The phylogeography of the darkling beetle, *Hegeter politus*, in the eastern Canary Islands

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We investigated the phylogeography of *Hegeter politus*, a saprophagous, flightless darkling beetle endemic to the eastern Canary Islands, using a fragment of the mitochondrial *COI* gene. Distance and parsimony based gene trees of the mitotypes identified revealed a striking association between mitotype clades and sampling locations. The branching order of the clades suggested that the colonization of the islands by *Hegeter politus* proceeded from the southern part of Fuerteventura in a north-northeast direction to Lanzarote and the smaller islands. Based on this, a colonization scenario compatible with the reported geological ages and volcanisms of the various parts of the islands has been proposed. The high divergence of the beetles collected from the extreme south of Fuerteventura (the Jandía peninsula) from all other samples has led us to propose that they may be from a new species that has not been described previously. The ecological isolation of Jandía from the rest of Fuerteventura by the sand dunes that cover its narrow isthmus in the north, and the existence of many plant and animal endemisms unique to Jandía, lend supportive evidence to our proposal. The similarities between the evolution of island endemics in the Hawaiian and Canary archipelagos have been discussed. We conclude that many endemics in the Canary archipelago, like the Hawaiian Islands, are most likely to have originated from post-colonization differentiation and divergence.

Keywords: cytochrome oxidase I, Canary Islands, phylogeography, speciation, beetles

1. INTRODUCTION

Intraspecific mitochondrial DNA mitotypes can be ordered phylogenetically into gene trees and when geographical locations are overlaid on these, often, significant associations between tree topology and location are detected. The study of the spatial and phylogenetic aspects of genetic variability in this manner has been referred to as phylogeography (Avise 1994). In addition to selective forces, the main factors that contribute to these associations are past events such as colonization history and current demography. Populations on islands, particularly those of known geological or climatic history present clear models to investigate the impact of the former.

The islands of the Canary archipelago, which have become progressively available for colonization during the last 15–20 million years, provide an unusually good opportunity to examine the contribution of colonization history to phylogeography and the various associated evolutionary hypotheses. The reports of Thorpe *et al.* (1993, 1994) on the distribution and phylogenetic differentiation of the lizard species, *Gallotia gallotia*, demonstrate effectively the inferences and deductions about colonization history that can be made from such studies. This lizard species is endemic to the western Canary Islands of Tenerife, La Palma, Hierro and Gomera. These islands,

which originated from independent underwater volcanic eruptions have never been joined above sea-level. Thus, their colonization is essentially the result of dispersal among islands and any spatial patterns observed are not complicated by vicariance. Thorpe *et al.* (1994) used several DNA markers and showed that the lizards spread from Tenerife in the east to Hierro and Gomera in the west.

Using similar techniques, Juan *et al.* (1995) showed that beetle species of the genus *Pimelia* also colonized all the islands of the Canary archipelago sequentially from Fuerteventura in the east to El Hierro in the west. Again, this sequential colonization was found to be compatible with the geological history of the islands. Within the island of Tenerife, the distribution of *Pimelia* mtDNA haplotypes and their phylogenetic relationships revealed two ancient lineages which coincide strikingly with the disjunct volcanic evolution of the different parts of the island (Juan *et al.* 1996).

In this paper, we report on the phylogenetic differentiation of *Hegeter politus*, a saprophagous, flightless darkling beetle which is endemic to the eastern Canary Islands, where it occupies xerophylic habitats at altitudes of up to 800 m above sea-level. In Lanzarote and the northern islets of La Graciosa, Montaña Clara and Alegranza, the species occurs everywhere except on recent barren lavas and sand dunes. No other *Hegeter* species shares this distribution except for the nitrophilous *H. tristis* occurring in disturbed lowland places. In Fuerteventura, at altitudes over 350 m *Hegeter politus* is less abundant and coexists with *H. plicifrons*, and has been displaced totally by the

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latter over 450 m. Other *Hegeter* species on this island include: *H. fernandezi* and *H. gonzalezi*, both of which are allopatric with respect to *H. politus*.

The eastern Canary Islands of Fuerteventura, Lanzarote, La Graciosa, Montaña Clara, and Alegranza, lie on a submarine ridge roughly parallel to the African coast. Recent age determinations by the potassium–argon (K–Ar) method have been carried out in many locations in these islands (Le Bas *et al.* 1986; Coello *et al.* 1992; Ancochea *et al.* 1996). They show that the basal rock of the islands is 48 ± 2 million years (Ma) old. It comprises a mixture of plutonic rocks and dykes of subcrust origin as well as submarine pillow lavas and sedimentary strata. The uplift of this complex formed the bedrock of the islands. This basal complex was later overlaid by subaerial volcanic layers; continued uplift and erosion have, however, exposed basal plutonic rocks in parts of Fuerteventura (Anchochea *et al.* 1996).

We have investigated the gene genealogies of mitochondrial haplotypes of *Hegeter politus* and its population genetic structure across the eastern Canary Islands in relation to reported ages, and, the history of volcanic activities subsequent to the formation of the islands. In addition, estimates of genetic divergence between mtDNA mitotypes from various sites within the eastern Canary Islands have been used to evaluate the taxonomic status of the sampled populations.

2. METHODS

Nine populations, four in Fuerteventura, two in Lanzarote and one in each of the smaller islands of La Graciosa, Montaña Clara and Alegranza were sampled (figure 1). The number of beetles collected from each site ranged from 3 to 12; the limiting factor being the abundance of the beetles at each locality and the ease with which they could be collected. The total number of beetles analysed was 53. The actual sample sizes from each population are shown in table 1.

DNA was extracted and purified by methods described in Juan *et al.* (1995). The oligonucleotides used to amplify the *COI* DNA fragment (255 bp long, including primers) were: 5′CCTACAGGAATTAAAGTTTTTAGATGATTAGC3′ and 5′ATAGGGGGAATCAGTGAACTAGTCC3′, their 5′ end positions correspond to sites 2410 and 2665 in the *D. yakuba* mitochondrial genome. Each PCR cycle comprised: denaturation at 94 °C for 30 s, annealing at 50 °C for 1 min, and primer extension at 72 °C for 1 min. Purification of single stranded DNA was made by using one of the two primers biotinilated at its 5′ end in the PCR reaction. The two DNA strands were separated with magnetic beads coated with streptoavidin (DYNAL) according to the manufacturer's recommendations. The sequences were generated using the dideoxy Sanger method with a fluorescinated sequencing primer and the Pharmacia ALF automatic sequencer. Sequences were aligned using the Lasergene program, MegAlign (DNASTAR).

All the unique mtDNA haplotypes were identified and compared pairwise to estimate the fraction of nucleotide sites that differ between them. Following Lynch & Crease's (1990) notation, if this value, π_{xy} is small ($\leqslant 0.05$), it is approximately equal to the average number of substitutions per nucleotide site, π_{xy} (Nei 1987; Kumar *et al.* 1993). For higher values, π_{xy} is an underestimate and can be corrected by a number of methods which differ in the models of nucleotide substitution. For

example, the Jukes–Cantor method assumes that sites mutate independently and with equal probability to any of the four bases while Kimura's 'two-parameter' model adds to these differences in rates of transition and transversion (Kimura 1980). We used the latter to convert π_{xy} to δ_{xy}. The program NEIGHBOR in Felsenstein's (1993) phylogeny inference package (PHYLIP) was used to obtain an UPGMA tree of mitotype using δ_{xy} as a distance measure.

By averaging the estimates for paired sequences within populations or between populations, it is possible to estimate the within (V_i) or the among (V_{ij}) population variation as described in Nei (1987), and Lynch & Crease (1990). The statistical significance of these estimators were tested by randomizing the allocation of sequences to populations as recommended by Hudson *et al.* (1992).

For highly diverged sequences, the Kimura two-parameter model based on estimates of genetic distance may underestimate the times of divergence because of mutation saturation at the most variable third codon positions. The level of substitutional saturation was assessed by graphic evaluation of transitional and/or transversional substitutions at third positions versus the Kimura two-parameter genetic distance. Estimates of distances based on either the silent substitutions (K_s) or the four-fold degenerate sites (K_4) only provide more linear relationships with time since divergence; these were computed using Li's (Li *et al.* 1985; Li 1993) programmes.

We carried out maximum parsimony analysis using PAUP version 3.1.1 (Swofford 1993). Heuristic searches with 100 replications of random addition of taxa were performed. The strict consensus option was used to produce a single tree. Confidence in the nodes of the tree was assessed by calculating decay indices as the number of extra steps necessary for a monophyletic clade not to be unequivocally supported in the strict consensus tree (Bremer 1994).

3. RESULTS

A 200 bp region of the mitochondrial *COI* gene was sequenced in a total sample of 53 beetles, collected from nine sites in the Eastern Canary Island. Pairwise and multi-alignment sequence comparisons revealed 50 variable sites (figure 1), and 35 unique mitotypes whose distribution in the collection site (figure 2) is shown in table 1. All the variable sites involved single base pair silent substitutions of which 39 were transitional, 3 transversional and 8 multiple. The haplotypes have been deposited at the EMBL data library under accession numbers Z93891–Z93926.

The maximum estimate of divergence between a pair of mitotypes, based on Kimura's 'model-2' with 2:1 expected transition to transversion ratio, was 0.1385. This is rather high for intraspecifc divergence. However, the maximum within population (collection) divergence, V_i, was 0.0365 and the average was 0.019 (table 2); both are within the ranges of intraspecific variability reported in other insect populations (Cooper *et al.* 1995; Zhang & Hewitt 1996).

Figure 2 shows a UPGMA tree of the 35 *Hegeter politus* mitotypes and a sequence from *Pimelia grandis* of North Africa, used as an outgroup. This was obtained using the Kimura two-parameter distance between the haplotypes in the program NEIGHBOR in Felsenstein's (1993) phylogeny inference package (PHYLIP). The grouping of the

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               3469112223333334445566667778889999911111111111111111111111111111
               3814703469258183692561480136900011112222334445556667778888899
                          2581478036925147039251470367912
          F0   TGTATTTGAAGTAACTATTCTAAATCAGTCAACACCCTATACCGCCTCTCCATAACACATTTGT
          F1   .....C..................C.....................................A
          F2   .......A......................................................
          F3   .......A......G................G.........T....................
          F4   ......A.......G...........T...G.........T...................C
          F5   ......A.......G.C..............G.........T....................
          F6   ......A.........................G.....G..T.C..................
          F7   ......A.........................G.....G..AT.C.................
          F8   ..C............T........................T........G........
          F9   ......A.......................................T.............C
          F10  ..............................................T.............C
          F11  .......A............................C.........................
          F12  ......CA............G.....................A...................
          F13  ......A......................G...T.CT..........G.....
          F14  ..C....A..................G.....G..AT.........G........C
          F15  ......A......................G...T..T.........G.....
          F16  C.C.............................G.....G..AT.........G........C
          F17  .......A...............G.........G...T..T.........G.....
          F18  ..C.............................G.....G..AT.........G........C
          F19  ......CA.........C.......A...........CG.G...T.........T..GT......
          F20  ......CA.....G...C.......A...........CG.G.T.T........T..G.......
          F21  ......CA.........C.......A...........CG.G...T.........T..G.......
          F22  ......CA.........C.......A...........CG.G...T.........T..G.......
          F23  ..A.CC...............GT.CT...T....TTT.G....ATT.........G.TT.....A
          F24  ..A.CC.A...C..T..C...TC.CT...T....TTT......AT..T...G....GT...C.A
          F25  ..A.CC.A.............GT.C....T....TTT.G....ATT...........CT...C.A
          F26  ..A.CC...............GT.C....T....TTT......AT.......C...CT.....A
          F27  ..A.CC.A.............GT.C....T....TTT.G....AT.......C.G.CT......
          F28  ..A.CC.A.............GT.C....T....TTT.G....ATT......CTG.CT.....G
          F29  ..A.CC.A.............GT.C....T....TTT.G....AT.......C...CT......
          F30  ..A.C..A...C..T..C...TTGC....T....TTT......AT..T........GT...C.A
          F31  ..A.C..A...C.....C...TTGC....T....TTT......AT..T........GT...C.G
          F32  ..A.C..A...C..TC.C...TTGC....T....TTT......AT..T........GT...C.G
          F33  ..A.C..A...C..T..C...TTGC....T....TTT......AT..T........GT...C.G
          F34  ..C..CC..CA....A.....G..CTG.C..T..TT...C...A.....TT.....G....C..
          Pim  CCACCCC.TCACT.TA.C.TCTT..T.A..TTTCA.T....T.C.TATC.T.GT.T.TTCAACA
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Figure 1. The 35 mitochondrial sequence variants (F0 to F34) of *Hegeter politus* detected in this study and the corresponding sequence from *Pimelia grandis* (Pim), the outgroup species. Only variable sites are shown; their base positions along the sequence are to be read vertically downwards in the first three rows.

Table 1. *Distribution of mitochondrial sequence variants in the nine collection sites shown in figure 2.*

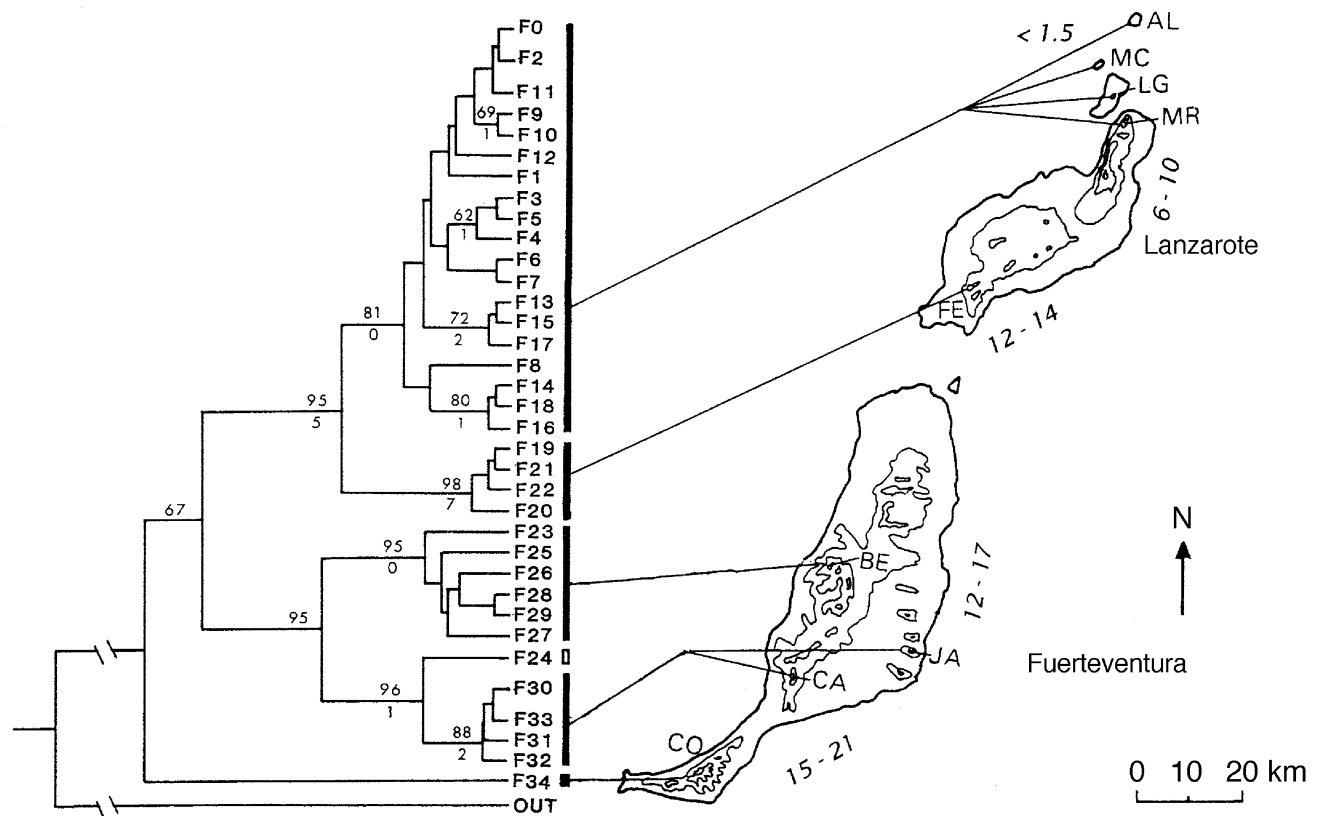| code	location	mtDNA haplotypes	*N*
AL	Alegranza	F0(2), F1(2), F2(3), F3(2), F4(1), F5(1)	11
LG	La Graciosa	F6(2), F7(1), F8(1)	4
MC	Montaña Clara	F9(1), F10(1), F11(4)	6
MR	Mirador del río (L)	F12(1), F13(1), F14(2), F15(1), F16(1), F17(1), F18(1)	8
FE	Femés (L)	F19(1), F20(3), F21(2), F22(1)	7
BE	Betancuria (F)	F23(2), F24(1), F25(1), F26(1), F27(1), F28(1), F29(1)	8
JA	Jacomar (F)	F30(1), F31(1), F32(1)	3
CA	Montaña Cardón (F)	F30(2), F33(1)	3
CO	Cofete (F)	F34(3)	3

Figure 2. A UPGMA tree of *Hegeter politus* mitotypes and an outgroup sequence from *Pimelia grandis* of North Africa obtained using the Kimura two-parameter model-based estimates of genetic distance. The map shows the sites in the eastern Canary Islands from which samples were collected. The 200, 400 and 600 m contours and the ages in millions of years (Ancochea *et al.* 1996) of the different parts of the islands are shown. The full names of the collection site are given in table 1. The association between the phylogenetic clustering of haplotypes in the UPGMA tree and their geographic locations are shown by vertical bars. Bootstrap values from 500 random replications are shown above and Bremer Support (Bremer 1994) for a consensus tree from a parsimony analysis (see text) are shown below the branches next to the relevant nodes. Fuerteventura: CO, Degollada de Cofete; CA, Montaña Cardón; JA, Jacomar; BE, Bentancuria. Lanzarote: FE, Femés; MR, Mirador del río. LG, La Graciosa. MC, Montaña Clara. AL, Alegranza.

Table 2. *Values of within (V) and among (V$_{ij}$) collection sites estimates of divergence (see §2)*

(Full names of the two letter collection site codes are given in table 1.)

	V_i	V_{ij}							
		MC	LG	MR	FE	BE	JA	CA	CO
AL	0.0181	0.0029	0.0085	0.0110	0.0267	0.0711	0.0896	0.0907	0.0977
MC	0.0099	—	0.0153	0.0099	0.0277	0.0742	0.0951	0.0974	0.1002
LG	0.0205	—	—	0.0004	0.0180	0.0723	0.0937	0.0958	0.1114
MR	0.0385	—	—	—	0.0099	0.0677	0.0782	0.0801	0.0970
FE	0.0342	—	—	—	—	0.0768	0.0887	0.0908	0.1057
BE	0.0352	—	—	—	—	—	0.0340	0.0346	0.0962
JA	0.0103	—	—	—	—	—	—	0.0006	0.1316
CA	0.0034	—	—	—	—	—	—	—	0.1368
CO	0.0000	—	—	—	—	—	—	—	—

mitotypes clearly reflects their geographic location. Five clades that correspond to distinct geographic locations are identified: the collection site Degollada de Cofete (CO) at 350 m in the Jandía peninsula; sites Montaña Cardón (CA) and Jacomar (JA) in southern Fuerteventura; Betancuria (BE) in the central part of the same island;

site Femés (FE) of south Lanzarote; and, the remaining four collection sites, all of which are within a radius of 20 km from the northern tip of Lanzarote. Furthermore, the branching order of these clades suggests that colonization may have proceeded from the southern part of Fuerteventura in a north-northeast direction to Lanzarote
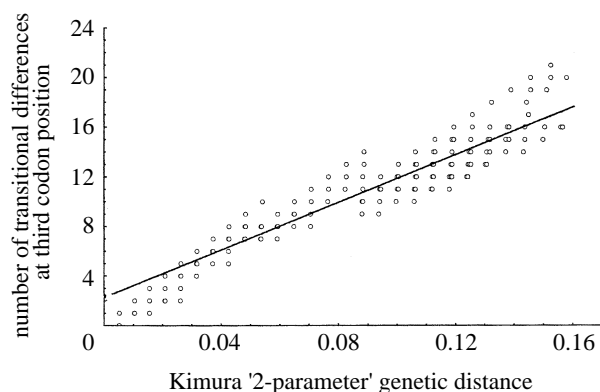
Figure 3. A plot of number of third codon position transitional differences versus Kimura's (1980) two-parameter genetic distance between pairs of *Hegeter politus* haplotypes. A linear trend line was fitted to the data; a correlation coefficient, *r*, of 0.958 was obtained.

and the smaller islands (figure 2). The only exception to the striking matching of mitotypes to localities is mitotype F24. Although F24 was identified in an individual from the Betancuria (BE) collection site, it is on average 50% less diverged from the CA and JA mitotypes than the other Betancuria mitotypes.

The parsimony analysis produced 5700 equally parsimonious trees of 138 steps. The topology of the strict consensus tree was very similar to that shown in the distance tree (figure 2). As is the case in the distance tree, the deepest node resolved all the mitotypes except F34 of CO as a monphyletic clade. Again, similar to the distance tree, the next internal nodes resolve mitotypes of central Fuerteventura, and, those from Lanzarote and the northern islets, as two monophyletic groups. Within the former, the CA and JA mitotypes are monophyletic and within the later the FE mitotypes are most strictly monophyletic (figure 2). F24 is again an exception as discussed above.

There was no indication of saturation in the K_4 and K_s distances between the 35 mitotypes computed using Li's (Li *et al.* 1985; Li 1993) programme. Comparisons with the outgroup sequence however indicated saturation; thus the outgroup branch length shown in figure 2 is not to the same scale as the other branches. The plot of genetic distance versus total number of transitional substitutions at third codon position was clearly linear, and hence, did not indicate any saturation effect (figure 3). This was the case when A-G and T-C transitions were considered separately or when total number of transitions was plotted.

4. DISCUSSION

The genealogy of the mitotypes identified in this study has clearly shown associations between specific clades and geographic location. Is it possible to infer the colonization history of *Hegeter politus* from the topology of this geographically structured gene tree?

The most parsimonious colonization history that can be deduced from the branching order in figure 2 is a stepwise south to north colonization. Thus, *Hegeter politus* either first arose as a species or colonized the Jandía peninsula and proceeded to expand northwards. The south-central part of Fuerteventura (CA, JA and BE) and southern

Lanzarote were colonized next, followed by colonization from the latter of northern Lanzarote and the smaller islands of La Graciosa, Montaña Clara and Alegranza more or less sequentially.

The above colonization scenario takes into account only the phylogeographic patterns revealed by the distance and parsimony based gene trees of the mitotypes (figure 2). However, if the divergence times represented in the gene trees are comparable with the ages of volcanic activities on the islands, either during their formation or subsequently, both the geological history of the islands and the observed phylogeographic patterns need to be taken into account when proposing a specific colonization scenario.

Coello *et al.* (1992) conducted extensive K–Ar based age determinations throughout the eastern Canary Islands. Their data show that in these islands, subaerial volcanic activity has been almost continuous in the last 20–22 Ma. This took place during two distinct cycles, series I, aged 12–21 Ma; and series II, III and IV, of ages ranging from 6 Ma to prehistoric and historic eruptions. The current top layers of the islands originate predominantly from these volcanic eruptions and show a south-southwest to north-northeast age gradient similar to the trend observed in the mitotype genealogy (figure 2). An exception to this is the age of 20.7 Ma given for sites at the Jandía peninsula compared to the 22.5 Ma and 21.1 Ma obtained at Montaña Cardón (CA) and Jacomar (JA), both located to the north of this peninsula (Anchochea *et al.* 1996).

We propose that the history of subaerial volcanic activity on the islands has had a major impact on the current phylogeography of *Hegeter politus*. Thus, the CO population on the series I layers of the southern edifice of Fuerteventura represent the oldest population of the species in the islands. While the phylogeny based colonization scenario described above identified this region as the first to be colonized, it is equally possible that the reason it outdates the populations to the north is because late series I, as well as series II and III, volcanic activities wiped out the beetle populations in these parts of the island. Series II and III volcanic activities which were absent in Jandía are known to have had a significant impact in these parts of the island. Subsequent colonization could have established the central Fueteventura (CA, BE and JA) populations as well as the southern Lanzarote (FE) population. Northern expansion from FE established the MR, LG, MC and AL populations. The estimates of divergence between these populations (table 2) are compatible with this volcanism-constrained colonization history. The divergence of 9–13% of CO from the rest of the populations which is approximately equal to 4.5–6.5 Ma using a 2% Ma^{-1} molecular clock (DeSalle *et al.* 1987) is compatible with a post-series I separation. Similarly, the divergence between the central Fuerteventura samples is probably of post-series II and III origin. More recent, series IV separation is reflected in the branch lengths between the samples from northern Lanzarote and the islands of La Graciosa, Montaña Clara and Alegranza.

As stated above, the 9–13.85% estimated divergence between the Jandía mitotype, CO, and the other mitotypes is rather high for intraspecific variability. Moreover, the fact that this mitotype is unique to Jandía peninsula and

these samples appeared to be morphologically distinguishable from the beetles from the other collection sites suggest that the Jandía samples may represent a distinct species that has evolved in allopatry. It is certainly true that for many organisms Jandía is ecologically isolated from the rest of Fuerteventura by the low altitude sand dunes that cover its narrow isthmus in the north.

Several endemisms unique to Jandía indicate that this isolation may have been effective in evolutionary terms. On the other hand, these species may be endemic to the peninsula because of extinction elsewhere. Examples of species endemic to Jandía include two higher plants: *Echium handiense* at mountain tops and *Euphorbia handiensis* in the desert lowlands. Insects endemic in Jandía include *Hegeter fernandezi* in the mountains, *Hegeter gonzalezi* exclusive to the beaches and sand dunes (Oromí 1982), the darkling beetle *Oreomelasma ormii* Español as well as the ground beetle *Calathus gonzalezi* Mateu. The spider species *Dysdera longa* Wunderlich and the slug *Parmacella susanae* Hutterer, are endemic to the Jandía mountains (Hutterer 1989). It is most likely that for all the mountain species the narrow, low lying, sandy isthmus has been an effective barrier to northern expansion.

In conclusion, it is worthwhile to draw attention to comparisons that have been made between European studies of evolution on Atlantic islands and the extensive American work done on the fauna and flora of the Hawaiian archipelago (Wagner & Funk 1995). Carlquist (1995) points out that many European workers have ascribed Atlantic island endemisms to relicts of original colonists (Berry 1992; Cronk 1992). Having analysed the results of our studies in light of K–Ar geological datings, we would argue that many endemics in the Canary archipelago, like the Hawaiian Islands, are more likely to have originated from post-colonization divergence.

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