

THE DYNAMICS OF NATURAL SELECTION AND VICARIANCE IN THE DOMINICAN ANOLE: PATTERNS OF WITHIN-ISLAND MOLECULAR AND MORPHOLOGICAL DIVERGENCE

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Abstract.—The larger islands of the Lesser Antilles are ecologically and geologically complex and are inhabited by single, but morphologically variable, *Anolis* species. Although earlier work has indicated that a large part of the morphological variation in *Anolis oculatus* from Dominica can be attributed to selection, a history of recurrent volcanic activity over the last few million years suggests that vicariance may have also played a significant role. We report a study of variation in the cytochrome *b* gene of mitochondrial DNA across the island to address this issue. We uncovered a very high degree of polymorphism, with an overall gene diversity of 0.97 and a nucleotide diversity of 0.04. Sequences, on average, differ by 3.82% and the maximum pairwise divergence (corrected for multiple hits) is 9.29%. Most haplotypes are restricted to single localities (a pattern not changed by increasing the sample size). Phylogenetic analysis revealed the presence of two distinct lineages on the island with strong phylogeographic structure. One of these is geographically restricted to a relatively small part of the central Caribbean coast. Sublineages were also discernible within the other more widely distributed lineage, but resolution within and support for these sublineages was poor. The phylogeographic pattern is not congruent with generalized body shape and scalation, but is significantly correlated with color pattern. Even when correcting for this lineage effect with partial Matrix correspondence tests, the relationship between color pattern and vegetation is reaffirmed, suggesting that although both vicariance and selection have played a role in the morphological differentiation of this species, selection for current environmental conditions has been more important. We discuss the causes of the phylogeographic structure in light of the volcanic history of the island and highlight the exceptional instance of congruence between all morphological character systems and lineage boundaries, which occurs at the transition between the northern and southern Caribbean ecotypes.

Key words.—*Anolis oculatus*, cytochrome *b*, Dominica, gene flow, Lesser Antilles, mitochondrial DNA, phylogenetic analysis, phylogeography, selection, speciation, vicariance, volcanism.

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The complex Greater Antillean *Anolis* communities have been a paradigm for community ecologists and evolutionary biologists for many years (Williams 1983; Losos 1995; Losos et al. 1998). In contrast, the anole communities of the Lesser Antilles (a chain of smaller, volcanic islands fringing the eastern margin of the Caribbean) are simple, with no island having more than two species of anole occurring naturally. Williams (1972) suggested that this simpler structure resulted from them still being at an early stage of community evolution, but this is now thought not to be the case (Losos and de Queiroz 1997). Based on an examination of species-area relationships in the Caribbean islands, Losos (1996) concluded that the Lesser Antillean islands are probably below the critical minimum size required to allow speciation. However, at least some of the Lesser Antilles do display considerable geological, geographic, and environmental heterogeneity despite their comparatively small size. The larger central islands (Guadeloupe, Martinique, and Dominica) are each occupied by a single species of anole (according to currently recognized taxonomic arrangements) that is endemic to that island (or island bank) and that all show considerable within-island geographic variation in morphology (Lazell 1972). These may yield some insights into the processes involved in divergence in *Anolis* lizards, with possible wider implications for understanding the processes underlying the radiation of this speciose group. This study focuses on *Anolis oculatus* from Dominica.

The complex geological history and topography of Dominica suggests the possibility that both history and variation

in the strength and direction of natural selection may be responsible for patterns of geographic variation in *A. oculatus*. Disentangling the relative effects of these factors and the interactions between them has been a goal of evolutionary biology for years. *Anolis oculatus* presents a particularly rich and informative situation to examine these possibilities.

Volcanic activity began in the Lesser Antilles approximately 40 million years ago (mya) in the Eocene (Morris et al. 1990; Pindell and Barrett 1990). However, Dominica belongs to a younger, still volcanically active section where activity is widely considered to have begun in the late Miocene (≈ 15 mya), becoming widespread approximately 5 mya, in the Pliocene (Maury et al. 1990). In terms of subaerial volume of volcanic products, Dominica is by far the most active island in the chain (Carr and Stoiber 1990). Only a few published radiometric dates are available for Dominican rocks, the oldest of which are K-Ar dates of 1.2 my and 1.8 my (Briden et al. 1979) for lava in southeast Dominica. However, some pre-Pliocene rocks are exposed on the east coast (based on unpubl. K-Ar dates, quoted in Maury et al. 1990). On much of the west coast this has been overlain by sedimentary deposits (observed up to 30–60 m above present-day sea level) and later volcanic material, particularly that associated with the Pleistocene explosive eruptions of the main volcanic cones present today (Sigurdsson and Carey 1990). One of these, the Morne Trois Piton/Morne Micotrin complex, is thought to have been responsible for one of the largest eruptions in the Lesser Antilles in the last 200,000 years, which took place 28,000–30,000 years ago. In terms

of the volume of ejected material, this event was almost five times more severe than the 1883 Krakatau eruption (Carey and Sigurdsson 1980; Mandeville et al. 1996; H. Sigurdsson, pers. comm.). Pyroclastic flows originating from the Morne Trois Piton/Micotrin center have been detected extending 300 km to the south into the Grenada basin, whereas ash deposits on the sea bed extend 650 km to the east (Carey and Sigurdsson 1980). The extent of destruction of the habitat would have been severe, extending far beyond the present position of the pyroclastic flow deposits due to the effects of hot dilute surges that accompanied the channelled flows. It is thought that within the last 700,000 years there have been 12 other major explosive eruptions originating from Dominica (Sigurdsson and Carey 1981). Thus, the volcanic history of the island has been turbulent and is likely to have had major and repetitive effects on the biota.

Although one of the largest of the Lesser Antilles, Dominica is only 45 km long and 16 km at its widest point. As a geologically young island, it has a rugged topography, reaching a maximum altitude of 1421 m. The presence of a number of peaks over 1000 m, from the extreme northern to the southern tip of the island, creates a barrier to the prevailing northeasterly trade winds, which results in a very high rainfall (Lang 1967). The uneven distribution of rainfall, in turn, determines the distribution of habitats (Beard 1948). Rainforest covers much of the center of the island, whereas the leeward (western or Caribbean) coast, which has both the lowest annual rainfall and the most pronounced dry season, has much more xerophytic vegetation. The vegetation of the windward (eastern or Atlantic) coast shares some species with both Caribbean coast xeric woodland and rainforest, but the salt-laden onshore winds have a strong influence and produce a distinct vegetation type (termed "littoral woodland") with a characteristic dense, asymmetric canopy. The marked differences in vegetation type present an opportunity for strong natural selection acting in different directions in different parts of the island.

Morphological Differentiation in Anolis oculatus

Anolis oculatus is a small, semi-arboreal lizard that feeds mainly on insects (Bullock et al. 1993), although fruits, soft-bodied invertebrates, and even small vertebrates are occasionally taken (pers. obs.). It inhabits the entire island, primarily at elevations lower than 900 m, and is very geographically variable (pictured in Malhotra and Thorpe 1992), most obviously in color pattern and body size, but also in scalation characters. Lazell (1972) reflected this variability by naming four subspecies. Geographic variation also exists in other aspects of its biology such as population density, basking behavior (Ruibal and Philibosian 1970), prey composition (Bullock et al. 1993), and activity patterns (J. T. Reardon and R. S. Thorpe, unpubl. data).

A quantitative description of the pattern of geographic variation in morphology was previously obtained by measuring body proportions, scale counts, and color pattern characters across the island and summarized by multivariate ordination methods (Malhotra 1992; Malhotra and Thorpe 1997a,b). Considerable geographic variation was found in almost all characters studied, typically taking the form of a variety of

clines including east-west clines and altitudinal clines (for examples, see Malhotra and Thorpe, 1997a,b). North-south clines are also frequently seen, but tend to be restricted to the Caribbean coast and are usually superimposed on the other types of cline. This produces a rather sharp and congruent transition between the northern and southern Caribbean coast populations, in contrast to the rest of the island, which is characterized by low congruence between the patterns shown by different characters. The result is that overall variation is far from categorical (Fig. 1) and thus we have described the variation as ecotypic rather than subspecific (Malhotra and Thorpe 1991a).

A number of hypotheses could explain the morphological divergence in *A. oculatus*. First, much of the observed variation appears to be associated with habitat differences (Malhotra 1992; Thorpe et al. 1994; Malhotra and Thorpe 1997a,b), thus suggesting selection by geographically varying environmental conditions as one possible cause. Further evidence was obtained from parallel patterns of morphological variation in the closely related *Anolis marmoratus* from Basse Terre, an island that is known to be historically independent of Dominica, but that shares common climatic patterns (Malhotra and Thorpe 1994; Thorpe and Malhotra 1996). We concluded that an explanation involving the common climatic conditions found on both islands was more parsimonious than independent historical vicariant events with similar geographic relationships to environmental gradients (see also Brown et al. 1991). Moreover, we obtained direct evidence that natural selection acts on these populations from a series of controlled and replicated manipulative field experiments. When populations from various habitats were translocated into a set of enclosures situated in a single habitat, we were able to demonstrate a detectable difference in the multivariate morphology of survivors and nonsurvivors within each translocated population over a very short time scale (Malhotra and Thorpe 1991b). Furthermore, the selection intensity was dependent both on the magnitude of the ecological change experienced by translocated populations and the season when the manipulation took place (Malhotra and Thorpe 1991b; Thorpe and Malhotra 1996; J. T. Reardon, R. S. Thorpe and A. Malhotra, unpubl. data).

Alternatively, patterns of seemingly adaptive differentiation among populations are being increasingly revealed to be the result of phenotypic plasticity. Examples include tadpoles (Van Buskirk and Relyea 1998), snails (DeWitt 1998), fish (Day et al. 1994; Holopainen et al. 1997), and reptiles (Shine et al. 1997; Elphick and Shine 1998; Qualls and Shine 1998; Queral Regil and King 1998; but see Forsman and Shine 1997). Because closely related species may share a similar norm of reaction, this cannot be ruled out by the study of parallels among species. However, the adaptive/plastic hypotheses are not mutually exclusive. To measure the relative contribution of genetically determined variation to the pattern of morphological variation observed in *A. oculatus*, eggs were hatched and young reared from a number of different localities under common environmental conditions. The pattern of geographic variation among parental populations was also exhibited by the offspring raised in a common environment (J. T. Reardon and R. S. Thorpe, unpubl. data), thus suggesting that variation resulting from phenotypic plasticity is

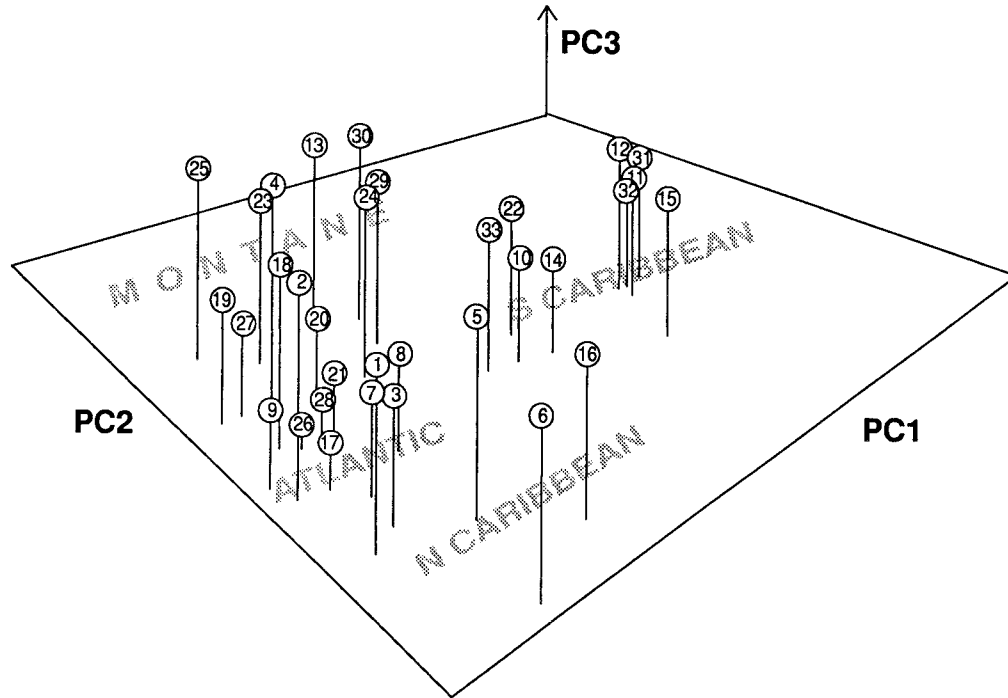


FIG. 1. Patterns of overall morphological variation in male *Anolis oculatus* summarized by principal component analysis (plot of first three principal components illustrating 62% of total variation). It is clear that the variation is not categorical as implied by the former subspecific nomenclature (see text for details). However, the ordination of the localities does make geographic sense as indicated on the base of the plot. It is also clear that the southern Caribbean coast populations appear to be more differentiated than the others. Females show very similar patterns (Malhotra 1992).

considerably less important than genetically controlled variation in this case.

Another factor affecting morphological divergence of *A. oculatus* is the volcanic history of Dominica, which may have created the necessary conditions for divergence in allopatry followed by secondary intergradation (see Schneider 1996). The vicariance/secondary contact and selection hypotheses are not mutually exclusive, but their relative importance and the way in which they interact is of considerable interest. The atypically congruent differences observed between the northern and southern Caribbean coast populations in almost all characters, despite the relative similarity of the habitat (compared to the rest of the island), is consistent with secondary contact. Malhotra and Thorpe (1994) showed that the transition in morphology between the two forms was very sharp, occurring over a distance of only a few kilometers. A parallel cline in some aspects of the morphology of *A. marmoratus* was also observed along the Caribbean coast of Basse Terre. However, a preliminary analysis of divergence in the mitochondrial cytochrome *b* gene revealed that in both cases these morphological clines were congruent with a major transition in cytochrome *b* sequence. This leads to a paradox in which the within-island congruence suggested the action of historical factors, but the between-island congruence (using the logic outlined above) suggested this was unlikely. One hypothesis that could not be rejected by these paradoxical findings was that mitochondrial patterns might themselves be influenced by environmental gradients (Malhotra and Thorpe 1994; Thorpe et al. 1995). Because this hypothesis is contentious (Schneider 1996), an island-wide study of

mitochondrial patterns was clearly required to adequately address the issues raised (Thorpe et al. 1995).

Aims and Hypotheses

Here we report the results of a study of mitochondrial sequence divergence in the cytochrome *b* gene in *A. oculatus* across Dominica. We first test for the presence of phylogeographic structure by reconstructing a phylogeny of mitochondrial haplotypes and determining the geographic distribution of the resulting clades. If phylogeographic structure is present, we can then test the hypothesis that morphological variation observed in the species is a result of divergence of traits while in allopatry, with limited introgression following secondary contact producing the clinal variation observed (this will be referred to hereafter as the "phylogenetic" hypothesis). A similar phylogenetic hypothesis has been proposed for other *Anolis* species (Schneider 1996) and is implied by the categorical nature of the four subspecies proposed by Lazell (1972). Under this scenario, the geographic distribution of phylogeographic groups should correspond with that of morphologically similar groups. Even if they do not correspond, it is still possible that historical events contribute to patterns of divergence. The extent of the contribution of historical events can be evaluated by testing patterns of morphological differentiation against phylogenetic relationships. If there is no significant relationship between patterns of phylogenetic relationship and morphological similarity, we can conclude that the historical signal has largely been overwritten, with selection remaining as a strong con-

tender as the main influence on morphological diversification. Alternatively, a significant relationship with phylogeny would require a reevaluation of the relationship between morphology and environmental variation, which can be accomplished by incorporating phylogenetic information into comparative statistical analyses.

MATERIALS AND METHODS

DNA Isolation, Amplification, and Sequencing

Tail-tip biopsies (preserved in 80% ethanol) were taken from individuals from each of the original 33 populations represented in the morphological study (Malhotra and Thorpe 1991a, 1997a,b). An additional 10 localities situated along the central part of the Caribbean coast were also sampled (Fig. 2) to determine the distribution of lineages more accurately in the region where the northern and southern Caribbean ecotypes meet. Whole genomic DNA was extracted from approximately 0.02 g of ethanol-preserved muscle tissue using standard protocols (Sambrook et al. 1989). Primers L14841 and H15149 (Kocher et al. 1989) were used to amplify a 307-bp section of the cytochrome *b* gene. Reaction conditions were standard (Innis et al. 1990). A negative (blank) control was always included to exclude the possibility of contamination. Unincorporated nucleotides and primers were removed using a variety of commercially available kits (e.g., Prep-a-gene [Biorad, Hercules, CA] or Wizard minicolumns [Promega, Madison, WI]) and the double-stranded product was then sequenced using a modification of the Sequenase v2.0 protocol. Both strands were sequenced, in most cases using several different polymerase chain reaction products.

The high haplotype diversity uncovered did not lend itself to screening methods (e.g., Hillis et al. 1996; Thorpe et al. 1996) that would have allowed the rapid typing of multiple individuals per population. Instead, between one and three individuals were sequenced from each population. To determine how much this low sample size might affect the patterns detected, a larger number of individuals (between eight and 10) were sequenced from three equidistant populations from the west coast. In total, 81 individuals of *A. oculatus* were sequenced.

Sequence Analysis

Sequences were aligned by eye. They were translated into amino acid sequences to check for the unexpected occurrence of stop codons, which might indicate that pseudogenes (Sorenson and Fleischer 1996; Zhang and Hewitt 1996) had been amplified. Maximum divergence levels between haplotypes are relatively high, thus it is possible that some degree of saturation is present in the sequences. If this is the case, then it may be appropriate to apply corrective weighting regimes (e.g., downweighting of transitions). The number of pairwise differences due to transitions were plotted against overall genetic divergence, using Jukes-Cantor corrections to produce a crude estimation of time because divergence (Krajewski and King 1996; Milinkovitch et al. 1996). Plots were produced for all codon positions and also for third position

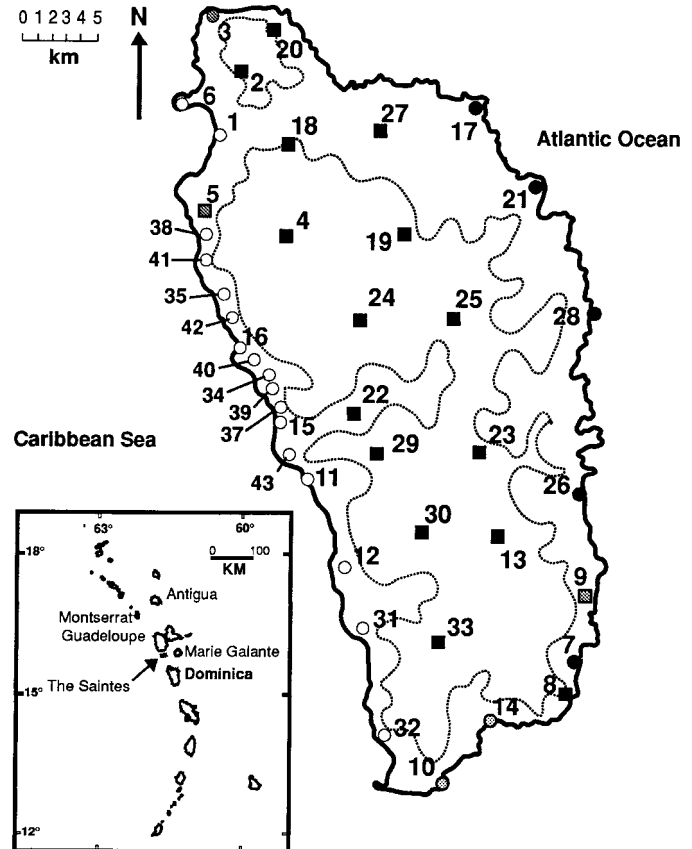


FIG. 2. Map of Dominica showing localities represented in this study, with the locality numbers. Localities 1–33 are those used in the original morphological studies (see text). Localities 34–42 are additional localities not used in matrix correlation tests (because morphological data for these latter localities were not collected in the same way), but included to determine the distribution of mitochondrial lineages more accurately in the region where the southern and northern Caribbean ecotypes meet. The symbols marking localities indicate the habitat type at that locality. Empty circles denote xeric woodland, solid circles denote littoral woodland, and solid squares denote rainforest. Gray circles denote a vegetation type that is transitional between littoral woodland and xeric woodland, and gray squares indicate a transition between rainforest and the adjacent coastal vegetation type. The inset map of the Lesser Antilles indicates the central position of Dominica and the relative position of other islands mentioned in the text.

transitions separately, because the latter are the most likely to show saturation effects.

A preliminary analysis was carried out on a selection of *A. oculatus* sequences and sequences from *Anolis* populations from all the Guadeloupean islands, Montserrat, and Antigua (A. G. Stenson, A. Malhotra and R. S. Thorpe, unpubl. data) to confirm the monophyly of *oculatus* sequences and to choose the most appropriate outgroups. Populations on islands to the south of Dominica can be ignored because they belong to a distantly related group of anoles (Gorman et al. 1980). *Anolis oculatus* sequences were found to be monophyletic and on the basis of this analysis, one sequence each from the Saintes and Marie Galante were retained for use as outgroups.

Both distance-based and character-based methods were used to reconstruct a haplotype phylogeny, following the ar-

gument that the application of different methods allows the consistency of phylogenetic estimation to be evaluated (Kumar et al. 1993; Avise 1994). PHYLIP (Felsenstein 1993) was used to construct trees based on the least-squares algorithm (using the program FITCH, with random addition of OTUs and no global rearrangements) and neighbor-joining (using the program NEIGHBOR, on Jukes-Cantor distances). The Fitch-Margoliash tree was bootstrapped 100 times and the neighbor-joining tree 500 times to obtain a measure of relative support for nodes. A greater number of bootstraps were not possible because of computational limitations associated with the large dataset. A maximum-likelihood tree was also produced, using quartet puzzling (Strimmer and von Haeseler 1996; Strimmer et al. 1997), a fast tree-search algorithm implemented in the program PUZZLE. Empirical transition:transversion ratios and base frequencies and the HKY substitution model (Hasegawa et al. 1985) were used. Maximum-parsimony trees were constructed using PAUP 3.1.1 (Swofford 1993) using a heuristic search with 1000 random-addition replications to adequately sample the large tree space. Five hundred bootstraps of the parsimony tree were performed. Decay indices, sometimes called Bremer support values (Bremer 1988), were also calculated for the nodes defined by the resulting strict consensus tree using the program Autodecay (Eriksson 1977). Farris et al. (1996) recently discussed problems associated with bootstrapping and proposed a new procedure, parsimony jackknifing. It has the major advantage that, because its aim is to reveal the strongly supported groups only (which in fact is the information of interest), it runs very rapidly on large datasets (i.e., seconds as opposed to hours). Parsimony jackknifing (using the program Jac, available from A. Kluge, Museum of Zoology, University of Michigan) was carried out using 10,000 jackknives.

Given the possibility of nonneutral evolution of the cytochrome *b* gene in this species suggested by Malhotra and Thorpe (1994), various tests of neutrality were carried out using the program DnaSP, version 2.5 (Rozas and Rozas 1997). These included Fu and Li's D^* and F^* , which are based on neutral model predictions concerning the difference between the number of singletons and the total or average number of nucleotide differences between pairs of sequences, respectively (Fu and Li 1993). The modifications of these tests, for use with an outgroup sequence, were also carried out (Fu and Li 1993). However, Fu and Li's tests have been shown not to be particularly powerful at detecting some types of selection (Wayne and Simonsen 1998). Consequently, alternative tests were also carried out. McDonald and Kreitman's (1991) test is a goodness-of-fit test that compares ratios of synonymous to replacement substitutions within and between species, with the expectation under neutrality that these should be the same.

The hypothesis of rate constancy can be tested by the likelihood-ratio test (Felsenstein 1988), which compares the likelihood of a tree using an assumption of clocklike evolution and a tree calculated without this assumption. However, there are potential statistical problems with this test (Goldman 1993; Hillis et al. 1996). Moreover, it tests the overall "clockness" of the tree, whereas it would be of interest to compare rates of substitution in subsets of the data (such as

the clades revealed by phylogenetic analyses). Relative rate tests between the various lineages were therefore carried out using the two-cluster test of Takezaki et al. (1995) as implemented in PHYLTEST, which tests the null hypothesis that the average number of substitutions in two lineages, relative to that of an outgroup lineage, will be equal. It is similar to Li and Bosquet's (1992) test, but allows more than one outgroup sequence to be used. Each of the clades (represented by clusters of monophyletic sequences) was tested against its sister clade, using the clade immediately interior to them as the outgroup (because the power of the test increases when the outgroup is more closely related). When the most basal ingroup clade was involved in the test, the outgroup was taken from the sister taxa identified by the preliminary phylogenetic analysis (see above).

To further investigate the impact of the volcanic event of 28,000 years ago, we computed gene and nucleotide diversity (Nei 1987) within the three populations from which larger samples had been sequenced using the program DnaSP version 2.5 (Rozas and Rozas 1997). Our expectation was that diversity should be greatly reduced in the population closest to the center of the eruption (population 12) relative to those further away (populations 16 and 6).

Hypothesis Testing

Partial matrix correlation tests (PMCT; Manly 1991; Thorpe 1996; Thorpe and Malhotra 1996; and references therein) were carried out to test the congruence between patterns of phylogenetic history and potential selective factors on the one hand and current patterns of morphological variation on the other. These tests compare observed patterns of the dependent variable (e.g., of geographic variation in individual or multivariate suites of morphological characters) to several hypothesized patterns (independent variables) simultaneously. Considering hypotheses simultaneously may be important because, (a) putative causes that generate non-independent patterns (intercorrelated independent variables) are frequently encountered and (b) putative causes (e.g., different selective forces, or selective forces and historical factors) may be additive in their effect rather than simply mutually exclusive. Where effects are additive, it can be useful to estimate the relative contribution of each (Thorpe et al. 1994). Because the matrix elements are not independent, degrees of freedom are unknown and standard parametric tests are inappropriate for PMCTs. Thus, the probability of rejecting the null hypotheses of no association is obtained by comparing the statistic measuring the association (e.g., partial regression) with its distribution obtained after randomizing rows and columns. PMCT's were performed using Thorpe's adaptation of Manly's RT-MANT program, which can handle up to nine independent matrices (commercially available from B. J. F. Manly, University of Otago, New Zealand).

Phylogenetic structure can be represented as one of the independent variables by the patristic distance matrix (i.e., the sum of the branch lengths on the path between each pair of taxa on the phylogenetic tree). The morphology (dependent variable) can then be compared against various putative selective forces, independent of these patristic phylogenetic relationships. However, if the history of the populations is

characterized by extensive intermingling via gene flow, then a model of dichotomous branching (a phylogenetic tree) may not be appropriate to represent the history of the populations and, instead, a distance model may be more appropriate. Because it is very likely that, once again, the actual situation is somewhere between the two extremes, we conducted the analyses using both patristic and genetic distance matrices.

Although independent contrasts (Felsenstein 1985) also removes the phylogenetic component of between-taxa comparisons, matrix correspondence tests have distinct advantages in studies of geographic variation in contiguous populations (for a full explanation, see Thorpe 1996; Daltry et al. 1996). Briefly, PMCTs have the advantage of being able to take out the effect of geographic proximity (representing the ability to exchange genes between populations and unspecified geographic effects) and consider other sets of variables that are also essentially matrix in form (i.e., not one-dimensional).

To calculate patristic distances, it was necessary to account for intralocality variation. Previous studies have used the modal haplotype (Daltry et al. 1996; Thorpe et al. 1996), but due to the high proportion of unique haplotypes in the current dataset, modal haplotypes were impossible to define. Instead, in cases where more than one haplotype was present in a locality, appropriate ambiguity codes were inserted in polymorphic positions producing a composite haplotype. The patristic distance matrix was then calculated from a strict consensus of all maximum-parsimony trees calculated for these composite haplotypes, using the appropriate option in PAUP. The use of composite haplotypes is not recommended in situations where divergent haplotypes are present in the same locality, but in *A. oculatus*, all haplotypes present in any one locality are closely related. Genetic distance matrices were Jukes-Cantor distances with intrapopulation variability accounted for by taking the average interpopulation distance.

Dissimilarity matrices (Euclidean distances) between the localities were calculated for each morphological character set separately (using a program written by R. S. Thorpe). These are based on normalized population means for 12 (10) size-adjusted body dimensions (Malhotra and Thorpe 1997a), eight (seven) scalation characters (Malhotra and Thorpe 1997b), and 38 (29) color pattern characters (Malhotra 1992) for males (females in parentheses), respectively. Overall morphological variation is not calculated as the larger number of color pattern characters relative to the other character sets means that the results are largely similar to those of color pattern alone. Only the original 33 localities (see Fig. 2) were used for these tests, because the morphological data were collected in a different way for the remaining localities and are not strictly comparable.

Matrices were also constructed to represent patterns of environmental variation. These were vegetation type (VEG), altitude (ALT), mean annual temperature (TEMP), and mean annual rainfall (RF). These variables were chosen because accurate and comparative data could be obtained for all sites. Temperature (in °C) and rainfall (in inches, converted to mms) were obtained from maps in Lang (1967). Altitude, in meters above sea level, was measured with a Thommen Classic altimeter accurate to ± 10 m, most measurements being verified on separate occasions. Because the complexity of

vegetation patterns seen in Dominica precluded a simple scoring system as in Thorpe and Brown (1989), in which only two main biotopes existed, vegetation was scored as three variables corresponding to xeric woodland, rainforest, and littoral woodland, respectively (Beard 1948). A rainforest site would thus be scored as 0, 1, 0; a xeric woodland site as 1, 0, 0; and a littoral woodland site 0, 0, 1. A site in the transition between two vegetation types would given a score that reflected this (e.g., a transition between xeric woodland and rainforest would be scored as 0.5, 0.5, 0). The score for any particular site thus reflects a number of aspects of the vegetation type including its physical structure (height and degree of closure of canopy, girth of trees) as well as species composition. These variables were then summarized in a single "vegetation" dissimilarity matrix. Maps illustrating the distribution of these variables can be found in Malhotra and Thorpe (1991a). Finally, a geographic proximity matrix was calculated from the latitude and longitude of each locality.

We first tested for significant geographic structure in the mitochondrial lineages by testing the patristic distance matrix against geographic proximity. We then tested whether mitochondrial patterns were congruent with environmental gradients, as suggested by our earlier work, by comparison to environmental matrices. Next, we tested for a significant association between phylogeny and morphological variation. Finally, we tested the correspondence between morphological differentiation and environmental gradients first omitting and then including the matrix of patristic/genetic distances. Geographic proximity was included in all cases. Probabilities were derived from 10,000 randomizations (Jackson and Somers 1989) and Bonferroni-adjusted for multiple comparisons using Rice's (1989) sequential correction procedure (for a fuller explanation of the latter procedure, see Malhotra and Thorpe, 1997b).

RESULTS

Patterns of Molecular Variation in Cytochrome b

A total of 306 bp were aligned between sequences, and a large amount of variation was found to be present in the samples analyzed. Of the 81 individuals sequenced, 43 different haplotypes were identified in the ingroup, only eight of which are present at more than one locality. It is worth noting that increasing the sample size in some populations did not substantially increase their overlap with neighboring populations. For example, all haplotypes sampled from population 6 were unique to this population, and only one haplotype in each of the other populations (12 and 16) were found at any other locality. The small sample size representing many localities may therefore not be a problem in this dataset.

Four sequences were eliminated from further study because they had a significant amount of missing data at the 3' end. Among the 39 remaining ingroup sequences, there were 48 variable sites (15.7%). Pairwise differences between haplotypes were between 0.0033 and 0.0929, the latter being between haplotypes from localities 40 and 24. The mean number of differences between sequences is 11.69 (3.82%), the gene diversity is 0.9719 (SE \pm 0.0088), and the nucleotide di-

versity is 0.0362 (\pm 0.0186). The mean distance to the closest outgroup is 0.1426.

The number of studies that have detected copies of cytochrome *b* inserted into the nuclear genome have increased dramatically (Zhang and Hewitt 1996), but we are confident that our sequences represent the expressed, mitochondrial sequence. Amplification and sequencing results were unambiguous and sequences from several independently amplified products that were sequenced in both directions always gave the same sequence. Moreover, no insertions, deletions, or stop codons are present. The substitutional and base composition biases are similar to those reported for the cytochrome *b* gene for other taxa (e.g., Krajewski and King 1996, Milinkovitch et al. 1996). Over all positions, transitions exceeded transversions by a factor of 5.35 and pyrimidine transitions exceeded purine transitions by a factor of 1.56. The saturation plots indicated no departure from linearity, even for third position transitions, which are expected to saturate rapidly (plots not shown). All phylogenetic analyses were therefore performed on equally weighted data.

Phylogenetic Analysis of Haplotypes

With outgroups included, there were 80 variable and 46 parsimony informative sites. All the methods, which make different underlying assumptions, produce very similar results in terms of the main groups. In all the trees, sequences fall into two main lineages (Figs. 3, 4). These two lineages are strongly supported by all methods. The distribution of these lineages, when plotted onto a map of Dominica, has clear geographic structure (Fig. 5). One of the lineages has a restricted distribution in the central part of the Caribbean coast. This central Caribbean lineage contains a large number of very divergent haplotypes, which are strongly supported as monophyletic in all the trees except the parsimony jackknife. The latter, while not supporting the monophyly of this clade, does support its distinctness from all other sequences present on the island (Fig. 4). The other, more widespread, lineage contains further groupings of haplotypes that receive support as monophyletic clades. Two of these (to be referred to as the northern and southern Caribbean, respectively) are supported in all the trees, although to varying extents. In particular, the methods vary on the support for sequences from populations 10, 32, and 33 (all from the southwestern area of the island) being grouped with other southern Caribbean populations. Neighbor-joining and Fitch-Margoliash bootstraps support their monophyly strongly, but only weak support is found in the maximum-likelihood and maximum-parsimony trees. The remaining haplotypes will be referred to, for convenience, as the central-Eastern group although this is a "catch-all" category rather than a well-supported group. In particular, haplotypes from localities 17 and 27 frequently appear to be more closely related to the southern Caribbean lineage than to their geographic neighbors, although this relationship is never well supported. With this exception, the remaining central-eastern haplotypes are supported as a monophyletic group, albeit weakly, by the decay index (Fig. 4). In the tests that require the haplotypes to be grouped into clades, 17 and 27 have been included with the other central eastern haplotypes because this is consistent

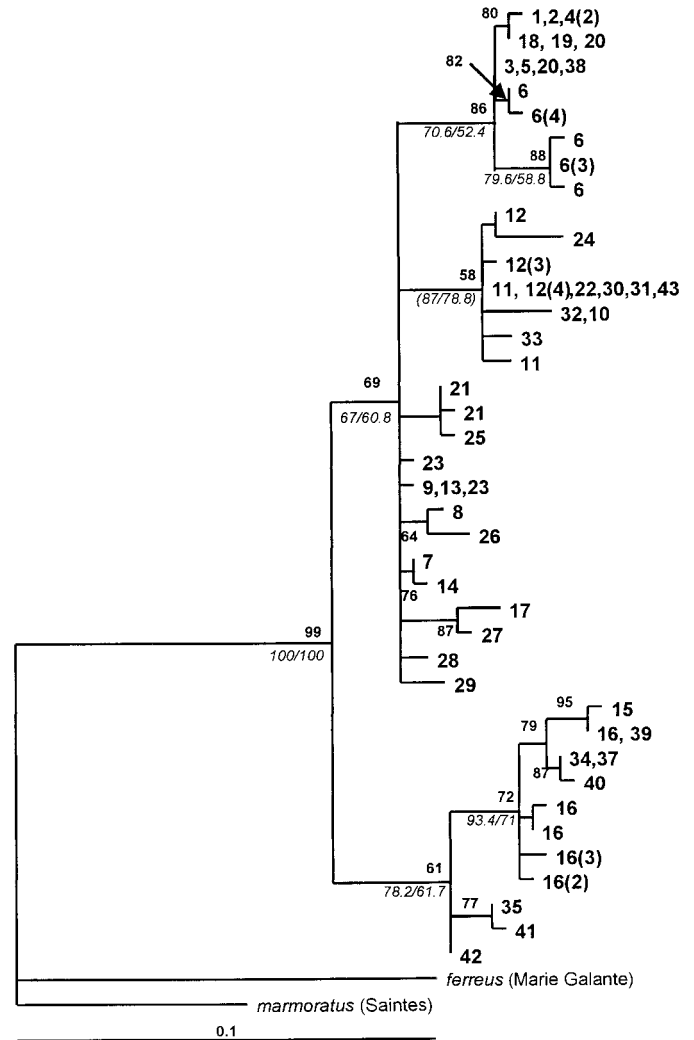


FIG. 3. Maximum-likelihood phylogram. Support values for internal branches from the maximum-likelihood analysis are given above the branches in bold type. Support values given below the branch in italics are percent bootstrap values (exceeding 50%) from 500 and 100 bootstraps of neighbor-joining and Fitch-Margoliash trees, respectively. These are given for major nodes only, which are consistent between all the trees. The tips are labeled with the localities from which the haplotypes derive, and the number of individuals (where more than one) bearing that haplotype at that locality is indicated in parentheses.

with their geographic distribution. The haplotype diversity seen within this group, and their relatively wide distribution makes it likely that more substructure might emerge with additional sampling of haplotypes from this area. Representative sequences from all lineages have been deposited in GenBank (accession nos. AF178577–80).

Tests of neutrality do not allow the null hypothesis of neutral evolution to be rejected in *A. oculatus* (for a similar result for a subsample of *Anolis marmoratus* sequences, see Schneider 1996). The null hypothesis of no difference between rates of evolution in different clades, which was tested by the relative rates test, could also not be rejected in any of the pairwise comparisons. These were: southern Caribbean versus central-eastern (with northern as outgroup); southern

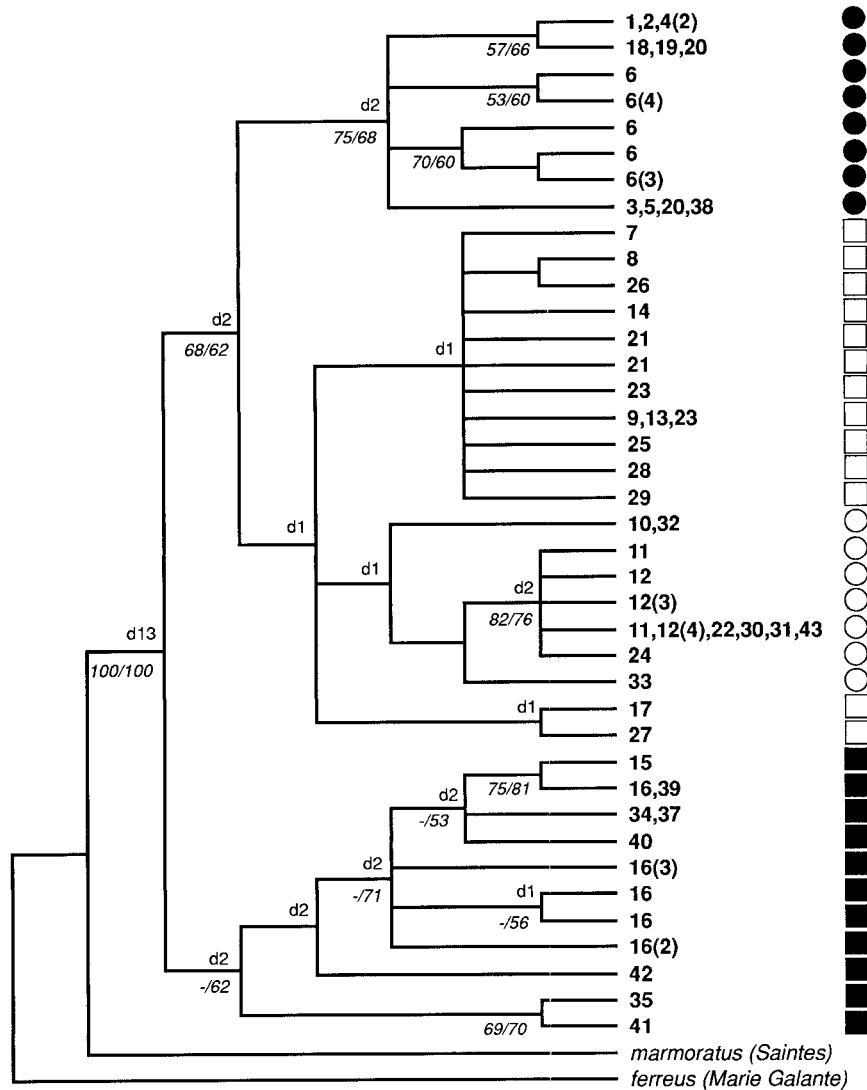


FIG. 4. Strict consensus of 16 most parsimonious trees. Bremer support values (decay indices) are given above and parsimony jackknife/ bootstrap frequencies > 0.5 are given below the branch leading to the node to which they refer. Tips are labeled as in Figure 3. Symbols next to tips identify the major clade to which they belong: central Caribbean (solid squares), northern (solid dots), southern (empty dots) and central-eastern (empty squares).

Caribbean plus central-eastern versus northern (with central Caribbean as the outgroup); central Caribbean versus southern Caribbean plus central-eastern plus northern (with the sister species as the outgroup).

Within-Population Comparisons

Because some of the sequences from population 12 had missing data at the 3' end, not all the haplotypes were used in the phylogenetic analysis. However, for the within-population analysis, all haplotypes were used and sites at which more than 5% of the data was missing (47 sites) were excluded from the data. The gene diversity of locality 16, in which six of nine individuals sequenced have different haplotypes, is 0.8889 (± 0.0910). These haplotypes are relatively divergent from each other, with a mean number of pairwise differences of 2.41 (± 1.44) and a nucleotide diversity of 0.0093 (± 0.0063). Locality 6 (northern Caribbean) has five

haplotypes (of 10 sequences), corresponding to a gene diversity of 0.8000 (± 0.1001). There are 2.45 mean pairwise differences and the nucleotide diversity is 0.0088 (± 0.0059), which is comparable with locality 16. The southern Caribbean population from locality 12 also has five haplotypes (of 10 individuals sequenced), but only 0.87 (± 0.66) mean pairwise differences exist between them, corresponding to a substantially lower nucleotide diversity of 0.0033 (± 0.0028).

Hypothesis Testing

The population tree connecting composite haplotypes from each locality was completely consistent with the haplotype tree, which is unsurprising because most sequences within localities are closely related. The patristic distance matrix calculated from this tree is significantly correlated with geography, but not with any environmental gradients (Table 1). The patristic distances are also highly correlated with the

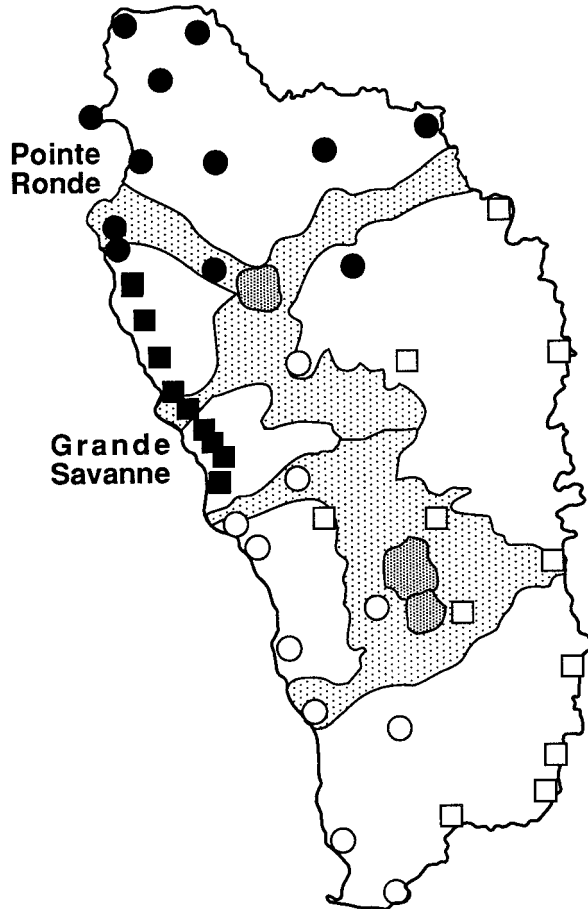


FIG. 5. Geographic distribution of the four clades revealed by the phylogenetic analysis, superimposed on the distribution of major young pyroclastic flow deposits on Dominica, after Sigurdsson and Carey (1980). Heavy stippling represents the major domes and light stippling represents pyroclastic flows. Although the geographic correspondence is visually striking, considerable temporal discrepancy exists (see text for further details).

averaged genetic distances ($r = 0.94$, $P < 0001$), indicating that the phylogenetic structure of the data is strong. PMCT results (Table 2) show that the pattern of distribution of mitochondrial lineages (represented by the patristic distance matrix) are not congruent with the pattern of variation in generalized shape or scalation across the island, but are congruent with color pattern variation in both sexes. Although results using the genetic distance matrix are broadly similar, a slight increase in the strength of the relationship with male shape makes it significant at 5%.

In spite of the latter significant association, regressing out phylogeny does not change the significant correlation between morphological variation and environmental gradients (Table 3) described in previous work (Malhotra and Thorpe 1997a,b). Generalized shape, scalation and color pattern are all significantly correlated with vegetation type in males. In addition, generalized scalation is also significantly correlated with rainfall in males. In females, generalized shape shows no significant correlations, whereas scalation is correlated with rainfall only and color pattern with both vegetation type and rainfall. These conclusions do not change when genetic

TABLE 1. Partial regression coefficients from partial matrix correspondence tests showing the congruence over the whole island between environmental patterns (vegetation, VEG; altitude, ALT; temperature, TEMP; rainfall, RAIN), and patterns of mitochondrial lineages (represented by the patristic distance matrix, PAT). PRO represents geographical proximity and is included to control for spatial effects. Significance of the standardized partial regression coefficients is Bonferroni corrected (by row). Figures in parentheses indicate the number of characters contributing to multivariate character matrices.

CHARACTER	PRO	PAT
VEG (3)	0.2266**	0.0323
ALT	0.2318**	0.0093
TEMP	0.2370**	0.0549
RAIN	0.2365**	0.0487

* $P < 0.05$; ** $P < 0.01$.

distances are used instead of the patristic distances; however, some weaker relationships between female shape and altitude and female scalation and temperature also become significant when genetic distance is used.

DISCUSSION

Historical Vicariance in Anolis oculatus populations

What produces the strong phylogeographic structure that we have shown to be present in the cytochrome *b* sequences of *A. oculatus*? No readily obvious geographic feature that might act as a barrier to anole dispersal exists today (although the central/southern Caribbean lineage boundary appears to be coincident with the position of the Layou River estuary, the largest in Dominica, samples collected from its northern and southern banks to test whether it acts as a barrier only differ from each other by one base pair and both clearly belong to the southern Caribbean lineage). The spatial distribution of mitochondrial lineages over the whole island has

TABLE 2. Partial regression coefficients from partial matrix correspondence tests showing the congruence over the whole island between multivariate morphological patterns and patterns of mitochondrial sequence relatedness (represented by the patristic distance matrix, PAT, on the first line and the genetic distance matrix, GEN, on the second line) for males (A) and females (B). PRO represents geographic proximity and is included to control for spatial effects. Significance of the standardized partial regression coefficients is Bonferroni corrected (by row). Figures in parentheses indicate the number of characters contributing to multivariate character matrices.

CHARACTER	PRO	PAT/GEN
(A)		
Generalized shape (12)	0.0704	0.2167
	0.0880	0.2369*
Generalized scalation (8)	0.2137**	0.0322
	0.2149**	0.0449
Color pattern (38)	0.1984**	0.2598**
	0.2222**	0.2636**
(B)		
Generalized shape (10)	0.0177	0.0699
	0.0339	0.0983
Generalized scalation (7)	0.0318	0.0381
	0.0348	0.0425
Color pattern (29)	0.0230	0.3521*
	0.0454	0.4296**

* $P < 0.05$; ** $P < 0.01$.

TABLE 3. Partial regression coefficients from partial matrix correspondence tests showing that including lineage effects does not change the correlation between morphological and environmental variation. The first row gives the standardized partial regression coefficients and their Bonferroni-corrected significance (sequential by row) with the various causal hypotheses; geographical proximity (PRO), vegetation (VEG), altitude (ALT), temperature (TEMP), and rainfall (RAIN), but without lineage being included. At the next step, the patristic distance matrix (PAT) or the genetic distance matrix (GEN) was included (second and third rows, respectively). PRO is included in all analyses to control for spatial effects. Figures in parentheses indicate the number of characters contributing to multivariate character matrices. (A) males; (B) females.

CHARACTER	PRO	PAT/GEN	VEG	ALT	TEMP	RAIN
(A)						
Generalized shape (12)	0.0979	—	0.2579**	0.2046	-0.0942	-0.0468
	0.0476	0.2157	0.2556**	0.2313	-0.1183	-0.0592
	0.0695	0.2259	0.2415**	0.2259	-0.0981	-0.0619
Generalized scalation (8)	0.2057**	—	0.2231**	0.0461	-0.2427	0.2867*
	0.2001**	0.0238	0.2228**	0.0490	-0.2454	0.2853*
	0.2027*	0.0237	0.2213**	0.0483	-0.2431	0.2851*
Color pattern (38)	0.2336**	—	0.1975*	0.0815	-0.1029	0.0121
	0.1730*	0.2598*	0.1947*	0.1137	-0.1319	-0.0028
	0.2017**	0.2538**	0.1790*	0.1055	-0.1072	-0.0048
(B)						
Generalized shape (10)	0.0512	—	0.1209	0.3024	-0.2336	0.1726
	0.0353	0.0681	0.1201	0.3109†	-0.2412	0.1687
	0.0509	0.0942	0.0984	0.3136*	-0.2342	0.1309
Generalized scalation (7)	0.0758	—	0.1113	0.2121	-0.3127	0.4825**
	0.0697	0.0260	0.1110	0.2153	-0.3156†	0.4810**
	0.0725	0.0260	0.1094	0.2145	-0.3132*	0.4807**
Color pattern (29)	-0.0076	—	0.3934**	0.0641	-0.2270	0.3185*
	-0.0033	0.3380*	0.3898**	0.1059	-0.2647	0.2991*
	0.0255	0.3985**	0.3644**	0.1017	-0.2338	0.2919*

* $P < 0.05$; ** $P < 0.01$.

† $0.06 > P > 0.05$.

also been shown to be incongruent with environmental gradients. This lack of congruence over the whole island contrasts with the patterns described for the central Caribbean coast in Malhotra and Thorpe (1994) and highlights the desirability of considering patterns throughout the species' range and not just a limited part.

Given the geological background of the island already described, historical vicariant events resulting from volcanic activity seem the most likely candidate. Figure 5 shows the distribution of major pyroclastic flows on the island (all of which have occurred within the last 50,000 years) in relation to the distribution of the lineages. Although some congruence exists with the lineage distribution (e.g., the boundary between the northern and central Caribbean lineages roughly matches the position of the Pointe Ronde flow, one of the main channels of the Morne Diablotin eruptions), some marked spatial and temporal discrepancies also exist. An example of the former is the Grande Savanne flow (Fig. 5), which has apparently not affected the distribution or divergence of mtDNA haplotypes in its vicinity. To evaluate temporal congruence, we need to estimate a time scale for the evolution of the lineages. However, the geology of the island has yielded few absolute dates (see Introduction), especially of the older formations. We do know that all the younger islands were formed at a similar time in the late Miocene-early Pliocene (Maury et al. 1990). Even the older islands of the northern Lesser Antilles are only likely to have emerged from a period of submergence (during which extensive limestone deposits were laid down) at around the same time. We assume an upper limit of the time of origin of *A. oculatus* of 10 mya and use the average distance between sequences of *A. oculatus* and its closest relative to give a minimum estimate

of the rate of evolution. This is not biologically unrealistic because it is known that volcanic islands may be colonized relatively soon after they are formed (Carson et al. 1990; Thornton 1996). This gives a rate of 1.43% pairwise divergence per million years, a figure not out of line with rates calculated for other reptiles (Zamudio and Greene 1997), although the small body size and short generation time of *Anolis* might lead one to expect a higher rate (Martin and Palumbi 1993). No evidence suggests that the lineages evolve at different rates, so we can extrapolate to a date for the initial divergence between the central Caribbean lineage and the northwestern lineage of 4.46 ± 0.08 my (calculated from the average linearized distance between the lineages and its standard error, using PHYLTEST). Similarly, the divergence between the southwestern and central-eastern lineages dates to 2.16 mya (± 0.47 my). It is clear that these estimates are very speculative. Higher rates of evolution may have occurred if the islands are younger than assumed (e.g., 5 my suggested by Maury et al. 1990) or if a substantial time-lag existed between the origin of the island and its colonization. However, unrealistic rates of pairwise evolution (an order of magnitude higher than that suggested above) would be required for the divergence between the clades to match the dates of the lava flows depicted in Figure 5. It is therefore clear that these lava flows cannot explain the formation of the highly distinct lineages on the island. This is a similar situation to organisms from various parts of the world whose current distribution has been explained by expansion from putative Pleistocene climatic refugia, but whose divergence has been found to considerably predate these events (Joseph et al. 1995; Taberlet et al. 1998).

The map in Figure 5 also does not give any indication of

the relative magnitude of the volcanic events associated with each deposit. The Grande Savanne event was a comparatively minor block and ash flow (Sparks et al. 1980), whereas the flows around Morne Trois Piton/Morne Macaque resulted from the extremely violent eruption of 28,000 years ago. It seems at first sight highly unlikely that anything on the island would have survived such a cataclysmic event, but there is no disputing the fact that the island cannot have been sterilized by this event. Apart from *A. ocellatus*, there are several other endemic animals and plants, including two species of *Amazona* parrots. Nevertheless, life in the immediate vicinity is likely to have been exterminated, and all life on the island is likely to have been reduced to isolated pockets. If volcanic events have been influential in shaping the phylogeographic structure of this species, this eruption is a good candidate for detecting these effects. Although the lower within-population diversity within locality 12 and the wider geographic distribution of haplotypes in the southern Caribbean lineage are suggestive of a recent expansion from a period of isolation, the loss of diversity is not as great as one might expect from the recency of the event. This suggests that even bottlenecks that follow such catastrophic events may not be severe. Even small pockets of habitat may harbor large populations of anoles, given their potential to exist in high population densities (Schoener and Schoener 1980, 1982). Because volcanic deposits can weather very quickly in the moist tropics and may be recolonized rapidly on an evolutionary timescale, these bottlenecks may also be short in duration. This is certainly likely to be true for small-scale events, such as the Grande Savanne flow; even events that are perceived as disastrous from a human perspective (such as the 1903 Morne Pelee eruption on Martinique) may be relatively insignificant in terms of the population history of anoles. This study therefore raises interesting questions about what did produce the strong genetic structure seen in this, and other, species of anole. Rather than any single event being responsible, it may be due to repeated cycles of population expansion and contraction associated with different volcanic events (possibly, but not necessarily, involving the same centers).

Another interesting pattern observed is the large between-locality heterogeneity in mitochondrial haplotypes, especially given the small spatial scales associated with this study. Sex-biased gene flow is an appealing explanation because it could also explain the maintenance of the strong mitochondrial phylogeographic structure in the absence of current barriers to gene flow. A similar situation arises in macaques (Hoelzer et al. 1994) among other species, as a result of extreme site fidelity in females with most of the dispersal being carried out by males. Little direct data supports the existence of male-biased gene flow in anoles (but see Schoener and Schoener 1980). However, female anoles from a number of species are known to have smaller territories than males, and these may overlap at high female densities (Stamps 1973; Fleming and Hooker 1975; Schoener and Schoener 1982), whereas male territories tend to be larger and nonoverlapping. Assuming a 1:1 sex ratio, this could force males to move further afield to establish territories. Migration is likely to occur mostly at the juvenile stage, because adults of both sexes appear to be largely sedentary (Ruibal and Philibosian 1974). We are currently critically

examining the hypothesis of sex-differential gene flow by screening hypervariable microsatellites to measure gene flow at nuclear loci.

Extent of Congruence between Morphological and Mitochondrial Patterns

Although the correlations between morphological and environmental gradients described in earlier work strongly suggested that selection was important in driving morphological evolution in this species, it remained possible that the lack of a phylogenetic framework in these studies may have confounded this interpretation. The incorporation of this framework has continued to support the significance of the role of selection. The incongruence of phylogenetic relationships with the patterns of variation in body dimensions and scalation leads to the conclusion that vicariance has played a minor role in morphological divergence in this species. However, this interpretation has to be modified for color pattern characters, which appear to retain the phylogenetic signal to a greater extent (although it is important to note that this still does not negate the importance of environmental selection). No a priori reason suggests that different characters under selection must evolve in the same way, because the selection pressures on different systems may be different. However, the nature of color pattern lends itself to categorical divisions more than the other character systems (Malhotra 1992), and these categories may at least partly coincide with lineages. Nevertheless, a real underlying relationship between color pattern and phylogeny may exist as a result of the role of color pattern as a signal for both attracting and escaping attention. The selection pressure acting on it is a function of the visual systems of the intended recipient of the signal (Endler 1992; Johnstone 1997); thus, there may be several alternative ways of achieving the same visual balance (between conspicuousness and crypsis). However, there are not likely to be as many optimal solutions to a water balance or locomotor efficiency problem. Indeed, the many parallels that are found between anole scalation and body shape between species evolving in parallel on independent islands suggests that multiple optima do not exist (Malhotra and Thorpe 1994, unpubl. data; Losos et al. 1998). Color pattern variation may be freer to reflect small differences that originate during periods of isolation even among populations in similar environments. This may come to characterize different lineages, thus leading to an association between phylogeny and color pattern. However, some differences in scalation and body shape between northern and southern Caribbean populations do exist, although they occupy the same habitat (by the criteria used to assess this here). The above logic implies that one of the character combinations is nonoptimal for that environment. Apart from the observation that southern Caribbean populations have unusually low densities, we have no evidence to support or refute this.

The abrupt change between the two morphological types in the central Caribbean coast, which is congruent with the boundary between deeply divergent lineages, suggests restricted gene flow at nuclear as well as mitochondrial loci. If the morphological differences between the populations extend to behavioral characters also, it may be sufficient to

cause some degree of reproductive isolation because visual and behavioral cues are important in courtship in anoles (Jenssen 1970; Case 1990; Giannasi 1997). Further work on the island will focus on this transition zone. Finally, we note that the existence of a series of independent islands in the Lesser Antilles, each with similarly complex ecological and geological backgrounds but its own endemic anole species, offers the prospect of independent replicates with which to explore the processes leading to population divergence and speciation.

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