Historical volcanism and within-island genetic divergence in the Tenerife skink (*Chalcides viridanus*)

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Running title.-Within-island divergence in the Tenerife skink

Abstract

This study examines the genetic diversity of the Tenerife skink (*Chalcides viridanus*) within the context that Tenerife is now thought to have arisen as a single island as opposed to two/three precursor islands. DNA sequences were obtained from two mitochondrial regions and five nuclear loci. MtDNA divergence was substantial with four geographical clusters being detected. Two of these corresponded to two ancient areas that have undergone little recent eruptive activity (Anaga, Teno), while two further clusters were found within the more volcanically active central region. Nuclear divergence was low and revealed no strong geographical pattern. Estimated divergence of the Anaga group was 0.4-2.1 Ma ago (95% posterior interval), while the divergence time of the Teno cluster was 0.1-1.2 Ma ago. Phylogeographic distributions correspond well with ancient edifices, but divergence times postdate those expected under the previous ‘unification of ancient islands’ geological scenario. There is evidence of a major recent expansion of the central group following a decline in eruptive activity in this region, which also fits well with current geological hypotheses. Previously-described within-island evolutionary patterns in other Tenerife species also need to examined within this new geological context.

Introduction

Islands have played a key role in our understanding of biogeographical processes chiefly through studies of inter-island dispersal, see review by de Queiroz (2005). Less well-known is the fact that they have also provided examples of divergence of contiguous populations over small discrete geographical areas. While several insular regions offer good examples of within-island diversity, including arthropods on Hawaii, reviewed by Roderick and Gillespie (1998), Anole lizards in the Caribbean, e.g., Malhotra and Thorpe (2000) and giant tortoises from the Galápagos (Ciofi *et al.*, 2006), the Canary Island archipelago has become a key area for these studies. Research has addressed quite a broad range of taxonomic groups (Rees *et al.*, 2001; Moya *et al.*, 2004; Macías-Hernández *et al.*, 2013; Mairal *et al.*, 2015), including several lizards (Thorpe & Baez, 1987; Thorpe *et al.*, 1996; Gübitz *et al.*, 2000; Bloor *et al.*, 2008; Suárez *et al.*, 2014). Lizards seem to provide good vertebrate models for within-island diversity, possibly because within-island dispersal is easily interrupted due to their terrestrial habit. One paradigm that has emerged is the close relationship between historical island volcanism and genetic diversity. It has been facilitated by geological research that has established quite detailed reconstructions of the subaerial histories of the Canaries.

The island of Tenerife provides some of the more interesting examples of within-island divergence. It is the largest (2032km^2) and highest (3718m a.s.l.) Canary island and one of the tallest volcanic structures on earth (as measured from the ocean floor). It has a long and complex geological history and also harbours major ecological heterogeneity. Four endemic lizards are found on Tenerife, with three of these being widespread. MtDNA haplotype distributions within the widespread species (Thorpe *et al.*, 1996; Brown *et al.*, 2000; Gübitz *et al.*, 2000) show associations, to a greater or lesser extent, with areas that correspond to the three ancient volcanoes that make up the island: Anaga in the north-east, Teno in the north-west and the large central edifice between them (Fig. 1).

Anaga and Teno have shown relatively little recent volcanic activity since their respective subaerial appearances 4.9–3.9 Ma and 6.2–5.6 Ma (Ancochea *et al.*, 1990; Ancochea *et al.*, 1999). In contrast, three major eruptive cycles 3.5-2.7 Ma, 2.5–1.4 Ma and 1.1–0.2 Ma affected the older (11.9-8.9 Ma) central edifice. These eruptions raised the height of the island and also produced huge flank collapses (Martí *et al.*, 1997; Carracedo *et al.*, 2011). It was first thought that the three main volcanic regions arose as independent islands (Ancochea *et al.*, 1990; Ancochea *et al.*, 1999) and phylogeographic studies have generally interpreted within-island patterns within this context. However, following geochronological and isotope studies, a new geological consensus has emerged. Teno and Anaga appear to have formed as smaller shield volcanoes on the flanks of the large central subaerial shield and not as separate islands (Guillou *et al.*, 2004; Carracedo *et al.*, 2007; Deegan *et al.*, 2012; Delcamp *et al.*, 2012). Under this new scenario, within-island divergences of animal/plant populations require updated interpretations, i.e., genetic diversity needs to be considered as potentially having arisen from population fragmentation within one island rather than dispersal between independent islands.

The Tenerife skink (*Chalcides viridanus*) is one of three widespread endemic lizards found on this island. It has two close relatives, both from the Canary Islands: *C. sexlineatus* from Gran Canaria and *C. coeruleopunctatus* from El Hierro and La Gomera (Brown & Pestano, 1998). An analysis of two short fragments of mtDNA with relatively slow substitution rates indicated phylogeographical structuring and provided some support for divergent mtDNA groups within two of the ancient volcanic areas of Anaga and Teno (Brown et al., 2000). However, one of these groups was detected at only one Anaga site, the other was detected at only two Teno sites, and statistical support was weak. Also, it remains unknown whether or not similar patterns of divergence are evident in the nuclear DNA. A reanalysis of genetic patterns in this species is therefore timely, particularly given: i) the new geological context and ii) the availability of new statistical techniques such as Bayesian multispecies coalescent approaches for estimation of population/species divergence times (Heled & Drummond,

2010) and advances in methods for analyses of spatial genetic patterns (Corander *et al.*, 2003; Manolopoulou *et al.*, 2011).

Assessment of whether or not the phylogeographic pattern in *C. viridanus* fits the former "three ancient islands" interpretation or the current "single island with three volcanic regions" geological interpretation must be largely centre around timing. Under the former, divergence occurred after transmarine dispersal between the two/three ancient putative islands and would be supported by divergence times that correspond to the period before these islands were joined. The subaerial ages of the Teno, Anaga and Roque del Conde edifices are well-established (Ancochea *et al.*, 1990). If ancient island colonization occurred soon after appearance, as established in other Canary Island lizards (Cox *et al.*, 2010), then late Miocene/early Pliocene within-island divergence times are predicted. Spatial genetic structuring should also show an association with the ancient islands. The alternative hypothesis of divergence within a single island would more likely favour Pleistocene divergence times. The rationale is that, similar to patterns observed on other islands (e.g., Suarez et al., 2004), they should coincide with, or postdate, the major Cañadas I and II eruptions that affected large parts of the centre of the island (Ancochea *et al.*, 1990). These eruptions are expected to have extinguished populations and created dispersal barriers. Under this scenario, a spatial pattern is predicted in which older lineages are also associated with the more peripheral populations that were found in regions that were inactive at the time. Unfortunately this pattern does not differ much from that under the three ancient island scenario because both geological interpretations essentially recognize the three ancient areas in Tenerife. Nonetheless, under this hypothesis, post-eruptive colonization of the central shield is expected to have required major population expansion by central populations, which may be detectable using coalescent approaches.

We analysed mtDNA and nuclear loci from *C. viridanus* sampled from a large number of sites across Tenerife in order to describe and assess patterns and causes of within-island intraspecific genetic diversity. We specifically examined whether the timing of within-island genetic divergence was likely to fit the timescale expected under the hypothesis of dispersal between ancient islands, or a more recent period that coincided with major central eruptions and landslides on a single island.

Materials and methods

Fieldwork and DNA sequencing

Chalcides viridanus (*n*=64) were captured by hand from 36 Tenerife sites in April 2010 and a 5 mm tail tip was removed before release at the site of capture (authorized by the Cabildo Insular de Tenerife)(Fig. 1). Latitudes and longitudes of each site were recorded using a Garmin eTrex GPS (see S1 in Supporting Information for more information). Sampling was designed to cover all main parts of the distribution of this species. Despite being found at altitudes of at least 2100m on Tenerife the species is scarce above 1100m (Brown et al., 1992). Hence most sample sites were low altitude, with the exception of sites 7 and 30. Also, despite intensive searching, no individuals were obtained from an area of the east coast, south of the Güímar valley. Homologous sequences from closely-related species *C. coeruleopunctatus* and *C. sexlineatus* were also obtained from specimens collected during previous studies (Brown and Pestano, 1998; Suarez et al., 2014). *C. coeruleopunctatus* specimens were from Frontera (*n*=1) and Virgen de los Reyes (*n*=1) in El Hierro, and from Hermigua (*n*=1) and Calera (*n*=1) in La Gomera. *C. sexlineatus* were from Tafira Baja (*n*=2) in Gran Canaria. Inclusion of these specimens allowed use of a calibration in phylogenetic dating analyses.

DNA was extracted and two mtDNA fragments were sequenced in both directions for all individuals to obtain: 1) 581 bp of cytochrome b gene sequence, 2) 982 bp of sequence incorporating partial sequences from the ND1 and the ND2 subunits of the NADH dehydrogenase gene and three intermediate tRNAs, which will be referred to as the ND sequence (see S2).

Suitable primers for amplification of nuclear sequences were examined by testing those published by four previous studies on reptiles/amphibia including one study that attempted to identify rapidly-evolving nuclear markers (see S2 in Supporting Information). This yielded good-quality sequences for ten nuclear loci in *C. viridanus*. These sequences were tested on individuals from 3–4 sites (always including site 5 in Anaga and site 1 in the south) but only five loci contained variable sites (four of these had previously been shown to show within-species polymorphism in other species: see references in S2). Up to one specimen for each of the 36 sample sites was then sequenced for each of these five loci: PRLR (534bp, *n*=31), Rag-1 (849bp, *n*=32), RELN (583bp, *n*=35), EXPH (796bp, *n*=30) and SELT (399bp, *n*=30) (see S2).

Statistical analyses

Relationships among mtDNA haplotypes and alleles from nuclear loci were examined using median joining networks, as implemented within the program Network 4.6.0.0 (Fluxus engineering).

The program BPEC (Manolopoulou *et al.*, 2011; Manolopoulou & Hille, 2016) is a Bayesian MCMC approach that identifies genetic clusters that are geographically coherent, and was applied to the mtDNA haplotypes only (see below). The haplotype tree and clustering parameter values, including the number and location of genetic clusters, are updated at each step in the MCMC algorithm. Our primary aim was to visualize the geographical distributions (and associated uncertainty) of mtDNA clusters. This approach is based on a migration model and might be less appropriate for inferring structuring under population fragmentation (for this reason an alternative approach was also used, see below). The parsimony criterion was not relaxed due to high levels of divergence between haplotypes, and the maximum number of migrations was specified as six. One thousand five hundred posterior samples were taken from an MCMC chain of 3×10^6 steps.

Spatial clustering of individuals was also analysed using the program BAPS v. 6 (Corander *et al.*, 2003). Two BAPS analyses were carried out: i) mtDNA sequences alone and ii) the five nuclear loci (together) with prior geographical information input as latitudes and longitudes. A linked model was used for mtDNA sequences and an unlinked model for the nuclear loci.

The program BEAST 1.8.3 (Drummond & Rambaut, 2007) was used to simultaneously date divergence times and investigate relationships between the main mtDNA groups (identified by BAPS) using the *BEAST multispecies coalescent approach (Heled & Drummond, 2010). The mtDNA sequence was split *a priori* into five partitions (all containing reasonable numbers of variable sites) to accommodate likely differences in rate and other features of the evolutionary process. The partitions and respective models were: i) cytochrome b codon positions 1 and 2 (HKY+G), ii) cytochrome b codon position 3 (HKY), iii) NADH positions 1 and 2 (from both subunits)(TN93+G), iv) NADH position 3 (HKY+G), and v) tRNA regions (JC+G). The most appropriate model available in BEAST was selected after comparing corrected Akaike information criterion scores of different models. The inclusion of specimens from La Gomera and El Hierro provided a time calibration. Skinks from these two islands have previously been established as monophyletic and must have diverged sometime after the island of El Hierro appeared about 1.12 mya (Guillou *et al.*, 1996; Brown & Pestano, 1998). The ancestral node for these two island populations (on the species tree) was monophyly-constrained and its age specified from the gamma distribution G(110, 0.001) which has a median of 0.11 (time units were 10 Ma). The root of the species tree was loosely specified from G(5.3, 0.1) (median: 0.497) based on previous analyses of divergence times in this group (Brown & Pestano, 1998; Carranza *et al.*, 2008; Brown *et al.*, 2012). A strict clock was used due to the shallow root (Brown & Yang, 2011). The hyperprior on the gamma distributed population sizes was specified from the uniform distribution U(0, 0.06). The MCMC chain was run for 40 million generations, with a sampling interval of 5000. The first 4 million generations were discarded as burn-in, and the run repeated three times from different starting positions to ensure convergence on the same posterior.

Historical demographic changes in the individual mtDNA spatial clusters were analysed using Bayesian skyline plots (BSPs) under the piecewise-constant model in BEAST (Drummond *et al.*, 2005; Drummond & Rambaut, 2007). Only the third codon positions (ND and cytochrome b) were used for these analyses due to low sequence diversity within each genetic cluster. For each analysis, a gamma prior was placed on the most basal node which matched the 95% Highest Posterior Density (HPD) of the posterior determined by the *BEAST analysis. This allowed assessment of demographic changes on the *BEAST timescale.

Results

Spatial structuring of mtDNA

The 581bp of aligned cytochrome b sequences (GenBank accession numbers KX909600-KX909663) contained 94 variable sites and the 982 bp of ND2 sequence contained 141 variable sites (KX909822-KX909885). The MJ network of all mtDNA sequences revealed considerable within-island divergence, and suggested four clusters of haplotypes (Fig. 2). Two of these clusters corresponded to groups of sites within Teno (three sites) and Anaga (four sites) and were very divergent both from one another and from the remaining two clusters. These remaining clusters were separated by a smaller number of mutational steps and corresponded to the central areas of Tenerife (the more widespread one will be referred to as Central I and the other as Central II) but also extended into Teno and Anaga.

The network patterns were supported by spatial clustering using BAPS. The highest likelihood corresponded to four mtDNA clusters, and each cluster had an identical haplotype composition to those described in the MJ network.

BPEC phylogeography analyses favoured either three (posterior probability = 0.382) or four (0.306) migrations. Four clusters contained haplotypes with high assignment probabilities (each cluster having one or more haplotypes assigned with probabilities of 0.61 or higher) and were interpreted, while the three additional clusters had low assignment probabilities and were not analysed further. One of the clusters was centred on the ancient area of Anaga while a second cluster corresponded to Teno (Fig. 3). Both of these clusters had the same haplotype compositions as two of the clusters detected by BAPS. The remaining two BPEC clusters corresponded to central areas but had slightly different compositions to the central groups detected by BAPS: one was confined to the north-western region (including Anaga) and the west coast (Fig. 3A). The other was found across the central part of Tenerife but does not extend significantly into Anaga (Fig. 3B).

Spatial structuring of nDNA

Low levels of sequence diversity were found within the five nuclear loci (see S3 in Supporting Information; EXPH5: KX909664-KX909693, PRLR: KX909694-KX909724, RAG1: KX909725-KX909756, RELN: KX909757-KX909791, SELT: KX909792-KX909821). MJ network analyses of all markers revealed no evidence of geographical structuring. Numbers of mutational steps were very small and there was no clear evidence of associations between alleles and Teno/Anaga regions.

The BAPS analysis of all nuclear loci revealed only one genetic cluster (P=0.999). This suggested little or no phylogeographic information was contained within these loci and so no further analyses were carried out.

Divergence time dating

MtDNA alone was used for dating analyses due to the lack of phylogenetic information within the nuclear loci. The posterior median divergence time for the most basal Tenerife split between north-eastern Anaga and the remaining haplotypes was 1.25 Ma (95% HPD: 0.4-2.1 Ma)(Fig. 4). The next branching event represents the separation of Teno from the central clades with a posterior median divergence time of 0.44 Ma (95% HPD: 0.1-1.2 Ma). The split between the Central I and Central II clades was the most recent within-island divergence (posterior mean 0.05 Ma, 95% HPD: 0.0-

0.1 Ma). Posterior support for different groups within the topology was generally high (Fig. 4), although not for the (Teno, (Central I and II)) node, which had a posterior probability of 0.55.

Demographic history

Historical demographic changes were analysed in the large and widespread Central II clade (see Fig. 2) as this contained many individuals (*n*=43) allowing examination of a reasonable number of temporal groups (five were used and reported here, but analyses were also performed with between 2–10 groups to ensure the findings were robust). A clear signature of historical population expansion was seen between approximately 0.01-0.02 Ma ago (Fig. 5). The remaining three clusters from the smaller of the two Central clades, Anaga and Teno, contained too few individuals (*n*=6–8) to allow decisive analyses. However none of the tests using just 2 or 3 temporal groups provided any indications of population changes (results not shown).

Discussion

Studies of the effects of volcanism on island diversity have tended to focus on the effects of island appearance and transmarine dispersal between islands, e.g., Juan *et al.* (2000), Caccone *et al.* (2002),Thorpe *et al.* (2005), Garb and Gillespie (2006), Benavides *et al.* (2009), Cox *et al.* (2010). The within-island diversity of *C. viridanus* could have originated in this way if Tenerife originally comprised more than one independent island. Given the ages of the ancient edifices (see Introduction) and assuming that colonization occurred soon after island appearance, hypothesized transmarine dispersal would correspond to the Pliocene or Miocene. Instead, we detect Pleistocene divergence times that seem incompatible with the hypothesis that the genetic diversity originated through dispersal between putative independent islands. We therefore favour the hypothesis that genetic diversity originated from local extinctions and creation of dispersal barriers within the intermediate central shield volcano, leading to isolation of populations in the extreme north-east and the north-west (discussed in more detail below). Hence, the origin of the genetic diversity in *C. viridanus* appears quite similar to that in lizards on the neighbouring islands of Gran Canaria (Gübitz *et al.*, 2005; Suárez *et al.*, 2014) and Lanzarote (Bloor *et al.*, 2008) and also to giant tortoises on the Galápagos (Beheregaray *et al.*, 2003).

A previous study also contested the 'dispersal between ancient islands' hypothesis of population divergence in another Tenerife lizard, *Gallotia galloti* (Brown *et al.*, 2006). Divergence in this species was not dated by the same method used here. In particular, ancestral polymorphism was not taken into account, and this generally leads to estimation of older divergence times. However, much lower sequence divergence was detected (~1.4% between lineages for cytochrome b, compared with ~5% here) and so divergence almost certainly postdated that in *C. viridanus*. In the other widespread Tenerife lizard, the gecko *Tarentola delalandii*, divergence is much greater (~13% between lineages, cytochrome b) and is likely to have occurred much earlier (Gübitz *et al.*, 2000). Estimations of within-island divergence times in insects show a similar level of variation, with Anaga/Teno forms potentially having arisen at the same time as in *C. viridanus* in some groups (Contreras-Díaz *et al.*, 2007) while others show much deeper divergence, similar to *T. delalandii*, see review by Mairal *et al.* (2015). The next phase of analysis of within-island diversity for Tenerife will be to provide an explanation of these differences between species, some proportion of which may be attributed to technical differences in divergence estimation. In the next three paragraphs we offer an updated interpretation of within-island evolution of *C. viridanus*, in relation to current theories on the geological formation of the island.

Both the current and previous studies support an original colonization of Tenerife that considerably predates the first within-island divergence (i.e., Anaga), possibly by more than 3 Ma (Brown & Pestano, 1998; Suárez *et al.*, 2014). Given the long post-colonization period, it is reasonable to assume that skinks would have had a fairly widespread distribution at this time. Hence the question is: how did the Anaga populations become isolated in north-east Tenerife? Our estimated time frame includes the end of the *penultimate eruptive cycle and the final eruptive cycle that affected the* central region of the island after the Anaga and Teno volcanoes were fully formed and quiescent (Ancochea et al., 1990). A recently well-documented feature of Tenerife's volcanic history is the north-east rift zone, which was active ca. 1.1 and 0.83 Ma and situated on the island's dorsal spine which runs from the centre to the north east (Carracedo *et al.*, 2011). Eruptions along this dorsal ridge, with associated lava flows on the north and south-facing slopes, would have effectively isolated the north-eastern region, including Anaga, from the rest of the island (Carracedo *et al.*, 2011). This intense eruptive activity was followed by major debris avalanches, which occurred both sides of the ridge, 0.83 Ma ago (Carracedo *et al.*, 2011). The landslide on the south eastern side of the ridge was also invoked to explain a phylogeographic break in the sympatric lizard *G. galloti* (Brown *et al.*, 2006). In summary, there is geological evidence that eruptions and landslides that could have cleaved the original distribution in a manner that is spatially and temporally consistent with the current phylogeographic pattern.

Following the isolation of Anaga, the subsequent split occurred in the late Pleistocene leading to divergence of Teno from the more central populations (broadly ~0.5 Ma ago). The distribution of this group is clearly centred on the ancient Teno volcanic edifice. Eruptions and landslides in the north-west rift zone could have isolated Teno populations, but these events occurred mainly during the Holocene (Carracedo *et al.*, 2007) and therefore seem too recent. Some eruptions did occur around the periphery of Teno at times that fit the divergence time, such as the Tierra del Trigo (0.2 Ma) and Montaña de Taco (0.7 Ma) eruptions, but current geological evidence suggests they were quite localised (Carracedo *et al.*, 2007) and so do not provide convincing explanations of long-term vicariance. Nevertheless, the ongoing activity and location of this rift zone within the recently-active Cañadas edifice seems to provide the most likely cause of the isolation of Teno populations.

The origins of the two clusters detected within the central clade are more difficult to interpret, especially as cluster compositions (and therefore geographical distribution) are dependent on the method of analysis. A geographically coherent genetic cluster that is associated with the region between the central volcano and Teno is detected by BPEC. Its distribution and low level of divergence suggest that it could have originated through population fragmentation caused by the Holocene volcanism in the north-east rift zone, as discussed previously. The population expansion detected in the larger of the two central clusters post-dated the major explosive period that marked the end of the formation of the central Teide volcano (Carracedo *et al.*, 2007). A similar signature of post-eruptive expansion has been documented in Galápagos tortoises from the island of Isabela (Beheregaray *et al.*, 2003). In summary, extinctions that gave rise to refugia populations, which recolonized central regions of the island when suitable habitat became available, could account for the origins and demographic histories of the two central clades.

Finally, nuclear sequences provided little information on the phylogeographic pattern. This was likely due to low substitution rates leading to incomplete lineage sorting between regions. While microsatellite regions might intuitively appear to be better markers, a recent study that used microsatellites to examine genetic diversity in *C. sexlineatus* on Gran Canaria (Suárez *et al.*, 2014) found only a small proportion of the twelve markers to be informative (although divergence time was older than within *C. viridanus*, i.e., ~ 2Ma) (Suárez *et al.*, 2014). This suggests that in some cases within-island divergence might be too old to render microsatellites a useful marker but too recent for a small number of nuclear sequences to be informative. Future genome-wide SNP analyses are likely to resolve this issue, once approaches become financially accessible to more researchers. For example, a very recent RADseq study by one of the authors detected evidence of historical isolation in the genome of the Tenerife lizard *Gallotia galloti* which has a similar divergence time to that observed here (Brown *et al.*, 2016).

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Figure Legends

Fig. 1. The 36 sample sites on Tenerife (Google Earth, Grafcan), showing the regions containing exposed ancient rocks in Anaga and Teno (delimited by white lines) and other geological features mentioned in the text.

Fig. 2. Median-Joining (MJ) network showing relationships between Tenerife skink mtDNA haplotypes, and also their geographical locations. Corresponding colours are used on the MJ figures and the Tenerife image (which is from Google Earth: Grafcan).

Fig. 3. Posteriors on the distributions of the main Teno (NW) and Anaga (NE) geographical mtDNA clusters, detected using BPEC. A. and B: Posteriors for the distributions of the two "central" clusters. Background Tenerife image is from Google Earth (Grafcan).

Fig. 4. *BEAST multispecies coalescent tree for Western Canary Island *Chalcides* (*C. sexlineatus* from Gran Canaria, *C. coeruleopunctatus* from La Gomera and El Hierro, and the four identified clades within *C. viridanus* from Tenerife (Anaga, Teno, Central I and II). Values in parentheses are the 95% highest posterior densities on divergence times in units of 10 Ma. Italicised values on branches are posteriors for the terminating node on the branch.

Fig. 5. Bayesian skyline plot for the large central II group of *Chalcides viridanus* haplotypes (see Figure 2), indicating rapid recent population growth starting around 20 ka ago (1 time unit = 10 Ma). The intermediate line is the median, upper and lower lines give the 95% HPDs from the posterior for population size.

Appendices

Appendix 1. Table of latitudes and longitudes of the 36 sample sites in Tenerife obtained in the field using Garmin eTrex GPS (see also Fig. 1 in main paper).

Site	*Latitude*	*Longitude*
1	N 28 01.387'	W 16 41.398'
2	N 28 02.397'	W 16 40.625'
3	N 28 17.968'	W 16 22.810'
4	N 28 31.449'	W 16 09.324'
5	N 28 30.437'	W 16 11.725'
6	N 28 24.986'	W 016 19.615'
7	N 28 24.720'	W 016 24.420'
8	N 28 33.597'	W 16 09.382'
9	N 28 33.641'	W 16 12.849'
10	N 28 31.215'	W 16 17.613'
11	N 28 32.267'	W 16 21.420'
12	N 28 32.505'	W 16 21.237'
13	N 28 29.865'	W 16 25.142'
14	N 28 29.483'	W 16 16.439'
15	N 28 22.923'	W 16 22.615'
16	N 28 26.746'	W 16 28.036'
17	N 28 24.035'	W 16 31.284'
18	N 28 23.714'	W 16 35.773'
19	N 28 23.615'	W 16 39.365'
20	N 28 21.984'	W 16 51.368'
21	N 28 21.769'	W 16 50.554'
22	N 28 22.157'	W 16 49.414'
23	N 28 22.226'	W 16 46.804'
24	N 28 22.309'	W 16 43.131'
25	N 28 05.985'	W 16 44.148
26	N 28 07.715'	W 16 45.691
27	N 28 10.235'	W 16 47.906
28	N 28 11.965'	W 16 49.428
29	N 28 13.011'	W 16 49.998
30	N 28 17.925'	W 16 48.900
31	N 28 18.970'	W 16 51.319
32	N 28 20.178'	W 16 51.094
33	N 28 19.778'	W 16 24.901
34	N 28 07.009'	W 16 34.894
35	N 28 04.331'	W 16 39.241
36	N 28 17.702'	W 16 25.054

Appendix 2. Primers used for amplification of mtDNA and nuclear DNA sequences.

Locus	Primers	Reference
MtDNA – Cytochrome b	Forward 5′-AAAACTTAATGGCCCACAACC-3′ Reverse 5′-TGGGTGGAATGGAATTTTGT-3′	This study
MtDNA – NADH	Forward 5′-GCCCCATTTGACCTTACAGAG-3′ Reverse 5′-ATTGCTGCTATTCAGCCTAGGTGGGC-3′	Forward: Macey et al., 1998 Reverse: Suarez et al., 2014
EXPH5	Forward (*F1**) 5′-AATAAACTKGCAGCTATGTACAAAACAAGTC-3′ Reverse (*R1)* 5′-AAYCGCCCTTCTGTGAGTGACCTCT-3′	Portik et al., 2012
RELN	Forward (*RELN61F*) 5′-GAGTMACTGAAATAAACTGGGAAAC-3′ Reverse (*RELN62R*) 5′-GCCATGTAATYCCATTATTTACACTG-3′	Pinho et al., 2010

PRLR	Forward (*PRLR-F15*) 5’-GACARYGARGACCAGCAACTRATGCC-3’ Reverse (*PRLR-F35*) 5’-GACYTTGTGRACTTCYACRTAATCCAT-3’	Townsend et al., 2008
RAG-1	Forward (*RAG-1F*) 5’-TGCACTGTGACATTGGCAA-3’ Reverse *RAG-1R* 5’-GCCATTCATTTTYCGAA-3’	Townsend et al., 2004
SELT	Forward (*SELT-F6*) 5’-GTTATYAGCCAGCGGTACCAAGACATCCG-3’ Reverse (*SELT-R6*) 5’-GCCTATTAAYACTAGTTTGAAGACTGACAG-3’	Jackson & Austin, 2012

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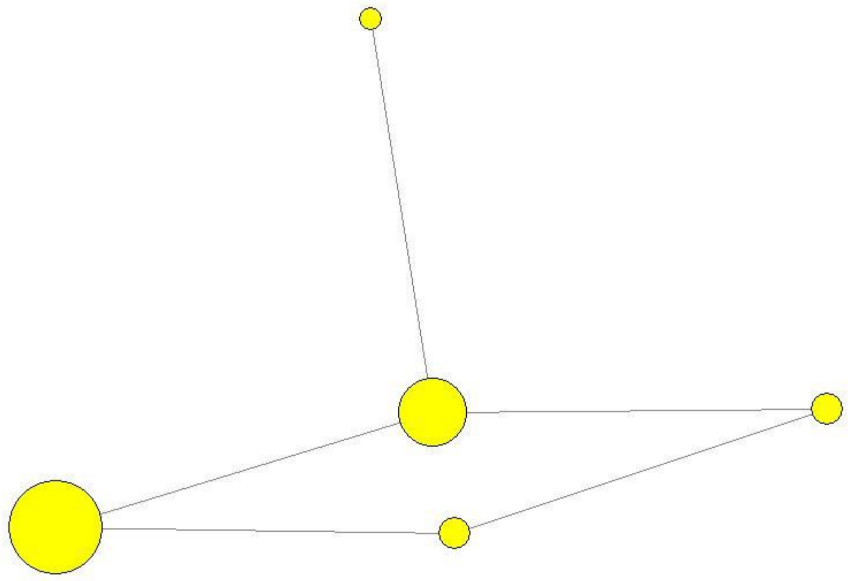
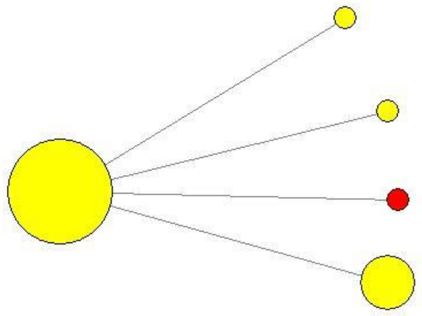
Figs. S1-S5 Median joining networks indicating levels of polymorphism within the five nuclear loci. Node areas are proportional to numbers of individuals, with the smallest nodes containing only one individual in all cases. Nodes indicated in blue or red are those that contain only individuals from within either the Teno or Anaga regions, respectively (all other nodes are indicated in yellow).

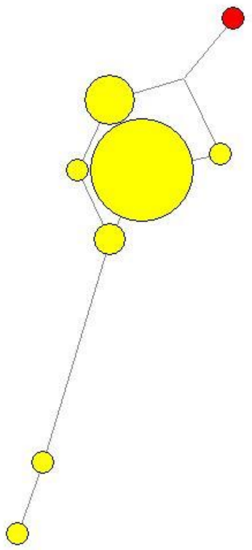
S1 PRLR gene



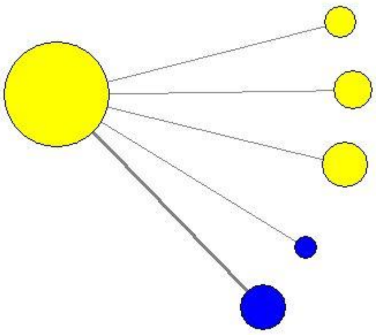
S2 EXPH gene



S3 RAG-1 gene



S4 RELN gene



S5 SELT gene