Recent volcanism and mitochondrial DNA structuring in the lizard *Gallotia atlantica* from the island of Lanzarote

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Abstract

The phylogeography of the lacertid lizard *Gallotia atlantica* from the small volcanic island of Lanzarote (Canary Islands) was analysed based on 1075 bp of mitochondrial DNA (mtDNA) sequence (partial cytochrome *b* and *ND2*) for 157 individuals from 27 sites (including three sites from neighbouring islets). Levels of sequence divergence were generally low, with the most distant haplotypes separated by only 14 mutational steps. MtDNA divergence appears to coincide with formation of the middle Pleistocene lowland that united formerly separate ancient islands to form the current island of Lanzarote, allowing rejection of a two-island model of phylogeographical structure. There was evidence of large-scale population expansion after island unification, consistent with the colonization of new areas. A nested clade phylogeographical analysis (NCPA) revealed significant phylogeographical structuring. Two-step and higher-level clades each had disjunct distributions, being found to the east and west of a common area with a north–south orientation that extends between coasts in the centre-east of the island (El Jable). Other clades were almost entirely restricted to the El Jable region alone. Bayesian Markov chain Monte Carlo analyses were used to separate ongoing gene flow from historical associations. These supported the NCPA by indicating recent (75 000–150 000 years ago) east–west vicariance across the El Jable region. Lava flows covered El Jable and other parts of the central lowland at this time and likely led to population extinctions and temporary dispersal barriers, although present-day evidence suggests some populations would have survived in small refugia. Expansion of the latter appears to explain the presence of a clade located between the eastern and western components of the disjunct clades. Direct relationships between mtDNA lineages and morphology were not found, although one of two morphological forms on the island has a disjunct distribution that is broadly concordant with east–west components of the phylogeographical pattern.

This work demonstrates how recent volcanic activity can cause population fragmentation and thus shape genetic diversity on microgeographical scales.

Keywords: Canary Islands, incomplete lineage sorting, morphology, phylogeography, vicariance, volcanism

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Introduction

Volcanic islands are dynamic environments. They have undergone changes in size and shape during their formation. Surfaces have been buried and reburied by new lava flows, destroying habitats and creating new ones. Such events modify the genetic structure and content of populations through repeated local extinctions, fragmentation, and range changes. The fact that populations on volcanic islands may frequently be subject to such effects means that they offer ideal models for testing evolutionary processes underlying genetic variation in relation to changing geographical area at the population level, particularly in the presence of detailed data on the chronology of geological events. A number of examples have been analysed in sufficient detail to provide insights into the processes underlying their evolution. For example, the union of formerly separate ancient islands...
(by volcanic activity) containing differentiated populations may explain the presence of several mitochondrial DNA (mtDNA) lineages in some species (e.g. Thorpe et al. 1996; Gübitz et al. 2000), while vicariance (with local extinction) associated with lava flows may explain similar patterns in others (e.g. Carson et al. 1990; Pestano & Brown 1999; Malhotra & Thorpe 2000; Behereregay et al. 2003; Vandergast et al. 2004; Gübitz et al. 2005). This study investigates a species in which both of these processes have potentially had an impact on within-island geographical variation.

The volcanic islands of the Canary Islands lie off the northwest coast of Africa. The two large islands of Gran Canaria and Tenerife have provided some of the clearest examples of the association between island evolution and intraspecific/intrageneric patterns of within-island diversity in several different groups (e.g. Brown et al. 2000, 2006; Juan et al. 2000; Contreras-Diaz et al. 2003; Moya et al. 2004; Emerson et al. 2006). Situated in the east of the archipelago are the oldest islands of Lanzarote and Fuerteventura (15–20 million years; Coello et al. 1992). They have received relatively little attention possibly because of lower altitudes and the consequent narrower range of habitats that prevail. Although most of the current island of Lanzarote is relatively young (middle Pleistocene), there are two areas of ancient volcanic deposits that represent separate precursor islands that had formed by about 8.7–10.2 million years ago (mya) (Coello et al. 1992): Los Ajaches in the southwest and Famara in the northeast (Fig. 1a). Volcanic activity joined these ancient (Miocene-Pliocene) islands approximately 0.8 mya by an extensive volcanic lowland to form a single island (Carracedo & Rodríguez-Badiola 1993). Recurrent volcanic activity over the central lowland continued until quite recently (i.e. 130 000–75 000 years ago), with the area entering a quiescent phase during or just after the last interglacial (Zazo et al. 1997, 2002; Dunai & Wijbrans 2000; Meco 2003). Some of the most recent basaltic lava flows on the island have left large areas of malpais (or volcanic badlands) habitat in the centre–west (Timanfaya lava field) and north–east (Malpais de la Corona lava field) of the island (Fig. 1b) (Carracedo et al. 1992, 2003). The existence of both malpais and arid habitat creates considerable ecological variation across the island.

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The complexity of the geological history of Lanzarote produces several competing (but nonmutually exclusive) hypotheses to explain phylogeographical structuring and genetic diversity for species on this island. First, the ‘two-island’ hypothesis assumes both precursor islands (Los Ajaches and Famara) contained differentiated populations (as a result of transmarine colonization prior to island union) that met once the ancient islands were joined by the volcanic lowland. Colonization of the newly formed central lowland and dispersal between the formerly isolated areas would have followed. This hypothesis predicts substantial divergence and phylogeographical structure in some species on Lanzarote. The same scenario could explain the presence of two or more distinct within-island lineages in each of the lizards Gallotia galloti, Chalcides viridanus, and Tarentola delalandii on Tenerife (Thorpe et al. 1996; Brown et al. 2000; Gübitz et al. 2000). Second, postcolonization expansion of a population from a single precursor island (the ‘single-island’ hypothesis) predicts little or no divergence, or phylogeographical structure. Third, more recent volcanic activity has likely led to local extinction and the creation of temporary dispersal barriers (i.e. lava flows) associated with the dispersal of local populations across the newly formed central lowland (the ‘mid-Pleistocene vicariance’ hypothesis), modifying the basic pattern of postcolonization dispersal. This hypothesis leads to the prediction of shallow divergence and weak phylogeographical structure in some species on Lanzarote.

The lacertid lizard, Gallotia atlantica, is endemic to the Eastern Canary islands and represents a model species to assess hypotheses of phylogeographical structuring and genetic diversity on Lanzarote. This is, in part, because it is distributed throughout the island, which allows very complete sampling of all geological areas, but more importantly because it appears to have colonized one or more of the two ancient islands prior to their union (González et al. 1996). Moreover, Lanzarote populations are divergent and reciprocally monophyletic with respect to Fuerteventura populations (González et al. 1996), so potentially confounding multiple colonization events do not appear to contribute to within-island diversity. The first aim of this paper was to test the a priori predictions of phylogeographical structuring and genetic diversity based on the geological history of Lanzarote for the lizard G. atlantica.

In addition, G. atlantica exhibits within-island variation in morphology (Bloor & Brown 2005). Two morphological forms have been related to the different habitat types within the island: a ‘malpais’ form from areas of malpais habitat, and a nonmalpais form from habitats elsewhere (López-Jurado & Mateo 1992). Subspecies have been assigned that broadly reflect these differences (Castroviejo et al. 1985; López-Jurado et al. 1995): G. a. laurae to populations on the Malpais de la Corona lava field, with G. a. atlantica to populations across the rest of the island. A more detailed analysis supported the dichotomous pattern of within-island differentiation, but the association with malpais areas was not as clear as previously thought (Bloor & Brown 2005), with the putative malpais form having a disjunct distribution, being found on the Malpais de la Corona lava field and small habitat ‘islands’ in the southern extension of the Timanfaya lava field. However, not all populations studied from the Timanfaya lava field exhibited the malpais form, nor are the habitat islands themselves covered by recent lava flows (although populations within them are intimately associated with areas of malpais around the edge of the habitat islands), running counter to the habitat association hypothesis.

This morphological variation could be explained by historical relationships between populations (rather than being a result of current processes). Under this hypothesis, correspondence between phylogeography (i.e. distribution of distinct mtDNA lineages) and morphological variation would be predicted. Similar patterns have been found in lizards on other islands (e.g. Pestano & Brown 1999), although morphological variation is discordant with phylogeography in others (e.g. Gübitz et al. 2005). Alternatively, morphological variation may be the result of adaptation (e.g. Malhotra & Thorpe 1991), phenotypic plasticity (e.g. Losos et al. 2000), or both, in relation to the habitat types on the island. This would be supported by the finding of little mtDNA structuring, and/or otherwise incompatible morphological-mtDNA patterns. The second aim of this work was to assess the causes of morphological variation in G. atlantica.

These aims are addressed by comprehensive island-wide sampling, and subsequent phylogeographical and coalescent-based analyses based on sequence data from rapidly evolving regions of mtDNA. In this way, our results can provide insights into the processes underlying genetic and morphological variation on volcanic islands.

Materials and methods

Sampling and molecular data

Gallotia atlantica were sampled in August/September 1999 and October 2002 from 24 sites representing all ecological and geological regions of Lanzarote (Fig. 1a; sample details available on request). The sites included 18 of those used in the previous analysis of morphological variation (Bloor & Brown 2005) with the addition of sites S21–S25 and S27 to allow accurate description of lineage distributions across the island.

Whole genomic DNA was extracted from tail samples (stored in 95% ethanol) using standard phenol-chloroform extraction and ethanol precipitation protocols (Hillis et al. 1996). We designed the primers Cytb.Gat.L (5′-CCACCGTACATCACCAACCCT-3′) and Cytb.Gat.H (5′-TACTGCTGTCCCTCCGATTTC-3′) to amplify a 563-base pair (bp) fragment of the cytochrome b gene, and ND2.Gat.L (5′-CACCCACGTGC-
CACTGAAG-3′) and ND2Egr.H (5′-AATGAGCGGGGA-
TAGACCTC-3′) to amplify a 591-bp fragment of the NADH
dehydrogenase subunit II (ND2) gene. The cytochrome b
primers were designed from G. atlantica sequence (accession
no. AY762378; this study), and the ND2 primers designed
from published Eremias graminea sequence (accession no.
U71331; Macey et al. 1997). Polymerase chain reactions (PCR)
were carried out in 30-μL reaction volumes containing
1× ABgene PCR Master Mix (2.0 mM MgCl2, 0.2 mM of each
dNTP, and 0.75 U Taq Polymerase) and 0.4 μM of each
primer. Cycling conditions were 1 min at 94 °C followed by
35 cycles of 30 s at 94 °C, 30 s at 55 °C, and 30 s at 72 °C. The
light strand was sequenced for six individuals from each
site except for S27 for which a single individual was
sequenced. Where haplotypes were represented by a single
sequence in the sample, both strands were sequenced. In
addition to 139 Lanzarote G. atlantica individuals, 18 indi-
viduals were sequenced from the same
G. atlantica clade
(González et al. 1996) from three islets to the north of
Lanzarote (La Graciosa, Montaña Clara, and Alegranza: one
site per islet, six individuals per site) and two individuals
from the reciprocally monophyletic G. atlantica clade
(González et al. 1996) on the island of Fuerteventura (two
sites: Tesjuate and Triquivijate, one individual per site).
Sequences were aligned using the program clustal w
(Thompson et al. 1994) and fragments combined. The
neutrality of mutations in the mtDNA fragments used here
was tested using the McDonald–Kreitman test (McDonald
& Kreitman 1991) using G. atlantica sequence from Fuert-
eventura as an ‘outgroup’ (program dnasp version 4.10;
Rozas et al. 2003).

Analyses
Phylogenetic relationships among haplotypes were estimated
using a network based on the 95% probability under
parsimony criterion (program tcs version 1.21; Clement
et al. 2000). In resolving ambiguous connections, we followed
the usual criteria described in Crandall & Templeton (1993)
and Posada & Crandall (2001). Clades were nested using
standard nesting rules (Templeton et al. 1987; Crandall 1996),
and a nested clade phylogeographical analysis computed
(NCPA; Templeton et al. 1995) using the program geodis
version 2.5 (Posada et al. 2000). This procedure revealed
several clades each with east–west disjunct distributions
(these will be referred to hereafter as the ‘disjunct clades’) separated by a clade located between them in the centre–
est of the island (Fig. 2). The common area that separates
the east–west components will hereafter be referred to as 'El Jable': it broadly corresponds with a region of sand deposits and sparse vegetation known locally by this name. There is no formal interpretative framework for making biological inferences from such a pattern in NCPA, so the east–west components of the disjunct clades were separated for the analysis. The present geographical analysis focused on processes underlying geographical associations within Lanzarote and so samples from the islets to the north of Lanzarote were not included. Analyses were organized into two sets (A and B), with the relative frequency of haplotypes at each site maintained for the separate analyses: (A) eastern components of disjunct clades and the centre–eastern clade (hereafter referred to as the El Jable clade) and (B) western components of disjunct clades and the El Jable clade. Although computing NCPA in this way could lead to lower statistical power (because of reduced sample sizes) it should minimize inference errors. Latitudes and longitudes of sites were obtained from large-scale maps. Inferences were based on statistically significant distances within clades and geographical distribution of clades (www.darwin.uvigo.es/download/geodisKey_11Nov05.pdf).

Divergence times between Lanzarote and Fuerteventura lineages were estimated (based on cytochrome b fragments only) with the aid of data on island age and emergence to provide a calibration point for the estimation of mutation rate per year per sequence ($\mu$) for the combined sequence. The cytochrome b fragments were aligned with sequences from the islands of La Palma (G. galloti — three individuals; S. Carranza, unpublished), El Hierro and La Gomera (G. caesaris — three individuals from each island; S. Carranza, unpublished), G. simonyi (GenBank) AF101217, G. stehlini (three individuals; P. Bloor, unpublished), and Psammodromus algirus (GenBank) AF101217 (outgroup). This resulted in 343 bp of homologous sequence. The obtained tree was in agreement with the widely accepted tree topology for Gallotia (e.g. González et al. 1996). Posterior distributions of rates and node dates for this topology were generated using a Bayesian Markov chain Monte Carlo method as implemented by the program MULTIDIVTIME (Thorne et al. 1998) using the approach described by Rutschmann (2004) (see also Thorne & Kishino 2002; Yang & Yoder 2003). Node constraints followed those in Brown et al. (2006). They were based on the emergence of El Hierro approximately 1.12 mya (Guillou et al. 1996) (G. caesaris appears to have colonized El Hierro very soon after its appearance; see Brown et al. 2006) and an upper limit to the colonization of La Palma constrained by its subaerial appearance 1.77 mya (Guillou et al. 2001 and references therein). The final run was based on $2 \times 10^6$ generations, sampled at 100 generation intervals, and with the first $1 \times 10^5$ cycles discarded as 'burn-in'.

A Bayesian Markov chain Monte Carlo method was used to distinguish between ongoing gene flow and historical association (after recent vicariance) as possible causes of the closely related east–west components of the disjunct clades: west ($n = 67$) and east ($n = 28$) (program IM, Hey & Nielsen 2004). Individuals with haplotypes corresponding to the El Jable clade (3–4) were regarded as representing admixture so were excluded from the analysis. To examine the effects of unequal sample sizes, runs were also made with a sub-sample of individuals from the western component. Runs using both full and reduced datasets produced similar results, so results from full analyses are presented here. Starting values for the prior distributions were empirically obtained by running IM with large parameter intervals and the Hasegawa-Kishino-Yano mutation model (Hasegawa et al. 1985) to ensure that posterior distributions fell completely within the prior distributions. Three final runs (model parameter values: $m_1 = 3, m_2 = 1.5, q_1 = 120, q_2 = 45, q_3 = 5, t = 3$) were made using different starting points (random number seeds) and a single chain ($5 \times 10^7$ steps, sampling interval 10 000) with the first 1 000 000 samples discarded as 'burn-in'. Assessment of autocorrelation parameter values over the course of each independent run showed that the Markov chain simulations converged on the true stationary distribution. The peaks of the resulting marginal posterior distributions were taken as estimates of the parameters (Nielsen & Wakeley 2001).

Finally, changes in population size (as expected from the colonization of the newly formed central lowland and dispersal between the formerly isolated areas) were estimated from the mismatch distribution using a generalized least-square approach (program ARLEQUIN version 3.1; Excoffier et al. 2005). This allowed estimation of the time since expansion, $\tau = 2ut$, where $t$ is the time in generations and $u$ is the mutation rate of the sequence. This distribution is typically multimodal for sequences sampled from populations at demographic equilibrium, but typically unimodal for populations having passed through a recent demographic expansion (Slatkin & Hudson 1991; Rogers & Harpending 1992) or through a range expansion with high levels of migration between adjoining populations (Ray et al. 2003). Using the same procedures and program, bootstrap support values for the fit of the estimated model and confidence intervals for associated parameter estimates were determined using 5000 replicates. The parameter $\tau$ was scaled to mutation rate per year by dividing the estimate by $2u$ (where $u$ is the mutation rate of the entire fragment per generation) and multiplied by the length of a generation. Mismatch distributions were computed separately on samples from east and west components of the disjunct clades, on clades (as defined by NCPA) when there were statistically significant geographical associations within clades, and on the island-wide sample. To correct for homoplasy (convergent site changes or reverse mutations), the number of mutational steps along the resolved parsimony network (NCPA) between any two haplotypes was used.
Results

Molecular variation

A total of 1075 bp of homologous sequence was obtained for all individuals (GenBank accessions: AY762379-AY762456 and AY762457-AY762534). Eighty-seven variable sites were detected in the Lanzarote G. atlantica clade, with synonymous substitutions at all but 19 of these (Table S1). Seventy different haplotypes were detected among the 139 Lanzarote samples (Table S2), of which only 12 haplotypes were detected at more than one site: only haplotypes '1', '43', and '71' were detected in samples on either side of El Jable (Fig. 3). Haplotypes were connected into a single network (Fig. 4) under the 95% probability of parsimony criterion (14 steps allowed). 'Outgroup' haplotypes could not be connected under the limits of parsimony. Five three-step clades were detected (Fig. 4) (islet haplotypes were nested well within the Lanzarote haplotypes). The most divergent clades were 3–4 and 3–5 (mean and maximum uncorrected sequence divergences 0.75% and 1.40%, respectively). Clades 3–1 and 3–3 were the least divergent clades (mean and maximum uncorrected sequence divergences 0.31% and 0.74%, respectively). Mean and maximum uncorrected sequence divergences averaged over all clades were 0.56% and 1.04%, respectively. There was no evidence of selection in the sequence under study (McDonald–Kreitman test; Fisher’s exact test, P = 0.81).

There is clear geographical structuring in G. atlantica within Lanzarote. Four of the main clades (3–1, 3–2, 3–3, and 3–5) are mostly or completely overlapping with east–west disjunct components, separated by a common area (El Jable) in the centre–east of the island (although the western limit of clade 3–3 is represented by a single specimen at site S18) (Fig. 2). Clade 3–4 (El Jable clade) is a centre–eastern clade. It is mostly or completely nonoverlapping with the east–west components of the disjunct clades, but some sites have representatives from both: site S1 is typically centre–western but with El Jable haplotypes (two specimens), while S18 is typically northern but with El Jable haplotypes (two specimens). The El Jable clade shows some north–south structuring with mostly overlapping subclades (clades 2–12 and 2–11). The disjunct clade 3–1 has further east–west structuring, with geographically separated subclades on both sides of the El Jable region: clade 2–4 in the south and centre–west, and clade 2–2 in the north. Together, these analyses point to a recent east–west separation of populations with close historical relationships on either side of the centre–east of the island. The long-term stability of this pattern suggests restrictions on contemporary dispersal.

A NCPA revealed statistically significant associations between haplotypes and geography in five clades representing two separate analyses (A and B; Table 1). Geographical associations in the western component (geographical regions south and centre–west) of the disjunct clades were compatible with restricted gene flow with isolation by distance (clade 3–1) and allopatric fragmentation (clade 3–5): the fragmentation event was identified between the southerly coastal clade (2–13) and northerly coastal clade (2–14) that are separated by lava flows from historical Timanfaya eruptions. In the centre–east, geographical associations supported restricted gene flow with isolation by distance (clade 3–4). The pattern of clade distributions at the ‘cladogram’ level
(analyses A and B) was compatible with past fragmentation followed by range expansion (rather than long-distance colonization possibly linked with subsequent fragmentation). No statistically significant geographical associations were detected in the eastern component of the disjunct clades.

The Bayesian time estimate for the G. atlantica Lanzarote/Fuerteventura node was 1.88 mya (95% confidence interval: 1.28, 3.27), which is in keeping with estimates published by other studies (e.g. Maca-Meyer et al. 2003; Arnold et al. 2007). We were concerned about the reliability of the estimate of mutation rate per year per sequence based on this point.
The results of the Ibm analysis are summarized in Fig. 5. Bayesian estimates of migration rates (scaled for mutation rate) revealed strong peaks at zero for both migration rate parameters (from east to west and west to east), indicating strong restrictions on gene flow between the eastern and western components of the disjunct clades. The sharp peak in the marginal posterior probability distribution of the divergence time parameter strongly indicates a recent east–west vicariance of populations over the El Jable region. When converted to a scale of years for the given range of mutation rates, the location of the peak (t = 1.05) corresponds to 75 107–150 214 years (49 821, 111 536; 99 643, 223 071: 90% highest posterior density intervals for upper and lower mutation rates, respectively). The position of the peaks of the marginal posterior densities for both population size parameters suggest that present-day populations of the east–west disjunct clades have substantially larger effective populations than the ancestral population (congruent with changes in population size estimated by other methods, see later).

Mismatch distribution estimates based on Lanzarote haplotypes provide clear evidence of population growth both in the east–west components of the disjunct clade group and the island-wide sample (Fig. 6; not all mismatch distributions are shown due to the redundancy of patterns at lower clade levels). The broad similarity between the mismatch distributions for samples on either side of the El Jable region supports the idea that they belong to the same period of expansion. Although the bimodal distribution of El Jable clade (3–4) does not differ significantly from the model, the subsequent estimate of τ is likely an overestimate. Given the range of mutation rates (and form of the mismatch distribution), τ for the island-wide sample suggests expansion of a historically unsundered precursor population about 86 000–172 000 years ago.

Morphology

Individual populations are classified into one of two discrete morphological categories, referred to as ‘malpais’ (four sites corresponding to recent lava fields) or ‘widespread’ (populations from the rest of the island: only a small number of which are associated with recent lava fields) (Bloor & Brown 2005). Haplotypes associated with the morphological categories are distributed throughout the haplotype network (not shown). Moreover, several haplotypes were shared between morphotypes. These included the ancestral haplotype ‘I’ that was detected in seven individuals from sites on Lanzarote for which morphology had previously been recorded: three from sites assigned to the malpais form (sites S2 and S26, Malpais de la Corona lava field), and four from sites assigned to the widespread form (sites S17, S20, and S9). No haplotypes detected in more than one individual were confined to sites assigned to the malpais form. This indicates a lack of association between the two morphological variants and distinct evolutionary (mtDNA) lineages.

Discussion

The phylogeographical structure in Lanzarote G. atlantica does not appear to be explained by predifferentiated lineages from the ancient precursor islands of Los Ajaches and Famara (the absence of separate Los Ajaches and Famara lineages either reflecting colonization failure or subsequent extinction). Not only are the ancient islands extremely old relative to the extent of divergence, but also Lanzarote lineage coalescence (i.e. average divergence from haplotype ‘I’) occurs at or after the time of island union. Moreover, Lanzarote has been exhaustively sampled, so deep lineages would be revealed by the phylogeographical analysis if they were present. Consequently, the ‘two-island’ hypothesis can be rejected in favour of the ‘single-island’ hypothesis, as it appears that G. atlantica lineages diverged in situ on Lanzarote soon after the precursor population dispersed across the newly formed central lowland joining the ancient precursor islands (see later).
More detailed consideration of the results suggests that the phylogeographical structure in Lanzarote *G. atlantica* is the result of a three-stage process: (i) a single Pleistocene postcolonization expansion that occurred after the two ancient precursor islands were united by a volcanic lowland. The substantial population growth detected in the mismatch distribution for the island-wide sample is consistent with the postcolonization range expansion of a historically unsundered precursor population from one of the ancient islands into areas of the volcanic lowland about 180,000 years ago. This scenario of postcolonization expansion following the union of formerly separate islands is similar to that proposed for other species. For example, distinct lineages of both the gecko *Tarentola delalandii* (Gübitz *et al.* 2000) and the skink *Chalcides viridanus* (Brown *et al.* 2000) show evidence of population expansions after the precursor islands of Teno, Anaga, and Roque del Conde were joined to form Tenerife. (ii) Subsequent east–west vicariance across the centre–east (El Jable) of the island. Several lines of evidence support the recent (mid-Pleistocene) vicariance hypothesis. Allopatric fragmentation at the cladogram level leading to the east–west disjunct clade distributions was detected by the NCPA and supported by the **m** analysis. Lavas from the last interglacial covered large areas of the centre–east of the island (Zazo *et al.* 1997, 2002; Meco 2003). Detailed information on the extent of this volcanic activity is not available but the boundaries between the El Jable clade (3–4) and the east–west components of the disjunct clades do appear to coincide quite well with the known distributions of these lava flows. We propose that this volcanic cycle caused extinctions over the immediate area, with some local populations isolated in small refugia, separating eastern and western subpopulations on either side of an inhospitable volcanic barrier and initiating their independent evolution. This is not biologically unrealistic because it is exactly what is currently observed in the Timanfaya lava field in the centre–west of the island (Bloor & Brown 2005). In addition, the divergence estimate (**m**) for east–west components (75,000–150,000 years ago) fits particularly well with the ages of these lava flows, providing further support for this hypothesis. (iii) Postvicariance dispersal of surviving populations and re-establishment of a continuous distribution. Past fragmentation followed by range expansion at the ‘cladogram’ level (analyses A and B) was detected by the NCPA. Small isolated refugia populations from the centre–east could have subsequently expanded to form the current El Jable clades, located between the east–west components of the disjunct clades.

Although the NCPA did not provide statistical support for fragmentation within clade (3–4) from El Jable, the larger than average number of steps connecting El Jable two-step clades, together with evidence of recent expansion by other methods (mismatch distributions; Fig. 6e, f), does point to fragmentation; rather than restricted gene flow with isolation by distance, as inferred from the NCPA. The analysis presented here is a microgeographical application of NCPA and anything other than an extreme restriction on movement of individuals of formerly isolated populations...
will lead to considerable overlap between clades within such a small area, making the inference key less able to discriminate isolation-by-distance from allopatric fragmentation. Analysis using more rapidly evolving markers combined with more detailed sampling is required to resolve the processes underlying the clade distributions in this area.

While the distinct east–west phylogeographical structure may be explained by recent within-island volcanism, there are several possible explanations for the negligible degree of admixture between the east–west components of the disjunct clades and the El Jable clade (3–4). It is possible that an important component of the pattern of clade admixture revealed here is attributable to sex-biased dispersal with female philopatry. This is an appealing explanation because it could also explain the maintenance of strong phylogeographical structure over small distances in the absence of any readily obvious present-day physical barriers to lizard dispersal. The possible effects of sex-biased dispersal on
phylogeographical structure have been put forward to explain similar patterns in the gecko Tarentola boettgeri on the island of Gran Canaria (Gübitz et al. 2005), while discordant patterns of mtDNA and nuclear variation (indicative of sex-biased dispersal) in the lacertid G. galloti on the island of Tenerife (Thorpe & Richard 2001) and the anole Anolis oculatus on the island of Dominica (Stenson et al. 2002) are also suggestive of this. However, habitat preference would also seem plausible given that the boundaries broadly coincide with the transition from jable habitat (areas of sand deposits and sparse vegetation) to adjacent habitat. Alternatively, it could result from differential selection against individuals with mtDNA from the east–west components and El Jable nuclear genomes (or vice versa) because of the disruption of co-evolved interactions between them (Rand et al. 2004; Ellison & Burton 2006). Similar explanations have been given for negligible admixture between distinct mtDNA clades in the skink C. sexlineatus on Gran Canaria (Pestano & Brown 1999) and the gecko T. delalandii on Tenerife (Gübitz et al. 2000). However, lack of information from nuclear markers means that it is currently difficult to distinguish between these alternatives. In a future study, we aim to examine critically the hypothesis of sex-biased dispersal through estimation of nuclear gene flow, using microsatellite markers.

The analysis of mtDNA variation allows evaluation of the basis of the two main morphological forms within Lanzarote. The malpaís morphology is found in the centre–west of the island (from the southern extension of the Timanfaya lava field) and the northeast (Malpaís de la Corona lava field) (Fig. 1b), and therefore separated by the El Jable region. The hypothesis that morphological variation could reflect historical relationships between populations (rather than being a result of current processes) predicts correspondence between phylogeography (i.e. distribution of distinct mtDNA lineages) and morphological variation. Although the lack of general association between morphology and mtDNA lineages (i.e. there was not a close concordance on a site-by-site basis) allows rejection of the ‘historical’ hypothesis, the fact that both systems exhibit similar disjunct distributions over an intermediate area that incorporates the El Jable region is intriguing. If we assume that the coincidence of these patterns is merely a chance effect, then ecotypic contributions over an intermediate area that incorporates the El Jable region, but no longer shows a close concordance with the mtDNA distribution due to differences in subsequent processes, such as rates of gene flow, which could differ substantially between nuclear and mtDNA markers (e.g. García-París et al. 2003).

This detailed analysis demonstrates how recurrent within-island volcanism may explain phylogeographical structuring over small geographical areas within a terrestrial species with relatively low vagility. It also provides new insights into the genesis of phylogenetically deeper, clearer patterns (i.e. distinct clades with strong clade localization) found on other islands. Subsequent divergence, together with lineage extinctions, could lead to a reduced number of differentiated lineages within G. atlantica on Lanzarote, which would mirror the patterns found in lizards from Tenerife and Gran Canaria.

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**Supplementary material**

The following supplementary material is available for this article:

**Table S1** Variable sites for 76 mtDNA haplotypes based on 1075 bp of cytochrome b and D2N sequence from 157 individuals of *G. atlantica*.

**Table S1** Number of *Gallotiaatlantica* haplotypes detected among sites and regions on Lanzarote.

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-294X.2007.03575.x

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