

Palaeo-islands as refugia and sources of genetic diversity within volcanic archipelagos: the case of the widespread endemic *Canarina canariensis* (Campanulaceae)

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Abstract

Geographical isolation by oceanic barriers and climatic stability has been postulated as some of the main factors driving diversification within volcanic archipelagos. However, few studies have focused on the effect that catastrophic volcanic events have had on patterns of within-island differentiation in geological time. This study employed data from the chloroplast (cpDNA haplotypes) and the nuclear (AFLPs) genomes to examine the patterns of genetic variation in *Canarina canariensis*, an iconic plant species associated with the endemic laurel forest of the Canary Islands. We found a strong geographical population structure, with a first divergence around 0.8 Ma that has Tenerife as its central axis and divides Canarian populations into eastern and western clades. Genetic diversity was greatest in the geologically stable ‘palaeo-islands’ of Anaga, Teno and Roque del Conde; these areas were also inferred as the ancestral location of migrant alleles towards other disturbed areas within Tenerife or the nearby islands using a Bayesian approach to phylogeographical clustering. Oceanic barriers, in contrast, appear to have played a lesser role in structuring genetic variation, with intra-island levels of genetic diversity larger than those between-islands. We argue that volcanic eruptions and landslides after the merging of the palaeo-islands 3.5 Ma played key roles in generating genetic boundaries within Tenerife, with the palaeo-islands acting as refugia against extinction, and as cradles and sources of genetic diversity to other areas within the archipelago.

Keywords: ancestral areas, extinction, oceanic islands, palaeo-islands, volcanic refugia

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Introduction

Due to their small size, discrete geographical boundaries, substantial environmental heterogeneity and buffered climates, oceanic islands represent excellent natural laboratories to examine the role of ecological adaptations vs. geographical isolation as drivers of diversification (Carson & Templeton 1984; Gillespie 2004). Patterns of genetic variation are expected to be hierarchical in islands, with between-island genetic differentiation stronger than within-islands (Bottin *et al.* 2005; García-

Verdugo *et al.* 2010), because oceanic barriers are generally more effective than topographic barriers at promoting isolation in insular systems (Gillespie & Clague 2009). However, the birth and development of volcanic islands is usually followed by a large number of destructive events in the form of secondary eruptions, landslides, merging of palaeo-islands, etc. (Carracedo 1994; Fernández-Palacios *et al.* 2011). These events promote habitat fragmentation and the subsequent genetic isolation of populations, and they ultimately drive differentiation and speciation within-islands (Carson *et al.* 1990; Gillespie & Roderick 2002, 2014; Macías-Hernández *et al.* 2013). Therefore, volcanic archipelagos represent an ideal framework for studying patterns of diversification at dif-

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ferent spatial scales (between- and within-islands) and also over different temporal levels (allopatric speciation, extirpations and recolonizations).

The Canary Islands are a volcanic archipelago formed by a chain of seven islands, located 110 km from the north-western coast of Africa. Their geological history and highly endemic biota have interested scientists since the early 19th century (von Humboldt 1814; Lyell 1855). The islands were formed in the last 21 Ma with an east to west pattern of chronological emergence due to a mantle plume (Carracedo *et al.* 1998; Zaczek *et al.* 2015) (Fig. 1a). They are separated by deep oceanic trenches and have never been connected to the mainland. The Canary Islands have long been considered refugia for continental lineages that have survived the climatic changes of the Late Cenozoic, but also as cradles of biodiversity where multiple in situ diversification events have taken place (Francisco-Ortega *et al.* 2000; Juan *et al.* 2000). In addition, these islands are regarded as a hotspot for plant diversity (Médail & Quézel 1997): approximately 40% of Canarian vascular plants are endemics (Santos-Guerra 2001) and the flora is generally characterized by high levels of interpopulation differentiation in comparison with other archipelagos (Francisco-Ortega *et al.* 2000; de Paz & Caujapé-Castells 2013). Recently, they have been proposed as reservoirs and sources of genetic diversity (García-Verdugo *et al.* 2015; Patiño *et al.* 2015).

Most studies on the Canarian flora have focused on the pattern of interisland diversification, particularly at the species level (Francisco-Ortega *et al.* 2002; Kim *et al.* 2008; Vitales *et al.* 2014a,b). Nevertheless, the complex topographies and long histories of avalanches and secondary eruptions of many of these islands are likely to have favoured within-island diversification (Juan *et al.* 2000; Brown *et al.* 2006). Tenerife has the most complex history of all the islands. It existed at first as three separate islands, dating back to the Late Miocene: Roque del Conde (11.9–8.9 Ma) in the southwest, Teno (6.2–5.6 Ma) in the northwest, and Anaga (4.9–3.9 Ma) in the northeast. Eruptive central volcanic episodes fused these islands 3.5 Ma and gave rise to the present island of Tenerife (Ancochea *et al.* 1990; Fig. 1b). The palaeo-islands remained thereafter relatively stable, whereas the central part of Tenerife continued to be active until 0.13 Ma (Ancochea *et al.* 1990, 1999; Cantagrel *et al.* 1999; Guillou *et al.* 2004; Carracedo 2014; see Fig. 1b). Interestingly, the three palaeo-islands of Tenerife, together with La Gomera—which has also remained geologically stable since the Pliocene—are presently home to the highest phylogenetic diversity and endemic richness of the Canarian Archipelago (Reyes-Betancort *et al.* 2008). These areas also harbour the best-preserved laurel forests, considered as an ancient, unique flora restricted to Macaronesia, and they share several endemic and restricted species (see Table S1, Supporting information). Besides their geological

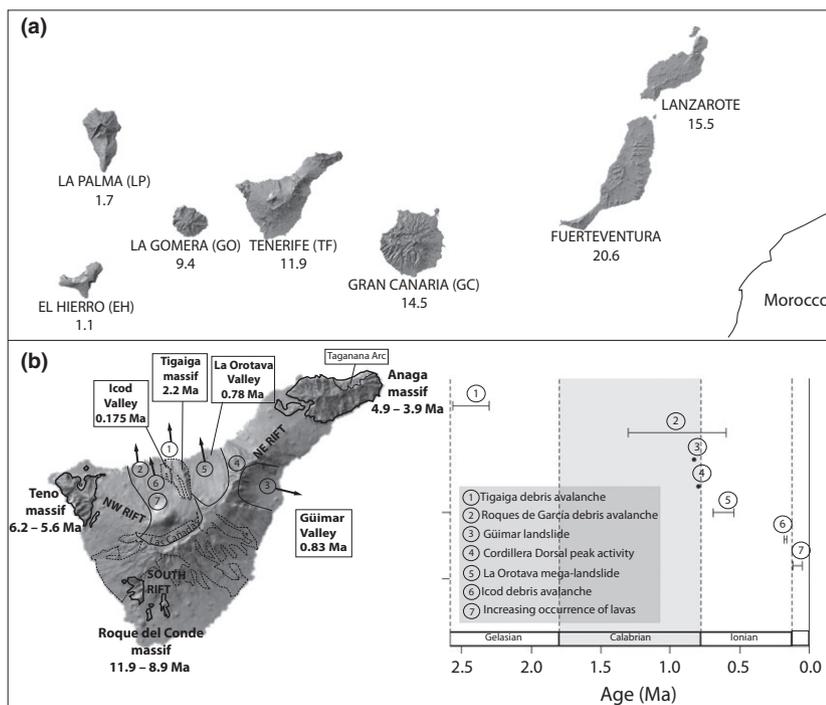


Fig. 1 (a) Geographical map of the Canary Island archipelago, with the code for each island used in the text and their age of emergence (million years, Ma). (b) Map of Tenerife showing main Pleistocene landslides and other geological events cited in the text. The old basaltic series corresponding to the palaeo-islands are shown in the three contoured massifs at the edges of the island. Lines show valleys created after landslides. Dashed lines indicate the post-erosional Las Cañadas volcano. Arrows indicate the directions of the landslides. The graph on the right shows the main Tenerife catastrophic events that are commented in the text; the numbers in the legend refer to those in the map. Ages and maps adapted from: Carracedo *et al.* 1998, 2007; Ancochea *et al.* (1999); Cantagrel *et al.* (1999); Guillou *et al.* (2004); Boulesteix *et al.* (2012); Zaczek *et al.* (2015).

(volcanic) stability, the three palaeo-islands of Tenerife also exhibit a topographic complexity and variety of micro-climates that might have favoured their role as micro-refugia against climate- or human-induced extinction (Harter *et al.* 2015).

In reviewing the role of Tenerife palaeo-islands as refugia across several plant lineages, Trusty *et al.* (2005) found that species endemic to the palaeo-islands often occupied a derived position in the lineage's phylogeny. This position argued against the idea of these massifs as ancient refugia. However, other studies, especially in animals, have reported ages for divergence events between taxa endemic to these palaeo-islands, either at the species or at the intraspecies level (Juan *et al.* 1996, 2000; Dimitrov *et al.* 2008; Macías-Hernández *et al.* 2013; Puppo *et al.* 2014) that are contemporaneous or predate the age of merging of the precursor palaeo-islands 3.5 Ma (Ancochea *et al.* 1990). Few plant studies (Gómez *et al.* 2003; García-Verdugo *et al.* 2010) have focused on patterns of within-island genetic variation for widespread Canarian endemics, and none of them have provided estimates of lineage divergence times, which is necessary to relate within-island patterns to the island geological history. Species that are present in multiple islands ('multiple island endemics' or MIEs) are especially relevant to understand the role of palaeo-islands as undisturbed areas that have acted as reservoirs and sources of genetic diversity not only within- but between-islands.

Here, we study patterns of genetic diversity and the demographic and spatial history of a multiple island endemic, *Canarina canariensis* (L.) Vatke. This 'flagship' species of the Canary Islands, elected as its 'national flower' (Kunkel 1991), is a diploid ($2n = 34$) herbaceous plant that grows mostly in cleared areas surrounding the endemic laurisilva forest. It presently occurs in the central and western Canary Islands: Gran Canaria, Tenerife, La Gomera, La Palma and El Hierro. *Canarina canariensis* is a herbaceous plant that occasionally climbs on nearby plants; it is pollinated by generalist birds (Rodríguez-Rodríguez & Valido 2011) and its fleshy fruits are dispersed by vertebrates (Valido *et al.* 2003; Rodríguez *et al.* 2008). Genus *Canarina* belongs to tribe Platycodoneae, a basal group within family Campanulaceae (Mansion *et al.* 2012; Olesen *et al.* 2012; Wang *et al.* 2013; Mairal *et al.* 2015). In addition to *C. canariensis*, the genus comprises two other species inhabiting the Afrotropical forests of Eastern Africa, *C. eminii* and *C. abyssinica*, being this an example of a wide, continental-scale disjunction of 7000 km spanning across the Sahara. Mairal *et al.* (2015) recently reconstructed the phylogeny and spatiotemporal evolution of *Canarina*. They inferred that *C. canariensis* diverged from its African relatives at the end of the Miocene (c. 7 Ma). This

extraordinary temporal and spatial disjunction was explained as the result of vicariance and climate-driven extinction resulting in the fragmentation of an ancient widespread distribution. The colonization of the Canary Islands by the ancestors of *C. canariensis* apparently occurred much later, in the Pleistocene, probably from a now extinct and geographically closer North African population: the earliest event of population divergence is dated at only around 1 Ma (Late Pleistocene) and involved an east-west vicariance within Tenerife (Mairal *et al.* 2015).

This age and the presence of *C. canariensis* in several islands makes it an ideal candidate to evaluate patterns of within-island diversification in relation to the recent geological history of the archipelago. Our main aims were to: (i) determine the geographical distribution of genetic variation within *C. canariensis*, (ii) find evidence of extinction and diversification processes that may be related to geological events, (iii) find ancestral areas and reconstruct interisland migration events and (iv) examine the putative role of the palaeo-islands of Tenerife as refugia of genetic diversity, both relictual and recent.

Haplotype networks are commonly used in population-level studies because they provide a clearer picture of the reticulate relationships between genetic pools than a branching tree, especially when gene flow is present. These networks are often inferred using Statistical Parsimony (SP, Templeton *et al.* 1992) implemented in the software TCS (Clement *et al.* 2000), which allows estimation of the haplotype network while minimizing the number of mutation events differing among haplotypes. However, this method fails to incorporate the uncertainty associated with the network inference and therefore does not allow for statistical evaluation of alternative phylogeographical scenarios (Bloomquist *et al.* 2010). Moreover, unobserved events such as local population growth or past extinction of haplotypes may mislead inference in parsimony-based methods. Here, we compare results from TCS with those obtained from a model-based, Bayesian statistical method, Bayesian phylogeographic and ecological clustering (BPEC, Manolopoulou *et al.* 2011), which allows estimating the posterior probabilities for haplotype tree networks under a coalescent-based migration-mutation model (Manolopoulou & Emerson 2012). To our knowledge, this is the first study to use this method for island phylogeography.

Materials and methods

Population sampling and DNA extraction

Canarina canariensis has a significantly greater presence on Gran Canaria (GC) and Tenerife (TF), in comparison

with La Gomera (LG), La Palma (LP) and El Hierro (EH). Seventeen populations of *C. canariensis* were sampled in several fieldtrips between 2009 and 2012: four in GC, eight in TF, two in LG, two in LP and one in EH. Where possible, we collected a minimum of 10 samples per population. To reduce inflation in gene descriptors due to biased sampling (Caujapé-Castells 2010), samples were collected from individuals scattered across the whole occupancy area of each population. DNA from 160 individuals and preserved in silica gel was extracted using the DNeasy Plant Mini Kit (QIAGEN Inc., Valencia, CA, USA), from 20 to 25 mg of silica-gel-dried leaves obtained from the fresh plant tissue collected from the field expeditions.

Chloroplast DNA sequencing

We selected three cpDNA intergenic spacers regions for sequencing; these markers have proven to be useful for intraspecific analyses of population structure (Mairal *et al.* 2015). We generated 432 new sequences: *rpl32-trnL*^{UAG} (144 sequences), *trnS*^{GCU}-*trnG*^{UCC} (144 sequences) and *petB*¹³⁶⁵-*petD*⁷³⁸ (144 sequences). PCR and sequencing protocols followed those of Mairal *et al.* (2015). The sources of the material examined, the GenBank Accession nos and full references are detailed in Table S2 (Supporting information).

Haplotype analyses

Sequences for each region were aligned using MAFFT 6.814b (Katoh *et al.* 2002), implemented in the software GENEIOUS PRO 5.4.4. (Biomatters Ltd., Auckland, New Zealand). Sequences were checked and manually adjusted where necessary by following alignment rules described in Kelchner (2000). We analysed the three sequenced regions as three data partitions to perform phylogenetic analyses. MRMODELTEST v.2.2 software (Nylander 2004) was used to determine the best fitting model of sequence evolution of each data partition.

Summary statistics for within-population genetic diversity were calculated as follows: the number of haplotypes $H(n)$, haplotype diversity (H_d), nucleotide diversity π , nucleotide heterozygosity θ , and G_{ST} and the number of migrants per generation (N_m) were estimated for each population using DnaSP (version 5.10; Librado & Rozas 2009).

The relationships among lineages were investigated through haplotype network analysis, using 6–12 individuals from different populations and examining the three sequenced regions. Genealogical relationships among haplotypes were inferred via the statistical parsimony algorithm (Templeton *et al.* 1992) implemented in TCS 1.21 (Clement *et al.* 2000). The number

of mutational steps resulting from single substitutions among haplotypes was calculated with 95% confidence limits, and gaps were represented as missing data.

The BPEC method (Manolopoulou *et al.* 2011; Manolopoulou & Emerson 2012) was implemented in the R package to identify genetically distinct geographical population clusters and ancestral locations. Like TCS, BPEC relies on parsimony in order to reduce the number of candidate trees to a manageable set. The BPEC method, unlike Standard Parsimony, fits a prior over all possible trees in order to identify trees with high posterior probability in a fully model-based framework, thus accommodating for uncertainty in haplotype relationships, which is one of the main criticisms of TCS (Knowles 2008). Each possible tree defines a set of possible migration events that may have led to the observed population substructure. Different scenarios of trees and migration events are explored through Markov chain Monte Carlo (MCMC), similar to the method proposed by Sanmartín *et al.* (2008) for estimating rates of interisland dispersal. Migration events were assumed to occur when a haplotype (with or without a mutation from its parent haplotype) migrates to a new geographical cluster. MCMC simultaneously estimates high probability trees, number of migration events and corresponding clusters. The method assumes that the migration rate and the population growth are constant. BPEC requires two main user-defined inputs: the maximum number of migrations (denoted as 'Max-Mig' in the software) and the parsimony relaxation parameter used to reconstruct the set of possible trees (denoted as 'ds' in the software). MAXMIG allows the user to set the upper bound for the number of migration events and hence the maximum number of clusters (Max-Mig + 1). Larger values include more models but require much greater computation time. As the ds value is increased, the parsimony assumption is relaxed: if two observed sets of sequences have an unobserved intermediate missing sequence (an unobserved mutation), then any pair of sequences of distance $\{1, \dots, ds\}$ nucleotides will be considered as the 'missing path'. Two MCMC chains were run for 3 million iterations. The results were stable, with $ds > 3$ having no effect on the inferred tree, and similarly, any number of migrations above four converging to a 5-cluster model. The phylogeographical clustering obtained was superimposed upon a haplotype tree and used to estimate ancestral locations for migration events. As a further exploration, we divided the data set into two groups (eastern and western clades—for details, see *coalescent dating* section in results). Haplotypes sampled from Roque del Conde were quite divergent; thus, separate analyses were run in which we included and excluded this location from the two groups. We ran BPEC analysis on each of these four data sets

(eastern and western groups with and without Roque del Conde).

Haplotype divergence times were estimated in BEAST v.1.7 (Drummond & Rambaut 2007). We carried out a first analysis under a strict clock model and a coalescent constant population tree prior, using a secondary age estimate (Mairal *et al.* 2015; normal prior: mean = 0.76 Ma, standard deviation (SD) = 0.327 Ma) to calibrate the root node of the '*C. canariensis* data set'; this included all haplotypes detected in our sampling ($N = 10$). This analysis gave us very large 95%HPD (high posterior density) credibility intervals and poor ESS for posterior age estimates, probably due to the low information content at the population level (see Results) and the presence of a single calibration point. We carried out a second analysis applying the 'nested dating approach' described in Mairal *et al.* (2015), in which a higher-level data set including representatives of all three species of *Canarina* and nine outgroup taxa was used to inform the clock rate of a linked population-level data set (*C. canariensis*) under a *mixed Yule-coalescent model* (Ho *et al.* 2005; Pokorny *et al.* 2011). The higher-level data set was calibrated with fossil-derived secondary age estimates (see Mairal *et al.* 2015), while the tree prior was unlinked to apply a coalescent constant size model to the population-level data set and a stochastic birth–death (Yule) prior to the species-level one (Mairal *et al.* 2015). The clock model was set to an uncorrelated log-normal prior to accommodate the change in mutation rate from species to populations, with a uniform distribution for the *uclid.mean* (10^{-4} – 10^{-1}) and a default exponential distribution for the *uclid.stdev*; the substitution model was set to GTR+G; choice of priors was based on Bayes Factor comparisons using the path sampling method in BEAST (Baele *et al.* 2012); see Table S3 (Supporting information) for results from exploratory analyses to assess the reliability of our date estimates with reference to these settings. Two MCMC chains were run for 50 million generations, sampling parameters every 1000 generation. We used Tracer v1.6 (Rambaut *et al.* 2007) to verify the following: whether a stationary distribution was attained, whether there was convergence among chains and whether effective sample sizes (ESS values) were >200 for all parameters. A 10% burn-in of the sampled populations was discarded (5 million). Post-burn-in trees were summarized into a maximum clade credibility tree using TREEANNOTATOR v.1.6.1, with mean values and 95% credible intervals for nodal ages, and were visualized in FIGTREE 1.3.1 (Rambaut & Drummond 2009). The resulting age estimates from this second analysis exhibited considerably larger ESS values and narrower 95%HPD intervals than in the first and are the ones reported here.

Demographic history

Statistics used to describe demographic patterns may be biased by a strong genetic structure or lack of panmixia among populations. As we detected strong genetic structure in our data set (see results), we performed demographic analyses in subsetted data sets that were less genetically structured (including approximate panmictic populations). Three groups previously recognized by the haplotype network and BEAST analyses were used: ET-GC (including populations in east Tenerife and Gran Canaria), TENO-GO (including populations in Teno and La Gomera) and LP-EH (including populations in La Palma and El Hierro). For the same reasons as described above, the Roque del Conde population was included and discluded from the ET-GC and TENO-GO groups. Overall, we ran the analyses on five groups.

We used three different approaches to infer the demographic processes shaping the genetic structure of *C. canariensis*. First of all, to test for evidence of population expansion, we carried out a neutrality test—Fu and Li's tests (Fu & Li 1993; Fu 1996) and Tajima's *D* test (Tajima 1989)—for each population group. We used the DNAsp program, version 5.0 (Librado & Rozas 2009), and assessed the significance of each test with 10 000 coalescent simulations. Second, we plotted the mismatch distribution for each group using the observed number of differences between all pairs of sequences with the ARLEQUIN v.3.0 software (Excoffier *et al.* 2005). The goodness of fit of the observed mismatch distribution to the theoretical distribution under a constant population size model was tested with the raggedness index (*HRag*) (Harpending 1994). Third, (i) we created the extended Bayesian skyline plot (EBS), implemented in BEAST, for each population group, and (ii) we performed EBS analyses for each of the 16 population groups. For each group from (i) and (ii): two independent chains were run simultaneously for 150 million generations, sampling every 1000 generations; a strict clock model was used, whereas all other parameters were set identical to those described above for the nested dating analysis; the root node was calibrated using a normal prior with a mean age estimate and 95% high posterior density (HPD) credible intervals obtained from this analysis.

AFLP fingerprinting

For the AFLP analysis, we used a total of 97 individuals from 10 populations, which covered all of the islands: one population from Gran Canaria, five from Tenerife, one from La Gomera, two from La Palma and one from El Hierro. Laboratory molecular protocols for the AFLP

analysis (Vos *et al.* 1995) were implemented using the AFLP plant mapping kit (Applied Biosystems[®], Foster City, CA, USA). To select the appropriate primers, we first carried out a pilot study combining fluorophores and restriction enzymes for five geographically distant individuals (one per island), using in total 32 primer combinations. One sample from each individual was duplicated as a blind sample to test for reproducibility and contamination. Reproducibility and the number of alleles per sample were calculated by choosing three combinations of primers: 1-*EcoRI*_{6-FAM}-ACT/*MseI*-CAA, 2-*EcoRI*_{VIC}-AGG/*MseI*-CTA and 3-*EcoRI*_{VIC}-AGG/*MseI*-CTT and using the GENEMAPPER v3.7 software (Applied Biosystems). These three primer pairs showed high reproducibility and homogeneously scattered bands and produced polymorphic AFLP profiles and clear fragments. In the digestion phase, samples of DNA were digested with the restriction enzymes *EcoRI* and *MseI* and linked to the primers *EcoRI* 5'-CTCGTAGAC TGCGTACC-3'/5'AATTGGTACGCAGTCTAC-3') and *MseI* (5'-GACGATGAGTCCTGAC-3'/5'-ATCTCAGGA CTCAT-3'). The three different AFLP reactions were as follows: (i) restriction and ligation in a single reaction; (ii) and (iii) consecutive PCR amplifications (preselective and selective). PCR products were checked on 1% agarose gels.

AFLP data analyses

The resulting AFLP fragments were analysed using the GENEMAPPER 3.7 software. Peaks were recorded in 100–500 base pairs ranges. Shorter fragments were discarded because the majority of this size class have a high chance of being nonhomologous fragments (Vekemans *et al.* 2002). For each primer combination, an automated size detection and peak binning was employed followed by manual editing of bins to exclude shoulder peaks and unreliable loci (variation between replicates). Peak height data were then exported and loaded into the R package AFLPScore version 1.4a (Whitlock *et al.* 2008), and the AFLP profiles were scored and the error rates were estimated. These rates were below the critical bound of 5% indicated in previous reports (Bonin *et al.* 2004) for each primer combination. Data reliability was assessed through comparison of duplicates, from one or two individuals per population. Data were converted into binary presence/absence scores for each locus. The resulting AFLP presence/absence matrix was analysed using a selection of different analyses. The AFLPSURV v.1.0 software (Vekemans *et al.* 2002) was used to estimate demographic statistics such as Nei's gene diversity (H_j), pairwise differentiation among subpopulations (F_{ST}) and the percentage of polymorphic fragments per population (P) (Nei & Li 1979; Lynch & Milligan 1994).

This was done under the assumptions of either the Hardy–Weinberg equilibrium or partial self-fertilization, based on a previous study on the reproductive biology of *C. canariensis* (Rodríguez-Rodríguez & Valido 2011). A Bayesian method in AFLPSURV was also used to estimate allelic frequencies through employment of a nonuniform prior distribution (Zhitovovsky 1999). Ten thousand permutations were run to calculate the F_{ST} parameter from which genetic distances between individuals, populations and geographical groups were calculated. To locate genetic clustering of individuals within the AFLP data set, a pairwise similarity matrix for all individuals using the Dice's coefficient as similarity distance was constructed, and the resulting matrix subjected to a principal coordinates analysis (PCA) implemented in NTSYS v.2.1 (Rohlf 1998). Next, genetic relationships among samples were visualized in SPLITS-TREE v.4.10 (Huson & Bryant 2006) using neighbour-net analysis through the use of the split decomposition method. Finally, to quantify the amount of genetic differentiation attributable to geographical and population subdivision, a hierarchical analysis of molecular variance was performed using ARLEQUIN v.3.0 software (Excoffier *et al.* 2005). Exploratory analyses were performed considering, alternatively, islands and palaeo-islands as geographical units in order to investigate the distribution of genetic variance attributable to oceanic barriers.

Bayesian clustering methods implemented in STRUCTURE v.2.3 (Pritchard *et al.* 2000; Falush *et al.* 2007) were used to assess the genetic structure of populations. This model-based approach assumes that loci are in Hardy–Weinberg equilibrium and linkage equilibrium within populations. Analyses were performed under admixture conditions and correlated allele frequencies between groups. 500 000 MCMC generations (plus a burn-in of 100 000) were run for K values of 1–10, with 10 repetitions for each. The most likely K value was determined by the method from Evanno *et al.* (2005), which is implemented in STRUCTURE HARVESTER (Earl 2012). We explored other values of K to detect further genetic substructure of populations, especially in Tenerife. To test the effect of the spatial distance on the genetic structure of the populations, correlations between genetic (measured as F_{ST}) and spatial distances between pairs of populations were determined using the Mantel permutation procedure implemented in NTSYS v. 2.1. The genetic distance matrix used was based on the presence/absence matrix; the geographical distance matrix was based on the absolute distances between the geographical coordinates for each collected population. In addition, to identify possible geographical locations acting as major genetic barriers among *C. canariensis* populations, we computed barriers on a Delaunay triangulation using Monmonier's algorithm in BARRIER

v.2.2 (Manni *et al.* 2004). The significance was examined by the mean of 1000 bootstrapped distance matrices obtained using AFLPSURV. Only barriers with support >96% were considered as significant.

Results

Haplotype network analysis and coalescent dating

The *pet* B¹³⁶⁵–*pet* D⁷³⁸ region consisted of 937 sites, *rpl32*–*trnL*^{UAG} of 654 sites and *trnS*^{GCU}–*trnG*^{UCC} of 658 sites. The final concatenated data matrix consisted of 144 sequences and 2249 nucleotide sites, of which nine were polymorphic (Table S4, Supporting information). Among the 144 individuals sampled from the 16 populations, we observed 10 different plastid DNA haplotypes (H1 to H10, Fig. 2a) with haplotype diversity of 0.6632. There were three haplotypes that dominated and were clearly geographically delimited: H1, H9 and H3. The most frequently observed haplotype was H1 (52.08%), which was dominant in GC and east Tenerife (ET). Haplotype H9 was restricted to West Tenerife (WT) and LG. Haplotype H3 was present in the western islands of LP and EH. Populations with the commonest and rarest haplotypes (frequencies ≤ 2%) were in the most rugged areas of Tenerife: the palaeo-islands of Anaga (An-W, An-C, An-E—exclusive haplotypes H2 and H5), Teno (Ten-A, Ten-Ep—exclusive allele H8 and allele H10 shared with LG) and Roque del Conde (Conde—exclusive H7). The small western islands presented one unique haplotype, each: LP (LP-Lt—H4) and EH (H6).

Summary statistics for within-population genetic diversity are shown in Table 1. Populations situated in the three palaeo-islands [Anaga (An-W), Teno (Ten-A) and Roque del Conde (Conde)] showed the highest

values for the number of haplotypes $H(n)$, haplotype diversity $H(d)$, nucleotide diversity π and nucleotide heterozygosity θ . The least diverse population areas were found in Gran Canaria and outside the palaeo-islands regions of Tenerife. G_{ST} and N_m values indicated high genetic cohesion between some islands: east Tenerife populations were highly connected with Gran Canaria populations; west Tenerife with La Gomera; and La Palma with El Hierro (Table S5, Supporting information). Interestingly, the highest G_{ST} values and lowest N_m were found between populations situated east and west of Tenerife, with the exception of Anaga An-W. This is the only population in east Tenerife that showed some genetic cohesion with populations in west Tenerife, in particular with the Teno and Adeje massifs.

The haplotype network constructed with TCS (Fig. 2b) shows a loop involving three dominant haplotypes, with haplotype H1 as the ancestral root haplotype according to coalescent criteria on haplotype frequency (Templeton *et al.* 1992). Dominant haplotypes at each side of Tenerife (East H1 and West H9) were separated by five mutational steps, with haplotype H10 as intermediate. Haplotype H3, present in the western islands, was separated by four mutational steps from H1 and three from H9.

BPEC results are summarized in Table 2. With mutational step limit equal to 10 and the maximum number of migrations equal to 8, we allowed for high gene flow, genetic divergence and numerous hidden mutations, which separate the haplotype clusters. The clustering with high posterior probabilities ($pp = 1$) showed the existence of five clusters (contour regions in Fig. 3a). The total number of haplotypes was 10 plus one missing haplotype (Fig. 3b). Location of the ancestral populations for migration events and inference of the root node were consistent across exploratory analyses of the

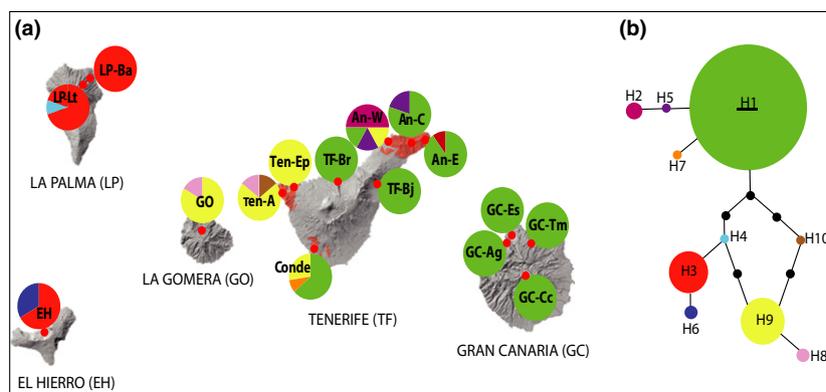


Fig. 2 Haplotype distribution and network inferred for the chloroplast markers by TCS. (a) Pie charts show the geographical location of populations and the frequency of occurrence of each haplotype, and circle size is proportional to population size. Population codes are given in Table S2 (Supporting information). (b) Statistical Parsimony network inferred by TCS. Each haplotype is shown in a different colour; circle size is proportional to its frequency among populations.

Table 1 Descriptors of within-population genetic diversity in the cpDNA haplotypes and AFLP markers for each population studied of *C. canariensis*

Haplotypes		AFLPs									
Island (Palaeo-island)	Population	No samples	Haplotype	H (n)	H (d)	π	θ	No samples	No of polymorphic fragments (% in brackets)	Hj (SE)	Number of private fragments
Gran Canaria	GC-Cc	8	H1	1	0	0	0	8	409 (68.7)	0.2344 (0.0085)	0
Gran Canaria	GC-Tm	11	H1	1	0	0	0	NA	NA	NA	NA
Gran Canaria	GC-Es	10	H1	1	0	0	0	NA	NA	NA	NA
Gran Canaria	GC-Ag	11	H1	1	0	0	0	NA	NA	NA	NA
Tenerife (Anaga)	An-W	6	H1, H2, H5, H9	4	0.8	0.00112	0.00136	7	401 (67.4)	0.2677 (0.0088)	4
Tenerife (Anaga)	An-E	10	H1, H2	2	0.2	0.00018	0.00031	10	345 (58.0)	0.2303 (0.0080)	0
Tenerife (Anaga)	An-C	11	H1, H5	2	0.327	0.00015	0.00015	11	375 (63.0)	0.2453 (0.0078)	1
Tenerife	TF-Bj	4	H1	1	0	0	0	NA	NA	NA	NA
Tenerife	TF-Br	6	H1	1	0	0	0	NA	NA	NA	NA
Tenerife (Roque del Conde)	Conde	11	H1, H9, H7	3	0.618	0.00121	0.00091	NA	NA	NA	NA
Tenerife (Teno)	Ten-Ep	8	H9	1	0	0	0	9	351 (59.0)	0.2449 (0.0082)	0
Tenerife (Teno)	Ten-A	7	H8, H9, H10	3	0.524	0.00038	0.00054	7	415 (69.7)	0.2997 (0.0088)	2
Gomera	GO	12	H8, H9	2	0.303	0.00013	0.00015	12	473 (79.5)	0.3012 (0.0077)	1
La Palma	LP-Ba	10	H3	1	0	0	0	9	285 (47.9)	0.1923 (0.0080)	0
La Palma	LP-Lt	10	H3, H4	2	0.2	0.00009	0.00016	10	348 (58.5)	0.2309 (0.0079)	0
El Hierro	EH	9	H3, H6	2	0.5	0.00022	0.00016	9	303 (50.9)	0.2115 (0.0082)	1

H(n), number of haplotypes; H(d), haplotype diversity; π , nucleotide diversity; θ , nucleotide heterozygosity; Hj (se), Nei's gene diversity (standard error). Geographical locations for population codes are shown in Fig. 2. The most diverse population in each palaeo-island is shown in bold.

complete data set. The root node was inferred as missing (H11—Fig. 3b) and carried a high uncertainty. Haplotypes H1, H2 and H8 also carried significant posterior mass probabilities (Table 2). The most likely source for ancestral migration events within- and between-islands were populations located on the following: Conde (pp = 0.13), An-W (pp = 0.10) and LP-Lt (pp = 0.086). The subsetting analysis, including Conde, always recovered this population as the ancestral area with the highest probability. Without Conde, other palaeo-island populations were recovered as ancestral areas: Ten-A for the Western clade; and the three populations of Anaga for the Eastern clade, with An-W the most probable (Table 2; Fig. S1, Supporting information).

The Bayesian chronogram of haplotypes showed a geographical pattern of divergence congruent with the groups detected by TCS and BPEC (Fig. 4). The crown

age or first divergence event among *Canarina* haplotypes was estimated to be 0.878 Ma (95% HPD = 0.452–1.365), corresponding to the Mid-Pleistocene period; it divided haplotypes into an eastern and western clade. The first divergence event in the eastern clade was estimated at 0.255 Ma (95% HPD = 0.041–0.633), while that in the western clade was dated older: 0.522 Ma (95% HPD = 0.161–0.967), separating La Palma and El Hierro from Teno and La Gomera. Mean age values and 95% HPD intervals for the BEAST analysis are shown in Table S6 (Supporting information).

Demographic analyses

Negative values of Fu's and Tajima's indicated a recent population expansion, although these values were only significant for the Fu's test of the East group (Table S7, Supporting information). The frequencies of pairwise

Table 2 Summary of results from the Bayesian phylogeographic and ecological clustering (BPEC) analyses. Selected values for parameters MaxMig (maximum number of migrations) and ds (relaxed parsimony assumption) are shown. Ancestral locations (Anc. Loc.) for migration events are shown with their associated posterior probabilities

	Maxmig	ds	Anc. Loc. 1	Anc. Loc. 2	Anc. Loc. 3	Root node	Root node probability	No of clusters
Total	8	10	Conde (0.13)	An-W (0.10)	LP-Lt (0.086)	H11 (missing)	0.17	5
Western Clade (WT + LP + GO + EH)	4	3	Ten-A (0.40)	Lp-Lt (0.31)	EH (0.18)	H11 (missing)	0.18	3
Eastern Clade (ET + GC)	1	3	An-W (0.212)	An-C (0.122)	An-E (0.116)	H11 (missing)	0.16	2
Western Clade + Conde	4	3	Conde (0.416)	Ten-A (0.197)	GO (0.190)	H11 (missing)	0.18	3
Eastern Clade + Conde	1	3	Conde (0.23)	An-W (0.17)	An-C (0.086)	H11 (missing)	0.16	3

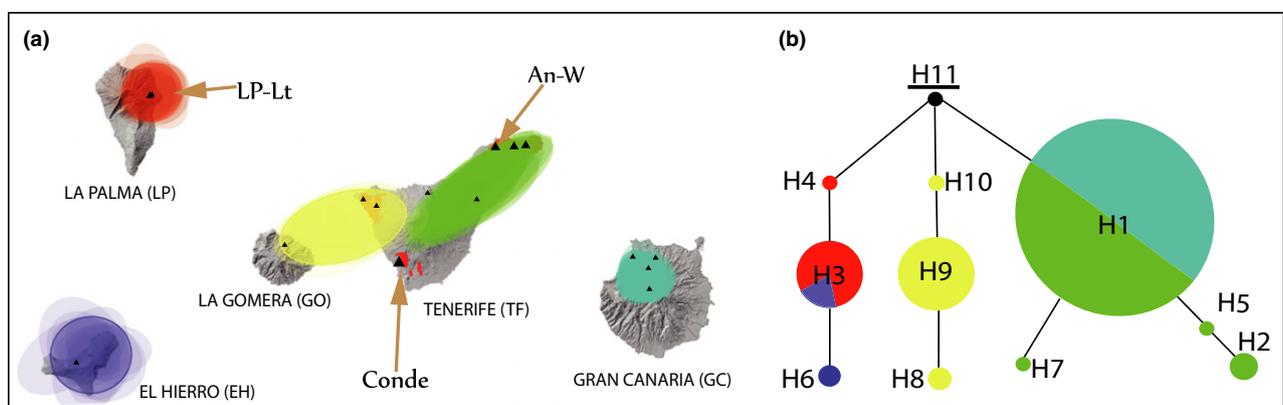


Fig. 3 Results from the Bayesian phylogeographic and ecological clustering (BPEC) analyses for the cpDNA markers. (a) Phylogeographical clusters (coloured blobs) and ancestral location for migration events (denoted by arrows). The contour regions are centred at the 'centre' of each population cluster, and the shaded areas show the radius of 50% concentration contours around it. Locations situated beyond the clusters could also belong to these clusters, but with low probability; in the case of Conde, it suggests a mixed composition, with this population as source of migrant alleles to the east and western clusters. (b) Haplotype network receiving the highest posterior probability. The small black circle H11 indicates an unobserved (missing) ancestral haplotype.

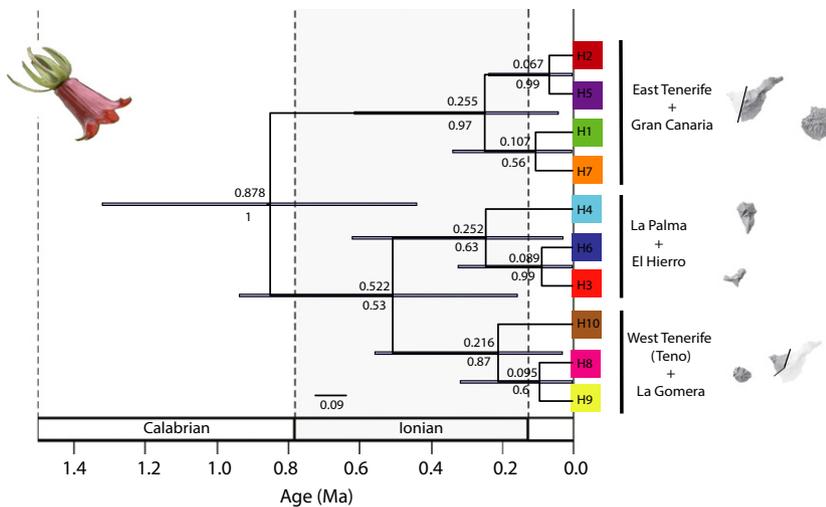


Fig. 4 Maximum clade credibility (MCC) tree obtained from the BEAST analysis of cpDNA haplotypes, showing mean ages (above branches) and 95% HPD credible intervals. Numbers below branches indicate Bayesian posterior clade support values. Codes for tips (H1 to H10) correspond to the haplotypes shown in Fig. 2.

differences in the mismatch distribution analysis resulted in unimodal distributions that were consistent with an expansion model. When we include the population of Conde in the East group, we obtained a second small peak. The raggedness statistics derived from the mismatch distribution were not significant and thus failed to reject the null hypothesis of recent population expansion. EBSF indicated a constant population size (Fig. S2, Supporting information). EBSF for each of the individual populations also obtained a constant population size (results not shown).

AFLP polymorphism, genetic diversity and structure

These results are summarized in Table 1. The final data set, after scoring, comprised 572 loci from 97 individuals and 10 populations. For AFLP analyses, we removed populations from Gran Canaria and Roque del Conde, which had been extracted with a different (less rigorous) method (CTAB) to standardize the quality of extracted DNA. H_j and P were higher in the La Gomera (GO) population, followed by the Teno (Ten-A) population in west Tenerife. The largest number of private fragments was detected in Tenerife (seven fragments). In La Gomera and El Hierro islands, a single fragment was detected. In Tenerife, five private fragments were detected in the Anaga massif (specifically in the An-W population—four fragments) and two in the Teno massif (Table 1). F_{ST} values with the nuclear data (Table S8, Supporting information) were consistent with the results obtained from the chloroplast markers (see above G_{ST} and Nm) and showed the same genetic cohesions between-islands and palaeo-islands.

A PCA differentiated three groups (Fig. S4, Supporting information): (i) east Tenerife populations; (ii) west Tenerife populations; and (iii) the An-W population, which demonstrate an intermediate position between (i)

and (ii). The split network analysis outlined the same divergences between the two well separated groups, including the West group An-W population (Fig. 5b).

Hierarchical AMOVA analyses showed the largest proportion of genetic variation to be found among groups 4 and 5 (Table 3; Table S9, Supporting information). By analysing each island separately, we observed that differentiation within-islands (24.27%) is greater than between-islands (3.62%). A further analysis considering islands and palaeo-islands as separate units showed that differentiation within-islands and within-palaeo-islands is lower (11.30%) than between-islands and between-palaeo-islands (16.63%). According to the method of Evanno *et al.* (2005), STRUCTURE indicated that the most likely number of genetic clusters $K = 2$ ($\Delta K = 460$) represented the optimal number of Bayesian groups within *C. canariensis*, separating the east populations from the west, but also detected the presence of admixture in the intermediate populations. K levels $K = 4$ and $K = 5$ (the latter identified as $K = 6$, with 5 defined clusters plus one 'ghost' cluster with no individuals assigned so it was ignored, see Guillot *et al.* 2005) revealed a more complex genetic structure in these intermediate populations, resulting in additional clusters (Fig. 5a; Fig. S5, Supporting information). The STRUCTURE analysis using only Tenerife populations also delimited two groups (inset $K = 2$ in Fig. 5a; Fig. S5b, Supporting information). Overall, the results revealed a strong interisland genetic structure (Gran Canaria – Anaga massif and La Palma–El Hierro), admixture phenomena (among An-W, Teno populations and La Gomera) and an even more complex substructure within the island of Tenerife. The STRUCTURE sublevels ($K = 4$ and $K = 5$) were consistent with the AMOVA analyses. Only La Gomera differed between analyses; this is explained due to its high genetic variability and mixed composition. These groups are also congruent

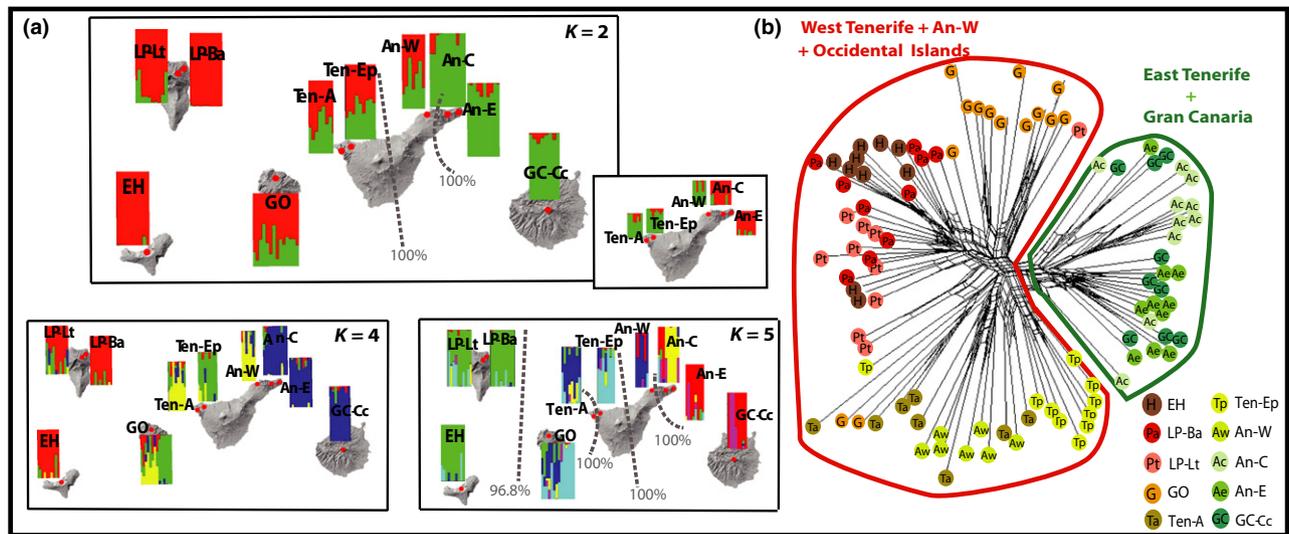


Fig. 5 Results from the analysis of AFLP markers. (a) Histograms showing the Bayesian clustering of individuals within populations (STRUCTURE); colours represent the proportion of individual membership to each inferred Bayesian group. Dotted lines indicate barriers to gene flow and their percentage, as inferred by BARRIER. (b) Split network with points coloured according to location, as indicated in the legend. Codes for populations found in Table S2 (Supporting information).

with the BARRIER results, which revealed two or four major boundaries (Fig. 5a for $K = 2$ and $K = 5$). All barriers showed values of 100% except LP-EH (96.8%). No linear relationship was found between pairwise F_{ST} and geographical distance with the Mantel analysis ($r = 0.42$, $P = 1$).

Discussion

Palaeo-islands as refugia against volcanic (catastrophic) extinctions

The strong genetic structure often detected in island endemics has been postulated to respond to several interconnected physical and biotic factors (Stuessy *et al.* 2014); foremost among them are geographical isolation and extinction-recolonization processes (Carson *et al.*

1990; Gübitz *et al.* 2005; Macías-Hernández *et al.* 2013). The strongest genetic structure and levels of genetic variation in *C. canariensis* were detected between two of the ancient palaeo-islands that currently form Tenerife: Teno in the west and Anaga in the east. Populations in other islands were genetically associated with these two lineages: La Palma, La Gomera and El Hierro to Teno; and Gran Canaria to Anaga. The fact that these patterns are shared between chloroplast (cpDNA, Fig. 2) and nuclear (AFLPs, Fig. 5) markers supports a long history of isolation among populations (Zink & Barrowclough 2008). Furthermore, the hierarchical AMOVA analysis based on the AFLP data showed that genetic variation was notably higher among populations within Tenerife than those located in different islands (Table 3). This pattern has been found in a few other Canarian endemics (*Olea europaea guanchica* and *Pinus canariensis*), but

Table 3 Hierarchical analysis of molecular variance (AMOVA) for *C. canariensis* based on allelic variation at different levels: (A) among groups, (B) among populations within groups and (C) within populations

AMOVA groups	No of groups (K)	Levels			F-statistics		
		A	B	C	Fsc	Fst	Fct
[GC + Anaga] [An-W + Ten-A] [Ten-Ep] [GO + LP + EH]	4	17.44	13.06	69.5	0.15820	0.305	0.17439
[GC+ An-E] [An-C] [Ten-Ep] [An-W + Ten-A] [GO + LP + EH] [GC] [TF] [GO] [LP] [EH]	5	18.92	11.21	69.88	0.13819	0.30123	0.18918
[GC] [An-E + An-C] [An-W] [Ten-Ep] [Ten-A] [GO] [LP] [EH]	8	16.63	11.30	72.07	0.13558	0.27934	0.16631

Geographical locations of populations are shown in Fig. 2.

usually not as marked as here (<10%: Gómez *et al.* 2003; García-Verdugo *et al.* 2010; see review in García-Verdugo *et al.* 2014). Interestingly, considering the palaeo-islands as separate areas reversed the pattern (Table 3). Together with our BARRIER analyses (Fig. 5a in $K = 5$), these results support the hypothesis that the geographical distribution of genetic diversity in *C. canariensis* is structured around the palaeo-islands and that these ancient massifs could be considered as separate units in phylogeographical analyses, a sort of 'islands within-islands'. It indicates that geological barriers within-islands—for example, those associated to catastrophic volcanic events—have probably been more important in structuring patterns of genetic differentiation within species than the oceanic barriers separating the islands (García-Verdugo *et al.* 2014).

Unfortunately, we could not obtain AFLP data for the population in Roque del Conde. Given the mixed composition of the chloroplast compartment in this population (Fig. 2), it is possible that inclusion of this population in our analysis would have led to higher levels of genetic admixture and lower K values in the STRUCTURE analyses—though given the marked east/west split among the remaining populations (Fig. 5), this is unlikely. On the other hand, the two most frequent haplotypes, H1 and H9, in the Conde population are also the most frequent or dominant within the eastern and western clades, respectively, while the divergence of these two haplotypes in the BEAST tree (Fig. 4) can be traced back to the basalmost split in *C. canariensis* (0.8 Ma). This, together with the presence of a unique haplotype (H7) and the fact that the Conde population is identified as the ancestral source of westward and eastward migration events in the BPEC analyses, suggests that the admixture detected in the chloroplast compartment for Roque del Conde is of ancient rather than recent origin. It is thus possible that including this population in the AFLP analysis would have increased rather than decreased levels of genetic diversity within Tenerife for *C. canariensis*.

Which might have caused this level of within-island genetic structure? Many Canarian plant and animal taxa include sister lineages endemic to the Tenerife palaeo-islands (Juan *et al.* 2000; Báez *et al.* 2001), especially among laurel forest species from Teno and Anaga (Table 4). While in some cases, the divergence between these taxa is found at the species level (e.g. *Trechus*, Contreras-Díaz *et al.* 2007; *Pericallis*, Jones *et al.* 2014) and/or predates the merging of the palaeo-islands (*Micromeria*, Puppo *et al.* 2014; *Pholcus*, Dimitrov *et al.* 2008); in others, it is observed within species (e.g. *Tarentola delalandii*, Gübitz *et al.* 2000) and/or postdates the merging of the palaeo-islands (*Eutrichopus*, Moya *et al.* 2004; *Calathus abaxoides*, Emerson *et al.* 1999). The

fact that the pattern of divergence is not contemporary across taxa suggests that the role of Tenerife palaeo-islands in structuring genetic variation has been long lasting. In *C. canariensis*, the basal divergence between the basal eastern and western lineages was dated as 0.878 Ma (Fig. 4) substantially, postdating the age of the merging of the palaeo-islands (c. 3.5 Ma, Ancochea *et al.* 1990). This 'young' east–west divergence might be explained by historical dispersal events between forest patches followed by in situ diversification. Alternatively, it could be explained by allopatric speciation (vicariance) driven by catastrophic volcanic events within a previously more widespread distribution with low or no reconnection. This might explain the 'young' (<3.5 Ma) east–west Tenerife divergence found in *Canarina*, and seen in other taxa (Table 4). The reciprocal monophyly, similar haplotype diversity levels and deep temporal divergence found between the eastern and western clusters of *C. canariensis* in Tenerife (Fig. 4) favour the vicariant, allopatric scenario. In the last 1 million years, several major landslides and volcanic events have affected the north of Tenerife, extending from the summit to the coast (Boulesteix *et al.* 2012, 2013; Carracedo 2014), for example the Güimar and La Orotava mega-landslides (Fig. 1b). These events could have fragmented the ancient laurel forest corridor that extended across the northern flank of the island (Moya *et al.* 2004), interrupting gene flow within species associated with this laurel forest (e.g. *C. canariensis*) and promoting differentiation among populations. Several studies have supported debris avalanches as important factors driving diversification within terrestrial Canarian organisms (Juan *et al.* 2000; Brown *et al.* 2006; Macías-Hernández *et al.* 2013); others have reported a temporal divergence within northern Tenerife (Thorpe *et al.* 1996; Moya *et al.* 2004) that is similar to the one found in *C. canariensis* and corresponds well with the time of the La Orotava mega-landslide (Ancochea *et al.* 1990; Boulesteix *et al.* 2013).

Whereas central Tenerife was almost completely covered by catastrophic events until as recently as 0.13 Ma (Ancochea *et al.* 1999), the three palaeo-islands of Teno, Anaga and Roque del Conde remained geologically stable since the mid-Pliocene (see Fig. 1). This suggests that these areas could have acted as refugia, allowing the survival of populations that otherwise disappeared in other parts of the island that were affected by volcanic activity. Support for this suggestion comes from the population genetic analysis. According to the central-marginal hypothesis (Eckert *et al.* 2008), spatial structure and genetic diversity should be higher in areas that have acted as refugia for the preservation of genotypes that went extinct in other areas and generally for the long-term persistence of populations (Hewitt 2000;

Table 4 Phylogeographical breaks with divergence times reported in the literature between the palaeo-islands of Tenerife

Organism	Phylogeographical disjunction and groups	Divergence (Ma)	Markers	References
Species complex in darkening beetle <i>Pimelia</i>	East Tenerife/West Tenerife	5.5 Ma	Mitochondrial (COI) and nuclear (ITS-1)	Juan <i>et al.</i> (1996)
Lizard <i>Gallotia galloti</i>	Western/North-eastern lineages	0.7 Ma	Mitochondrial cytochrome b	Thorpe <i>et al.</i> (1996)
Beetle <i>Calathus abaxoides</i>	Teno/Anaga	350 000 years	Two mitochondrial (COI and COII)	Emerson <i>et al.</i> (1999)
Skink <i>Chalcides viridanus</i>	Teno/Anaga	1.1 Ma	Two mitochondrial (12S and 16S)	Brown <i>et al.</i> (2000)
Gecko <i>Tarentola delalandii</i>	Clade 1. Teno + Roque del Conde/Anaga. Clade 2. Teno/Roque del Conde	Clade 1. 9–10 Ma Clade 2. 7.6 Ma	Mitochondrial cytochrome b	Gübitz <i>et al.</i> (2000)
Mite <i>Steganacarus carlosi</i>	Clade 1. Teno/Anaga. Clade 2. Roque del Conde/Anaga	Clade 1. 3.2 Ma Clade 2. 25–3.6 Ma	Mitochondrial cytochrome oxidase 1	Salomone <i>et al.</i> (2002)
Beetles <i>Eutrichopus</i>	Teno (<i>E. gonzalezi</i>)/Anaga (<i>E. canariensis</i>)	0.7 Ma	Mitochondrial (COII marker)	Moya <i>et al.</i> (2004)
Species complex in beetle <i>Tarphius</i>	Teno/Anaga	1.2 Ma (1–1.4)	Two mitochondrial (COI and COII)	Emerson & Oromí (2005)
Beetle <i>Trechus</i>	Clade 1. Anaga + Teno (<i>T. antonii</i> , <i>T. tenoensis</i> , <i>T. felix</i>)/Anaga (<i>T. fortunatus</i>) Clade 2. <i>T. flavocintus</i> ; Teno/East Tenerife	Clade 1. 1.73 Ma (HPD: 1.48–2.01) Clade 2. approx 0.75 Ma	Part of mitochondrial genes cytochrome oxidase I and II (Cox1 and Cox2), and nuclear (ITS 2)	Contreras-Díaz <i>et al.</i> (2007)
Spider <i>Pholcus</i>	Anaga (<i>P. malpaisensis</i> , <i>P. knoeseli</i>)/Teno (<i>P. intricatus</i> , <i>P. mascaensis</i> / <i>P. tenerifensis</i> and <i>P. roquensis</i>)	3.93 Ma (HPD: 2.2–5.88)	Four mitochondrial (CO1, 16S, NADH and tRNA ^{Leu}). Morphological data	Dimitrov <i>et al.</i> (2008)
Grasshopper <i>Arminda brunneri</i>	Anaga + Güimar/Teno + Roque del Conde	1–0.17 Ma	Two mitochondrial (12s rRNA, ND5) and two nuclear gene fragments (28s rRNA, ITS2)	Hochkirch & Goerzig (2009)
Spider <i>Dysdera verneuui</i>	Teno/Anaga	3.94 Ma (HPD: 5.1–2.7)	Mitochondrial (<i>cox1</i>)	Macías-Hernández <i>et al.</i> (2013)
Plant <i>Pericallis</i>	Teno (<i>P. echinata</i>)/Anaga (<i>P. tussilaginis</i>)	2.87 Ma (HPD: 1.55–4.76)	Nuclear ITS	Jones <i>et al.</i> (2014)
Plant <i>Micromeria</i>	Teno (<i>M. densiflora</i>)/Anaga (<i>M. teneriffae</i> , <i>M. glomerata</i> and <i>M. rivas-martinezii</i>) + Central group	5.2 Ma	8 nuclear loci	Puppo <i>et al.</i> (2014)

Tzedakis *et al.* 2013; Feliner 2014; Gavin *et al.* 2014). The higher number of ancestral and endemic alleles, private fragments and larger heterozygosity levels exhibited by the populations of the palaeo-islands of Tenerife (Table 1) are congruent with the idea that these massifs acted as reservoirs of ancient genetic diversity and as refugia against volcanically induced extinction. Interestingly, La Gomera, an island that has been geologically quiescent since the Pliocene (Carracedo & Day 2002), shows the highest H_j and percentage of polymorphic

nuclear DNA fragments (Table 1), suggesting that this island might have acted in a similar way to the palaeo-islands of Tenerife.

Further support for the extinction hypothesis comes from the BPEC analysis. Theoretical predictions of coalescent theory states that high-frequency haplotypes have been present for a long time, and more recent ones are rare and derived from the commonest haplotypes (Posada & Crandall 2001). Additionally, a root haplotype is expected to have a higher number of haplotype

connections in the network, rather than being close to the tips. However, past extinction of haplotypes can obscure the inference, with younger haplotypes becoming the most prevalent, so accurately identifying the root haplotype is a challenging task. The fact that BPEC does not provide a single estimate of the haplotype network like Statistical Parsimony, but a finite (probability) distribution of haplotype trees—as well as the existence of the underlying migration model—allows this method to incorporate the uncertainty in the haplotype rooting. BPEC estimates the missing, extinct haplotype H11 as the most probable root of the haplotype network (Fig. 3b), although haplotypes H1, H2 and H8 are also associated with high posterior probabilities.

Have palaeo-islands acted as sources of genetic diversity within and towards other islands?

The theory of Pleistocene climate refugia (Hewitt 2000) states that historically environmentally stable areas can act as sources of genetic diversity exporting migrant alleles to other, disturbed regions (Gavin *et al.* 2014). Palaeo-islands could have played the same role in volcanic archipelagos, although in this case catastrophic geological events rather than climatic changes might be responsible for the observed patterns. BPEC provides support to this hypothesis, identifying the populations in the palaeo-islands of Tenerife as the source areas of ancestral migration events to other adjacent islands, such as from Teno to La Gomera or from Anaga to Gran Canaria (Fig. 3a, b; Table 2). A third dispersal event from west Tenerife/La Gomera to La Palma is supported by the BEAST tree (Fig. 4), and a fourth dispersal event from La Palma to El Hierro is inferred by the BPEC analysis (Fig. 3). Similar patterns with the central Canaries as centres of dispersal events within the archipelago have been described in other animal and plant studies (Francisco-Ortega *et al.* 2002; Gómez *et al.* 2003; Sanmartín *et al.* 2008; Mairal *et al.* 2015; Puppo *et al.* 2015). Moreover, a spatio-temporal pattern of colonization comparable to *Canarina*, showing Tenerife as the centre of dispersal events to adjacent islands in the last 1 Ma, can be found in the Canarian lineage of *Cistus* [0.33 (0.88–0.07) Ma; Guzmán & Vargas 2010], *Cheirolophus* (1 Ma; Vitales *et al.* 2014a), *Cistus monspeliensis* (0.93–0.20 Ma; Fernández-Mazuecos & Vargas 2011) and *Gallotia galloti* (0.8–0.9 Ma; Cox *et al.* 2010). This might be a consequence of the central geographical position of Tenerife within the archipelago—acting as a crossroad for dispersal events—but also of the concentration of plant genetic diversity in the palaeo-islands.

The case of Gran Canaria is especially interesting. It shares the same haplotype with Tenerife (H1, Figs 2 and 3). Low haplotypic diversity (Hn and Hd in

Table 1) could be explained by a recent colonization after a catastrophic event. The island was subject to intense volcanic activity during the Holocene (24 eruption events; Rodríguez-Gonzalez *et al.* 2009), so extinction might explain its present low genetic diversity. Another possibility is related to the topography of Gran Canaria, where a network of ravines (locally known as 'barrancos') connecting at their summits could have facilitated gene flow among populations. Dispersal of *C. canariensis* seeds by *Gallotia* lizards (Rodríguez *et al.* 2008), probably using forest gaps and edges of roads as dispersal corridors (Delgado *et al.* 2007), might have helped to connect populations in the highly altered laurel forest of Gran Canaria.

In addition to dispersal events between-islands, the palaeo-islands of Tenerife might have acted as sources of genetic diversity within Tenerife, exporting migrant alleles to other geologically unstable, disturbed areas. Our demographic analyses indicated a recent population expansion in two populations of east Tenerife close to Anaga (TF-Br and TF-Bj). Although this result should be taken with caution (the EBSF analysis supported a constant effective population size; Fig. S2, Supporting information), these two populations exhibited also a single cpDNA haplotype, which agrees with the idea of a recent colonization. The areas where these populations are located (La Orotava Valley and Güimar Valley, respectively) have been subjected to catastrophic volcanic events (Fig. 1b). Some authors (Thorpe *et al.* 1996; Gübitz *et al.* 2000) have proposed the existence of a corridor of suitable habitat along the northern coastal fringe of Tenerife to explain migrations of the reptiles *G. galloti* and *T. delalandii* from Anaga to the west. These dispersal events could also explain the patterns found in our BPEC analyses, which suggest Anaga populations as sources of migration events to other populations in eastern Tenerife and Gran Canaria (Fig. 3; Table 2).

Ongoing genetic connectivity between the populations in the palaeo-islands of Tenerife and those from other areas is supported by the nuclear genome, which shows genetic admixture between west-Anaga, Teno and La Gomera (Fig. 5a). Admixture could be explained by the carrying of pollen by nectar-feeding birds between forest patches (e.g. ringing techniques have confirmed migration of the main pollinator (*Phylloscopus canariensis*) between Teno and Anaga; A. González, personal communication). The fact that this connectivity is to some extent lost in the cpDNA might be explained by the cpDNA not being transported via pollen or, alternatively, by the small size and haploid nature of the chloroplast genome, which imply shorter coalescent times and less time to fix novel mutations for chloroplast markers (Avice 2000; Jakob & Blattner

2006). On the other hand, the widespread distribution of some cpDNA haplotypes across the archipelago (H1, H3 and H9, Fig. 2a) supports some gene flow among populations driven by seed dispersal. In the Canary Islands, birds have been cited as important vectors for the dispersal of fleshy fruits (Arevalo *et al.* 2007; Padilla *et al.* 2012), and the latter has been associated with frequent gene flow preventing speciation within widespread island endemics (García-Verdugo *et al.* 2014), such as in *Canarina*. This fits with what is known on the reproductive biology of *C. canariensis*, which is pollinated by generalist birds (Rodríguez-Rodríguez & Valido 2011), while its fleshy fruits are dispersed by vertebrates such as *Gallotia* lizards (Valido *et al.* 2003; Rodríguez *et al.* 2008). An additional factor to explain frequent dispersal between-islands are the eustatic sea level shifts during the Pleistocene, which might have decreased geographical distance between-islands (Rijsdijk *et al.* 2014).

Finally, in addition to exporting migrant alleles to other islands and disturbed areas, the palaeo-islands of Tenerife might have acted as cradles or sources or new genetic diversity. The higher haplotype and nucleotide diversity and higher Nei's gene diversity H_j exhibited by populations in these areas (Table 1) agree with their role as ancient refugia but also as sources of novel genetic diversity. Maximum topographic complexity is one of the main factors explaining species richness and high speciation rates within-islands (Whittaker *et al.* 2007). The rugged nature of the Tenerife palaeo-islands has likely promoted genetic differentiation within these massifs. For example, divergences found between populations in east and west forest ranges within Anaga have been explained by the existence of deep ravines and the volcanic arc of Taganana (Fig. 1b), acting as geographical barrier to gene flow (Macías-Hernández *et al.* 2013). These divergences were also detected in *C. canariensis*.

Conclusions

Traditionally, the distribution of genetic diversity within archipelagos is assumed to be structured around oceanic barriers, with between-island divergences expected to be larger than within-island differentiation. Here, we showed that within-island genetic patterns might be as strong as or stronger than those observed between-islands when they are associated with historical volcanic events. In *C. canariensis*, geographical patterns of genetic variation are structured around the palaeo-islands of Tenerife, with a minor secondary effect due to oceanic barriers. Carine & Schaefer (2010) argued that although relatively short oceanic distances separate the Canary Islands, they might be responsible

for the high diversity levels found in the archipelago, acting as effective barriers to dispersal and promoting allopatric speciation. However, our results suggest that this hypothesis might not be valid for endemic species with widespread distributions across several islands (e.g. *Canarina*), for whom stretches of ocean are apparently less of a barrier than topographic relief within volcanic islands. Phylogeographical studies on other MIEs (multiple island endemics) are needed to confirm this hypothesis. The palaeo-islands of Tenerife have probably acted as both genetic refugia and sources of new diversity within- and between-islands. The preservation of genotypes that became extinct everywhere else and the topographic complexity of the palaeo-islands makes them potential 'phylogeographical hotspots' (Médail & Diadema 2009) and reservoirs of unique genetic diversity, whose conservation should be prioritized.

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M.M., M.A., J.J.A. and I.S. designed the study; M.M. carried out the field work, M.A. and J.J.A. contributed some samples; M.M. performed the research and analysed the data under the supervision of M.A. and I.S.; I.M., M.M., I.S. and V.C. performed the BPEC analyses; M.M. wrote the paper with help from I.S., M.A. and J.J.A., M.M. and I.S. revised the manuscript. All authors contributed with comments and approved the final version.

Data accessibility

DNA sequences: Genbank Accession nos KP797991–KP798432.

GenBank accessions, sampling locations and/or online-only appendices uploaded as online supplemental material.

NEXUS files for the concatenated cpDNA data set and original AFLPs Matrix of *Canarina canariensis*, Dryad doi:10.5061/dryad.j4103.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Plant species which are restricted and shared between two or more of the ancient static areas considered: the three paleo-islands of Tenerife and La Gomera.

Table S2 Sampling information and GenBank accession numbers for all of the taxa included in this study.

Table S3 Results from the sensitivity analyses to assess confidence in our age estimates.

Table S4 Haplotypes of *Canarina canariensis*, as determined by using the variable sites and indels found in the sixteen *C. canariensis* populations with markers *PetB-PetD* (937 bp), *Rpl32-trnL* (654 bp) and *trnS-trnG* (658 bp).

Table S5 G_{ST} and number of migrants (in parentheses) obtained from chloroplastic data in *Canarina canariensis*.

Table S6 Mean ages and 95% HPD confidence intervals from the BEAST analysis.

Table S7 Results from the DNA polymorphism, neutrality test and mismatch raggedness for *Canarina canariensis* haplotypes.

Table S8 Pairwise F_{ST} between populations of *Canarina canariensis* with nuclear data.

Table S9 Hierarchical analysis of molecular variance (AMOVA) for *C. canariensis* based on allelic variation at different levels: (a) amongst groups, (b) amongst populations within groups and (c) within populations.

Fig. S1 Clusters and networks obtained with bayesian phylogeographic and environmental clustering (BPEC) with plastid DNA sequences.

Fig. S2 Mismatch distribution (left handside) and Extended Bayesian Skyline Plot analysis (right handside) for the different groups.

Fig. S3 The MCC tree, from the *BEAST* analysis, showing nodes with mean ages and the 95% HPD confidence intervals (values specified in Table S5).

Fig. S4 Principal coordinates analysis of AFLP data for individuals of *C. canariensis*.

Fig. S5 The estimated probability of the likelihood function according to the Evanno method for: (a) *STRUCTURE* analysis for *C. canariensis*; (b) *STRUCTURE* analysis using only Teneriffean populations.