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Gene flow, barriers, speciation and hybridization in *Parolinia* species (Brassicaceae) endemic to Gran Canaria

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Oceanic islands are natural laboratories where evolutionary processes can be studied more readily. In the present work we use nuclear microsatellites to quantitatively assess the roles of hybridization, colonization, gene flow and geographical barriers in four *Parolinia* spp. endemic to Gran Canaria in connection with known geological events throughout the dynamic geological ontogeny of the island. Our genetic analyses show that these *Parolinia* spp. share 69% of all alleles detected and display low genetic divergence among populations, suggesting a close genetic relationship and recent differentiation. This close relationship is more evident between *Parolinia platypetala* and *Parolinia ornata*, which were included in the same genetic pool in the STRUCTURE analysis, and probably represent the early stages of an incipient speciation process, as suggested by the absence of gene flow barriers between them. NEWHYBRIDS, STRUCTURE and MIGRATE analyses unequivocally suggest frequent past migration and hybridization from *P. ornata* to *P. filifolia*, but contemporary migration is low overall. Despite the overall genetic closeness among the *Parolinia* spp. assessed, microsatellites dispelled doubts regarding the appropriate name/s for two taxonomically conflicting populations. In line with the predictions of the surfing syngameon hypothesis, ancestral hybridization, vicariance and dispersal events linked to the complex ontogeny of the island may have been key factors to shape the current genetic diversity and structure of *Parolinia* in Gran Canaria.

ADDITIONAL KEYWORDS: Canary Islands – colonization – endemic species – gene flow – hybridization – island evolution – speciation.

INTRODUCTION

Understanding how selection, gene flow and genetic drift shape the divergence of closely related species has been of long-standing interest in evolutionary biology (Feder *et al.*, 2013). Allopatric speciation driven by physical barriers that completely restrict inter-populational gene flow and trigger divergence through genetic drift or selection is conventionally considered to be the prevalent mode of speciation (Carson & Clague, 1995). However, hybridization followed by introgression (i.e. the transfer of alleles from one lineage into the gene pool of another lineage via hybridization and backcrossing: Anderson & Hubricht, 1938; Harrison & Larson, 2014) can often lead to diversification and speciation (Arnold *et al.*, 1991; Fogelqvist *et al.*, 2015). These processes might also explain some traits of insular radiations, thereby providing the opportunity to study evolutionary processes associated with interactions between species (Harrison, 1990).

Oceanic islands represent true evolutionary biology laboratories where colonization, speciation and hybridization processes can be detected and recognized in endemic taxa more readily than on continents (Saro *et al.*, 2015, 2018; Puppo *et al.*, 2016; Silva *et al.*, 2016). The Canary Islands are a good example of oceanic

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islands where these processes have been developing for millions of years, and the archipelago is included into the Mediterranean basin biodiversity hotspot (Myers *et al.*, 2000).

Gran Canaria is one of the central Canary Islands and provides an excellent geographical framework in which to address questions about how gene flow, geographical barriers, colonization and hybridization may have shaped the genetic structure of the current endemics throughout its prolonged and dynamic geological ontogeny. Gran Canaria is also one of the biggest (1560 km^2) and oldest islands of the archipelago (14.5 My: Carracedo & Day, 2002; Anderson *et al.*, 2009; Rodríguez-González *et al.*, 2018), with a maximum elevation of 1949 m a.s.l. and a high topographic complexity per unit area. This suite of geographical characteristics has enabled the island to harbour a high plant biodiversity, with >100 single island endemics (Reyes *et al.*, 2008) and multiple island endemics shared with other islands in the archipelago.

Parolinia Webb (Brassicaceae) is a genus endemic to the Canary Islands comprising seven endemic species distributed in four of the seven islands of the archipelago (Gran Canaria, Tenerife, La Palma and La Gomera). Except for Gran Canaria, where four island-exclusive species occur (*Parolinia ornata* Webb, *Parolinia platypetala* Kunkel, *Parolinia filifolia* Kunkel and *Parolinia glabriuscula* Montelongo & Bramwell), the other islands of distribution harbour only one endemic species each.

The four *Parolinia* spp. on Gran Canaria show contrasting distribution patterns (Fig. 1). Whereas *P. ornata* and *P. filifolia* are variably widespread (100-500 m a.s.l. in the south-west, and west of the island, respectively), *P. glabriuscula* is restricted to a volcanic crater 1000 m in diameter and 200 m deep in the north-east of the island (the Caldera de Bandama), which is the most recent Holocene eruption in Gran Canaria (< 2000 years BP; Carracedo & Troll, 2016); likewise, *P. platypetala* is only known in the Barranco de Guayadeque, a ravine with an estimated age of 3.6 My (Anderson *et al.*, 2009; Rodríguez-González *et al.*, 2018), situated in the Post Roque Nublo volcanism area in the south-east of the island. According to the latest edition of the IUCN catalogue of endangered taxa (IUCN, 2012), two of these species are critically endangered (*P. platypetala* and *P. glabriuscula*) and one is endangered (*P. filifolia*).

In Gran Canaria, different stochastic and geological events throughout the complex ontogeny of the island have probably isolated and reconnected populations and species and may have promoted recurrent cycles of hybridization and genetic differentiation, as suggested by the surfing syngameon hypothesis (Caujapé-Castells, 2011; Caujapé-Castells *et al.*, 2017). The south-western half of Gran Canaria dates from the Miocene ('Palaeocanaria') and the north-eastern half from the Pliocene ('Neocanaria') (Fig. 1). This general chronological division was shown to have had a high influence on the evolutionary history of species (Mairal *et al.*, 2015; Puppo *et al.*, 2016), resulting in higher genetic diversity, but lower differentiation in species distributed in the younger part of the island (Curto *et al.*, 2017).

Although interspecific hybridization has not been described or suggested in *Parolinia*, we hypothesize that this process could underlie the taxonomic debate that exists for some populations (Fernández-Palacios, 2010). Indeed, the populations from Agaete and Veneguera (Fig. 1) especially represent prominent examples of taxonomic uncertainty (Fernández-Palacios, 2010). Although traditionally described as *P. ornata* (Kunkel, 1969; Bramwell & Bramwell, 2001), Fernández-Palacios (2010) highlighted that these populations cannot be assigned clearly to any of the four species and suggested the need for a thorough taxonomic and chorological review of the genus. Furthermore, the overlap of the areas of distribution of *P. ornata* and *P. filifolia* (Fig. 1) also makes it feasible that hybrid zones between these two taxa exist in the south-west of the island.

The detection of hybrids using morphological characters generally assumes that hybrid individuals will be phenotypically intermediate with respect to the parents. However, this is often not the case, because individuals from a hybrid swarm sometimes express a mosaic of parental phenotypes (Campton, 2000; González-Pérez & Sosa, 2009) or are often morphologically indistinguishable from the parental taxa if they inherited most of their genes from one of the parents (Allendorf *et al.*, 2001). In these situations, the application of hypervariable genetic markers and statistical tools may greatly facilitate the quantification of the levels and effects of gene flow and the probabilistic detection of hybridization in populations that show no morphological signs of it.

Studies carried out with allozymes (Fernández-Palacios, 2010) suggested a monophyletic origin of *Parolinia* spp. on Gran Canaria, and great intra-population variation but low inter-population genetic differentiation (Fernández-Palacios, 2004, 2006, 2010). In addition, all *Parolinia* spp. show high micro-morphological diversity (Fernández-Palacios, 2010). These studies also indirectly suggest recent speciation and high levels of gene flow among geographically close populations; however, the low resolution revealed by these techniques makes it appropriate to use more highly polymorphic molecular markers to provide insight into the various populational and taxonomic uncertainties described.

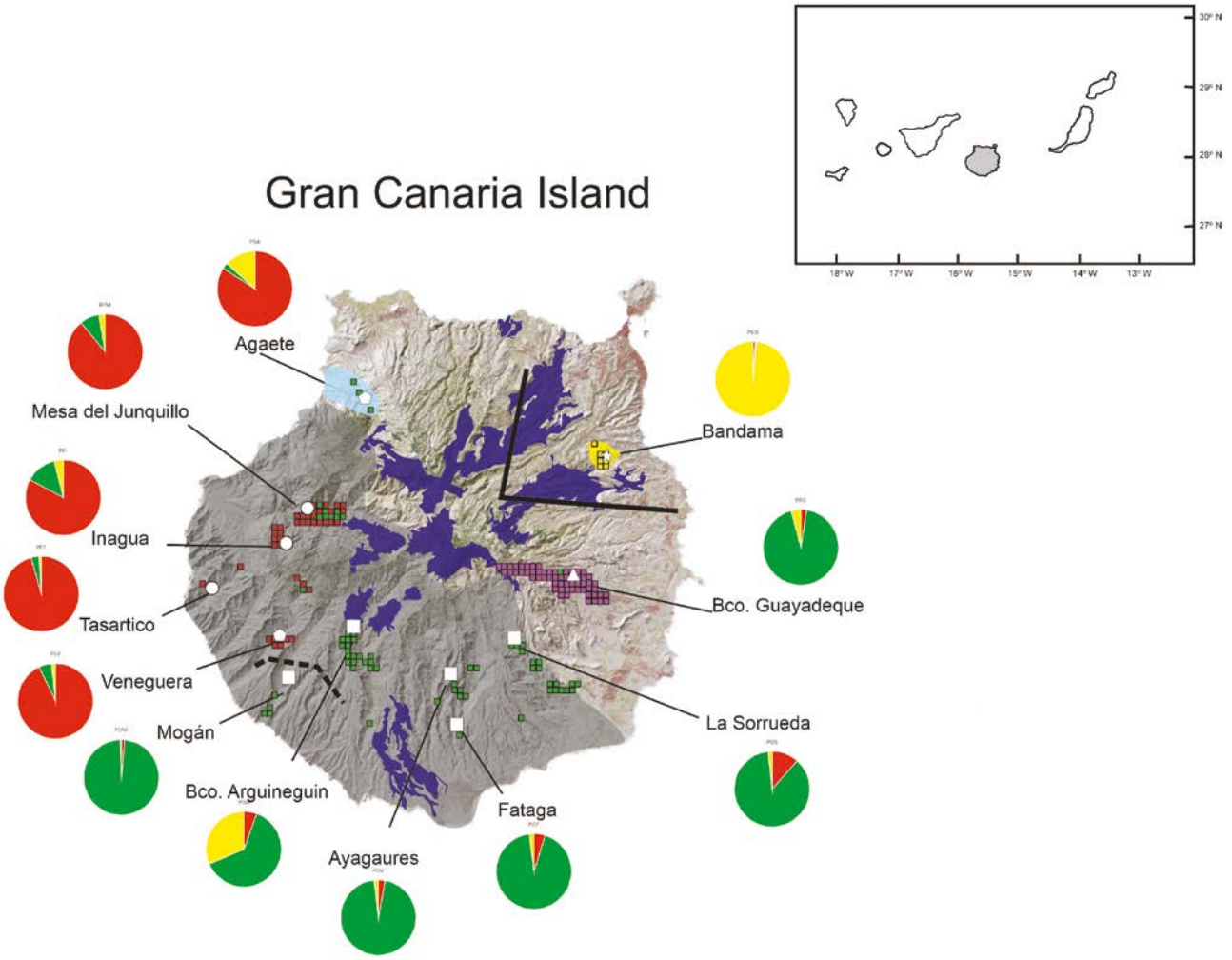

Figure 1. Geographical location of the sampled *Parolinia* populations, geological map of Gran Canaria and genetic clustering obtained with the Bayesian approach in STRUCTURE assuming three clusters (K = 3). Coloured squares represent the species distribution in the Biodiversity Data Bank of the Canary Islands (green: *P. ornata*; red: *P. filifolia*; yellow: *P. glabriuscula*; purple: *P. platypetala*). Lines represent barriers to gene flow with > 70 % support, as identified by the BARRIER software, of potential first order (solid line) and second order (dashed line). The geological map [adapted from Pérez-Torrado *et al.* (2006) and Rodríguez-González *et al.* (2018) shows the division of Gran Canaria into Neocanaria (light grey) and Palaeocanaria (dark grey) and the main geological events that have affected the current distribution areas of *Parolinia* populations: the Roque Nublo volcanism (dark blue) and the Bandama eruption (yellow). The area of Agaete affected by a tsunami is indicated in light blue.

We use eight nuclear microsatellites (González-Pérez & Caujapé-Castells, 2014) on an exhaustive population sampling of these taxa to address three challenging questions related to their evolution and conservation genetics. (1) Has the chronological division of Gran Canaria (Palaeocanaria/Neocanaria) influenced the genetic diversity and differentiation of *Parolinia* spp.? (2) Do microsatellites support a role for hybridization in the diversification of *Parolinia* in Gran Canaria? (3) Were the genetic relationships among these taxa influenced by major changes throughout the dynamic ontogeny of the island?

Under the hypothesis that the dynamic geological ontogeny of the island triggered different processes of within-island colonization, divergence, gene flow and hybridization in *Parolinia* spp., we expect to retrieve population genetics clues to help in answering these questions and to relate the levels of populational genetic structuring to the relative importance of ancestral vs. contemporary gene flow within and among taxa.

MATERIAL AND METHODS

Plant material

Plants of *Parolinia* are woody perennials up to 2.5 m high occurring on dry and sunny slopes of the xerophytic Canarian zone from the coast to 700 m a.s.l. All species are diploid ($2n$ = 22, Febles, 1989) and have hermaphrodite flowers with homomorphic sporophytic self-incompatibility (Fernández-Palacios, 2010). Fernández-Palacios (2010) also suggested three types of flower morphology: closed flowers (*P. ornata* and *P. platypetala*); open flowers (*P. glabriuscula*); and intermediate flowers (*P. filifolia*). Diverse insects (flies, beetles, bees, wasps) visit the flowers and presumably carry out pollination. Seed dispersal is anemochorous (iBañares *et al.*, 2004; Fernández-Palacios, 2010). Seeds are flat, light (1.8 mg), have an ovate to elliptic shape (1.44 × 1.02 mm), and are surrounded by a thin transparent wing (0.07 mm) that enables them to glide several kilometres (Bramwell, 1986; Fernández-Palacios, 2010). *Parolinia* spp. are a conspicuous example of the high relevance of reproductive characters (flower, fruit, seeds) over vegetative characters (leaves, growth form) in taxonomic identification (Fernández-Palacios, 2010).

To estimate the levels of genetic diversity, differentiation and relationships between the four species, an extensive and intensive population sampling was carried out (Table 1), using the distributional information for *Parolinia* available in the Banco de Datos de Biodiversidad de Canarias (Gobierno de Canarias, https://www.biodiversidadcanarias.es/biota). We collected samples from the only known localities of *P. glabriuscula* and *P. platypetala*, and the more widely distributed *P. ornata* and *P. filifolia* were extensively sampled throughout their distribution areas, including the taxonomically conflicting populations of Agaete and Veneguera (see Introduction).

DNA isolation and microsatellite analysis

DNA was extracted from silica-gel dried young leaves that were ground in a Mixer-Mill (RETSCH MM 301, Haan, Germany), with two glass balls in each microtube. We followed a slightly modified 2 × CTAB protocol (Doyle & Doyle, 1987). Approximately 150 µL of each total DNA sample was purified using a GenElute PCR Clean-Up Kit (Sigma-Aldrich, Saint-Louis, USA).

Forward and reverse primers specifically developed for *P. ornata* (González-Pérez & Caujapé-Castells, 2014) were used to amplify eight polymorphic microsatellite loci. Polymerase chain reactions (PCRs) were performed in a final volume of 12 µL that contained *c*. 20 ng DNA, 10 pmol each primer and 7 µL PCR Master Mix (Premix Taq, Takara Bio Inc., Otsu, Japan).

Table 1. Genetic diversity indices of the *Parolinia* populations studied, with voucher numbers at the LPA Herbarium (Jardín Botánico Canario "Viera y Clavijo"-Unidad Asociada al CSIC: LPA) and population codes

Population	Voucher	Code	N	A	NE	H_o	H_e	%P	F_{IS}
P. ornata									
Fataga	38735	POF	20	4.125	0	0.506	0.523	100	0.043
Ayagaures	38734	POV	19	4.875	1	0.447	0.499	100	0.103
Bco. Arguineguín	38746	POA	20	5.125	0	0.463	0.648	100	0.127
Mogán	38874	POM	20	3.875	2	0.453	0.464	100	0.041
Bco. La Sorrueda	38742	POS	20	4.750	1	0.394	0.495	100	0.098
Overall			99	8.250	12	0.454	0.600	100	
P. filifolia									
Inagua	38745	PFI	20	5.000	0	0.500	0.533	100	0.057
Mesa del Junquillo	38733	PFM	20	4.500	1	0.504	0.514	100	0.018
Tasartico	38740	PFT	19	4.000	2	0.467	0.494	100	0.054
Overall			59	7.000	5	0.495	0.603	100	
P. glabriuscula									
Bandama	38739	PGB	20	2.625	0	0.443	0.417	100	0.021
P. platypetala									
Bco. Guayadeque	38736	PPG	20	4.250	2	0.430	0.484	100	0.099
Parolinia sp.									
Agaete	38991	PSA	20	6.250	1	0.619	0.704	100	0.096
Veneguera	38992	PSV	20	5.125	0	0.483	0.643	100	0.063

N: number of individuals analysed, A: average number of alleles per locus, NE: number of alleles exclusives, H_o: observed heterozygosity, H_e: expected heterozygosity, %P: polymorphic loci percentage.

PCR products were detected using an ABI 3130XL Genetic Analyzer and fragment sizes were determined using GENEMAPPER v.4.0 (Applied Biosystems, Inc.). This allowed us to identify allele peak profiles at each locus and to assign genotypes to each individual, considering their diploid nature.

DATA ANALYSIS

The genotypic data matrix was entered into Transformer-4 v.2.0.2 (Caujapé-Castells *et al.*, 2013) which allowed us to export the data to different software programs. The standard within-population genetic diversity statistics were calculated with GENALEX v.6.5 (Peakall & Smouse, 2006): mean number of alleles (A), number of exclusive alleles (AE), observed (H_o) and expected (H_e) heterozygosity. The genetic differentiation coefficient (R_{ST}) between all possible population pairs was estimated using SPAGeDi v.1.5 (Hardy & Vekemans, 2002). As null alleles can overestimate F_{IS} values, INEST 2.2 (Chybicki & Burczyk, 2009) was used to simultaneously estimate the presence of null alleles and get corrected values of F_{IS}. Using a Bayesian approach, with 50 000 burn-in cycles and 500 000 Markov chain Monte Carlo (MCMC) iterations in total. To detect the eventual existence of inbreeding effects in our data set, INEST was run using the models 'nfb' (null alleles, in breeding coefficients and genotyping failures) and 'nb' (null alleles and genotyping failures).

To represent the genetic relationships among populations, a principal coordinate analysis (PCoA) was performed in GENALEX v.6.5 (Peakall & Smouse, 2006) based on allele frequencies of populations as implemented. The analyses of differentiation patterns were conducted following population-based approaches. To test the extent of genetic drift in the sampled populations, the occurrence of recent bottlenecks was explored using INEST 2.2 software (Chybicki & Burczyk, 2009), under the infinite allele model (IAM), the stepwise mutation model (SSM) and an intermediate two-phased model (TPM).

An analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992) was performed using the software ARLEQUIN 3.0 (Excoffier & Heckel, 2006) to calculate variance components and partitioning the variation ('between species', 'among populations within species' and 'within populations') for the entire data set. Subsequently, separate AMOVA analyses were used to test the distribution of genetic variance among and within populations of the species with more than one population (*P. filifolia* and *P. ornata*). Finally, an additional AMOVA was carried out considering the two taxonomically doubtful populations within their putative parental species.

Population structure was inferred using the Bayesian clustering procedure implemented in STRUCTURE 2.2 (Pritchard *et al.*, 2000) to identify the K (unknown) genetic clusters of origin of the sampled individuals and to assign individuals to the inferred clusters. The most likely value of K was assessed by comparing the likelihood of the data for different values of K. We used Markov chain Monte Carlo (MCMC) iterations as implemented in the software algorithm to explore a parameter space considering individual memberships to the K clusters, ranging from K = 1 (null hypothesis of panmixia) to K = 12 (the total number of sites sampled). Each MCMC search was replicated ten times for each K, with burn-ins of 10^5 and 10^6 replicates. We assumed an admixture model and correlated allele frequencies, which means that frequencies in the different populations are similar probably due to migration or shared ancestry. The optimum K, indicating the number of true clusters in the data, was also determined using ΔK criterion applying the methods described by Evanno *et al.* (2005) as implemented in STRUCTURE HARVESTER (Earl & von Holdt, 2012).

To investigate the effects of geographical barriers in the distribution of genetic variability and structuring of the subpopulations, we used the approach implemented in BARRIER 2.2 (Manni *et al.*, 2004). The spatial organization of subpopulations was modelled by Voronoi tessellation, and a maximum-difference algorithm (Monmonier's algorithm) identified the borders between neighbouring populations that exhibited the highest levels of genetic differences (Manni *et al.*, 2004). One hundred permutations of pairwise matrices of population differentiation based on Nei's distance (Nei, 1978) were used to assess the consistence of the boundaries detected by the algorithm. Only barriers with support > 70% were considered significant.

NEWHYBRIDS (Anderson & Thompson, 2002) was used to assign the multilocus genotypes to a putative parental species, F_1, F_2 or backcross categories, among all *Parolinia* species pairs. We used default genotypic classes with no prior information on allelic frequencies and included uniform and Jefferey's priors for θ and π, following default settings (Anderson & Thomson, 2002). Five separate analyses were conducted with 10^5 sweeps of burn-in and 10^7 sweeps of data collection. Threshold values (Tq) of 0.90 and 0.75 were assigned separately for the genotypic categories if $q \geq Tq$, and they were left unassigned if $q < Tq$.

STRUCTURE and NEWHYBRIDS give complementary information about genetic pool admixture (Arntzen *et al.*, 2016). Therefore, we plotted the proportion of membership (qi) from STRUCTURE

vs. posterior probabilities (*pp*) from NEWHYBRIDS to visualize the relationship between these two analyses. In addition, we calculated Pearson's correlation and carried out a subsequent significance test between both variables using XLSTAT v.7.5.2 (XLSTAT, 2004).

Contemporary rates of gene flow among species and migration directions were estimated with BAYESASS v.3.0 (Wilson & Rannala, 2003). The BAYESASS program was run multiple times with different seeds, and we compared the posterior mean parameter estimates for concordance, as recommended by the authors. In all cases, runs consisted of 20 000 000 generations with a burn-in of 5 000 000 and sampling increments of 200. Parameters for migration rates, inbreeding coefficients and allele frequencies were set to 0.1, 0.4 and 0.2, respectively, to ensure adequate mixing and acceptance rates between 0.2 and 0.6 (Wilson & Rannala, 2003).

Historical gene flow was estimated using the Bayesian inference and Brownian motion approach implemented in the MIGRATE 3.7.2 software (Beerli & Felsenstein, 2001). Following Beerli's recommendations, MIGRATE was run considering the three genetic clusters of *Parolinia* spp. identified by STRUCTURE, which converge better than single populations. One long chain was run saving 50 000 generations with sampling increments of 100 generations after a burn-in step of 10 000 generations. Prior distributions were uniform with a range from 0.00 to 20.00 for Theta (Θ) and for migration rate (M). A static heating scheme was applied with four temperatures of 1, 1.5, 3 and 1×10^6 (Beerli & Palczewski, 2010), due to uneven sample size populations. The MIGRATE analysis was performed on the high-capacity server of the Instituto Tecnológico de Canarias (ITC).

RESULTS

Genetic diversity

Overall, 94 different alleles were observed for the eight polymorphic loci surveyed, ranging from seven to 17 per locus. At the species level, the two more widely distributed species (*P. ornata* and *P. filifolia*) showed the highest genetic diversity values (H_e = 0.600 and H_e = 0.603, respectively), and the two more narrowly distributed species (*P. glabriuscula* and *P. platypetala*) the lowest (H_e = 0.417 and H_e = 0.484, respectively) (Table 1). The populations with the highest values of genetic diversity (H_e = 0.704 and H_e = 0.643) were those about which taxonomic doubts have been expressed (PSA and PSV, respectively; see Introduction).

Estimates of F_{IS} varied from 0.018 (population PFM) to 0.127 (population POA, Table 1). However, there were no significant differences between the two models used (nfb and nb), and the posterior 95% probability intervals included zero in all the estimates, indicating non-significant inbreeding in these populations. Null allele frequencies ranged from 0 to 0.14, and therefore the bias introduced by null alleles could be considered negligible (Dakin & Avise, 2004). In addition, Wilcoxon tests implemented in the INEST 2.2 software detected heterozygosity excesses in POA, PGB, PSA and PSV under the IAM model, indicating recent bottlenecks in these populations.

The AMOVA found that the largest proportion of genetic variation corresponded to the within-population component (74.19%), and only 12.78% to the among-species component (Supporting Information, Table S1). At the species level, most genetic variation was maintained within populations in *P. filifolia* (81.96%) and in *P. ornata* (86.97%). However, when we considered the taxonomically doubtful populations (PSA and PSV), the percentage of variation among populations increased slightly in *P. ornata* (14.42%) and decreased in *P. filifolia* (12.55%).

Genetic differentiation among populations

The number of exclusive alleles per species (Table 1) ranged from 0 (*P. glabriuscula*) to 12 (*P. ornata*). Seven of the 12 populations analysed showed private alleles in frequencies > 5% (POV, POM, POS, PFM, PFT, PPG and PSA).

Sixty-five out of 94 alleles (69%) were shared between intraspecific (6%) and interspecific (63%) population pairs, suggesting a close genetic relationship among all four species. Thus, the populations of *P. ornata* and *P. filifolia* shared 41 alleles, 12 of them exclusively. *Parolinia glabriuscula* shared four alleles with *P. ornata*, but none with *P. filifolia* or *P. platypetala*. Similarly, *P. platypetala* shared two alleles with *P. filifolia* and two with *P. ornata*.

The values of the genetic differentiation coefficient (R_{ST}) between population pairs (Supporting Information, Table S2) ranged from R_{ST} = 0.008 between POV and POF, to R_{ST} = 0.581 between PFM (*P. filifolia*) and POV (*P. ornata*). The high number of shared alleles between *P. filifolia* and *P. ornata* was also reflected in the moderate average pairwise R_{ST} between both species (R_{ST} = 0.259), in contrast with the considerably higher genetic differentiation coefficient between *P. glabriuscula* and *P. platypetala* (R_{ST} = 0.438).

Spatial patterns of genetic diversity

Following the method of Evanno *et al.* (2005) in the STRUCTURE analysis with the total data set (238 individuals, eight microsatellite loci, 12 populations),

the probability of the data reaches its maximum at K = 3 (Fig. 1; Supporting Information, Fig. S1), corresponding to: all individuals of *P. filifolia* plus *Parolinia* sp. individuals from the populations of Agaete and Veneguera (cluster I); all individuals of *P. glabriuscula* (Bandama) with some individuals of *P. ornata* from Agaete and Arguineguín (cluster II); and the remaining individuals of *P. ornata* and all those of *P. platypetala* (cluster III). We note that individuals from Arguineguín were placed by this analysis in either cluster II or cluster III. These results are consistent with the geographical pattern detected in the PCoA, which accounts for 59.51% of the total variance (Fig. 2).

Gene flow and hybrid detection

The BARRIER software detected a primary order barrier between *P. glabriuscula* and the rest of populations and a secondary order barrier between POM (*P. ornata*) and neighbouring populations (Fig. 1).

Overall, BAYESASS showed minimal contemporary migration among species, with migration rates below 0.05 for most of the comparisons (Supporting Information, Table S3). The only exceptions were migration rates from *P. filifolia* to PSA (m = 0.216) and from *P. ornata* to *P. platypetala* (m = 0.071).

In contrast, the historical migration rates calculated by MIGRATE between the three clusters defined by STRUCTURE varied between 0.644 and 7.605 individuals per generation (Fig. 3), with cluster I (*P. filifolia*, PSA and PSV) and cluster III (*P. ornata* and *P. platypetala*) as the main sources of inter-cluster gene flow (i.e. it had the highest migration rate). On the other hand, *P. glabriuscula* (cluster II) exhibited the lowest historical migration rate, indicating highly asymmetrical migration between clusters. MIGRATE revealed considerable historical migration between cluster I and cluster III and more intense migration from cluster III (*P. ornata* + *P. platypetala*) to cluster I (*P. filifolia*, PSA and PSV).

NEWHYBRIDS only detected putative hybrids between *P. ornata* and *P. filifolia* populations. The analysis identified 18 individuals as hybrids with PP > 0.90: eight belonging to the Veneguera population (PSV), eight in different populations of *P. filifolia* (PFI, PFM and PFT) and two in *P. ornata* (POV and POF). Likewise, 13 other potential hybrids were detected with 0.75 < PP < 0.90. All these 31 hybrids were classified as F_2 individuals by NEWHYBRIDS; no individual was identified as a F_1 hybrid or backcross, possibly due to the limited number of loci, that results in insufficient statistical power to unequivocally assign the hybrid individuals to specific backcross categories.

Of the 31 putative hybrid individuals detected, 12 belonged to *P. filifolia* populations (four from PFI, five from PFM and three from PFT), nine to *P. ornata* populations (four from POS, one from POV, two from POF and two from POA) and ten to the taxonomically doubtful populations (one from PSA and nine from PSV). Thus, 40% of the individuals analysed in

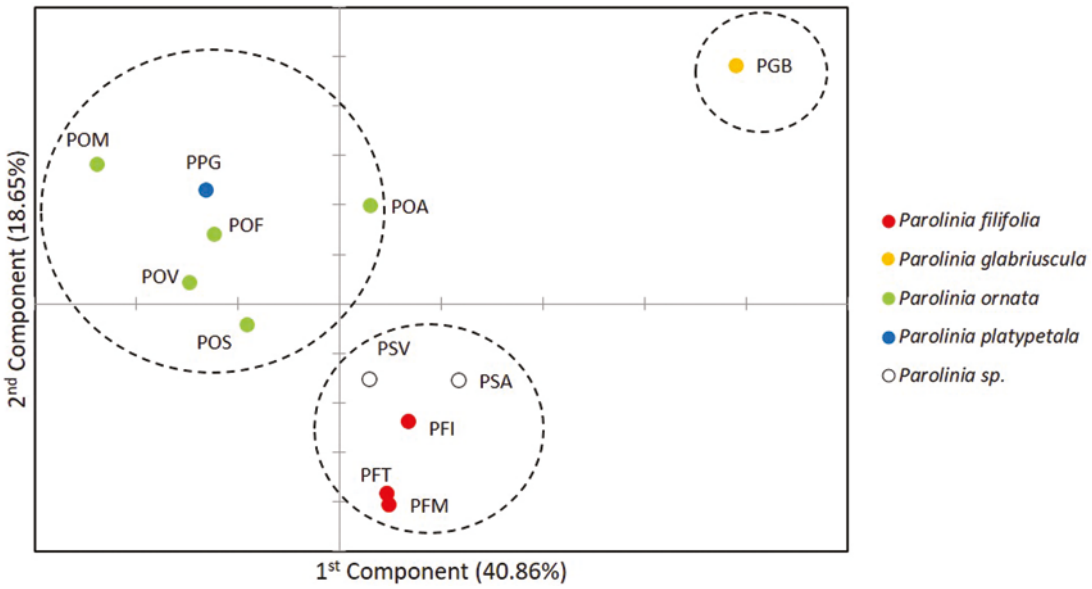

Figure 2. PCoA analyses based on allele frequency of eight scored microsatellite loci genotypes on populations of *Parolinia* sampled. The percentage of explained variance of each axis is given in parenthesis. Populations are coded as in Table 1. Dashed circles represent the three clusters inferred by Bayesian cluster analysis.

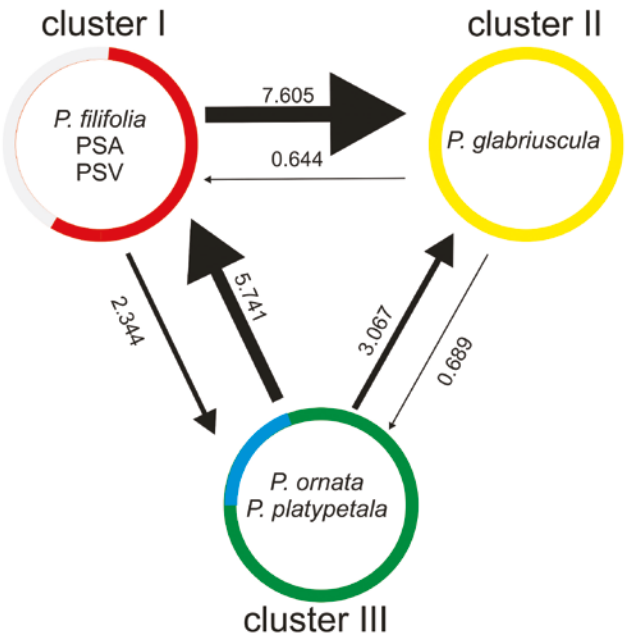

Figure 3. MIGRATE estimates of historical migration rates between the genetic clusters of *Parolinia* species identified by STRUCTURE. The thickness of the arrows and the numbers denote migration rates from one cluster to another. The colours in the circles represent the proportion of each taxon in the groups inferred by STRUCTURE.

Veneguera (PSV) showed genetic evidence of a hybrid origin.

Furthermore, the NEWHYBRIDS analysis retrieved ten individuals from PSA (Agaete) and one from PSV (Veneguera) as *P. filifolia* (PP > 0.90) and identified four individuals from either population as probable *P. filifolia* (0.75 < PP < 0.90). Notably, no individual of these two taxonomically doubtful populations was identified as *P. ornata*.

NEWHYBRIDS identified 82 of the 99 individuals analysed in *P. ornata* (83%) as pure (PP > 0.75). However, only 41% (24 of 59) of the individuals of *P. filifolia* analysed were assigned with a high posterior probability to this taxon. The other 12 individuals from *P. filifolia* populations (20%) were identified as F_2 hybrids.

The plot of proportion memberships (STRUCTURE) against the PP of falling in the pooled hybrid class (NEWHYBRIDS) showed a typical unimodal distribution, suggesting that both analytical results are strongly correlated (Supporting Information, Fig. S2). In addition, Pearson correlation analysis showed a highly significant correlation between both analysis ($r = 0.952$; $P > 0.0001$). Overall, all populations showed low hybridity values (PP < 0.5), except for Veneguera (PSV; PP > 0.6).

Most populations showed a high proportion of membership ($qi > 0.8$) to one of the clusters resolved by STRUCTURE. Only POA (Arguineguín) showed a qi value < 0.8; however, the posterior probability of belonging to the hybrid pool inferred by NEWHYBRIDS was low (PP < 0.2). Importantly, POA was the only population with individuals of both *P. filifolia* (30%) and *P. ornata* (55%), as well as putative hybrids (15%).

In general, except for the POA population, *P. ornata* displayed a highly cohesive genetic pool, since it showed a high proportion of membership to one group and a low PP of belonging to the hybrid pool. Although *P. filifolia* exhibited a high proportion of membership to one group, it also showed clear introgression signs, since its populations displayed a substantial hybrid inheritance. Several individuals could be considered hybrids, having a high PP for belonging to the hybrid pool and signs of admixture between the two clusters considered (Supporting Information, Fig. S2).

DISCUSSION

Genetic diversity

In general, higher genetic diversity associated with widespread distributions on islands is explained by the accumulation of neutral genetic variation by mutation, immigration and recombination (García-Verdugo *et al.*, 2015). Overall, *Parolinia* spp. studied here showed average genetic diversity values similar to those of other endemic plant species from the Canary Islands (Sosa *et al.*, 2013) and higher values than those described for endemic species ($H_e = 0.420$; Nybom, 2004); the two species with a wider distribution

(*P. filifolia* and *P. ornata*) show higher genetic diversity values (H_e = 0.603 and H_e = 0.600, respectively) than the two narrowly distributed ones (*P. glabriuscula* and *P. platypetala*; H_e = 0.417 and H_e = 0.484, respectively). In addition, the higher proportion of genetic variation in *P. ornata* and *P. filifolia* was found within populations (86.97% and 81.96%, respectively, Supporting Information, Table S1), in agreement with expectations for predominantly outcrossing species (Hamrick & Godt, 1990; Fernández-Palacios, 2010). In line with García-Verdugo *et al.* (2015), the overall high levels of variation detected in *Parolinia* provide additional evidence against the long-standing idea that island populations should be less genetically diverse than mainland populations, largely stemming from studies on animals [see e.g. White & Searle (2007)].

The three populations of *Parolinia* with higher genetic diversity (PSA, POA and PSV) were those where hybridization was detected, and two of them (PSA and POA) are localized in the older part of Gran Canaria (Palaeocanaria). A generalized hybridization context also helps explain that in some cases populations in Neocanaria exhibit higher levels of genetic variation than expected from their recent origin alone [Puppo *et al.* (2016); Curto *et al.* (2017); see also Caujapé-Castells *et al.* (2017) for more general context).

The three main clusters (*P. glabriuscula*, *P. filifolia* and *P. ornata*-*P. platypetala*) detected by the PCoA and STRUCTURE analyses (Figs 1, 2) coincide with the groups described by Fernández-Palacios (2010) on the basis of a single morphological reproductive character (open flower, closed flower and intermediate flower). Sixty-five alleles out of 94 (69%) were shared by all sampled *Parolinia* populations, which suggests close genetic relationships between them. In addition, both the absence of barriers and the high percentage of shared alleles (82%) between *P. ornata* and *P. platypetala* suggest that these taxa have diverged recently. Therefore, they may represent the early stages of an incipient speciation process in *Parolinia* on Gran Canaria, because the contemporary migration rate estimate (m = 0.071) still supports the existence of ongoing gene flow from *P. ornata* to *P. platypetala*.

The convergence of high allozyme genetic differentiation and low genetic diversity in *P. glabriuscula* was hypothesized to stem from a recent origin of this taxon (Fernández-Palacios, 2010), currently restricted to the crater of the Bandama volcanic complex, formed after the most recent Holocene eruption in Gran Canaria (< 2000 years BP, Carracedo & Troll, 2016). Alternatively, it could be feasible that this species represents a relict of a more widely distributed taxon that colonized the Bandama crater after the eruption and subsequently disappeared from its more pristine distribution areas. Our estimates of historical migration rates do not support the latter possibility, as they detected considerable gene flow from cluster I (*P. filifolia* + PSA + PSV; m = 7.605) and cluster III (*P. ornata* + *P. platypetala*; m = 3.067) to cluster II (*P. glabriuscula*), but only a limited migration rate between cluster II (where *P. glabriuscula* belongs) and clusters I and III (m = 0.644 and m = 0.689, respectively) (Fig. 3).

Furthermore, *P. glabriuscula* has no exclusive alleles, and its genetic make-up is a subset of that of its congeners from Gran Canaria. Finally, evidence of a bottleneck was detected in this population, which is also compatible with a founder effect. On the basis of these findings, we hypothesize that *P. glabriuscula* is a recent taxon that shares a close ancestor with *P. filifolia*, the genetic footprint of which is also significantly present in the populations of Agaete (PSA) and Bco. Arguineguín (PFA) (see below).

Past hybridization in *Parolinia* on Gran Canaria

The taxonomically doubtful populations of *Parolinia* in Gran Canaria (PSA and PSV) had been earlier described as *P. ornata* (Kunkel, 1969; Bramwell & Bramwell, 2001), but their morphological differences with respect to that species led to the suggestion of a thorough taxonomic reassessment (Fernández-Palacios, 2010). While the needed review develops, PCoA, STRUCTURE and AMOVA analyses of our microsatellite data unequivocally suggest that both populations should be considered genetically as *P. filifolia*. In addition, NEWHYBRIDS detected that 40% of the individuals analysed in Veneguera (PSV) show genetic evidence of hybridization.

Although a barrier to gene flow was detected between the only population of *P. glabriuscula* and the other species (Fig. 1), the Bayesian cluster analysis reveals an unexpectedly close relationship among *P. glabriuscula*, *P. filifolia* from Agaete and, especially, *P. ornata* from Bco. Arguineguín (Fig. 1), with which it has the lowest pairwise population genetic differentiation coefficients (R_{ST} = 0.069 and R_{ST} = 0.188, respectively; Supporting Information, Table S2). Notably, the populations from Agaete (PSA) and Arguineguín (POA) have the highest genetic diversities (H_e = 0.704 and H_e = 0.648, respectively).

On the whole, these results suggest that these populations share a recent ancestor, and they were also affected by hybridization, especially in the case of Arguineguín (see Supporting Information, Fig. S2, and below). Indeed, Arguineguín is the only population of *P. ornata* that showed a positive value in the first component of the PCoA (Fig. 2), a result that could reflect its localization in the contact zone between *P. ornata* and *P. filifolia*. In agreement with these findings, NEWHYBRIDS detected hybrid individuals

in this population, and putative *P. filifolia* individuals. In addition, the INEST software detected significant heterozygosity excess, in line with the expected results for populations that possess different allele frequencies in adjacent regions of allopatry (Kulikova *et al.*, 2004; Zalapa *et al.*, 2010), thus further supporting the influence of hybridization in Arguineguín.

The populations of Agaete (PSA, *P. filifolia*), Bandama (*P. glabriuscula*) and Bco. Guayadeque (*P. platypetala*) are localized in the youngest parts of Gran Canaria (Neocanaria) (Fig. 1). Agaete is much closer to other *P. filifolia* in the PCoA analysis (Fig. 2), probably due to a recent colonization (the migration rate from *P. filifolia* to PSA is m = 0.216, Supporting Information, Table S3), which is bound to have occurred after the tsunami originated by a giant landslip on the east coast of Tenerife, that flooded the whole Agaete valley *c*. 0.84 Mya (Pérez-Torrado *et al.*, 2006).

Hybridization and introgression can lead to diversification and speciation (Arnold *et al.*, 1991; Rieseberg *et al.*, 2003; Baack & Rieseberg, 2007; Fogelqvist *et al.*, 2015), and these processes may help explain the apparent monophyly in many Canarian plant radiations (Herbén *et al.*, 2005; Saunders & Gibson, 2005). As laid out in the surfing syngameon hypothesis (Caujapé-Castells, 2011; Caujapé-Castells *et al.*, 2017), hybridization and introgression also underlie many diversification processes on oceanic islands. Briefly, this hypothesis posits a landscape of frequent migration among and within islands, where genetic diversity is shaped by alternating constructive and destructive cycles in the geological ontogeny of the islands, that foster (a) gene flow, hybridization and introgression leading to the formation of syngameons (i.e. populations with high genetic diversity) and (b) gene flow suppression conducive to genetic diversity loss and speciation, respectively.

Several major volcanic upheavals have occurred throughout the geological ontogeny of Gran Canaria (Fig. 1): the Roque Nublo vulcanism *c*. 4.4-3.4 Mya, that affected a large part of the central part of the island (MacDougall & Schmincke, 1976); more recently, the flooding of the Agaete valley as a consequence of a giant landslip in the valley of Güímar in Tenerife (*c*. 0.84 Mya, Pérez-Torrado *et al.*, 2006), and the formation of the Bandama crater (barely 2000 years BP, Carracedo & Troll, 2016). Consequently, extreme biotic range contractions (or extinctions) possibly happened in these regions, which could be of especial relevance in explaining the origins and current distribution of *Parolinia* and of other genetically distinctive species in Neocanaria.

If there is a relationship between the geological ontogeny of Gran Canaria and the diversification processes in *Parolinia*, we should detect an overall prevalence of ancestral gene flow and hybridization in Palaeocanaria and of contemporary gene flow in the regions of Neocanaria facilitated by the recent removal of earlier barriers to gene flow, or by the appearance of new distribution areas.

Do our genetic data fit these expectations? We detected hybrids not only in many contact zones between *P. ornata* and *P. filifolia* (see above) but also in secluded populations that are not yet genetically isolated, tentatively as a consequence of the high ancestral migration rates (Fig. 3) and subsequent hybridization (Supporting Information, Fig. S2). Indeed, the population from Arguineguín (POA), described as *P. ornata* and localized in the contact zone of this species and *P. filifolia* (Fig. 1) also showed 15% of hybrids, despite the absence of taxonomic conflict. All these were classified as F_2 individuals, which is often the case in relatively old contact zones where many generations of backcrossing complicate a precise assignment of individuals to specific hybrid classes (Arntzen *et al.*, 2016). In addition, this population showed the highest genetic diversity (H_e = 0.648, Table 1). Thus, the formation of hybrids in the contact zone may have contributed to: (i) the increase of genetic variation, facilitating adaptation to changing conditions, (ii) ecological niche shifts and (iii) range shifts after secondary contact, as described in *Micromeria hyssopifolia* Webb & Berthel. (Lamiaceae, Puppo *et al.*, 2016). Accordingly, we believe that conservation management in *Parolinia* should not only focus on pure populations of the endemics, but also consider those in the contact zones where these evolutionary processes are developing.

Barriers to hybridization between species are usually weak on oceanic islands (Carr, 1985; Crawford *et al.*, 1987; Herbén *et al.*, 2005; Saunders & Gibson, 2005). Consistent with this expectation, NEWHYBRIDS and STRUCTURE analyses show the hybrid nature of many of the individuals assessed, not only in the taxonomically conflictive populations (Agaete and Veneguera), but also in other populations of *P. filifolia* and *P. ornata*, thus suggesting an overall relevance of hybridization and, presumably, introgression. The detected lack of full reproductive isolation strongly suggests a considerable permeability of species boundaries in *Parolinia* (Fig. 3); most of the hybridization events probably happened from the most widespread species (*P. ornata*) to the less widespread species (*P. filifolia*), but the narrowly distributed *P. platypetala* and *P. glabriuscula* may also have contributed.

Conversely, recent or contemporary migration is low (Supporting Information, Table S3), thus intimating that only ancestral hybridization must have been important in generating the great genetic diversity detected in most populations (Table 1), a finding that had remained elusive to detection and assessment

until this investigation. The clear contrast between ancestral and contemporary migration rates is also relevant to construe the genetic structure of *Parolinia* in connection with the complex geological ontogeny of this island.

In line with the predictions of the surfing syngameon hypothesis [Caujapé-Castells *et al.* (2017), and above], our data strongly suggest that two kinds of processes seem to have stimulated the current genetic structure of *Parolinia* in Gran Canaria and its high population genetic diversity [Table 1; see also Fernández-Palacios *et al.* (2004, 2006) (allozymes)].

On the one hand, ancestral hybridization (Fig. 3) probably followed by introgression fostered the generation of high population genetic diversity in most distribution areas (especially in Palaeocanaria, but also in some regions of Neocanaria), which was further enhanced by migration over intermediate or long distances (Fig. 3), thus facilitating range expansion. Seeds of *Parolinia* have several morphological features to enable wind dispersal (see Material and Methods), which probably aided inter-population gene flow and colonization of new areas as they became available. These processes leading to expansion may have encompassed the current distribution ranges of *P. ornata* and *P. filifolia* (Palaeocanaria, Fig. 1), where admixture could increase especially during periods of geological stasis.

The absence of detectable hybridization in some populations adjacent to contact zones between species (e.g. *P. ornata* POM; Fig.1) suggests that vicariance and dispersal events during the more recent ontogenetic episodes of the island probably set the stage for two non-exclusive processes in both Palaeo- and Neocanaria: (i) the appearance of new geographical or ecological discontinuities determining barriers to gene flow in the original ranges of *P. ornata* and *P. filifolia* (see the low values of contemporary migration rates in Supporting Information, Table S3); and (ii) the colonization of new distribution ranges as they became available (feasibly the cases of *P. glabriuscula* and *P. platypetala*).

Finally, although this investigation has allowed us to relate the geological ontogeny of Gran Canaria to major events in the diversification of *Parolinia* and to dispel some morphological and taxonomical conflicts, extreme caution has to be used with any inference until the sequencing analysis and divergence time estimates under way provide a robust phylogenetic hypothesis for all the species in this Canarian endemic genus.

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REFERENCES

Allendorf FW, Leary RB, Spruell P, Wenburg JK. 2001. The problems with hybrids: setting conservation guidelines. *Trends in Ecology and Evolution* **16:** 613–622.

Anderson CL, Channing A, Zamuner AB. 2009. Life, death and fossilization on Gran Canaria – implications for Macaronesian biogeography and molecular dating. *Journal of Biogeography* **36:** 2189–2201.

Anderson E, Hubricht L. 1938. Hybridization in *Tradescantia*: III. The evidence for introgressive hybridization. *American Journal of Botany* **25:** 396–402.

Anderson EC, Thompson EA. 2002. A model-based method for identifying species hybrids using multilocus genetic data. *Genetics* **160:** 1217–1229.

Arnold ML, Buckner CM, Robinson JJ. 1991. Pollen-mediated introgression and hybrid speciation in Louisiana irises. *Proceedings of the National Academy of Sciences of the United States of America* **88:** 1398–1402.

Arntzen JW, Trujillo T, Butôt R, Vrieling K, Schaap O, Gutiérrez-Rodríguez J, Martínez-Solano I. 2016. Concordant morphological and molecular clines in a contact zone of the common and spined toad (*Bufo bufo* and *B. spinosus*) in the northwest of France. *Frontiers in Zoology* **13:** 52.

Baack EJ, Rieseberg LH. 2007. A genomic view of introgression and hybrid speciation. *Current Opinion in Genetics and Development* **17:** 51318.

Bañares Á, Blanca G, Güemes J, Moreno JC, Ortiz S, eds. 2004. *Atlas y Libro Rojo de la flora vascular amenazada de España*. Madrid: Dirección General de Conservación de la Naturaleza.

Beerli P, Felsenstein J. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in *n* subpopulations by using a coalescent approach. *Proceedings of the National Academy of Sciences* **98:** 4563–4568.

Beerli P, Palczewski M. 2010. Unified framework to evaluate panmixia and migration direction among multiple sampling locations. *Genetics* **185:** 313–326.

Bramwell D. 1986. Contribución a la biogeografía de las Islas Canarias. *Botánica Macaronésica* **14:** 3–34.

Bramwell D, Bramwell Z. 2001. *Flores silvestres de las Islas Canarias*. Madrid: Editorial Rueda.

Campton DE. 2000. Natural hybridization and introgression in fishes: methods of detection and genetic interpretations. In: Ryman N, Utter F, eds. *Population genetics and fishery management*. Seattle: University of Washington Press, 161–192.

Carracedo JC, Day S. 2002. *Canary Islands. Classic geology in Europe series. Vol.* **4**. Harpenden: Terra Publishing.

Carracedo JC, Troll V. 2016. *The geology of the Canary Islands*. Amsterdam: Elsevier.

Carson H, Clague D. 1995. Geology and biogeography of the Hawaiian Islands. In: Wagner WL, Funk VA, eds. *Hawaiian biogeography: evolution on a hot spot archipelago*. Washington: Smithsonian Institution Press.

Caujapé-Castells J. 2011. Jesters, red queens, boomerangs and surfers: a molecular outlook on the diversity of the Canarian endemic flora. In: Bramwell D, Caujapé-Castells J, eds. *The biology of island floras*. Cambridge: Cambridge University Press, 284–324.

Caujapé-Castells J, Castellano JJ, Ramos R, Henríquez V, Sabbagh I, Quintana-Trujillo FM, Rodríguez JF. 2013. Transformer-4 version 2.0.1, a free multi-platform software to quickly reformat genotype matrices of any marker type, and archive them in the Demiurge information system. *Molecular Ecology Resources* **13:** 484–493.

Caujapé-Castells J, García-Verdugo C, Marrero-Rodríguez Á, Fernández-Palacios JM, Crawford DJ, Mort ME. 2017. Island ontogenies, syngameons, and the origins and evolution of genetic diversity in the Canarian endemic flora. *Perspectives in Plant Ecology, Evolution and Systematics* **27:** 9–22.

Carr GD. 1985. Monograph of the Hawaiian Madiinae (Asteraceae): *Argyroxiphium*, *Dubautia*, and *Wilkesia*. *Allertonia* **4:** 1–123.

Chybicki IJ, Burczyk J. 2009. Simultaneous estimation of null alleles and inbreeding coefficients. *Journal of Heredity* **100:** 106–113.

Crawford DJ, Whitkus R, Stuessy TF. 1987. Plant evolution and speciation on oceanic islands. In: Urbanska, K. ed. *Patterns of differentiation in higher plants*. London: Academic Press, 183–199.

Curto M, Puppo P, Kratschmer S, Meimberg H. 2017. Genetic diversity and differentiation patterns in *Micromeria* from the Canary Islands are congruent with multiple colonization dynamics and the establishment of species syngameons. *BMC Evolutionary Biology* **17:** 198.

Dakin EE, Avise JC. 2004. Microsatellite null alleles in parentage analysis. *Heredity* **93:** 504–509.

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin, Botanical Society of America* **19:** 11–15.

Earl DA, vonHoldt BM. 2012. Structure harvester: a website and program for visualizing structure output and implementing the Evanno method. *Conservation Genetics Resources* **4:** 359–361.

Excoffier L, Heckel G. 2006. Computer programs for population genetics data analysis: a survival guide. *Nature Reviews Genetics* **7:** 745–758.

Excoffier L, Smouse PE, Quattro JM. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes-application to human mitochondrial DNA restriction data. *Genetics* **131:** 479–491.

Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology* **14:** 2611–2620.

Febles R. 1989. Estudios de la flora Macaronésica: algunos números de cromosomas VI. *Botánica Macaronésica* **17:** 57–76.

Feder JL, Flaxman SM, Egan SP, Comeault AA, Nosil P. 2013. Geographic mode of speciation and genomic divergence. *Annual Review of Ecology, Evolution, and Systematics* **44:** 73–97.

Fernández-Palacios O. 2010. *Biodiversidad morfológico-reproductiva y genética del género endémico canario* Parolinia *Webb (Brasicaceae)*. PhD Thesis, Universidad de Las Palmas de Gran Canaria.

Fernández-Palacios O, Pérez de Paz J, Febles R, Caujapé-Castells J. 2004. Diversidad genética en *Parolinia*: *P. glabriuscula* y *P. platypetala* (Brassicaceae: Matthioelae). *Botánica Macaronésica* **25:** 147–164.

Fernández-Palacios O, Pérez de Paz J, Febles R, Caujapé-Castells J. 2006. Duplicaciones y diversidad genética de *Parolinia ornata* (Brassicaceae: Matthioelae) endemismo de Gran Canaria en relación a dos congéneres más restringidos y otros taxones isleños y continentales. *Botánica Macaronésica* **26:** 19–54.

Fogelqvist J, Verkhozina AV, Katyshev AI, Pucholt P, Dixelius C, Rönnberg-Wästljung AC, Lascoux M, Berlin S. 2015. Genetic and morphological evidence for introgression between three species of willows. *BMC Evolutionary Biology* **15:** 193.

García-Verdugo C, Sajeva M, La Mantia T, Harrouni C, Msanda F, Caujapé-Castells J. 2015. Do island plant populations really have lower genetic variation than mainland populations? Effects of selection and distribution range on genetic diversity estimates. *Molecular Ecology* **24:** 726–741.

Gobierno de Canarias. *Banco de Datos de Biodiversidad de Canarias*. Available at: http//:www.biodiversidadcanarias.es\biota. Accessed August, 2020.

González-Pérez MA, Caujapé-Castells J. 2014. Development and characterization of nuclear microsatellite markers for *Parolinia ornata* Webb (Brassicaceae), and cross-species amplification in all species described in the Canarian endemic genus *Parolinia*. *Conservation Genetics Resources* **6:** 705–706.

González-Pérez MA, Sosa PA. 2009. Hybridization and introgression between the endemic *Phoenix canariensis* and the introduced *P. dactylifera* in the Canary Islands. *The Open Forest Science Journal* **2:** 78–85.

Hamrick JL, Godt MJW. 1990. Allozyme diversity in plant species. In: Brown HD, Clegg MT, Kahler AL, Weir BS, eds. *Plant population genetics, breeding and genetic resources*. Sunderland: Sinauer.

Hardy OJ, Vekemans X. 2002. SPAGeDi: a versatile computer program to analyse spatial genetic structure at

the individual or population levels. *Molecular Ecology Notes* **2:** 618–620.

Harrison RG. 1990. Hybrid zones: windows on evolutionary process. *Oxford Surveys in Evolutionary Biology* **7:** 69–128.

Harrison RG, Larson EL. 2014. Hybridization, introgression, and the nature of species boundaries. *Journal of Heredity* **105:** 795–809.

Herben T, Suda J, Munclinger P. 2005. The ghost of hybridization past: niche pre-emption is not the only explanation of apparent monophyly in island endemics. *Journal of Ecology* **93:** 572–575.

IUCN. 2012. *IUCN red list of threatened species. Version 2012.1.* World Conservation Union. Available at: http://www.iucnredlist.org

Kulikova IV, Zhuravlev YN, McCracken KG. 2004. Asymmetric hybridization and sex-biased gene flow between eastern spot-billed ducks (*Anas zonorhyncha*) and mallards (*A. platyrhynchos*) in the Russian Far East. *The Auk* **121:** 930–949.

Kunkel G. 1969. Sobre la distribución de *Parolinia ornata* (Cruciferae) en Gran Canaria. *Cuadernos de Botánica Canaria* **7:** 1–4.

MacDougall I, Schmincke HU. 1976. Geochronology of Gran Canaria, Canary Islands: age of shield building volcanism and other magmatic phases. *Bulletin of Volcanology* **40:** 1–21.

Mairal M, Sanmartín I, Aldasoro JJ, Culshaw V, Manolopoulou I, Alarcón M. 2015. Palaeo-islands as refugia and sources of genetic diversity within volcanic archipelagos: the case of the widespread endemic *Canarina canariensis* (Campanulaceae). *Molecular Ecology* **24:** 3944–3963.

Manni F, Guerard E, Heyer E. 2004. Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by using Monmonier's algorithm. *Human Biology* **76:** 173–190.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J. 2000. Biodiversity hotspots for conservation priorities. *Nature* **403:** 853–858.

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89:** 583–590.

Nybom H. 2004. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology* **13:** 1143–1155.

Peakall R, Smouse PE. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6:** 288–295.

Pérez-Torrado FJ, Paris R, Cabrera MDC, Schneider JL, Wassmer P, Carracedo JC, Rodríguez-Santana A, Santana F. 2006. Tsunami deposits related to flank collapse in oceanic volcanoes: the Agaete Valley evidence, Gran Canaria, Canary Islands. *Marine Geology* **227:** 135–149.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155:** 945–959.

Puppo P, Curto M, Meimberg H. 2016. Genetic structure of *Micromeria* (Lamiaceae) in Tenerife, the imprint of geological history and hybridization on within-island diversification. *Ecology and Evolution* **6:** 3443–3460.

Reyes-Betancort A, Santos-Guerra A, Guma R, Humphries CJ, Carine M. 2008. Diversity, rarity and the evolution and conservation of the Canary Islands endemic flora. *Anales del Jardín Botánico de Madrid* **65:** 25–45.

Rieseberg LH, Raymond O, Rosenthal DM, Lai Z, Livingstone K, Nakazato T, Durphy JL, Schwarzbach AE, Donovan LA, Lexer C. 2003. Major ecological transitions in wild sunflowers facilitated by hybridization. *Science* **301:** 1211–1216.

Rodríguez-González A, Pérez-Torrado FJ, Fernández-Turiel JL, Aulinas M, Paris R, Moreno-Medina C. 2018. The Holocene volcanism of Gran Canaria (Canary Islands, Spain). *Journal of Maps* **14:** 620–629.

Saro I, García-Verdugo C, González-Pérez MA, Naranjo A, Santana A, Sosa PA. 2018. Genetic structure of the Canarian palm tree (*Phoenix canariensis*) at the island scale: does the 'island within islands' concept apply to species with high colonisation ability? *Plant Biology* **21:** 101–119.

Saro I, González-Pérez MA, García-Verdugo C, Sosa PA. 2015. Patterns of genetic diversity in *Phoenix canariensis*, a widespread oceanic palm (species) endemic from the Canarian archipelago. *Tree Genetics & Genomes* **11:** 815.

Saunders NH, Gibson DJ. 2005. Breeding system, branching processes, hybrid swarm theory, and the humped-back diversity relationship as additional explanations for apparent monophyly in the Macaronesian island flora. *Journal of Ecology* **93:** 649–652.

Silva LB, Sardos J, de Sequeira MM, Silva L, Crawford D, Moura M. 2016. Understanding intra and inter-archipelago population genetic patterns within a recently evolved insular endemic lineage. *Plant Systematics and Evolution* **302:** 367–384.

Sosa PA, González-Pérez MÁ, González-González EA, Rivero E. 2013. Genetic diversity of Canarian endemisms revealed by microsatellites: knowledge after one decade of analysis. In: Caujapé-Castells J, Nieto Feliner G, Fernández-Palacios JM, eds. *Proceedings of the Amurga international conferences on island biodiversity*. Maspalomas: Fundación Canaria Amurga, 94–100.

White TA, Searle JB. 2007. Genetic diversity and population size: island populations of the common shrew *Sorex araneus*. *Molecular Ecology* **16:** 2005–2016.

Wilson GA, Rannala B. 2003. Bayesian inference of recent migration rates using multilocus genotypes. *Genetics* **163:** 1177–1191.

XLSTAT. 2004. *Statistical software, version 7.5.2 for Microsoft Excel*. New York: Addinsoft Inc.

Zalapa JE, Brunet J, Guries RP. 2010. The extent of hybridization and its impact on the genetic diversity and population structure of an invasive tree, *Ulmus pumila* (Ulmaceae). *Evolutionary Applications* **3:** 157–168.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site:

Table S1. Results of hierarchical analysis of molecular variance (AMOVA) conducted at two hierarchical levels considered. The test of significance was based on 1023 permutations.
Table S2. Pairwise differentiation (R_{ST}) between the 12 *Parolinia* populations analysed.
Table S3. Contemporary migration rates between *Parolinia* species calculated by BAYESASS. Individuals from species on the left originated from species on the top. Values for migration within taxa are highlighted in grey. Values > 0.05 are represented in bold. Standard deviation values are in parentheses.
Figure S1. ΔK calculated by the Evanno method. The test was performed with all *Parolinia* populations from K = 1 to K = 12.
Figure S2. Dot chart of the proportion memberships identified by STRUCTURE (*x*-axis) vs. the posterior probabilities of belonging to the hybrid pooled class inferred by NEWHYBRIDS (*y*-axis) of *P. ornata* (POS, POF, POM, POA and POV), *P. filifolia* (PFI, PFM and PFT) and the putative hybrid populations (PSA and PSV) analysed. Data points represent individuals (solid round circles) or population means (open round circles).