CHAPTER
8

Testing ecological and phylogenetic hypotheses in microevolutionary studies

R.S. THORPE, R.P. BROWN, M. DAY, A. MALHOTRA,
D.P. McGREGOR & W. WÜSTER

CONTENTS

Keywords: Geographic variation – Mantel tests – DNA phylogenetics – field experiments – parallel selection.

Abstract

The patterns of variation among populations may reflect ecogenetic adaptation by natural selection for current ecological conditions. However, the geographic variation may also reflect phylogenesis when past events, such as vicariance, have had an influence on the population differentiation. The influence of these causative factors can be difficult to disentangle. A range of procedures for determining the causative factors in morphological variation are investigated in relation to geographic variation in reptiles. These include: quantitative tests (including tests of anagenesis in trees, randomization tests of congruence, Mantel tests, partial Mantel tests and canonical correlations); parallel patterns on adjacent islands which have parallel ecological

---

Phylogenetics and Ecology
ISBN 0-12-232990-2

differentiation but independent geological histories; large-scale field experiments which indicate the role of natural selection in relation to ecological conditions; and the role of molecular phylogenies in helping to determine the cause of geographic variation in morphology.

INTRODUCTION

The interaction between ecology and phylogeny has a particular meaning for studies of geographic variation, because it is at this intraspecific level that these two forces can directly combine to influence allele frequency, and hence evolution. Previous publications (Thorpe, 1991; Thorpe *et al.*, 1991) have expanded on the idea that one can usefully think of geographic variation within a species as being influenced by these two forces, that is ecogenesis (natural selection for current ecological conditions) and phylogenesis (past, historical events, including the effects of genetic drift and selectively neutral mutations).

This conceptualization can be facilitated by examples. Species recently introduced to new areas that have clearly differentiated geographically supply examples of ecogenesis, as historical events can have had little, or no, influence on the geographic pattern of differentiation in their new regime. Examples of this are sparrows in North America (Johnston & Selander, 1971) and New Zealand (Baker, 1980), Mynas in New Zealand (Baker & Mooed, 1979) and *Drosophila buzzatii* in Australia (Barker & Mulley, 1976; Thorpe *et al.*, 1991).

On the other hand, constellations of species pairs, or parapatric races, which have contact zones with coincident direction (rather than exactly coincident position) and a position that does not relate to a present barrier, or ecotone, may be best interpreted in light of past events. For example, a number of species pairs and parapatric races have north-south orientated contact zones aggregating around central Europe. These species include the grass snakes (*Natrix natrix*), hedgehogs (*Erinaceus*), crows (*Corvus*) and water snakes (*N. maura* and *tessellata*) (Thorpe, 1979). One historical factor affecting all these species was the Pleistocene ice caps, which at their maxima would have split the distribution of the precursor species into south-east and south-west refugia. These would have differentiated over time, and on expanding in post-glacial times have met their sister-taxa along contact zones. If one takes the grass snake example, one can find numerous racial differences between forms either side of the contact zone, but these do not coincide with an ecotone and cannot be ascribed to selection for current ecological conditions.

While these examples may represent the two extremes, in most cases these two factors interact in a complex manner, and to understand the relative importance of these forces and their role, one needs to disentangle them. Methods that may elucidate the causes of geographic variation will be discussed under four headings: quantitative tests; natural situations; natural experiments; and molecular data.

QUANTITATIVE TESTS

Some of this area has recently been reviewed elsewhere (Thorpe, 1991; Thorpe *et al.*, 1991) and these aspects will not be treated in depth here. One can look at tests for: (a) pattern congruence; (b) pattern of anagenesis in phylogenetic trees; and (c) tests of association between observed and hypothesized patterns.

Pattern congruence

If a major historical process, such as a vicariance event, influences the racial differentiation of a species, then the entire genome is subject to the same event, and one has the expectation that most major character systems will reflect this process. This predicts high congruence between patterns of geographic variation in different characters and character systems caused by 'phylogenesis'. On the other hand, different characters and character systems may be predominantly adapted to different facets of the environment (e.g. rainfall *versus* vegetation) by natural selection This predicts the possibility of low congruence between patterns of geographic variation caused by 'ecogenesis'.

One may therefore be able to elucidate the cause of the variation by inspecting the levels of congruence. This, however, is complicated by the fact that congruence between character systems is influenced by the number of characters employed. A random re-sampling procedure (Thorpe, 1991, and references therein) enables one to compare the congruence between character sets for a given number of characters. It is predicted that the congruence-against-character-number curve should be higher for phylogenesis than ecogenesis. When tested using models, which on the basis of independent evidence are thought to represent ecogenetic and phylogenetic differentiation (geographic variation in the Palaearctic grass snake, *Natrix natrix*, is the 'phylogenetic' model; Thorpe, 1984; and geographic variation in the Tenerife gecko, *Tarentola delalandii*, is the 'ecogenetic' model; Thorpe, 1991), then, as predicted, the former has higher congruence levels than the latter (Thorpe, 1991).

Pattern of anagenesis in phylogenetic trees

One can (erroneously) subject taxa to a phylogenetic analysis even if their differentiation is not caused by phylogenesis, and even in these cases, one would expect the position of the representative population on the tree to be related to its geographic origin. Consequently, the existence of a geographically interpretable 'tree' does not necessarily indicate a phylogenetic cause. However, the pattern of anagenesis in a tree should indicate causation because only if the differentiation is phylogenetically caused should one expect the extent of anagenesis to be related to the time of divergence, that is the branches arising near the root should be longer than branches arising further from the root. This is what one finds in trees based on largely selectively neutral nucleic acid data (Fig. 1) (Thorpe *et al.*, 1993). Consequently, when morphological trees have a clear pattern of anagenesis related to time of divergence, phylogenesis is implicated (as in the *N. natrix* example; Thorpe, 1984). When no such pattern is evident, as in the Tenerife gecko (Thorpe, 1991), then ecogenesis is implicated. This is discussed in greater depth in Thorpe (1991) and references therein.

Tests of association between observed and hypothesized patterns

Correlating an observed pattern of geographic variation in a character with a pattern derived from a hypothesized cause (e.g. rainfall) can be useful. Of course,

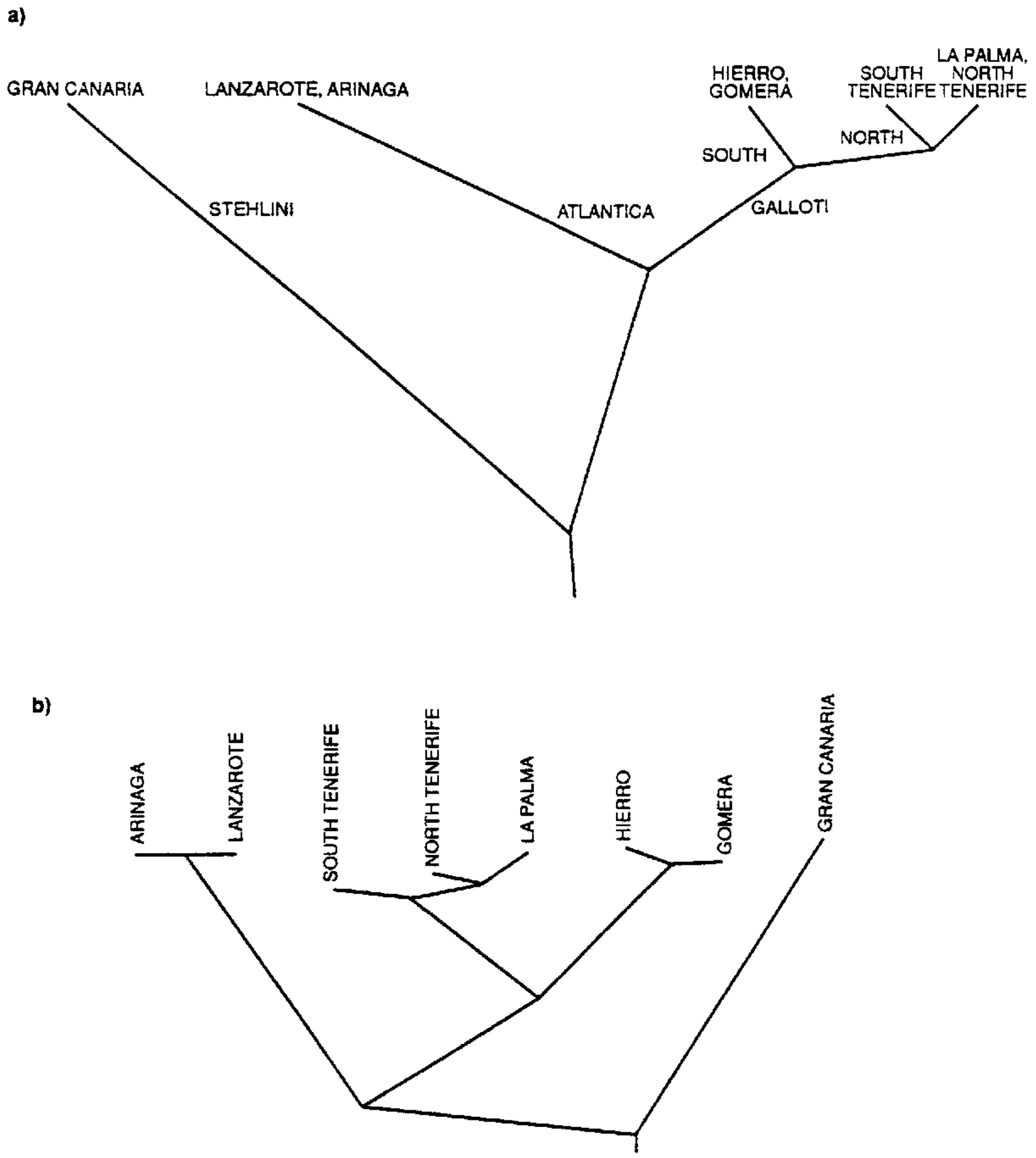

Figure 1 Fitch-Margoliash phylogenetic trees derived from: (a) mtDNA RFLPs (Thorpe *et al.*, 1993); and (b) a 1005 base pair sequence of mtDNA (Thorpe *et al.*, in press) of *Gallotia* populations listed by island name (Arinaga, Lanzarote = *G. atlantica*: Gran Canaria = *G. stehlini*: Tenerife, La Palma, Gomera, Hierro = *G. galloti*). Note that these trees, derived from 'selectively neutral' data, have branch lengths which tend to be related to time (position) of origin, unlike those perturbed by ecogenesis (Thorpe, 1991).

correlation does not prove causation, but the lack of a correlation can enable one to reject a hypothesis.

Partial correlations

A comprehensive range of hypotheses have to be tested for this procedure to be useful, but this raises the problem that the hypothesized patterns may be intercorrelated. This can be overcome by using partial correlation when both the observed and hypothesized patterns are unidimensional. This is the procedure used by Thorpe & Brown (1989a,b) to relate colour pattern in the Tenerife lacertid, *Gallotia galloti*, to several putative causal hypotheses.

Mantel tests

However, some multivariate patterns and hypotheses (e.g. geographic distance between sites) cannot readily be expressed in single dimensions and so matrices are compared by Mantel tests (Mantel, 1967; Dietz, 1983; Manly, 1986, 1991; Schnell, Douglas & Hough, 1986; Smouse, Long & Sokal, 1986; Dow, Chevereud & Friedlaender, 1987; Cheverud, Wagner & Dow, 1989; Brown & Thorpe 1991a,b; Brown, Thorpe & Baez, 1991; Malhotra & Thorpe, 1991a; Sokal, Oden & Wilson, 1991; Thorpe, 1991: Thorpe & Brown, 1991; Thorpe & Baez, 1993). A matrix representing the affinity between localities based on the observed pattern is compared to a matrix representing the affinities between localities based on the hypothesized cause of variation. The units of these matrices are not independent so instead of using tabulated degrees of freedom the probability of association in a Mantel test is derived from randomizing one of the matrices numerous times. The observed coefficient of association (e.g. correlation or regression) is then compared to the distribution of the coefficient derived from the randomizations.

An example of the use of a simple Mantel Test is given by the comparison of generalized ecological conditions to generalized morphology in the Dominican anole, *Anolis oculatus* (Malhotra & Thorpe, 1991a). This showed a significant relationship between generalized morphological divergence and generalized ecological conditions.

Simultaneous (partial) Mantel tests

Mantel tests suffer from the same drawback as ordinary correlations in that several causes may actually, or potentially, influence the pattern of divergence, and the various hypothesised patterns (independent variables) may be inter correlated. Consequently, one needs to partial out the effects of the various effects with a simultaneous (partial) Mantel test. Until recently, software that could consider more than two independent matrices simultaneously was not (readily) available and this was a major limitation. However, one can now use Mantel tests to compare simultaneously a matrix of observed differences among sites to numerous matrices of hypothesized differences between sites. Three example of the use of simultaneous Mantel tests are given here: Philippine cobras, Tenerife lacertids and Dominican anoles.

Philippine cobras Sixteen scalation characters were recorded from 44 specimens of Asiatic cobras (*Naja naja* species complex) from the southern Philippines and Borneo, and the affinities between individuals expressed as a taxonomic distance. Three causative factors, each expressed as a dissimilarity matrix between individuals, were considered. There is a longitudinal climatic gradient across the Philippines with the eastern sector being wetter than the western sector. Since rainfall and humidity can influence geographic variation in reptilian scalation (Thorpe & Baez, 1987), this climatic gradient was considered as a putative causal factor. In contrast to this ecogenetic hypothesis reflecting current conditions, one could hypothesize that the affinities are determined by a historical (phylogenetic) factor, that is their origin on the Pleistocene mega-islands (Fig. 2) that resulted from the lower sea levels during

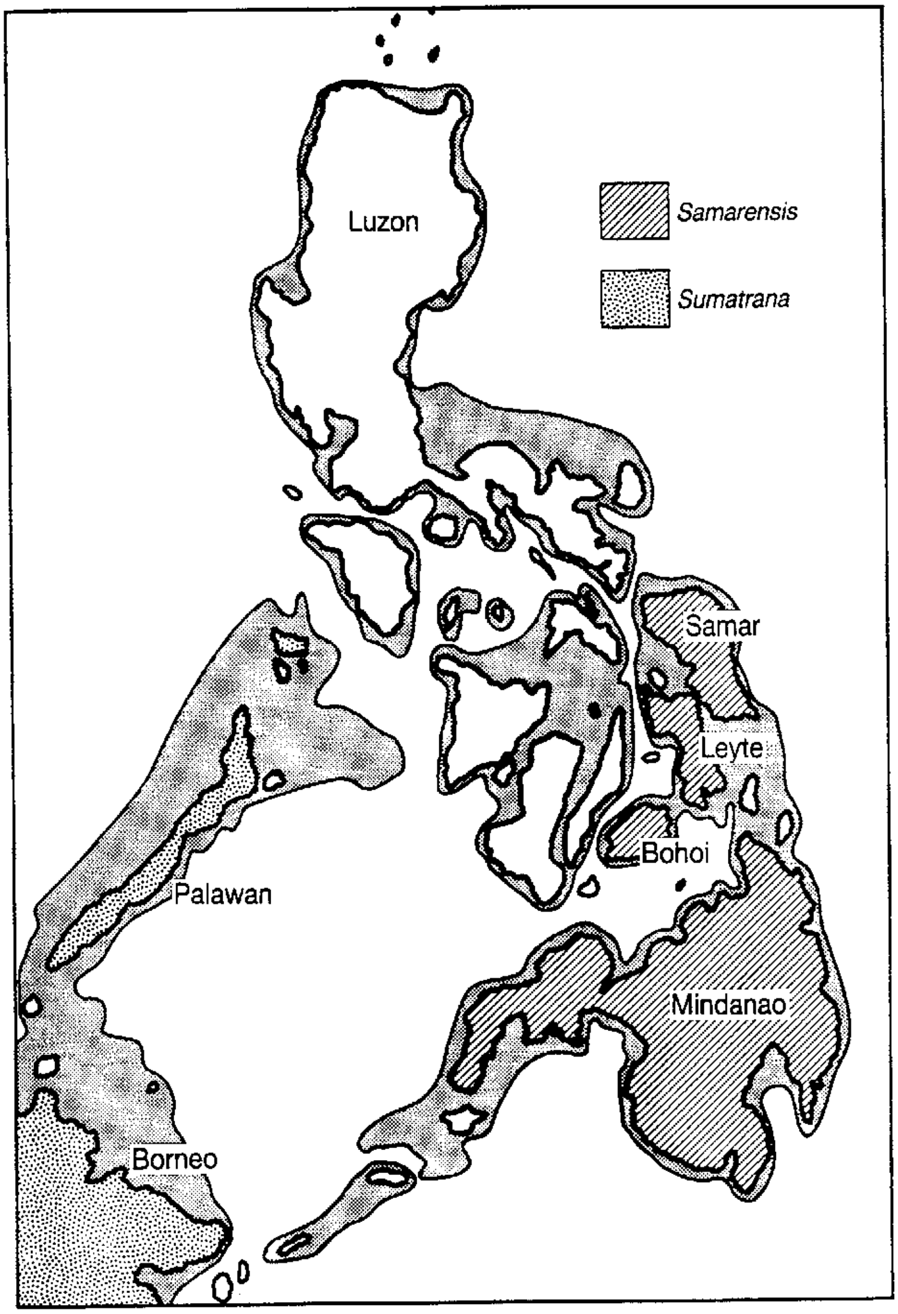

Figure 2 Distribution of Philippine cobras (*Naja* sp.) on current islands and Pleistocene mega-islands (enclosed by shaded area). The scalation variation of cobras (*samarensis* and *sumatrana*), distributed across the southern two mega-islands, was subjected to a partial Mantel test. This shows that past mega-island origin, rather than current climate, influences the geographic variation (see text for explanation).

the Pleistocene glaciations (Heaney, 1985, 1986). Finally, one can test whether the affinities are determined by their current island origin. The results of the simultaneous Mantel test (Table 1) show that even when the current island origin is taken into account the scalation is still significantly influenced by the historical factor of Pleistocene mega-island origin, whilst the ecogenetic factor (climate) has no significant effect.

Tenerife lacertids The above example was based on only three hypotheses, but one may have to test more hypotheses than this. A previous study of *Gallotia galloti*

Table 1 Philippine Cobras. Absolute partial correlations and probabilities from simultaneous Mantel tests of association between observed variation in scalation and three putative causal hypotheses. See text for explanation

Pleistocene mega-islands (phylogenesis)	Climate (ecogenesis)	Current islands
0.13 ($P<0.01$)	0.02 insignificant	0.34 ($P<0.001$)

on Tenerife used partial correlations to test the association between colour pattern (67 locality means) and a wide range of inter-correlated ecogenetic and phylogenetic hypotheses (Thorpe & Brown, 1989a,b, 1991). Here, these hypotheses are tested using simultaneous Mantel tests, with geographic proximity as one of the independent matrices. Briefly, the causative hypotheses that can be tested to see if they account for the colour pattern differentiation within Tenerife are as follows: (1) that the precursor islands of Anaga and Teno had phylogenetically divergent populations on them which met to produce the observed pattern after the eruption of Teide joined the islands; (2) that the cloud layer around Tenerife has separated high and low altitude populations long enough for them to be phylogenetically divergent; (3) that the colour pattern, like the scalation, is ecogenetically adapted to altitude; (4) that the colour pattern is ecogenetically adapted to the distinct climatic/vegetational biotypes which meet in a sharp ecotone, the north being humid with lush vegetation and the south being hot, arid and barren; and (5) that the affinities are determined by geographic proximity, that is, opportunity for gene flow. A fuller explanation of these hypothesized patterns is given in Thorpe & Brown (1989b).

The results (Table 2) clearly show that the multivariate generalized ('total') colour pattern is significantly associated only with the ecotone hypothesis (hypothesis 4). All other hypotheses, both ecogenetic and phylogenetic, can be rejected. However, considerable care must be used when interpreting multivariate patterns when ecogenesis is thought to be the causative factor, because different individual characters may be selected for in different facets of the environment. These

Table 2 Tenerife lacertids. Absolute partial correlations and probabilities from simultaneous Mantel tests of association between individual colour pattern characters plus 'total-multivariate' pattern and numerous causal hypotheses. See text for explanation.

Character	An/Teno (phylo)	Cloud (phylo)	Altitude (ecogen)	Ecotone (ecogen)	Proxim	Sel/GF
1	0.1061	0.0495	0.0166	0.6029***	0.1583	
2	0.0201	0.0753	0.0461	0.0639	0.0182	
3	0.0268	0.0070	0.0296	0.5422***	0.0191	
4	0.0924	0.0289	0.0320	0.6360***	0.1506	
5	0.0045	0.0595	0.0579	0.2462**	0.2141*	
6	0.0273	0.0087	0.0488	0.6240***	0.0181	
Total	0.0608	0.0770	0.0024	0.6400***	0.1517	4.2

* indicates significance beyond $P<0.01$ (Bonferroni correction of 0.05), ** $P<0.0005$, *** $P<0.0001$.

individual adaptations may be obscured by multivariate generalization. Mantel tests on each of the six individual characters (Table 2) indicate that, in this case, multivariate generalization has not obscured adaptation of individual characters to different environmental factors as five of the six characters give the same result as the generalized pattern (although proximity is just significant for character 5) and the sixth character is not significantly related to any hypothesis. A biological interpretation of the geographic variation in the colour pattern, in relation to natural selection for cryptic dorsal coloration *versus* sexual selection for blue lateral coloration, is given in Thorpe & Brown (1989b).

Dominican anoles The mountainous island of Dominica (Lesser Antilles) is subject to prevailing easterly winds which result in pronounced environmental differentiation within the island, both with longitude and altitude. Generalized morphology is significantly related to generalized ecology when subjected to a simple Mantel test (Malhotra & Thorpe, 1991a). When generalized body dimensions (13 characters adjusted for size independence), generalized scalation (10 characters) and generalized colour pattern (22 characters) are tested (using simultaneous Mantel tests) against the potential causative environmental factors broken down into the biotic environment (vegetation), temperature, rainfall, altitude and geographic proximity, one can see that in females shape is significantly associated with the biotic environment, scalation with rainfall and colour pattern with both (Malhotra, 1992) (Table 3). Once again as ecogenesis is the cause of the variation individual characters may be associated with different facets of the environment which would be obscured by multivariate generalization. This can be seen to be the case when the individual scalation characters are tested against these hypotheses. Whilst rainfall is the predominant 'cause' of scalation variation, the variation in the scales between the orbits is also associated with temperature and altitude, and the number of enlarged white scales is also associated with the vegetation type (biotic environment) (Table 4). This emphasizes the need to treat each character separately when dealing with ecogenetically caused variation.

Canonical correlations

Not only can the various components of the character variation be coalesced by multivariate generalization, or treated separately, the various components of the causative factors can also be rather arbitrarily coalesced, or separated. For example, in the Dominican anole case, the various facets of the physical environment may be

Table 3 Dominican anoles. Absolute partial correlations and probabilities from simultaneous Mantel tests of association between character set patterns (females) and numerous causal hypotheses. See text and Malhotra (1992) for explanation.

Character set	Proximity	Veg. type	Temp.	Altitude	Rainfall
Body proportions	0.06	0.14*	0.02	0.05	0.12
Scalation	0.07	0.07	0.11	0.02	0.34**
Colour pattern	0.10	0.34**	0.09	0.04	0.25**

* indicates significance beyond $P<0.05$, ** $P<0.01$, *** $P<0.001$

Table 4 Dominican anoles. Absolute partial correlations and probabilities from simultaneous Mantel tests of association between individual scalation characters plus 'total-multivariate' pattern (females) and numerous causal hypotheses. See text and Malhotra (1992) for explanation.

Character	Proximity	Veg. type	Temp.	Altitude	Rainfall
BSC	0.05	0.13	0.14	0.11	0.34***
LAM	0.11	0.01	0.02	0.10	0.07
VENTSC	0.08	0.04	0.00	0.04	0.14*
SUPLABS	0.10	0.04	0.06	0.05	0.29**
SUBLABS	0.06	0.06	0.01	0.08	0.23*
SCBORB	0.10	0.06	0.18*	0.23**	0.26*
SS	0.10	0.26**	0.10	0.06	0.25*
Total	0.07	0.07	0.11	0.02	0.34**

* indicates significance beyond $P<0.05$, ** $P<0.01$, *** $P<0.001$.

treated separately, or coalesced by multivariate generalization. This underlines the utility of being able to treat each character and causative factor separately *a priori* and then look for constellations of characters that are related to constellations of causative factors. Canonical correlation attempts to do this.

To illustrate this the Dominican anole case study is used. Six ecological factors (three biotic and three physical) were canonically correlated with 46 individual morphological characters (Malhotra, 1992) in males. Table 5 summarizes the results for the second canonical variate which shows that occurrence of littoral woodland is

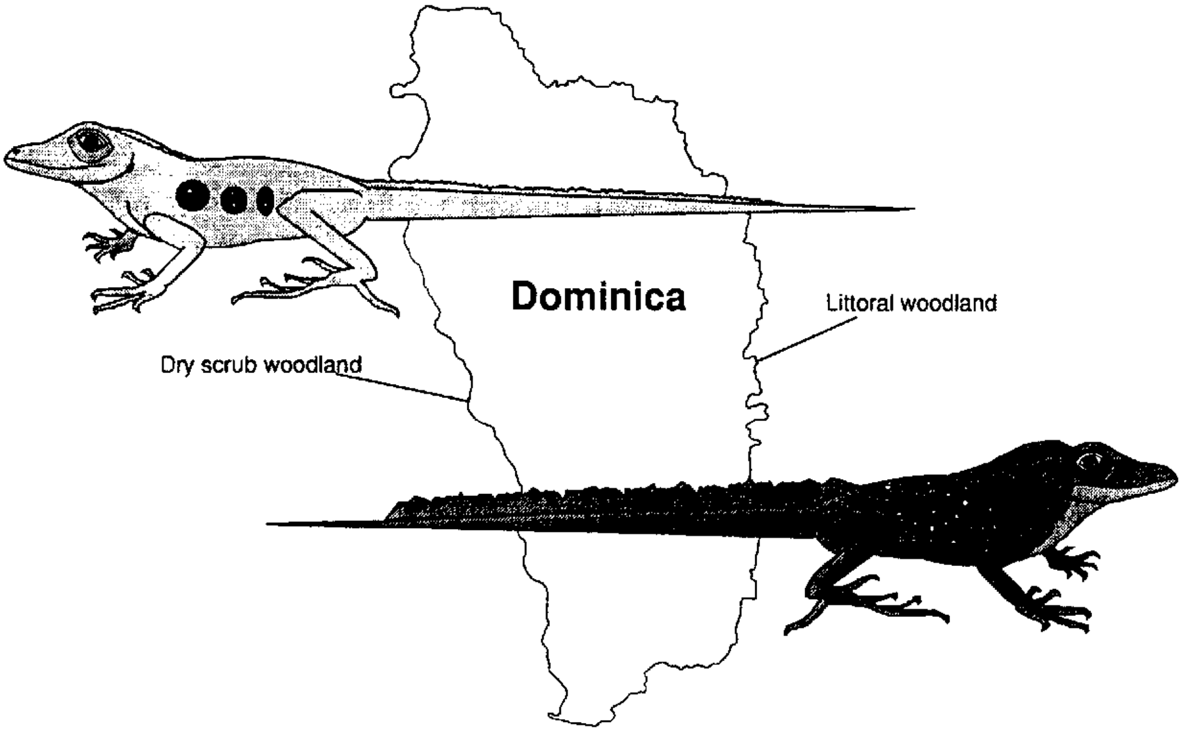

Figure 3 Dominican *Anolis oculatus* from the Caribbean dry scrub woodland (left) and Atlantic wet littoral forest (right). A functional complex of scalation, body proportion and colour characters give a different visual image for each of these anoles living in the different habitats. The anole living in the more open dry scrub woodland are light coloured with black blotches, whilst those living in the denser littoral woodland are more saturated in colour and have enlarged light lateral scales and a profile dominated by large neck crest and tail crest (Malhotra, 1992).

Table 5 Canonical correlation loadings on the first two variates for: (A) ecological variables; and (B) selected morphological variables, of Dominican *Anolis* (after Malhotra, 1992).

A

Ecological factor	CV1	CV2
Temperature	0.699	0.445
Altitude	−0.755	−0.430
Rainfall	−0.880	−0.256
Dry scrub woodland	0.896	−0.420
Rainforest	−0.878	−0.301
Littoral woodland	0.072	0.911

B

Character systems	Morphological character	CV1	CV2
Body shape	Head width	0.485	−0.256
	Lower leg length	−0.469	0.014
	Toe length	−0.524	−0.150
	Tail depth	0.101	0.358
Scalation	Number of scales at mid-body	0.533	0.123
	Number of ventral scales	0.146	0.355
	Enlargement of white scales	−0.186	0.351
Colour	Dorsal colour – magenta	−0.287	0.379
	Dorsal colour – cyan	−0.399	−0.045
	Dewlap colour – cyan	−0.364	−0.265
	Eyeskin colour – cyan	−0.681	−0.120
	Ventral colour – magenta	−0.224	0.519
	Ventral colour – cyan	−0.428	0.313
	Number of black patches	−0.118	−0.533

associated with 'visual effect' characters from each of the character systems, that is size of the tail crest (body dimensions), enlargement of white lateral scales (scalation) and intensity of magenta and absence of black spots (colour pattern). These characters produce an overall functionally coherent effect in adapting the male lizards to the different visual background offered by the littoral woodland compared to the other vegetation types (Fig. 3). While canonical correlations are successful in showing *a posteriori* constellations of characters (and causes) that cut across the subdivisions into 'man-made' character systems, they have two limitations. They are non-probabilistic and are unable to take into account multidimensional patterns and hypotheses represented by matrices, for example geographic proximity in two dimensional space. This problem is addressed by Malhotra & Thorpe (in press).

NATURAL SITUATIONS

In some circumstances the natural situations are such that a cause of geographic variation can be deduced. If, within a region, such as an island, two species show

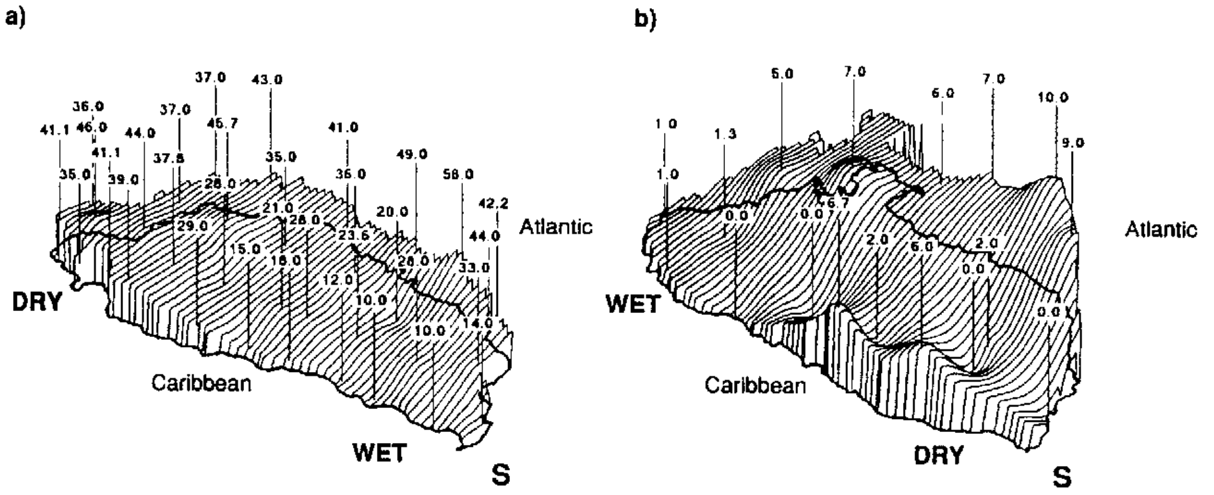

Figure 4 Parallel variation in the Lesser Antillean anoles. Isometric plots of intensity of dorsal magenta in: (a) *Anolis oculatus* on Dominica; and (b) *Anolis marmoratus* on Basse Terre (Guadeloupe) showing parallel variation. The intensity of the magenta component of the dorsal colour is greatest in the Atlantic littoral woodland and least in the wettest part of the Caribbean dry scrub woodland for both Dominica and Basse Terre anoles (Malhotra, 1992).

parallel patterns of variation, this does not allow one to deduce cause, because these species may have both ecogenetic factors and historical factors in common. However, in a situation where two islands have parallel patterns of environmental differentiation within them, but independent histories, then parallel character variation in these allopatric species can be ascribed to ecogenesis as they have only parallel ecological conditions in common.

Two examples of this are given here. To the north of Dominica the adjacent island of Basse Terre (Guadeloupe) is another mountainous island subject to the same weather patterns. Consequently it has parallel environmental differentiation to Dominica, for example wet littoral woodland on the Atlantic coast, hot dry scrub woodland on the Caribbean coast and very wet cooler rainforest at higher altitudes. A series of characters from the scalation, colour pattern and body dimensions show parallel patterns of geographic variation in the Dominican anole, *Anolis oculatus*, and the Guadeloupean anole, *Anolis marmoratus* (Malhotra, 1992). Their parallel variation in the increased intensity of the magenta component of dorsal hue in association with Atlantic littoral woodland is illustrated here (Fig. 4). This parallel variation can be ascribed to ecogenesis rather than phylogenesis for the reasons given above.

The second example comes from Canary island skinks (Brown, Thorpe & Baez, 1991). Gran Canaria and Tenerife are adjacent mountainous islands subject to the same prevailing northerly trade winds which result in a humid lush northern sector and a hot arid barren southern sector on these islands. On Gran Canaria the skink, *Chalcides sexlineatus*, has a northern form with a brown tail and a southern form with a bright blue tail. This is thought to reflect alternative anti-predator strategies as the tail can be autotomized and bright colour will attract a predator's attention away from the vulnerable head to the disposable tail. Parallel variation in the tail colour (Fig. 5) and multivariate generalized colour pattern can be seen in *Chalcides viridanus* on the adjacent island of Tenerife. In both cases simultaneous Mantel tests are used to test colour pattern variation against various alternative causal hypotheses.

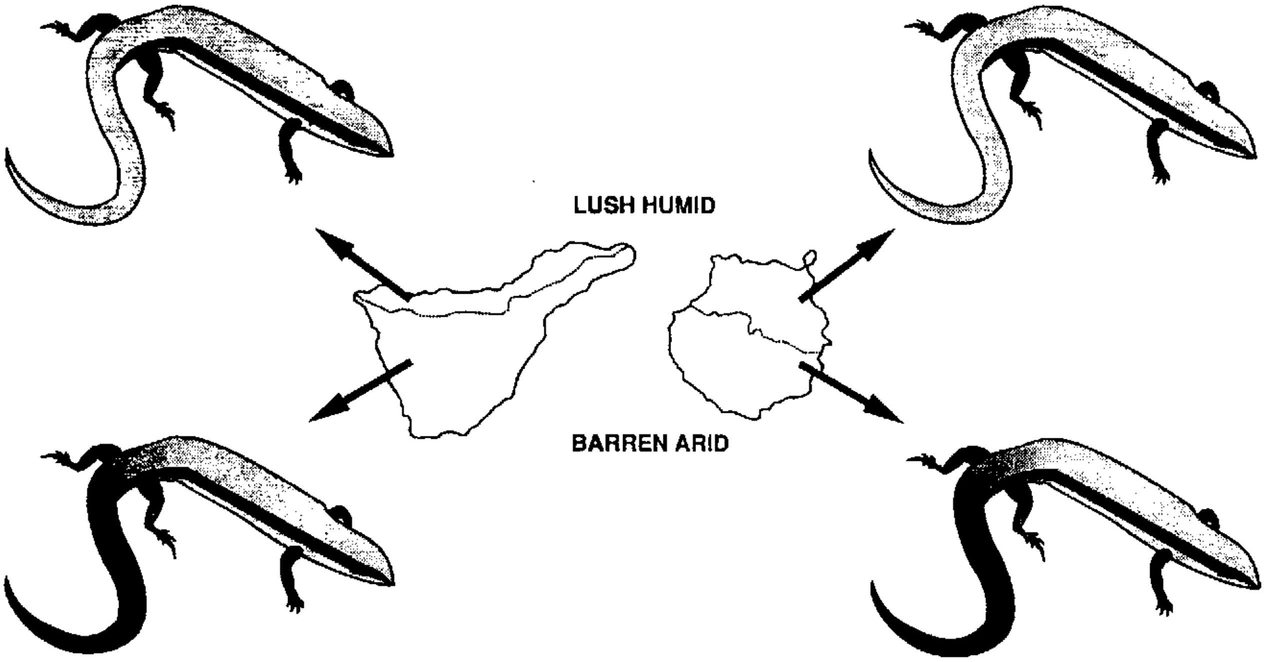

Figure 5 Parallel variation in Canary Island skinks. The colour pattern variation in the Gran Canarian skink (*Chalcides sexlineatus*) (right) is associated with climatic differences within the island. These differences are paralleled by colour pattern variation by a related skink (*Chalcides viridanus*) on the adjacent island of Tenerife (left). In particular, skinks from the dry barren south of both islands tend to have blue tails (Brown, Thorpe & Baez, 1991).

These tests, and the parallel variation, indicated ecogenetic adaption to the two biotic regimes (Brown, Thorpe & Baez, 1991).

NATURAL EXPERIMENTS

For several reasons field experiments on evolution are very difficult and consequently, rarely carried out. The time scale of evolutionary processes generally exceeds that of a study and there can be considerable practical difficulties in obtaining comparable, discrete, experimental entities. However, Endler's outstanding study of the balance between natural selection for crypsis and sexual selection for bright coloration of guppies in Trinidadian stream pools has shown the value of field experiments in investigating causative factors in microevolution (Endler, 1980), as have a few other studies (e.g., Halkka & Raatikainen, 1975; Knights, 1979).

Terrestrial species, such as anoles, may not be naturally separated into convenient experimental units. Consequently, Malhotra & Thorpe separated natural forest into four large experimental enclosures and utilized a lizard-proof fence to segregate populations of the Dominican anole (Malhotra & Thorpe, 1991b, 1993; Malhotra, 1992; Thorpe & Malhotra, 1992). The lizards were cleared from each of the four enclosures and replaced by examples of *Anolis oculatus* from the four ecotypes (including a control). The multivariate morphology of each specimen was recorded before translocation. In as brief a time as two months there was a difference in the morphology of the survivors compared to the non-survivors within an enclosure. For the montane ecotype (which come from the locality with an environment most different to that of the enclosures) the difference was significant for both males and females, and the extent of morphological difference was significantly correlated to the extent of ecological difference between enclosure and locality of origin across the ecotypes (Malhotra & Thorpe, 1991b; Thorpe and Malhotra, 1992). This indicates the importance on natural selection in determining the pattern of geographic variation and the surprising rapidity of its action. Partial repetitive experiments in different ecozones, and allochronic repeats have confirmed this conclusion.

MOLECULAR DATA

The study of evolution has been strongly influenced by investigations of the differences among island populations in archipelagos, but often little thought has been given to the relative importance of the underlying causes. Both phylogenesis (e.g. the pattern of colonization) and ecogenesis (natural selection for different environmental conditions on the different islands) are likely to have contributed to the pattern of inter-island differentiation. Their relative contribution can be very difficult to determine. If one wishes to test to what extent a pattern of morphological divergence among islands is due to ecogenetic adaptation one needs to take into account the phylogenetic history. However, at the intraspecific level, if all one has is the phylogenetic tree based on morphology, one will not be able to do this in case the tree is corrupted by this convergence. A way out of this circular problem is to erect a tree on an independent information system which is least likely to be perturbed by ecogenesis. Mitochondrial DNA is such an information system (Wilson *et al.*, 1985).

The Canary island lizards of the genus *Gallotia* provide a model for this approach. The islands are volcanic in origin and arose from the sea floor. The western islands are separated by deep sea and have not been joined to one another, the islands to the east, or the African mainland, in the time scale pertinent to this study. The ages of origin of the islands can be estimated. The eastern islands are oldest with those to the west being progressively younger (Abdel-Monem, Watkins & Gast, 1971, 1972; Anguita & Hernan, 1975, 1986; Carracedo, 1979; Anchocea *et al.*, 1990; and references in Thorpe, Watt & Baez, 1985 and McGregor, 1992). These western islands are occupied by one extant species, *Gallotia galloti* (except for a small relict population of *G. simonyi* on Hierro). For the reasons given above their distribution can only be explained by dispersal. These islands differ in form and position, and consequently in climate. Any morphological differences among island populations could therefore be due the dispersal history (phylogenesis), or adaptation to the different ecological conditions (ecogenesis).

Numerous morphological differences exist among the island populations of *G. galloti* (Thorpe, 1985a,b; Thorpe, Watt & Baez, 1985), but we can take body size as an illustration (Fig. 6). Adult body size differs among island populations even when raised under constant laboratory conditions. In north Tenerife and south Tenerife the lizards are large whilst in the west the lizards from La Palma are smaller and those from Gomera and Hierro are smallest. Attempts to relate body size to island size have been shown to be flawed by Thorpe (1985b), but reduced body size could either reflect ecogenetic adaptation to similar ecological conditions on far western islands (La Palma, Hierro and Gomera), or phylogenesis (i.e. a clade of smaller animals occupying La Palma, Gomera and Hierro).

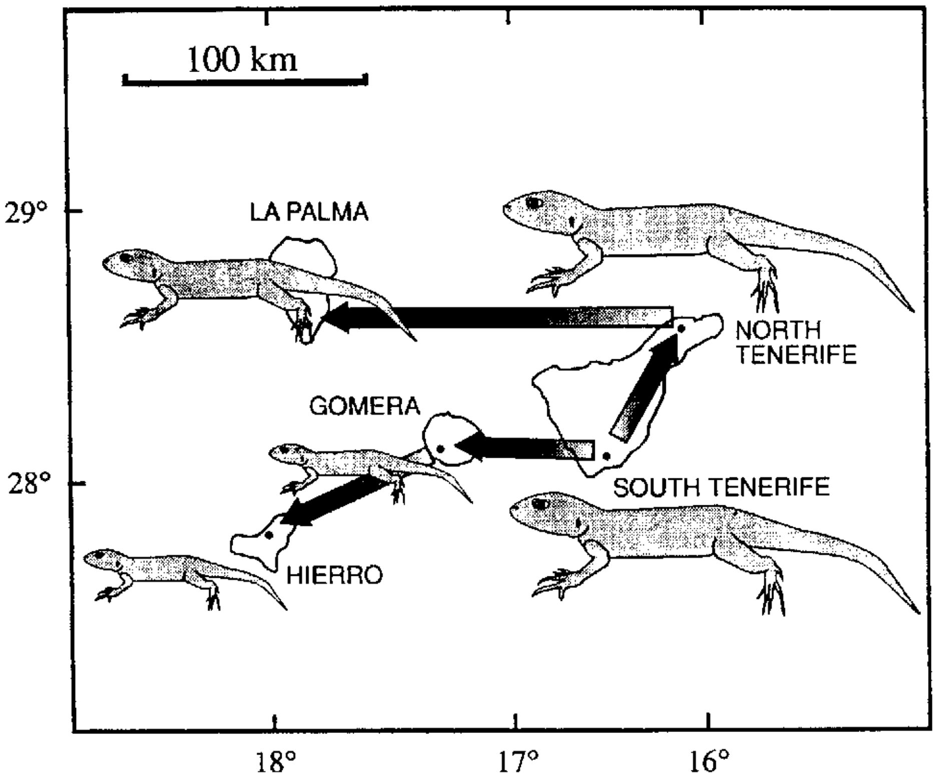

Figure 6 *Gallotia galloti* on the western Canary islands vary in size. Those from Tenerife are largest while on islands to the west they are smaller, particularly in Gomera and Hierro. When the colonization path is deduced from a DNA phylogenetic tree (Thorpe *et al.*, in press) it is apparent that these smaller lizards on La Palma, Hierro and Gomera do not constitute a separate 'small-body' clade, but represent ecogenetic adaptation of the northern and southern lineages that separately colonized westwards.

Phylogenetic trees (Fitch-Margoliash and Wagner trees) were derived from a range of mitochondrial DNA data, that is six-cut restriction fragment length polymorphisms (RFLPs), 4-cut RFLPs, cytochrome b sequence, cytochrome oxidase I sequence and 12s rRNA sequence (Thorpe *et al.*, in press). Data from a novel RAPD (random amplified polymorphic DNA) analysis of nuclear DNA was also used to construct a tree (Thorpe *et al.*, in press). None of these 6 trees indicated that the populations of La Palma, Hierro and Gomera formed a separate clade (Thorpe *et al.*, in press).

When the colonization sequence amongst the islands was deduced from either geography plus tree topology, or topology plus anagenesis, using rules explained in Thorpe *et al.* (in press), then an origin in southern Tenerife is indicated with two independent expansions westward from older to progressively younger islands. Hierro is colonized from south Tenerife via Gomera; and La Palma is colonized from north Tenerife. Various estimates for the time of geological origin of the islands (McGregor, 1992; Thorpe *et al.*, in press) can be compared to the estimates for time of colonization based on molecular clock assumptions, whether taken overall (Thorpe *et al.*, in press), or for each DNA data type separately (Table 6) (McGregor, 1992). These procedures agree in so far as they indicate that in each case the hypothesized time of colonization of an island is only after its estimated geological origin.

One can see that this phylogenetic analysis, independent of the morphology, allows one to reject the hypothesis that the evolution of small size is primarily due

Table 6 Origin (oldest rocks) of western Canary Islands (Anguita & Hernan, 1975) and divergence times for their *Gallotia galloti* populations (in millions of years) based on various mitochondrial DNA data. DNA dates derived from McGregor (1992), but see also Thorpe *et al.* (1993) and Thorpe *et al.* (in press).

Island	Origin	RFLP	Cyt b	Cyt ox I	12s rRNA
Tenerife	15.7	5.5	5.5	8.0	11.0
Gomera	12.0	2.0	2.5	4.0	3.5
La Palma	1.6	0.4	1.1	0.2	–
Hierro	0.8	0.5	0.6	–	–

to 'phylogenesis'. Small size appears to be an ecogenetic adaptation which occurs independently in the two lineages.

This approach has been combined with the use of Mantel tests (Thorpe, in press). The observed matrix of morphological differences (single characters or multivariate generalizations) are the dependent matrix, with independent matrices representing various hypothesized causes. Phylogenesis, for example, is represented by patristic distances on the molecular phylogenetic tree, whilst ecogenesis is represented by several, potentially important ecological variables. This approach is related to the 'comparative method' (Cheverud, Dow & Leutenegger, 1985).

Inter-island variation in the endangered *Iguana delicatissima* provides a further example of how DNA data may aid in the interpretation of morphological variation. This species has a patchy distribution across the Lesser Antilles from Martinique and Dominica in the south to Anguilla in the north. Morphological variation between (and in some cases within) islands is revealed by multivariate analysis of 60 variables from the scalation, colour pattern and body dimensions. Is this geographic variation due to adaptation to the ecological differences between islands, or does it reflect the historical colonization sequence? Analysis of the sequence of the mitochondrial gene cytochrome b in other iguanids reveals substantial inter-population variation (11–14% in *Anolis oculatus*; Malhotra, 1992). There are clear differences in cytochrome b sequence between the two species of iguana in this area (*Iguana iguana* and *I. delicatissima*). However, in *I. delicatissima* there are no cytochrome b sequence differences among the island populations of Desirade, Anguilla and Dominica, even though they are from opposite ends of the species range. This implies that the populations of this species have been dispersed recently, probably by human factors (pre-Columbian Indians) and the observed morphological differences are largely, or solely, due to ecogenetic adaptation to the different ecological conditions on each island.

SUMMARIZED DISCUSSION

It is evident, that whilst it may be difficult to determine the relative importance of 'phylogeny' and 'ecology' in causing a pattern of geographic variation, there are several procedures which may help us do so. Simultaneous Mantel tests appear to be a particularly useful technique, even though there is an arbitrary element in how the observed and hypothesized patterns are considered. Parallel patterns found in independent situations may be useful where they are found, and when possible manipulative field experiments can be very revealing. However, these two procedures will not be of widespread utility. DNA data can now play a very important role in revealing intraspecific phylogenesis and aiding us in interpreting variation in morphological data.

ACKNOWLEDGEMENTS

The senior author (RST) wishes to thank Bryan Manly for access to his Mantel test programs, and the following bodies for financial support: Science and Engineering Research Council (GR/F/19968 to RST and A. Cumming); Natural Environment Research Council (GR3/6943 to RST and A. Cumming); The Leverhulme Trust; The Carnegie Trust for the Universities of Scotland and The Royal Society. We acknowledge additional support from SERC (research studentships to WW, AM, MD) and NERC (research studentship to RB and a postdoctoral fellowship to WW).

REFERENCES

ABDEL-MONEM, A., WATKINS, N.D. & GAST, P.W., 1971. Potassium–Argon ages, volcanic stratigraphy, and geomagnetic polarity history of the canary islands: Lanzarote, Fuerteventura, Gran Canaria, and La Gomera. *American Journal of Science, 271*: 490–521.

ABDEL-MONEM, A., WATKINS, N.D. & GAST, P.W., 1972. Potassium–argon ages, volcanic stratigraphy and magnetic polarity history of the Canary Islands: Tenerife, La Palma and Hierro. *American Journal of Science, 272*: 805–825.

ANCHOCEA, E., FUSTER, J.M., IBARROLA, E., CENDRERO, A., COELLO, J., HERNAN, F., CANTAGREL, J.M. & JAMOND, C., 1990. Volcanic evolution of the island of Tenerife (Canary Islands) in the light of new K-ar data. *Journal Volcanolology Geothermal Research, 44*: 231–249.

ANGUITA, F. & HERNAN, F., 1975. A propagating fracture model versus a hot spot origin for the Canary Islands. *Earth and Planetary Science Letters, 27*: 11–19.

ANGUITA, F. & HERNAN, F., 1986. Geochronology of some Canarian dike swarms: contribution to the volcano-tectonic evolution of the archipelago. *Journal Volcanolology Geothermal Research, 30*: 155–162.

BAKER, A.J., 1980. Morphometric differentiation in New Zealand populations of the house sparrow (*Passer domesticus*). *Evolution, 34*: 638–653.

BAKER, A.J. & MOOED, A., 1979. Evolution in the introduced New Zealand populations of the common myna, *Acridotheres trictis* (Aves: Sturnidae). *Canadian Journal of Zoology, 57*: 570–584.

BARKER, J.S.F. & MULLEY, J.C. 1976. Isozyme variation in natural populations of *Drosophila buzzatii*. *Evolution, 30*: 213–233.

BROWN, R.P. & THORPE, R.S., 1991a. Description of within-island microgeographic variation in body dimensions and scalation of the skink *Chalcides sexlineatus*, with testing of causal hypotheses. *Biological Journal of the Linnean Society, 44*: 47– 64.

BROWN, R.P. & THORPE, R.S., 1991b. Within-island microgeographic variation in the colour pattern of the skink, *Chalcides sexlineatus*: pattern and cause. *Journal of Evolutionary Biology, 4*: 557–574.

BROWN, R.P., THORPE, R.S. & BAEZ, M., 1991. Lizards on neighboring islands show parallel within-island microevolution. *Nature, 352*: 60–62.

CARRACEDO, J.C., 1979. *Paleomagnetismo e Historia Volcanica de Tenerife.* Tenerife: Aula de Cultura.

CHEVERUD, J.M., DOW, M.M. & LEUTENEGGER, W., 1985. The quantitative assessment of phylogenetic constraints in comparative analyses: sexual dimorphism in body weight among primates. *Evolution, 39*: 1335–1351.

CHEVERUD, J.M., WAGNER, G.P. & DOW, M.M., 1989. Methods for the comparative analysis of variation patterns. *Systematic Zoology, 38*: 201–213.

DIETZ, E.J., 1983. Permutation tests for association between two distance matrices. *Systematic Zoology, 32*: 21–26.

DOW, M.M., CHEVERUD, J.M. & FRIEDLAENDER, J., 1987. Partial correlation of distance matrices in studies of population structure. *American Journal of Physical Anthropology, 72*: 343–352.

ENDLER, J.A., 1980. Natural selection on color patterns in *Poecilia reticulata. Evolution, 34*: 76–91.

HALKKA, O. & RAATIKAINEN, M., 1975. Transfer of individuals as a means of investigating natural selection. *Hereditas, 80*: 27–34.

HEANEY, L.R., 1985. Zoogeographic evidence for Middle and Late Pleistocene landbridges to the Philippine Islands. *Modern Quarternary Research in South-East Asia, 19*: 127–143. HEANEY, L.R., 1986. Biogeography of mammals in SE Asia: estimates of rates of colonization, extinction and speciation. *Biological Journal of the Linnean Society, 28*: 127–165.

JOHNSTON, R.F. & SELANDER, R.K., 1971. Evolution in the house sparrow. II. Adaptive differentiation in North American populations. *Evolution, 25*: 1–28.

KNIGHTS, R.W. 1979. Experimental evidence for natural selection on shell shape in *Cepaea hortensis* (Mull). *Genetica, 50*: 51–60.

McGREGOR, D.P., 1992. 'Mitochondrial DNA Evolution in Canary Island Lizards (genus: *Gallotia*)'. Unpublished Ph.D Thesis, University of Aberdeen.

MALHOTRA, A. 1992. 'What Causes Geographic Variation: a Case Study of *Anolis oculatus*'. Unpublished Ph.D Thesis, University of Aberdeen.

MALHOTRA, A. & THORPE, R.S., 1991a. Microgeographic variation in Anolis oculatus on the island of Dominica, West Indies. *Journal of Evolutionary Biology, 4*: 321–335.

MALHOTRA, A. & THORPE, R.S., 1991b. Experimental detection of rapid evolutionary response in natural lizard populations. *Nature, 353*: 347–348.

MALHOTRA, A. & THORPE, R.S., 1993. An experimental field study of an eurytopic anole, *Anolis oculatus. Journal of Zoology, London, 229*: 163–170.

MALHOTRA, A. & THORPE, R.S., in press. An evaluation of Mantel tests: testiry geographic patterns in the Dominican anole.

MANLY, B.F.J., 1986. Randomization and regression methods for testing associations with geographical, environmental and biological distances between populations. *Researches on Population Ecology, 28*: 201–218.

MANLY, B.F.J., 1991. *Randomization and Monte Carlo methods in biology.* New York: Chapman & Hall.

MANTEL, N., 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research, 27*: 209–220.

SCHNELL, G., DOUGLAS, M. & HOUGH, D.J., 1986. Geographic patterns of variation in offshore spotted dolphins (Stenella attenuata) of the eastern tropical Pacific Ocean. *Marine Mammal Science, 2*: 186–213.

SMOUSE, P.E., LONG, J. & SOKAL, R.R., 1986. Multiple regressions and correlation extensions of the Mantel test of matrix correspondence. *Systematic Zoology, 35*: 627–632.

SOKAL, R.R., ODEN, N.L. & WILSON, C., 1991. Genetic evidence for the spread of agriculture in Europe by demic diffusion. *Nature, 351*: 143–145.

THORPE, R.S., 1979. Multivariate analysis of the population systematics of the ringed snake *Natrix natrix. Proceedings of the Royal Society of Edinburgh, 78*: 1–62.

THORPE, R.S., 1984. Primary and secondary transition zones in speciation and population differentiation: a phylogenetic analysis of range expansion. *Evolution, 38*: 233–243.

THORPE, R.S. 1985a. Relative similarity between subspecies of the western Canary Island lizard, *Gallotia galloti. Bonner Zoologische Beitrage, 36*: 529–532.

THORPE, R.S., 1985b. Body size, island size and variability in the Canary Island lizards of the genus *Gallotia. Bonner Zoologische Beitrage, 36*: 481–487.

THORPE, R.S., 1991. Clines and cause: microgeographic variation in the Tenerife gecko *Tarentola delalandii. Systematic Zoology, 40*: 172–187.

THORPE, R.S., in press. The use of DNA divergence to help determine the correlates of evolution of morphological characters.

THORPE, R.S. & BAEZ, M., 1987. Geographic variation within an island: univariate and multivariate contouring of scalation, size and shape of the lizard *Gallotia galloti. Evolution, 41*: 256–268.

THORPE, R.S. & BAEZ, M., 1993. Geographic variation in scalation of the lizard *Gallotia stehlini* within the island of Gran Canaria. *Biological Journal of the Linnean Society, 48*: 75–87.

THORPE, R.S. & BROWN, R.P., 1989a. Testing hypothesized causes of within-island geographic variation in the colour of lizards. *Experientia, 45*: 397–400.

THORPE, R.S. & BROWN, R.P., 1989b. Microgeographic variation in the colour pattern of the lizard *Gallotia galloti* within the island of Tenerife: distribution, pattern and hypothesis testing. *Biological Journal of the Linnean Society, 38*: 303– 322.

THORPE, R.S. & BROWN, R.P., 1991. Microgeographic clines in the size of mature male *Gallotia galloti* (Squamata: Lacertidae) on Tenerife: causal hypotheses. *Herpetologica, 47*: 28–37.

THORPE, R.S. & MALHOTRA, A., 1992. Are *Anolis* lizards evolving? *Nature, 355*: 506.

THORPE, R.S., WATT, K. & BAEZ, M., 1985. Some interrelationships of the Canary Island lizards of the genus *Gallotia. Bonner Zoologische Beitrage, 36*: 577–584.

THORPE, R.S., BROWN, R.P., MALHOTRA, A. & WÜSTER, W., 1991. Geographic variation and population systematics: distinguishing between ecogenetics and phylogenetics. *Bollettini di Zoologia, 58*: 329–335.

THORPE, R.S, McGREGOR, D. & CUMMING, A.M., 1993. Molecular phylogeny of the Canary Island lacertids (*Gallotia*): mitochondrial DNA restriction site divergence in relation to sequence divergence and geological time. *J. Evol. Biol., 6*: 725–735.

THORPE, R.S., McGREGOR, D.P., CUMMING, A.M. & JORDAN, W.C., in press. DNA evolution and colonization sequence of island lizards in relation to geological history: mtDNA RFLP, cytochrome b, cytochrome oxidase, 12s rRNA sequence, and nuclear RAPD analysis. *Evolution.*

WILSON, A.C., CANN, L.C., CARR, S.M., GEORGE, M., GYLLENSTEN, U.B., HELM-BYCHOWSKI, K.M., HIGUCHI, R.G., PALUMBI, S.R., PRAGER, E.M., SAGE, R.D. & STONEKING, M., 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. *Biological Journal of the Linnean Society, 26*: 375–400.