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Molecular and morphological evolution within small islands

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SUMMARY

Although explicit rules can be used to hypothesise an inter-island colonization sequence from a molecular phylogeny, and statistical methods can be used to assist in partitioning historical factors from current selective factors, this latter process has limited efficacy where there are a limited number of islands. Studying numerous populations within small, but heterogenous, islands allows a better understanding of the factors causing geographic variation. Three main approaches have been used to study within-island geographic variation using lizards on Canarian and Lesser Antillean archipelagos: (i) matrix correspondence tests and their partial regression/correlation extensions on morphological and molecular data; (ii) identification of within island patterns of morphological geographic variation paralleled on independent islands; and (iii) large scale field experiments on selection. These studies reveal that, even on small islands, 'island populations' may not be homogeneous in morphology, or molecular phylogeny, and that natural selection for current ecological conditions appears to be a primary force influencing morphological population differentiation, irrespective of phylogenetic history.

1. INTRODUCTION

Many studies of evolution on islands, from Darwin's (1859) time onwards, have tended to emphasise inter-island differences. However, inter-island studies (although very popular) are generally not in a position to contribute much to an understanding of relative importance of historical processes and selection on their own. There have been some efforts to distinguish between the roles of historical factors and current selection pressures (Snell *et al.* 1984; Gardner 1986), but they do not use the appropriate methodology to make much headway with this problem.

Some of the difficulties involved are exemplified by the inter-island differences in the endemic western Canary Island lacertid *Gallotia galloti*. The distinct differences in scalation, colour-pattern and body dimensions could be due to historical factors like founder effects and drift, or to adaptation to the current ecological conditions that differ among islands (Thorpe 1996). Molecular data, such as mtDNA sequence and restriction fragment length polymorphism (RFLPS) (which are hopefully minimally confounded by selection effects) can be used to reconstruct a phylogeny (Thorpe *et al.* 1994*a*). The western Canaries have not been joined to one another or the mainland (Carracedo 1979, and references therein). Consequently, any organism naturally distributed across them must have undergone inter-island dispersal. Given this geological background a rigorous set of rules can be used to convert this phylogeny into a colonization sequence (Thorpe et al. 1994*a*; Juan *et al.* 1995). *G. galloti* appears to have arisen on the oldest western island, and colonized the younger islands further to the west. The colonization time for each island inferred from the DNA divergence is appropriately less than the geological time of origin of each island (Thorpe *et al.* 1994*a*).

Once a quantifiable perspective of the historical relationships (with minimal selection effects) is obtained, one can test for adaptation taking into account these historical relationships using partial regression based matrix correspondence tests (see below). Historical relationships may be represented in a variety of ways, including as a matrix of patristic distances among taxa along the branches of a molecular phylogeny (Thorpe *et al.* 1995, 1996; Thorpe 1996; but see also Douglas & Matthews 1992). When there are only a few islands, as in the western Canaries, and each island is generally being treated as an evolutionary entity, one can only obtain a preliminary and limited answer to the roles played by various evolutionary processes (Thorpe 1996). For example, the presence or absence of blue leg spots in western Canarian lacertids appears to reflect phylogenetic relationships, irrespective of selection for environmental/climatic conditions; yellow dorsal bars appear to be associated with selection for wet climates, irrespective of history or other ecological conditions; and body size appears to associated with how depauperate the environment is, irrespective of history or climatic factors (Thorpe 1996).

Although inter-island studies will remain popular and important, there are inescapable limitations to using a few islands as single entities when trying to investigate the relative contribution of current natural selection and historical factors. More progress can be made by considering islands with habitat differentiation or zonation within them as the heterogeneous entities that they are, and investigating population differentiation among numerous local populations (Thorpe & Brown 1989; Brown *et al.* 1991; Malhotra

& Thorpe 1991*a*; Castellano *et al.* 1994; Prentice *et al.* 1995; Thorpe *et al.* 1996). Small islands may offer distinct advantages for these studies of microgeographic variation. Biotic and physical factors pertinent to natural selection may vary substantially over very short geographic distances, which gives considerable logistic advantages over mainland systems where considerable distances may be involved. The general trend is to have a depauperate fauna with few species, which may mean that individual species are found in densities far greater than on mainland systems. This facilitates sampling and may allow exhaustive coverage of the species range over all habitat types.

This paper surveys our studies of within-island microgeographic variation that use lizards as model organisms. This work has primarily been carried out in the Canarian and Lesser Antillean archipelagos and can be considered under three rubrics: (i) matrix correlation tests for association between observed patterns and patterns generated by putative causal factors; (ii) investigations of parallel patterns of variation; and (iii) large scale field experiments on natural selection.

2. MATRIX CORRESPONDENCE (MANTEL) TESTS AND MOLECULAR STUDIES

Tests of a null hypothesis of no association between an observed pattern of geographic variation and a pattern generated from one or more causal hypotheses are useful in that they enable hypotheses to be rejected. When dealing with geographic patterns, both patterns and hypotheses can conveniently (and in some cases must) be represented as a matrix of dissimilarities between entities (e.g. local demes). The correspondence between the matrices can be measured by a statistic such as a correlation or regression, but the probability of the null hypotheses cannot be tabulated because the elements of a matrix are not independent and the degrees of freedom are unknown. With large matrices, which cannot be exhaustively permutated, the rows and columns of one of the matrices (Manly 1986*a*, *b*, 1991) can be randomized and the statistic recomputed. This is repeated a large number of times (e.g. 10000-fold in Thorpe *et al.* 1996) to give a distribution of the statistic so that the probability of the null hypothesis of no association can be found. Where there are several hypotheses, the patterns generated by them may be intercorrelated. To overcome this, partial correlation, or partial regression extensions of the test are used (Smouse *et al.* 1986) where the observed pattern (e.g. in morphology) is taken as the dependant variable and patterns generated from the hypotheses are taken as the independent variables (Manly 1986*b*; Thorpe & Baez 1993).

These tests are making an important contribution to studies at the intraspecific level and have recently had a high profile (Brown *et al.* 1991; Sokal *et al.* 1991; Waddle 1994; Daltry *et al.* 1996; see also a review by Smouse & Long 1992). Two examples of their application to within island geographic variation are given here: (i) Tenerife lacertids; and (ii) Dominican anoles.

(*a*) *Geographic variation of the lacertid* G. galloti *within Tenerife*

The colour pattern of sexually mature male lacertids varies markedly across Tenerife. The variation in six colour pattern characters which can be treated individually or combined by multivariate analysis (Thorpe & Brown 1989; Thorpe *et al.* 1994*b*). Thorpe *et al.* (1994*b*) used partial regression matrix correspondence (PRMC) methods to test the association of the pattern of geographic variation in the colour pattern, across 67 localities, against several hypotheses including: (i) historical separation of ancient precursor islands; (ii) a cloud layer around Teide inducing separation of high altitude populations from low altitude populations; (iii) adaptation to altitude; (iv) adaptation to two latitudinal climatic/vegetational biotopes which meet along a sharp ecotone; and (vi) geographic proximity representing the opportunity for gene flow and unspecified geographical components. All hypotheses except for adaptation to the climatic/vegetational biotopes can be rejected. It appears that the colour pattern of sexually mature males may be a balance between crypsis to avoid predation (overhead avian predators see the dorsum with its disruptive yellow bars in the north) and sexual selection for lateral, blue, display markings (for laterally positioned conspecifers).

Later studies of the molecular affinities of these Tenerife populations (across largely the same set of localities) by Thorpe *et al.* (1996), using cytochrome b sequence data, revealed three main haplotypes which, when subjected to an outgroup rooted phylogenetic analysis, revealed eastern and western lineages. Historical relationships, whether derived from molecular or other data (Sokal *et al.* 1991; Waddle 1994), can also be tested against alternative historical scenarios. In this case the historical relationships are represented by the molecular phylogenetic affinities (patristic distances among populations on the molecular phylogenetic tree). These were treated as the observed (dependant) pattern. Several alternative historical hypotheses can be tested. These hypotheses are primarily based on the concept of populations existing on the ancient precursor islands that formed Tenerife (Ancochea *et al.* 1990). Patterns generated from these hypotheses are represented as independent variables in a PRMC test. A set of three similar patterns represented hypotheses involving a single western precursor and an eastern precursor, a fourth pattern represented separate northwestern and southwestern precursors, a fifth pattern represented three separate precursors, one on each of the three ancient areas, a sixth pattern representing cloud induced vicariance, with a seventh matrix representing geographic proximity. A series of pairwise matrix correspondence tests followed by a PRMC tests allows one to reject all hypotheses other than that the pattern was formed by expansion from an ancient western and an ancient eastern precursor island (Thorpe *et al.* 1996).

The earlier colour pattern studies made no allowance for different molecular phylogenetic lineages being present on the island. When this is allowed for by: (i)

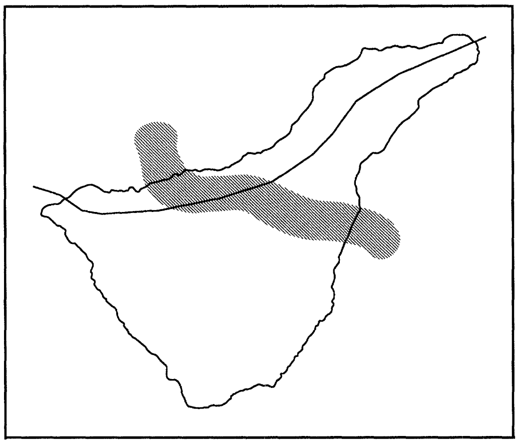

Figure 1. Colour pattern variation and DNA lineages of *G. galloti* on Tenerife. The thin line indicates the transition between northern and southern colour morphs (Thorpe & Brown 1989) which is associated with an ecotone between different climatic/vegetational biotopes. The shaded band indicates the western and northeastern mtDNA lineages, bearing in mind that some populations contain different haplotypes (Thorpe *et al.* 1996).

testing colour pattern against biotope and geographic proximity within the eastern and western lineage separately; and (ii) testing colour pattern against climatic biotopes, geographic proximity and phylogenetic patristic distances in a PRMC test (see Thorpe *et al.* 1996, but also Thorpe *et al.* 1995; Daltry *et al.* 1996; Thorpe 1996) then there is still an association between colour pattern of sexually mature males and biotope.

The eastern and western lineages revealed in the clonal mtDNA appear to have introgressed completely as there is no indication of reproductive isolation between these east–west lineages in previous quantitative morphological studies of scalation, shape (Thorpe & Baez 1987), size (Thorpe & Brown 1991), or colour (Thorpe & Brown 1989). Indeed, natural selection of the colour pattern for current biotopes appears to have largely eradicated historical effects. The molecular lineages have an east–west pattern while colour pattern has a marked north–south pattern irrespective of lineage (figure 1). DNA times (which are compatible with geological times; Thorpe *et al.* 1996) suggest this introgression occurred after ca. 700000 years separation.

(*b*) *Dominican anole*, Anolis oculatus

Anolis oculatus is the only anoline lizard on Dominica, a young volcanic island in the Lesser Antillean island chain fringing the eastern Caribbean. Dominica possesses a diverse set of climatic and vegetational regimes with littoral woodland on the Atlantic coast, xeric woodland on the Caribbean coast and montane rain forest and cloud forest in the extremely mountainous centre. Anoles were sampled from 33 localities across the island and showed pronounced geographic variation in the 47 morphological characters (body shape, scalation and colour pattern) studied (figure 2).

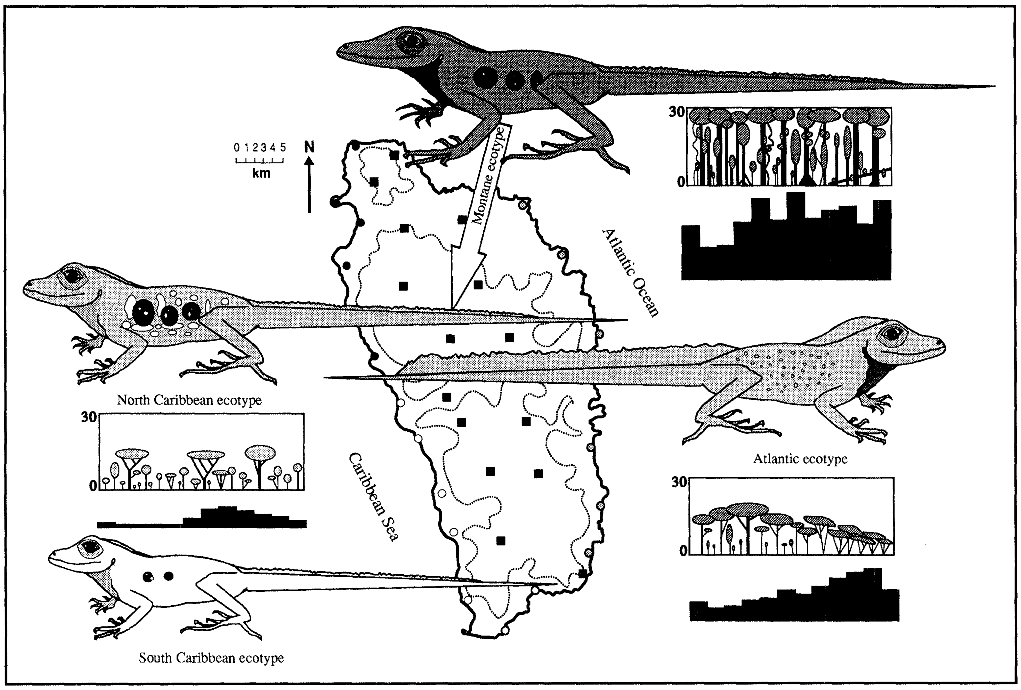

Figure 2. Map of Dominica, indicating the sites from which *Anolis oculatus* morphology and DNA were sampled. The type of vegetation at these sites is illustrated by the following symbols: a filled square indicates evergreen rainforest; filled circles indicate evergreen littoral woodland; open circles indicate seasonal xeric woodland; and grey circles indicates a transition between the latter two. The dashed line on the map indicates the 300 m contour. Around the map some of the visually obvious geographic variation in the size, shape and colour pattern of the lizards is illustrated; there is also significant variation in other characters such as number of scales around the body. A diagrammatic representation of the canopy height (in metres) and structure in the different vegetation zones associated with these ecotypes is given and barcharts illustrate the relative amount and seasonal distribution of rainfall (Lang 1967).

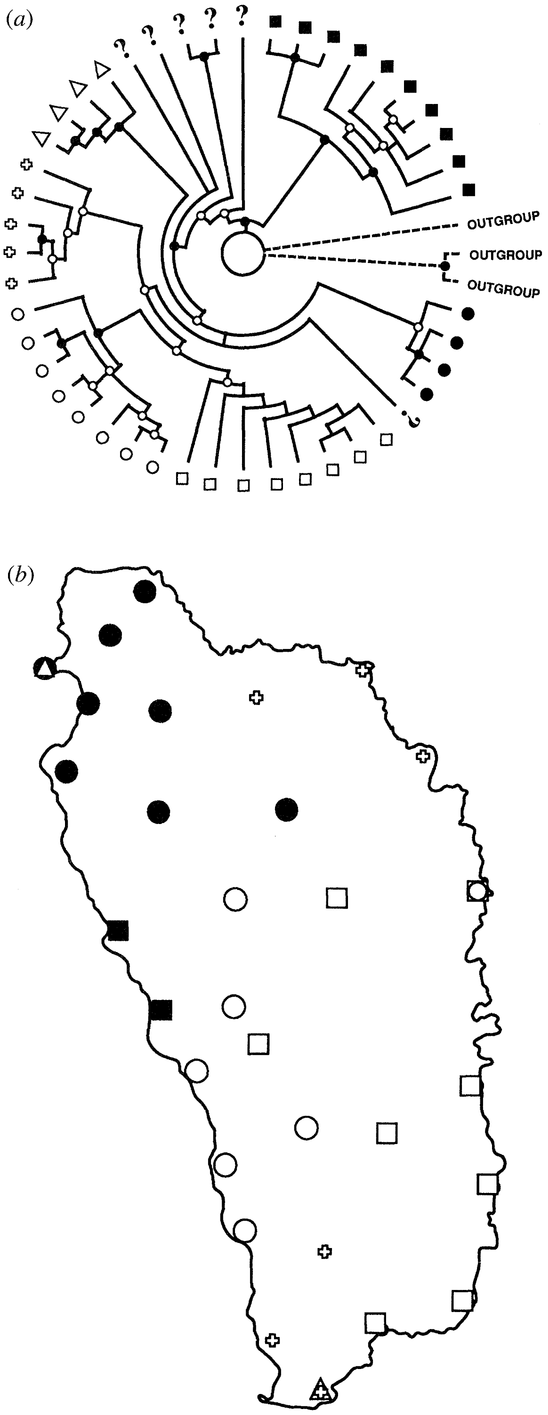

Figure 3. (*a*) 50% consensus parsimony tree, showing relationships between mitochondrial cytochrome b haplotypes of *Anolis oculatus*. Although there are a very large number of equally parsimonious trees, most of the nodes are well supported. The level of support for the different nodes is indicated by a black (100%) or white dot (> 80%). Nodes shown as resolved but without a symbol are represented in between 50 and 80% of trees. (*b*) Map of Dominica illustrating the geographic position of the haplotype lineages shown in (*a*). Individual haplotypes not clearly belonging to any major group (indicated by ? in figure 3*a*) are not plotted. Filled squares indicate central Caribbean; filled circles indicate northwest; open circles indicate southwest; open squares indicate central-east; open crosses indicate south/northeast; and open triangles indicate south/northwest.

The 'overall' similarity among samples of females using 47 morphological characters is strongly associated ($P < 0.0001$) with the overall ecological similarity among localities (using altitude, rainfall, temperature and vegetation; Malhotra & Thorpe 1991*a*) when tested with a partial matrix correspondence test which also includes geographic proximity.

A more detailed picture can be obtained by using PRMC tests to test multivariate character sets such as body shape, scalation and colour pattern (as dependant variables) against a series of independent variables i.e. geographic proximity, altitude, temperature, rainfall and vegetation type. This shows that, using locality means, general body shape and colour are related to vegetation and general scalation is related to rainfall (Thorpe *et al.* 1994*b*, Malhotra & Thorpe 1997*a*, *b*; A. Malhotra & R. S. Thorpe, unpublished data). When individual characters are tested they generally conform to the multivariate sets, but with some exceptions. For example, relative size of enlarged lateral scales is associated with vegetation type, not rainfall (Malhotra & Thorpe 1997*a*), whereas the cyan element of body hues is related to rainfall, not vegetation type (A. Malhotra & R. S. Thorpe, unpublished data) (see also Thorpe *et al.* 1994*b*).

Generally there is rather limited congruence in geographic variation in individual characters and the extent of morphological difference is commensurate with the extent of ecological difference. However, along the Caribbean coast the morphological change is marked while the ecological change is subtle. Moreover, there is a higher degree of congruence among characters in this area. This raises the question as to whether there is a phylogenetic/historical component to this geographic variation along the Caribbean coast.

A 267 base pair section of the mtDNA cytochrome b gene was sequenced which showed 22% variability across the 33 localities. A phylogenetic tree (figure 3) was reconstructed using both parsimony and distance-based methods, which gave very similar results. Because of the high variability, there were many equally parsimonious reconstructions, but a 50% consensus tree was resolved to a surprisingly high degree (figure 3*a*), and revealed the presence of several lineages. The most basal split is the best supported (appearing in 100% of all trees) and would have occurred 4 Ma BP (using a rate of 2.5% Ma^{-1}). Populations belonging to this lineage are found in the central part of the Caribbean coast (figure 3*b*) and the southern edge of its range may be close to the transition between north and south Caribbean ecotypes. Other lineages are more closely related, but also have a degree of geographical coherence when mapped. Nevertheless, the phylogenetic relationships do not adequately explain the morphological difference between north and south Caribbean coast populations. The geographical distribution of lineages shows no relationship to current or known past barriers to gene flow (i.e. position of lava flows). The most divergent populations in terms of mitochondrial sequence are completely introgressed morphologically with populations further north. Although the morphologically differentiated southern Caribbean coast populations do belong to a single lineage, it appears to be relatively recently derived, and its only peculiar feature is that it has a lower haplotype diversity compared to other lineages, but this may simply be a consequence of the somewhat lower population densities in this part of the island.

Moreover, when a 'phylogenetic' distance matrix derived from this sequence information was added to partial matrix correspondence tests comparing generalized morphological (47 characters of females) with generalized ecology and geographic proximity, then morphology remains associated with ecology ($P < 0.0001$ with both the inclusion and exclusion of phylogeny), whereas generalized morphology is not closely associated with phylogeny ($P = 0.0168$), or proximity ($P = 0.2385$). The null hypotheses of no association has to be accepted for the last two variables after Bonferroni correction across the three independent variables ($0.05/3 = 0.0167$). Each character system is associated with an aspect of the ecology. This association is not changed when a partial matrix correspondence test is used to test each system in turn against the pertinent ecological factor together with geographic proximity and DNA phylogeny i.e. colour pattern and body proportions are still associated with vegetation ($P < 0.0001$, $P < 0.0011$, respectively), and scalation is associated with rainfall ($P < 0.0001$). These tests show no association between the DNA phylogeny and scalation ($P = 0.7200$), or body proportions ($P = 0.7200$), although there is a significant (but slight) association between DNA phylogeny and colour pattern ($P = 0.0151$, respectively) even after Bonferroni correction.

Consequently, the population phylogeny may contribute something to the understanding of the morphological differentiation, but the overwhelming factor appears to be natural selection for current ecological conditions.

3. PARALLELS

Several species on the same island may show similar patterns of geographic variation. For example in Tenerife the lacertid, *G. galloti* (Thorpe & Brown 1989, 1991), the gecko, *Tarentola delallandi* (Thorpe 1991), and the skink, *Chalcides viridanus* (Brown *et al.* 1993) show similar latitudinal patterns. In Dominica the anole, *Anolis oculatus* (Malhotra & Thorpe 1991), the iguana, *Iguana delicatissima* (M. Day, unpublished data), and the ground lizard, *Ameiva fuscata* (Malhotra & Thorpe 1996) show, to varying degrees, a longitudinal pattern of geographic variation with differences between the Caribbean and Atlantic coasts. Similarly, on Gran Canaria the lacertid, *G. stehlini* (Thorpe & Baez 1993), the gecko, *Tarentola boettgeri* (R. S. Thorpe, unpublished data) and the skink, *C. sexlineatus* (Brown & Thorpe 1991*a*, *b*) all show a latitudinal pattern of geographic variation.

These similar patterns within a single island are, on their own, of limited utility in differentiating between historical vicariance and selection for current ecological conditions. This is because organisms on a common island may have been subject to both the same historical/geological processes at a given time, and the same ecological differentiation. However, when there are islands within archipelagos which have independent histories but common ecological zonation, then parallel patterns of geographic variation and directions of character state change, among different species on different islands argue for natural selection for adaptation to this zonation because it is the ecological zonation they have in common, not their internal history. Two examples have been elucidated using lizards, the Canary Island skinks and the Lesser Antillean anoles.

(*a*) *Canary Island skinks*

The high elevation islands of Tenerife and Gran Canaria, in the Canarian archipelago, both receive wind-borne rain predominantly from the north. This results in similar ecological zonation in both islands, with a lush, warm, humid habitat on the north facing slopes (below the 1500 m inversion level in Tenerife) and a barren, hot, arid habitat in the south. The skink, *C. sexlineatus*, shows very pronounced geographic variation across 47 localities within Gran Canaria, in its scalation (Brown & Thorpe 1991*a*), body shape and size (Brown & Thorpe 1991*a*) and colour pattern (Brown & Thorpe 1991*b*). Similarly, the skink, *C. viridanus* shows geographic variation across 17 localities within Tenerife (Brown *et al.* 1993). The colour pattern variation is particularly noticeable, and in both islands the pattern of geographic variation is shown only to be associated with these climatic biotopes when PRMC tests are employed (Brown *et al.* 1991). In both islands, skinks (both males and females) from the north have brown tails, whereas those in the south have bright blue tails. Tail autotomy is known to be an antipredator mechanism in lizards, which may be more effective when the tail is conspicuously coloured (Cooper & Vitt 1986). This parallel change in character state, in concert with parallel change in ecological conditions on two independent islands, provides support for the role of natural selection in adapting tail colour for different antipredator strategies in the different habitat types.

(*b*) *Lesser Antillean anoles*

The central Lesser Antilles are a series of high altitude islands with parallel ecological zonation that offers opportunities to investigate parallel patterns in their endemic anoles. Parallel patterns in the morphology of *Anolis oculatus* on Dominica and *Anolis marmoratus* on the neighbouring island of Basse Terre (Guadeloupe) were investigated. Basse Terre belongs to the same period of orogenesis as Dominica and is very similar in topography, climate and vegetation. In essence, it is a mirror image of Dominica, as the highest mountain is in the south of Basse Terre, but in the north of Dominica. Thus the rain shadow effect results in the southwest coast of Basse Terre, and the northwest coast of Dominica, being the driest regions of the respective islands. As well as being closely related, these anoles are ecologically similar. Both are solitary species which are widely distributed in a number of different habitats and show a wide range of morphological variation.

The 17 morphological characters (colour pattern, scalation and body dimensions) recorded were selected from an analysis of morphological variation in *A. oculatus* on the basis of high between-locality F-ratios and large squared multiple correlations with ecological variables. Finally, characters showing clear homologies with *A. oculatus* were favoured. This was particularly relevant for colour pattern characters. Males from 25 localities in Basse Terre were investigated and morphological distance matrices representing multivariate generalizations of the three character systems were derived. These dependant variable matrices were each compared with several independent variable matrices representing environmental variation (as described above) using a PRMC test.

The relationship between morphology and environmental variation found in *A. marmoratus* were similar to those found in *A. oculatus*. In both species, generalized scalation is correlated with rainfall and generalized colour pattern with vegetation type ($P < 0.0005$). There are also parallel state changes in individual characters; body size is significantly correlated with both altitude and rainfall, the number of body scales is significantly correlated with rainfall and altitude, and the number of spots with the occurrence of dry scrub woodland (Malhotra & Thorpe 1994).

The parallel variation strongly suggest that natural selection is responsible for determining morphological geographic variation in these anoles. It may also give us some insight into the cause of differentiation of southern Caribbean coast populations of *A. oculatus* in Dominica, as a corresponding parallel cline is observed on the Caribbean coast of Basse Terre (Malhotra & Thorpe 1994). This suggests a non-historical cause common to both species, and raises the possibility that an ecological factor that is important to the lizard, but is not obvious to humans, does vary along the Caribbean coast of both islands. An intriguing twist to this parallel variation is added by the parallel variation in cytochrome b sequence of these two species along the Caribbean coast (Malhotra & Thorpe 1994). The sequence variation is congruent with some morphological and ecological clines. At first sight this conflicts with the conventional interpretation of mtDNA variation as reflecting historical changes rather than selection effects. However, a fuller understanding will require a detailed study of cytochrome b variation across both islands.

Other islands in the Lesser Antilles present the opportunity for further tests of parallel variation. A series of islands of independent origin, sharing the same climatic patterns, and having a single endemic species of anole, are present. However, the orogenetic history is more complex for some (e.g. St Lucia, Martinique) and the contrast between habitat types may be less pronounced for smaller, less mountainous islands (e.g. Montserrat). Nevertheless, preliminary results from studies in progress on these islands indicate that there are some parallels in common across all these species. For example, male *Anolis luciae* (St Lucia) show a parallel association between patterns of variation in generalized colour pattern and vegetation ($P < 0.0100$) as do both sexes of *Anolis lividus* (Montserrat) ($P < 0.0052$ males, $P < 0.0004$ females). The latter species also shows a parallel association between scalation and moisture levels in females ($P < 0.0090$).

4. FIELD EXPERIMENTS ON SELECTION

Relatively few rigorously tested, direct demonstrations of current selection in natural populations exist (Endler 1986; but see Halkka & Raatikainen 1975; Knights 1979; Endler 1980; Price *et al* 1984). The aim of this manipulative field experiment was to provide such a demonstration of the action of natural selection on morphological variation in *A. oculatus*.

Anolis oculatus, despite being a relatively *k*-selected anole (Andrews 1979), has a relatively short generation time, with juveniles reaching sexual maturity in under a year. Other features of its population structure that make it especially suitable for such a study are its extremely high population density, territorial and relatively terrestrial behaviour and striking degree of phenotypic variation. Because the geographic variation in morphology relates to the four ecological zones, the extremes of the continuum are referred to as ecotypes. The relative ecological difference between the habitats indicates the north and south Caribbean coast habitats are very similar, and the Atlantic coast habitat is somewhat intermediate between the former and that of the Montane habitat.

In this experiment, large-scale lizard-proof enclosures were constructed in two different habitats. Four enclosures were constructed (Malhotra & Thorpe 1993) in dry scrub woodland on the northern Caribbean (west) coast, and samples of four source populations (representing the four ecotypes) were translocated into these enclosures. One enclosure contained a 'resident' control, which was subjected to the same procedures as translocated 'foreign' ecotypes. Similarly, two enclosures were constructed on the Atlantic (east) coast containing one resident control population and one translocated population from the Caribbean coast, thus providing a partial reciprocal experiment. Before marking and releasing into the appropriate enclosure, ten morphological characters (Malhotra & Thorpe 1991*b*) were recorded from each lizard (which was individually marked by toe clipping). This multivariate phenotypic profile was later used to compare morphology of survivors and non-survivors (lizards were not remeasured). The west-coast enclosures (1 to 4) were stocked in June/July 1990 (at the start of the wet season) and monitored in September 1990. The two east-coast enclosures (5 and 6) were stocked in September 1990 and monitored in February 1991.

Multivariate morphological differences between survivors and non-survivors were tested. As the morphological differences between sexes and between ecotypes also needed to be taken into account, a three-way multivariate analysis of variance (MANOVA) was used initially for the west-coast experiment. The model included interactions between sex (male or female), survival (survivor or non-survivor), and ecotype (North Caribbean, South Caribbean, Montane, Atlantic). The interaction between survival and ecotype reveals whether the magnitude of morphological difference between survivors and non-survivors is greater in some enclosures than others. A canonical variate analysis was then performed on all groups for each experiment (4 ecotypes × 2 sexes × survival/non-survival = 16 groups for the west-coast experiment and 8 groups for the east-coast experiment). Because the ecotypes differ considerably in size, a possible bias may be introduced into the analysis. Although canonical analysis takes into account the intercorrelating effects of size, this was checked by size-adjusting the linear measurements before the analysis, and repeating this with SVL included and excluded. In all cases, the results were unaltered (Thorpe & Malhotra 1992).

(*a*) *West-coast experiment*

The results of a three-way MANOVA show a highly significant interaction exists between enclosure and survival versus non-survival ($P < 0.001$). This implies the existence of varying selection intensity between the ecotypes. The multivariate distance (Mahalonobis D^2) between the morphology of survivors and non-survivors of each ecotype was obtained from the canonical analysis. After only two months, the montane population was already showing significant differences between morphology of survivors and non-survivors ($P < 0.01$ for males, and $P < 0.001$ for females) (Malhotra & Thorpe 1991*b*).

To examine the relationship between selection intensity (represented by the extent of morphological separation between survivors and non-survivors of each ecotype) and the extent of ecological change experienced by the translocated populations more rigorously, the ecological dissimilarity was plotted against the morphological dissimilarity (D^2) averaged across the sexes (see also Malhotra & Thorpe 1991*b*). A curve of best fit to the four data points gave a correlation of 1.0000, which is significant ($P < 0.01$) even with the one degree of freedom left by adopting this curvilinear model (Thorpe & Malhotra 1992). This suggests that the intensity of selection on the different population was strongly dependent on the magnitude of ecological change experienced.

(*b*) *East-coast experiment*

Few animals in the transferred population survived till the first monitoring session. Even so, in males, there is a significant difference between the morphology of survivors and non-survivors of the translocated North Caribbean ecotype ($D^2 = 8.46$, $P < 0.05$), but not in the control littoral woodland ecotype ($D^2 = 1.20$, $P > 0.05$) (Malhotra 1992).

There is much discussion of the role, mode of action, and rate of natural selection in evolution (Endler 1989). This experiment, designed to run over a long-term period, unexpectedly demonstrated that significant mortality selection can occur over a very short time scale within single generations of perturbed populations. Two points argue strongly for the differences among ecotypes being maintained by natural selection for current ecological conditions. First, both the west and east-coast experiments indicate a significant difference in morphology of survivors and non-survivors of critical translocated ecotypes but no difference in the control ecotypes. Second, the west-coast experiment indicates a correlation between the extent of morphological difference between survivors and non-survivors and the extent of ecological difference between the enclosure habitat and the habitat from where they were translocated.

5. CONCLUSIONS

This overview reveals that even on small islands a species may show pronounced morphological differentiation and distinct, sometimes deep, molecular phylogenetic divisions. A more complete understanding of nature of the geographic variation comes from a combination of molecular and morphological studies. However, patterns may only be revealed by sampling at numerous localities across the entire island. Sampling restricted areas (Malhotra & Thorpe 1994) may not be capable of fully revealing the pattern or underlying process. Coarse sampling, or using conventional subspecies as operational entities is also unlikely to give a sufficiently detailed picture to be of much value and may be positively misleading.

Evidence from hypothesis testing, using matrix correspondence (Mantel) tests of both molecular and morphological data, together with a study or parallel patterns on independent islands and translocation experiments all suggest that natural selection for current ecological conditions is the primary factor determining morphological geographic differentiation in small islands. This appears to be the case irrespective of molecular phylogenetic history.

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