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DNA EVOLUTION AND COLONIZATION SEQUENCE OF ISLAND LIZARDS IN RELATION TO GEOLOGICAL HISTORY: MTDNA RFLP, CYTOCHROME B, CYTOCHROME OXIDASE, 12S RRNA SEQUENCE, AND NUCLEAR RAPD ANALYSIS

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Abstract.—A novel source of nuclear DNA information from random amplified polymorphisms (RAPD) and a wide-range mitochondrial DNA information (cytochrome b, cytochrome oxidase, and 12s rRNA sequence, RFLP from 4-base and 6-base recognition endonucleases) are used to reconstruct the population phylogeny of the western Canary Island lizard, *Gallotia galloti*, which, for geological reasons, has been subject to dispersal but not vicariance. Interpretation of DNA phylogenies in terms of colonization sequence indicates that *G. galloti* arose in Tenerife and dispersed westward in two independent pathways: north from north Tenerife to La Palma, and south from south Tenerife to Gomera to Hierro. The direction and timing of colonization by DNA divergence is entirely compatible with geological time and sequence of island origin.

Key words.—Cytochrome b, cytochrome oxidase, direct sequencing, dispersal biogeography, *Gallotia* lizards, island colonization, mitochondrial DNA, nuclear DNA, PCR, RAPD, restriction fragment length polymorphisms, 12s rRNA.

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Almost all species must disperse and extend their range at some stage in their existence, but, with few exceptions (Thorpe 1984), their population phylogeny is generally not rigorously interpreted in terms of dispersal or range expansion. Two main problems that occur with such rigorous interpretation: (1) both natural selection for current ecological conditions (ecogenesis) (Endler 1980, 1983, 1986; Thorpe and Brown 1989; Brown et al. 1991; Malhotra and Thorpe 1991; Thorpe 1991; Thorpe and Malhotra 1992) and differentiation occurring over time on account of historical factors (phylogenesis) (Endler 1983; Thorpe 1984; Wilson et al. 1985) can influence population differentiation; and (2) both dispersal and vicariance can influence phylogenesis. Here, (1) is countered by using DNA data that are unlikely to be strongly influenced by ecogenesis, and (2) is countered by using a model (western Canary Island lizard, *Gallotia galloti*), which, for geological reasons, has been subject to dispersal but not vicariance.

Gallotia galloti belongs to a genus endemic to the Canary Islands. Phylogenetic analysis of morphological and molecular data indicates that it is a distinct species occupying Tenerife and all Canary Islands to the west, that is, La Palma, Hierro, and Gomera (Thorpe et al. 1985, 1993a,b). Its complete (not relict) distribution and its morphological (Thorpe 1985a,b) and molecular differentiation among islands indicate its intermediate stage in the taxon cycle (Wilson 1961).

The islands occupied by this species, the western Canary Islands, erupted from the sea independently, are separated from one another by deep channels, and are not thought to have been joined above sea level to one another or to the African mainland. This holds irrespective of whether one follows the hot-spot, or propagating-fracture hypothesis of western Canary Island origin (Abdel-Monem et al. 1971, 1972; Anguita and Hernan 1975, 1986; Carracedo 1979; Feraud et al. 1985; Ancochea et al. 1990). Consequently, the distribution and phylogenetic differentiation of species such as *G. galloti* must be interpreted in terms of dispersal among islands, that is, colonization from one island to another. However, attempts to interpret a morphological phylogenetic tree (Thorpe 1985b) in terms of colonization sequence are compromised by ecogenetic adaptation to current selective pressures influ-

encing the tree. Consequently, this study is based on DNA data, including nuclear DNA (random amplified polymorphisms) and a wide range of mitochondrial DNA data (cytochrome b, cytochrome oxidase, and 12s rRNA sequence, 4-cut and 6-cut RFLPs), which is not likely to produce a phylogeny perturbed by ecogenetic adaptation. Restriction fragment-length polymorphisms in particular are thought to be near neutral (Wilson et al. 1985; DeSalle and Templeton 1988).

Materials and Methods

Specimens.—Ten specimens of *Gallotia galloti* were collected from each single locality on the islands of Hierro, Gomera, La Palma, and from both north and south Tenerife (fig. 2B). The congener *G. stehlini* from the adjacent island of Gran Canaria was consistently used as an outgroup, and *G. atlantica* (Lanzarote), and the lacertids *Psammodromus hispanicus* (southern France) and *Podarcis muralis* (southern France) were also used when data were available.

Restriction fragment-length polymorphisms of mtDNA: 4 cut.—Data from 4-base-pair recognition, and 6-base-pair recognition, enzymes were treated separately. Mitochondrial DNA samples were prepared by a modification of Lansman et al.'s (1981) procedure. Fragments for the 4-cut enzymes *Hae*III and *Mva*I were characterized by electroblotted digested genomic DNA, hybridized with purified mtDNA (Thorpe et al. 1993a). This gave 56 fragments, 48 of which were polymorphic among the *G. galloti* and *G. stehlini* populations. There was no heteroplasmy and only one haplotype per sample locality. The relationships among the populations from La Palma and north and south Tenerife (*G. stehlini* outgroup) were confirmed by additional analysis of 132 restriction fragments from four additional 4-cut enzymes (*Hinf*I, *Taq*I, *Msp*I, *Dsa*V). Data are given in McGregor (1992) for this and other sources of DNA information.

Restriction fragment-length polymorphisms (RFLPs) of mtDNA: 6 cut.—Fragments for the 6-cut enzymes (*Apa*I, *Asp*700, *Asp*718, *BamH*I, *Cla*I, *Dra*I, *Eco*RI, *Hind*III, *Ksp*I, *Pst*I, *Pvu*II, *Sac*I, *Sal*I, *Sma*I, *Xba*I, *Xho*I) and *Sty*I (C/CWWGG) were detected by the above hybridization technique (Thorpe et al. 1993b), or by an antibody technique (Chapman and Brown 1989; Thorpe et al. 1993b) after Southern blotting from an agarose gel. Sixty restriction sites were detected in the *G. galloti, G. stehlini,* and *G. atlantica* samples.

Cytochrome-b sequence.—PCR amplification (Saiki et al. 1988) and direct sequencing of this mtDNA gene (Kocher et al. 1989), using both L14641 and H15149 primers, gave information on 291 base pairs. Seventy-six base pairs differed among taxa (*G. galloti, G. stehlini, G. atlantica* and *P. hispanicus*), only 14 of which were at the first or second base position, and only one of which produced an amino acid substitution within the species of interest (*G. galloti*). Transversions are greatly outnumbered by transitions at the third base position and are absent from first and second base positions.

Cytochrome-oxidase-I sequence.—PCR amplification and direct sequencing of subunit I of the mtDNA gene cytochrome oxidase (Kessing et al. 1989) gave information on 465 base pairs. One hundred and twenty-three base pairs differed among taxa (*G. galloti, G. stehlini, G. atlantica,* and *P. hispanicus*), none of which result in an amino-acid substitution.

12s rRNA sequence.—A 249-base-pair section of the mitochondrial gene 12s rRNA was cloned and sequenced after PCR amplification (Medlin et al. 1988). Fifty-four base pairs differed among taxa (*G. galloti, G. stehlini, G. atlantica,* and *P. hispanicus*). The high transition/transversion ratio observed in the protein-coding genes, for example, cytochrome b, is not seen in the region of 12s rRNA that was sequenced.

Random amplified polymorphisms of nuclear DNA (RAPD).—Short primers (10 base pairs) of arbitrary sequence are used by PCR to amplify nuclear DNA products (Williams et al. 1990). Six such primers were used (McGregor 1992), which gave 121 bands, 120 of which varied among taxa (*G. galloti, G. stehlini, G. atlantica,* and *Podarcis muralis*). Previously, this genomic mapping procedure has been used to identify hybrids (Williams et al. 1990), but here the banding pattern is used for phylogeny reconstruction.

Genetic Distance and Phylogeny Reconstruction.—Genetic distances among taxa were constructed for each sequence, and RFLP mtDNA data-set (Nei and Li 1979; Nei 1987) Fitch-Margoliash trees (Fitch and Margoliash 1967), without the assumption of a molecular clock, were derived from these distance matrices using the Phylip 3.3 program (Felsenstein 1990). For the RAPD data, the Fitch-Margoliash trees were computed from a mismatch distance matrix. Wagner parsimony trees (Farris 1970) were also

TABLE 1. Molecular clock times (based on 2% per my) in relation to geological origin of islands (see references in the text) in millions of years before present.

Island	Molecular clock			Geological origin
	RFLP data	Sequence data	Weighted mean	
S. Tenerife (*Gallotia stehlini* common ancestor)	3.01	7.45	6.29	15.7
S. Tenerife (*G. atlantica* common ancestor)		6.18		15.7
Gomera (from S. Tenerife)	1.63	3.27	2.84	12.0
La Palma (from N. Tenerife)	0.30	0.50	0.45	1.6
Hierro (from Gomera)	0.55	0.20	0.29	0.75

computed from each original data set, applying Fitch's (1971) adaptation for sequence data, using the Phylip 3.3 program which employs bootstrapping (Felsenstein 1985).

Although data sets were usually kept separate, genetic distance and divergence times were computed treating all RFLP data as one set and all sequence data as another set. Divergence times between selected island populations (table 1) were then computed for each set on the assumption of a 2% change per million years (Wilson et al. 1985). Since the RFLP data surveyed, on the average, 351 bases per taxon, and the sequence data covered 1005 bases, weighted average divergence times were computed across both these data sets (table 1). A Fitch-Margoliash tree (without the assumption of a molecular clock) was derived for all sequence data pooled.

PHYLOGENIES

Wagner trees derived from all six data sets (fig. 1) give topologies for *Gallotia galloti* that are identical to those of the Fitch-Margoliash trees. These trees all indicate two primary lineages within *G. galloti,* a "southern" lineage comprised of Gomera and Hierro and a "northern" lineage comprised of north Tenerife, south Tenerife, and La Palma. The 12s rRNA sequence (fig. 1A) and nuclear DNA RAPD analysis (fig. 1B) did not differentiate at lower taxonomic levels within these northern and southern *G. galloti* lineages, but the cytochrome-oxidase sequence (fig. 1C) differentiated the northern lineage samples and the 4-cut RFLP (fig. 1D), 6-cut RFLP (fig. 1E), and cytochrome-b sequence (fig. 1F) differentiate among samples in both the northern and southern lineage. The latter four data sets indicate that La Palma and north Tenerife populations are sister groups.

The trees (figs. 1C–F) that give the finest resolution amongst the least differentiated populations allow a common topology for *G. galloti* (fig. 2A), which can be interpreted in terms of colonization sequence. In addition, the sequence data from cytochrome b, cytochrome oxidase, and 12s rRNA can be combined (1005 bp) to give a Fitch-Margoliash tree with a greater information content (fig. 2D) which is used to determine colonization sequence. The relative branch lengths of the tree based on all sequence data is corroborated by the relative branch lengths of a comparable Fitch-Margoliash tree based on pooled RFLP data presented elsewhere (Thorpe et al. 1993b).

COLONIZATION SEQUENCE AND TIMING

We suggest two ways of interpreting a tree in terms of colonization sequence. The first process depends on tree topology (which is robust) and geographic distance (fig. 2A–C). The second relies on the branch length (which is labile) and topology, and is independent of geographic distance (figs. 2B,D).

Process 1: Topology Plus Geography.—Process 1 is based on the proposition that an island is more likely to be colonized from a close island than from a distant one. For this process, the tree topology for *Gallotia galloti,* which is common

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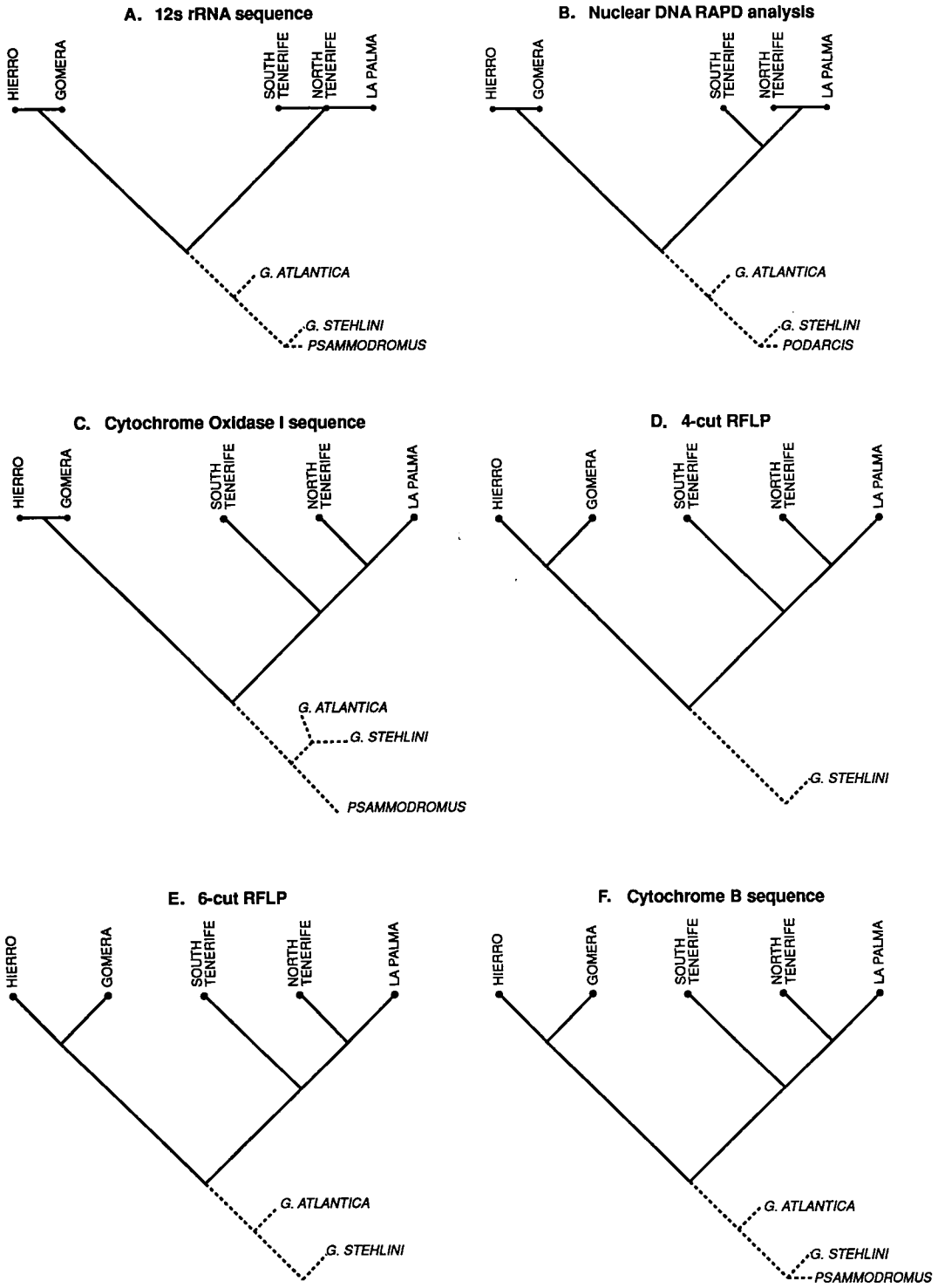
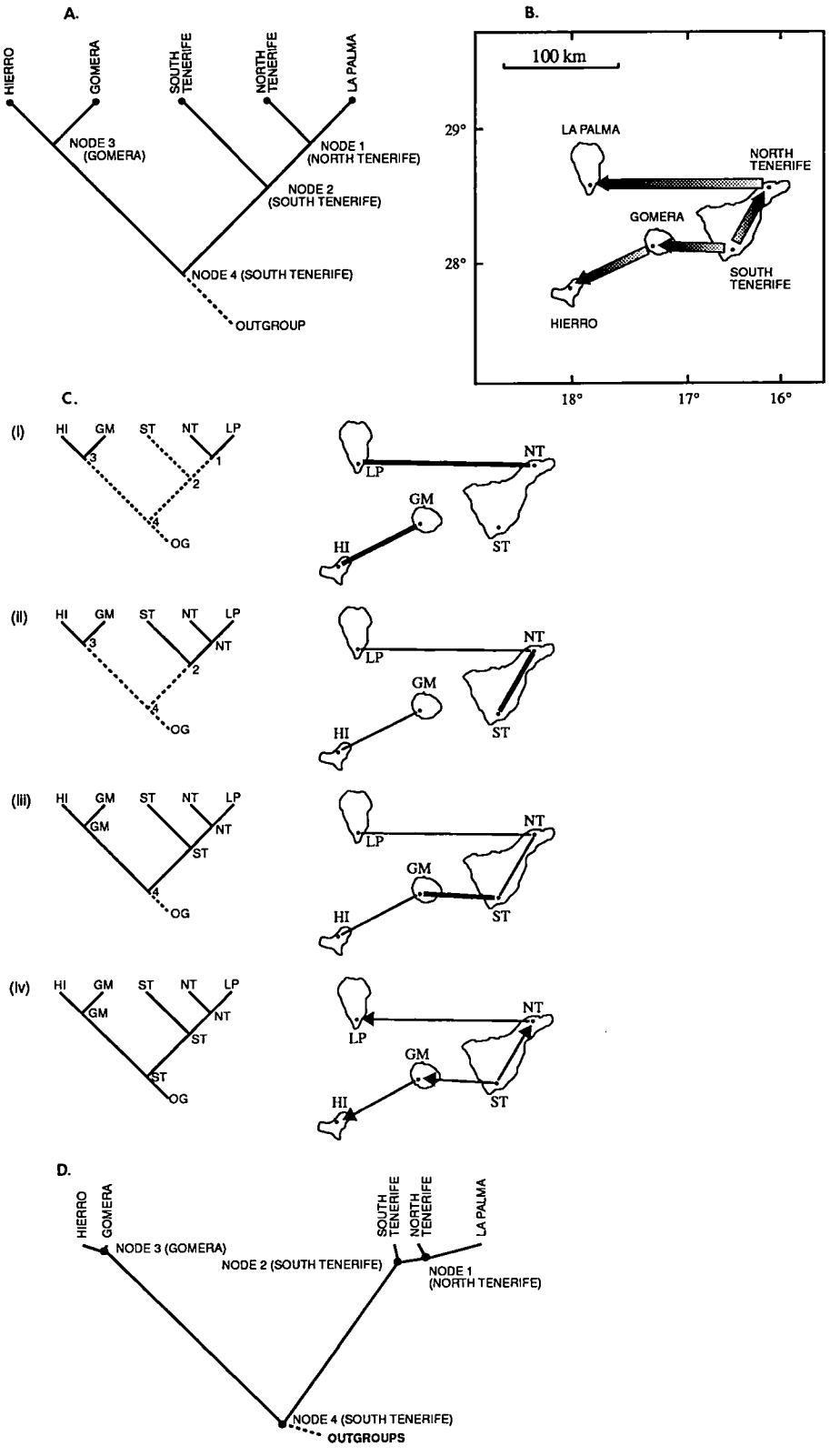
FIG. 1. Topologies of Wagner and Fitch-Margoliash phylogenetic trees (without the assumption of a molecular clock) of *Gallotia galloti* populations constructed from nucleic-acid data and outgroup rooted. A. 12s rRNA sequence data, southern (Hierro, Gomera) and northern (north Tenerife, south Tenerife, La Palma) lineages within *G. galloti* are apparent, but the more closely related populations within these lineages are not differentiated. B. RAPD nuclear DNA data give a similar tree as 12s rRNA except that south Tenerife is differentiated from



north Tenerife/La Palma. C. Cytochrome oxidase I data show the northern and southern *G. galloti* lineages and differentiate among the groups of the northern lineage. Four-cut RFLP analysis of mtDNA (D), six-cut RFLP analysis of mtDNA (E), and cytochrome-b sequence data (F) show the northern and southern lineages of *G. galloti* and differentiate among all groups within these lineages.


A.
HIERRO
GOMERA
SOUTH TENERIFE
NORTH TENERIFE
LA PALMA
NODE 3 (GOMERA)
NODE 1 (NORTH TENERIFE)
NODE 2 (SOUTH TENERIFE)
NODE 4 (SOUTH TENERIFE)
OUTGROUP
B.
100 km
29°
28°
LA PALMA
NORTH TENERIFE
GOMERA
SOUTH TENERIFE
HIERRO
18°
17°
16°
C.
(I)
(II)
(III)
(IV)
HI
GM
ST
NT
LP
OG
D.
HIERRO
GOMERA
SOUTH TENERIFE
NORTH TENERIFE
LA PALMA
NODE 3 (GOMERA)
NODE 2 (SOUTH TENERIFE)
NODE 1 (NORTH TENERIFE)
NODE 4 (SOUTH TENERIFE)
OUTGROUPS

to all data sets that differentiate all groups, is used (fig. 2A). The dispersal or colonization sequence (fig. 2B) is obtained by joining these node and population localities on a map with the direction of colonization being away from the root of the tree toward its terminal branches. Node 4 (fig. 2A) in south Tenerife indicates the origin of the species by a founder from the east, with a colonization to Gomera (node 3) and then from Gomera to Hierro in the south. A separate colonization of La Palma from north Tenerife (node 1) occurs after north Tenerife has been colonized from south Tenerife (nodes 2 to 1).

The colonization sequence was obtained from the tree topology and geographic proximity by the following process illustrated in figure 2C.

Step 1 (fig. 2Ci).—Join pairs of sister populations on the map. Because Hierro and Gomera are sister populations on the tree, they are joined on the map; and north Tenerife and La Palma are sister populations on the tree, and they are also joined on the map.

Step 2 (fig. 2Cii).—Move down to the next level on the tree (nearer the root) and recognize the junction of a pair of sister clades, or the junction of a single population to a clade (in this case, south Tenerife joins the north Tenerife-La Palma clade). Join these on the map by the shortest geographic distance. In this case, south Tenerife is joined to the north Tenerife-La Palma clade (fig. 2Cii) in the geographic sequence La Palma—north Tenerife—south Tenerife, because south Tenerife is geographically closer to north Tenerife than La Palma. The next step is to allocate a geographic locality to the node within the first clade (i.e., the north Tenerife-La Palma clade). This is done by allocating the node the same geographic location as whichever population arising from it (i.e., north Tenerife or La Palma) is closest to its sister taxon (i.e., south Tenerife). Consequently, node 1 is located in north Tenerife (fig. 2Cii) because the sister taxon to the north Tenerife-La Palma clade is south Tenerife, which is closer to north Tenerife than to La Palma. Once a node is given a geographic location, it is treated as a "taxon."

Step 3 (fig. 2Ciii).—Repeat step 2. The Hierro-Gomera clade joins the south Tenerife-node 1 (north Tenerife) clade by a connection between Gomera and south Tenerife on the map (fig. 2Ciii), as Gomera and south Tenerife are the geographically closest of the three possibilities (i.e., Gomera to south Tenerife is less distance than Hierro to south Tenerife or Gomera to north Tenerife). The colonization pathway is now complete. Node 2 is located at south Tenerife (fig. 2Ciii) because, of the two members of the south Tenerife-node 1 (north Tenerife) "taxa," it is the closest to the Gomera-Hierro taxa. Similarly, node 3 is located at Gomera (fig. 2Ciii) because, of the two members of the Hierro-Gomera taxa, it is geographically closest to node 2 (south Tenerife).

Step 4.—Repeat step 2. Node 4 is located at south Tenerife (fig. 2Civ) because, of the two members of the node 3 (Gomera)-node 2 (south Tenerife) "taxa," south Tenerife is geographically nearest to whichever of the outgroups (*G. stehlini* in Gran Canaria and *G. atlantica*) is used. *Gallotia galloti* arose in south Tenerife, having been founded from an ancestor from the islands to the east.

Process 2: Topology Plus Branch Length.—This method is based on the proposition that when an ancestral population $\{a\}$ on island A colonizes island B, the subsequent populations, $\{a'\}$ on island A and $\{b\}$ on island B, will not be equally divergent from ancestral population $\{a\}$. Founder effects (DeSalle and Templeton 1988) will render $\{b\}$ more divergent than $\{a'\}$. In a given tree, which is not constrained to have equal branch lengths (i.e., a Fitch-Margoliash tree without the assumption of a molecular clock), a node is given the geographic location of the anagenically closest taxa above it in the tree. Using the Fitch-Margoliash tree based on all mtDNA sequence data (fig. 2D), which is corroborated by an overall RFLP tree (Thorpe et al. 1993b), this procedure gives the same results as the previous procedure (fig. 2B). That is, in figure 2D, node 1 is anagenically closer to north Tenerife than to La Palma and is therefore given a location in north Tenerife; node 2 is anagenically closest to south Tenerife; node 3 is anagenically closest to

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FIG. 2. Colonization sequence of *Gallotia galloti* in the western Canary Islands. A. Common topology of *G. galloti* groups based on trees in figure 1. B. Colonization pathway of *G. galloti* among western Canary Islands deduced from the two procedures explained in the text. C. Deduction of colonization sequence from the topology of the tree in figure 2A and geographic distance, as explained in the text. D. Fitch-Margoliash tree of all sequence data used to deduce the colonization sequence based on topology and branch length.

Gomera; node 4 is anagenically closest to south Tenerife.

Discussion

When the phylogeny is interpreted in terms of colonization sequence (range expansion or dispersal), by either procedure, there is an origin for the species in south Tenerife (perhaps by a founder from the islands to the east), dispersal to north Tenerife and then a westward colonization from north Tenerife to La Palma. In the south, an independent colonization sequence resulted in Gomera being colonized from south Tenerife and Hierro subsequently being colonized from Gomera.

This origin in Tenerife and subsequent westward colonization is compatible with the geological history (table 1) of these islands as Tenerife, the island on which the species first arose, is the oldest (15.7 mybp) and the islands to the west are progressively younger, with the last to be colonized being youngest of all (i.e., La Palma at 1.6 my and Hierro at 0.75 my) (Abdel-Monem et al. 1971, 1972; Anguita and Hernan 1975, 1986; Carracedo 1979; Feraud et al. 1985; Ancochea et al. 1990). If one accepts the assumption of a 2% divergence rate per million years (Brown et al. 1982; Wilson et al. 1985) for mtDNA (averaged across sequence and RFLP data), then the hypothesized time of colonization is compatible with geological history, irrespective of whether one uses times computed from RFLP data, sequence data, or their weighted average (table 1). The origin of the species in Tenerife (ca. 6.3 mybp) postdates the geological origin (15.7 my) of the island; the colonization of La Palma from north Tenerife (ca. 0.45 my) postdates its origin (1.6 my); the colonization of Gomera from south Tenerife (ca. 2.84 my) postdates its origin (12 my); and the colonization of Hierro from Gomera (ca. 0.29 my) postdates its origin (0.75 my). Consequently, not only does the east to west colonization of the islands match their east to west origin, no island is hypothesized to be colonized before its origin, even though the founder effect (DeSalle and Templeton 1988) may cause an overestimation of divergence time. Alternative explanations for the times of geological origin exist, and the DNA divergence times may be estimated separately for RFLPs, cytochrome b, cytochrome oxidase, and 12s rRNA (McGregor 1992; Thorpe et al. 1994), but the pattern and conclusions remain the same.

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APPENDIX

Base-pair sequences from the mitochondrial genome of *Gallotia* species and populations compared to those of the outgroup *Psammodromous hispanicus*. Island codes are: LP, La Palma; NT, north Tenerife; ST, south Tenerife; GM, Gomera; HI, Hierro. See the Methods section for details.

Cytochrome b sequences (291 bp)

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Psammodromus    TCATTACTAG GACTTTGCCT AGTAACCCAA ATTATTACAG GGTTATTCTT AGCAATACAC
G. stehlini     ...C.T.... ....C..... .A.C.TT... ..C..C.... .CC.C...C. ...C..G...
G. atlantica    ...C.T.... .......... .A.T.TT... ..C....... .CC....... ......G...
G. galloti(LP)  ...C.T.... .......... .A.T.T.... ..C....... .CC....... G.........
G. galloti(NT)  ...C.T.... .......... .A.T.TT... ..C....... .CC....... G.........
G. galloti(ST)  ...C.T.... .......... .A.T.T.... ..C....... .CC....... G.....G...
G. galloti(GM)  ...C.T.... .......... .A.T.T.... ..C....... .CC....... G.....G...
G. galloti(HI)  ...C.T.... .......... .A.T.T.... ..C....... .CC....... G.....G...

Psammodromus    TATAATGCAG ACATTAACTC CGCATTCTCA TCAGTAGCCC ACATCCATCG AGACGTTCAA
G. stehlini     ..C..C.... .......... .......... ..CA.T.... .......C.. T..T..C...
G. atlantica    ..C..C.... .......... .......... ..CA.T.... .......C.. T..T......
G. galloti(LP)  ..C..C.... ....C..... .......... ..CA.C.... ....T..... T.....C...
G. galloti(NT)  ..C..C.... ....C..... .......... ..CA.C.... ....T..... T.....C...
G. galloti(ST)  ..C..C.... ....C..... .......... ..CA.C.... ....T..C.. T.....C...
G. galloti(GM)  ..C..C.... ....C..... .......... ..CA.C.... .......C.. T.....C...
G. galloti(HI)  ..C..C.... ....C..... .......... ..CA.C.... ....T..C.. T.....C...

Psammodromus    CACGGATGAC TAATCCGAAA TATTCACGCC AACGGAGCAT CACTATTCTT TATCTGTATT
G. stehlini     .......... .C..T..C.. .G.C...... .....C..T. .......... C.....C..C
G. atlantica    ..T..T.... .T........ ...C...... ........C. .......T.. ......C..C
G. galloti(LP)  .....T.... .......... ...C...... ........C. .......T.. ......C..C
G. galloti(NT)  .....T.... .T..T..... ...C...... ..T.....C. .......T.. ......C..C
G. galloti(ST)  .....T.... .T........ ...C...... ..T.....C. .......T.. ......C..C
G. galloti(GM)  .....T..G. .T........ C..C...... ..T.....C. .......T.. ......C..C
G. galloti(HI)  .....T..G. .T........ C..C...... ..T.....C. .......T.. ......C..C

Psammodromus    TATATGCACA TTGGACGGGG CTTGTACTAC GGATCTTACT TATTTACTGA AACCTGAAAC
G. stehlini     ..CGC...T. .C.....T.. .C....T... ..C..A...C .......... ..........
G. atlantica    ..C....... .......T.. .C........ ..C..A.... .....GT... ...T......
G. galloti(LP)  ..C..A.... .......... .C....T... ..C..A.... .G........ .........T
G. galloti(NT)  ..C..A.... .......A.. .C.A...... ..C..A.... .......... .........T
G. galloti(ST)  ..C..A.... .......A.. .C.A...... ..C..A.... .......... ..........
G. galloti(GM)  ..C..A.... .......... ...A..T... ..C..A..TC ......T... ...A.....T
G. galloti(HI)  ..C..A.... .......... T..A..T... ..C..A..TC .G....T... ...A.....T

Psammodromus    ATTGGAGTTC TCCTCCTTCT ACTAGTCATA GCCACAGCTT TCATAGGCTA T
G. stehlini     ........C. .......... G.....T... ........C. .T........ .
G. atlantica    ..C.....A. .T..A..A.. T......... .......... ....G..... .
G. galloti(LP)  ........A. .T..T..C.. .T........ .......... .......... .
G. galloti(NT)  ........A. .T..T..C.. .T........ .......... .......... .
G. galloti(ST)  ..C.....A. .T..T..C.. .T........ .......... .......... .
G. galloti(GM)  ..C.....A. .T.....C.. ...G...... .......... ....G..... .
G. galloti(HI)  ..C..G..A. .T.....C.. ...G...... .......... ....G..... .
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APPENDIX. Continued.

12s rRNA sequences (249 bp)

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Psammodromus    GAAAGGCCAA AAATAACGAC AAACGGCGTA AAATGTGACT AGAGAAACTA AATATAATAA
G. stehlini     .......... .G.....AG. .......... .......... ....G.CTAT ....CTTA..
G. atlantica    .......... ........G. C......... .......... ......CTAT .....TTA..
G. galloti(LP)  .......... ........G. .......... .......... ......CTAT .....CTA..
G. galloti(NT)  .......... ........G. .......... .......... ......CTAT .....CTA..
G. galloti(ST)  .......... ........G. .......... .......... ......CTAT .....CTA..
G. galloti(GM)  .......... ........G. .......... .......... ....T.CTAT .....CTA..
G. galloti(HI)  .......... ........G. .......... .......... ....T.CTAT .....CTA..

Psammodromus    AAATTTTAAC TCACACGTAG TTGTAAAATA CTAAACTAAT GAGGAAAACC TCAAAAATCT
G. stehlini     .G...C.... ...T..AC.. .A........ .........C ......G... C..C......
G. atlantica    ..T..C.... C...G.AC.A .A........ T.....CGG. .G....GC.. A.C....AT.
G. galloti(LP)  ..C..A.... ...T.T.... .A...G.... .......... .......C.. C.C.C..GT.
G. galloti(NT)  ..C..A.... ...T.T.... .A...G.... .......... .......C.. C.C.C..GT.
G. galloti(ST)  ..C..A.... ...T.T.... .A...G.... .......... .......C.. C.C.C..GT.
G. galloti(GM)  ..C..G.... ...T.TA... .A...G.... .....T.... .G....CC.. C.C....GT.
G. galloti(HI)  ..C..G.... ...T.TA... .A...G.... .....T.... .G....CC.. C.C....GT.

Psammodromus    TTTAATACTA TTTCTTGACC ACACGAAAGC TTAGACACAA ACTAGGATTA GATACCCTAC
G. stehlini     .......T.. .......... .......... .....A.... .......... ..........
G. atlantica    .......T.G C.C....... .......... .....A.... .......... ..........
G. galloti(LP)  .......T.. ..C....... .......... .....A.... .......... ..........
G. galloti(NT)  .......T.. ..C....... .......... .....A.... .......... ..........
G. galloti(ST)  .......T.. ..C....... .......... .....A.... .......... ..........
G. galloti(GM)  .......T.. ..C....... .......... .....A.... .......... ..........
G. galloti(HI)  .......T.. ..C....... .......... .....A.... .......... ..........

Psammodromus    TATGCTAAGC CATAAACAGA CGGTAAAAAT ACAATACCCT CCGCCAGAGA ACTACAAGTG

G. stehlini     .......... .......... GA...T.... ....C..T.. .......... ..........
G. atlantica    .......... .G........ GA...TCT.. .......TT. .......... ..........
G. galloti(LP)  .......... .......... TA.....T.. .......TT. .......... ..........
G. galloti(NT)  .......... .......... TA.....T.. .......TT. .......... ..........
G. galloti(ST)  .......... .......... TA.....T.. .......TT. .......... ..........
G. galloti(GM)  .......... .G........ TA........ ....C..TT. .......... ..........
G. galloti(HI)  .......... .G........ TA........ ....C..TT. .......... ..........

Psammodromus    AAAAACTTA

G. stehlini     ....*....
G. atlantica    .........
G. galloti(LP)  .........
G. galloti(NT)  .........
G. galloti(ST)  .........
G. galloti(GM)  .........
G. galloti(HI)  .........
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Cytochrome oxidase sequences (465 bp)

Psammodromus	TTTTTTGGTC	ACCCAGAAGT	CTACATTTTA	ATTTTACCAG	GATTTGGAAT	AATTTCCCAC
G. stehlini	..C.....	....	T.....C...	..CC.G....	....	...C......
G. atlantica	..C.....A.	....	......C..G	..CC.T..C.	.......T..	...C..A..T
G. galloti(LP)	........G.	....	...T...C..	..CC.G..C.	.......G..	...C..G...
G. galloti(NT)	........A.	....	...T...C..	..CC.G..C.	.......G..	...C..G...
G. galloti(ST)	........A.	....	.......C..	..CC.G..C.	.......G..	...C..G...
G. galloti(GM)	........G.	....	......CC..	...C....T.	....C.....	...C..G...
G. galloti(HI)	........G.	....	......CC..	...C....T.	....C.....	...C..G...

Psammodromus	ATTGTTACCT	ACTACGCAGG	AAAAAAAGAA	CCATTCGGGT	ATATAGGTAT	AGTATGGGCT
G. stehlini	..C..C....	.T........	....	........A.	.C..G..C..	...C..A..C
G. atlantica	.....A....	....T.....	....	..T.....C.	....G..C..	G..T.....C
G. galloti(LP)	.....C....	....T.....	....	..C.....A.	....G..C..	...T..A...
G. galloti(NT)	.....C....	....T.....	....	..C.....A.	....G..C..	...T..A...
G. galloti(ST)	.....C....	....T.....	....	..C.....A.	....G..C..	...T..A...
G. galloti(GM)	.....C....	....T.....	....	..C.....A.	.C..G..C..	...T..A...
G. galloti(HI)	.....C....	....T.....	....	..C.....A.	.C..G..C..	...T..A...

Psammodromus	ATAATATCTA	TTGGATTTCT	AGGGTTTATT	GTATGGGCAC	ACCACATATT	TACCGTGGGT
G. stehlini	........A.	....C..C..	T..C.....C	.....A....	.......G..	...A..C..C
G. atlantica	....	.C.....C..	T..C......	..G..A....	.......G..	...T..A...
G. galloti(LP)	..G.....C.	....G..C..	G..C..C...	....	....	C..A.....G
G. galloti(NT)	..G.....C.	....G..C..	G..C..C...	....	....	C..A.....G
G. galloti(ST)	..G.....C.	.......C..	G..C..C...	....	....	C..A.....G
G. galloti(GM)	........C.	.C.....C..	G..C..C...	.....A....	....	C..A..A..A
G. galloti(HI)	........C.	.C.....C..	G..C..C...	.....A....	....	C..A..A..A

Psammodromus	ATAGATGTTG	ATACACGAGC	CTACTTTACA	TCAGCCACTA	TAATTATTGC	TATCCCGACC
G. stehlini	.....C....	.C..C.....	......C..C	........A.	....C..C..	C.....A..A
G. atlantica	.....C....	.C..T..G..	....	........A.	....C..C..	C.....T...
G. galloti(LP)	........C.	.C..T.....	.........T	..G..T..A.	....C..C..	C..T..A..G
G. galloti(NT)	........C.	.C..T.....	.........T	..G..T..A.	....C..C..	C..T..A..G
G. galloti(ST)	........C.	.C..T.....	.........T	.....T..A.	....C..C..	C..T..A..G
G. galloti(GM)	........C.	.C..C.....	......C..C	.....T..A.	....C..C..	...T..A..A
G. galloti(HI)	........C.	.C..C.....	......C..C	.....T..A.	....C..C..	...T..A..A

Psammodromus	GGAGTAAAAG	TGTTTAGCTG	ATTAGCAACA	CTGCATGGAG	GCACAATCAA	ATGAGACGCC
G. stehlini	..G.......	.A..C.....	.C.C......	..C.......	.......T..	...G......
G. atlantica	..G.......	.T..C.....	.C.T......	..T..C....	.G.....T..	G........T
G. galloti(LP)	.....T....	.C.....T..	.C.T.....G	..T..C....	....	....
G. galloti(NT)	.....T....	.C........	.C.T.....G	..T..C....	....	....
G. galloti(ST)	.....T....	.C........	.C.T.....G	..T..C....	....	....
G. galloti(GM)	..G..T....	.C........	.C.T......	..T..C..G.	.G........	......T...
G. galloti(HI)	..G..T....	.C........	.C.T......	..T..C..G.	.G........	......T...

Psammodromus	GCTATACTAT	GGGCCCTCGG	ATTTATTTTT	CTATTTACGG	TTGGAGGTCT	TACCGGAATC
G. stehlini	..C..G..C.	.A.....T..	C	..C.....A.	.C..C..A..	A.........
G. atlantica	..C.......	.A.....T..	C.........C	..T.....A.	.A..T..A..	...A.....T
G. galloti(LP)	..C.....C.	.A...T.G..	G.........C	..C.......	.C.....G..	C..A..C...
G. galloti(NT)	..C.....C.	.A...T.G..	G.........C	..C.......	.C.....G..	C..A..C...
G. galloti(ST)	..C.....C.	.A...T.G..	G.........C	..C.......	.C.....G..	C..A..C...
G. galloti(GM)	..C.......	.A.....G..	G.........C	..C.....A.	.C..T..A..	C..A..T..T
G. galloti(HI)	..C.......	.A.....G..	G.........C	..C.....A.	.C..T..A..	C..A..T..T

Psammodromus	ATTTTAGCCA	ACTCATCATT	AGACATTGTA	TTACACGATA	CATACTATGT	AGTAGCCCAC
G. stehlini	..CC......	.T.....CC.	C.........	C.T..T..C.	.C........	......T...
G. atlantica	..CC......	.......C..	C	C.T..T..C.	.C..T..C..	...T......
G. galloti(LP)	..CC......	.......CC.	T.........	C.C.....C.	.C.....C..	....
G. galloti(NT)	..CC......	.......CC.	T.........	C.C.....C.	.C.....C..	....
G. galloti(ST)	..CC......	.......CC.	T.........	C.C.....C.	.C.....C..	....
G. galloti(GM)	...C......	.......CC.	C.....C...	C.C.....C.	.C.....C..	....
G. galloti(HI)	...C......	.......CC.	C.....C...	C.C.....C.	.C.....C..	....

Psammodromus	TTTCATTATG	TCCTATCAAT	AGGAGCAGTC	TTTGCCATTA	TGGCA
G. stehlini	..C..C....	..T.......	G..G..T..A	.........C.	....T
G. atlantica	........C.	....	...T..T..A	....	.....
G. galloti(LP)	....	.A........	...C..T..A	.........C.	.A...
G. galloti(NT)	....	.A........	...C..T..A	.........C.	.A...
G. galloti(ST)	....	.A........	...C..T..A	.........C.	.A...
G. galloti(GM)	....	.GT.......	G..C..T..A	....	.A..C
G. galloti(HI)	....	.GT.......	G..C..T..A	....	.A..C