# COLONIZATION AND DIVERSIFICATION OF THE SPECIES *BRACHYDERES RUGATUS* (COLEOPTERA) ON THE CANARY ISLANDS: EVIDENCE FROM MITOCHONDRIAL DNA COII GENE SEQUENCES

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*Abstract.*—The genus *Brachyderes* Schonherr (Coleoptera: Curculionidae) is represented by the species *B. rugatus* Wollaston on the Canary Islands, with one subspecies on each of the islands of Gran Canana, Tenerife, La Palma, and El Hierro. These four subspecies are associated with the endemic pine tree *Pinus canariensis,* and their distnbutions are broadly coincident. Eighty-eight individual Canarian *Brachyderes,* sampled from across the distnbutions of each subspecies, have been sequenced for 570 bp of the mitochondrial DNA (mtDNA) cytochrome oxidase 11 gene (COII). No mitotypes are shared among islands. Both maximum-likelihood and distance-based phylogenetic analyses have shown that: Tenerife is composed of a single monophyletic clade of mitotypes, El Hierro is composed of a single monophyletic clade occurring within a larger clade compnsing all the La Palma mitotypes. and the mitotypes of these three islands form a monophyletic group distinct from Gran Canana. New methods for estimating divergence times without the assumption of rate constancy have been used to reconstruct the direction and approximate timing of colonizations among the islands. Colonization has occurred from older to progressively younger islands, and these colonizations are estimated to have occurred less than 2.6 million years ago, although the timing of the initial colonization of the archipelago is not discernable. New methods for the estimation of diversification rates that use branching times as the analyzed variable have been applied to each island fauna. Hypothesized effects of different levels of recent volcanism among islands were not apparent. All islands exhibit a gradually decreasing rate of genetic diversification that is marked by periodic sudden changes in rate.

*Key words.*—Canary Islands, Coleoptera, colonization, diversification, extinction, mitochondrial DNA.

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Volcanic island archipelagos are increasingly being used as model systems for studying evolution, particularly the Hawaiian and Canary Islands. The attractiveness of these archipelagos stems from: (1) their isolation and the consequent insular nature of speciation; (2) their tremendous range of environmental diversity; and (3) the known geological history of island formation, which provides a chronological template for evolution. Although many studies have sought to reconstruct evolutionary histories above the species level, little attention has been paid to population-level processes. This study addresses issues of colonization and subsequent diversification within islands for recent colonists by looking at genetic diversity within a species of weevil, *Brachyderes rugatus,* inhabiting on the Canary Islands.

The genus *Brachyderes* Schonherr (Coleoptera: Curculionidae) is found predominantly in the Iberian Peninsula and North Africa, with some species extending their ranges further north into France and northern Italy. The genus is also represented on four of the seven Canary Islands (Fig. 1). Each of these islands was accorded its own species by Lindberg and Lindberg (1958; *B. rugatus* on La Palma, *B. calvus* on Gran Canaria, *B. sculpturatus* on Tenerife, and *B. hierroensis* on El Hierro). In his revision of the genus, de Viedma (1966) recognizes 14 distinct species of *Brachyderes,* divided into four subgenera, and considers the four Canary Island taxa to be only geographically separated subspecies of *B. rugatus,* one of nine species comprising the subgenus *Brachyderes.* A subsequent taxonomic study of the Canary Island *Brachyderes* supports de Viedma's subspecific designations (Palm 1976).

The Canary Island *Brachyderes* are primarily associated with the endemic pine tree *Pinus canariensis.* The absence of *Brachyderes* on La Gomera, Lanzarote and Fuerteventura is correlated with the absence of *P. canariensis* in native woods on these islands. The distribution of *Brachyderes* on each island is broadly correlated with the range of *P. canariensis,* but the weevils can sometimes be found on the introduced *Pinus radiata.* Several recent studies have used mitochondrial DNA (mtDNA) sequence data to examine phylogeographic pattern among Coleoptera endemic to the archipelago, both above the species level (Juan et al. 1995, 1996a, 1997; Emerson et al. 1999) and below (Juan et al., 1996b, 1998). Those studies below the species level revealed a phylogeographic pattem concordant with the known geological history. On Tenerife, darkling beetles of the genus *Pirnefia* exhibit two divergent mtDNA lineages (Juan et al. 1996b). These lineages do not correspond to the two described species, but do suggest a recent expansion into central Tenenfe from isolated massifs following a recent eruptive period connecting the two. On Fuerteventura and Lanzarote another darkling beetle, *Hegeter politus,* revealed an mtDNA phylogeography consistent with an ancestral population in the south that colonized in a northerly direction, following the progressive northward cessation of volcanic activity (Juan et al. 1998).

The distribution of *B. rugatus* provides an ideal situation for a comparative study of genetic diversity on different islands. Potentially confounding factors such as phylogenetic and ecological differences are minimized. The principal differences among the island populations will relate to the time of colonization, the availability of suitable pine forest habitat, and the geological history of each island. Gran Canaria has
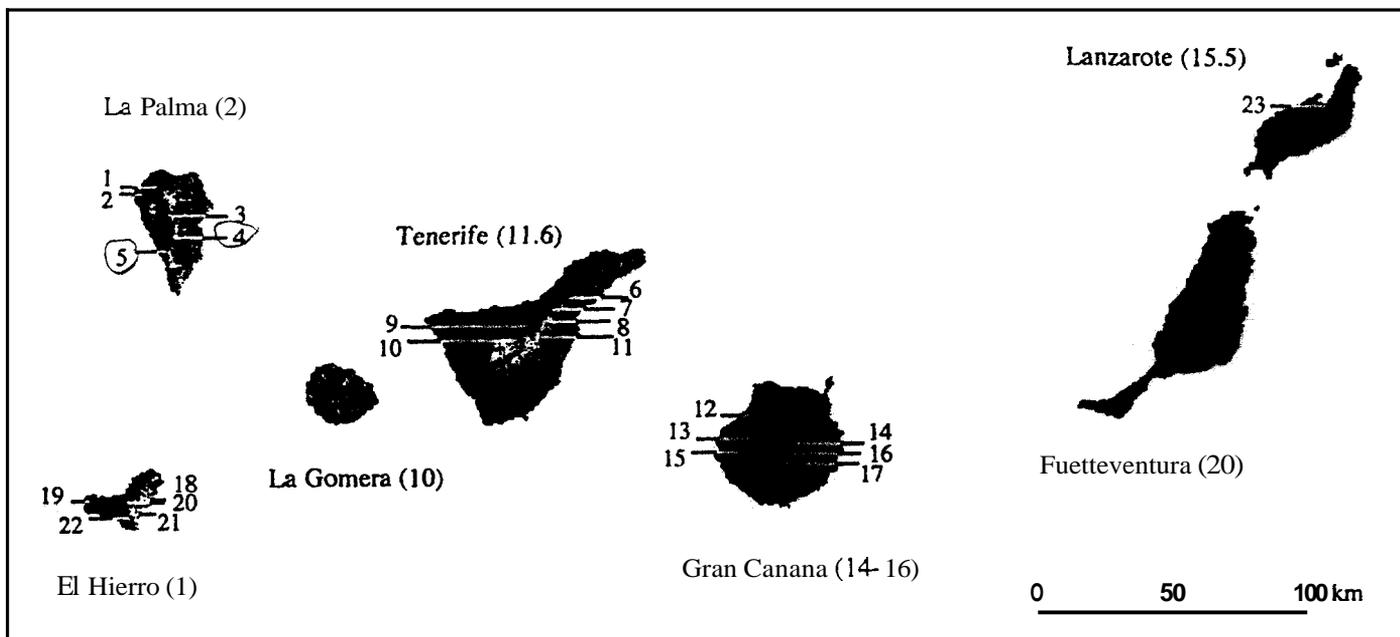
FIG. 1. Sampling locations and number of individuals sequenced (in parentheses) for *Brachyderes* on the Canary Islands (maximum age of islands in millions of years shown in parentheses on the figure): (1) Garafía (4); (2) Puntagorda (4); (3) El Bejenado (4); (4) El Jable (4); (5) Lomo María (4); (6) Las Lagunetas (4); (7) Fuente Joco (4);(8) Ayosa (4); (9) Caldera de Aguamansa (4); (10) Pinar de la Fortaleza (4); (11) Barranco de Badajoz (4);(12) Tamadaba (7); (13) La Calderilla (2); (14) Las Nieves (4); (15) Inagua (4); (16) Juncal (4); (17) Pinar de Tirajana (4); (18) Mirador de las Playas (4); (19) Pinar de la Ermita (1); (20) Cruz de los Reyes (4); (21) Hoya del Morcillo (4); (22) Montaña la Empalizada (2); (23) Haría (4).

been comparatively quiescent in the last few million years compared to Tenerife (Ancochea et al. 1990), La Palma *(An-cochea* et al. 1994), and the recently emerged El Hierro (Guillou et al. 1996), all of which have undergone substantial volcanic activity in the last 1–2 million yr.

Recent efforts in phylogenetics have been directed toward making inferences about diversification rates (Hey 1992; Kirkpatrick and Slatkin 1993; Sanderson and Bharathan 1993; Sanderson and Donoghue 1994; Kubo and Iwasa 1995; Nee et al. 1995; Wollenberg et al. 1996). Tests of the constancy of a population size through its history have been developed using statistics derived from the frequency distribution of pairwise differences between sequences and the number of segregating or polymorphic sites (e.g., Slatkin and Hudson 1991). Nee et al. (1995. 1996) have applied coalescent theory from population genetics to intraspecific gene genealogies to make inferences about population dynamic history. Wollenberg et al. (1996) have used simulation analysis to generate cumulative distribution functions of branching times with constant and equal branching and extinction probabilities. These cumulative distribution functions were then used to statistically test for nonrandomness in temporal cladogenetic pattern. However, the method does not extend beyond this to test other models of diversification. Paradis (1997. 1998a,b) has developed a method that uses survival models to estimate variation in diversification from phylogenies. The method is set within a statistical framework enabling rigorous hypothesis testing of models for constant diversification, gradual change in diversification, or sudden change. It is also possible to test the homogeneity of diversification among different clades against specifically defined

alternative models. The method was primarily developed to examine the phylogenies of recent species, but is equally suited to intraspecific gene genealogies (E. Paradis, pers. comm.) and is the method of choice for our analysis.

It is perhaps premature to raise simple unifying hypotheses for the evolutionary events on several different islands over several million years, but we would predict that diversification rate should be greatest on islands that undergo disturbance such as periodic volcanic activity compared to islands that are volcanically quiescent. Volcanically active islands will undergo more population disjunction, thus creating a favorable environment for divergence of mitotypes. Also, local reductions in population size may increase the chance acquisition of new alleles into the population by random drift (Kimura and Ohta 1969). In this context, we would expect to find greater rates of diversification on the volcanically active islands of El Hierro, La Palma, and Tenerife relative to the comparatively quiescent island of Gran Canaria.

We have sequenced 88 individual Canarian *Brachyderes* for 570 bp of the mtDNA cytochrome oxidase 11 gene (COII), plus two outgroup species also belonging to the subgenus *Brachyderes, B. pubescens* and *B. grisescens,* both. occurring in Iberia and North Africa. On an unrelated field trip to Lanzarote to collect specimens from the genera *Calathus* and *Nesotes,* we discovered *Brachyderes* among a mixed stand of *P. canariensis* and *P. radiata* near the town of Haría. *Pinus canariensis* on Lanzarote is the result of a human introduction from Gran Canaria in the early 1960s, but the origin or possible endemism of the *Brachyderes* is unknown. Specimens are included in the present study for clarification.

## MATERIALS AND METHODS

### Sampling

*Brachyderes* were collected from the Canary Islands during the years 1996, 1997, and 1998. We aimed to sample multiple locations across each island, collecting four individuals at each collection site. However, at some locations this was impossible, particularly on the island of El Hierro, which resulted in smaller sample sizes. Figure 1 shows sampling locations and sample sizes. All samples were stored in pure ethanol prior to extraction of DNA. *Brachyderes pubescens* was collected in Forêt d'Azrou of the Mid-Atlas Mountains of Morocco and *B. grisescens* was collected from Punta Paloma in Tarifa, Spain.

### DNA Extraction and Polymerase Chain Reaction Amplification

For each individual beetle, DNA was extracted from the head and pronotum. The head and pronotum were placed in liquid nitrogen for 20 min, removed, ground, then resuspended in 500 μl of homogenization buffer (20mM Tris, 10mM EDTA, 0.5% SDS), and digested overnight at 40°C with proteinase K at a concentration of $100 \mu g \ ml^{-1}$. The DNA was then purified with Wizard DNA Clean-Up System (Promega. Southhampton, U.K.) minicolumns following the manufacturer's recommendations. The primers used for amplification were TL2-J-3038 (designed in the laboratory of GMH) 5' TAATATGGCAGATTAGTGCATTGGA 3' and C2-N-3668 (alias C2B-605, Simon et al. 1994) 5' GCTCCA-CAAATTTCTGAGCA 3'. Together they amplify an mtDNA fragment of 672 bp. Polymerase chain reactions (PCRs) were carried out in 100-μl volumes including 2.5 μl of each 10 μM primer and in a 2-mM concentration of $MgCl_2$. Two microlitres of a 1:20 dilution of DNA extraction was used for amplification. Each of 40 PCR cycles comprised denaturation at 94°C for 1 min. annealing at 48°C for 1 min, and extension at 72°C for 1 min.

### Sequencing

PCR products were purified with Promega's Wizard PCR Clean-Up System minicolumns following the manufacturer's recommendations. Sequences were generated using both a Pharmacia (Hertfordshire, U.K.) ALF automated sequencer and a Perkin-Elmer (Cheshire, U.K.) ABI automated sequencer. For both systems, sequencing reactions were performed using a Thermo Sequenase cycle sequencing kit from Amersham Life Science (Buckinghamshire, U.K.). Sequences were aligned by eye against the *Pissodes strobi* sequence (Langor and Sperling 1997).

### Phylogenetic Analysis

Both maximum-likelihood (ML) and neighbor-joining (NJ) methods were employed to reconstruct phylogenetic relationships among the *Brachyderes* mitotypes. ML analyses were performed using the DNAML program of PHYLIP version 3.573c. It was possible to perform an ML analysis of all taxa by running the PHYLIP software on a DEC alpha unix machine. An estimate of the transition:transversion ratio
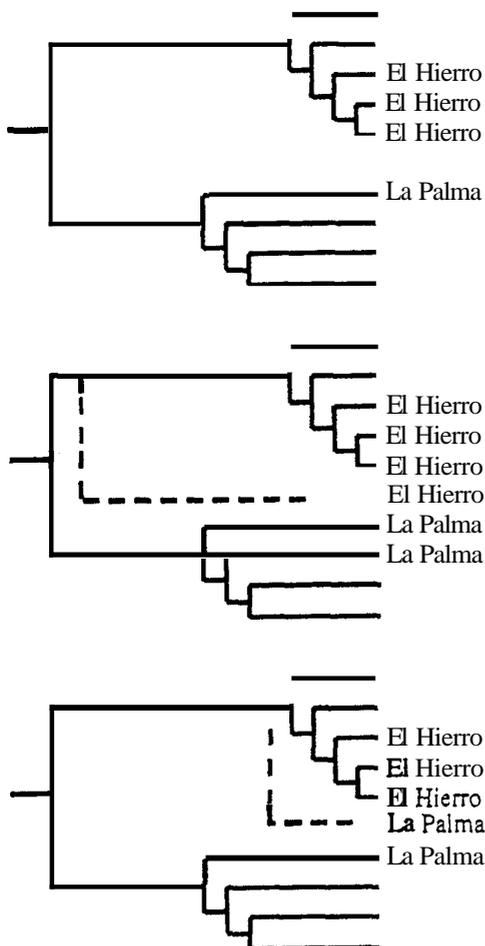
was calculated using PUZZLE 4.0 (Strimmer and von Haesler 1996; http://www.zi.biologie.uni-muenchen.de/~strimmer/puzzle.html); empirical base frequencies were used with one category of substitution rates and the global rearrangements option was not used. Distance analyses were carried out using PAUP* test version 4.0d64, written by Swofford (1998). Genetic distances were corrected for multiple substitutions using Kimura's two-parameter method (Kimura 1980) with a gamma correction, and a tree was generated from a distance matrix using the NJ algorithm of Saitou and Nei (1987).

### Estimation of Divergence Times

The estimation of divergence times among genes has two requirements: (1) for all lineages, the gene in question (in this case mtDNA COII) evolves with a constant clocklike rate of substitution: and (2) this rate is calibrated against time (e.g., the fossil record). Under these two conditions, an ultrametric tree can be constructed and the divergence times for nodes on the tree can be estimated. Studies by DeSalle et al. (1987) and Brower (1994) have shown that mtDNA in arthropods does evolve in a clocklike manner. Both authors calibrated the rate of sequence evolution using age estimates for splitting events among arthropod groups, and their rate estimates are very similar (2% and 2.3% per million yr, respectively).

Several features of intraspecific gene genealogies complicate the direct application of the aforementioned clock calibrations to estimate divergence times. A fundamental difference between inter- and intraspecific phylogenies is the treatment of ancestral types (Crandall and Templeton 1996). Ancestors are generally assumed to be extinct in interspecific systematics. However, within a population, when one copy of a mitotype (existing as numerous copies within the population) mutates to form a new mitotype, the "ancestral" mitotype is still present within the population. An ultrametric topology will be an erroneous summary of divergence times in this situation. A related point is that at the intraspecific level lineage evolution will be less than clocklike because of stochastic variation, and this can manifest itself in significant branch length differences. That is, at a substitution rate of approximately 2.0–2.3% per million yr there will be a "sampling time" error that will increase as divergence times approach zero. Because of this, even if a clock were not rejected across the whole tree, it would still be expected that between closely related lineages substantial branch length differences would exist. This too would generate an incorrect summary of divergence times under the constraint of an ultrametric topology.

A more appropriate method for estimating divergence times would be to obtain estimates directly from a tree constructed without the constraint of having contemporaneous tips. Sanderson (1997) has developed algorithms for estimating divergence times from nonultrametric trees, and here we use his method of nonparametric rate smoothing. This method smoothes local transformations in rate as rates change over the tree. Essentially this method replaces the constraint of a constant rate across a tree with a much weaker constraint on how rates vary, but one that is still sufficient to allow the estimation of divergence times. The program r8s (anonymous

cestral population was first addressed by Nei and Li (1979) and more recently by Takahata and Satta (1997). A component of the divergence between two populations as estimated by DNA sequence data may include genetic divergence that was already present in the common ancestral population. The more general problem of the need to take into account the possibility of extinct lineages when estimating colonization times is illustrated in Figure 2. We have generated estimates of colonization times that incorporate both these sources of error.

### Analysis of Clade Diversification

Several approaches have been developed for making inferences about diversification rates from sequence data (Hey 1992; Kirkpatrick and Slatkin 1993; Sanderson and Bharathan 1993; Sanderson and Donoghue 1994; Kubo and Iwasa 1995; Nee et al. 1995; Wollenberg et al. 1996). The method of Paradis (1997, 1998a,b) analyses divergence times among a group of taxa as estimated from a phylogenetic tree with the statistical models used to analyze survival data (Cox and Oakes 1984). The number of taxa within a phylogeny increases with time, but if the time axis is reversed, it can be seen as a representation of survival data, with one taxon dying at each dichotomous node. The ages of the divergences measured on the phylogeny from present to past are failure times. Survival analysis is centered on individuals for each of which there is a failure event. The failure rate is the probability of failure at a point in time conditioned on survival until this time (Cox and Oakes 1984). Analogously, a node observed on a tree is a divergence event conditioned on future survival (Paradis 1997). Using the ages of the divergences it is possible to estimate diversification rate ($\delta$) with an ML approach outlined in Paradis (1997, 1998a). This method assumes that for each lineage there is an instantaneous diversification rate $\delta(t)$, which has two components, an instantaneous speciation rate $\delta(t)$ and an instantaneous extinction rate $\epsilon(t)$. Thus, at any given point in time, all lineages are exposed to extinction and speciation. It is assumed that $\delta(t)$ and $\epsilon(t)$ cannot be separately estimated, and the method allows for multifurcations in tree topology. The analysis of diversification software package Diversi version 0.1 enables one to test for temporal variations in diversification rate within a clade and also to test for diversification rate variation among clades.

### Analysis of Temporal Variation within a Clade, and Among-Clade Variation

As its input data, Diversi uses diversification times as estimated from a tree. Three models are fitted by Diversi using the ML method. Model A assumes constant diversification through time, model B assumes a diversification rate that is either increasing or decreasing through time, and model C assumes a breakpoint in time with different diversification rates before and after. As its output, Diversi generates both likelihood values and Akaike information criteria (AIC;

ftp from public directos, at loco.ucdavis.edu) also allows for one or more nodes to be assigned ages. This then allows absolute ages for other nodes to be inferred. For all analyses using r8s, final solutions were perturbed using a factor of 0.05 and searches were restarted from 10 different initial times.

Interpreting' a gene tree in terms of species divergence events and subsequently infemng estimates of species divergence times (colonization events) is complicated by two factors: (1) the effect of existing genetic diversity in the ancestral population; and (2) the possibility of lineage extinction. The problem of genetic divergence within the an-

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FIG. 3. Maximum-likelihood tree for mitotypes of Canary Island *Brachyderes* using cytochrome oxidase II sequence data. Approximate ages for numbered nodes are estimated by calibration with the known 1.1 million yr geological age of El Hierro (see text and Table 1).

B. grisescens

Gran Canaria

2

*calvus* (Pinar de Tirajana 2)
*calvus* (Pinar de Tirajana 4)
*calvus* (Pinar de Tirajana 1)
*calvus* (Juncal 3)
*calvus* (Pinar de Tirajana 3)
*calvus* (Juncal 4)
*calvus* (Juncal 2)
*calvus* (Juncal 1)
*calvus* (Inagua 1)
*calvus* (Inagua 2)
*calvus* (Inagua 3)
*calvus* (Inagua 4)
*calvus* (Tamadaba 3)
*calvus* (Tamadaba 1)
*calvus* (Tamadaba 6)
*calvus* (Tamadaba 4)
*calvus* (Tamadaba 7)
*calvus* (Las Nieves 2)
*calvus* Las Nieves 3)
*calvus* [Las Nieves 4)
*calvus* (Las Nieves 1)
*calvus* (La Calderilla 2)
*calvus* (La Calderilla 1)
*calvus* (Tamadaba 5)
*calvus* (Tamadaba 2)

3

Tenerife

4

*sculpturatus* (Las Lagunetas 2)
*sculpturatus* (Bco. de Badajoz 3)
*sculpturatus* (Fuente Joco 3)
*sculpturatus* (Las Lagunetas 4)
*sculpturatus* (Pinar de la Fortaleza 3)
*sculpturatus* (Caldera de Aguamansa 4)
*sculpturatus* (Pinar de la Fortaleza 4)
*sculpturatus* (Bco. de Badajoz 4)
*sculpturatus* (Bco. de Badajoz 1)
*sculpturatus* (Pinar de la Fortaleza 2)
*sculpturatus* (Ayosa 1)
*sculpturatus* (Ayosa 3)
*sculpturatus* (Ayosa 4)
*sculpturatus* (Ayosa 2)
*sculpturatus* (Fuente Joco 4)
*sculpturatus* (Bco. de Badajoz 2)
*sculpturatus* (Las Lagunetas 3)
*sculpturatus* (Las Lagunetas 1)
*sculpturatus* (Fuente Joco 2)
*sculpturatus* (Pinar de la Fortaleza 1)
*sculpturatus* Fuente Joco 1)
*sculpturatus* [Caldera de Aguamansa 1)
*sculpturatus* (Caldera de Aguamansa 3)
*sculpturatus* (Caldera de Aguamansa 2)

La Palma

5

6

7

rugarus (Puntagorda 4)
*hierroensis* (hoya del Morcillo 4)
*hierroensis* (Hoya del Morcillo 2)
*hierroensis* (Mirador de las Playas 3)
*hierroensis* (Mirador de las Playas 1)
*hierroensis* (Hoya del Morcillo 1)
*hierroensis* (Mont. la Empalizada 2)
*hierroensis* (Cruz de los Reyes 3)
*hierroensis* (Mont. la Empalizada 1)
*hierroensis* (Cruz de los Reyes 1)
*hierroensis* (Mirador de las Playas 2)
*hierroensis* (Hoya del Morcillo 3)
*hierroensis* (Pinar de la Ermita)
*hierroensis* (Mirador de las Playas 4)
*hierroensis* (Cruz de los Reyes 4)
*hierroensis* (Cruz de los Reyes 2)

El Hierro

*rugatus* (Lomo María 1)
*rugatus* (Lomo María 3)
*rugatus* (Lomo María 4)
*rugatus* (Lomo María 2)
*rugatus* (Bejenado 1)
*rugatus* (El Jable 4)
*rugatus* (El Jable 3)
*rugatus* (Bejenado 3)
*rugatus* (Bejenado 2)
*rugatus* (Bejenado 4)
*rugatus* (El Jable 2)
*rugatus* (El Jable 1)

*rugatus* (Garafía 4)
*rugatus* (Garafía 3)
*rugatus* (Garafía 1)
*rugatus* (Puntagorda 3)
*rugarus* (Puntagorda 2)
*rugarus* (Puntagorda 1)
*rugarus* (Garafía 2)

Akaike 1973) for the fit of the data to each of the three models. Two different approaches are then available to test which model better explains the data. Likelihood-ratio tests (LRT) can be computed to compare two nested models, in this case model A is nested within both models B and C. When there is no nesting relationship, as is the case between models B and C, the AIC can be used to reject one model in favor of another because the AIC requires no relationship of nestedness. The model with the lowest AIC (AIC = −2 $\log L + 2np$, where $np$ is the number of identifiable parameters of the model) is selected as the one that best describes the data. The β parameter of model B is indicative of either increasing or decreasing diversification rates. If β > 1 (<1) then this signifies that diversification is decreasing (increasing) through time.

Specifically defined models of diversification can be constructed for this analysis of among-clade variation. With several clades under analysis, models can range from the null model where all clades have the same 6 to a model where all clades have a different 6. The models are fitted to the data using ML and, as in the temporal variation analysis, models are evaluated using the LRT and AIC. All LRTs were performed at the 5% probability level.

## RESULTS

Within the ingroup 15% of nucleotide sites were variable. Across all sites AT richness was 70% and, as is typical in mtDNA, third codon positions exhibited very low G composition. The maximum ingroup genetic divergence observed was 7.7% (gamma corrected Kimura two-parameter). Within islands maximum genetic divergences were 1.9% for El Hierro, 3.3% for La Palma, 2.1% for Tenerife, and 7.4% for Gran Canaria. Divergences of ingroup taxa from the closest outgroup, *B. grisescens*, ranged from 14.9% to 18.7%. An ML tree is presented in Figure 3. An NJ tree constructed from gamma corrected Kimura 2-parameter distances is presented in Figure 4, and bootstrap values are given for nodes with more than 70% support. Both trees show essentially the same features: (1) there are no mitotypes shared between islands; (2) Tenerife is composed of a single monophyletic clade of mitotypes; (3) El Hierro is composed of a single monophyletic clade; (4) the El Hierro clade occurs within a larger clade comprising all the La Palma mitotypes; (5) within La Palma there are three clear geographic groupings: El Jable and Bejenado, Lomo Maria, and Garafia and Puntagorda; (6) the earliest divergence appears to have occurred on Gran Canaria; and (7) Tenerife, La Palma, and El Hierro are monophyletic with regard to Gran Canaria.

The four individuals from Lanzarote were identified as having come from Gran Canaria. They have mitotypes identical to the four individuals of *B. r. calvus* from Las Nieves and two from La Calderilla. They are not included on Figures 3 and 4 and are excluded from subsequent analyses because

it is clear that their presence on Lanzarote is the result of a very recent introduction, most certainly anthropogenic.

### Divergence Times

Although both ML and NJ trees are very similar, there are differences between the two, particularly with regard to branch lengths within island lineages. Yang (1994) has shown that when relatively short sequences are used, as is the case here, the ML method has a higher probability of recovering the true tree. This is further supported by Swofford et al. (1996), who view distance methods as less desirable approximations to a full ML approach. Thus, for the estimation of divergence times we have used topologies generated from the ML analysis.

An LRT for a molecular clock was performed using PUZZLE 4.0 (Strimmer and von Haesler 1996) and the presence of a treewide clock was rejected at the 1% significance level. A departure from clocklike behavior, particularly at lower divergence levels, is demonstrated with the branch-length test of Takezaki et al. (1995). This test examines the deviation of the branch length between the tree root and a tip from the average length. A test of branch length differences across the entire gene tree detected only one significant departure from the average branch, that for a *B. rugatus* mitotype from La Palma. However, the same analysis for individual island gene trees generated greater deviation from average branch length, particularly for La Palma, where all but three mitotypes were different from the average branch length at the 5% level of significance. This confirms our earlier supposition that deviation from a molecular clock is to be expected at lower levels of divergence.

An analysis of the phylogenetic pattern for all four subspecies was performed using the calibrate option of r8s (Sanderson 1997). With this option a tree node can be assigned an age and the ages of other nodes in the tree are calibrated from this. The island of El Hierro is very young (1.1 million yr; Guillou et al. 1996), with a level of mitochondrial divergence (1.9%) suggestive of colonitation almost immediately after its emergence based on the rate estimates of DeSalle et al. (1987) and Brower (1994). Nodes 6 and 7 (Fig. 3) represent the earliest and latest possible times for the colonization of El Hierro when allowing for the effect of existing genetic diversity in the ancestral population and the possibility of lineage extinction between these nodes (Fig. 2). Both these issues need to be accounted for when using a gene genealogy to infer population divergence. Thus, two analyses were performed assigning an age of 1.1 million yr to nodes 6 and 7 (Table 1). La Palma is also a relatively young island (2.0 million yr; Ancochea et al. 1994), with a level of divergence (3.3%) indicative of colonization soon after emergente, but perhaps not as immediate as for El Hierro. However, for La Palma the interval between nodes 1 and 5 (Fig. 3) represents the interval of uncertainty (Fig. 2) for the time

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FIG. 4. Neighbor-joining tree for mitotypes of Canary Island *Brachyderes* using gamma corrected distances for the Kimura two-parameter model calculated using cytochrome oxidase II sequence data. Bootstrap values are indicated for nodes gaining more than 70% support (1000 replications).
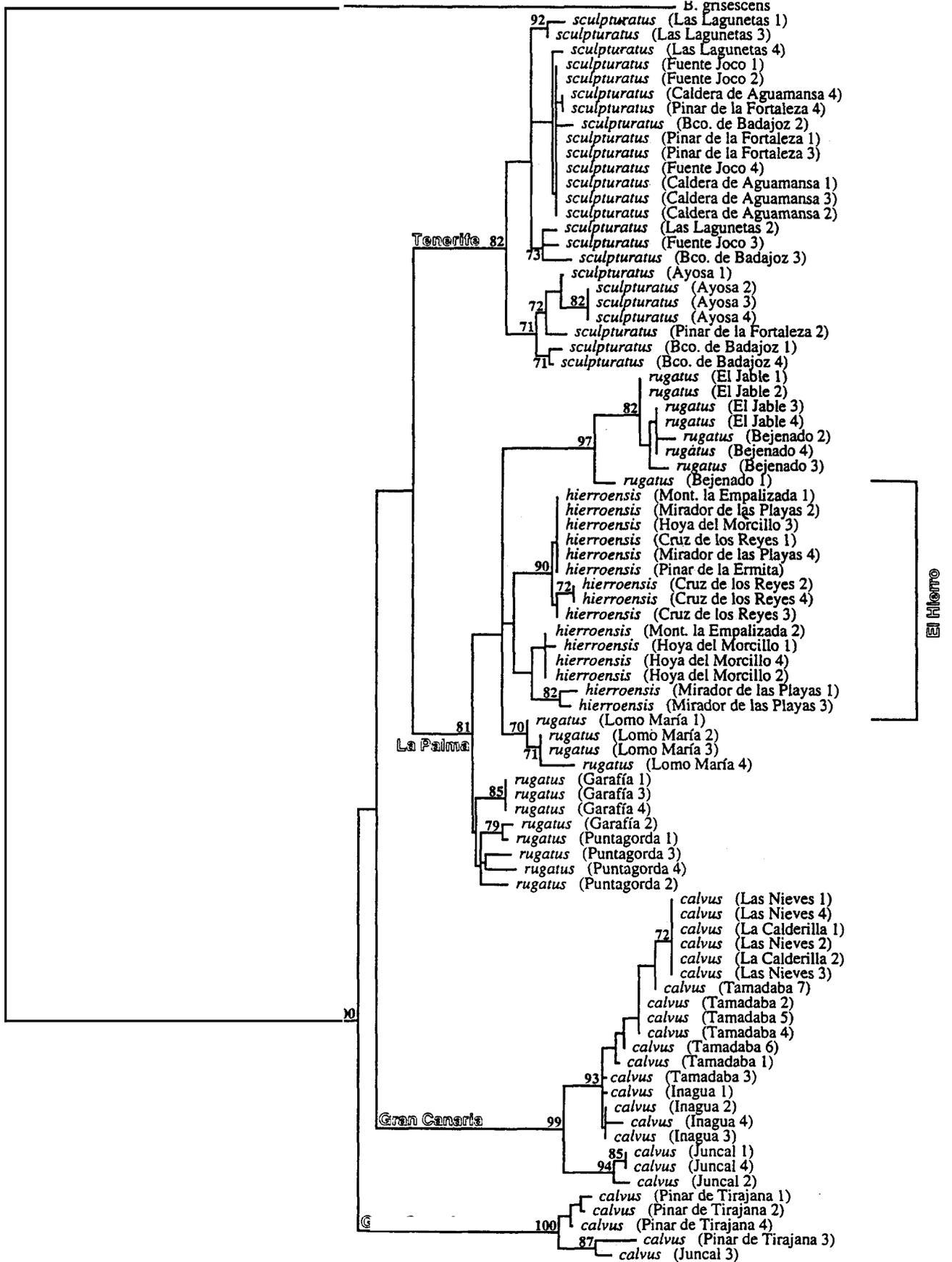
B. grisescens
92 — *sculpturatus* (Las Lagunetas 1)
*sculpturatus* (Las Lagunetas 3)
*sculpturatus* (Las Lagunetas 4)
*sculpturatus* (Fuente Joco 1)
*sculpturatus* (Fuente Joco 2)
*sculpturatus* (Caldera de Aguamansa 4)
*sculpturatus* (Pinar de la Fortaleza 4)
*sculpturatus* (Bco. de Badajoz 2)
*sculpturatus* (Pinar de la Fortaleza 1)
*sculpturatus* (Pinar de la Fortaleza 3)
*sculpturatus* (Fuente Joco 4)
*sculpturatus* (Caldera de Aguamansa 1)
*sculpturatus* (Caldera de Aguamansa 3)
*sculpturatus* (Caldera de Aguamansa 2)
*sculpturatus* (Las Lagunetas 2)
*sculpturatus* (Fuente Joco 3)
Tenerife 82 — 73 *sculpturatus* (Bco. de Badajoz 3)
*sculpturatus* (Ayosa 1)
*sculpturatus* (Ayosa 2)
72 — 82 *sculpturatus* (Ayosa 3)
*sculpturatus* (Ayosa 4)
71 *sculpturatus* (Pinar de la Fortaleza 2)
*sculpturatus* (Bco. de Badajoz 1)
71 *sculpturatus* (Bco. de Badajoz 4)
*rugatus* (El Jable 1)
*rugatus* (El Jable 2)
82 *rugatus* (El Jable 3)
*rugatus* (El Jable 4)
*rugatus* (Bejenado 2)
97 *rugatus* (Bejenado 4)
*rugatus* (Bejenado 3)
*rugatus* (Bejenado 1)
*hierroensis* (Mont. la Empalizada 1)
*hierroensis* (Mirador de las Playas 2)
*hierroensis* (Hoya del Morcillo 3)
*hierroensis* (Cruz de los Reyes 1)
*hierroensis* (Mirador de las Playas 4)
*hierroensis* (Pinar de la Ermita)
90 72 *hierroensis* (Cruz de los Reyes 2)
*hierroensis* (Cruz de los Reyes 4)
*hierroensis* (Cruz de los Reyes 3)
*hierroensis* (Mont. la Empalizada 2)
*hierroensis* (Hoya del Morcillo 1)
*hierroensis* (Hoya del Morcillo 4)
*hierroensis* (Hoya del Morcillo 2)
82 *hierroensis* (Mirador de las Playas 1)
*hierroensis* (Mirador de las Playas 3)
El Hierro
81 70 *rugatus* (Lomo María 1)
*rugatus* (Lomo María 2)
71 *rugatus* (Lomo María 3)
La Palma *rugatus* (Lomo María 4)
*rugatus* (Garafía 1)
85 *rugatus* (Garafía 3)
*rugatus* (Garafía 4)
79 *rugatus* (Garafía 2)
*rugatus* (Puntagorda 1)
*rugatus* (Puntagorda 3)
*rugatus* (Puntagorda 4)
*rugatus* (Puntagorda 2)
*calvus* (Las Nieves 1)
*calvus* (Las Nieves 4)
72 *calvus* (La Calderilla 1)
*calvus* (Las Nieves 2)
*calvus* (La Calderilla 2)
*calvus* (Las Nieves 3)
*calvus* (Tamadaba 7)
*calvus* (Tamadaba 2)
*calvus* (Tamadaba 5)
*calvus* (Tamadaba 4)
*calvus* (Tamadaba 6)
*calvus* (Tamadaba 1)
93 *calvus* (Tamadaba 3)
*calvus* (Inagua 1)
*calvus* (Inagua 2)
*calvus* (Inagua 4)
Gran Canaria 99 *calvus* (Inagua 3)
85 *calvus* (Juncal 1)
94 *calvus* (Juncal 4)
*calvus* (Juncal 2)
*calvus* (Pinar de Tirajana 1)
*calvus* (Pinar de Tirajana 2)
G 100 *calvus* (Pinar de Tirajana 4)
87 *calvus* (Pinar de Tirajana 3)
*calvus* (Juncal 3)

**TABLE 1.** Age estimates for nodes on Figure 2 using the 1.1 million yr age of El Hierro to calibrate nodes **6** and **7**.

| Calibration point (1.1 million yr) | Estimated ages (million yr) | | | | | | |
|---|---|---|---|---|---|---|---|
| | node 1 | node 2 | node 3 | node 4 | node 5 | node 6 | node 7 |
| node 6 | 2.12 | 1.86 | 1.89 | 1.34 | 1.58 | — | 0.93 |
| node 7 | 2.56 | 2.75 | 2.27 | 1.61 | 1.89 | 1.3 | — |

**TABLE 2.** Log-likelihood and Akaike information criteria (AIC) values for models **A** and **B** for each of the **four** island groups. For each island, model B is better supported than rnodel **A** as assessed through log-likelihood ratio tests and comparison of AIC values (see text for details).

| Island | Log likelihood (AIC) values | | | |
|---|---|---|---|---|
| | model A | | model B | |
| Gran Canaria | 1.814 | (−1.628) | 4.526 | (−5.052) |
| Tenerife | −6.886 | (15.773) | −1.775 | (7.550) |
| La Palma | −3.578 | (9.155) | −1.145 | (6.289) |
| El Hierro | −2.733 | (7.466) | −0.253 | (4.507) |

of colonization, as it is possible that La Palma was colonized before Tenerife. Thus, calibrations were not done for La Palma because the interval between calibration points was too great.

### Analysis of Diversification

MtDNA clades for each island were analyzed separately by calibrating the roots to an arbitrary value of one to generate relative divergence times on a scale of zero to one to carry out analyses of temporal variation within a clade and among-clade variation. For all four islands model B is always better supported than model A (log likelihood tests and AIC values; Table 2), indicating gradual change rather than constant diversification. Beta values are more than one suggesting that diversification rates are decreasing (Tenerife, $\beta$ = 2.45; La Palma, $\beta$ = 1.73; El Hierro, $\beta$ = 2.38; Gran Canaria, $\beta$ = 1.64). For each island fauna a series of analyses of temporal variation were run with sequential breakpoints (Tc) for model C (assumes a Tc with different diversification rates before and after) along the relative time scale from zero to one. The results of these analyses are given in Figure **5** along with the **AIC** values for the fit of the data for each island to model B. The model with the lower AIC is the one that best describes the data. For much of the time axis, diversification on all islands is best described by a gradual decrease in rate (model **B),** but there are breakpoints for both Gran Canaria and Tenerife when model C is favored, thus indicating sudden changes in rate.

In an analysis of among-clade variation in diversification rate we tested homogeneity in diversification rate among islands by comparing a model with different diversification rates for each island (model 1, Table 3) and a model with a similar diversification rate among islands (model 2, Table **3).** RTs could not reject the hypothesis of a hornogenous diversification rate among islands.

### DISCUSSION

The colonization pattern of Brachyderes can be clearly deduced from the mtDNA phylogeny within the context of the known geological ages of the Canary Islands and divergence times approximated using the combination of nodes **6** and **7** as calibration points (Fig. 6). It is possible that these values are overestimates for two reasons. First, colonization of El Hierro may postdate the emergence of the island. However, the maximum genetic divergence of 1.9% argues against this (DeSalle et al. 1987; Brower 1994). A second factor leading to overestimation is that a component of the divergence between two populations as estimated by DNA sequence data

may include genetic divergence that was already present in the common ancestral population. For our colonization time estimates we have effectively included this error by estimating a time interval for colonization that extends from the node of divergence between two populations (e.g., node **3** of Fig. 3 between Tenerife and La Palma) and the node of the first divergence within the recently colonized population (e.g., node **5** of Fig. 3).

The actual age of colonization of the Canary Islands by Brachyderes is unknown and lies somewhere between node 1 and the root (Fig. 3). The deep divergence on Gran Canaria of 1.9–2.3 million yr (Table 1) suggests this to be the oldest island assemblage and the first island colonized. The divergente between Gran Canaria and the clade including Tenerife and La Palma is estimated to have occurred between nodes 1 and **3,** which equates to an approximate age for this event 1.9–2.6 million yr ago (Table 1). The fact that La Palma has a rnaximum age of only 2 million yr, coupled with the geographic proximity of Tenerife to Gran Canaria, suggests that Tenerife was the next island colonized from Gran Canaria. Colonization then occurred from Tenerife to La Palma and this was estimated to have occurred sometime between nodes 3 (**2.3** millian yr ago) and **5** (1.6 million yr ago). Given the 2 million yr age for La Palma, we can narrow this estimate to between 2 million yr ago and 1.6 million yr ago, thus suggesting colonization occurred relatively soon after island emergence. Finally, and most recently, El Hierro was colonized from La Palma. This combination of approximated divergence times and known geological ages of islands provides a satisfactory result without the restnctive assumption of a constant clocklike rate of nucleotide substitution.

### Analysis of Diversification

For all islands the pattern of mitotype diversification is better described by a decreasing rate of diversification. In all four cases, rnodel B is significantly better at explaining the pattern of mtDNA lineages than model A, and $\beta$-values are greater than one. However, at specific points in time for Gran Canaria and Tenerife, there is a better fit to model C (Fig. **5).** There is no apparent difference between the volcanically inactive Gran Canaria and the other three volcanically active islands with regard to diversification rate. This is verified by the test of among clade rate variation that could not reject the null hypothesis of equivalent diversification rates across islands in favor of an alternative hypothesis that diversification rates vary across the four islands (Table 3).

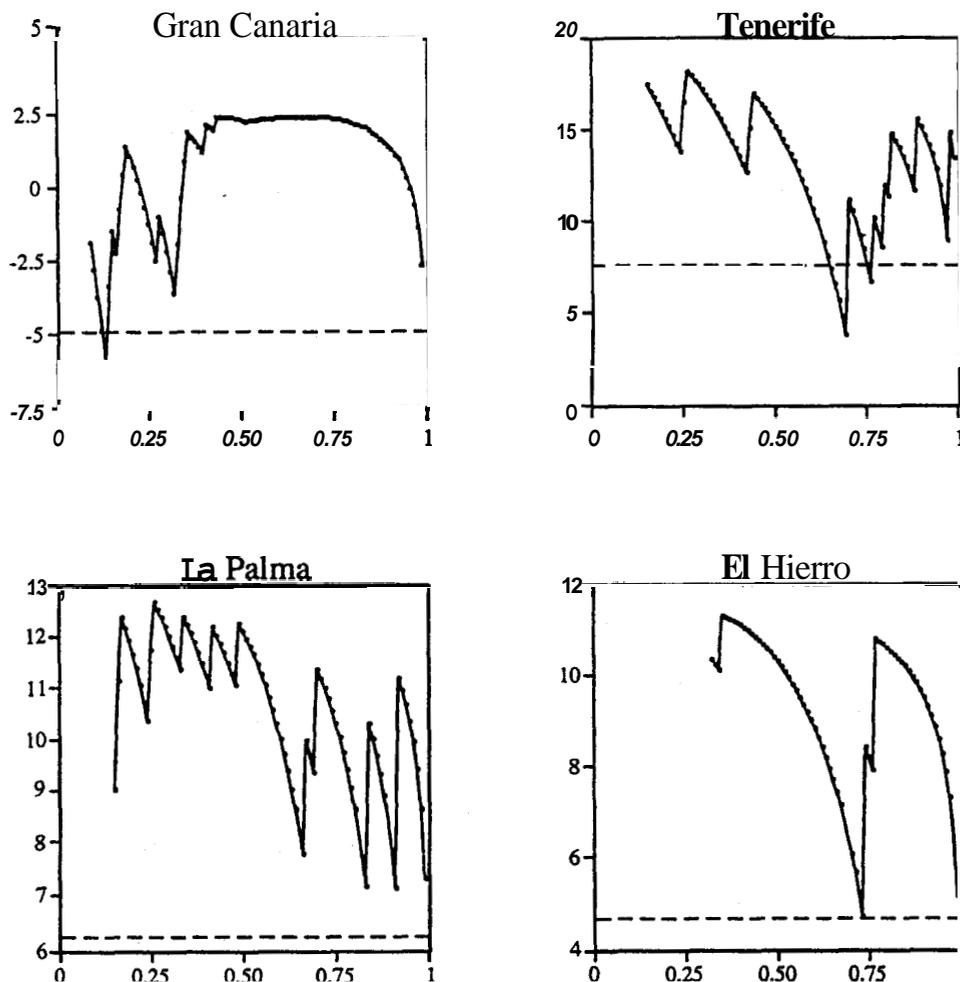An immediate observation from Figure 3 is that apart from

FIG. 5. Distribution of Akaike information criteria (AIC) values for models B (gradually increasing or decreasing diversification rate; broken line) and C (a breakpoint in time with different diversification rates before and after; solid line). The x-axis represents breakpoints for model C, and the y-axis represents AIC values. Model C was evaluated against model B for 100 breakpoints. The model with the lowest AIC is selected as the one that best describes the data.

El Hierro, island clades are characterized by a lack of older lineages, that is, diversity is recent relative to colonization. One possible mechanism for this is the action of population bottlenecks and/or reductions in effective population size in the past. Whereas localized population disjunctions and bottlenecks may favor the acquisition of new alleles (Kimura and Ohta 1969), particularly in unstructured populations, larger extinction events will succeed in removing much of the existing diversity, particularly when the diversity is geographically structured. The genetic effect of a catastrophic

TABLE 3. Log-likelihood and Akaike information critena (AIC) values for models 1 and 2 testing for a different diversification rate for each island. A log-likelihood ratio test and comparison of AIC values (see text for details) could not reject the model of all islands having the same diversification rate.

| Model | Log-likelihood (AIC) values |
|---|---|
| 1 (different rate for each island) | −11.382 *(30.765)* |
| 2 (all islands have the same rate) | −12.981 *(27.962)* |

extinction event can be easily demonstrated from our data. Consider what the result would have been if we were to have sampled on La Palma and there had previously been an extinction event resulting in the survival of only the two northwestern sampling locations, the two central sampling locations, or the southwestern sampling location (Fig. 7).

We propose that island populations of *Brachyderes* are periodically subject to reductions in mtDNA diversity. Initially it would have been due to the colonization of an island by one or a few founders, but reductions subsequent to this have also occurred. Our results suggest that the rate of mtDNA diversification following a reduction slows down with time and that this rate is not constant, but is sometimes marked by periodic sudden changes. We can be certain that volcanic activity will be a significant mechanism, but this does not explain the similarity of pattern between Gran Canaria and the other islands. It is without question that volcanic activity on El Hierro, La Palma, and Tenerife has been extensive in the last 1–2 million yr (Ancochea et al. 1990, 1994; Guillou et al. 1996) with most, if not all island building occurring within this period. The recent volcanic activity on
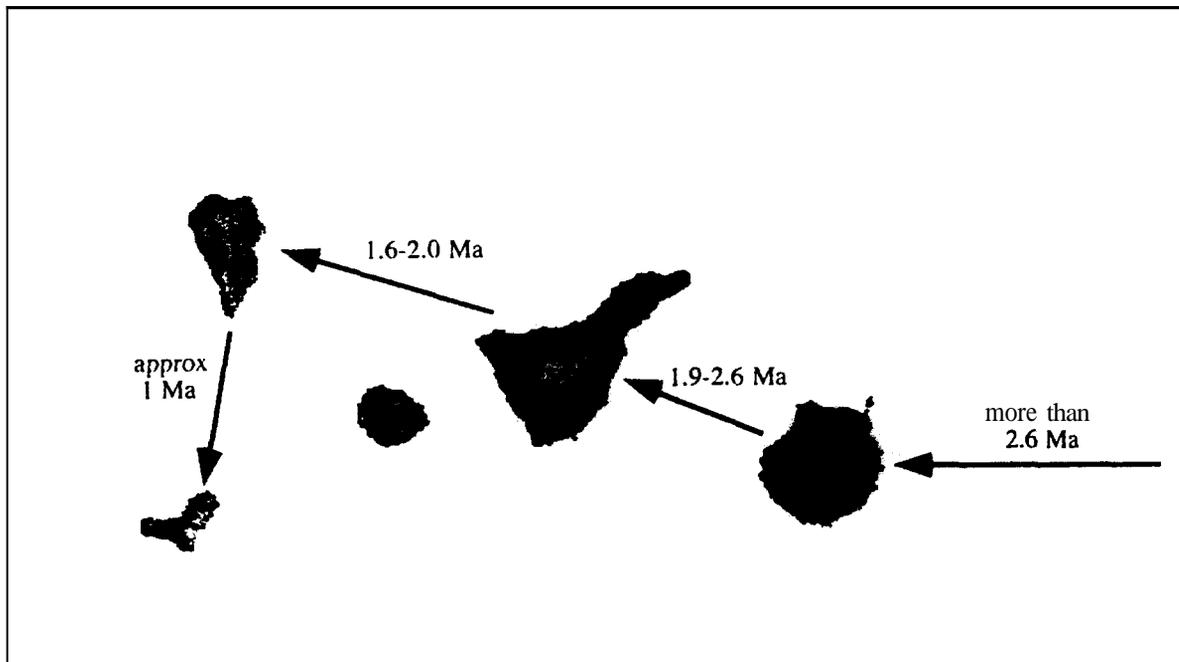
FIG. 6. **Proposed colonization** sequence **and approximate timing of colonization** events for Brachyderes on the **Canary Islands.**

Gran Canaria (last 3 million yr) has been substantially less (for a comparison with Tenerife, see Ancochea et al. 1990), emanating from vents scattered across the whole island (Hoernle et al. 1991), with the eruptive rate increasing from **1.8** million yr ago to the present (Hoernle and Schmincke 1993). We propose that this level of volcanic activity, combined with the dramatic erosional events evidenced on the island, has been high enough to periodically diminish mtDNA diversity generating a similar overall pattern of diversification to the other islands.

### Intraisland Phylogeography

A nonrandom distribution of mitotypes with regard to island geography is clearly demonstrated for *B. r. calvus* on Gran Canaria (Fig. 8). Although *B. r. calvus* appears to have been present on Gran Canaria for at least 2.6 million yr (Fig. 6), the geographic pattern and level of genetic divergence indicates a recent reduction in distribution followed by an expansion. We interpret the deep lineage divergence as the persistence of an earlier diversification, and the presence of both lineages at Juncal is consistent with this being the source area for a recent expansion into the rest of the island. The phylogeographic pattern suggests a recent range expansion, originating from the area of Juncal and Pinar Tirajana, that circumscribed the island clockwise through Inagua, Tamadaba, Las Nieves, and La Calderilla. It is possible that the pattern observed for *B. r. calvus* is part of a more general pattern for the *P. canariensis* ecosystem, but until phylogeographic data is obtained for other groups restricted to the pine forest, the generality of this pattern remains uncertain.

La Palma also exhibits geographic structuring of mitotype distribution. The progressive volcanic evolution of La Palma is well understood (Ancochea et al. 1994). There has been a general north-south migration of volcanic activity with time,

and one might expect this to be reflected in the mtDNA, as has been observed for *Hegeterpotitus* on the islands of Fuerteventura and Lanzarote (Juan et al. 1998). *Hegeter politus* appears to have colonized in a northerly direction, following the progressive northward cessation of volcanic activity on these islands. The phylogeographic pattern of *B. r. rugatus* does not correspond with the pattern of volcanism on La Palma, but there are three distinct geographic groupings of mitotypes, as can be seen in Figure 3. Although no clear barriers to dispersal between these three groupings are apparent, the groupings themselves may be indicative of historical habitat disjunctions. Both Tenerife and El Hierro show no clear correspondence between geography and mitotype distribution.

The contrasting pattern of mitotype distribution on the four islands is perhaps not surprising given the complex nature and independent history of each. The pattern for each island can be viewed as a snapshot of the continuing process of population dynamics, contingent upon the biotic and abiotic factors that have been predominant on each island. The geographic structuring of mitotype diversity found on the islands of Gran Canaria and La Palma indicate that gene flow among populations within each island is limited, whereas on Tenerife and El Hierro the lack of structure suggests the opposite. The generality of these patterns within each island requires the phylogeographic study of other species specific to the pine forest. This would be particularly interesting for Gran Canaria, where it appears that recent range expansion has occurred from a localized part of the island.

### Conclusion

This study demonstrates the utility of applying recent advances in phylogenetics to further unravel the history of island colonizations below the species level. The phylogenetic
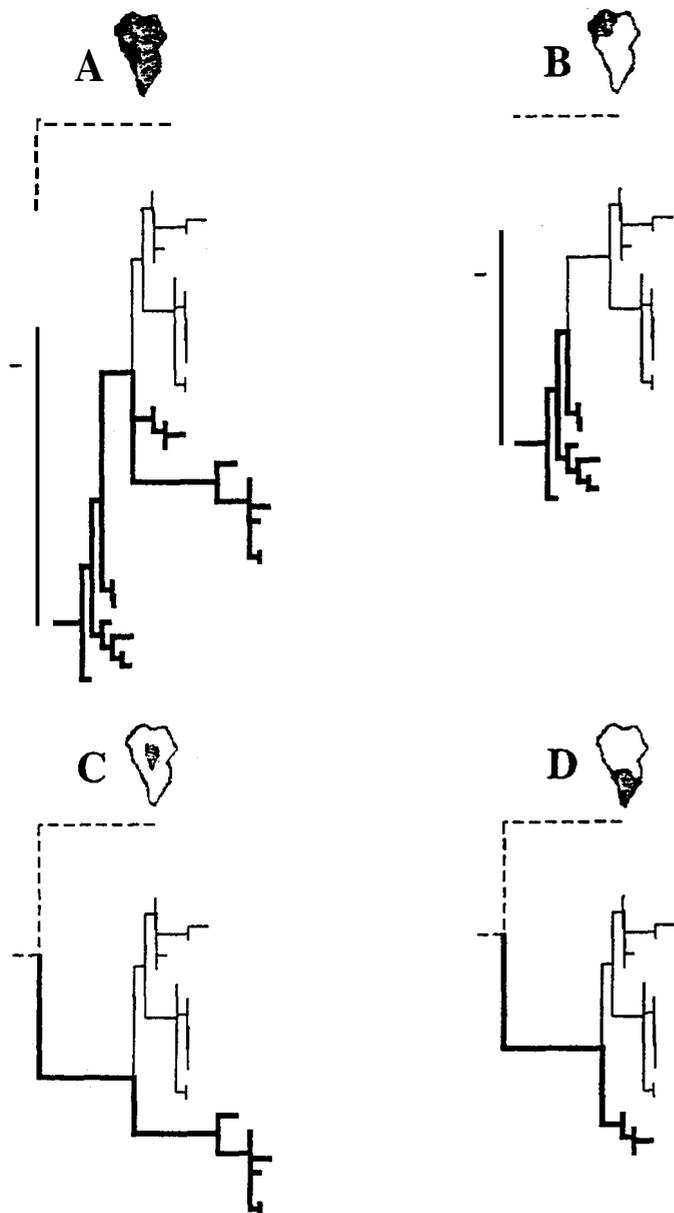
FIG. 7. Lineage extinction and phylogenetic pattem. Hypothesizing regional extinction based on empirical data for La Palma mitotypes (bold lines) demonstrates the potential narrowing effect on genetic diversity. El Hierro mitotypes are shown as solid lines and the Tenerife clade is represented by the broken line. **(A)** Phylogeny of extant lineages; (B) survival of only northwestern Puntagorda and Garafía populations; (C) survival of central Bejenado and El Jable populations; (D) survival of the southwestern Lomo Maria population.

FIG. 8. Phylogeography of Brachyderes *rugatus calvus* mitotypes on Gran Canaria. Branch lengths are not proportional to maximum-likelihood branch lengths (Fig. 2).

relationships among Canary Island *Brachyderes* beetles suggest recent colonizations among islands within the archipelago. The rate of mitotype diversification on all islands appears to gradually decrease with time, but there are intervals marked by sudden rate change. We conclude that lineage extinction has been an important factor in shaping phylogenetic pattern and that for the more distantly colonized islands of Gran Canaria, Tenerife, and to some extent La Palma, signatures of past diversifications have been progressively

lost. A mechanism for this loss, by localized extinction in geographically structured populations, can be described using empirical data. The uniformity of the pattem of diversification across the four islands is interesting and provides a platform for testing the generality of this trend among other species. We are currently obtaining similar sequence data for a species of colydiid beetle, *Tarphius canariensis,* which inhabits the monteverde (laurel forest and fayal-brezal, the vegetation growing around the laurel forest proper) on the islands of Gran Canaria, Tenerife, and La Palma. We hope this study stimulates further investigation of island colonization below the species level, an area that has received little attention to date.

LITERATURE CITED

Akaike, H. 1973. Information theory and an extension of the maximum likelihood principle. Pp. 267–281 in B. N. Petrov and F. Csaki, eds. Proceedings of the second international symposium on information theory. Akadémia Kiado, Budapest.

Ancochea, E., J. M. Fuster. E. Ibarrola, A. Cendrero, J. Coello, F. Hernán, J. M. Cantagrel, and C. Jamond. 1990. Volcanic evolution of the island of Tenerife (Canary Islands) in the light of new K-Ar data. J. Volcanol. Geotherm. Res. 4: 231–249.

Ancochea, E., F. Hernán, A. Cendrero. J. M. Cantagrel. J. M. Fuster, E. Ibarrola, and J. Coello. 1994. Constructive and destructive episodes in the building of a young oceanic island, La Palma, Canary Island, and genesis of the Caldera de Taburiente. J. Volcanol. Geotherm. Res. 60:243–262.

Brower, A. V. Z. 1994. Rapid morphological radiation and convergence among races of the butterfly Heliconius erato inferred from patterns of mitochondrial DNA evolution. Proc. Nati. Acad. Sci. USA 91:6491–6495.

Crandall, K. A.. and A. R. Tempelton. 1996. Applications of intraspecific phylogenetics. Pp. 187–202 in P. Harvey, A. Leigh Brown, J. Maynard Smith, S. Nee, eds. New uses for new phylogenies. Oxford Univ. Press, Oxford, U.K.

Cox, D. R., and D. Oakes. 1984. Analysis if survival data. Chapman and Hall, London.

DeSalle, R., T. Freedman, E. M. Prager. and A. C. Wilson. 1987. Tempo and model of sequence evolution in mitochondrial DNA of Hawaiian Drosophila. J. Mol. Evol. 26:157–164.

de Viedma, M. G. 1966. Revisión del género Brachyderes Schönherr, con especial referencia al estudio de su edeago y a su distribución geográfica dentro de la Península Ibérica. Eos XLII: 575–596.

Emerson, B. C., P. Oromí, and G. M. Hewitt. 1999. MtDNA phylogeography and recent intra-island diversification of Canary Island Calathus beetles (Carabidae). Mol. Phylogenet. Evol. 13: 149–158.

Guillou, H., J. C. Carracedo, F. Pérez Torrado, and E. Rodríguez Badiola. 1996. K-Ar ages and magnetic stratigraphy of a hotspot-induced, fast grown oceanic island: El Hierro, Canary Islands. J. Volcanol. Geotherm. Res. 73:141–155.

Hey, J. 1992. Using phylogenetic trees to study speciation and extinction. Evolution 46:627–640.

Hoernle, K., and H.-U. Schmincke. 1993. The role of partial melting in the 15-Ma geochemical evolution of Gran Canaria: a blob model for the Canary hotspot. J. Petrol. 34:599–626.

Hoernle, K., G. Tiiton, and H.-U. Schmincke. 1991. Sr-Nd-Pb isotopic evolution of Gran Canaria: evidence for shallow enriched mantle beneath the Canary Islands. Earth Planet. Sci. Lett. 106: 44-63.

Juan, C., P. Oromí, and G. M. Hewitt. 1995. Mitochondrial DNA phylogeny and sequential colonization of Canary Islands by darkling beetles of the genus Pimelia (Tenebrionidae). Proc. R. Soc. Lond. B Biol. Sci. 261:173–180.

———. 1996a. Phylogeny of the genus Hegeter (Tenebrionidae, Coleoptera) and its colonization of the Canary Islands deduced from cytochrome oxidase 1 mitochondrial DNA sequences. Heredity 76:392–403.

Juan, C., K. M. Ibrahim, P. Oromí, and G. M. Hewitt. 1996b. Mitochondrial DNA sequence variation and phylogeography of Pimelia darkling beetles on the island of Tenerife (Canary Islands). Heredity 77:589–598.

Juan, C., P. Oromí, and G. M. Hewitt. 1997. Molecular phylogeny of darkling beetles from the Canary Islands: comparisons of inter island colonization patterns in two genera. Biochem. Syst. Ecol. 25:121–130.

Juan, C., K. M. Ibrahim, P. Oromí, and G. M. Hewitt. 1998. The phylogeography of the darkling beetle, Hegeter politus, in the eastern Canary Islands. Proc. R. Soc. Lond. B Biol. Sci. 265: 135–140.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16:111–120.

Kimura. M.. and T. Ohta. 1969. The average number of generations until fixation of a mutant gene in a finite population. Genetics 61:763–771.

Kirkpatrick, M., and M. Slatkin. 1993. Searching for evolutionary patterns in the shape of a phylogenetic tree. Evolution 47: 1171–1181.

Kubo, T., and Y. Iwasa. 1995. Inferring the rates of branching and extinction from molecular phylogenies. Evolution 49: 694–704.

Langor, D. W., and F. A. H. Sperling. 1997. Mitochondrial DNA sequence divergence in weevils of the Pissodes strobi species complex (Coleoptera, Curculionidae). Insect Mol. Biol. 6: 255–265.

Lindberg, H., and H. Lindberg. 1958. Coleoptera Insularum Canariensium. 1. Aglycyderidae und Curculionidae. Commentationes Biologicae. 30:278–287.

Nee, S., E. C. Holmes, A. Rambaut, and P. H. Harvey. 1995. Inferring population history from molecular phylogenies. Phil. Trans. R. Soc. Lond. B Biol. Sci. 349:25–31.

——— 1996. Inferring population history from molecular phylogenies. Pp. 66–80 in P. Harvey, A. Leigh Brown, J. Maynard Smith and S. Nee, eds. New uses for new phylogenies. Oxford Univ. Press, Oxford, U.K.

Nei, M., and W.-H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. (USA) 76:5269–5273.

Palm, T. 1976. Zur kenntnis der kaferfauna der Kanarischen Inseln, 20. Die Gattung Brachyderes Schonherr (Coleoptera: Curculionidae). Entomol. Scand. 7:309–311.

Paradis, E. 1997. Assessing temporal variations in diversification rates from phylogenies: estimation and hypothesis testing. Proc. R. Soc. Lond. B Biol. Sci. 264:1141–1147.

——— 1998a. Detecting shifts in diversification rates without fossils. Am. Nat. 152:176–187.

——— 1998b. Testing for constant diversification rates using molecular phylogenies: a general approach based on statistical tests for goodness of fit. Mol. Biol. Evol. 15:476–479.

Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406–425.

Sanderson, M. J. 1997. A nonparametric approach to estimating divergence times in the absence of rate constancy. Mol. Biol. Evol. 14:1218–1231.

Sanderson, M. J., and G. Bharathan. 1993. Does cladistic information affect inferences about branching rates? Syst. Biol. 42: 1–17.

Sanderson, M. J., and M. J, Donoghue. 1994. Shifts in diversification rate with the origin of angiosperms. Science (Wash.) 264: 1590–1593.

Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. Flook. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Entomol. Soc. Am. 87: 651–701.

Slatkin, M., and R. R. Hudson. 1991. Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. Genetics 129:555–562.

Strimmer, K., and A. von Haeseler. 1996. Quartet puzzling: a quartet maximum likelihood method for reconstructing tree topologies. Mol. Biol. Evol. 13:964–969.

Swofford, D. L. 1998. PAUP*, Phylogenetic analysis using parsimony (* and other methods). Vers. 4, Sinauer Associates, Sunderland, MA.

Swofford, D. L., G. J. Olsen, P. J. Waddell, and D. M. Hillis. 1996. Phylogenetic inference. Pp. 407–514 in D. M. Hillis, C. Moritz, B. K. Mable, eds. Molecular systematics. 2d ed. Sinauer, Sunderland, MA.

Takahata, N., and Y. Satta. 1997. Evolution of the primate lineage leading to modern humans: phylogenetic and demographic inferences from DNA sequences. Proc. Natl. Acad. Sci. (USA) 94: 4811–4815.

Takezaki, N., A. Rzhetsky, and M. Nei. 1995. Phylogenetic test of

the molecular clock and linearized trees. Mol. Biol. Evol. 12: 823–833.

Wollenberg. K.. J. Arnold, and J. C. Avise. 1996. Recognising the forest for the trees: testing temporal patterns of cladogenesis using a null model of stochastic diversification. Mol. Biol. Evol. 13:833–849.

Yang. Z. 1994. Statistical properties of the maximum likelihood method of phylogenetic estimation and comparison with distance matrix methods. Syst. Biol. 43:329–342.