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RESEARCH PAPER

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?

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ABSTRACT

- Oceanic islands are dynamic settings that often promote within-island patterns of strong population differentiation. Species with high colonisation abilities, however, are less likely to be affected by genetic barriers, but island size may impact on species genetic structure regardless of dispersal ability.
- The aim of the present study was to identify the patterns and factors responsible for the structure of genetic diversity at the island scale in Phoenix canariensis, a palm species with high dispersal potential. To this end, we conducted extensive population sampling on the three Canary Islands where the species is more abundant and assessed patterns of genetic variation at eight microsatellite loci, considering different within-island scales.
- Our analyses revealed significant genetic structure on each of the three islands analysed, but the patterns and level of structure differed greatly among islands. Thus, genetic differentiation fitted an isolation-by-distance pattern on islands with high population densities (La Gomera and Gran Canaria), but such a pattern was not found on Tenerife due to strong isolation between colonised areas. In addition, we found a positive correlation between population geographic isolation and fine-scale genetic structure.
- This study highlights that island size is not necessarily a factor causing strong population differentiation on large islands, whereas high colonisation ability does not always promote genetic connectivity among neighbouring populations. The spatial distribution of populations (*i.e.* landscape occupancy) can thus be a more important driver of plant genetic structure than other island, or species′ life-history attributes.

INTRODUCTION

Understanding the genetic structure of wild populations has been a long-standing focus of biological research, since it results from the interaction of some of the main forces that drive evolution (Loveless & Hamrick 1984). Thus, the differentiation observed in neutral markers across a spatial range of populations is determined by the balance between migration and genetic drift, which in turn are influenced by the effective sizes, spatial distribution and degree of geographic/ecological isolation of populations, in addition to life-history traits such as mating system (Epperson 2003; Petit & Hampe 2006). In geographic areas where effective long-distance dispersal is severely limited by habitat suitability, including multi-island systems, the patterns of gene flow are particularly affected by the spatial scale considered. At broad scales, the intuitive assumption that genetic variation is more structured between than within islands has received considerable support over the years, and relies on the notion that over-water dispersal is less frequent than local, within-island dispersal in species with widespread distributions (Nielsen 2004; Bottin *et al.* 2005; García-Verdugo *et al.* 2017; Jaros *et al.* 2018; but see García-Verdugo *et al.* 2014 for discussions on dispersal syndromes and genetic structure).

Nevertheless, despite their often small geographic size, oceanic islands typically contain a wide variability of spatio-temporal restrictions to gene flow in the form of geographic and/or ecological barriers that may increase the genetic structure of populations. Extinction–recolonisation dynamics due to catastrophic events (Gómez *et al.* 2003; García-Verdugo *et al.* 2010; Mairal *et al.* 2015), oscillation in sea level caused by climate changes (Mayol *et al.* 2012), internal physical barriers (Nielsen 2004) or human impacts (Muller *et al.* 2009) modify the shape, size and connections of plant populations, thus restricting intraspecific gene flow and enhancing the divergence of populations at the within-island scale. The concept of 'islands within islands' defines well these situations, but research on this topic has focused on island species with substrate restrictions (*e.g.* Jestrow *et al.* 2012; Crawford & Stuessy 2016), for which limitations in gene flow are clearly associated with habitat preferences.

In plants, life form is thought to be another trait that significantly affects species′ population structure (Loveless & Hamrick 1984). For instance, trees usually display a predominantly outcrossed mating system, mechanisms for long-distance dispersal and large population sizes, which often result in high levels of intra-population genetic diversity coupled with a relatively weak population genetic structure (Petit & Hampe 2006). From this perspective, trees with widespread distributions in island systems may counter the idea of low genetic variation associated with islands (Payn *et al.* 2008; García-Verdugo *et al.* 2015). However, because large-scale studies typically involve limited sampling effort on particular islands, genetic information to hand is not sufficiently robust to predict whether tree species with high colonisation abilities may overcome strong within-island barriers (Slatkin 2005).

The Canary Islands are a good geographic framework in which to address questions about patterns of genetic variation and the importance and extent of genetic drift and gene flow as drivers of evolutionary divergence between populations. This archipelago comprises diverse island sizes and levels of orographic complexity, as well as being affected by historical geological processes that determine different spatio-temporal frameworks of within-island isolation (Gómez *et al.* 2003; Mairal *et al.* 2015). Due to these facts and following previous studies, an excellent candidate in which to investigate genetic patterns across geographic scales is the widespread, endemic palm, *Phoenix canariensis* H. Wildpret (*Arecaceae*; Rivera *et al.* 2013). Thus far, molecular studies on *P. canariensis* have revealed that oceanic barriers play an important role in the genetic structuring of populations at broad (*e.g.* archipelago) scales, although a substantial degree of genetic variation is also suspected at the within-island scale (Saro *et al.* 2015). According to previous phylogeographic analyses, the central islands represent the area of the archipelago with larger numbers of populations and substantial levels of genetic diversity in comparison to peripheral islands (Saro *et al.* 2015), thus providing an interesting framework for addressing patterns of within-island variation using replicated sampling units. Furthermore, despite large potential for pollen dispersal, recent results have shown that seed dispersal could be more limited than expected for a fleshy-fruited species, with small populations exhibiting clear patterns of fine-scale spatial genetic structure (Saro *et al.* 2014). Taken together, the available information suggests that, apart from oceanic barriers, intrinsic factors may account for the structuring of genetic diversity in *P. canariensis* at different scales.

In this study, we use nuclear microsatellite markers and extensive population sampling to analyse the spatial organisation of the genetic diversity of *P. canariensis* at within-island scales. With this approach, we aim at bridging the gap of most plant studies dealing with widespread insular species, in which even a limited sampling at the island scale often suggests complex patterns, most notably on large islands (Nielsen 2004; Mairal *et al.* 2015). Understanding how genetic diversity is structured within islands would provide valuable information for both conservation-oriented and biogeographic studies, particularly in oceanic archipelagos where the ontogeny of each island is thought to have an idiosyncratic imprint on its terrestrial biota (Crawford & Stuessy 2016; Caujapé-Castells *et al.* 2017). Specifically, we addressed the following aims: (i) to compare the spatial patterns of genetic diversity among *P. canariensis* island populations, and (ii) to identify the factors that may impact on the observed genetic patterns.

MATERIAL AND METHODS

Study species, sampling design and genetic analysis

Phoenix canariensis is a long-lived, arborescent palm endemic to the Canary Islands. It is a species with potential for large dispersal distances, since pollination is predominantly anemophilous and several animals consume its fleshy fruits, including *Gallotia* lizards and some passerine species (Nogales *et al.* 2016). However, *P. canariensis* bears large fruits that also may be disseminated over short distances by barochory (I Saro, personal observation). Its current natural distribution consists of patchy palm groves that formerly presented a wider distribution within the thermophilous woodland zone, most of which has been converted to agricultural use (Sosa *et al.* 2007). Furthermore, the allochthonous congener *Phoenix dactylifera* L., massively introduced into the Canarian archipelago, has threatened the genetic conservation of *P. canariensis* as they form fertile hybrids (González-Pérez *et al.* 2004). *P. canariensis* is regarded as threatened by the Canarian Government (BOC 1991), and its habitat is recognised by Annex I of the European Directive 92/43/EEC under category 'priority habitat 9370 – Palm groves of *Phoenix*'.

The present study focused on the central islands of the Canarian archipelago, where *P. canariensis* is more abundant than on peripheral islands (Del Arco 2006). The extensive occurrence of populations on the central islands provides an appropriate sample for investigating how genetic diversity is structured across nearly continuous distributions (*i.e.* under the assumption of high genetic connectivity). In addition, the central islands display substantial levels of genetic diversity, thus allowing representation of areas less likely affected by recent stochastic or human-mediated factors (*e.g.* founder effects, human introduction; see Saro *et al.* 2015). Since population sizes and the spatial distribution of *P. canariensis* individuals vary considerably between sites, we did not follow a particular sampling scheme. Rather, a similar number of adult individuals were randomly sampled in each site, but the mean distance between samples ranged from 83 to 390 m, depending on whether individuals followed a clumped or scattered distribution, respectively. Overall, leaf material from 30 to 40 individuals was collected in 24 sites from the islands of Gran Canaria (N = 9), Tenerife (N = 5) and La Gomera (N = 10), thus sample size was 935 individuals in total (see Table 1, Figure S1). Sampled specimens were also mapped with a GPS (GPSmap 76CS; Garmin, Kansas City, KA, USA) projected to Universal Transverse Mercator coordinate system (UTM) with datum WGS84, except for samples from four sites on Gran Canaria (CTF, CFA, CSO, CAT), which were collected in 2004 without individually georeference data.

Total DNA samples were extracted using the Invisorb® DNA Plant HTS 96 Kit (STRATEC Molecular, Berlin, Germany), following the manufacturer′s protocol. Subsequently, the DNA

Table 1. Sampling site code, coordinates, sample size (N), mean elevation (alt), number of neighbouring individuals within a 600-m radius around each site (Nneigh) and summary statistics of within-population genetic variation obtained with eight nuclear microsatellites in *Phoenix canariensis*: number of different alleles (N_a), number of effective alleles (N_e), number of private alleles (N_{pa}), observed heterozygosity (H_o), unbiased expected heterozygosity (H_e), fixation index (F_{IS}) following Weir & Cockerham (1984), and probability of heterozygote excess with respect mutation-drift equilibrium ($P_{He>Heq}$). Mean genetic differentiation among each site and the rest of the sites sampled on the same island is expressed as AvF_{ST}.

island	code	coordinates (N/W)	N	alt (m)	nneigh	N_a	N_e	N_{pa}	H_o (±SE)	H_e (±SE)	F_{IS}	$P_{He>Heq}$	AvF_{ST}
Gran Canaria	CAC	27°59′52″/15°41′32″	40	554	187	6.2	3.2	2	0.68 (0.10)	0.62 (0.09)	−0.107 n.s.	0.81	0.100
Gran Canaria	CAR	28°07′46″/15°32′03″	40	135	952	7.0	3.5	2	0.62 (0.09)	0.61 (0.09)	−0.008 n.s.	0.97	0.156
Gran Canaria	CTF	28°04′28″/15°27′17″	40	285	1595	7.8	4.3	2	0.70 (0.08)	0.69 (0.08)	0.008 n.s.	0.77	0.093
Gran Canaria	CTE	28°00′30″/15°28′07″	40	294	831	5.4	2.9	0	0.54 (0.11)	0.52 (0.10)	−0.031 n.s.	0.66	0.147
Gran Canaria	CSO	27°53′11″/15°32′08″	40	455	1571	5.6	2.9	0	0.57 (0.09)	0.60 (0.09)	0.053***	0.29	0.062
Gran Canaria	CST	27°54′15″/15°33′06″	40	514	2345	5.1	2.2	0	0.55 (0.08)	0.51 (0.07)	−0.074 n.s.	0.98	0.090
Gran Canaria	CAT	27°50′46″/15°34′04″	33	359	313	5.7	2.8	2	0.55 (0.09)	0.58 (0.08)	0.064**	0.87	0.079
Gran Canaria	CFA	27°52′52″/15°34′04″	40	525	1135	6.0	2.3	0	0.55 (0.09)	0.53 (0.08)	−0.029 n.s.	0.98	0.079
Gran Canaria	CMO	27°54′20″/15°41′49″	40	449	441	5.2	3.0	0	0.58 (0.11)	0.55 (0.10)	−0.061 n.s.	0.41	0.113
Tenerife	TMA	28°18′11″/16°50′20″	40	557	784	3.1	1.8	0	0.35 (0.10)	0.36 (0.10)	0.026 n.s.	0.71	0.197
Tenerife	TLS	28°21′47″/16°48′11″	40	264	172	5.1	2.9	0	0.57 (0.12)	0.52 (0.10)	−0.079 n.s.	0.63	0.159
Tenerife	TCA	28°23′50″/16°35′42″	40	80	352	3.1	2.0	1	0.40 (0.08)	0.43 (0.08)	0.082 n.s.	0.47	0.191
Tenerife	TSU	28°26′09″/16°29′14″	40	222	437	3.4	1.8	0	0.34 (0.10)	0.35 (0.10)	0.025 n.s.	0.63	0.237
Tenerife	TEC	28°31′51″/16°12′27″	40	166	355	4.9	2.6	1	0.51 (0.10)	0.50 (0.10)	0.006 n.s.	0.68	0.184
La Gomera	GVR	28°06′28″/17°19′18″	40	188	1808	5.1	2.7	0	0.52 (0.12)	0.52 (0.11)	0.001 n.s.	0.59	0.092
La Gomera	GTG	28°08′32″/17°20′31″	40	179	2018	4.6	2.9	0	0.49 (0.13)	0.49 (0.13)	0.004 n.s.	0.41	0.080
La Gomera	GTZ	28°11′14″/17°18′56″	40	367	2873	5.2	2.7	1	0.50 (0.12)	0.48 (0.12)	−0.027 n.s.	0.85	0.082
La Gomera	GLH	28°07′44″/17°17′46″	30	995	1288	4.8	2.8	0	0.55 (0.12)	0.51 (0.12)	−0.075 n.s.	0.65	0.085
La Gomera	GMA	28°10′15″/17°16′25″	40	284	1001	5.2	2.6	1	0.49 (0.12)	0.50 (0.12)	0.015 n.s.	0.95	0.080
La Gomera	GSI	28°11′22″/17°14′24″	40	357	1366	4.6	2.6	1	0.49 (0.13)	0.46 (0.12)	−0.073 n.s.	0.85	0.096
La Gomera	GHE	28°09′10″/17°12′21″	38	424	1845	4.8	2.9	0	0.46 (0.12)	0.47 (0.12)	0.016 n.s.	0.87	0.109
La Gomera	GEL	28°06′30″/17°09′02″	40	170	1073	5.7	2.9	3	0.52 (0.08)	0.53 (0.08)	0.023 n.s.	0.99	0.157
La Gomera	GJE	28°05′44″/17°11′18″	40	788	2096	4.7	2.8	0	0.47 (0.13)	0.47 (0.13)	−0.007 n.s.	0.76	0.101
La Gomera	GPA	28°03′51″/17°12′58″	34	202	2171	5.1	2.6	1	0.41 (0.11)	0.45 (0.12)	0.081 n.s.	0.92	0.114

P-value after Bonferroni correction: n.s., not significant; $^{**}P < 0.01$; $^{***}P < 0.001$; SE, standard error.

templates were amplified with eight nuclear microsatellite loci successfully assayed in a subset of samples and following the same procedure detailed in Saro *et al.* (2015). A combination of all loci was used to define an exclusive multilocus genotype for each sample.

Within-population genetic variability and differentiation estimates

We first checked if selection might be affecting allelic diversity at particular loci by conducting the coalescent-based simulation test implemented in the software LOSITAN (Antao *et al.* 2008). Following parameterisation, this approach has been shown to perform well under isolation-by-distance (IBD) patterns (Lotterhos & Whitlock 2014; García-Verdugo *et al.* 2015), which is the scenario identified for *P. canariensis* in previous analyses (Saro *et al.* 2015). Presence of null alleles was tested with Micro-Checker software (van Oosterhout *et al.* 2004). Standard genetic diversity parameters were then estimated for each sampling site using the program GenAlEx v6.5 (Peakall & Smouse 2006): number of different alleles (N_a), number of effective alleles (N_e), percentage of polymorphic loci (p), number of private alleles (N_{pa}), observed heterozygosity (H_o) and unbiased expected heterozygosity (H_e). Weir and Cockerham′s fixation index (F_{IS}) and a probability test (Fisher exact tests) of within-population deviations from Hardy-Weinberg equilibrium (HWE) were assessed as implemented in GENEPOP version 4.2 (Raymond 1995). A Bonferroni correction was applied to control for the occurrence of Type-I error. To gain insights into recent demographic processes affecting sampling sites, deviations from mutation-drift equilibrium were assessed considering the two-phase mutation model implemented in Bottleneck (Cornuet & Luikart 1996). Genetic differentiation between sites (pair-wise F_{ST}) according to Weir & Cockerham (1984) was also estimated with GENEPOP. The average F_{ST} of each site, based on pair-wise F_{ST} estimates between each site and the rest of the sampling sites of a given island, was calculated to examine the degree of population divergence experienced by each site (Mayol *et al.* 2012). FSTAT version 2.9.3 (Goudet 1995) was used to estimate global within-island genetic diversity and differentiation indices (H_o, H_e, F_{IS} and F_{ST}).

Within-island genetic structure

The genetic structure was analysed independently for each study island using different approaches. First, genetic structure was analysed using Bayesian clustering methods implemented in STRUCTURE version 2.2 (Pritchard *et al.* 2000). The program was run assuming both the admixture ancestral model and correlated allele frequencies between sites (Falush *et al.* 2003), since we expected that migration and/or common ancestry in allele frequencies could be similar across sites. First, to estimate the number of potential source populations (*K*) with maximum likelihood, ten runs of simulations were performed for each value of *K* from 1 (thus testing the null hypothesis of panmixia) to the maximum number of sampling sites on each island (*K* = 10 on La Gomera, *K* = 5 on Tenerife and *K* = 9 on Gran Canaria). We used a burn-in period and a run length for the MCMC of 1×10^5 and 1×10^6 iterations, respectively. The optimum *K* (number of true clusters in the data) was obtained following the method described in Evanno *et al.* (2005) and implemented in STRUCTURE HARVESTER (Earl & vonHoldt 2012). Second, we tested for isolation by distance (IBD) to check for the association between genetic and geographic distances. The correlation between the matrix of pair-wise linearised F_{ST} values [expressed as $F_{ST}/(1-F_{ST})$] and a log-transformed geographic distance was analysed with Mantel tests (10 000 permutations) using the IBDWS software (Jensen *et al.* 2005). Then, to highlight geographical areas at the within-island scale with pronounced genetic discontinuities between sampling sites (*i.e.* genetic barriers), we used the program BARRIER version 2.2 (Manni *et al.* 2004). The population connection into a geographic network was modelled by the Delauney triangulation (derived from the Voronoï tessellation), in which each edge is then associated with the estimated genetic distance (in our case, pair-wise Nei's values between sites; Nei 1978). Using the maximum-difference algorithm (Monmonier's algorithm), the borders between neighbouring sites that exhibited the largest genetic differences were identified (Manni *et al.* 2004). One hundred matrices of pair-wise genetic distances were bootstrapped for each island to strengthen the consistency of the boundaries detected by the algorithm. Only barriers with strong support (>75) were considered significant, but we also examined barriers with less support. Finally, a spatial analysis of molecular variance (SAMOVA) based on a simulated annealing procedure was used to define groups of sampling sites that were geographically homogeneous and maximally differentiated from each other. The program (SAMOVA 2.0; Dupanloup *et al.* 2002) iteratively seeks the composition of a user-defined number *K* of groups of geographically adjacent sites that maximises F_{CT}, *i.e.* the proportion of total genetic variance resulting from differences between groups of sites. Each *K* (from *K* = 2 to *K* = n−1, where n is the maximum number of sites sampled on each island) was iterated 10 000 times and the combination with largest F_{CT} values after the 100 independent simulated annealing processes was retained as the best grouping of sampling sites. The clustering outcomes, considering whether there were geographic constraints between sites or not, were explored.

Fine-scale spatial genetic structure (SGS)

The family structure (fine-scale SGS) within each of the sampled sites was estimated using the Kinship coefficient (F_{ij}) described in Loiselle *et al.* (1995). Genetic co-ancestry indices were averaged over a set of ten distance classes, automatically defined to contain equal numbers of pair-wise comparisons within each distance interval, and regressed over the natural logarithm of spatial distance between individuals ($\ln d_{ij}$) to calculate the regression slope (b_{log}). To obtain 95% confidence intervals around the null hypothesis of random genetic structure, 10 000 random permutations of individual locations were tested. Standard errors of observed F_{ij} and b_{log} values were calculated using 10 000 times the jackknife procedure over loci. All the spatial autocorrelation tests were performed using SPAGeDi version 1.3 (Hardy & Vekemans 2002). Bearing in mind that a moderate to low number of sampled individuals ($n \leq 40$, in our study) strongly affects the significance of kinship values, the strength of isolation by distance was additionally evaluated through the Sp statistic (Vekemans & Hardy 2004), estimated by the ratio $-b\ (1-F_1)$, where F_1 is the average kinship coefficient over the first distance interval. Samples without georeferenced data were excluded from the fine-scale SGS analysis, which resulted in the exclusion of the four sites sampled in 2004 (CTF, CFA, CSO, CAT).

Effect of abundance of *P. canariensis* across the landscape

We anticipated that levels of genetic diversity and differentiation may be related to the types of habitat occupied by *P. canariensis*. Sampling sites located in areas not associated with the most common habitat (Sosa *et al.* 2007) may enhance population structure, *e.g.* by limiting levels of gene flow due to habitat filtering. To test this idea, we estimated the number of *P. canariensis* individuals occurring around a 600-m radius from each sampling site using digital cartographical maps of geo-referenced specimens of *P. canariensis* (Figure S1) and the ARCGIS version 10 platform (ESRI, Redlands, CA, USA). Thus, relationships among the number of neighbouring individuals (Nneigh), sampling site mean elevation (alt), genetic diversity indices (H_o and H_E), mean genetic differentiation (AvF_{ST}) and estimates of fine-scale structure (Sp_{log}) were assessed using Spearman's rank correlation tests implemented in R (R Development Core Team 2008).

RESULTS

Population genetic diversity and differentiation estimates

Following an initial run of model parameterisation, the outlier-F_{ST} test conducted with LOSITAN did not identify any candidate locus affected by selection at the island level (results not shown). Evidence for null alleles or large allele dropout in the dataset was negligible, as it was only detected in 2 out of 192 tested 'loci × site' combinations. All subsequent analyses were therefore conducted with the data revealed by the eight loci assayed. Allelic diversity was generally high, with alleles among loci ranging from 4 to 28, and an average of 14.3 over all loci (results not shown). The mean number of different alleles (N_a) varied across sites, while the number of effective alleles (N_e) remained virtually constant among sites. In addition, exclusive alleles were found in some sampling sites of each island, especially in those sampled on Gran Canaria. In each site, H_o and H_e were very similar and only two populations (CSO and CAT, from Gran Canaria) showed significant departures from HW equilibrium (Table 1). Interestingly, the range of H_e values observed across Tenerife (0.355–0.524) and Gran Canaria (0.512–0.692) sites suggested smaller overall levels of genetic diversity on the former. Most sampling sites were shown to be demographically stable, since heterozygosity excess tests performed with BOTTLENECK were not significant in any case (Table 1). However, these tests detected significant heterozygosity deficiency in three sampling sites on Gran Canaria (CAR, CST, CFA) and two sites on La Gomera (GMA, GEL). Estimates of within-island genetic differentiation varied depending on the island considered (Table 1): sampling sites on Tenerife displayed the highest mean value ($AvF_{ST} = 0.198$

across all sites), whereas Gran Canaria and La Gomera exhibited the same, lower estimates ($\mathrm{AvF}_{ST} = 0.105$, in both cases). In addition, patterns of geographic distance and pair-wise genetic differentiation were not similar among islands. The most geographically distant sampling sites on La Gomera were the most genetically differentiated (pair-wise $\mathrm{F}_{ST} = 0.207$, for the GVR–GEL pair; Table S1). In contrast, the highest pair-wise genetic distances detected in the other islands were between nearby sampling sites on Tenerife (pair-wise $\mathrm{F}_{ST} = 0.331$, for the pairs TSU–TCA), and similarly, between two geographically close sites (CAR and CTE) on Gran Canaria (pair-wise $\mathrm{F}_{ST} = 0.218$).

Population genetic structure and spatial analyses

The Bayesian clustering analysis carried out independently for each island showed a more fragmented scenario for Gran Canaria ($K = 6$; Fig. 1, Figure S3) than for the other two islands (both with $K = 2$; Fig. 1, Figure S3). For Gran Canaria and Tenerife, two clusters with an east-to-west geographic pattern were detected.

Regarding the spatial patterns of the observed genetic structure, population differentiation fitted an IBD pattern on Gran Canaria (Mantel test: $r = 0.652$, $P < 0.001$) and La Gomera (Mantel test: $r = 0.780$, $P < 0.001$). However, this pattern was not detected across populations sampled on Tenerife ($r = -0.078$, $P > 0.05$). BARRIER analyses revealed significant within-island genetic structure, with at least one major barrier strongly supported on each island. Furthermore, considering a less restrictive analysis (<75 bootstrap for significant barriers), the observed pattern was highly consistent with the inferred clustering (Table S2, Fig. 1). Thus, SAMOVA revealed the following partitions: on Gran Canaria, sampling sites were grouped into six groups: 'CAR', 'CTE, 'CTF', 'CAC', 'CMO', with the remaining sites included in a single group (CST, CFA, CSO and CAT); sites from La Gomera were partitioned in three groups following an east–west progression: the easternmost site 'GEL' in its own group, 'GHE-GPA-GJE' together in a central group, and the westernmost sites all together in a single group

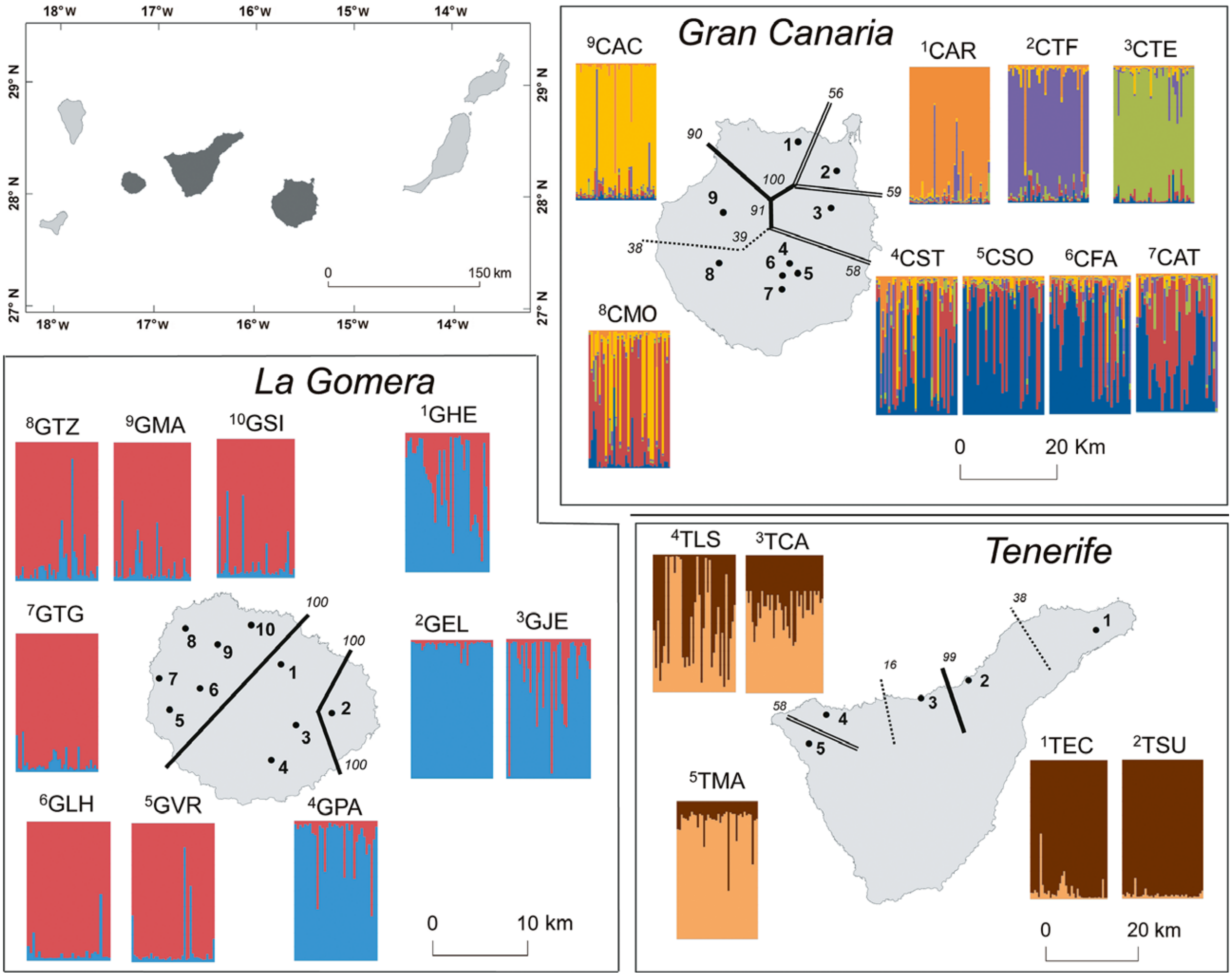

Fig. 1. Distribution of sampling sites of *P. canariensis* on the central islands of the Canarian archipelago, with spatial patterns of within-island genetic differentiation detailed. The degree of genetic isolation detected by BARRIER between neighbouring sites is indicated by different lines: black solid lines (>75% of bootstrap support), double black lines (50% <bootstrap support <75%) and dotted lines (<50% bootstrap support). For each sampling site, diagrams represent the proportional assignment of individuals to each within-island cluster, following STRUCTURE results and assuming six clusters ($K = 6$) on Gran Canaria, and two clusters ($K = 2$) each on Tenerife and La Gomera. Sites codes are indicated in Table 1.

Table 2. Fine-scale spatial genetic structure parameters within each sampling site analysed in *Phoenix canariensis* for this study: average kinship coefficient between individuals at the first distance class (F_1); regression slope of kinship coefficient values as a function of the logarithmic distance between individuals (b_{log}), Sp statistic reflecting the extent of SGS calculated as −b (1−F_1) (Sp_{blog}). We present the standard error (SE) of b (calculated by jackknifing over loci) as an estimate of the variability of Sp statistics. Site codes are the same as in Table 1.

island	code	n	F_1	b_{log}	Sp_{blog} (±SE)
La Gomera	GVR	40	0.0211 n.s.	−0.0082 n.s.	0.0084 (0.00847)
La Gomera	GTG	40	−0.0039 n.s.	−0.0054 n.s.	0.0054 (0.00427)
La Gomera	GTZ	40	0.0166 n.s.	−0.0037 n.s.	0.0038 (0.00378)
La Gomera	GLH	30	−0.0034 n.s.	0.0044 n.s.	−0.0044 (0.00248)
La Gomera	GMA	40	0.0045 n.s.	0.0011 n.s.	−0.0011 (0.00732)
La Gomera	GSI	40	−0.0105 n.s.	−0.0018 n.s.	0.0018 (0.00484)
La Gomera	GHE	38	0.0374 n.s.	−0.0070 n.s.	0.0073 (0.00437)
La Gomera	GEL	40	0.0418***	−0.0283***	0.0295 (0.00598)
La Gomera	GJE	40	0.0250 n.s.	−0.0063 n.s.	0.0064 (0.00734)
La Gomera	GPA	34	0.0013 n.s.	0.0066 n.s.	−0.0066 (0.00454)
Tenerife	TMA	40	0.0273 n.s.	−0.0175 n.s.	0.0180 (0.00983)
Tenerife	TLS	40	0.0346**	−0.0303***	0.0314 (0.01601)
Tenerife	TCA	40	0.0229 n.s.	−0.0083 n.s.	0.0085 (0.00226)
Tenerife	TSU	40	0.0163 n.s.	0.0023 n.s.	−0.0024 (0.00299)
Tenerife	TEC	40	0.0715***	−0.0238***	0.0256 (0.00840)
Gran Canaria	CAC	40	0.0212 n.s.	−0.0043 n.s.	0.0044 (0.00242)
Gran Canaria	CAR	40	−0.0008 n.s.	−0.0059 n.s.	0.0059 (0.00376)
Gran Canaria	CTE	40	0.0588***	−0.0267***	0.0283 (0.00927)
Gran Canaria	CST	40	0.0037 n.s.	0.0021 n.s.	−0.0021 (0.00352)
Gran Canaria	CMO	40	0.0236 n.s.	−0.0149*	0.0153 (0.00571)

n.s., not significant.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

(GSI, GMA, GTZ, GTG, GLH and GVR); finally, on Tenerife, each sampling site was virtually separated into a unique group, except that formed by populations 'TMA' and 'TCA'. A significant proportion of genetic variation was shared between groups of Gran Canaria and La Gomera (10.13% and 11.89%, respectively; Table S2). On Tenerife, the proportion of genetic variation between groups was larger than that observed between the two other islands (15.85%), but without statistical significance (Table S2).

Fine-scale spatial genetic structure (SGS)

Patterns of SGS were different across study sites, with Kinship coefficient values between individuals at the first distance class (F_1) ranging widely from −0.0008 to 0.0588 (Table 2). However, most of the F_1 estimations were not significant, with some exceptions such as GEL (La Gomera), TLS, TEC (Tenerife) and CTE (Gran Canaria) (Table 2, Figure S2.1–3). These sites were also the most genetically divergent in comparison with the rest of the sampled sites on each island (*i.e.* high AvF_{ST} values; Table 1). In agreement with this trend, negative and significant values of the regression slope (b_{log}) were only observed in the same sites mentioned above, in addition to CMO (Table 2). Significant fine-scale SGS in these sites was accompanied by high Sp values, which ranged from 0.015 (CMO) to 0.0314 (TLS), but these estimates revealed weak structure in the rest of sites (Table 2).

Effect of landscape occupancy in *P. canariensis*

Mean elevation of sampling sites (alt) did not result in significant correlations with any of the genetic statistics (results not shown). However, the number of individuals around sampling sites (Nneigh) showed some significant relationships with genetic parameters. First, estimates of intra-population genetic variation (H_o, H_e) did not correlate with the observed number of neighbouring individuals (Nneigh|H_o: $r_s = -0.194$, $P = 0.364$; Nneigh|H_e: $r_s = -0.214$, $P = 0.316$). In contrast, we found that population mean F_{ST} showed a significant negative correlation with the abundance of neighbouring individuals (AvF_{ST}|Nneigh: $r_s = -0.507$, $P < 0.01$), and a positive correlation with Sp statistics (AvF_{ST}|Sp: $r = 0.589$, $P < 0.01$). Lastly, the number of individuals in the landscape also affected the intensity of fine-scale SGS detected across populations (Nneigh|Sp_{log}: $r_s = -0.481$, $P < 0.05$).

DISCUSSION

Our results suggest that long-lived species with high colonisation abilities are affected by barriers to dispersal within large oceanic islands, although levels of genetic structure at this scale vary depending on conditions other than island size. In our study, *P. canariensis* showed a moderate degree of genetic structure on islands with contrasting geographic extensions, such as La Gomera and Gran Canaria. Despite similar island size, genetic structure on Tenerife was, however, substantially larger than that inferred for Gran Canaria.

Similar levels of genetic differentiation on Gran Canaria and La Gomera can be attributed to factors common to both islands. Extensive habitat availability in species with high dispersal potential is often associated with efficient population connectivity regardless of island size (Payn *et al.* 2008; García-Verdugo *et al.* 2017). In the case of *Phoenix*, these two islands are home to 86% of the total number of natural specimens estimated for the entire archipelago (42% on Gran Canaria, and 44%, on La Gomera; see Figure S1). Gran Canaria and La Gomera share a similar age and topography (Fernández-Palacios *et al.* 2011), with networks of deep ravines connecting at their summits that provide adequate habitats for population establishment. Hence, even though the current natural distribution of this species is rather fragmented at the island scale, palaeoecological evidence suggests a more continuous presence of *P. canariensis* on both islands in the past (Nogué *et al.* 2013; De Nascimento *et al.* 2016). The fact that these two islands provide adequate conditions for *P. canariensis* is further supported by the significant deviation from mutation-drift equilibrium (*i.e.* heterozygosity deficiency) found in several populations of these two islands, which is associated with population expansion (Cornuet & Luikart 1996). In addition, Mantel tests revealed similar patterns of gradual change in allele frequencies within these islands, with genetic drift only playing a significant role among geographically distant populations (Hutchison & Templeton 1999). While this pattern may be partially explained by the relatively recent divergence of *P. canariensis* populations (especially on La Gomera; see Saro *et al.* 2015), this result also supports the idea that gene flow has been extensive among populations at local to medium scales.

Apart from similar genetic patterns, our study also pinpointed differences between Gran Canaria and La Gomera populations that may be attributable to human-mediated activity. For instance, the large number of clusters inferred for Gran Canaria, in addition to the main barriers detected by BARRIER and SAMOVA, indicated that the demographic history of these populations appears to have been more complex than that accounting for population structure on La Gomera. This pattern would agree with the idea of genetic differentiation caused by historical factors, since the correlated model of our clustering approach allows detection of differences among closely related populations within short evolutionary time scales (Falush *et al.* 2003; Rosenberg *et al.* 2005). Indeed, paleoecological studies have documented a decline in fossil pollen records of *P. canariensis* on Gran Canaria associated with human activity during the Late Holocene (De Nascimento *et al.* 2016). Such a scenario is therefore compatible with loss of genetic diversity derived from the rapid deforestation of the Gran Canaria thermophilous forests, an activity that dramatically increased on the island since human colonisation (Naranjo 2002; Morales *et al.* 2009). Comparatively, declines in palynological records on La Gomera are smaller for the same period, which has been attributed to episodes of Quaternary climate change rather than to human-mediated activity (Nogué *et al.* 2013).

Human-mediated impacts on the genetic structure of island populations were also evidenced on La Gomera from different results. Our analyses consistently showed that one sampling site (GEL) was highly differentiated from all other neighbouring sites. Such an unexpected result does not follow the general IBD pattern described above, and may well respond to a certain degree of genetic introgression from the allochthonous congener, *Phoenix dactylifera*. Although population sampling was conducted to avoid areas of suspected hybrid origin (González-Pérez *et al.* 2004; Del Arco 2006), further admixture analyses revealed a significant contribution (40% of membership; results not shown) of *P. dactylifera* gene pools when samples from several African locations were included. The genetic pattern detected for this population matches that observed in mixed stands composed of continental congeners and *P. canariensis* individuals (González-Pérez *et al.* 2004).

The strong genetic structure found in the case of Tenerife can be explained in the context of limited habitat availability. First, the central part of the island was affected by catastrophic events of secondary volcanism and mega-landslides during the Pleistocene (Ancochea *et al.* 1999; Boulesteix *et al.* 2013). The geological configuration of Tenerife after such episodes was noticeably different from its neighbouring islands, and deep ravines providing suitable conditions for *P. canariensis* were virtually absent from the central region. Second, following the post-erosional episode of Las Cañadas volcano, much of the southern region was covered by phonolitic lava (Ancochea *et al.* 1999), which produced soils with acidic and permeable characteristics that are inappropriate for the establishment of *P. canariensis*. Limited habitat availability on Tenerife during the Holocene is also supported by the few pollen fossil records documented to date (De Nascimento *et al.* 2009) and may account for the strong east-to-west pattern of differentiation clearly detected on this island (cf. García-Verdugo *et al.* 2010; Mairal *et al.* 2015).

Lastly, spatial fragmentation in the distribution of *P. canariensis* also appears to have had some effect on population-level processes. The potential for extensive pollen-mediated flow of *P. canariensis* (Saro *et al.* 2014), together with the high levels of genetic diversity (*e.g.* Gran Canaria and La Gomera), may indicate strong resilience to loss of genetic diversity due to past demographic fluctuations, and a considerable participation of historical gene flow (Petit & Hampe 2006; Wang *et al.* 2011). Lack of correlation between genetic diversity (H_e) and extant spatial abundance (Nneigh) in our study also supports this view. In contrast, fine-scale genetic structure may be detectable in only a few generations after modification of the physical environment and population sizes (De-Lucas *et al.* 2009; Browne *et al.* 2015). In this study, populations of *P. canariensis* with remarkable local structure displayed *Sp* values similar to those detected in other species with intrinsic high dispersal abilities located in fragmented areas, such as *Pinus pinaster* (Sp = 0.026; De-Lucas *et al.* 2009) or *Sorbus terminalis* (Sp = 0.017; Oddou-Muratorio *et al.* 2004). Moreover, those populations were also the most genetically differentiated and isolated from the rest. It has been suggested that fluctuations in population density and/or gradual population isolation are associated with the loss of favourable sites for seedling establishment (Oddou-Muratorio *et al.* 2004). Indeed, progressive fragmentation and isolation cause variation in pollen and seed dispersal kernels (*e.g.* by increasing non-random movement of dispersers), thus promoting the clumped spatial distribution of related genotypes (Oddou-Muratorio *et al.* 2004; De-Lucas *et al.* 2009; Wang *et al.* 2011; Browne *et al.* 2015; Heer *et al.* 2015). Considering the wide ecological range of *P. canariensis*, the effect of historical disturbance on SGS patterns will depend on the extent of the perturbation and the particular resilience of each population, *i.e.* different patterns in spatial aggregation of adult palms to counteract the effects of limited gene flow, resulting in diverse levels of SGS among sites (Gonzales *et al.* 2010; Eliades *et al.* 2018). For instance, populations occurring in habitats with favourable conditions, *i.e.* those with high soil moisture availability, may favour considerably the recruitment of new seedlings of *P. canariensis*. Favourable areas may thus promote fast recruitment and blur the genetic pattern associated with the aggregation of related genotypes (Troupin *et al.* 2006), which in turn will result in higher connectivity across the landscape and weak population structure at the regional scale. However, conclusions on particular *Phoenix* populations are constrained by the limited sample size considered at this scale, and a more robust assessment of fine-scale genetic structure is needed to unambiguously identify the main factors driving the distribution of genetic variation within specific populations.

In summary, our study shows that genetic structure at within-island scales can be determined by factors other than island size or the species′ colonisation ability. A regular pattern of distribution of individuals across the landscape greatly promotes population connectivity, even for large islands subjected to human-mediated pressures, producing moderate levels of differentiation within islands. Such information could serve to make recommendations for effective *in situ* conservation measures. For instance, we suggest the definition of management units that match the observed patterns of genetic structure; *i.e.*

western and eastern areas on Tenerife and La Gomera, and the genetic clusters detected on Gran Canaria. These basic units could help to focus further research on particular factors affecting the species' conservation, such as identification of the degree of genetic introgression with *P. dactylifera* or the drivers of within-island genetic fragmentation. In addition, the preliminary information generated in this study could inform programmes of germplasm collection, as well as provide a basis for genetic identification and restriction on movement of plant material obtained for ornamental purposes. Overall, our study illustrates that increasing sampling effort at the island scale can reveal areas of special interest that may have been overlooked by studies dealing with broader spatial scales, since our results on *Phoenix* show that high colonisation ability does not always promote genetic connectivity among neighbouring populations.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Current natural distribution of *Phoenix canariensis* (areas coloured in green) across the central islands of the Canarian archipelago. Red dots indicate locations of sampling sites, which are named following the site codes in Table 1.

Figure S2.1. Individual-based analysis of spatial genetic structure of *Phoenix canariensis* for each sampling site located in Gran Canaria island. Average kinship coefficient values (F_{ij}) were plotted against geographic distance between individuals (d_{ij}). Dashed lines delimit 95% confidence intervals around the null hypothesis of spatial randomness, as determined by permutations.

Figure S2.2. Individual-based analysis of spatial genetic structure of *Phoenix canariensis* for each sampling site located in Tenerife island. Average kinship coefficient values (F_{ij}) were plotted against geographic distance between individuals (d_{ij}). Dashed lines delimit 95% confidence intervals around the null hypothesis of spatial randomness, as determined by permutations.

Figure S2.3. Individual-based analysis of spatial genetic structure of *Phoenix canariensis* for each sampling site located in La Gomera island. Average kinship coefficient values (F_{ij}) were plotted against geographic distance between individuals (d_{ij}). Dashed lines delimit 95% confidence intervals around the null hypothesis of spatial randomness, as determined by permutations.

Figure S3. Distribution of Δ K values across different clusters as estimated using Structure Harvester for each island scenario.

Table S1. Matrix of FST values estimated from eight nuclear microsatellite loci for pair-wise comparisons of *Phoenix canariensis* sites sampled on each of the islands considered in this study. See Fig. 1 and Table 1 for further details on geographic locations and site codes.

Table S2. Table S2. Results of hierarchical spatial analysis of molecular variance (SAMOVA) in *Phoenix canariensis* at the island level. Significance test are based on 1023 permutations and site codes are the same as in Table 1.

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