

Population evolution of western Canary Island lizards (*Gallotia galloti*): 4-base endonuclease restriction fragment length polymorphisms of mitochondrial DNA

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The mitochondrial DNA evolution of the western Canary Island lacertid, *Gallotia galloti*, was studied using six restriction enzymes (*Hae* III, *Mva* I, *Hin* II, *Taq* I, *Msp* I and *Dsa* V). The population phylogeny (*G. stehlini*, from the adjacent island of Gran Canaria, was used as an outgroup) based on 56 restriction fragments from the first two enzymes indicates two distinct lineages; a 'northern' lineage of La Palma, north Tenerife and south Tenerife populations and a 'southern' lineage of populations from Gomera and Hierro. The relationships within the northern lineage are also supported by an additional analysis of 132 restriction fragments from the remaining four enzymes. This molecular phylogeny argues for the recognition of two subspecies corresponding to the two main lineages and against the recognition of the six current subspecies which are based on inadequate 'conventional' criteria.

ADDITIONAL KEY WORDS:—Molecular phylogeny – geographic variation mtDNA – Canary Island lizards.

CONTENTS

Introduction	219
Materials and methods	220
Results	222
Discussion	225
Acknowledgements	226
References	226

INTRODUCTION

The western Canary Island lizard, *Gallotia galloti*, is a member of a lacertid genus that is endemic to the Canary Islands. This species is found on Tenerife and all islands to the west, i.e. La Palma, Gomera and Hierro. It is the only lacertid on these islands except for a single minute relict population of *G. simonyi* on the cliffs of Hierro. It is at an intermediate stage in the taxon cycle (Wilson, 1961) in that it has a complete (no uninhabited islands) distribution and shows inter-island geographic variation in morphology (Thorpe, 1985a–c). It also exhibits a strong pattern of within-island geographic variation of the colour pattern in Tenerife with associated variation in the scalation and body dimensions (Thorpe & Baez, 1987; Thorpe & Brown, 1989a, b, 1991). This

within-island variation has been subject to quantitative hypothesis testing with Mantel tests (and partial correlations) and is thought to be due to natural selection for current ecological conditions. However, the cause of the inter-island variation in morphology (Thorpe, Watt & Baez, 1985) is likely to be composed of both ecogenetic (natural selection for current ecological conditions) and phylogenetic (historical) components (Thorpe, 1991; Thorpe *et al.*, 1991).

In an attempt to reconstruct the phylogeny of this species, free from the confounding effects of current natural selection, we studied the mitochondrial DNA using a set of restriction endonucleases (recognizing 4-bases) to indicate restriction fragment length polymorphisms (RFLPs). These are 'selectively neutral', maternally inherited and rapidly evolving (Wilson *et al.*, 1985) and thus are suitable for elucidating the phylogenetic relationships among closely related island populations.

MATERIALS AND METHODS

Specimens. Ten specimens were collected from single localities on each of the islands of La Palma, Hierro and Gomera, as well as from north and south Tenerife (Fig. 1). The outgroup was a population of *G. stehlini* from Gran Canaria (adjacent to Tenerife).

Sample preparation. Mitochondrial DNA samples were prepared by the method of Lansman *et al.* (1981), by sucrose step-gradient purification of mitochondria, following differential centrifugation of liver homogenate (liver tissue was homogenized in 0.25 M sucrose, 50 mM Tris-HCl pH 7.5, 10 mM EDTA). Mitochondrial DNA was released by 1% SDS lysis in STE buffer (200 mM NaCl, 100 mM Tris-HCl pH 8.0, 10 mM EDTA) prior to proteinase K digestion

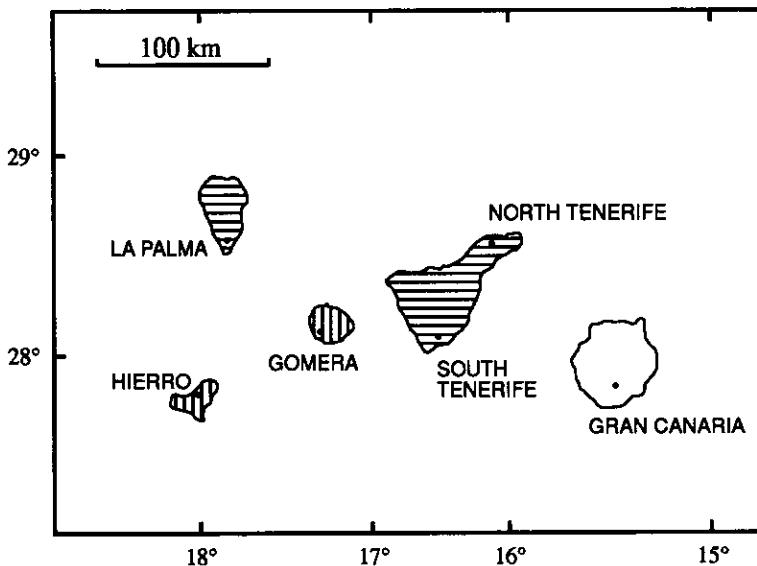


Figure 1. Sample sites in the western and central Canary Islands. *Gallotia galloti* was sampled from La Palma, Hierro, Gomera and north and south Tenerife, while the outgroup, *Gallotia stehlini*, was sampled from Gran Canaria. The geographic distribution of northern (horizontal hatching) and southern (vertical hatching) lineages of *G. galloti* is illustrated.

(20 mg/ml of sample) for two hours at 37°C. Samples were extracted three times with phenol–chloroform–isoamyl alcohol (25:24:1) and once with chloroform–isoamyl alcohol (24:1). Mitochondrial DNA was precipitated with one-tenth of a volume of 7.5 M ammonium acetate and three volumes of absolute ethanol. Total DNA samples were prepared simultaneously by the same DNA extraction procedure with the exception that proteinase K incubation was increased to 16 hours.

End-labelling of mitochondrial DNA. The restriction fragments for *Hin* II, *Taq* I, *Msp* I and *Dsa* I, on samples from La Palma, north Tenerife, south Tenerife and *G. stehlini*, were characterized using the standard end-labelling procedure (Dowling, Moritz & Palmer, 1990). Fragments for digested mitochondrial DNA samples were incubated in 1 incubation buffer (6 mM KCl, 100 mM Tris–HCl, 10 mM MgCl₂, 7 mM β-mercaptoethanol) with 3 μCi of the appropriate α-labelled dNTP and 0.5 units of Klenow fragment for 20 minutes at room temperature (Dowling, *et al.*, 1990). The reaction was stopped by the addition of loading dye [6 × loading dye: 0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol, 69.5% deionized H₂O, (Maniatis, Fritsch & Sambrook, 1982)].

Gel electrophoresis. Samples digested with 4-base restriction enzymes were electrophoresed on 0.75 mm acrylamide, 1 × TBE (90 mM Tris–HCl pH 8.3, 90 mM boric acid, 10 mM EDTA) gels in an LKB2001 vertical electrophoresis assembly at 25 mA per gel until the bromophenol blue dye had travelled to 1 cm from the bottom of the gel. Following electrophoresis, unlabelled, digested total DNA in acrylamide gels was electrophoretically transferred (Reed & Mann, 1985), in an LKB Transphor semi-dry blotter, onto a nylon filter (Biotrace RP, Gelman Sciences) in 0.25 × TBE buffer at 0.8 mA cm⁻² for 30 minutes prior to hybridization with mitochondrial DNA probe. Gels containing end-labelled mitochondrial DNA samples were dried down on filter paper at 80°C under a vacuum for 1 hour prior to autoradiography.

Probe preparation. Restriction fragments for *Hae* III and *Mva* I were characterized by an alternative technique using, however, the electrophoretic transfer described above. Linearized mitochondrial DNA, for use as a probe, was purified from an agarose gel using the Prep-a-gene kit (Bio-Rad). The probe was boiled for 10 minutes, snap-cooled on ice/water and the volume adjusted to 9 μl and labelled using the BCL Random Primed Labelling kit and [α-³²P]dCTP.

Hybridization. Nylon filters with total DNA digests were pre-hybridized in 5 × SSPE (0.6 M NaCl, 0.3 M NaH₂PO₄, 0.004 M EDTA pH 7.4), 0.3% SDS and 0.5 mg ml⁻¹ sheared, denatured herring sperm DNA in sealed bags in a shaking waterbath for 2–4 hours at 65°C. Hybridization was carried out overnight under the same conditions after the addition of boiled radiolabelled probe. Filter washing was carried out as follows: two cycles of 10 minutes in 5 × SSPE, 0.3% SDS at room temperature followed by 10 minutes in 1 × SSPE, 0.3% SDS at 65°C. The washed filter was partially dried on filter paper prior to autoradiography for a period of between 24 hours and 1 week. The film was then developed and the banding pattern analysed.

Tree reconstruction. Genetic distances were calculated using the McElroy *et al.* (1991) Restriction Enzyme Analysis Package (REAP) from a data matrix constructed from all locality samples analysed with *Hae* III and *Mva* I to examine all *G. galloti* populations while a second matrix was constructed from all six enzymes to analyse *G. galloti* populations on Tenerife and La Palma. In both

cases *G. stehlini* data were included as the outgroup. Phylogenetic trees were reconstructed from the data by Wagner parsimony, and from the genetic distance matrix by the Fitch-Margoliash method without the assumption of a molecular clock (Swofford & Olsen, 1990), using programs BOOT and FITCH from PHYLIP 3.3 (Felsenstein, 1990).

RESULTS

The two methods of visualization, end-labelling and hybridization, were successful for 3–5 samples per locality. End-labelling (Fig. 2) appeared to be more sensitive than hybridization (Fig. 3), detecting down to less than 300 bp

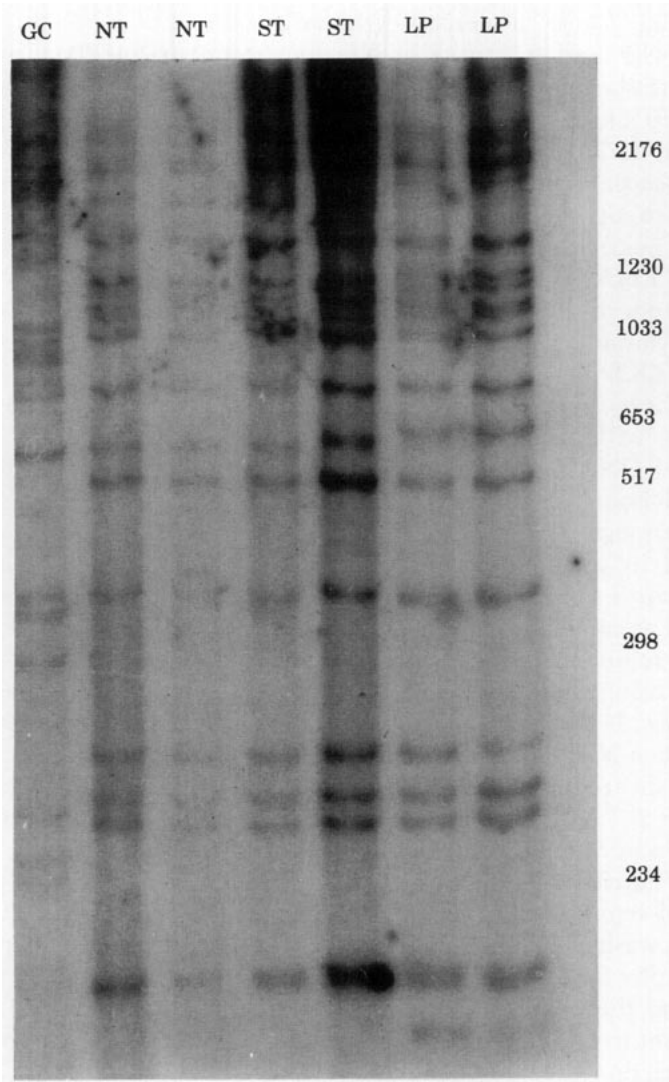


Figure 2. ^{32}P -end-labelled *Taq*I-digested mitochondrial DNA. The samples are of *G. stehlini* from Gran Canaria (GC) and of *G. galloti* from La Palma (LP), north Tenerife (NT) and south Tenerife (ST). The size of fragments, in base pairs, is given on the right.

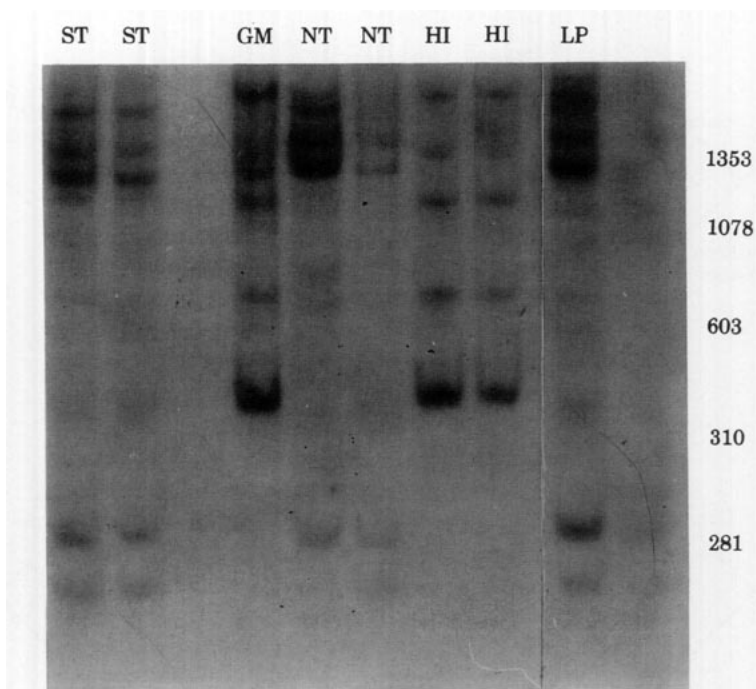


Figure 3. Electroblotted *Hae*III digested genomic DNA, hybridized with purified mitochondrial DNA. Codes are as for Fig. 2, but also include *G. galloti* samples from Gomera (GM) and Hierro (HI).

fragments. While the electroblotting and hybridization should be capable of detecting fragments of the same size as end-labelling, in practice it detected fewer bands (Fig. 3). The samples studied showed no heteroplasmy, no major variation in the length of the mtDNA, only one haplotype per locality and different haplotypes at each of the 5 localities.

*Hae*III and *Mva*I yielded 56 restriction fragments, 48 of which were polymorphic among La Palma, north Tenerife, south Tenerife, Hierro, Gomera and the outgroup. The evolutionary distance among populations is computed from this restriction fragment data (Nei & Li, 1979; Nei, 1987) and is given, together with standard errors (Upholt, 1977) in Table 1.

TABLE 1 Evolutionary distance (lower diagonal) among populations based on *Hae*III and *Mva*I and its standard error (upper diagonal). Sample codes are *G. stehlini* from Gran Canaria (GC) and *G. galloti* from south Tenerife (ST), north Tenerife (NT), Hierro (HI), Gomera (GM) and La Palma (LP)

	GC	LP	NT	ST	GM	HI
GC		0.020	0.021	0.018	0.021	0.020
LP	0.068		0.009	0.011	0.017	0.018
NT	0.078	0.012		0.012	0.017	0.017
ST	0.056	0.018	0.0920		0.015	0.015
GM	0.073	0.044	0.046	0.032		0.011
HI	0.073	0.053	0.055	0.035	0.016	

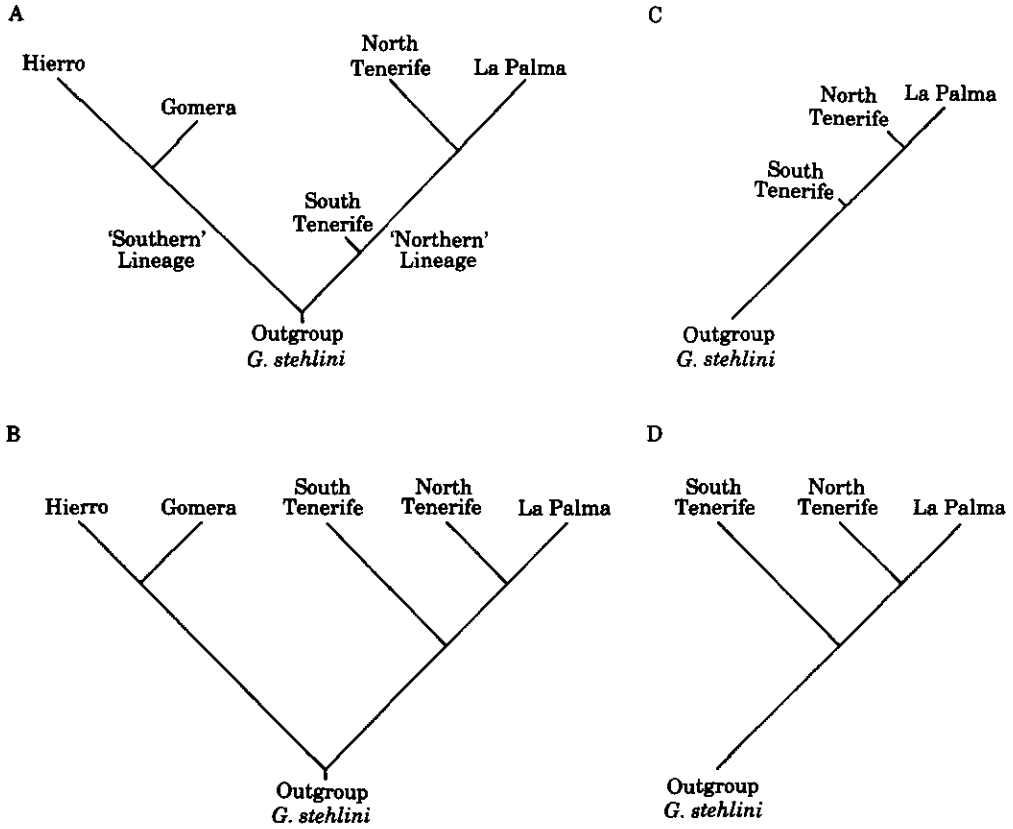


Figure 4. Fitch-Margoliash phylogenetic tree (A) and Wagner tree (B) of all *G. galloti* populations (outgroup rooted with *G. stehlini*) based on two endonucleases and showing southern and northern lineages for *G. galloti*. Fitch-Margoliash tree (C) and Wagner tree (D) of northern lineage of *G. galloti* (outgroup rooted with *G. stehlini*) based on all six endonucleases and showing north Tenerife and La Palma as sister groups. The branch lengths of the Fitch-Margoliash trees (A, C) are to scale.

The Fitch-Margoliash tree and the Wagner tree have the same topology. There are two distinct lineages within the *G. galloti* species (Fig. 4A, B). One occupies Tenerife (north and south) and La Palma and is referred to as the northern lineage, while the other occupies Gomera and Hierro and is referred to

TABLE 2. Evolutionary distance (lower diagonal) among populations and its standard error (upper diagonal) based on *Hae* III, *Mva* I, *Taq* I, *Msp* I, *Hinf* I, and *Dsa* V. Sample codes are as for Table 1

	GC	LP	NT	ST
GC		0.012	0.012	0.012
LP	0.100		0.004	0.005
NT	0.103	0.006		0.004
ST	0.094	0.011	0.008	

as the southern lineage. In the northern lineage La Palma and north Tenerife are sister groups.

The restriction enzyme *Hinf*I, *Taq*I, *Msp*I and *Dsa*V yielded 132 restriction fragments, but were studied only in the samples from north Tenerife, south Tenerife, La Palma (i.e. the northern lineage) and the outgroup. When this information is added to the previous RFLP data (Table 2), the northern lineage (in both the Wagner tree and the Fitch-Margoliash tree) has the same topology as in the previous trees (Fig. 4C, D).

DISCUSSION

Heteroplasmic samples were not found in this study despite their occurrence in other vertebrate species (Densmore, Wright & Brown, 1985; Bermingham, Lamb & Avise, 1986). This is possibly due to the low sample number; however, a study carried out on 55 lizard samples using a wide range of 6-base pair recognition restriction enzymes has not detected heteroplasmic states in *Gallotia* (McGregor, 1992). Major mitochondrial DNA length variations have also been observed in a variety of vertebrate species (Densmore *et al.*, 1985; Bermingham *et al.*, 1986; Moritz & Brown, 1987), but were not found in the *Gallotia*. However, the tendency for one haplotype to predominate in a particular population or geographical area has been found in other studies (Bermingham & Avise, 1986). Frequent founder effects in the evolutionary history of a species, as must occur with this island group, will reduce mitochondrial variability within populations (Wilson *et al.*, 1985).

The two restriction endonucleases (that gave information across all samples) surveyed, on average, 109 bases per sample. This level of information was sufficient to show that *G. galloti* is comprised of two distinct lineages. These lineages are geographically coherent; the southern lineage occupies the south western islands of Gomera and Hierro, while the northern lineage occupies Tenerife and the north-western island of La Palma (Fig. 1). It is the island populations of La Palma and north Tenerife that are sister groups, rather than those of north and south Tenerife (this is supported by the analysis across all six enzymes which surveys, on average, 399 bases per sample). At first sight this may appear counter intuitive, but it only implies that the northern and southern populations on Tenerife differentiated prior to La Palma being colonized from north Tenerife (Thorpe *et al.*, in press).

Currently, there are six subspecies recognized for *G. galloti*, one for each island (La Palma, *G. g. palmae*; Hierro, *G. g. caesaris*; Gomera, *G. g. gomerae*) except Tenerife, where there are three, one in the south (*G. g. galloti*), one in the north (*G. g. eisentrauti*) and one on a tiny islet on the north coast, Roque de Fuera de Anaga (*G. g. insulanae*) (Klemmer, 1976; Bischoff, 1982; Martin, 1985; reviewed by Baez, 1987). Subspecies recognized on 'conventional' criteria generally have little, or no, value (Thorpe, 1980, 1981, 1987; Malhotra & Thorpe, 1981; Wüster *et al.*, 1992) and these are no exception. They are not predictive (Thorpe, 1987) and do not reflect the major phylogenetic subdivision within the species. Consequently, they need revision in light of this information on DNA phylogeny. A superior arrangement would be to have two subspecies which correspond to the two lineages (Fig. 1); i.e. a northern subspecies *Gallotia galloti galloti* from Tenerife (and its islets) and La Palma; and a southern

subspecies *Gallotia galloti caesaris* from Gomera and Hierro. This taxonomic revision is further supported by phylogenetic analysis of data from 6-base pair recognition restriction enzymes, base pair sequence of cytochrome b, cytochrome oxidase and 12 s rRNA, together with phylogenetic analysis of random amplified polymorphism of nuclear DNA (RAPD) (Thorpe *et al.*, in press). Analysis of these phylogenies in light of the geology of the Canarian archipelago (Abdel-Monem, Watkins & Gast, 1971, 1972; Anguita & Hernan, 1975, 1986; Carracedo, 1979) argues for these two subspecies having an origin early in the life of the species and separate colonization pathways across the archipelago (Thorpe *et al.*, in press.).

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