or subdioecy	89, 15, 6	102, 15, 6	70, 15, 6	100, 15, 6	63, 15, 5
Basic chromosome number [†]	1-596, 2-132***	0-397, 0-570*	0-117, 0-242***	0-145, 0-225***	0-264, 0-187
Small (X ≤ 10) vs. great (X > 10)	72, 38	85, 38	69, 22	83, 38	61, 22
Ploidy level [†]	1-701, 2-191*	0-421, 0-615*	0-139, 0-201*	0-157, 0-242**	0-267, 0-120*
Diploid vs. polyploid	92, 18	104, 19	79, 12	103, 18	70, 13
1C DNA amount	1-813, 1-598	0-454, 0-427	0-150, 0-133	0-172, 0-153	0-257, 0-231
Small (≤3 pg) vs. great (>3 pg)	94, 16	107, 16	76, 15	105, 16	67, 13
Pollination range	1-794, 1-647	0-451, 0-448	0-141, 0-187	0-171, 0-160	0-255, 0-148
Short (entomogamous taxa) vs. long (anemo + ornithogamous taxa)	96, 14	109, 14	79, 12	109, 12	74, 9
Seed-dispersal distance [†]	1-688, 1-698, 1-894 *	0-401, 0-439, 0-484***	0-115, 0-119, 0-191 ***	0-157, 0-149, 0-198 **	0-077, (0-310, 0-238) ***
Only short vs. only long vs. both (diplochory)	59, 103, 141	19, 56, 48	19, 35, 37	19, 54, 48	14, 38, 31
Seed dispersal agents	1-511, (2-191, 1-906)***	0-359, (0-656, 0-499)**	0-106, 0-155, 0-191***	0-134, (0-229, 0-195)***	0-295, 0-174, 0-215
Abiotic vs. biotic vs. both	44, 13, 53	57, 13, 53	44, 7, 40	57, 13, 51	36, 12, 35
Growth habit	1-810, 1-747	0-455, 0-447	0-156, 0-139	0-179, 0-161	0-250, 0-238
Phanerophytes vs. terophytes + chamephytes + hemicryptophytes	60, 50	60, 63	44, 47	60, 61	39, 44
OTHER BIOLOGICAL VARIABLES					
Population sizes [†]	1-456, 2-107***	0-327, 0-599***	0-102, 0-192***	0-137, 0-211***	0-306, 0-196**
Small/intermediate (≤500 individuals) vs. large (>500 individuals)	55, 55	67, 56	45, 46	67, 54	36, 47
Populations known per island of occurrence	1-711, 1-876	0-402, 0-520*	0-152, 0-141	0-167, 0-175	0-251, 0-237
A few (≤10) vs. many (>10)	63, 47	72, 51	53, 38	72, 49	40, 43
Non-radiating vs. radiating genera	2-025, 1-686*	0-581, 0-407*	0-191, 0-133*	0-206, 0-158*	0-159, 0-284*
≤2 vs. >2 Canary endemic species per genus	31, 79	31, 92	22, 69	29, 92	27, 56
Insular distribution range	1-782, 1-781	0-427, 0-490	0-141, 0-157	0-167, 0-175	0-230, 0-260
Single-island vs. multiple-island endemics	68, 42	77, 46	55, 36	77, 44	44, 39
Threat status	1-946, 1-629**	0-5531 0-364**	0-172, 0-124	0-185, 0-154	0-267, 0-209
Not threatened vs. some degree of threat (VU + EN + CR)	53, 57	64, 59	45, 46	62, 59	49, 34
ISLAND-DEPENDENT VARIABLES					
Island age	1-854, 1-681	0-501, 0-396*	0-151, 0-129	0-185, 0-158	-
Older (GCFL) vs. younger (TPH)	77,49	76, 54	69, 36	76, 54	-
Distance to the mainland [†]	1-815, 2-058, 1-595**	0-551, 0-577, 0-373***	0-187, 0-180, 0-117*	0-217, 0-206, 0-146**	0-223, 0-193, 0-284
Closer (FL), mean (C) vs. farther (TGPH)	7, 41, 62	7, 41, 75	6, 37, 48	7, 40, 74	7, 32, 44
Topographic complexity (number of ravines)	1-812, 1-675, 1-861	0-494, 0-392, 0-499	0-175, 0-130, 0-143	0-191, 0-160, 0-180	-
Lower (HFL) vs. moderate (PT) vs. higher (CG)	17, 46, 63	18, 50, 62	14, 34, 57	18, 50, 62	-
Ecological complexity (potential altitudinal vegetation zones)	1-822, (1-492), 1-849*	0-508, 0-342, 0-476	0-188, (0-102), 0-146**	0-206, (0-126), 0-180*	-
A few (FL) vs. some (GH) vs. many (TPC)	14, 21, 91	14, 21, 95	12, 18, 75	14, 22, 94	-

Continued

TABLE 1. Continued

Variables and categories tested	A	P	H _o	H _e	G _{ST} /F _{ST}
Number of bird species censused per island Lower (PGHFL) vs. higher (CT)	1-643, 1-894* 54, 72	0-402, 0-498* 55, 75	0-132, 0-152 43, 62	0-156, 0-187* 56, 74	-
Number of reptile species censused per island Lower (GFLP) vs. higher (TCH)	1-670, 1-866 51, 75	0-405, 0-485 51, 79	0-137, 0-147 41, 64	0-164, 0-180 52, 78	-
Number of insect species censused per island Lower (HGLF) vs. higher (TPC)	1-627, 1-846* 34, 92	0-408, 0-475 34, 96	0-138, 0-146 29,762	0-157, 0-180 35, 95	-
Island biotic density Lower (HFL) vs. higher (PGCT)	1-812, 1-783 17, 109	0-494, 0-452 18, 112	0-175, 0-138 14, 91	0-191, 0-171 18, 112	-
MISCELLANEOUS VARIABLES					
Population sampling	1-972, 1-623*** 50, 60	0-549, 0-384*** 50, 73	0-179, 0-109*** 50, 41	0-205, 0-145*** 50, 71	0-206, 0-277 39, 44
Intensive vs. extensive	1-659, 1-923** 59, 51	0-396, 0-502* 59, 64	0-117, 0-171* 40, 51	0-153, 0-185* 57, 64	0-258, 0-231 38, 45
Data set					
Taxa included in FOEA vs. only in this review					

Integer numbers below the test results are the corresponding sample sizes per category and genetic parameter. A hyphen means that the corresponding variable could not be estimated or tested. Island single-letter codes correspond to those in Fig. 1. The values of the categories for each taxon are based on Fig. 1, and on the genetic diversity digest at http://demiurge-project.org/matrix_digests/15 (see Annexes 1 and 2 on this site, and Pérez de Paz *et al.*, 2012 for biological traits).

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

[†]Variables used in multiple linear regression analyses (see the Materials and Methods and Fig. 2).

TABLE 2. Correlations (R^2) and values of the Akaike information criterion (AIC) for the MLRAs for the six variables and the three genetic parameters chosen (see Fig. 2 for the standardized values)

Parameter/model	Variables	R^2	R^2 (adjusted)	AIC
A				
1	X	0-181	0-143	-131-136
2	X/SI	0-468	0-443	-176-447
3	X/SI/DM	0-548	0-527	-192-588
4*	X/PL/SI/DM	0-603	0-584	-204-670
5	X/PL/SI/DD/DM	0-603	0-584	-202-732
H _o				
1	X	0-149	0-101	-370-744
2	X/PL	0-207	0-162	-375-220
3*	X/PL/DM	0-262	0-220	-379-752
4	X/PL/DD/DM	0-281	0-239	-380-073
5	X/PL/SI/DD/DM	0-288	0-246	-379-052
H _e				
1	X	0-163	0-127	-594-565
2	X/SI	0-383	0-357	-629-504
3	X/SI/DM	0-460	0-437	-643-726
4*	X/PL/SI/DM	0-527	0-506	-657-579
5	X/PL/SI/DD/DM	0-537	0-517	-658-253
G _{ST}				
1	PL	0-063	0-005	-258-445
2	PL/DM	0-097	0-040	-259-509
3	X/SI/DD	0-132	0-077	-260-844
4*	X/SI/DD/DM	0-177	0-125	-263-279
5	X/PL/SI/DD/DM	0-189	0-136	-262-427

The best regression model according to the AIC is labelled with an asterisk after the corresponding number of variables. The coding of the variable states used for this analysis were as follows: X = basic chromosome number (1, $X \leq 10$; 2, $X > 10$), PL = ploidy level (1, diploid; 2, polyploid), SI = partial or total self-incompatibility system (1, present; 0, absent), DD = seed dispersal distance (1, short; 2, long; 3, both or diplochory), DM = distance to the mainland (1, closer or taxa distributed only in F, L; 2, mean or taxa distributed only in C; 3, farther or taxa in T, G, P, H).

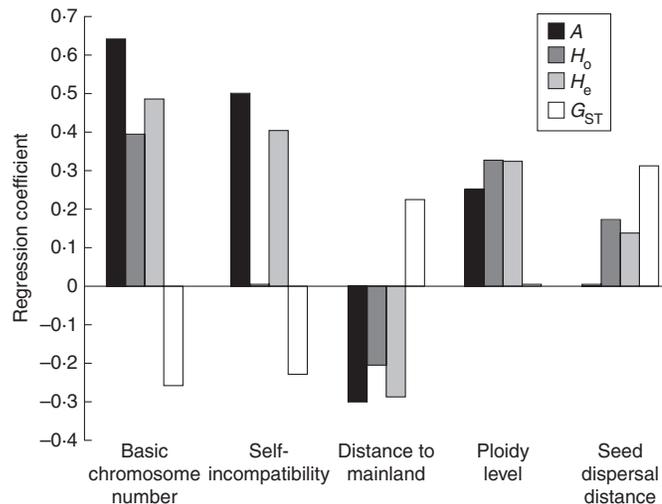


FIG. 2. Graphical representation of the standardized regression coefficients associated with each of the five variables subjected to the MLRAs (see the Methods and Table 2 for variable selection and coding). We selected only the parameters A, H_o, H_e and G_{ST} because of the extremely high correlation between A and the proportion of polymorphic loci ($R^2 = 0.923$, $P < 0.0001$).

Other authors have stated that admixture possibly influenced the generation of the high genetic variation in the Canarian flora (Herbén *et al.*, 2005; Saunders and Gibson, 2005); however, none of them emphasized the possible role of the easternmost islands in hosting these phenomena. The surfing syngameon hypothesis is compatible with the prevalent monophyly detected in molecular phylogenetic studies of Canarian groups, as recent studies (e.g. Currat *et al.*, 2008; Petit and Excoffier, 2009) emphasize a major role for enhanced gene flow in the maintenance of species cohesion, and in promoting rapid monophyly. Furthermore, it may be related to the hypothesis of anagenetic evolution of island plants (Stuessy *et al.*, 2006; Takayama *et al.*, 2012), whereby initial founder populations proliferate, and accumulate genetic variation through time by mutation and recombination, especially on low-elevation islands with little habitat heterogeneity. In the Canaries, the islands with both a few and many vegetation zones have significantly higher levels of allozyme genetic diversity than the islands with a moderate number of vegetation zones (see Table 1). This latter result is compatible with the hypotheses of both Sanmartín *et al.* (2008) and Caujapé-Castells (2011), and highlights that the current floristic make-up of the archipelago is the consequence of a broad diversity of colonization histories.

Biogeographical considerations notwithstanding, our tests unequivocally show that the distance to the mainland results in a genetic diversity discontinuity in the Canaries. If we consider each island as a ‘genetic diversity sink’ (similarly to Sanmartín *et al.*, 2008), the populations of taxa in the easternmost islands (Fuerteventura, Lanzarote and Gran Canaria) are significantly more diverse genetically than those in the westernmost islands. Thus, it seems genetically tenable to hypothesize a general subdivision between the easternmost and westernmost Canary islands, rather than the obvious geographical discontinuity between the islands and the mainland. Some recent works have further pointed out that gene flow between the mainland and the easternmost islands may be higher than between the easternmost and westernmost islands [e.g. García-Verdugo *et al.* (2009) with *Olea guanchica* (as *O. europaea* subsp. *Guanchica*), Oleaceae]. However, the low number of populations from Lanzarote and El Hierro may affect these results, and more investigations encompassing samples of insular endemics and mainland congeners are needed to test the generality of this pattern.

Biotic factors and genetic variation. I. Colonization of the islands and seed dispersal

Lineage-dependent biotic traits of Canarian taxa are important to explain the levels and structuring of population genetic diversity on islands, because many of these (especially reproductive features) determine the successful establishment of colonizing taxa, and they may also further facilitate or impede gene flow on and among islands, with some of the mentioned abiotic factors.

It is difficult to reconstruct the arrival and dispersal mode of oceanic island plant colonizers. However, in the first phase of insular colonization (on arrival at the islands), the adaptations that favour long-range dispersal are generally more important to generate genetic variation than the suitability of the

insular habitats to host the colonizers, or other biotic traits (Carlquist, 1966; Crawford *et al.*, 2011). The result that Canarian endemic plants with long-distance seed dispersal have significantly higher allozyme variation levels (Table 1) is consistent with this prediction. Thus, the importance of long-distance seed dispersal also applies in the Canarian archipelago [as noted by Nogales *et al.* (2012) and Vargas *et al.* (2012)], despite its much lower minimum distance to the mainland than other archipelagos.

Recent data for the Canarian flora (Valido and Olesen, 2007; Nogales *et al.*, 2012; Padilla *et al.*, 2012) show that the predatory birds that eat frugivorous lizards can also act as dispersers of the seeds eaten by the lizards, yielding a complex dispersal process known as diplochory. Diplochory is a much more common mechanism of oceanic island dispersal than it was hitherto assumed, and it pinpoints a significant gap concerning the knowledge of dispersal syndromes in the Canarian flora. Nogales *et al.* (2012) and Vargas *et al.* (2012) further suggested that (1) diplochory would be extensive to Canarian taxa with no particular seed dispersal syndrome (dry fruit) and two or more seed dispersers (e.g. wind and granivorous birds or other vertebrates); and (2) diplochory provides a much underestimated alternative to seed colonization of oceanic islands and between islands.

Notably, our tests show that diplochorous taxa (see Pérez de Paz *et al.*, 2012) have significantly higher genetic diversity than those with just long- or short-range dispersal (Table 1). Thus, with the abiotic factors mentioned earlier, diplochory must have played a crucial role in explaining the high population genetic diversity of the Canarian flora. Within the taxa exhibiting long-distance dispersal and diplochory, those that have only biotic dispersal vectors or both biotic and abiotic vectors display significantly higher genetic connectivities ($G_{ST} = 0.141$ and $G_{ST} = 0.212$, respectively) than taxa with only abiotic dispersal vectors ($G_{ST} = 0.420$) represented by anemochory (data not shown), indicating that biotic dispersal vectors also promote higher gene flow on Canary island plant endemics.

Quite unexpectedly, long-distance seed dispersal and diplochory also entail a significantly much lower genetic connectivity among populations ($G_{ST} = 0.310$ and $G_{ST} = 0.238$, respectively) than short-distance seed dispersal ($G_{ST} = 0.077$) (Table 1). This could be due to combinations of various causes, namely: (1) historical factors favouring inter-over intransland connectivity, as the frequent extinction–recolonization cycles associated with volcanism that may foster population divergence due to drift (García-Verdugo *et al.*, 2009, 2010); (2) the prevalence of evolutionary forces that promote divergence (such as natural selection and mutation) over homogenizing gene flow of pollen and seeds (e.g. Freeland *et al.*, 2012); (3) gene flow among extant populations and the founding of new populations increasing and decreasing G_{ST} values, respectively; or (4) possible differences in the geographical scale over which the population samples were taken. Nevertheless, we concur with, for example, Jordano (2007) that we still lack consistent hypotheses to explain crucial aspects of plant seed dispersal, gene flow or genetic structure, despite the advances in the use of molecular markers. Thus, the current information gaps about dispersal processes in the Canarian flora also could have biased this result.

Biotic factors and genetic variation. II. Post-colonization establishment and diversification

After arrival on the islands, reproductive systems largely determine plant establishment success, affecting the levels and structuring of population genetic diversity and the evolutionary responses to environmental changes (Barrett *et al.*, 2008; Barrett, 2011). The biotic characteristics that most significantly contribute to generate the high genetic diversity levels detected in the Canarian flora are related to total or partial self-incompatibility (xenogamy including mixed mating system), and to a high chromosome number (Tables 1 and 2, Fig. 2).

Vegetative reproduction, breeding systems and Baker's law. Mechanisms of reproductive assurance in outcrossers (such as selfing or vegetative reproduction) help promote the reproductive success of colonizing taxa, especially when opportunities for the mating of different individuals are non-existent or scarce (Lloyd, 1992; Holsinger, 2000; Barrett, 2011; Busch and Delph, 2012; Levin, 2012).

Although vegetative reproduction does not contribute to higher genetic diversity, it promotes the successful establishment of plant colonizers and helps maintain genetic diversity levels. This trait (often associated with sexual reproduction) especially benefits island species and ensures reproduction against episodes of environmental stochasticity, which can be quite frequent in oceanic islands (Brown and Eckert, 2005; Silvertown, 2008; Franks, 2010). Vegetative reproduction also confers an advantage during the founding and early expansion phases, when population sizes are still small (Lambrinos, 2001; Lloret *et al.*, 2005). In this context, the higher genetic connectivity detected for taxa with vegetative reproduction ($G_{ST} = 0.104$) than for taxa with no vegetative reproduction ($G_{ST} = 0.277$) is best presumed to be the effect of gene flow promoted by the already discussed association with sexual reproduction, or of recent colonization (Brzosko *et al.*, 2002; García-Verdugo *et al.*, 2009).

Self-compatible Canarian taxa exhibit much lower genetic variation and higher isolation levels than total or partial self-incompatible or predominantly xenogamous taxa (Table 1). This result is similar to the findings in previous analyses in other enclaves (Hamrick *et al.*, 1979; Hamrick and Godt, 1996; Weller *et al.*, 1996; Gitzendanner and Soltis, 2000; Crawford *et al.*, 2001; Duminil *et al.*, 2007, 2009) and agrees with the predictions of FOEA.

As noted by Crawford *et al.* (2008, 2009), the abundance of self-incompatible taxa in the Canarian flora may be construed as an exception to Baker's law, which states that self-compatible colonizers would be more likely to establish sexually reproducing colonies after long-distance dispersal (Baker, 1955). However, Baker and Cox (1984) also emphasized that insular factors that impede mating may help trigger the development of leaky self-incompatibility, also known as partial or pseudo-self-compatibility (see Levin, 1996; Leimu, 2004; Ferrer and Good-Avila, 2007; Busch and Schoen, 2008) or mixed mating system (Goodwillie *et al.*, 2005; Raduski *et al.*, 2012), by which self-incompatible colonizers may develop self-pollination capabilities and give rise to viable offspring. According to these authors, the transition from self-incompatibility to partial or pseudo-self-compatibility is spontaneous, reversible and may be influenced by the environment.

In fact, pseudo-self-compatibility can be considered as another reproductive assurance mechanism that explains the exception to Baker's law commented on above, because a single propagule would presumably carry sufficient genetic variation to establish a sexual population that may facilitate diversification (Crawford *et al.*, 2011; Busch and Delph, 2012; Levin, 2012).

Crawford *et al.* (2008, 2009, 2011) suggest that *Tolpis* and other Canarian Asteraceae (e.g. *Argyranthemum* and *Sonchus*) may derive from a pseudo-self-compatible allogamous ancestor, in a similar manner to *Dubautia* in Hawaii (Carr *et al.*, 1986), *Dendroseris* in the Robinson Crusoe islands (Anderson *et al.*, 2001; Bernardello *et al.*, 2001) or *Scalesia* in the Galápagos (Nielsen *et al.*, 2003). Similarly, we believe that this could be the case in many pseudo-, partial or total self-incompatible Canarian endemic Brassicaceae such as *Parolinia* (Fernández Palacios, 2010) or *Lobularia* (Borgen, 1996), and in other families with total or partial self-incompatibility or mixed mating systems (Poaceae, Cistaceae, Fabaceae, etc.).

Understanding the mechanisms that 'control' species genetic structure has always been a major objective in evolutionary studies. Based on allozyme and/or microsatellite data, it is generally accepted that the plant breeding system (self-compatibility or selfing) is the factor that most influences the degree of genetic isolation (e.g. Hamrick and Godt, 1996; Weller *et al.*, 1996; Gitzendanner and Soltis, 2000; Crawford *et al.*, 2001, 2011; Leimu *et al.*, 2006; Duminil *et al.*, 2007, 2009; Barrett, 2010). In the Canarian flora, self-compatibility or autogamy seem to be the best reproductive predictors of isolation as measured by G_{ST} or F_{ST} (see Table 1, and below), although they do not entail significant differences.

Concerning sexual systems (see Table 1, and Pérez de Paz *et al.*, 2012), lower genetic diversity in populations of dioecious Canarian endemic plants with obligate xenogamy (significant in the population level analyses, not shown), agrees with Bawa's (1980) and Charlesworth's (1999) hypothesis that dioecy is more related to effective resource allocation or assurance of progeny through sexual selection. Prospectively, we explain the detection of higher genetic diversity levels in hermaphrodite or monoecious and polygamous taxa by the close association of these traits with self-incompatibility (e.g. *Argyranthemum*, *Parolinia*, *Lobularia*, *Chamaecytisus*, *Lotus*, *Cistus*, *Limonium*, *Pinus*, *Olea* or *Echium*).

Chromosome number and genome size. Populations of Canarian taxa with greater chromosome numbers also have significantly higher allozyme genetic diversity (Table 1), as predicted by Hamrick *et al.* (1979) and, for the Canarian flora, by Pérez de Paz (2002) and Pérez de Paz *et al.* (2007). This is presumably due to the larger number of linkage groups (recombinations and segregations) as first argued by Stebbins (1950), and can be framed within Richards' (1997) hypothesis that high basic chromosome numbers may increase gametic and genotypic diversity. The higher genetic polymorphism of *Limonium dendroides* ($x = 9$) relative to *L. sventenii* ($x = 7$) despite the extremely low population sizes of the former taxon (Suárez-García *et al.*, 2009) could be related to this result (see Annex 1 in Caujapé-Castells and Pérez de Paz, 2011). Polyploid Canarian taxa also display significantly higher genetic variation levels than diploids (Table 1), as

found in other floras (e.g. Levin, 2002; López-Pujol *et al.*, 2006; Pickup and Young, 2008; Sampson and Byrne, 2012). However, there is considerable heterogeneity in the differences between congeneric taxa. For instance, the tetraploid *Limonium tuberculatum* has a much higher genetic polymorphism than its diploid congeners (see Annex 1 in Caujapé-Castells and Pérez de Paz, 2011), whereas in *Lotus* the differences are small. The nature of polyploidy may bring about different chromosomal and genome rearrangements in auto- and allopolyploids, or may generate selfing by breakdown or disruption of certain self-incompatibility systems, or promote apomixis or vegetative reproduction (Miller and Venable, 2000; Levin, 2002; Mable, 2004; Barringer, 2007; Husband *et al.*, 2008; Robertson *et al.*, 2011; Beest *et al.*, 2012). Tentatively, these differences may underlie the reported heterogeneity in genetic diversity indicators; however, the limited allozyme data about polyploid Canarian taxa, and the lack of knowledge about their origins, prevent us from commenting on these aspects further.

Leitch and Bennett (2004) and Kellogg and Bennetzen (2004) concluded that plant genome sizes (i.e. 1C DNA amounts) do not increase in direct proportion with ploidy. This observed genome downsizing with increasing ploidy (i.e. 'genome contraction') may be a widespread phenomenon of considerable biological significance, but its implications for plant diversification on oceanic islands continue to be poorly understood. In the Canaries, Kapralov and Filatov (2011) did not find a negative correlation between average genome size (1C DNA amount) and the number of endemic species per genus, in contrast to their results for Hawaii and the Marquesas islands. This finding led them to intimate that small genome sizes are not related to the high diversification rates observed in the Canarian flora. At odds with this hypothesis, the Canarian genera that show great radiation and for which allozyme data are available do have significantly smaller genome sizes (1C = 2.059 pg vs. 7.392 pg for non-radiating genera, P -value < 0.05; data not shown). Remarkably, smaller genome sizes are significantly associated with higher allozyme genetic variation levels in this flora (Table 1). Thus, our tests do not allow us to reject the hypothesis that a small genome size may have helped promote radiation in the Canaries.

Influences of biotic and abiotic factors on neutral genetic variation and structuring

In agreement with most empirical investigations (Hamrick *et al.*, 1979; Hamrick and Godt, 1996; Gitzendanner and Soltis, 2000; Leimu *et al.*, 2006; Duminil *et al.*, 2007, 2009; Barrett, 2010; Beest *et al.*, 2012), our tests suggest that allozyme genetic diversity levels in the Canarian flora are 'phylogenetically constrained'. Specifically, the MLRA (Fig. 2, Table 2) shows that the lineage-dependent features analysed (high basic chromosome numbers, partial or total self-incompatibility system and polyploidy) influence the generation of the high levels of genetic variation in the Canarian flora more than the abiotic factors (represented by a short distance to the mainland).

Biotic variables are also more influential than abiotic ones in the apportionment of genetic variation of the Canarian

flora as measured by G_{ST}/F_{ST} (Fig. 2, Table 2). However, in contrast to genetic diversity levels, short-distance seed dispersal prevails over high basic chromosome numbers, total or partial self-incompatibility and short distance from the mainland in the generation of high population connectivity (low G_{ST} values). As expected, higher G_{ST} values are directly related to a higher incidence of self-compatibility, and to a greater distance from the mainland. The latter effect may be associated with the greater habitat complexity of the western islands, which also may determine a greater genetic polymorphism and isolation, especially in taxa with long-distance seed dispersal (Table 1).

On the whole, these results indicate that (1) the interactions between the mentioned lineage-dependent biotic features of the colonizing taxa and the selective forces imposed by the abiotic features of each island are the drivers of plant diversification in the Canarian flora; and (2) long-distance gene flow does not suffice to counteract the geographical and ecological barriers that foster reproductive isolation, especially on the westernmost Canary islands (see also Franks, 2010; Freeland *et al.*, 2010, 2012).

Neutral genetic diversity, population size and endangerment

Most investigations based on neutral genetic markers assume that moderate to high levels of neutral genetic variation indicate a good conservation status. The fact that taxa with some degree of threat in general display lower levels of genetic variation than non-threatened species (Table 1) could possibly be construed as supporting evidence for this assumption in the Canarian flora. However, no significant differences were found between these categories in taxa sampled intensively (data not shown). Furthermore, critically threatened species according to IUCN categories (CR) hold levels of genetic diversity which are similar to (though lower than) those in species without threat; in all cases, EN taxa had the lowest genetic diversity and the highest G_{ST} values (data not shown).

Regardless of threat status, Canarian plant endemics with generally large population sizes have significantly higher genetic diversity levels than species with low and moderate or intermediate population sizes (Table 1). However, taxa with very small populations (<50) tend to be more polymorphic than those with moderate (51–500) sizes ($A = 1.523$, $P = 0.349$, $H_o = 0.117$, $H_e = 0.157$ vs. $A = 1.510$, $P = 0.353$, $H_o = 0.101$, $H_e = 0.134$; P -value < 0.001, respectively; data not shown). These results strongly suggest that population size is not directly related to genetic diversity levels in the Canarian flora. Notably, many Canarian endemic plants hold extremely high levels of genetic variation despite their high degree of endangerment, radically restricted distributions and very small population sizes, e.g. *Crambe tamadabensis*, *L. dendroides*, *Parolinia glabriuscula* and *Lotus kunkelii* (see Annex 1 in Caujapé-Castells and Pérez de Paz, 2011).

Apart from these results, many investigations have also reported no correlation between plant population size and genetic diversity (Maki *et al.*, 2003; Helenurm *et al.*, 2005; Riley *et al.*, 2010; Sosa *et al.*, 2010; Fernández-Mazuecos and Vargas, 2011; Gitzendanner *et al.*, 2012; Trapnell *et al.*,

2012), emphasizing the more predominant role of other factors such as breeding system, chromosome number and historical processes in the generation of genetic diversity.

Although allozymes have guided conservation efforts in many Canarian taxa (e.g. Batista *et al.*, 2001; González-Pérez *et al.*, 2004; Fernández-Palacios *et al.*, 2006; Oliva-Tejera *et al.*, 2006), we lack the needed comparisons to know if they furnish evidence of natural selection or evolutionary potential [but see Spielman *et al.* (2004) or Leinonen *et al.* (2008)]. Thus, it remains to be proven whether neutral genetic variation levels are positively correlated with levels of genetic variation in selectively relevant regions such as *S*-allele loci in self-incompatible taxa (De Mauro, 1993; Holderegger *et al.*, 2008; Young and Pickup, 2010; Young *et al.*, 2012).

In taxa with homomorphic self-incompatibility systems, a downward slump in population size can induce genetic diversity loss at the self-incompatibility locus (*S*-alleles), leading to a reduction in mate availability, with dramatic demographic implications. In heteromorphic self-incompatibility systems, a decrease in population size may carry about the loss of heteromorphism, preventing reproductive success and/or seed set. In the extreme case of *L. dendroides* (Plumbaginaceae), the loss of morph diversity associated with the heteromorphic sporophytic self-incompatibility system makes reproduction impossible, even in some of the genetically most variable populations (Suárez-García *et al.*, 2009).

It is also worth noting that not all the Canarian endemics with extremely small populations show evidence of an important reduction in *S*-allele diversity. Reproductive biology and demographic studies in some of these cases have revealed a high reproductive success and population stability, as in *P. glabriuscula* (Fernández-Palacios *et al.*, 2009; Fernández-Palacios, 2010). In these cases, endangerment would be more influenced by environmental factors (either natural or anthropogenic), because the dominance interactions among *S*-alleles in homomorphic sporophytic self-incompatibility systems may increase mate availability (and reproductive success) even when *S*-allele numbers are low, as shown in continental species such as *Senecio squalidus* and *Brassica insularis* (Brennan *et al.*, 2003; Busch and Schoen, 2008).

Neutral genetic structure, population size and endangerment

Genetic isolation can be a conservative, creative or destructive evolutionary force. Whereas weak genetic structuring (i.e. low G_{ST} values) generally indicates high species cohesion, strong genetic structuring (i.e. high G_{ST} values) is associated with a high isolation, and may trigger speciation or extinction. Especially on oceanic islands, genetic structuring is largely influenced by the inherent geographical fragmentation, and by the ruggedness of most of these enclaves. Not infrequently, these abiotic factors (and their interactions with biotic factors) foster genetic isolation, representing a powerful stimulus to speciation (see, for example, Franks, 2010).

In sharp contrast, the consequences of increasing human activities in the limited insular territory add to the fragility of the flora by introducing sudden fragmentation to which endemic plants cannot adapt readily. When threat is mainly due to human activities leading to generic habitat fragmentation or loss, the detected genetic signals should be better interpreted

as consequences of those activities, and not as the direct causes of threat.

It is therefore in order to assess the relationship between endangerment (as defined by the IUCN) and the neutral estimates of genetic isolation provided by allozymes (i.e. through G_{ST} or F_{ST} values). If neutral molecular markers were good predictors of endangerment, we would expect the endangered Canarian endemics to have the highest genetic isolation. Although the allozyme data set used contains a mixture of both natural and anthropogenic isolation, our general tests (Table 1) are at odds with this expectation, as taxa with some degree of threat are less genetically isolated than non-threatened taxa. However, taxa with small and intermediate population sizes are also significantly more isolated than taxa with generally large populations (Table 1). This result feasibly implies that in many cases drift is also reducing the *S*-allele diversity within small self-incompatible taxa (approx. 87% of our data set), thereby jeopardizing their short-term survival, and emphasizing the need for urgent action.

Although endangered taxa were significantly less genetically isolated than non-endangered taxa under extensive sampling ($G_{ST} = 0.153$ vs. $G_{ST} = 0.321$, respectively; $P < 0.05$), the reverse was true with intensive sampling ($G_{ST} = 0.235$ vs. $G_{ST} = 0.168$, respectively; non-significant) (data not shown). In agreement with Caujapé-Castells (2010), these tests further illustrate that the usefulness of neutral genetic data for predicting endangerment may be tightly and directly linked to a high sampling representativeness.

Conservation strategies, reproductive systems and neutral markers

Our results and other research advise extreme caution when interpreting the degree of neutral genetic variation in small populations in a conservation context, as high levels of neutral genetic variation do not necessarily relate to good conservation status.

The decrease in population sizes depends on the reproductive system and other life cycle traits (Aguilar *et al.*, 2006; Rocha and Gasca, 2007; Iriondo *et al.*, 2008), suggesting that conservation actions should ideally be taxon specific, and based on empirical data. Neutral genetic variation indicators may be misleading in the absence of other ancillary biological information. Since reproductive information often is more important than genetic diversity data for an adequate assessment of threat (Anderson *et al.*, 2001; Bernardello *et al.*, 2001; Crawford *et al.*, 2011), we suggest that reproductive evidence should always be effectively included in conservation strategies of Canarian plants.

We concur with, for example, Fay (2003) that it is feasible to use genetic methods in the most threatened species, where there are few populations of generally small or moderate size. However, considering our previous discussions, we also think that the data obtained would be of doubtful relevance for their urgent, effective conservation. Thus, we believe that habitat protection should be emphasized in all cases (whenever possible), and we agree with FOEA that *ex situ* genetic conservation should be urgently implemented in the worst cases. In addition, we contend that these actions should be undertaken together with reproductive biology studies, regardless of the

existence of estimates of genetic variation levels or other quantitative data. It is thus important to continue with the ongoing conservation programmes at the JBCVCSIC and other institutions to preserve these most endangered Canarian taxa *ex situ*, to monitor their populations in the wild and to eradicate grazing by introduced mammals if necessary.

This is not saying that genetic diversity studies are unimportant; we simply emphasize that, at least in the Canaries, they should not be prioritized for only the most critically endangered taxa, where the estimation of genetic diversity and the identification of other factors important for conservation might be difficult (and/or hardly relevant for their survival), and the populations could become extinct in the meantime. In these taxa, molecular and other studies are fitting if applied in a wide comparative context, encompassing their closest non-endangered congeners.

Rather, the joint use of demographic and molecular data would be far more important to avoid a decrease in population viability, genetic diversity and connectivity in common or recently rare taxa, known to be more susceptible to the effects of rapid, human-mediated fragmentation (see, for example, Barrett and Kohn, 1991; Aguilar *et al.*, 2008). Indeed, taxa with moderate population sizes (51–500) are also significantly more fragmented genetically ($G_{ST} = 0.300$) than taxa with very small (<50) and generally large (>500) populations ($G_{ST} = 0.281$ and 0.200; data not shown). Unlike critically endangered endemics, the factors that may threaten moderately widespread Canarian endemics with large population sizes could be more effectively mitigated by informed conservation strategies based on multidisciplinary research.

FOEA stressed that a decrease in population sizes may lead to increased inbreeding depression and reduced fitness in these species, lowering their ability to adapt to environmental changes, or to compete with introduced species. However, Angeloni *et al.* (2011) also found a significant increase in inbreeding depression (ID) with increasing population sizes, presumably because lower levels of inbreeding may impede the purging of deleterious alleles. These contrasting indications call for caution, highlighting the fact that we still need to understand better how different ecological and historical conditions influence the levels of ID in natural plant populations.

Adequate genetic structure indicators (Jost, 2008) and estimates of common and exclusive allelic diversity would also be instrumental in maximizing their genetic variation in seed and DNA banks (Bachetta *et al.*, 2008). These *ex situ* collections and reproductive biology studies may in turn facilitate further research and conservation action (for eventual reinforcements or reintroductions that maintain reproductive stability and population viability), and help refine *in situ* conservation priorities [e.g. through estimates of genetic diversity per unit area (Vane-Wright *et al.*, 1991; Faith, 1992)].

Especially in outcrossing and self-incompatible taxa, in which increased inbreeding may be more rapid and dramatic (Francisco-Ortega *et al.*, 2000; Aguilar *et al.*, 2008; Iriondo *et al.*, 2008; Angeloni *et al.*, 2011), the indicators obtained should always be complemented with other life cycle data that affect demographic dynamics and the maintenance of sexual reproduction. Notably, a high percentage of the widespread Canarian taxa tested in this investigation possess these reproductive characteristics.

Finally, our study reveals a number of gaps in the knowledge of the relationships among genetic variation, population size, fitness and other ecological traits. Future research on threatened Canarian endemics should therefore assess (1) the effects of genetic diversity on individual fitness through, for example, the estimation of *S*-allele diversity in SI species; (2) the adaptive potential of traits influencing life cycle in a context of global change (using quantitative trait locus mapping); or (3) the relationship between inbreeding depression, population size and species resilience (Reed and Frankham, 2001; Maki, 2003; Leimu *et al.*, 2006; Aguilar *et al.*, 2008; Angeloni *et al.*, 2011).

Conserving the genetic information relevant for conservation

We have found several important analytical limitations for rigorous, standardized statistical analyses, even for the data that we could use for our tests. Namely, in many cases: (1) not all the genetic diversity parameters are calculated in the data sources; and (2) the values of the genetic parameters are not reported in each population considered. These shortcomings are also incompatible with the targets set forth in the reformulated GSPC, which ideally require the preservation of (and access to) all existing genetic diversity information and associated data or meta-data in a standard format that facilitates further insight.

Iriondo *et al.* (2008) stressed that the multiple shifting factors involved in a crisis discipline such as conservation biology trigger the need for a constant critical reappraisal of assumptions. Concerning genetic diversity data, only by preserving and sharing the available genotype matrices in a standardized format may we reassess our present assumptions, and meet the dynamic knowledge challenges implicit in conservation science and in the reformulated GSPC.

As opposed to other web-based data repositories, the online information system *Demiurge* and its associated freeware T4 (http://demiurge-project.org/download_t4) provide a standard to facilitate the fast storage and analysis of genetic diversity data associated with any molecular technique, and ancillary biological information (Caujapé-Castells *et al.*, 2013). These new bioinformatic tools avoid the loss of the valuable information resident in genotype matrices, and allow their permanent availability to generate or reassess knowledge. Therefore, they may help meet the urgent reformulated GSPC targets 2, 3 and 16 (referring to conservation assessments, sharing of knowledge and protocols, and networking, respectively).

CONCLUSIONS

Both the high genetic diversity levels and the genetic structure of the Canarian endemic flora are phylogenetically constrained, as they are more influenced by biotic, lineage-dependent factors than by abiotic, island-dependent factors. The taxa best suited to diversify in the Canarian abiotic landscape are those with high basic chromosome numbers, polyploidy, partial or total self-incompatibility and long-distance seed dispersal. The analyses also highlight the fact that the current floristic make-up of the archipelago is the consequence of a broad diversity of colonization histories.

Our results advise extreme caution when interpreting the degree of neutral genetic variation in small populations in a conservation context, as high levels of neutral genetic variation do not necessarily converge with good conservation status. Furthermore, conserving the rich endemic plant diversity of the archipelago may be a complex endeavour distorted by the vagaries of extinction, by a possible lack of positive correlation between neutral and selectively relevant molecular markers and by important knowledge gaps. Consequently, conservation actions should be case specific and based on empirical data. In line with FOEA and the reformulated GSPC, our analyses indicate that plant conservation in the Canaries requires multidisciplinary approaches encompassing reproductive assessments and, whenever possible, effective habitat protection and dedicated monitoring.

Especially for the most extremely endangered endemics, reproductive studies and *ex situ* conservation should be urgently implemented without the need for prior ancillary data, the generation and analysis of which could be time-consuming, and add to the present endangerment status. In contrast, the application of multidisciplinary research prior to the suggestion of conservation strategies seems more adequate in non-endangered, moderately widespread Canarian plant endemics, which still have significantly higher population genetic diversity levels that need be preserved. Future conservation research should necessarily address the effects of genetic diversity on reproductive success and fitness to better understand the population dynamics of threatened Canarian endemics. Meeting the knowledge challenges implicit in the reformulated GSPC also entails conservation of the molecular information relevant for conservation in a standardized format to allow comparative re-assessments and insight.

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