Geographical structuring of mitochondrial DNA in *Chalcides sexlineatus* within the island of Gran Canaria

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The skink *Chalcides sexlineatus* shows clear north–south ecology-correlated variation in morphology within the island of Gran Canaria. Detailed study of this variation previously supported the hypothesis of *in situ* differentiation arising through climate-mediated variation in selection pressures. The more recent discovery of substantial within-island mitochondrial DNA (mtDNA) sequence divergence suggested an alternative historical vicariance scenario. This paper provides a detailed analysis of geographical structuring in mtDNA, based on a fragment amplified from the 12S rRNA region in 96 individuals. A very clear phylogeographic pattern was detected: three ‘deep’ lineages were evident corresponding to northern, south-eastern and south-western parts of Gran Canaria with generally low levels of mtDNA introgression. The pattern of among-site differentiation was highly concordant with the pattern of morphological variation. It also provided a close fit to a simple microevolutionary model based on population vicariance during volcanic eruptions known to have occurred during the past 2.8 million years in the north of the island. The minimum number of historical migration events required to explain the mtDNA tree was calculated and a novel randomization test used to show that there were a lower number of putative migrations across the morphology transition zone, relative to migrations within northern and southern zones. Differential adaptation to the two major habitat types and selection against hybrids may explain why the morphology transition zone coincides with the main ecotone and is relatively narrow, respectively.

Keywords: evolution; geographical variation; island; lizard; phylogeography; volcanism

1. INTRODUCTION

The use of mtDNA as a molecular marker has considerably enhanced knowledge of how organisms differentiate at the intraspecific level. A consequence of these studies has been recognition of the importance of historical population vicariance (followed by secondary introgression) as a cause of geographic variation, with emphasis being shifted away from explanations involving selection-mediated clines (Endler 1977; Avise *et al.* 1987; Avise 1994). Recent statistical developments have also contributed, with methods used in phylogenetics and population genetics being combined to enable a wider variety of hypotheses to be tested (e.g. Slatkin & Maddison 1989; Holsinger & Mason-Gamer 1996).

At the present time there is little evidence that potential advantages conferred by mtDNA mutations are environment-dependent and so where spatial structuring is substantial, explanations based on historical interruptions to gene flow are usually invoked (e.g. Avise *et al.* 1987; Avise 1994). Concordance with morphological

variation provides further support for a historical explanation (discordant patterns of morphological and mtDNA variation may provide evidence of additional current natural selection effects; Thorpe *et al.* 1996), although knowledge of environmental gradients, historical habitat distributions and/or geological events are also needed for a fuller assessment of cause (Riddle 1996).

Historical climate changes had an important impact on distributions of continental organisms and led to episodic cycles of range expansion or contraction (Riddle 1996). These events during the Pleistocene explain many of the observed patterns of geographic variation within European and North American fauna (Bartlein & Prentice 1989; Avise 1992; Hewitt 1996). Alternative historical causes of population differentiation not based on climate-induced range changes are also possible but have rarely been documented in vertebrates. Geographic variation within small oceanic islands is less likely to arise through climatic effects compared with continental species because of reduced geographical distances. Within-island differentiation–speciation of island insect faunas have received considerable attention (Carson & Kaneshiro 1976; DeSalle & Templeton 1992; Shaw 1996), and the contribution of historical volcanism may at least

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Figure 1. Isophene contour map depicting pattern of geographic variation in colour pattern of *Chalcides sexlineatus* in Gran Canaria. Contours are based on the first canonical vector (77.8% of among-population variation) converted to 1–10 scale from a canonical variates analysis of eight colour pattern characters (see Brown *et al.* 1991).

partially explain the observed biodiversity (Carson *et al.* 1990; Juan *et al.* 1996). There are now some suggestions that historical volcanism may have also played a role in the within-island differentiation of some vertebrates (Thorpe *et al.* 1996; Schneider 1996). This may yet prove to be relatively widespread, as many species described as showing geographic variation are from volcanic islands.

The primary aim of this study was to investigate geographical structuring of mtDNA for a species showing a clear pattern of phenotypic variation within an island with a relatively well-known geological history (Hansen-Machin 1987, 1988a,b; Hoernle & Schmincke 1993). In this way we could evaluate whether differentiation had occurred *in situ* or in historically vicariant populations with subsequent secondary contact.

The scincid lizard *Chalcides sexlineatus* has been the focus of several studies because it shows considerable variability within the small island (1532 km²) of Gran Canaria in the Canary archipelago (28° N, 16° W) (Pasteur *et al.* 1988; Mayer & Tiedemann 1991). Detailed analysis indicates a clear north-east–south-west spatial pattern of variation in coloration (Brown *et al.* 1991; Brown & Thorpe 1991a) (figure 1), scalation and body dimensions (Brown & Thorpe 1991b). A full range of morphologies are found and individuals within transition zone populations tend to be intermediate between northern and southern morphs. This pattern is tightly correlated with the substantial differences in habitat aridity found between the north and south of the island. The sister species, *C. viridanus*, shows a parallel pattern of north–south habitat-correlated differentiation on the similarly heterogeneous neighbouring island of Tenerife

(Brown *et al.* 1993). Both species show a tendency for conspicuous blue tail coloration (a trait of considerable functional importance in lizards; Cooper & Vitt 1986) in the xeric southern habitats and uniform brown dorsal and tail coloration in the more mesic northern areas, further strengthening the *prima facie* case of selection-mediated differentiation. Despite this, a recent study has shown that substantial mtDNA divergence does occur within Gran Canaria (Brown & Pestano 1998) indicating that historical population vicariance cannot be ruled out. Furthermore, substantial volcanic activity capable of causing local extinctions and giving rise to dispersal barriers is known to have occurred in the north-east of the island during the last 2.8 million years (Myr), a time period commensurate with the degree of within-island haplotype divergence (Brown & Pestano 1998).

2. MATERIALS AND METHODS

(a) *Sites, specimens and sample preparation*

Chalcides sexlineatus were caught by hand ($n=96$) from 24 evenly distributed sample sites covering the full range of morphologies within the island (figure 2). Minimum and maximum sample sizes were three and seven, respectively. The sample sites comprised a subset of the 46 sites used in previous morphology studies (Brown *et al.* 1991; Brown & Thorpe 1991a,b). Tail-tips were removed, placed in 99% alcohol and all individuals released.

Tissue samples were homogenized in a digest buffer and whole genomic DNA was extracted using chelex (Walsh *et al.* 1991). The L1091 and H1478 primers (Kocher *et al.* 1989) were used to amplify *ca.* 384 bp fragment of the 12S rRNA region of the mtDNA. Although this region is generally considered too conservative for intraspecific studies, substantial within-island sequence divergence is found in *Chalcides* (Brown & Pestano 1998), with the additional benefit of proving easier to amplify than D-loop and cytochrome *b* regions. PCR amplifications were based on 35 cycles with 94 °C (30 s) denaturing, 48 °C (30 s) annealing and 72 °C (30 s) extension temperatures and 2 mM MgCl₂. The light strand was sequenced for all individuals using an ABI 373 sequencer (University of Liverpool sequencing service), with 24 of the individuals being sequenced in both directions (this included all haplotypes that initially appeared only once). The first 22 bases were often ambiguous and so were not included in subsequent analyses.

(b) *Statistical analyses*

Sequences were aligned using Clustal W (Thompson *et al.* 1994), allowing identification of different haplotypes. Phylogenetic relationships among haplotypes were inferred using neighbour-joining (NJ) on Kimura two-parameter distances or from consensus parsimony trees (PARS) (Felsenstein 1993). As in previous cross-species comparisons in *Chalcides* (Brown & Pestano 1998), a transition:transversion weighting of 1:10 was used, approximating to observed values for pairwise comparisons in that study. Loops were down-weighted relative to stems in accordance with relative substitution rates in these two regions (see later). Note that application of these weightings had a negligible effect on the results presented here. Both *C. simonyi* from the eastern Canary island of Fuerteventura and the sister species *C. viridanus* from Tenerife were used as outgroups. A minimum spanning tree (MST) based on Jukes Cantor distances

Figure 2. Map of Gran Canaria (with altitude contours) showing the 24 sample sites and the haplotypes identified at each site. Northern morphology sites (defined as sites to the north of the mid-contour of the morphological variation) (figure 1) are prefixed N while southern sites are prefixed S. Similarly, the three main mtDNA haplotype lineages (see figure 4) are suffixed A, B, C.

was also used to elucidate distance-based relationships among haplotypes in relation to geography and morphology. Comparison of the MST with the NJ tree also allowed evaluation of whether different distance estimates affected phylogeographical groupings. The transition in morphology is sharp, so sites were classified as either northern morph (sites north of contour line 5 [N], figure 1) or southern morph (sites south of contour line 5 [S]).

Levels of between-site divergence were estimated using the N_{ST} statistic proposed by Lynch & Crease (1990). This measure of population subdivision is based on haplotype frequencies and degree of sequence divergence. Among-site congruence between colour pattern and mtDNA was evaluated by testing the matrix association between N_{ST} and Mahalanobis D^2 between-centroid distances using Pearson's product-moment correlation coefficient (c.f. Mantel's test; Mantel 1967), with significance determined by comparison of the observed value with values obtained after 4999 random matrix permutations. This provided a maximum achievable significance level of 0.0002. Significance of geographic structuring was evaluated using a recently proposed method which tests distances between daughter nodes of a tree constructed to estimate the hierarchical relationship among sites (Holsinger & Mason-Gamer 1996), thus avoiding statistical problems due to non-independence of sites.

The minimum number of migration events required to explain the phylogeny were calculated using the cladistic method described by Slatkin & Maddison (1989). The primary aim was a broad assessment of levels of gene flow across

morphological transition zones relative to that within zones, rather than to estimate Nm (where N is the population size and m is the immigration rate).

A randomization method written by R.P.B. evaluated the hypothesis that the migrations between site pairs was lower between northern and southern morph sites than within these respective areas. Presence or absence of between-site migrations were coded 1 and 0, respectively, in a between-site half-matrix. This matrix was compared with a between-site half-matrix with 1, 0 codes for site pairs in different or the same phenotype areas, respectively. The test statistic, which we will call M , was the number of putative between-site migrations across the transition zone (i.e. the sum of the occurrences of 1 in corresponding elements of the two matrices). Significance was determined by randomly reallocating observations to sites 999 times (maximum achievable significance level: 0.001) and recalculating M each time to allow comparison of the actual value with the distribution of the statistic under randomization (contact the corresponding author for further details).

The observed pattern of among site differentiation, as represented by N_{ST} , was tested against a simple model representing differentiation under a recent volcanic extinction scenario/secondary intergradation scenario (VA). Under this hypothesis eruptions that occurred in the north-east of the island during the last 2.8 Myr caused extinctions between present-day north-east-south-west populations, leaving refugia populations in the extreme north-east and the south-west (Hansen-Machin 1988b). The northern area that was volcanically active is quite clearly

delimited by a line traversing the island from positions close to site N23 in the north-west to site S10 in the south-east (figure 2) (Hansen-Machin 1988b). Distances between populations were coded 0, 1 according to whether they originated from the same or different refugia. The relationship between this and the N_{ST} distances, with current geographical proximity held constant, was evaluated using a randomization test based on that described by Manly (1986). This test uses a multiple regression approach and allows testing of the significance of the extra sums of squares explained for the dependent matrix after addition of each independent matrix. Data were standardized before use. It was preferred over other methods because use of a 0, 1 independent variable in a least-squared regression can be shown to give the same results as an ANOVA on the dependent with the independent as the grouping factor (which would appear a more logical approach), but with the additional advantage of allowing simultaneous comparison with geographical distance. A total of 4999 random matrix permutations were computed.

3. RESULTS

(a) Sequence divergence

A total of 34 haplotypes were identified in the 96 specimens (figure 2). The mismatch distribution of uncorrected pairwise differences summarizes the levels of sequence divergence found (figure 3). The distribution is clearly bimodal, indicating two levels of haplotype subdivision. Transition:transversion ratios were correlated with sequence divergence, with a maximum ratio of 15:1 being observed for the most divergent haplotypes. There were 41 variable sites, of which 26 were parsimony-informative (no indels were found within *C. sexlineatus*). Comparison with the secondary structure of bovine 12S rRNA indicated that 27 of these sites were situated in loops, while 14 were situated in stems.

(b) Relationships between haplotypes

Although not strongly supported by bootstrapping, very similar tree topologies were found by the NJ (figure 4) and PARS methods. Both provided evidence of three major lineages containing identical haplotype sets. The most geographically restricted lineage (B) contained haplotypes found only in populations from the centre-south-east of Gran Canaria. A second lineage (C) was confined to the centre-south-west of the island, while the most widespread lineage (A) was found only in the north. The MST depicted the relationship between morphology and mtDNA and also provided very similar results (figure 5), i.e. three main clusters containing the same haplotypes. Haplotypes found only at northern morphology sites were tightly clustered with ≤ 3 mutational steps between closest relatives. Four haplotypes found at southern sites (but close to the main morphology-transition area) were also found within this cluster (i.e. 11A, 14A, 15A, 19A) suggesting some limited introgression of northern mtDNA into a southern nuclear background. Haplotypes only found in the centre or south-west sites were less tightly clustered (≤ 6 mutational steps between closest relatives), while haplotypes confined to central south and south-east sites showed greater diversity (≤ 7 mutational steps between closest relatives). Within the main north and centre-south-west clusters, haplotypes 3A and 31B had the greatest number of mutational derivatives.

Figure 3. Mismatch distribution of haplotypes, as represented by pairwise differences ($\hat{d}$). The maximum pairwise difference of 17 represents 4.7% sequence divergence.

(c) Among-site differentiation and migration

Significant differentiation was detected between four main groups of sites, with the hierarchical pattern of differentiation showing a generally north-east-south-west trend (figure 6). The pattern of among-site differentiation was highly concordant with the pattern of among-site differentiation in morphology (males, $r=0.385$, $p=0.0002$; females $r=0.409$, $p=0.0002$).

Detailed conclusions cannot be drawn from the analysis of hypothesized migration events due to the small sample sizes. However, the overall pattern was clear: relatively few migrations between the north and south were necessary to explain the NJ tree (5 out of 31), while those that were detected all involved transition population-pairs. The number of migration events was significantly lower across the transition zone compared to within northern and southern sites ($M=5$, $p=0.001$). Note that this test strictly assumed independence of migration probability and geographical distance, and so was repeated with 53 matrix elements eliminated (i.e. those representing the most distant site pairs across the transition zone). In this second analysis the median across-transition-zone geographical distance was lower than the median distance within northern and southern morph areas, and so the results were not potentially biased towards erroneously detecting a lower number of migrations across the transition zone. Neither this second test nor use of the PARS instead of the NJ tree affected these conclusions.

The relationship between N_{ST} and geographical proximity was highly significant ($r=0.428$, $p=0.0002$), as was the association between N_{ST} and VA, with proximity constant (standardized partial regression coefficient, $b'_{N_{ST}/VA.DIST}=0.386$, $p=0.0002$). A significant extra sums of squares was explained by the addition VA to the matrix regression of N_{ST} against proximity and the pattern of lush or arid variation within the island.

4. DISCUSSION

Three main findings support the hypothesis of historical population vicariance due to volcanic eruptions across the north and centre of the island: (i) the

Figure 4. Bootstrapped NJ tree, with a schematic representation of the distributions of the three main haplotypes identified (see figure 1 for more details).

magnitude of the lineage divergence is substantial (up to 4.7% between haplotypes in a relatively conserved region of the mtDNA) and commensurate with the timing of the onset of the last eruptive cycle within the island (Brown & Pestano 1998); (ii) the current haplotype distribution shows a close fit to the pattern of volcanic activity during this period; and (iii) the present-day haplotype distribution is congruent with the morphological differentiation (and also shows similarities with *Gallotia stehlini* on the same island; Thorpe & Báez 1993) providing greater confidence in the described pattern. Furthermore, present-day mtDNA structuring in the lizard *G. galloti* reflects known events in the recent geological history of the neighbouring island of Tenerife (Thorpe *et al.* 1996).

How might differences in volcanic activity between the north and south have separated ancient populations? Interrelationships between volcanic activity and micro-evolutionary patterns have been previously documented in insects (Carson *et al.* 1990). Two main effects of volcanic eruptions on skink populations are likely: (i) the immediate extinction of populations due to the eruptions themselves; and (ii) the creation of long-term unsuitable habitat (lava fields) that could act as dispersal barriers. In support of the latter, Canary Island skinks generally

Figure 5. Minimum spanning tree connecting haplotypes. Line lengths are proportional to genetic distances: the shortest lines represent a single mutation difference.

prefer more densely vegetated habitats with humid substrates. The sister species *C. viridanus* also appears to be absent from recent lava flows on the neighbouring island of Tenerife (R. P. Brown, personal observation). Similar modes of diversification may also be applicable to insects inhabiting volcanic islands (Shaw 1996; Juan *et al.* 1996). Hansen-Machin (1988b) considers that two main sectors of activity began in the north of Gran Canaria approximately 2.8 Myr ago, one in the east–north-east and one in the north-west, while the area between them, i.e. the north-east segment of the island, remained largely unaffected. This latter area, around sample sites N4 and N15, could have provided a northern refugium. Secondary contact after expansion from northern and southern refugia is likely to have occurred much later because of the magnitude of the morphological divergence. In support of this, only one haplotype (3A) was found in this area and the MST suggested that this may be ancestral because it had a large number of mutational derivatives (Crandall & Templeton 1993) (although this conflicts with the NJ and PARS trees).

The reason for negligible mtDNA introgression between the main sites in the north and south is of interest as is the interrelated question of which factors have determined the position of the transition zone. Introgression of northern–southern mtDNA into ‘foreign’

Figure 6. Hierarchical relationships among sites, with probabilities representing the proportion of between-daughter node distances under randomization that were greater than or equal to the observed distance (Holsinger & Mason-Gamer 1996).

morphologies appears limited, unlike in other terrestrial vertebrates in which it can be considerable (Ruedi *et al.* 1997). One explanation could be selection against individuals with northern mtDNA and extreme southern nuclear genomes, or vice versa, due to coevolved interactions between them (Moritz *et al.* 1987).

Consideration of how the hybrid zone is maintained is limited by the lack of comparable data on nuclear markers, although two enzymes show a transition that closely corresponds to that in morphology, i.e. the majority of the change occurs over approximately 10 km (Mayer & Tiedemann 1991). There are two main alternatives: (i) the zone is a tension zone maintained by reduced fitness of hybrids (Key 1981; Hewitt 1989; Barton & Gale 1993); and (ii) the environmental gradient (which is highly correlated to morphological variation) leads to differential selection between northern and southern morphs. The evidence of reduced gene flow across the

zone supports the former. However, differential adaptation of phenotypes from the north to lush habitats and phenotypes from the south to arid habitats would also seem plausible given that the transition coincides so closely with the ecotone. Correlations with ecological heterogeneity have been observed in other species that have differentiated in allopatry (Schneider 1996; Patton & Smith 1994; Ruedi *et al.* 1997). Many continental European lineages that have come into post-glacial secondary contact also show characters that appear to adapt them to different lifestyles (Hewitt 1996). Furthermore, field experiments on island lizards suggest that phenotypic differences between populations are adaptive, providing further support for the environmental gradient hypothesis (Malhotra & Thorpe 1991). Differential adaptation would appear reasonable for *C. sexlineatus* as larger, more robust uniform-brown northern skinks appear suited to the more mesic northern areas while the

smaller bright blue-tailed skinks with relatively smaller heads and limbs appear suited to xeric southern areas (Brown *et al.* 1991; Brown & Thorpe 1991a,b).

The La Isleta population (N46) is the exception to the general trend of mtDNA variation (and therefore population history) explaining morphological variation. The morphological divergence between these skinks and neighbouring northern populations is considerable, but the mtDNA haplotype composition was identical to sites in neighbouring parts of the main island (i.e. haplotype 3A present only). La Isleta was formed independently from the rest of the island but was subsequently connected by a sand bar. The considerable habitat transition between La Isleta and the north of the island would indicate that differential selection regimes may be responsible, although similarity of mtDNA composition from introgression from the main island cannot be ruled out.

Despite the concordance with the volcanism model (which implies a single, major vicariance event which should give rise to two descendant lineages), there is evidence for the presence of three 'deep' mitochondrial lineages within the island. The model can only provide a simplified representation of the factors that led to within-island cladogenesis and so any more detailed explanations are purely speculative. Even so, it is interesting to note that there is geological evidence for substantial volcanic activity on Gran Canaria approximately 4.5–3.5 Myr ago (i.e. prior to vicariance events mentioned previously), and that this appears to have been sufficient to have almost extinguished life on the island (Hansen 1988a). The only area that was largely unaffected by this was the south-east, corresponding to the present-day distribution of lineage B, which might suggest that this is the ancestral lineage. However, weak support for relevant nodes on the trees means that we cannot yet determine whether this clade is the most basal or not.

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REFERENCES

- Avise, J. C. 1992 Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. *Oikos* **63**, 62–76.
- Avise, J. C. 1994 *Molecular markers, natural history and evolution*. New York: Chapman & Hall.
- Avise, J. C., Arnold, J., Ball, R. M., Bermingham, E., Lamb, T., Neigel, J. E., Reeb, C. A. & Saunders, N. C. 1987 Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *A. Rev. Ecol. Syst.* **18**, 489–522.
- Bartlein, P. J. & Prentice, I. C. 1989 Orbital variations, climate and paleoecology. *Trends Ecol. Evol.* **4**, 195–199.
- Barton, N. H. & Gale, K. S. 1993 Genetic analysis of hybrid zones. In *Hybrid zones and the evolutionary process* (ed. R. G. Harrison), pp. 13–45. Oxford University Press.
- Brown, R. P. & Pestano, J. 1998 Phylogeography of Canary Island skinks inferred from mtDNA sequences. *Mol. Ecol.* **7**, 1183–1191.
- Brown, R. P. & Thorpe, R. S. 1991a Within-island geographic variation in the colour pattern of the skink *Chalcides sexlineatus*: pattern and cause. *J. Evol. Biol.* **4**, 557–574.
- Brown, R. P. & Thorpe, R. S. 1991b Description of within-island microgeographic variation in the body dimensions and scalation of the Gran Canarian skink, *Chalcides sexlineatus*, with testing of causal hypotheses. *Biol. J. Linn. Soc.* **44**, 47–64.
- Brown, R. P., Thorpe, R. S. & Báez, M. 1991 Parallel within-island evolution in lizards on neighbouring islands. *Nature* **352**, 60–62.
- Brown, R. P., Thorpe, R. S. & Báez, M. 1993 Patterns and causes of morphological population differentiation in the Tenerife skink, *Chalcides viridanus*. *Biol. J. Linn. Soc.* **50**, 313–328.
- Carson, H. L. & Kaneshiro, K. Y. 1976 *Drosophila* of Hawaii: systematics and ecological genetics. *A. Rev. Ecol. Syst.* **7**, 311–345.
- Carson, H. L., Lockwood, J. P. & Craddock, E. M. 1990 Extinction and recolonization of local-populations on a grow shield-volcano. *Proc. Natl Acad. Sci. USA* **87**, 7055–7057.
- Cooper, W. E. Jr & Vitt, L. J. 1986 Blue tails and autotomy: enhancement of predator avoidance in juvenile skinks. *Zeitschrift für Tierpsychol.* **70**, 265–276.
- Crandall, K. A. & Templeton, A. R. 1993 Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. *Genetics* **134**, 959–969.
- DeSalle, R. & Templeton, A. R. 1992 The mtDNA genealogy of closely related *Drosophila silvestris*. *J. Hered.* **83**, 211–216.
- Endler, J. A. 1977 *Geographic variation, speciation and clines*. Princeton University Press.
- Felsenstein, J. 1993 PHYLIP (phylogeny inference package), version 3.5c. Distributed by the author. Seattle, WA: University of Washington.
- Hansen-Machin, A. 1987 *Los volcanes recientes de Gran Canaria*. Las Palmas, Spain: Cabildo Insular de Gran Canaria.
- Hansen-Machin, A. 1988a Guía de los volcanes de Gran Canaria I. *Aguayro (Las Palmas, Spain)* **175**, 19–26.
- Hansen-Machin, A. 1988b Guía de los volcanes de Gran Canaria II. *Aguayro (Las Palmas, Spain)* **176**, 21–26.
- Hewitt, G. M. 1989 The subdivision of species by hybrid zones. In *Speciation and its consequences* (ed. D. Otte & J. A. Endler), pp. 85–110. Sunderland, MA: Sinauer Associates.
- Hewitt, G. M. 1996 Some genetic consequences of ice ages, and their role in divergence and speciation. *Biol. J. Linn. Soc.* **58**, 247–276.
- Hoernle, K. & Schmincke, H. U. 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.
- Holsinger, K. E. & Mason-Gamer, R. J. 1996 Hierarchical analysis of nucleotide diversity in geographically structured populations. *Genetics* **142**, 629–639.
- Juan, C., Ibrahim, K. M., Oromi, P. & Hewitt, G. M. 1996 Mitochondrial DNA sequence variation and phylogeography of *Pimelia* darkling beetles on the island of Tenerife. *Heredity* **77**, 589–598.
- Key, K. H. L. 1981 Species, parapatry and the morabine grasshoppers. *Syst. Biol.* **30**, 425–458.
- Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., Villablanca, F. X. & Wilson, A. C. 1989 Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing of conserved regions. *Proc. Natl Acad. Sci. USA* **86**, 6196–6200.
- Lynch, M. & Crease, T. J. 1990 The analysis of population survey data on DNA sequence variation. *Mol. Biol. Evol.* **7**, 377–394.
- Malhotra, A. & Thorpe, R. S. 1991 Experimental detection of rapid evolutionary response in natural lizard populations. *Nature* **353**, 347–348.

- Manly, B. F. J. 1986 Randomisation and regression methods for testing for associations with geographical, environmental, and biological distances between populations. *Res. Popul. Ecol.* **28**, 201–218.
- Mantel, N. 1967 The detection of disease clustering and a generalized regression approach. *Cancer Res.* **27**, 209–220.
- Mayer, W. & Tiedemann, F. 1991 Proteinvariabilität und taxonomie des Gran Canaria skinks *Chalcides sexlineatus*. *Amphibia–Reptilia* **12**, 121–130.
- Moritz, C., Dowling, T. E. & Brown, W. M. 1987 Evolution of animal mitochondrial DNA: relevance for population biology and systematics. *A. Rev. Ecol. Syst.* **18**, 269–292.
- Pasteur, G., Keymar, P. F. & Perret, J. L. 1988 Canarian skink systematics: contrasting insular diversifications within a species subgroup. *Memoires et Travaux de l'Institut de Montpellier* **18**.
- Patton, J. L. & Smith, M. F. 1994 Paraphyly, polyphyly, and the nature of species boundaries in pocket gophers (genus *Thomomys*). *Syst. Biol.* **43**, 11–26.
- Riddle, B. 1996 The molecular phylogeographic bridge between deep and shallow history in continental biotas. *Trends Ecol. Evol.* **11**, 207–211.
- Ruedi, M., Smith, M. F. & Patton, J. L. 1997 Phylogenetic evidence of mitochondrial DNA introgression among pocket gophers in New Mexico (family Geomyidae). *Mol. Ecol.* **6**, 453–462.
- Schneider, C. J. 1996 Distinguishing between primary and secondary integradation among morphologically differentiated populations of *Anolis marmoratus*. *Mol. Ecol.* **5**, 239–249.
- Shaw, K. L. 1996 Sequential radiations and patterns of speciation in the Hawaiian cricket genus *Laupala* inferred from DNA sequences. *Evolution* **50**, 237–255.
- Slatkin, M. & Maddison, W. P. 1989 A cladistic measure of gene flow from the phylogenies of alleles. *Genetics* **123**, 603–613.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. 1994 CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucl. Acids Res.* **22**, 4673–4680.
- Thorpe, R. S. & Báez, M. 1993 Geographic variation in scalation of the lizard *Gallotia stehlini* within the island of Gran Canaria. *Biol. J. Linn. Soc.* **48**, 75–87.
- Thorpe, R. S., Black, H. & Malhotra, A. 1996 Matrix correspondence tests on the DNA phylogeny of the Tenerife lacertid elucidate both historical causes and morphological adaptation. *Syst. Biol.* **45**, 335–343.
- Walsh, P. S., Metzger, D. A. & Higuchi, R. 1991 Chelex® 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* **10**, 506–513.

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