

Analysis of Color Spectra in Comparative Evolutionary Studies: Molecular Phylogeny and Habitat Adaptation in the St. Vincent Anole (*Anolis trinitatis*)

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Abstract.—The use of color (as distinct from color pattern) in comparative evolutionary studies is important, and objective, independent characters are needed. A new method was employed to investigate geographic color variation in the small arboreal lizard *Anolis trinitatis* on the island of St. Vincent. The simple delta analysis (based on the difference between eigenvector coefficients for adjacent regions of the spectrum) is aimed at increasing the objectivity with which a spectrum is cut into independent segments and does not predetermine segment width or number. There are distinct habitat types within this small island and distinct phylogenetic lineages (based on a kilobase of cytochrome *b* sequence) within this species. A series of matrix correspondence (Mantel) tests indicate that aspects of color are associated with habitat type (e.g., green dorsum in rain forest lizards), molecular phylogeny, or both. Hence, both adaptation by selection and historical processes are implicated as causes of geographic variation in color. The dewlap variation (e.g., strong ultraviolet reflectance in some Atlantic coastal sites) is very pronounced and, contrary to some expectations, may result in reproductive isolation even within small Lesser Antillean islands. [*Anolis*; color spectrum; eigenvector coefficients; geographic variation; Lesser Antilles; natural selection; phylogeography.]

The colors and color patterns of organisms are of broad interest in comparative evolutionary studies because they may reflect phylogenetic relationships, habitat adaptation, and sexual selection and the interactions among these factors (Endler, 1990; Fleishman et al., 1993; Thorpe, 1996; Thorpe et al., 1996; Andersson and Amundsen, 1997; Andersson et al., 1998; Grill and Rush, 2000; Gübitz et al., 2000; Malhotra and Thorpe, 2000a, 2000b; Macedonia, 2001; Thorpe and Richard, 2001). Moreover, being visually orientated, humans often find it convenient to use color and color pattern in systematic definitions and descriptions, particularly at lower taxonomic levels.

There are various aspects of color and color pattern. Human perception of color includes brightness, chroma, and hue. Brightness is overall intensity, chroma is saturation, that is, how much light of other wavelengths (e.g., white light) is mixed with the focal color, and hue is the focal color at a particular wavelength range (e.g., red, green, blue) (see Endler [1990] and Grill and Rush [2000] for a fuller discussion). A color pattern may result from variation in any of these factors across the form of the organism and may, for example, result in quantitative characteristics such as the number of spots or the size of a blotch.

In a very real sense, all humans are color blind (regarding hue) when compared with many vertebrates. It is thought that the ances-

tral condition of vertebrates is for the SWS1 (short wavelength sensitive type 1) pigment to be ultraviolet (UV) sensitive (Yokoyama and Shi, 2000). An amino acid mutation in the SWS1 visual pigment of primates shifts the cone sensitivity to longer wavelengths and renders cones insensitive to UV light; hence, human color vision is restricted to ca. 400–700 nm (Yokoyama and Shi, 2000). Much has been made of the consequences of this restriction on human vision for behavioral studies of other vertebrates that have UV vision, but to what extent is this pertinent to comparative evolutionary studies using color?

Color matching or photographic techniques emulating human vision are not going to detect a difference in hue if none exists. However, these techniques may fail to detect a difference that does exist, such as the sexual difference in blue tits in the 360–400-nm section of the spectrum (Andersson et al., 1998). Similarly, differences in color pattern that can be visualized by humans, such as blotch number, are likely to be valid even though there is the possibility of having missed such a pattern if the marking is entirely below 400 nm. Even if the peak reflectance of a marking is below 400 nm, sufficient reflectance above 400 nm may exist to enable its pattern to be established satisfactorily. For example, in the Tenerife lizard (*Gallotia galloti*) the peak reflectance

of the lateral markings is clearly in the UV range (Thorpe and Richard, 2001). Nevertheless, humans see these markings as "blue," and a comparison of UV photography and ordinary color photography gives identical quantitative results for the pattern.

Hence, the limitations of human vision are likely to matter most in a quantitative comparison of color rather than in pattern identification. The objectivity and wider range of wavelength possible with a spectrometer (spectroradiometer) has distinct advantages over human vision-based color matching and description (Endler, 1990), particularly when the color has components below the 400-nm limit of human color vision. Spectrometers generally represent the color of a patch on an organism as the reflectance at a given nanometer value in a large number of arbitrary wavelength points along the electromagnetic spectrum (e.g., >1,000 points between 330 and 710 nm). There are currently two main approaches to using spectrometric data in comparative evolutionary studies. Endler (1990) divided the spectrum into a few (four) arbitrary, mutually exclusive adjacent units of equal nanometer range (e.g., violet, blue, green, red) and justified this approach in a visual biological context. Alternatively, the reflectance at a given wavelength, or small segment of the spectrum, can be treated as a character and subjected to principal component analysis (PCA). The vectors may then be interpreted as representing brightness, hue, chroma, and other aspects of color variation (Endler, 1990; Grill and Rush, 2000). These data may also be subsequently input into analyses such as discriminant function analysis and treated as characters (Cuthill et al., 1999).

Problems may exist with both these approaches in comparative evolutionary studies. The basic premise adopted here is that in comparative evolutionary studies such as systematics, independent unitary characters should be defined solely on the basis of the attributes of the organism irrespective of the perception of that organism by others with which it interacts, such as conspecifics, competitors, predators, or prey. Apart from the fact that color may have a nonvisual role, such as thermoregulation, any assumptions about the visual role of color are difficult to support. Consequently, dividing the spectrum into a few arbitrary, mutually exclusive adjacent units of equal nanometer range

may not be optimal for many comparative evolutionary studies. Even though PCA can be useful for investigating the importance of color, it is not suitable for intergroup comparison, where reflectances at numerous wavelengths are treated as independent "characters," because the measurements at adjacent or even close wavelengths are very strongly collinear. This problem cannot simply be overcome by methods that take into account the covariance among characters (e.g., canonical variate analysis), because (1) the pathological collinearity renders the (actual or implied) pooled within-group covariance singular and (2) there will not be adequate degrees of freedom. Cuthill et al. (1999) dealt with this problem by using PCA scores as input to discriminant function analysis (canonical variate analysis). Using the output from one multivariate analysis (orthogonal PCA scores) where among-group and within-group covariances are confounded to conduct another multivariate analysis, such as discriminant or canonical analysis (and related D^2), that is specifically designed to separate among-group and within-group covariance cannot be recommended and does not result in the unit characters often required in comparative evolutionary studies.

Hence, current methods for analyzing spectrometric output do not lend themselves to large-scale quantitative comparison of evolutionary groups. Here, I suggests procedures that can be used to derive, with some objectivity, a few relatively independent and informative spectral segments that are free of biological context (e.g., vision) and that can be used as unit characters in comparative evolutionary and systematic studies. These segments of the spectrum are nonoverlapping but may be of unequal size range, with gaps between them. These procedures were applied to a study of the geographic variation in hue of the St. Vincent anole (*Anolis trinitatis*).

Anolis trinitatis is the smaller of the two anoles endemic to the Lesser Antillean island of St. Vincent. This species does not have a complex color pattern (i.e., no large number of distinct spots, bars, or blotches), but regions of the body vary markedly in color across the island. For example, the dorsal surface may appear in human vision as bright green (montane), green/brown (Caribbean), or blue/green/gray (Atlantic), depending on geographic origin.

Lizard populations on small islands, far from being homogeneous evolutionary units, can and generally do show deep phylogenetic divisions commensurate with the island's age (Thorpe and Malhotra, 1996; Pestano and Brown, 1999; Gübitz et al., 2000; Malhotra and Thorpe, 2000a). Moreover, St. Vincent, like other high-altitude Lesser Antillean Islands, has pronounced ecological zonation (Beard, 1948), and several Lesser Antillean anoles have color patterns and hues that vary in relation to this ecological zonation (Thorpe et al., 2002). Color can vary with habitat because of both selection for crypsis and sexual selection to optimize signal transmission (Endler, 1993). Consequently, both selection processes can be implicated in this association between habitat and color. Sexual selection is, by original definition (Darwin, 1859; Futuyma, 1979), a type of natural selection, and the latter term is

used hereinafter to encompass both sexual selection and selection for crypsis. Although it is not the purpose of this study to focus on differentiating between types of selection, I have attempted to test quantitative assessments of the geographic variation in color against ecological zonation and phylogenetic lineage to elucidate broad causal hypotheses.

METHODS

Molecular Phylogeny

Two specimens from 11 localities (Fig. 1a) were sampled by noninvasive removal of tail tips (tissue preserved in 80% ethanol). Whole genomic DNA was extracted (Sambrook et al., 1989), and a 1,139-base pair (bp) segment of the cytochrome *b* gene was amplified with modified (Malhotra and Thorpe, 2000b) primers Mt-A (Lenk and

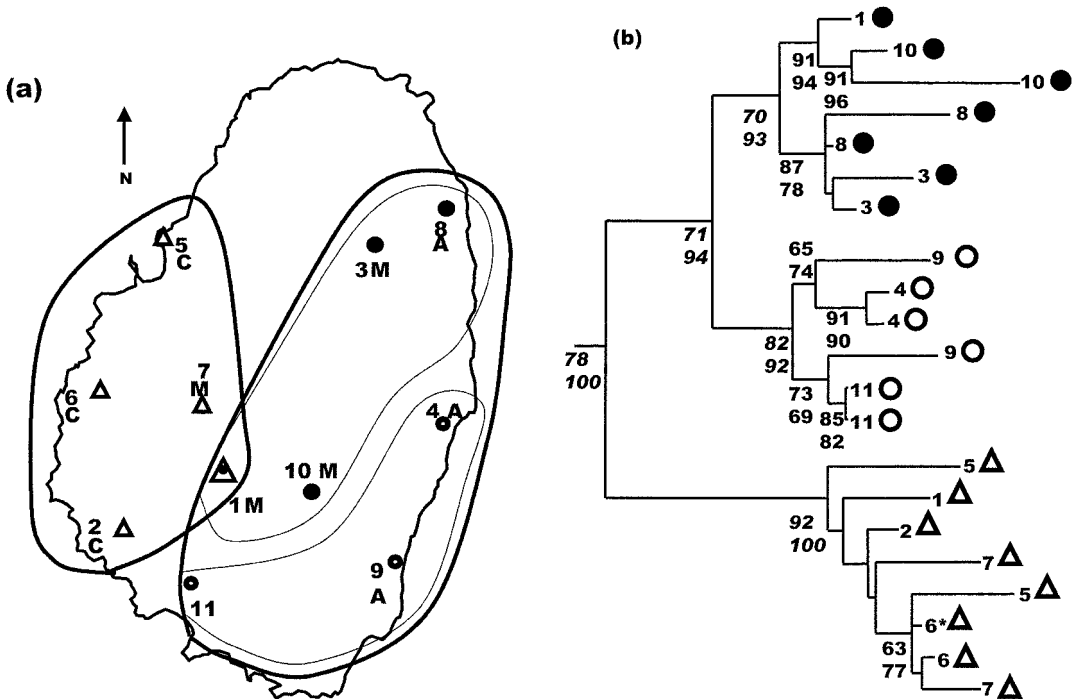


FIGURE 1. (a) Sample localities on St. Vincent. Letters C, M, and A are Caribbean xeric woodland, montane rain forest, and Atlantic coastal habitat sites, respectively, used in the spectrometry. The geographic distribution of lineages (thick lines) and main sublineages (thin lines) from the phylogram (b) are overlaid on the map. (b) ML gene tree with bootstrap supports given for ML (upper value) and MP (lower value) trees when nodes are identical and supported by bootstraps $>50\%$ for both trees. The major nodes defining the mapped clades are identified by italicized bootstrap values. The ML and MP topologies are identical except in the MP tree haplotype 6* belongs to a poorly resolved dichotomy, basal to the other three haplotypes in the trichotomy. The locality number of each individual is given at the terminal node. Δ = western lineage; \bullet = eastern lineage, north sublineage; \circ = eastern lineage, south sublineage. Within both eastern sublineages, there is even further north-south structuring.

Wink, 1997; 5'-CTCCCAGCCCCATCC AACATCTCAGCATGATGAACTTCG-3') and Mt-F (Wink, 1995; 5'-AGGGTGGAGTCTTCTGTTTTTGGTTTACAAGACCAATG-3'). Polymerase chain reactions (PCRs) were carried out in 50- μ l volumes containing 100–150 ng template DNA. The final reaction volumes consisted of 1 \times reaction buffer (50 mM KCl, 20 mM Tris-HCl, pH 8.4), 3 mM MgCl₂, 400 nM Mt-A and Mt-F primers, 400 μ M each dNTP, and 0.04 U/ μ l *Taq* DNA polymerase (Gibco BRL). After an initial denaturation step of 3 min at 94°C, 5 cycles of 30 sec denaturation at 94°C, 1 min annealing at 45°C, and 1 min elongation at 72°C were followed by 30 cycles with the annealing temperature increased to 51°C and then a final elongation step of 5 min at 72°C. Removal of excess primers, dNTPs, and nonspecific amplification products was achieved by electrophoresis through 1% agarose gels. The gel slice containing the desired amplification product was excised under UV illumination, and the DNA was recovered using Concert Rapid Gel Extraction System (Gibco BRL). After increasing the concentration of the recovered DNA by ethanol precipitation, Big-Dye terminator (PE Biosystems) cycle sequencing reactions were carried out following the manufacturer's protocol. The resultant reaction products were analyzed on an ABI 377 automatic sequencer. Both strands of the PCR product were sequenced.

Sequences were aligned by eye and translated into amino acid sequences using MEGA 1.02 (Kumar et al., 1993) to check for stop codons. There were no stop codons, insertions, or deletions, and pseudogenes were not suspected (Sorenson and Fleischer, 1996; Zhang and Hewitt, 1996). The full 987-bp sequence was available for all samples except one *A. trinitatis* replicate sample, which was excluded. In addition to 21 *A. trinitatis* samples, there were 5 samples from the same *roquet* species series (Underwood, 1959; Giannasi et al., 2000) (*A. griseus*, *A. extremus*, and southern, central, and northern *A. roquet*) and 1 outgroup (*A. oculatus*) from the distant *A. bimaculatus* series (Underwood, 1959). MODELTEST 3.0 (Posada and Crandall, 1998) was employed to test for the most appropriate model of sequence evolution. The parameters suggested by MODELTEST were then used to construct a maximum likelihood (ML) tree

using a heuristic search (specifying random addition of sequences and tree bisection-reconnection, with 100 replications) in PAUP* 4.0b8 (Swofford, 2001). Maximum parsimony (MP) was also used to reconstruct the haplotype tree using a heuristic search as above but with 5,000 replications. Using the same procedures and program, the bootstrap support (Felsenstein, 1985) for the nodes of these trees was assessed using 100 replicates. The patristic distance between a pair of terminal nodes is the sum of the branch lengths between them. The mean patristic distances were computed among the *A. trinitatis* sample localities for use in matrix correspondence tests to represent their phylogenetic relationships. Patristic distances, also called tree distances (see Page and Holmes, 1998: 26–28), are calculated in PAUP* 4.0b8 under the Describetrees option after saving the appropriate ML and MP trees.

Spectrometry and Associated Methods

The diffuse reflectance of a surface, as a percentage of a white tile standard calibrated against Spectralon (Labsphere, UK), was measured using an S2000 spectrometer (Ocean Optics Europe, The Netherlands) with dual deuterium and halogen light sources and SPECTRAWIN 4.1 software (Top Sensor Systems, The Netherlands). A standard (Ocean Optics) reflection probe with a 50 μ receptor fiber was presented to the surface at 45° with specially made matt black head screwed to the probe. The spectra were averaged across four independent recordings from each region on each lizard.

About five adult males were studied from each of 10 sites (Fig. 1a). These animals included those used for molecular analysis. Three sites were along the Atlantic coast in what is often originally littoral woodland habitat on high-elevation Lesser Antillean islands (Beard, 1948), three were along the Caribbean coast in xeric woodland habitat, and four were at high altitude in montane rain forest or associated areas. For each specimen, the reflectance spectrum was measured from seven regions of the body: dewlap, chin, temporal area (midpoint between the ear and eye), lighter marking above the forearm pit (oxter), middorsal trunk, midventral trunk, and lateral surface of the base of the tail. In addition, spectra were recorded from the dorsal and ventral surfaces of females from

montane sites 1 and 3. Although the reflectance of a small (2 mm) spot on a large flat surface can be recorded using this equipment configuration, the body area of females is too small to conveniently record several other body zones, such as dewlap and oster. All recordings were taken at the same temperature, and because anoles may darken under stress (metachrosis), care was taken to avoid stressing the animals during recording. All specimens were released unharmed at the site of capture.

The occurrence and distribution of UV reflectance on the anoles was also recorded using high-resolution macrophotography. A prefocused 100-mm Canon macro lens on an EOS1 body (mounted on a tripod) was fitted with a 360-nm UV pass filter, and the anole was illuminated against a UV-reflective background by a Metz flash modified for UV output, exposing 1,600-ASA Fuji Neopan monochrome negative film. This approach provided sufficiently sharp resolution for individual scales to be clearly defined. Matching Kodochrome 64 transparencies gave a photographic record of color in the approximate human visual range.

Data Analysis

Because the shape of the spectral profile tends to be far more consistent than the absolute reflectance (brightness), variability in reflectance can be overcome by standardization. Reflectance is standardized against the area under the entire curve, which effectively removes this brightness element. The reflectance percentage for the selected wavelength range was excised from SPECTRAWIN output files, standardized by area under the spectral curve, averaged across the four repeat measurements, cut into wavelength segments as desired, grouped by locality and character, and arranged for input into other programs by a suite of specially written programs, OMNISPEC (by R.S.T.). A one-way ANOVA (program 7D in BMDP Dynamic, release 7.0, SPSS) was run across the male sample sites using 10-nm fractions for each of the seven body zones, and the *F* ratios were plotted against wavelength. This analysis gives a measure of the strength of intergroup or geographic variation in the hue of a body zone at the various wavelengths and can be used to indicate which regions of the spectrum can be excluded be-

cause they contain relatively little intergroup information.

Within-group (locality) relationships among 19 fractions (20 nm wide) of the spectrum (330–710 nm) were investigated by multiple group PCA (MGPCA, by R.S.T.). The analysis was run separately for each of the seven body zones. MGPCA extracts eigenvectors and eigenvalues from the pooled within-group covariance matrix (Thorpe, 1983a, 1983b), treating each of the 19 spectrum fractions as a character and each of the 10 localities as a group. Segments 20-nm wide were selected for this analysis as a balance between degrees of freedom and detail (tests showed that 10-nm segments gave comparable results). The delta (δ) analysis procedure based on the eigenvector coefficient difference is introduced here as a means to investigate the relationship among sections of the spectrum. An eigenvector will have a series of coefficients, one for each spectral fragment treated as a character. The absolute difference between two coefficients adjacent on the spectrum is the eigenvector coefficient difference, δ . Hence, for a given eigenvector, the difference between the first and second, second and third, third and fourth, fourth and fifth, etc., coefficients is computed.

Put more formally, a given eigenvalue a_i has an associated eigenvector \mathbf{a}_i of length n (where n = the number of "characters" or small spectral fragments in this case) such that

$$\mathbf{a}_i = \begin{pmatrix} a_{i,1} \\ a_{i,2} \\ \cdot \\ \cdot \\ a_{i,n} \end{pmatrix}.$$

A derivative vector δ_i of length m (where $m = n - 1$) such that

$$\delta_i = \begin{pmatrix} \delta_{i,1} \\ \delta_{i,2} \\ \cdot \\ \cdot \\ \delta_{i,m} \end{pmatrix}$$

can be found from the associated eigenvector \mathbf{a}_i , where $\delta_{i,j} = |a_{i,j} - a_{i,j+1}|$ and j varies from 1 to m .

If there are sections of the spectrum under common control, they should have a low δ , if under independent control, they should have a high δ . An MGPCA run on a given body zone with 19 small spectral segments will yield 19 eigenvectors in this case, because there are ample individuals sampled. Each vector describes a component that expresses a progressively smaller proportion of the total within-locality variation until 100% of the accumulated variation is encompassed when the final (19th) vector is considered. However, most of the variation is expressed in the first few vectors. The coefficient, δ , can be averaged across several eigenvectors; the first four are chosen here because they generally expressed approximately 99% of the total within-locality variation for an MGPCA run on a given body zone. However, the procedure did not appear sensitive to the exact number of eigenvectors selected. Hence, a plot of mean δ (averaged over vectors) against spectrum should have peaks and valleys relating to segments of the spectrum if they are independent. Peaks (if they exist) can be used as points at which the spectrum can be objectively split into segments for that body zone. If different body zones have peaks in different but adjacent parts of the spectrum, then the peaks will be eradicated by generalizing across body zones, which is not advised. However, if it is appropriate to generalize these segments across the various body zones, then a grand mean δ (averaged across all body zones for selected vectors) will still show peaks and valleys. Workers tend to inspect eigenvector coefficients to detect patterns, and this approach gives this analysis a more formal basis.

The spectrum was split into segments using this delta analysis procedure, together with information from the F ratios indicating which sections of the spectrum do not show geographic variation. The generalized among-locality variation across (1) all body zones for a given spectral segment, (2) all spectral segments for a given body zone, and (3) all body zones and all spectral segments was analyzed by canonical variate analysis and associated Mahalanobis D^2 (relative similarity among sites). A spectral segment for a body zone was included in these analyses if the among-locality variation was significant using a one-way ANOVA (program 7M in BMDP Dynamic, release 7.0, SPSS) and it was not collinear with an included "character."

A two-way ANOVA (program 7D in BMDP Dynamic, release 7.0) across sites (localities 1 and 3) and sex was used to test for sexual dimorphism in dorsal and ventral hue in montane forms.

The relative similarity was computed among sites for (1) each spectral segment of each body zone above, (2) all body zones for a given spectral segment, (3) all spectral segments for a given body zone, and (4) all body zones and all spectral segments. These are the dependent variable matrices in the tests for causal hypotheses. Five causal hypotheses were tested (independent variable matrices).

Hypothesis 1 addresses geographic proximity, as represented by the geometric distance among localities. An isolation-by-distance model (Douglas and Endler, 1988) leads to the prediction that spatially close populations will have similar hues and that spatially distant populations will have dissimilar hues. Hence, there will be no distinct geographic pattern.

Hypotheses 2, 3, and 4 involve adaptation by natural selection to the habitat types on the island. Hypothesis 2 is that there is adaptation to a high-altitude montane rain forest habitat (binary coded to contrast localities 1, 3, 7, and 10 with other localities). This hypothesis leads to the prediction that these central montane populations will have hues different from those of the coastal populations in a "concentric" geographic pattern. Hypothesis 3 is that there is adaptation to the coastal Caribbean xeric woodland habitat (binary coded to contrast localities 2, 5, and 6 with others). This hypothesis leads to the prediction that these Caribbean coastal populations on the western edge of St. Vincent will have hues different from those of the other populations. Hypothesis 4 is that there is adaptation to the coastal Atlantic habitat (binary coded to contrast localities 4, 8, and 9 with others). This hypothesis leads to the prediction that these Atlantic coastal populations on the eastern edge of St. Vincent will have hues different from those of the other populations.

Hypothesis 5 addresses phylogenetic relationship, as represented by the average patristic distance among localities, based on the lengths along the branches of the ML tree. This distance was replicated in a subset of tests using patristic distances from MP trees and raw genetic distances to determine

whether they yielded results different from the ML patristic distances. Because the results are very similar, they are excluded here for conciseness. This hypothesis is that phylogenetically close populations have similar hues and phylogenetically distant populations have dissimilar hues, which leads to the prediction of a predominantly east-west pattern of geographic variation, as reflected in the geographic distribution of the lineages (Fig. 1a).

The (dependent) color variables were tested against the causal hypotheses (independent variables) using pairwise and stepped partial-regression matrix correspondence (Mantel) tests employing standardized regression coefficients and 10,000 randomizations (program by B. F. J. Manly, modified by R.S.T.; Manly, 1986; Daltry et al., 1996; Thorpe et al., 1996). In a pairwise test, the null hypothesis of no association is rejected when $P < 0.05$. When both adaptive (hypotheses 2-4) and historical (hypothesis 5) hypotheses are significant, a pairwise test is carried out on the adaptive hypothesis once the historical hypothesis has been regressed out a priori. Similarly, a pairwise test is carried out on the historical hypothesis once the adaptive hypothesis has been regressed out a priori. This approach is used to test the effects of natural selection irrespective of the effects of phylogenetic relationships and vice versa. In addition, if more than one independent variable is significant, these variables are entered into a stepped partial regression matrix correspondence test. The variable with the highest standardized regression/correlation is entered first, and subsequent variables are entered if they have the highest partial value after recalculation of included variables. This process is halted when a variable is entered that results in any included variable having significance level below that required by a sequential Bonferroni correction (Rice, 1989).

RESULTS

Molecular Phylogeny

Based on 987 bp, the uncorrected p distances have a maximum value of 6.2% (groups 7 and 10) among *A. trinitatis* haplotypes (GenBank accession numbers AF493583-AF493603). There are 76 parsimony informative sites within the *A. trinitatis*

ingroup. MODELTEST identified the general time-reversible plus gamma model for subsequent use in ML reconstruction with the rate matrix as [A-C] 3.317, [A-G] 11.434, [A-T] 4.493, [C-G] 2.155, [C-T] 22.211, and [G-T] 1.000, the ACGT base frequencies as 0.315, 0.219, 0.123, and 0.344, respectively, and the gamma shape parameter as 0.317. The ML and MP trees are very highly congruent; all nodes except one (near terminal) were identical. Consequently, only the ML tree is illustrated (Fig. 1b), but bootstrap support is shown for both reconstruction methods. The major (deeper) nodes (identified in Fig. 1b) have bootstrap support at $\geq 70\%$ for the ML tree and $\geq 82\%$ for the MP tree. *Anolis trinitatis* has a primary phylogenetic division between western and largely eastern lineages (Fig. 1), which overlap at one montane locality (locality 1). The largely eastern lineage is further divided into a sub-lineage occupying the northern and montane area (eastern lineage-north) and a sub-lineage occupying the southern coastal area (eastern lineage-south) with bootstrap support. Both of these eastern sublineages have further north-south structuring.

Spectral Segments

Actual spectra (representatives are illustrated in Fig. 2) show simple patterns with usually one or at most two peaks and provide no evidence of the need for the spectrum to be divided into large number of fine segments. There are marked differences among localities. For example, locality 4 (Atlantic coastal habitat, eastern lineage) shows high UV reflectance for all figured body zones, locality 7 (montane habitat, western lineage) has intense green temporal and dorsal surfaces and no UV component, and locality 6 (Caribbean xeric woodland habitat, western lineage) has a less intense temporal and dorsal surface with a relatively high red but no UV component.

The plot (Fig. 3) of F values from the ANOVA across localities for each character and each 10-nm fraction of the spectrum revealed (1) the extent to which segments of the spectrum showed geographic variation and (2) the extent to which the various regions of the body (body zones) show geographic variation in color. There is a clear indication (Fig. 3) that geographic variation in color differs substantially among body zones. The

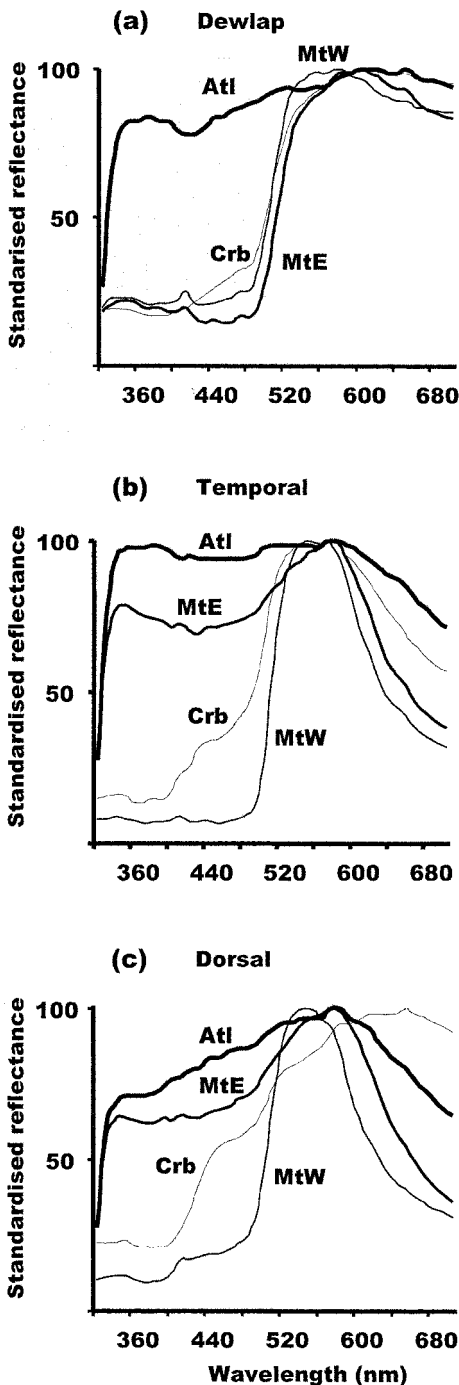


FIGURE 2. Representative spectra for three body zones from four localities across the center of St. Vincent: dewlap (a), temporal region (b), and dorsal surface of the trunk (c). From west to east (thin lines to thicker lines) the localities are Caribbean coast (Crb, locality 6), western montane (MtW, locality 7), eastern montane (MtE, locality 10), and Atlantic coast (Atl, locality 4). For ease of visualization, each spectrum is standardized with the maximum peak at 100%.

dewlap clearly has the most pronounced geographic variation in color, the temporal, chin, and oter show intermediate levels of geographic variation, the dorsal surface and tail show less geographic variation, and the ventral surface has the least variation. It is also evident from Figure 3 that geographic variation in reflectance is not consistent across the spectrum; there are distinct peaks and troughs. There is one very pronounced trough, that is, none of the body zones show much geographic variation around the 500-nm area of the spectrum. However, all characters show a peak close to this area at the 530+ (520–570) nm region of the spectrum, and some show peaks in the 360+ (340–400) and 420–460-nm regions of the spectrum. Hence, most geographic variation occurs in the UV, indigo/blue, and midgreen spectra, with relatively little in the short-wavelength green spectra (490–510 nm). The 490–510-nm segment of the spectrum was not included in later analyses because it showed little geographic variation.

The MGPCAs for each body zone for each analysis yield δ plots with distinct peaks and valleys, which indicates that the spectrum can be (generally) objectively divided into segments that have common control (covary) within them but independence between them. Moreover, these peaks and valleys are retained after averaging over all body zones (Fig. 4). Consequently, the same segmentation of the spectrum was used for each body zone. The delta analyses indicate five segments, 330–410, 410–490, 510–590, 590–630, and 630–710 nm. These are referred to, respectively and arbitrarily, as UV, blue, green, "yellow" (yellow/orange), and red. The position of the division between the UV and blue segments was chosen as 410 nm, but it could as well have been at the adjacent fraction, that is, 430 nm; all other divisions in Figure 4 are unequivocal.

Patterns and Tests

A canonical variate analysis, treating each of the five spectrum segments from each of the seven body zones as a character, ordinated the 10 local samples primarily according to habitat type and lineage after 13 of the characters were excluded because they showed insufficient among-locality variation (by ANOVA) or were collinear with included characters (Fig. 5).

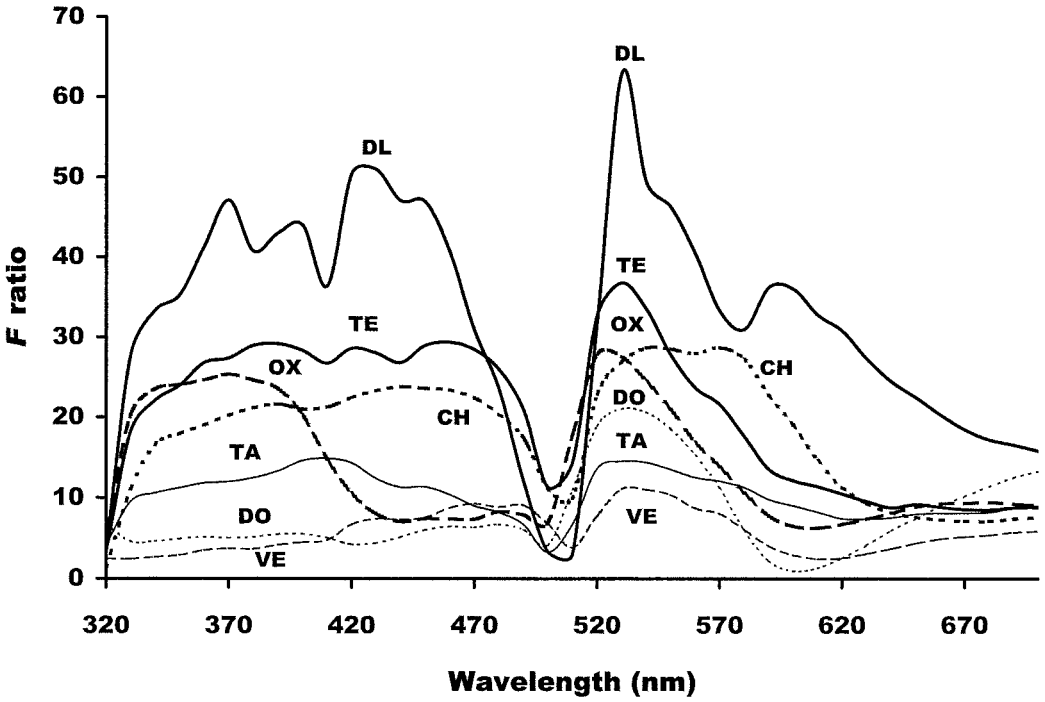


FIGURE 3. Magnitude of geographic variation in color. The extent of variation is shown at each wavelength among geographic localities for each body zone (expressed as *F* ratios, ANOVA). DL = dewlap; TE = temporal; CH = chin; OX = oxter; TA = tail; DO = dorsal; VE = ventral.

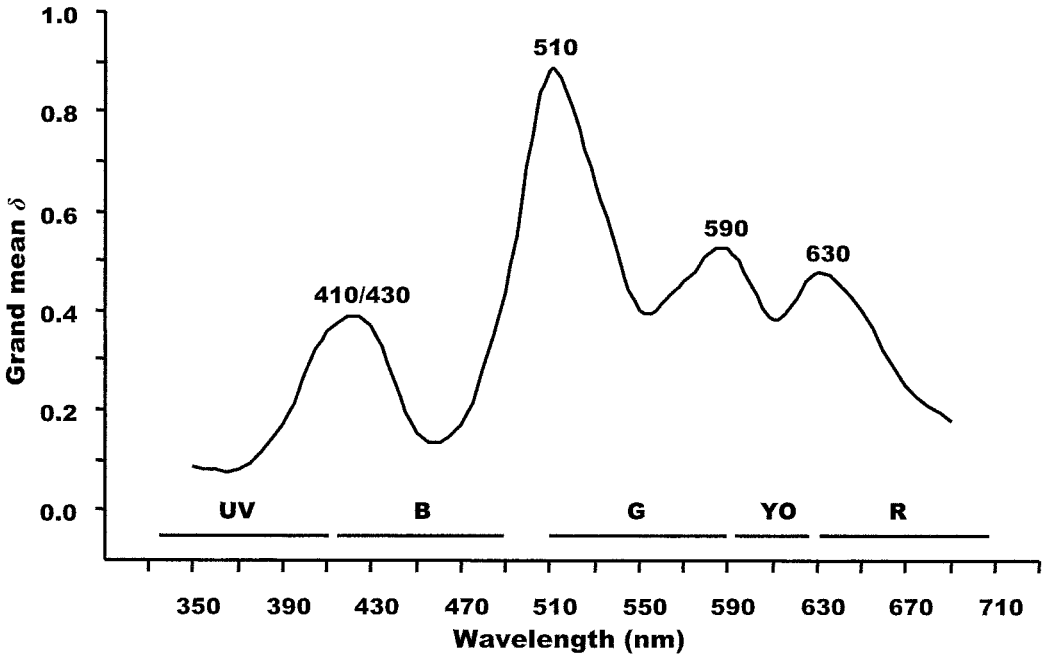


FIGURE 4. Delta analysis. Objective segmentation of the spectrum using the eigenvector coefficient difference (δ). The grand mean δ computed across the first four MGPCA eigenvectors for all body zones is plotted against wavelength. Peaks separate independent segments of the spectrum. In this example, they split the spectrum unequivocally at 630, 590, and 510 nm and at either the 410- or 430-nm division, giving five segments arbitrary referred to as ultraviolet (UV, 330–410 nm), blue (B, 410–490 nm), green (G, 510–590 nm), yellow/orange (YO, 590–630 nm), and red (R, 630–710 nm).

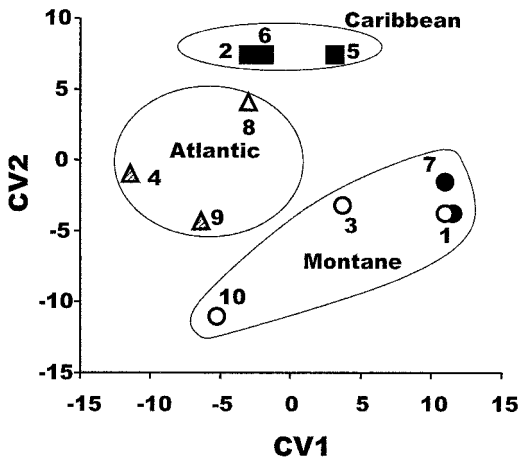


FIGURE 5. Population similarity based on generalized color. The canonical variate (CV) scatter diagram is based on spectrum segments from all body zones. Localities ordinate by habitat and lineage. Habitats are Caribbean coast (squares), montane (circles), and Atlantic coast (triangles). Phylogenetic lineages are western (solid symbols), eastern-north (open symbols), and eastern-south (shaded symbols).

Matrix correspondence tests (Table 1) of generalized character systems showed that the hue variation is associated with both habitat type and phylogeny. This association was apparent in the “total” analysis generalizing across all body zones/hues together, where both the phylogeny and an aspect of habitat were significant. Tests gen-

eralizing hue (across body zones) and body zones (across hue) also tended to show that both habitat factors and phylogeny are significantly associated with geographic variation in color. Principally, the analysis of hue (across body zones) indicated that even when phylogeny is taken into account the Atlantic coastal habitat is associated with low- and medium-wavelength hues, particularly high UV reflectance. When habitat is taken into account, phylogeny is associated with UV and “yellow” reflectance and with the hue of the temporal, chin, oxtar, and tail regions.

Tests of each hue for each body zone provided a more detailed picture (Table 2). The brilliant green dorsum of montane forms (irrespective of lineage) is reflected in a significant association between dorsal green and altitude. The duller, more reddish hue of the lowland Caribbean xeric woodland forms is also reflected in these tests. However, of the habitat types, the Atlantic coastal habitat is most frequently associated with geographic variation in color and is often linked to an association with phylogeny. Nevertheless, even when the phylogeny is taken into account the Atlantic coastal habitat is associated with low- and medium-wavelength color variation, particularly high levels of UV reflectance, on the dewlap, chin, tail, and ventral surfaces. When habitat is taken into account, phylogeny is associated with

TABLE 1. Matrix correspondence tests generalized across hue (for each body zone) and across body zones (for each hue) and all hues for all body zones (total). Values are significant pairwise standardized regressions (correlations) between character and hypotheses followed by the step number at which they were still significant (Bonferroni corrected) in a stepwise partial regression matrix correspondence test. The letter E following the values indicates when an adaptive hypothesis (B–D) is still significant in a pairwise test once the significant historical hypothesis (E) is regressed out *a priori*. Similarly, the letters B, C, and D indicate that the historical hypothesis (E) is still significant in a pairwise test once the respective significant adaptive hypothesis (B–D) is regressed out *a priori*.

	Proximity (A) ^a	Altitude (B)	Xeric (C) ^b	Atlantic coast (D)	Phylogeny (E) ^c
UV	0.39			0.55 (2, E)	0.52 (2, D)
Bl				0.54 (2, E)	0.35
Gr				0.48 (2, E)	0.26
Y	0.36			0.45 (2, E)	0.51 (2, D)
Dewlap				0.53	
Temporal					0.41
Chin					0.39
Oxtar		0.36 (2, E)	0.33		0.42 (2, B, C)
Tail		0.35 (E)	0.42 (2)	0.35 (2)	0.39 (B, C, D)
Dorsal		0.74 (2)	0.38 (2)		
Ventral		0.54			
Total		0.47 (2, E)			0.35 (2, B)

^aGeographic proximity.

^bCaribbean xeric woodland.

^cPatristic distances from ML phylogeny.

TABLE 2. Matrix correspondence tests. Spectrum segments for each body zone. Values are significant pairwise standardized regressions (correlations) between character and hypotheses followed by the step number at which they were still significant (Bonferroni corrected) in a stepwise partial regression matrix correspondence test. The letter E following the values indicates when an adaptive hypothesis (B–D) is still significant in a pairwise test once the significant historical hypothesis (E) is regressed out *a priori*. Similarly, the letters B, C, and D indicate that the historical hypothesis (E) is still significant in a pairwise test once the respective significant adaptive hypothesis (B–D) is regressed out *a priori*.

		Proximity (A) ^a	Altitude (B)	Xeric (C) ^b	Atlantic coast (D)	Phylogeny (E) ^c
Dewlap	UV	0.36			0.59	0.24
	Blue	0.33				
	Green				0.52	
	Yellor				0.57	
Temporal	UV	0.38			0.37	0.68 (2, D)
	Blue					0.34
	Green					0.34
	Yellor					0.37
Chin	UV	0.46			0.58 (3, E)	0.65 (3, D)
	Blue				0.42	0.53 (2, D)
	Green				0.53 (2, E)	0.39
	Yellor				0.51 (E)	0.53 (D)
	Red	0.42		0.39		0.64 (2, C)
Oxter	UV	0.30				0.53 (2)
	Tail					
Tail	UV	0.65			0.71 (2, E)	0.74 (2, D)
	Blue	0.45			0.65 (3, E)	0.50 (2, D)
	Green				0.74 (2, E)	0.35
	Yellor	0.60 (2)			0.56 (2, E)	0.57 (D)
	Red	0.66		0.56 (2, E)	0.42	0.79 (3, C, D)
Dorsal	UV	0.30				0.48
	Green		0.45			
	Red		0.38			
Ventral	UV	0.56 (2)			0.53	0.38 (D)
	Blue	0.36			0.56 (2)	
	Yellor	0.49			0.66 (2)	
	Red	0.35		0.43 (2)		

^aGeographic proximity.

^bCaribbean xeric woodland.

^cPatristic distances from ML phylogeny.

low- and medium-wavelength temporal hues, low-wavelength chin, dorsal, and oxter hues, and low- and high-wavelength tail hues.

The results of the two-way ANOVA on montane forms showed that males are significantly greener than females on both the dorsal ($P < 0.005$, df 1,10) and ventral ($P < 0.005$, df 1,10) surfaces.

DISCUSSION

Analysis of Spectra

In many comparative evolutionary studies, there is often a requirement to use unit characters with minimal collinearity and minimum assumptions as to the role of the character. This requirement extends to the use of color, and the delta analysis procedure was useful in segmenting the spectra, with some objectivity, to provide these independent unit characters. The delta anal-

ysis showed that the segments were relatively few in number and were not necessarily of equal size or of a predetermined number. Almost all the variation was expressed in the four vectors used to compute the mean δ , but the technique does not appear to be particularly sensitive to the exact number of eigenvectors used. Extracting the eigenvectors from the pooled within-group covariance matrix (MGPCA) would appear to be the preferred method to optimize the assessment of δ , but similar results were obtained from PCA. Even though standardization by area deletes the information on "brightness," this approach has considerable practical utility for comparing spectra where absolute brightness has poor repeatability, as with squamate skin with its irregular surface.

The delta analysis procedure is not absolutely objective, and there are alternatives. When the δ plots show a high level of

independence across a section of adjacent spectra (e.g., δ plots are rounded rather than sharp, as in the lower wavelengths in this study), judgement is required on the exact border of the segments. Similarly, when there are segments of the spectrum with low information level (as shown by ANOVA, Fig. 3), judgment is required on the exact extent of the wavelength range to be excluded. With regard to alternatives, one could employ a related method and use the correlation between adjacent segments of the spectrum, where $1 - r$ would give a plot comparable to δ . Although a rigorous comparison is beyond the scope of this study, these two approaches gave quite similar results with this data set. However, the delta analysis is preferred and employed in this study because (1) the eigenvectors on which δ is based are derived from a full set of correlations/covariances in a matrix of $n^2/(2 + n)$ values (where n is the number of spectral fragments) whereas the correlations are only between adjacent fragments (i.e., only $n - 1$ values) and (2) preliminary comparisons using this data set suggest that the delta analysis is better than the correlation analysis at retaining the detail of separate peaks when averaged across body zones. However, I suggest that what matters is to employ an objective method that divides the spectra into informative, independent segments free of assumptions about biological context rather than to worry about the precise details of the procedure.

Phylogeography

The kilobase of cytochrome *b* sequence used here appears to be particularly suitable for phylogenetic reconstruction because it results in very high congruence among reconstruction methods and in bootstrap support for the major nodes. The distinct phylogeographic structure within *A. trinitatis* is compatible with that observed in other such studies of lizard populations on small islands (Thorpe and Malhotra, 1996) in the Canaries (Thorpe et al., 1996; Pestano and Brown, 1999; Gubitz et al., 2000) and Lesser Antilles (Malhotra and Thorpe, 2000a) and probably reflects the relatively low vagility of lizards. Although molecular clock calibrations are fraught with difficulty (Rand, 1994; Nunn and Stanley, 1998), similar island lizard models suggest a rate of at least 1% of

the bases per million years (bpmy) (Gubitz et al., 2000) to 1.4% bpmy (Malhotra and Thorpe, 2000a). The uncorrected maximum pairwise divergence of approximately 6.2% in this species suggests a divergence time of approximately 4–6 million years, compatible with the suggested Pliocene volcanic origin of the island (Martin-Kaye, 1969; Sigurdsson and Carey, 1990).

Distinct phylogeographic structure on these small islands may be associated with subsequent coalescence of separate ancient islands joined by later volcanic activity, such as Tenerife (Thorpe et al., 1996; Gubitz et al., 2000) or with vicariance induced by lava flows within unitary islands, e.g., Gran Canaria (Pestano and Brown, 1999) and Dominica (Malhotra and Thorpe, 2000a). Here, I raise a third possibility to explain the east–west lineages of the St. Vincent anole. St. Vincent is entirely volcanic and latitudinally elongate, with a central ridge of mountains. From weathering of currently exposed rocks, the south appears to be older than the north (Martin-Kaye, 1960), and the far north is still volcanically active, with significant activity in 1979 (Sigurdsson and Carey, 1990). However, one northern center is old, with early Pliocene lavas (Sigurdsson and Carey, 1990). Whatever the direction, a series of volcanic centers have elongated the island. This process may have provided an opportunity for the development of separate eastern and western coastal lineages on either side of an uninhabitable active volcanic center. These haplotypes lineages may have expanded separately along the respective sides of the island as the island developed. The mountain area could have been colonized from the lower elevations to the east and west. Ongoing volcanic activity may have created numerous bottlenecks and temporary dispersal barriers, modifying the basic pattern of divergence. Certainly in the east there are well-supported secondary and tertiary splits that tend to have a north–south component. The existence of a sympatric species may have had an impact on this divergence if the distribution of the smaller *A. trinitatis* were largely restricted to lower elevations by the larger *A. griseus* (Roughgarden, 1995). However, we do not know whether the timing and sequence of colonization was such that *A. trinitatis* and *A. griseus* were sympatric at the time of the phylogeographic divergence within *A. trinitatis*, and currently

these species do not have mutually exclusive distributions.

Geographic Variation in Color

Because there are distinct lineages within the island and distinct habitat types, geographic variation in color could reflect past historical relationships and thus be associated with the phylogeny and/or it could be responding to current selection pressures associated with the distinct climatic/vegetation biotopes. The lineages are not entirely independent of biotope, as both have an east–west component, but appropriate statistical procedures, including regressing out the effects of selection or phylogenetic relationships *a priori*, may allow one to distinguish between these factors (but see Manly, 1997). Nevertheless, the description of biotope is based on simple vegetation zones rather than a detailed and exhaustive analysis of the habitats, and aspects of the environmental variation may have been missed, thus underestimating the role of habitat. Notwithstanding these considerations, both history and habitat appear to play a role in determining the geographic variation in the color of this anole.

Although color may have a nonvisual role (e.g., thermoregulation), vision is important in anole communication (males have large dewlaps, which they extend for conspecific communication), and vision may play an important role in interactions with both predators and prey (Macedonia, 2001, and references therein). Attempts to predict the relative importance of all potential predators and prey and their color vision may be doomed to failure, but for conspecific communication anoles can use UVA wavelengths and higher, having four cones with absorbance peaks at approximately 365, 450/460, 495/500, and 562/565 nm (Fleishman et al., 1993, 1997; Macedonia, 1999, 2001). Consequently, these anoles should be able to detect color across most, if not all, of the spectra illustrated in Figure 2, although there will be a decline in sensitivity at higher wavelengths (Fleishman et al., 1997).

The life of a mature male anole may largely be a balance between crypsis to avoid predation and the need to signal to conspecifics to maintain a territory and attract a mate (Malhotra and Thorpe, 2000a; Macedonia, 2001). However, the dewlap color is likely

to optimize signaling rather than crypsis because it is kept folded away when not employed in signaling. In Greater Antillean anole communities, dewlap color can differ markedly among species and is thought to be important in maintaining species integrity and may be important in speciation (Losos, 2002). However, evidence from comparison of dewlap color using color matching in the human visual range has previously led to the view that dewlap color shows little intraspecific variation within a Lesser Antillean island, even when there is marked geographic variation in other characteristics (Thorpe et al., 2002). This evidence supported the view that the situation was different in the Lesser Antillean anoles and that the dewlap was unlikely to be important for potential speciation within these small islands. However, the results here clearly show that the dewlap color varies greatly among habitat types and therefore may have the potential to facilitate differentiation. Studies of the extent of molecular gene flow among habitat types associated with different dewlap coloration may elucidate the actual extent to which dewlap coloration genetically isolates populations. In recent study of sexually mature male lizards (*Gallotia galloti*) from the Canary Islands (Thorpe and Richard, 2001), UV markings used in conspecific signaling were suggested to be more influential than historical separation in determining gene flow. The most striking aspect of geographic variation in dewlap color is the high reflectance in lower wavelengths, particularly UV, in Atlantic coastal habitat populations irrespective of phylogeny. The Atlantic coast of high-elevation Lesser Antillean islands tend to have greater cloud cover than the Caribbean coast (Beard, 1948) and hence will be subject to relatively higher UV levels in the ambient light of the woodland shade (Endler, 1993, pers. comm.). Consequently, on the Atlantic coast the high UV reflectance of the dewlap may be selected for because it allows a brighter signal in UV-rich ambient light. However, this hypothesis is very tentative and more needs to be done to test this link, such as establishing parallel trends on independent islands with similar environmental zonation (Brown et al., 1991; Thorpe and Malhotra, 1996; Thorpe et al., 2002).

Unlike the dewlap, surfaces such as the trunk dorsum are exposed all the time and so may be expected to be more influenced

than the dewlap by the requirements of crypsis. Anole dorsal surfaces may show parallel patterns of geographic variation, suggesting natural selection. For example, on Lesser Antillean islands montane anoles tend to be greener, which may be interpreted as selection for crypsis (Thorpe et al., 2002). However, even here the surface may play an important role in signaling. Several lines of evidence support this idea (see also Macedonia, 2001). First, in other species of Lesser Antillean anoles there can be strong sexual dimorphism in the dorsal color pattern, such as the presence of black blotches only in males (Malhotra and Thorpe, 1999). Second, in this species there is significant sexual dimorphism in the extent of greenness of the dorsal and ventral surfaces in montane anoles, with sexually mature males having more intense green than females. In contrast, females anoles have less emphasis on display (Jenssen et al., 2000) and a greater emphasis on crypsis (Macedonia, 2001) and in this species have significantly less green and more red on these surfaces, giving a less intense green coloration. Third, the peak reflectance of the montane anole dorsum (locality 7, Fig. 2) matches the peak irradiance of the ambient light in montane forest and hence would maximize the brightness of the signal (Endler, 1993). I am not suggesting that there is no cryptic component to dorsal coloration in adult male anoles. Although a bright green male anole may be conspicuous against the bark of a tree trunk, it will more closely match the color of leaves and be cryptic when hiding among them. Moreover, anoles may darken, which will substantially influence their conspicuousness. Male *A. trinitatis* may be seen in the early evening perched on a dark trunk displaying a bright dewlap, with their other surfaces darkened (pers. obs.). Under these conditions, the dewlap is very conspicuous but the rest of the body is not (at least to a human).

Many of the other surfaces may also have a role to play in signaling. Many anterior surfaces such as the chin, temporal region, and oter may be clearly visible to conspecifics. The temporal region may have irregular patches of skin with high UV reflectance in some Atlantic populations (e.g., 4 and 9) from one lineage. Similarly the chin, which grades into the anterior dewlap, would be readily

seen by a facing conspecific and also has high UV reflectance in some Atlantic populations (e.g., 4 and 9) from one lineage. A similar situation exists with the oter marking. Once again, the relatively high levels of UV light in cloudy Atlantic coast conditions may optimize the brightness of these marks if used for signaling. However, the analysis of the current data show that (except for the chin) there is not a strong link to habitat type irrespective of phylogeny.

The phylogeographic analysis suggests that there may have been separate coastal lineages that subsequently expanded up into the mountains. The question then becomes why some surfaces, but not the dewlap, of montane forms resemble those of the respective lowland forms from which the montane forms may have arisen. For example, the temporal surface of eastern and western montane forms resembles that of their respective coastal forms. This resemblance is reflected in a significant and predominant association with phylogeny rather than habitat. However, the dewlap of the eastern montane form more closely resembles that of the western montane form rather than that of the phylogenetically related coastal form (Fig. 2), and this pattern is reflected in a significant and predominant association with habitat. Why does the hue of one surface (e.g., temporal) still reflect its phylogenetic past while the hue of another surface (e.g., dewlap) does not? It may be that the dewlap, being the primary signaling surface (Jenssen, 1970; Echelle et al., 1971) is subject to much greater selection pressure and therefore has more rapidly (Malhotra and Thorpe, 1991) evolved away from its ancestral state. However, this situation is opposite that observed in *A. conspersus* by Macedonia (2001), who concluded that dewlap color has not kept pace with body color evolution as the species has colonized new habitats.

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