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Phylogeography and natural selection in the Tenerife gecko *Tarentola delalandii*: testing historical and adaptive hypotheses

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Abstract

Combining phylogeographic and matrix correspondence approaches in the analysis of geographical variation provides a fruitful approach to inferring the causes of molecular and morphological evolution within species. Here we present a study on the gecko *Tarentola delalandii* on the island of Tenerife, Canary Islands, which provides an outstanding model of an exceptionally high degree of phylogeographic differentiation in magnitude and pattern on a small spatial scale. We reconstruct the population history of *T. delalandii* using phylogeographic information, matrix correspondence tests and estimates of divergence times in conjunction with geological data. It appears that populations differentiated on three precursor islands and secondary contact followed the junction of these islands. The cytochrome *b* sequence appears to be evolving at least at ≈ 1% per million years in this species. Matrix correspondence tests indicate that morphological character systems may reflect ecological selection regimes (colour pattern), history (body dimensions) or both (scalation). The results imply that natural selection can override a historical legacy, but also underline the potential relevance of molecular phylogenetic data for the interpretation of geographical variation in morphology.

Keywords: Canary Islands, cytochrome *b*, geographical variation, molecular clock, population bottleneck, volcanism

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Introduction

Recent years have seen an enormous growth in phylogenetic studies based on molecular data, many of which are concerned with closely related species or variation within species. In particular, the use of mitochondrial DNA (mtDNA) as a molecular marker has considerably improved our knowledge about past events shaping the genetic diversity within species (Avise 1994; Riddle 1996). Phylogeographic studies have consolidated the view that historical changes in climate have had a major impact on the biota of continental Europe (Hewitt 1996), North America (Avise 1994), Australia (Joseph *et al*. 1995; Schneider *et al*. 1998) and New Zealand (Baker *et al*. 1995; Trewick 1998). Other explanations for large-scale phylogeographic patterns may involve tectonic events causing the isolation of populations (Macey *et al*. 1997; Walton *et al*. 1997; DaSilva & Patton 1998; Heads 1998; Shields 1998). Geographic variation is a common and well-known phenomenon in natural populations (Mayr 1942, 1963). However, in contrast to continental regions, intra-island variation on oceanic islands was neglected for a long time and the main interest focused on interisland or interspecific comparisons (Williamson 1981). Recent studies on reptiles and insects (among others) demonstrate that even relatively small oceanic islands can harbour considerable intraspecific morphological and genetic variation (Thorpe *et al*. 1995; Juan *et al*. 1996, 1998; Thorpe & Malhotra 1996; Giannasi 1997; Pestano & Brown 1999). In most cases, these islands are of volcanic origin and volcanism has been invoked to explain the intraspecific genetic structuring on such islands by either recurrent isolation and/or bottlenecking populations (Carson *et al*. 1990; DeSalle & Templeton 1992; Pestano & Brown 1999), or bringing previously allopatric populations into secondary contact (Thorpe *et al*. 1995; Thorpe & Malhotra 1996; Giannasi 1997). Thus, reconstructing recent population history may have an important bearing on the interpretation of evolution of island species.

The Tenerife gecko, *Tarentola delalandii,* is an outstanding model system for reconstructing population history

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and testing the effects of population history and adaptation on geographical variation. *T. delalandii* is a widespread, predominantly nocturnal, insectivorous lizard found on the volcanic islands of Tenerife and La Palma in the Canary Archipelago. The species can be abundant at low elevations and is rare or absent above 1500 m.

Tenerife has a complicated, but well-studied, geological history, consisting of two or three putative precursor islands with maximum ages of 15.7, 11.6 and 7.4 million years (Myr) for Anaga, Adeje and Teno, respectively. These were joined by volcanic events which began ≈ 1.9 million years ago (Ma) and lasted until ≈ 0.2 Ma (Abdel-Monem *et al*. 1972; Ancochea *et al*. 1990). La Palma emerged more recently and is thought to be 1.3 Myr (maximum 2 Myr) old (Ancochea *et al*. 1994). As a result of the local weather system, which is dominated by northeasterly trade winds (Fernandopulle 1976), and its topology (with Pico de Teide reaching 3718 m), Tenerife is an ecologically heterogeneous island providing a variety of different habitats along altitudinal and latitudinal clines. There is a particularly dramatic difference between the habitat along the southern coast, with desert-like conditions (annual precipitation < 200 mm), and the northern habitat, which is characterized by higher humidity, higher precipitation and lusher vegetative growth.

Widespread species occurring in such contrasting habitats are likely to experience different selection regimes and can be expected to show local adaptations (Endler 1977). Geographic variation in morphology corresponding to clines in climatic factors on Tenerife has been demonstrated for *T. delalandii* (Thorpe 1991) and two other sympatric lizard species, *Gallotia galloti* and *Chalcides viridanus* (Thorpe & Báez 1987; Thorpe & Brown 1989; Brown *et al*. 1991), suggesting ecogenetic adaptation. However, concordant patterns of morphological and molecular variation in *C. viridanus* have been taken as providing support for a historical cause (Pestano & Brown 1999). In contrast, discordance of morphological and molecular variation and a closer association of morphological variation with ecological (rather than phylogenetic) patterns may provide evidence for natural selection-mediated clines (Thorpe *et al*. 1996).

Here, we first infer the population history from phylogeography, based on mtDNA sequences from multiple localities. We then analyse patterns of morphological variation in relation to putative ecological and historical causes.

Materials and methods

Analysis of sequence data

Genomic DNA was extracted from autotomized tail tip samples of *Tarentola delalandii* from 39 localities on Tenerife and three localities on La Palma, and one sample each of *T. boettgeri* from Gran Canaria and *T. mauretanica* from Mallorca following standard protocols (Sambrook *et al*. 1989). A fragment of the mitochondrial cytochrome *b* gene was amplified by polymerase chain reaction (PCR) using modified versions of primers L14841 (Kocher *et al*. 1989) and MVZ16 (Moritz *et al*. 1992) (further details are available from the authors). PCR conditions were 92 °C for 1 min, 48 °C for 30 s and 72 °C for 1 min for 30 cycles with a final step of 72 °C for 3 min. Double-stranded PCR products were sequenced using the Sequenase kit (Amersham Life Science Inc.) according to the manufacturer's protocols. Primers used for sequencing were the modified L14841 and the internal primer 5′-GCCAACGGAGCATCATTATTCTTC-3′. Sequences were aligned by eye.

Evolutionary relationships among haplotypes were inferred using maximum parsimony and maximum likelihood as implemented in the program packages PAUP version 3.1.1 (Swofford 1990) and the beta test version (b2a) of PAUP* 4.0 (Swofford 1998). Most parsimonious trees were inferred by using unweighted characters in a heuristic search with tree-bisection-reconnection (TBR) branch-swapping with 100 random-addition replicates. Maximum likelihood trees were also calculated for unweighted characters. Transition : transversion ratios and base frequencies were estimated from the data, and the evolutionary model of best fit to the data was investigated using the procedure described in Malhotra & Thorpe (in press). Starting trees for heuristic searches were obtained by neighbour joining, with subsequent TBR branch swapping. Bootstraps were computed for both maximum likelihood and maximum parsimony (100 replicates) to evaluate support for nodes. However, nearest neighbour interchange (NNI) rather than TBR branch swapping was employed in ML bootstraps to reduce the computational time required.

Relative rate tests (Takezaki *et al*. 1995) among the major haplotype clades were carried out using the program PHYLTEST version 2.0 (Kumar 1996). Several tests, implemented in DnaSP 3.0 (Rozas & Rozas 1999), were used to test DNA polymorphism for deviation from the neutral expectation. Tajima's D (Tajima 1989a), Fu & Li's (1993) statistics with (D, F) and without (D*, F*) outgroups, are based on comparing different estimates of the parameter theta. However, recent investigations have found that these tests lacked the power to detect some forms of departure from neutrality (Wayne & Simonsen 1998). The McDonald–Kreitman contingency test (McDonald & Kreitman 1991) is an alternative, goodness-of-fit, test that compares ratios of synonymous to replacement substitutions within and between species, with the expectation under neutrality that these should be the same. For each of these tests, the outgroup used for each clade tested was as follows: for the entire *T. delalandii* dataset, *T. boettgeri*; for the Anaga clade, the Teno clade; for the Northern Anaga subclade, the SE and SW Anaga clade; for the Teno clade, the Anaga clade; and for the Teno clade, the Anaga clade was used. A molecular clock for *T. delalandii* was calculated based on the phylogenetic relationship and sequence divergence between haplotypes found on La Palma and their closest relatives on Tenerife using the younger island's age as a calibration point (Fleischer *et al.* 1998) and re-evaluated against the time of emergence of the Tenerife precursor islands.

Matrix correspondence tests

When testing causal hypotheses for patterns of geographical variation at the intraspecific level, whether molecular phylogenetic or clinal, one generally needs to consider the spatial proximity, which is best represented in matrix form. The application of matrix correspondence tests (MCT) offers one solution to the problem (Sokal 1979; Douglas & Endler 1982) and allows simultaneous testing of multiple hypotheses (Manly 1986; Smouse *et al.* 1986; Thorpe & Báez 1993). These tests also allow one to incorporate phylogenetic information when morphological (Thorpe *et al.* 1995, 1996; Thorpe & Malhotra 1996) and other data (Thorpe *et al.* 1995; Daltry *et al.* 1996) are tested against ecological variables. Both dependent (the observed pattern) and independent variables (representing hypotheses) were constructed as among-locality dissimilarity matrices (as described below). Pairwise (one dependent and one independent variable) and partial (simultaneous test of several independent variables) MCTs, based on standardized regressions, were carried out using R. S. Thorpe's adaptation of the RT-MANT program (distributed by B. F. J. Manly, University of Otago, New Zealand). Significance levels were derived from 10 000 randomizations and a sequential Bonferroni correction was applied (Rice 1989). A partial MCT was run in a stepwise regression procedure for variables which showed significant association in pairwise tests.

The phylogeographic pattern of major haplotype clade distribution was tested by relating the observed pattern to a geographical pattern derived from a specific hypothesis. The elements of the dependent matrix were given values of zero if the locality samples were from the same one of three distinct major clades, and a value of one for membership of a different clade. Alternatively, modal patristic distances between localities were tested instead of simple clade membership. The hypothetical scenario of population expansion from putative refuge areas, or precursor islands, was modelled as being the result of colonization from the presumed centres of the three old mountain ranges (Anaga, Teno and Adeje) to the more recently emerged intervening area of the island. The model assumed that the rate of expansion from each centre of dispersal was the same, and that there was no admixture of different haplotypes. The elements of the independent matrix were given values of zero if the locality samples were from the area occupied by the same precursor, and a value of one for membership of an area occupied by a different precursor. The hypothesized model was tested in a pairwise and partial matrix correspondence test including spatial proximity (a matrix of geographical distances) of sampling locations.

To test the cause of morphological variation considering phylogenetic, spatial and ecological information we used published morphological data from 14 low-altitude localities around Tenerife. Thorpe (1991) summarized morphological variables from three character systems by multivariate analysis: body dimensions (second canonical variate, CV2, of 15 characters was used instead of CV1 which primarily separated the sexes), scalation (CV1 of 14 characters) and colour pattern (CV1 of six characters). Where the localities used for the morphology did not exactly match those used for the mtDNA study, the haplotype was assumed to be similar to the closest locality for which mtDNA was known. In no case was this close enough to the contact between any of the three major haplotype ranges to be problematic. The genealogical relatedness among populations was represented as the patristic distance (i.e. the number of mutational steps along a phylogenetic tree) between modal haplotypes on the 50% majority rule consensus tree of the most parsimonious trees found in a heuristic search. As an alternative, we took into account the genetic relationships by using a genetic distance matrix (using the best distance model identified earlier), averaged among sequences sampled in different localities (Malhotra & Thorpe 2000). The ecological factors tested by matrix correspondence were: (i) mean annual rainfall; (ii) seasonal climate type (differentiated according to seasonal rainfall and temperature patterns); and (iii) potential natural vegetation type (as an indirect measure of actual vegetation type) (García Rodríguez *et al.* 1990). Mean annual rainfall of 0–250 mm was scored as 0, 250–500 mm as 1 and 500–750 mm as 2. Climate type was represented by 0 for dry summers and dry winters, 1 for dry summers and wet winters. The vegetation type characterized by *Euphorbia canariensis and E. obustifolia* was scored as 0, and the vegetation type characterized by *Juniperus phoenicea* and *Phoenix canariensis* was scored as 1 (García Rodríguez *et al.* 1990). Sites in the transition between two ecological categories were scored in order to reflect this (i.e. as 0.5 or 1.5). Spatial proximity was also considered in these tests of the association between morphological variables and ecological factors.

Results

A total of 30 haplotypes was found among the 102 sequences from Tenerife and three haplotypes were detected among the La Palma samples. Representative sequences have

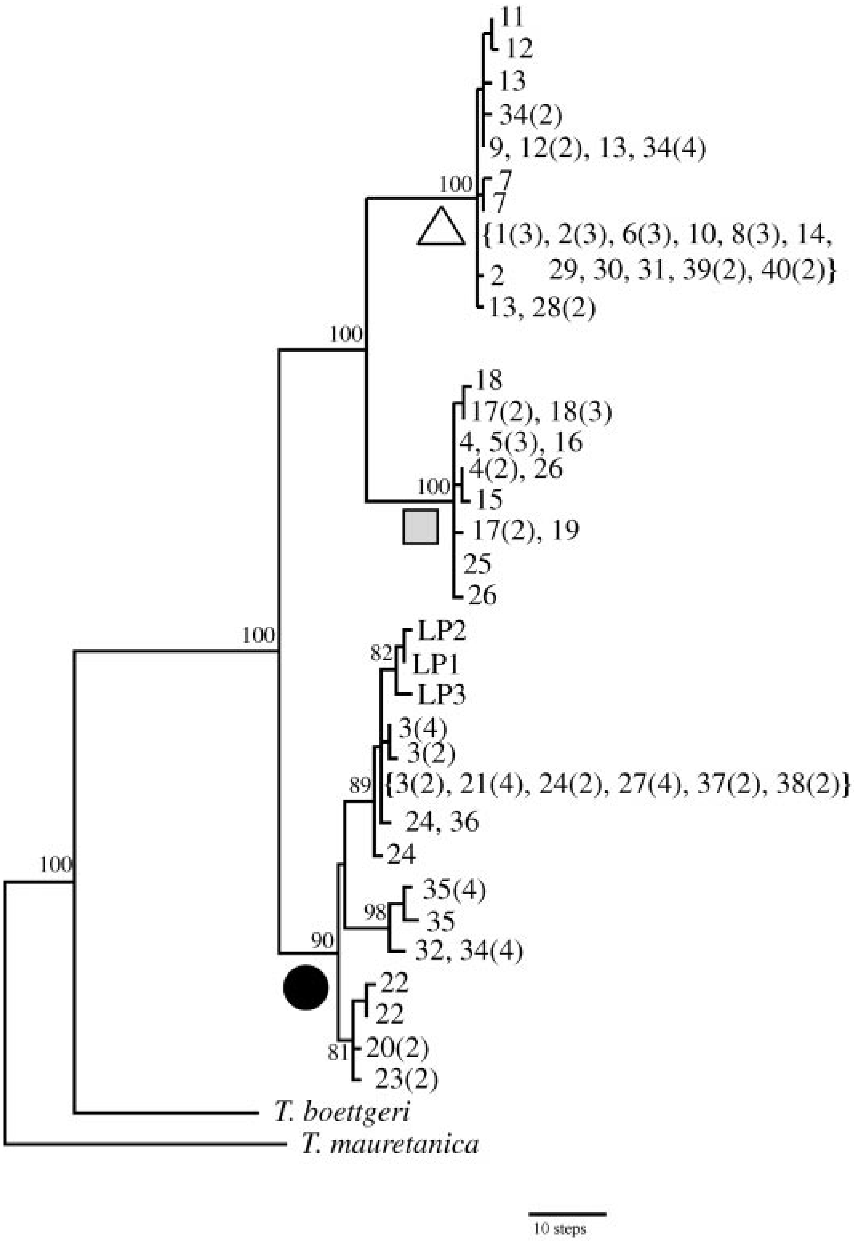

Fig. 1 Fifty per cent majority-rule consensus of 1440 most parsimonious gene trees. Tip labels indicate locality number where the haplotype is found with the number of specimens with that haplotype in plain brackets. Curled brackets enclose localities at which widespread haplotypes are found. Circles, squares and triangles indicate major clades. Numbers at branch nodes indicate bootstrap values (> 80) derived from 100 replicates.

been deposited in GenBank (Accession nos AF251297–251304). Analysis of 123 codons in the Tenerife sample showed 61 segregating sites with a total of 64 mutations, of which 53 were synonymous and 11 nonsynonymous. Of the several substitutional models that fit the data equally well using the criterion employed, the simplest was found to be the Kimura 2-parameter (K2P) model (Kimura 1980), without gamma correction. This was used for all subsequent distance-based analyses.

Phylogenetic analyses employing maximum parsimony and maximum likelihood consistently reveal the presence of three major clades in *Tarentola delalandii* (Fig. 1); moreover these are well supported by bootstrap analayses (> 70% support in each case). Geographically, each clade appears to be associated with one of the old mountain ranges/precursor islands (Fig. 2). Corresponding to their association with particular mountain ranges, we refer to them as the Adeje, Teno and Anaga clades (Adeje and Teno are sister groups and are jointly referred to as the western clade). Maximum pairwise (K2P) sequence divergence among the major clades was 8.63% between the two western clades (Adeje and Teno), and 12.84% between the western clade and Anaga (Table 1). Although intra- and interspecific molecular divergences in reptile and amphibian species tend to be higher than in mammals and birds, the sequence divergence levels within *T. delalandii* are still

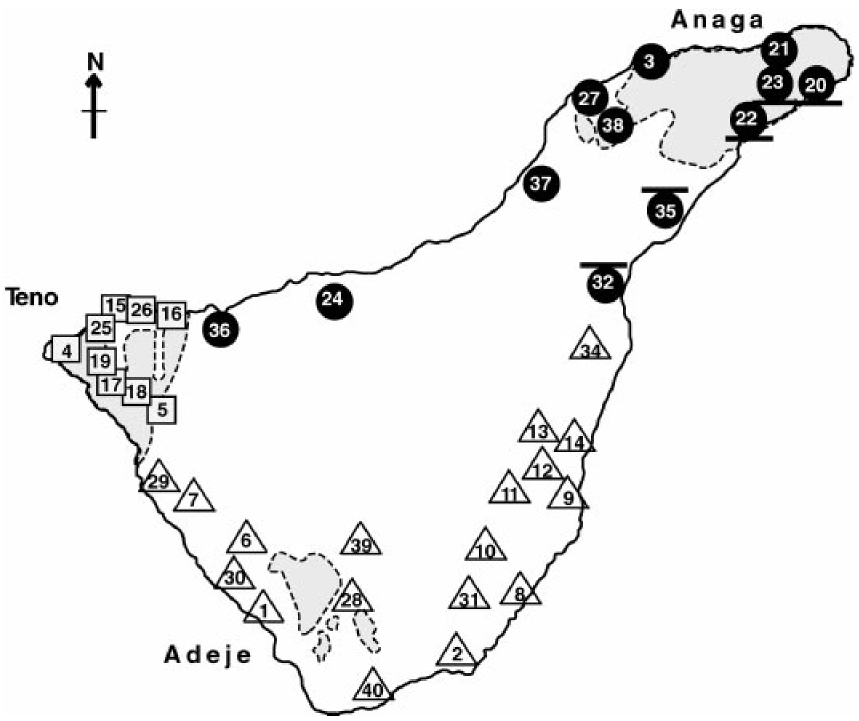

Fig. 2 Map of Tenerife (28°15′ N, 16°37′ W) showing sampling localities and locality numbers. Symbols indicate identity of modal haplotype lineages (corresponding to Fig. 1). Grey squares represent the Teno clade, open triangles represent the Adeje clade and circles represent the Anaga clade. For the Anaga clade three subclades are indicated by a bar over the circle (Southwest Anaga), a bar under the circle (Southeast Anaga), or by a circle only (North Anaga). The locations of the three old mountain ranges Anaga, Teno and Adeje are outlined in dashed lines.

Table 1 Corrected (K2P) pairwise distances between the three major clades of *Tarentola delalandii* on Tenerife, showing minimum, mean and maximum distances, respectively

Clade	Adeje	Teno
Anaga	0.1022/0.1163/0.1284	0.0930/0.1039/0.1227
Adeje		0.0739/0.0813/0.0862

exceptionally high and reach values more commonly associated with interspecific divergences (Avise *et al.* 1998; Johns & Avise 1998).

The localities sampled revealed no overlap in the geographical distribution of these major haplotype clades, with the exception of a single locality on the geographical border of the distribution of the Anaga and Adeje clades (locality 34, Fig. 2). While the Teno and Adeje clades show only minor phylogenetic substructuring, the Anaga clade comprises three distinct subclades, which are geographically localized (Fig. 2). One is widespread along the northern coast of Tenerife and two are localized in the southeast and the southwest of Anaga.

The three haplotypes from La Palma are nested within the northern Anaga clade (Fig. 1), indicating that the La Palma population stemmed from this, geographically closest, Anaga subclade. The geological age of 1.3–2 Myr for La Palma (Abdel-Monem 1972; Ancochea *et al.* 1994), and an average sequence divergence (based on K2P distances) of 0.0131 for the haplotypes from La Palma and northern Anaga, allows us to estimate a rate of at least 0.65–1.0% sequence divergence per Myr. These rates assume colonization of La Palma immediately after it emerged, therefore they indicate the slowest possible rates and a faster rate is more likely. Moreover, the slowest value of 0.65 per Myr is untenable, because extrapolation of this rate gives 17.6 Ma for the maximum divergence of the Anaga and western clades, which is before the origin of any part of Tenerife, including Anaga (15.7 Ma maximum) or Adeje (11.6 Ma maximum). Even assuming colonization of Adeje as soon as it arose (11.6 Ma), the maximum divergence of 12.84% (between Adeje and Anaga) argues for a rate of at least 1% (faster if it was colonized a substantial time after eruption). This estimated rate for cytochrome *b* may be compared with the range of estimates in other reptiles by Zamudio & Greene (1997) (0.5–1.3%), Malhotra & Thorpe (2000) (1.4%) and Giannasi (1997) (1–2%).

Although it is theoretically possible that very divergent haplotypes from genetically structured populations have become fixed by chance, this is less likely than allopatric divergence in this case, given the estimated age of clade divergence and the geological history of Tenerife and its precursor islands. Matrix correspondence was used to test the phylogeographical model that assumes divergence in allopatry on three putative precursor islands (Anaga, Teno and Adeje) and subsequent range expansion into the intervening area once these ancient precursors have been

Table 2 Result of (A) pairwise and (B) partial matrix correspondence test for the association of morphological variation patterns and causal hypotheses. These are proximity (Prox), patristic distance of modal haplotypes (Pat), mean Kimura 2-parameter distance among (K2P), potential natural vegetation (Veg), climate type (Clim) and rainfall (rain). For further details see text. Absolute standardized regression coefficients are followed by probabilities (in parentheses) which are derived from 10 000 randomizations. Regression coefficients and their *P*-values which are significant after sequential Bonferroni corrections are in bold

	Prox	Pat	K2P	Veg	Clim	Rain
A. Pairwise						
Body dimensions	0.2655 (0.0259)	**0.2997 (0.0184)**	**0.3662 (0.0100)**	0.2542 (0.0332)	0.1351 (0.1000)	0.1959 (0.0674)
Color pattern	0.0670 (0.7272)	0.1217 (0.0296)	0.0391 (0.5340)	**0.3316 (0.0075)**	0.0311 (0.8750)	0.0612 (0.6613)
Scalation	**0.4477 (0.0015)**	**0.6655 (0.0009)**	**0.6307 (0.0004)**	**0.5111 (0.0036)**	**0.5111 (0.0028)**	**0.6795 (0.0002)**
B. Partial						
Scalation		**0.4148 (0.0109)**				**0.4465 (0.0031)**
Scalation			**0.3763 (0.0125)**			**0.4800 (0.0015)**

joined by geological activity. This test strongly rejected the null hypothesis of no association of the hypothetical model with the observed pattern of clade distribution ($P < 0.0001$ pairwise and $P < 0.0001$ for a partial MCT with proximity regressed out; using genetic distances gave comparable results, $P < 0.0001$, $P < 0.0005$, respectively).

Geological studies strongly suggest that Anaga was an independent precursor island, but it is less clear from these studies whether Teno and Adeje represent a single or two separate precursor islands (Ancochea *et al*. 1990). From phylogeographic evidence, it is most parsimonious to assume that they were separate islands at the time of divergence of Teno and Adeje clades, with time of divergence (average sequence divergence at 1% per Myr) of the clades (7.6 Ma) consistent with the time of origin of Adeje (11.6 Ma) and Teno (7.4 Ma). The alternative, of fixation of divergent haplotypes without bottlenecking/expansion and coexistence without mixing for 7.4 Myr, is less likely but not impossible. An additional hypothesis is that the presence of divergent clades on Tenerife is the result of multiple colonization from other islands within the archipelago. This hypothesis can be rejected as *T. delalandii* does not occur on islands other than Tenerife and La Palma, and other islands within the archipelago appear to have been colonized early in their history by other taxa of *Tarentola* (Nogales *et al*. 1998).

Fu and Li's D, D*, F and F* test statistics (i.e. with or without outgroup) for the whole data set of Tenerife sequences showed significant deviations from the neutral expectation. However, when different data partitions based on the clades revealed by the phylogenetic tree were tested, significant deviation from neutrality was shown only for the Anaga clade, but not for the Adeje or Teno clades (or northern Anaga subclade). Tajima's D for non-neutrality showed a similar significant result for the whole set of Tenerife sequences, but no significant deviation for any smaller data partition. The significant positive values for the D, D*, F and F* statistics for the whole data set merely indicate a substructured population, as shown by the phylogenetic analysis (Tajima 1989b). Moreover, the McDonald–Kreitman test did not show significant deviation from the neutral expectation for any data partition.

Given this reconstruction of the population history of *T. delalandii* and the ecological heterogeneity of Tenerife, we may expect that historical events on the one hand, and current ecological selection regimes on the other hand, may have shaped the morphological evolution of *T. delalandii* on Tenerife. Testing the association of patterns of geographical variation in the colour pattern, body dimensions and scalation, with various causal hypotheses showed a significant association of colour pattern with potential natural vegetation (pairwise MCT: $P < 0.0075$) and an association of body dimensions with population history (Table 2). Pairwise MCTs showed significant association for scalation with all independent variables (all pairwise MCTs: $P < 0.05$ after Bonferroni correction), but a partial MCT showed only associations with rainfall and population history (Table 2).

Discussion

Phylogeographic analyses and the analysis of sequence variation suggest that secondary contact of previously allopatric populations and recent bottleneck/population expansion events have shaped genetic diversity in the Tenerife gecko. A high degree of phylogeographic structuring in pattern and/or magnitude has also been reported in other reptile species on small oceanic islands (Thorpe *et al*. 1995; Thorpe & Malhotra 1996; Giannasi 1997; Pestano & Brown 1999; Malhotra & Thorpe 2000). These studies are all on islands of volcanic origin which may suggest that volcanism is an important factor in structuring genetic diversity. Among these cases, *Tarentola delalandii* is an example with a very distinct phylogeographic pattern and deep (up to 11.5% pairwise) divergence.

While early colonization and long-term allopatry on old precursor islands can explain the deep divergence among haplotype clades, the extremely low degree of spatial admixture of haplotype clades may be explained only in part by the relatively recent joining of the precursor islands and recent changes in population size. Given an estimated time of 0.6 Ma or less (perhaps even as recently as ≈ 0.2 Ma) since bottlenecking, a higher degree of admixture of mitochondrial haplotypes might be expected (Ibrahim *et al*. 1996). A higher degree of admixture is seen in the sympatric *Gallotia galloti*, which is thought to have undergone the same process of secondary contact after junction of the western and eastern precursor islands (Thorpe *et al*. 1996), although this species is admittedly more vagile than the gecko. Given that the western (Teno + Adeje) and Anaga clades are estimated to have been allopatric for ≈ 9–10 Myr (based on mean divergence), isolation mechanisms reducing or preventing gene flow across secondary contact zones may have evolved. The duration of the allopatric phase will be less if the molecular clock is faster than estimated and the Cañadas geological activity (Ancochea *et al*. 1999) allowed the precursor populations to join earlier than estimated, but it will still be several million years. If there are hybrid zones between the three clades, strong selection may also prevent intergradation of genetic markers.

While our study on a mtDNA marker raises questions about the introgression between, and subsequent taxonomic status of, the three major mtDNA clades in *T. delalandii*, additional data from unlinked (nuclear) loci and more intensive geographical sampling are required to answer these questions satisfactorily. Similarly, nuclear markers may also help to give a more conclusive answer to the question of whether the deep divergence between the Adeje and Teno clades is the result of random fixation of two very distinct haplotypes from a single population, or whether they originate from two previously allopatric populations on separate precursor islands. In the latter case, similar divergence times for independent loci would be expected when comparing the two populations. In the case of a single population, shorter and more variable divergence times for different, independent, loci would be expected.

Our suggestion that there were three precursor islands of Tenerife with populations of *T. delalandii*, is in contrast with earlier phylogeographic studies of the sympatric lizard *G. galloti* (Thorpe *et al*. 1996) and the darkling beetle genus *Pimela* (Juan *et al*. 1996). Both studies uncovered only two localized major mitochondrial haplotype clades within Tenerife (despite extensive sampling in the case of *G. galloti*) which could be seen as suggesting only two precursor islands at the time they diverged. The two clades of *G. galloti* on Tenerife show a much lower degree of sequence divergence than those of *T. delalandii*, suggesting that the clades diverged later, after the putative Teno and Adeje islands were joined, but while Anaga was still separate. In the case of *Pimela*, sequence divergence among the major clades is of a similar magnitude to *T. delalandii*. The absence of separate Teno and Adeje clades in the *Pimela* study may be a result of colonization failure, subsequent extinction, or inadequate sampling. Thus, our study represents the first phylogeographic support for the geological hypothesis of three precursor islands of Tenerife.

Although the phylogeographic pattern in *T. delalandii* is significantly associated with the pattern which would be expected under colonization of the intervening land from the three refugia at equal rates of expansion, the Teno clade does not appear to have expanded spatially at the same rate. This is very similar to the situation in the sympatric lizard *G. galloti* (Thorpe *et al*. 1996) insofar as the Anaga clade appears to have spread along the northern coast to a greater extent than the western/Teno clade. The unbiased assumption of equal rates of spread is based on the existence of equally suitable habitat without any partial (or complete) barriers. While this avoids circularity in hypothesis testing, it is perhaps too simple, for at least two reasons. First, where the cloud layer along northern Tenerife reaches ground level on the mountain at *c*. 1500 m, lizard densities are reduced dramatically (Thorpe & Brown 1989), creating a corridor of suitable habitat along the northern coast. Second, the steep Teno cliffs may be less suitable for habitation and reduce outward spread on the Teno clade. Hence, heterogeneity in habitat suitability may have resulted in the Anaga populations of both species (*G. galloti* and *T. delalandii*) being channelled west along the northern coastal corridor to a greater extent than the Teno populations were able to spread east to meet them.

Testing the causes of geographical variation in *T. delalandii*, while taking into account phylogenetic history, has shown that variation in its morphological character systems cannot be attributed solely to adaptation to current ecological conditions. The variation in body dimensions and scalation reflect the effects of population history. This may be due to either drift in long-term allopatry and/or adaptive differentiation to different precursor island habitats. While body dimensions appear to be strongly selected traits in arboreal lizards (Malhotra & Thorpe 1997a; Losos *et al*. 1998), this seems not to be the case in this particular terrestrial species. However, this study also provides support for a possible effect of natural selection on colour pattern and scalation in *T. delalandii*. Color pattern in lizards appears to be a highly variable character system, which responds strongly to natural selection (Thorpe *et al*. 1996). The association of potential natural vegetation with colour pattern may suggest a cryptic function of colour pattern. Despite its predominately nocturnal behaviour, the species can be seen foraging during dawn and dusk, and basking during the day in cooler habitats (T. Gübitz, personal observation) and hence may be subjected to predation by visually orientated predators. We also cannot reject the hypothesis that scalation is causally related to rainfall and that scalation may be of importance in controlling water loss in this species. These results are in agreement with studies on other squamate reptiles, indicating associations of potentially cryptic colouration with vegetation type and scalation with precipitation/humidity patterns (see Malhotra & Thorpe 1997b).

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One of the two main research themes led by R. S. Thorpe and A. Malhotra is the processes underlying population differentiation and speciation in lizards from the Canary Islands and Lesser Antilles (the other theme being the molecular evolution of Asian vipers). This work is part of the former theme and formed part of T. Gübitz's PhD. T. Gübitz is currently interested in the molecular basis of adaptive phenotypic differences within and between species.
