Color Variation, Habitat Light, and Background Contrast in *Anolis carolinensis* along a Geographical Transect in Florida[1]

Joseph M. Macedonia,[2] Arthur C. Echternacht, and Justin W. Walguarnery

Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, Tennessee 37996, USA

Abstract.—A hallmark trait of the lizard genus *Anolis* is the presence of remarkable interspecific variation in dewlap color patterns. Yet, considerable intraspecific variation also occurs in many *Anolis* species. In Florida different populations of the Green Anole (*Anolis carolinensis*) exhibit red, pink, magenta, and even greenish-gray dewlaps. To date, comparisons of color variation in this species have used subjective techniques based on human color perception. Instead, we used an objective method—reflectance spectrometry—to quantify *A. carolinensis* dewlap and body color variation along a transect from northeastern to southwestern Florida. Reflectance readings of local vegetation allowed us to calculate contrast of the lizards with their visual backgrounds. By incorporating local ambient light and *A. carolinensis* spectral sensitivity into additional calculations, we were able to estimate lizard-background contrast from the perspective of the lizards. Results revealed dewlap and dorsum spectra to differ significantly among most of our study populations, as well as between the lizards and background vegetation. Although increased resemblance of a color signal to the illuminating spectrum will increase the signal's broadcast effectiveness, dewlap colors in our study populations did not resemble the ambient light spectra in their habitats. We consider the potential effects of this species' peculiar spectral sensitivity on the evolution of its dewlap coloration and propose that this coloration may represent a selective compromise to the unusually broad range of light habitats in which *A. carolinensis* is found. Last, the "gray throated" population of southwest Florida proved to be extraordinary, and we devote special attention to discussing aspects of coloration and contrast in this enigmatic population.

Animal color signals are designed through selection by details of the visual systems that perceive them, attributes of the ambient spectra that illuminate them, and features of the background from which they must be discriminated. Adult males in many species of iguanian lizards exhibit colorful gular regions, or dewlaps, that have evolved exclusively for signaling (e.g., Cooper and Greenberg, 1992). In the largely arboreal genus *Anolis*, the dewlap is specialized as a thin, flat, and retractable skin fold (e.g., Losos and Chu, 1998; Fleishman, 2000). Given that light in forest habitats varies spectrally as a function of forest geometry (e.g., Endler, 1992, 1993, 1997), sexual selection should favor dewlap colors that reflect the strongest regions of the ambient spectrum while, at the same time, exhibit contrast with the visual background. Although the dewlaps of adult male anoles often are large and colorful (e.g., Schwartz and Henderson, 1985), few field studies have examined the potential influence of habitat light on dewlap coloration (e.g., Fleishman et al., 1993; Leal and Fleishman, 2001; Macedonia, 2001).

Unlike dewlap colors, body coloration in many species of anoles tends toward shades of green and brown that may reduce the probability of detection by predators against a background of vegetation. Nevertheless, body coloration may serve a secondary signaling function in at least some *Anolis* species (e.g., Medvin, 1990; Macedonia, 2001).

In arboreal lizards whose geographical distributions include more than one vegetational regime, differences in light environments may influence dewlap and body coloration on a local scale. With a distribution spanning the Carolinas westward into Texas, and from the Florida Keys northward into Tennessee, the only anole endemic to the United States—*Anolis* carolinensis—is an appropriate species for examining regional variation in coloration.

Anolis carolinensis is a member of the "carolinensis complex" (Williams, 1969), which arose on Cuba and includes the Cuban endemics *Anolis allisoni* and *Anolis porcatus*, plus *Anolis maynardi* on Little Cayman, *Anolis longiceps* on Navassa, and several *A. carolinensis* subspecies in the Bahamas. This assemblage contains mostly green, slender, long-snouted anoles of the "trunk-crown" ecomorph (e.g., Rand and Williams, 1969). Dewlaps in most of these taxa range from pink to red with varying degrees of violet to magenta overtones, although the dewlap of

---

[1] C. K. Dodd served as editor for this manuscript.
[2] Corresponding Author. Present address: Department of Life Sciences, Arizona State University West, Phoenix, Arizona 85069; E-mail: jmacedon@asu.edu
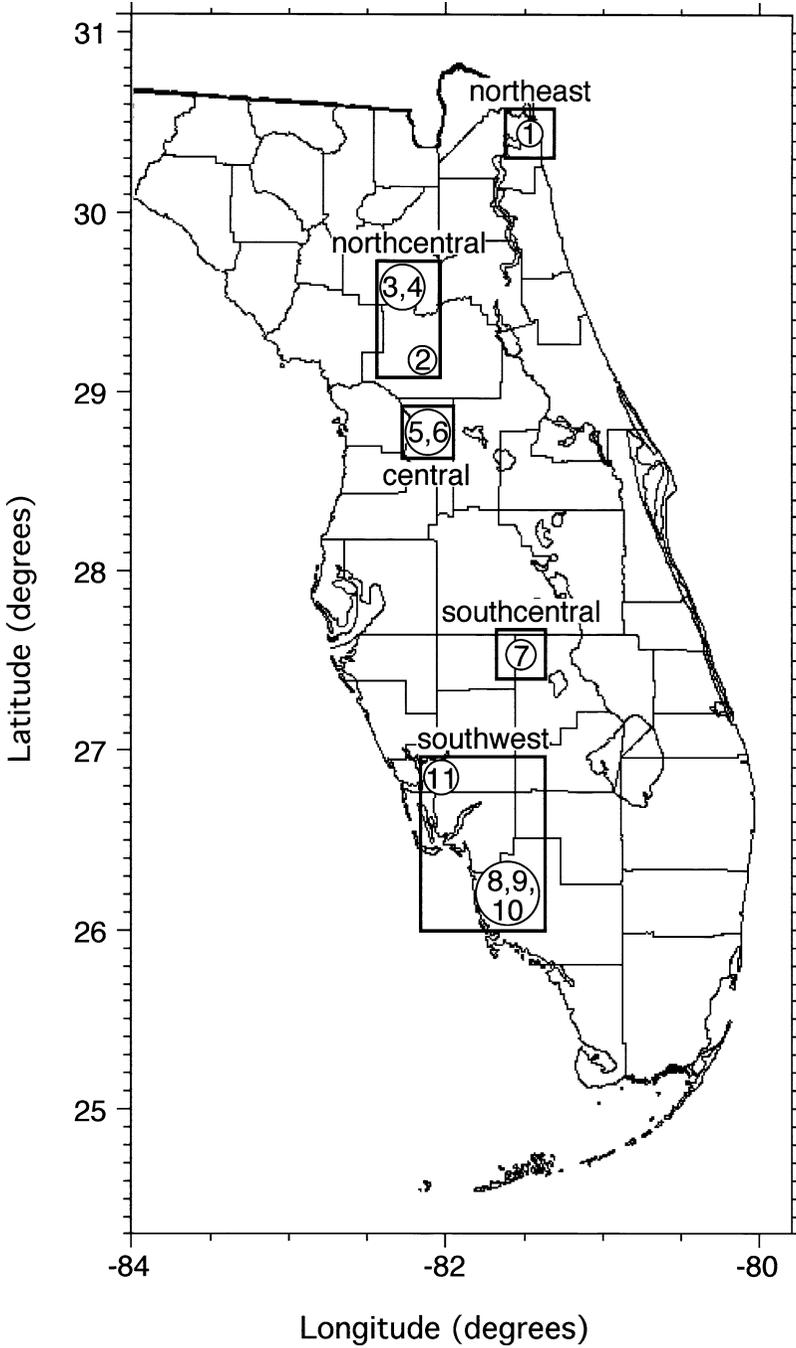
Fig. 1. Map of Florida, with the five geographical regions examined (boxes) and the study sites within them (circles).

A. longiceps is golden yellow and that of *A. maynardi* is pale yellowish-green (e.g., Schwartz and Henderson, 1985).

Throughout most of its range in the continental United States, *A. carolinensis* possesses dewlap coloration that is typical for the *carolinensis* complex (i.e., reddish). However, in an area roughly 10 km wide and reaching approximately 50 km north and south of Ft. Meyers, Florida, the dewlap is gray with greenish overtones

TABLE 1. Study sites and their geographical locations.

Site #	Site name	County	Geographical region
1	Black Hammock Island (Cedar Point)	Duval	Northeast
2	McIntosh (Sportsman's Cove)	Marion	Northcentral
3	Gainesville	Alachua	Northcentral
4	Paynes Prairie State Preserve	Alachua	Northcentral
5	Juniper Creek Swamp (Kettle Island Trail)	Sumter	Central
6	Juniper Creek Swamp (Bear Island Trail)	Sumter	Central
7	Highlands Hammock State Park	Highlands	Southcentral
8	Delnor-Wiggins Pass Recreation Area	Collier	Southwest
9	Lover's Key State Recreation Area	Lee	Southwest
10	Hickory Blvd. (South of Bonita Beach)	Lee	Southwest
11	Punta Gorda (Ponce de Leon Park)	Charlotte	Southwest

(Christman, 1980; Wade et al., 1983; Wilson and Echternacht, 1989). In documenting the distribution of this "gray-throated" form, Christman (1980) mapped variation in *A. carolinensis* dewlap coloration throughout southern Florida. Although Christman used a human color standard system to estimate the best match between dewlap color and the published color plates, his account of dewlap color variation ultimately was qualitative and subjective, and variation in body color was not reported.

In this study, we build on prior work by using spectrometric methods to quantify dewlap and body color variation in *A. carolinensis* along a northeast–southwest transect in Florida. We use several analytical procedures to assess the conspicuousness of dewlap and body colors by determining their contrast with the natural backgrounds of tree bark and leaves. We also estimate the influence of habitat light on coloration in our study populations by comparing dewlap spectral reflectance with local ambient illumination. Last, we discuss our findings in light of this species' unusual spectral sensitivity and speculate on the origins of the enigmatic gray throated *A. carolinensis* population restricted to southwest Florida.

MATERIALS AND METHODS

Definition of Terms.—In this paper, we use "intensity" to refer to the objectively measured amplitude of light spectra and define it as the sum of individual wavelength amplitudes over a specified set of wavelengths. "Color" refers to the spectral distribution of light independent of its intensity. We use "hue" to refer to color in the vernacular sense (e.g., red, blue), which is defined by the shape of the spectral curve, especially its peak. "Chroma" is color saturation and is a function of the relative difference in magnitude between strong and weak regions of a spectrum. Wavelengths from 600–700 nm are referred to as "long" wavelengths, those from 500–600 nm as "middle" wavelengths, those from 400–500 nm as "short" wavelengths, and those below 400 nm as "ultraviolet" or "UV" wavelengths. "Irradiance" is defined as light from a hemisphere striking a flat surface and is measured in μmols m^{-2} s^{-1} nm^{-1}. In this study, quantification of irradiance was restricted to downwelling ambient light (see below). "Reflectance" refers to the wavelength distribution of light reflected from an object with respect to a virtually perfect reflector (i.e., a white standard). We use "radiance" to refer to the spectrum of incident light reflected from an object, as measured over a small solid angle. In this study, radiance was calculated from irradiance and reflectance (see below). "Intensity contrast" is used to define amplitude differences between lizard and background spectral reflectance (or radiance), and "color contrast" refers to differences between lizard and background spectra independent of intensity.

Sampling Locations.—From 21–28 May 2001, we obtained spectrometric measurements of adult male *A. carolinensis* and of the habitats in which we found them in Florida (Fig. 1). We chose 11 study sites distributed across five geographic regions: northeast (NE), northcentral (NC), central (C), southcentral (SC), and southwest (SW) Florida (Table 1). Adult male lizards were captured with a pole and noose and by hand. Subjects were retained briefly in brown paper lunch bags following capture and kept in the shade until a sufficient sample (e.g., 5–10 individuals) had been obtained for spectrometric scanning. Following data collection, lizards were returned unharmed to their locations of capture.

Spectrometric Measurements of Lizards and Their Habitats.—Reflectance measurements of *A. carolinensis* were obtained in the field at an angle roughly perpendicular (approximately 90° ± 10°) to the skin surface through a fiber optic cable (400 μm) fitted with a collimating lens (Ocean Optics 74-UV) that was connected to an Ocean Optics

USB2000 portable spectrometer and a notebook computer. Data were displayed with OOIBase32 software (Ocean Optics, Inc.). A white standard (Labsphere Spectralon WS-1) was scanned and dark current removed from the signal immediately prior to obtaining a subject's reflectance readings. A small ruler attached to the side of the collimating lens maintained a constant 5 mm distance between the end of the probe and the target surface.

All spectrometric data were obtained outdoors during periods of relatively clear sky conditions, although high-altitude haze sometimes was present. The solar spectrum was used as the source of illumination. If low-altitude clouds were present, readings were not taken when a cloud was near to or obscuring the sun. Upon removal of a subject from its holding bag a scan immediately was made of its midbody dorso-lateral region. Roughly half of our subjects exhibited their "bright green" color state when removed from the bag. The remaining individuals exhibited either a full state of metachrosis (dark brown) or, more often, an intermediate state between bright and metachrosis coloration. Because we were interested in comparing variation in "bright state" coloration only, we did not gather spectral data from subjects exhibiting other dorsal color states. As a consequence, dorsum sample sizes typically are about half that of dewlap sample sizes, as follows: NE = northeast (dewlap: N = 11, dorsum: N = 7), NC = northcentral (dewlap: N = 14, dorsum: N = 5), C = central (dewlap: N = 10, dorsum: N = 5), SC = southcentral (dewlap: N = 10, dorsum: N = 6), SW = southwest (dewlap: N = 11, dorsum: N = 10).

Following the scan of the dorsum, the dewlap was extended with tweezers and a scan was taken from its approximate center. The dewlap then was permitted to retract, and the procedure was repeated to obtain a second reading. These two readings were averaged prior to data analysis. Reflectance readings of tree bark and leaves were obtained at the locations where lizards were captured, again using ambient light as the illumination source.

A cosine-corrected irradiance probe (Ocean Optics CC-3-UV) and Ocean Optics OOIIrrad software were used to quantify ambient light in the lizard habitats. Downwelling irradiance was measured by orienting the lens of the irradiance probe directly upward in locations were lizards were observed and/or captured. Irradiance spectra were collected at the time of, or ≤ 30 min following, lizard observation/capture. Prior to obtaining habitat light readings the irradiance probe was calibrated with a light source (Ocean Optics LS-1-CAL) designed for this lens. Irradiance data collection was restricted to times when the sky was more than one-third blue and the sun was not obscured by clouds (after Fleishman et al, 1997).

Analysis of Color Variation and Contrast with the Visual Background.—To determine whether dewlap and dorsum spectra differed among populations, as well as from background vegetation (bark and leaves), spectra were grouped into 10 nm bins from 340–700 nm and bin medians determined, resulting in 37 summary values per spectrum. Principal components analysis (PCA) was run separately on the dewlap-plus-vegetation and dorsum-plus-vegetation summary data sets, and components with eigenvalues > 1.0 were retained. One-way ANOVAs were run on each retained component to detect significant differences among population means. Where differences were significant in ANOVAs, Dunnett's T3 test for multiple comparisons revealed which populations differed from each other and from vegetation. In PCA of spectral data, PC1 represents variation in intensity, and subsequent PCs represent combinations of hue and chroma (Cuthill et al., 1999; Grill and Rush, 1999).

Two iterations of PCA were run. In the first set of analyses, reflectance spectra were used to calculate spectral relationships among lizard populations and between lizards and vegetation. Reflectance spectra are free from biases in the appearance of colors that arise from variation in illuminating spectra, as well as from differences among visual systems perceiving those colors. These "biases" often are precisely what one wants to include in analyses of color spectra, however, as they permit an estimation of how a given species is likely to perceive colors under natural conditions. Thus, in the second set of analyses, "perceived radiance" spectra were calculated by first multiplying each population's standardized (sum of wavelength intensities = 1) mean irradiance spectrum times the dewlap, dorsum, and vegetation reflectance spectra for that population. These calculations then were multiplied times *A. carolinensis* standardized spectral sensitivity (sum of wavelength intensities = 1), which had been determined previously (M. Frye and L. Fleishman, unpubl.) with electroretinographic flicker photometry (i.e., ERG; for methodological details, see Fleishman et al., 1997).

Discriminant (canonical) function analysis (DFA) was run on the reflectance data set as a complementary technique to PCA for assessing the spectral distinctiveness of the study populations and to determine their contrast with the visual background. PC scores were used as input data to the DFAs, given that they satisfy the parametric assumptions of DFA. Only principal components with eigenvalues > 1.0 found to be significant among groups in the ANOVAs (see
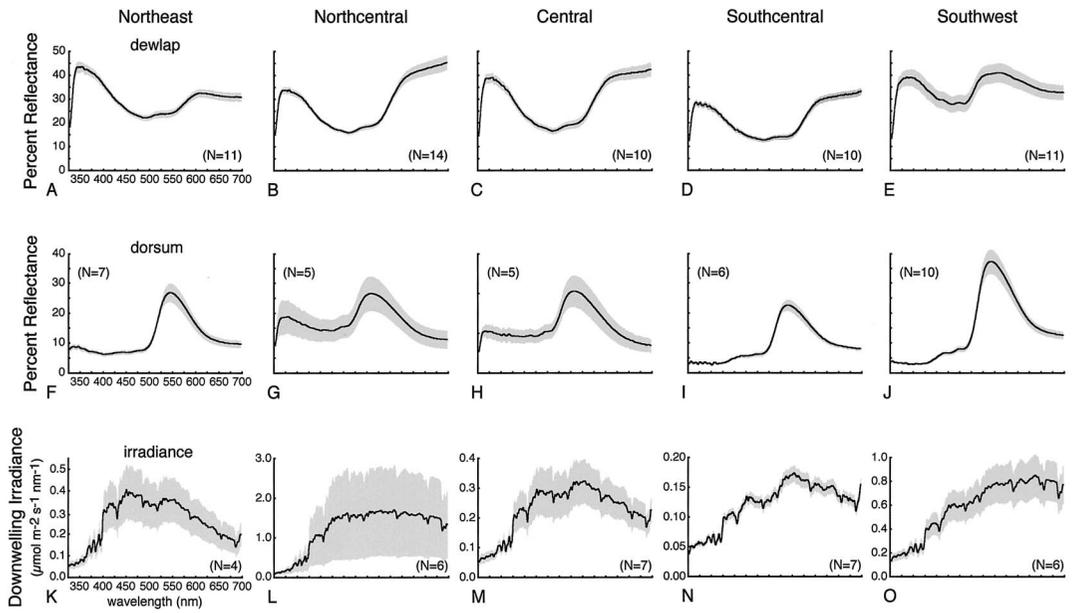
FIG. 2A–O. Mean (±SE) reflectance spectra from the dewlap (A–E) and dorsum (F–J) of adult male *Anolis carolinensis* from each of the five geographical regions sampled and downwelling irradiance spectra (K–O) from the study sites within those regions.

above) were used in the DFAs as input variables. "Leave-one-out" cross-validation DFA was used to construct the discriminant functions from all cases except the case being classified.

One final approach was taken to assess lizard-vegetation color contrast. We used our reflectance data with calculations in Fleishman and Persons (2001) that take into account each of the four classes of *A. carolinensis* visual pigments and their associated oil droplets (in Loew et al., 2002). This analysis of "chromatic contrast" was carried out as follows. Visual pigment absorption curves (standardized from 0–1) were generated from 340–700 nm using absorption peak (λ_{max}) values in Loew et al. (2002) and a nomogram template provided by L. J. Fleishman. In reptiles and birds, oil droplets associated with visual pigments serve as high-pass optical filters that absorb light below a particular cut-off wavelength (e.g., Loew et al., 2002). Oil droplet absorption spectra for *A. carolinensis* were multiplied by their associated visual pigments to calculate the response function of each photoreceptor class. These photoreceptor response functions then were multiplied by each *A. carolinensis* population's summary dewlap and dorsum reflectance spectra and summary bark and leaf reflectance spectra. The resulting "photoreceptor × target" spectra then were summed, producing a single value for each photoreceptor × target combination. To satisfy the assumption that "white"

light stimulates each cone class equally (Fleishman and Persons, 2001), each photoreceptor × target sum was corrected by multiplying it by the area under the curve of the relevant visual pigment. The relative stimulation of each cone class by a given target then was calculated by dividing the corrected sum of each cone class by the combined sum of the four cone classes. We refer to these relative stimulation values as Xuvs, Xsws, Xmws, and Xlws, where "uvs" is the UV-sensitive visual pigment, "sws" is the short wavelength sensitive pigment, "mws" is the medium wavelength sensitive pigment, and "lws" is the long wavelength sensitive pigment. Chromatic contrast then was calculated as:

$$C_c = [(T_{uvs} - B_{uvs})^2 + (T_{sws} - B_{sws})^2$$
$$+ (T_{mws} - B_{mws})^2 + (T_{lws} - B_{lws})^2]^{1/2} \quad (A1)$$

where T is the target body region (dewlap or dorsum) and B is the visual background type (bark or leaves).

RESULTS

Dewlap and Dorsum Reflectance Spectra.—Dewlap spectral reflectance (Fig. 2A–E) was of three basic types: (1) UV reflectance stronger than long wavelength reflectance (NE population); (2) long wavelength reflectance stronger than UV reflectance (NC, C, and SC populations), and (3) equally strong UV and middle wavelength reflectance (SW population). Dorsum reflectance
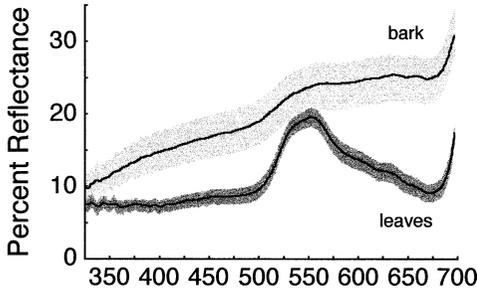
FIG. 3. Mean (± SE) reflectance spectra for bark (N = 26) and leaves (N = 9) analyzed in this study.

also was variable among the study populations, primarily in the relative intensity of middle to short/UV wavelengths (Fig. 2F–J).

Habitat Light and Vegetation Reflectance Spectra.—Habitat light differed greatly among the five geographic regions sampled (Fig. 2K–O). Downwelling irradiance spectra obtained at the NE site were biased toward short wavelengths (Fig. 2K), resembling Endler's (1992) "woodland shade" light habitat. This location was characterized by large mature trees and an open canopy, such that skylight had a major influence on the irradiance spectrum. The NC sites were more open, and the ambient light recorded in them resembles Endler's "large gap" light habitat (Fig. 2L). The C and SC sites (Fig. 2M–N) exhibited patterns of irradiance intermediate between Endler's "woodland shade" and "forest shade" light habitats. Finally, our SW sites were composed primarily of mangrove thickets with thin canopies, resulting in long-wavelength-biased irradiance spectra (Fig. 2O) that resemble Endler's "small gap" light habitat. Bark and leaf reflectance spectra (mean ± SE) recorded from our study sites are shown in Figure 3.

Reflectance Analysis.—A PCA created three factors that accounted for nearly 99% of the variance in the dewlap-plus vegetation reflectance data (Table 2A). The SC population was significantly weaker in dewlap spectral intensity than the NE and NC populations (Fig. 2A,B,D), and all populations differed significantly in intensity contrast with leaves whereas none differed from bark (PC1 in Table 2A, Fig. 4a). On PC2 the two geographically most distant populations (NE and SW) figured prominently in among-population dewlap color differences, and all populations except SW exhibited significant color contrast with bark and leaves (Table 2A, Fig. 4B). Dewlap coloration in the NE population was further separated from the other populations and from bark and leaves on PC3 (Table 2A, Fig. 4B).

A PCA produced three factors accounting for nearly 99% of the variance in the dorsum-plus-vegetation reflectance data (Table 2B). Although PC1 accounted for about 86% of that variation, considerable overlap in dorsum spectral intensity occurred among populations and the ANOVA was not significant (Table 2B, Fig. 4C). Population distinctiveness in dorsum coloration and in color contrast with vegetation was greatest for the SW population on PC2 and for the C population on PC3 (Fig. 4D).

Results of a DFA run on the dewlap-plus-vegetation PC scores (PC1-PC3) showed the NE population to be the most distinctive group, with misclassification errors of less than 20% (Table 3A). Most of the dewlap misclassifications for the NC and SC populations were PC scores assigned to each other (about 30% in each case). The C population exhibited particularly poor classification success (30% correct assignment), with most of its PC scores being assigned to the NC population (40%). With the exception of SW, no

TABLE 2. One-way ANOVAs on PCA factors derived from dewlap and dorsum reflectance spectra (340–700 nm) of *Anolis carolinensis* plus local vegetation (bark and leaves) in five geographical regions of Florida. Dewlap: df = 6, 84; dorsum: df = 6, 61; *** = $P \leq 0.001$; NS = ANOVA not significant. Multiple pairwise comparisons: Dunnett's T3 for unequal variances; $P \leq 0.05$. See text for dewlap and dorsum sample sizes. Bark: N = 26; leaves: N = 9.

| Factor	PC eigenvalue	% of variance	ANOVA F-ratio	Significant pairs
(A) Dewlap and vegetation				
PC1	31.1	81.9	7.3***	NE-SC, NC-SC; Bark: none; Leaves: all
PC2	4.2	10.9	55.3***	NE-NC, NE-C, NE-SC, NC-SW, C-SW, SC-SW; Bark: all *but* SW; Leaves: all *but* SW
PC3	2.2	5.8	17.0***	NE-NC, NE-C, NE-SC; Bark: all *but* NC; Leaves: NE
(B) Dorsum and vegetation				
PC1	32.7	86.2	1.7	NS
PC2	3.1	8.2	24.8***	NC-SC, NC-SW, C-SW; Bark: NE, SC, SW; Leaves: SC, SW
PC3	1.7	4.3	24.9***	NE-C, NE-SC, NE-SW, C-SC, C-SW; Bark: all *but* NC; Leaves: C
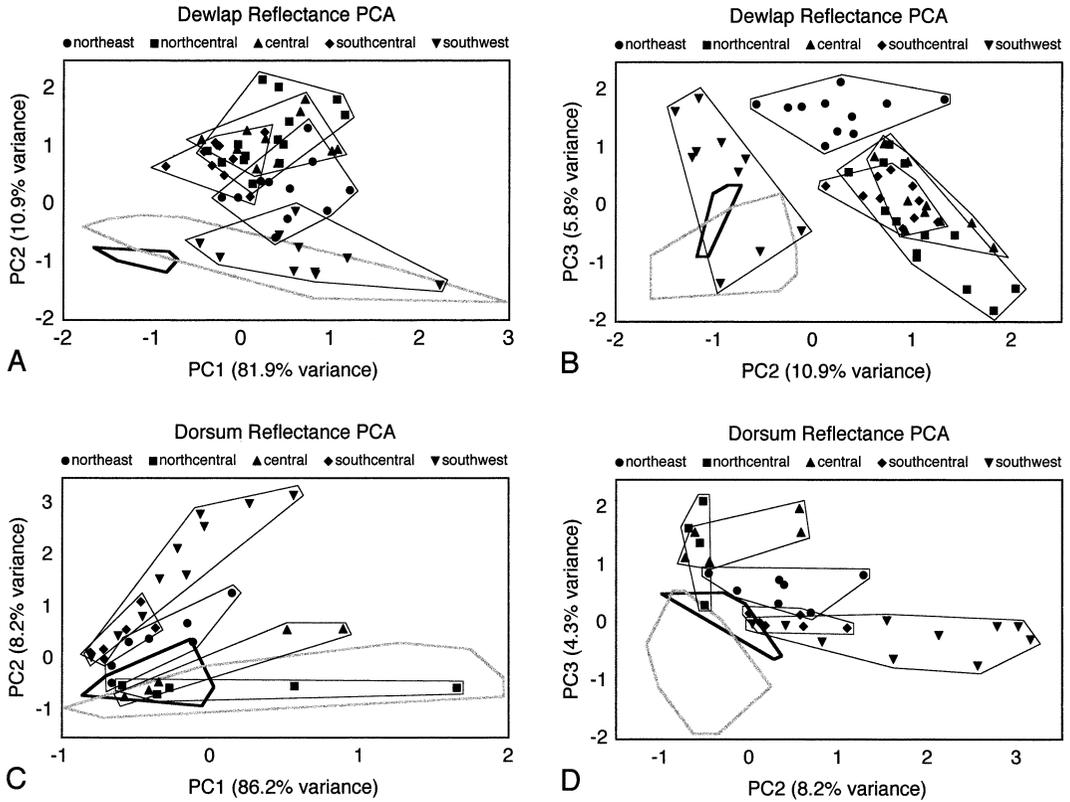
FIG. 4A–D. Plots of principle component scores of dewlap and dorsum reflectance spectra from 330–700 nm. NE = circles, NC = squares, C = triangles, SC = diamonds, SW = inverted triangles. Polygons with thick lines enclose PC scores for leaves (black lines) and for bark (gray lines). For clarity, individual vegetation PC scores not shown. Sample sizes in Fig. 2.

population had any dewlap PC scores misclassified with vegetation. Two of 11 dewlap PC scores from the SW population were classified with vegetation, however, and can be seen as the two PC scores falling within the bark polygon in Figure 4B. Interestingly, the single bark PC score misclassification was considered by the DFA to be a dewlap from the SW population (Table 3A).

In the DFA of dorsum-plus-vegetation PC scores (PC2 and PC3) the SW population was most successfully classified (70%)—a result underscoring the relative isolation of this population in PCA space (Fig. 4C,D). Although the four other populations exhibited variable degrees of misclassification with one another, the most interesting result of this DFA is the misclassification of dorsum PC scores with vegetation. Although less than 4% of the dewlap PC scores were incorrectly classified with vegetation (Table 3A), misclassifications of dorsum PC scores with vegetation ranged from 10–40%, and all of these incorrect assignments were with leaves (Table 3B).

"Perceived Radiance" Analysis.—Factoring *A.*

carolinensis spectral sensitivity and population-specific habitat light data into the calculations (i.e., perceived radiance) had a negligible effect on the distribution of lizard dewlap spectral intensity in PC space (Table 4A, Fig. 5A: PC1). Distribution of vegetation and lizard dewlap color (PC2 and PC3) differed slightly from the companion PCA on reflectance spectra in three ways. First, variance in bark PC scores was increased (Fig. 5B; note the slightly larger scales for PC2 and PC3 compared to Fig. 4). Because bark is low in chroma (i.e., shallow-sloped: Fig. 3) it was more strongly affected by habitat light than were dewlaps or leaves (e.g., Endler, 1990, 1997). Second, the NE and NC populations were more distantly located from each other and from the remaining populations in PC space. Third, color contrast increased between NC population and vegetation but decreased for the four remaining populations (Fig. 5B).

Distribution of vegetation and dorsum perceived radiance PC scores was astonishingly similar that seen in the PCA of reflectance spectra, with the primary difference being greater

TABLE 3. Cross-validation classification of lizard and vegetation reflectance PC scores by discriminant (canonical) functions. PCs significant in ANOVAs (PC1-PC3 for dewlap; PC2 and PC3 for dorsum) were used to generate discriminant functions. Values depict percentage (and quantity, in parentheses) of samples assigned to each category. Bold type on the diagonal indicates correct assignment. ''# Random Assignment'' equals the sample size for a given class divided by the total number of classes (i.e., seven), raised to the next whole number. Italics indicate classification assignment greater than expected by random chance.

Population (N)	NE	NC	C	SC	SW	Bark	Leaves	# Random assignment
(A) Dewlap								
NE (N = 11)	*81.8 (9)*	0	9.1 (1)	9.1 (1)	0	0	0	2
NC (N = 14)	7.1 (1)	*57.1 (8)*	7.1 (1)	*28.6 (4)*	0	0	0	2
C (N = 10)	20.0 (2)	*40.0 (4)*	*30.0 (3)*	10.0 (1)	0	0	0	2
SC (N = 10)	0	*30.0 (3)*	0	*70.0 (7)*	0	0	0	2
SW (N = 11)	9.1 (1)	0	0	0	*72.7 (8)*	18.2 (2)	0	2
Bark (N = 26)	0	0	0	0	3.8 (1)	*96.2 (25)*	0	4
Leaves (N = 9)	0	0	0	0	0	*44.4 (4)*	*55.6 (5)*	2
(B) Dorsum								
NE (N = 7)	*42.9 (3)*	0	0	14.3 (1)	14.3 (1)	0	*28.6 (2)*	1
NC (N = 5)	0	*40.0 (2)*	40.0 (2)	0	0	0	20.0 (1)	1
C (N = 5)	0	40.0 (2)	*20.0 (1)*	0	0	0	*40.0 (2)*	1
SC (N = 6)	0	0	0	*50.0 (3)*	16.7 (1)	0	33.3 (2)	1
SW (N = 10)	0	0	0	20.0 (2)	*70.0 (7)*	0	10.0 (1)	2
Bark (N = 26)	0	0	0	0	0	*92.3 (24)*	7.7 (2)	4
Leaves (N = 9)	0	0	0	0	0	11.1 (1)	*88.9 (8)*	2

overlap with bark PC scores in the latter analysis (Fig. 5D). This increased overlap, however, had little statistical effect on color contrast (PC2 and PC3 taken together: Table 4B).

"Chromatic Contrast" Analysis.—Within populations, the magnitude of dewlap-bark chromatic contrast was the same as or similar to that of dewlap-leaf contrast; this likewise was true for dorsum-vegetation contrast (Fig. 6). In four of the five populations, dewlap-vegetation contrast was greater than dorsum-vegetation contrast; the NE population was the most extreme in this regard. The SE population was unique in exhibiting stronger dorsum-vegetation contrast than dewlap-vegetation contrast, and the magnitude of its dewlap-vegetation contrast was virtually identical to that of dorsum-vegetation contrast in the NC population (Fig. 6).

DISCUSSION

The combined results of this study indicate that although each *A. carolinensis* population we examined possesses a unique dewlap-plus-dorsum spectral profile, some populations are more distinctive than others in coloration and in the magnitude of their contrast with vegetation. Excluding the SW population, *A. carolinensis* along our northeast-to-southwest transect exhibited a dewlap-dorsum dichotomy in color conspicuousness that is typical of many lizard taxa: highly conspicuous dewlap colors and relatively inconspicuous body colors. This dichotomy almost certainly provides a selective advantage against attracting the unwanted attention of predators (e.g., Endler, 1992). Nevertheless, dorsum reflectance PC scores revealed minimal overlap with those of bark and leaves

TABLE 4. One-way ANOVAs on ''perceived radiance'' (350–700 nm) PCA factors (see text). Legend as in Table 2.

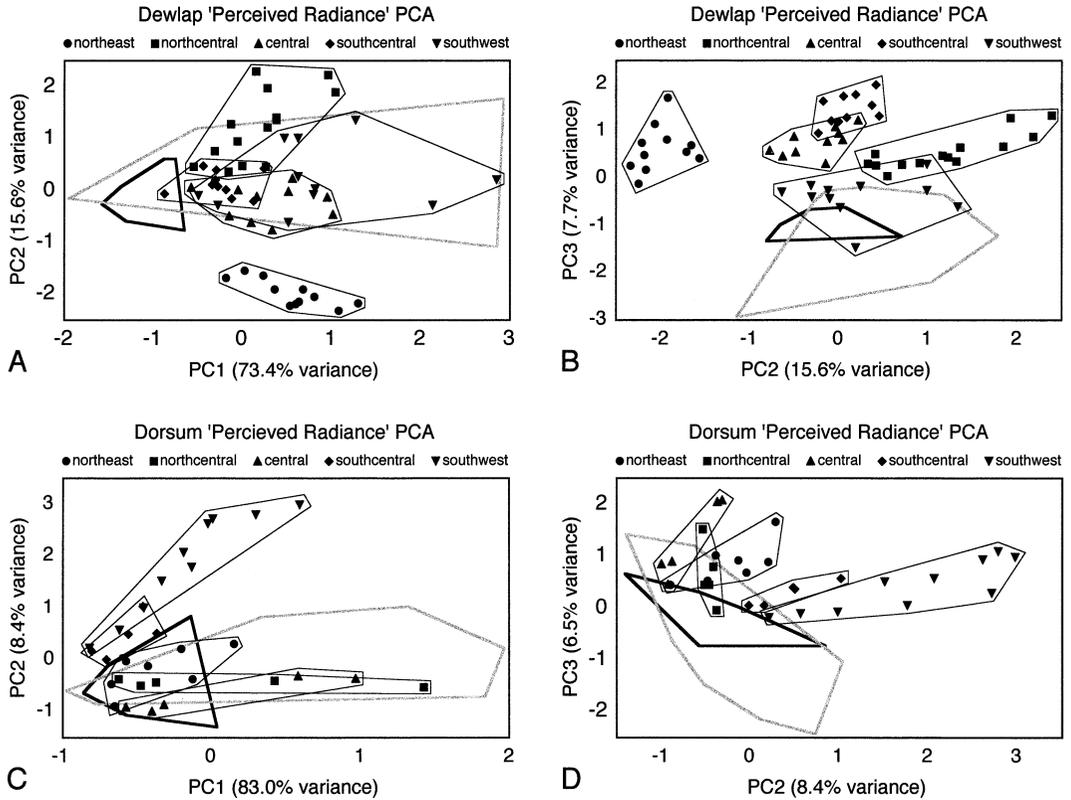| Factor	PC eigenvalue	% of variance	ANOVA F-ratio	Significant pairs
(A) Dewlap and vegetation				
PC1	26.5	73.6	6.4***	NE-SC; Bark: none; Leaves: all
PC2	5.6	15.6	40.6***	NE: all, NC: all; Bark: NE, NC; Leaves: NE, NC
PC3	2.8	7.7	45.4***	NE-SC, NE-SW; NC-SC, NC-SW; C-SC, C-SW; SC-SW; Bark: all *but* SW; Leaves: all
(B) Dorsum and vegetation				
PC1	29.9	83.0	1.5	NS
PC2	3.0	8.4	18.8***	C-SC, SW; all, Bark: SW; Leaves: SW
PC3	2.3	6.5	9.4***	Bark: all; Leaves: NE

FIG. 5A–D. Plots of principle component scores of dewlap and dorsum "perceived radiance" spectra. Legend as in Fig. 4.

(Fig. 4B,D). Interestingly, factoring habitat light (and spectral sensitivity) into the PCAs reduced lizard-background color contrast. Inclusion of local irradiance generally increased overlap between dorsum and bark PC score distributions (Fig. 5D), whereas dewlap coloration remained outside the polygons bounding the vegetation PC2 and PC3 scores for all populations except the SW population (Fig. 5B). Thus, natural lighting conditions might render *A. carolinensis* body coloration slightly less conspicuous against the visual background than their reflectance spectra would suggest.

Dewlap Color, Habitat Light, and Visual Contrast.—A dewlap that reflects the strongest regions of the ambient spectrum has the potential to maximize brightness contrast against a background of darker vegetation. For example, red and orange dewlaps usually occur in heliophilic anoles that reside in "no shade" (and to some degree "partial shade") habitats (e.g., Fleishman, 1992, 2000; Loew et al., 2002). Red and orange are favored here because direct sunlight is strong in long wavelengths. Yellow dewlaps are most common in closed canopy forest because sunlight filtered through and reflected from leaves is

rich in middle wavelengths. In this light habitat, a yellow dewlap exploits the strongest part of the ambient spectrum while differing sufficiently in hue from green leaves to be easily detected. In dark forest, where little light is available for color signals, whitish (low chroma and highly reflective) dewlaps prevail (e.g., Fleishman, 1992).

Brightness contrast (i.e., perceived intensity contrast) is more important than color contrast for detecting moving stimuli like dewlaps (e.g., Persons et al., 1999; Fleishman and Persons, 2001). Although all four classes of photoreceptors in the anoline retina are involved in perception of color contrast, the UVS and SWS cones do not contribute to the perception of brightness contrast. Dewlap spectral reflectance below about 450 nm (blue, violet, and UV), thus does not take advantage of the primary means by which anoles detect dewlaps. Brightness contrast and color contrast are, however, additive in their effects on the probability of stimulus detection.

Given the above, one could predict from our habitat light data that, to achieve strong reflectance as well as visual contrast, dewlaps in the NE population should be violet-blue (i.e., color contrast only), those in the C and SC
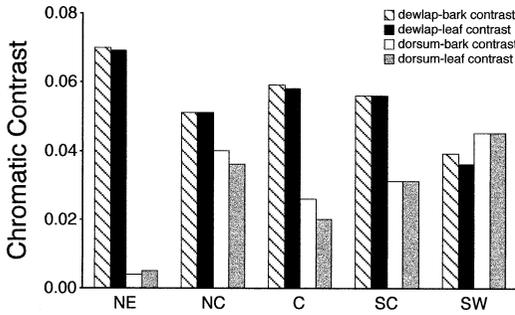
FIG. 6. "Chromatic contrast" between lizards and vegetation in the five study populations. Dewlap-bark contrast = hatched bars, dewlap-leaf contrast = black bars, dorsum-bark contrast = white bars, dorsum-leaf contrast = gray bars. For calculation of chromatic contrast, see text.
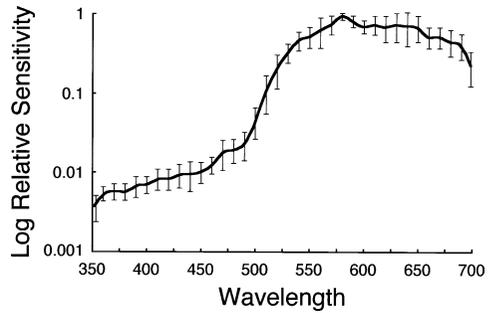

FIG. 7. Spectral sensitivity of *Anolis carolinensis* estimated from electroretinographic flicker photometry (M. Frye and L. Fleishman, unpubl.) using the methods described in Fleishman et al., 1997. The curve shown is the mean ($\pm$ 1 SD) ERG response of two males and two females. Each subject's response first was standardized to their peak value ($= 1$) and the mean (shown) calculated from the four response curves. Data kindly provided by L. J. Fleishman.

populations should be yellow, and those of the SW population should be orange-red (no clear prediction can be made for the NC population; see Fig. 2). We found, to the contrary, that dewlap reflectance spectra did not closely resemble local habitat light spectra in any of our study populations. The above prediction is based, however, on the premise that *A. carolinensis* spends much of its time in one particular light habitat. Although this may be true for many *Anolis* species, our data clearly indicate that *A. carolinensis* can be found in a diversity of light habitats.

Obviously, the more closely dewlap reflectance resembles one habitat light spectrum the less it can resemble another. For *Anolis* species that spend appreciable amounts of time in more than one light habitat, selection should favor a compromise. The bimodal dewlap reflectance pattern observed in *A. carolinensis* may be evidence of such a compromise. A dewlap that is highly reflective both in long wavelengths and in short wavelengths, but which is deficient in middle wavelengths, is well suited for signaling both in "small gap" (Fig. 2O) and "woodland shade" (Fig. 2K) forest light habitats. This dewlap also should be effective in sunny, open enviroments (Fig. 2L) against a background of UV-absorbing vegetation (e.g., Fleishman et al., 1993). All of our sample populations except SW exhibited a "reddish-violet" dewlap reflectance spectrum— a pattern that should broadcast well in all light habitats we recorded except closed canopy "forest shade," which is strongest in the middle wavelengths (e.g., Fig. 2N).

The fact that some of the populations along our geographical transect were observed in forest shade is interesting and may implicate the extraordinary spectral sensitivity properties of the *A. carolinensis* visual system. Likewise, it is curious that the light habitat in which we studied

the SW population was not one of forest shade— despite this being the one population whose dewlap reflectance spectrum would be ideally suited to the yellowish-green light of closed-canopy forest. We now turn our attention to a more detailed discussion of these two exceptional cases.

Visual Pigments and Perception of Reddish Dewlaps.—*Anolis carolinensis* exhibits a LWS visual pigment that is shifted approximately 60 nm further into the "red" end of the visible spectrum than any of its congeners examined to date (for data on 17 *Anolis* species, see Loew et al., 2002). The mechanism underlying this oddity is its use of vitamin A_2 to produce visual pigments: all other fully terrestrial vertebrates whose visual pigments are known use vitamin A_1 (Provencio et al., 1992; Loew et al., 2002). A consequence of this phenomenon is that long wavelength sensitivity in *A. carolinensis* (Fig. 7) is dramatically increased compared to other anoles (for a comparison of *A. carolinensis* with *Anolis cristatellus*— an anole with "typical" spectral sensitivity, see Fleishman, 2000).

Provencio et al. (1992) speculated that the red-shifted visual pigment in *A. carolinensis* may enhance the perception of red dewlaps. Given that many *Anolis* species with long-wavelength-reflective dewlaps do not have "red-enhanced" spectral sensitivity, such sensitivity obviously is not a corequisite of possessing a reddish dewlap (Loew et al., 2002). However, most red-dew-lapped *Anolis* species reside in habitats that are bathed in direct sunlight (e.g., Fleishman, 1992; Loew et al., 2002). The most common light habitat in which *Anolis* lizards are found, and the only one in which red-dewlapped species other than *A. carolinensis* do not regularly occur,

is the yellowish-green light of closed-canopy "forest shade". Enhanced "red" sensitivity in *A. carolinensis,* therefore, may provide an advantage in perceiving reddish dewlaps in light habitats that are weak in long wavelengths. This prediction might best be tested under controlled laboratory conditions (e.g., Persons et al., 1999; Fleishman and Persons, 2001).

Gray Throated Anolis carolinensis *in Southwest Florida.*—Morphological, physiological, and genetic studies have documented the uniqueness of *A. carolinensis* in southwest Florida (e.g., Wade et al., 1983; Wilson and Echternacht, 1989). For example, individuals in this population tend to be smaller in size and less cold tolerant than other populations (Wilson and Echternacht, 1989). Allozyme variation examined in populations from seven sites across the continental distribution of *A. carolinensis* revealed the SW Florida population to possess the most distinctive allele profile (Wade et. al, 1983). Moreover, genetic similarity between SW Florida individuals and those from New Orleans, Louisiana (Wade et. al, 1983) was only slightly more than between *A. carolinensis* and its putative Cuban ancestor, *A. porcatus* (Buth et al., 1980).

Alternative hypotheses have been proposed to explain among-population variation in *A. carolinensis* and the uniqueness of the SW Florida population. A "single origin" hypothesis suggests that the SW population was isolated from other populations during the Pleistocene when southern Florida alternated between being a peninsula and an archipelago (Duellman and Schwartz, 1958). In contrast, a "multiple origins" hypothesis suggests that variation observed in *A. carolinensis* is the manifestation of more than one successful colonization of Florida. The single origin hypothesis could be rejected if, for example, *A. carolinensis* from southwest Florida were found to be more closely related to a species in the *carolinensis* complex other than *A. porcatus*.

Within the *carolinensis* complex only one species—*A. maynardi* on Little Cayman—exhibits dewlap coloration at all resembling that of *A. carolinensis* from southwest Florida. These two taxa stand apart from the others by possessing a dewlap spectral reflectance peak around 550 nm. Compared to dewlaps in the SW population, the *A. maynardi* dewlap appears pale yellowish-green rather than gray because of decreased reflectivity of 25–30% from 400–500 nm and 5–15% from 600–700 nm (J. Losos and J. Endler, unpubl. data). As a result, the *A. maynardi* dewlap is more chromatic but less bright than those in our SW population, although both possess the same yellowish-green peak reflectance. Interestingly, although *A. porcatus* was not included in the large-scale phylogenetic study of Jackman et al. (1999), *A. carolinensis* and *A.*

maynardi were nested within the Cuban anoles as sister taxa in 100% of bootstrap replicates.

Can a functional explanation account for the greenish-gray dewlap color in *A. carolinensis* from southwest Florida? Pale dewlaps are most often seen in shade species, and white dewlaps usually are restricted to deep forest anoles where light levels are very low (e.g., Fleishman, 1992). The habitat of our SW population consisted primarily of coastal mangrove, which was anything but dark forest. We realize that this habitat is not representative of forest away from the coast, and it also could be quite different from the ecological setting in which the gray dewlap evolved. The absence of a reddish dewlap in the SW population cannot be attributed to some visual pigment anomaly, however, because these pigments and associated oil droplets do not differ from those occurring in "typical" *A. carolinensis* (E. Loew, pers. comm.). As we have shown, the bright green dorsum coloration of this population also is unique and contrasts more strongly with the visual background than does its greenish-gray dewlap color. We are unaware of any functional advantages these dewlap and dorsum colors might bestow on the SW population, and we remain open to the possibility that they may be retentions from an ancestor unshared with other *A. carolinensis* populations.

Christman (1980) concluded that the distribution of gray throated *A. carolinensis* in southwest Florida lacked any ecological or historical pattern with which he was familiar. Results of the present study likewise suggest that dewlap coloration in this population is unrelated to characteristics of the ambient light in locations where we found these anoles. A spectrometric investigation of coloration and habitat light variation that is focused on the SW population (and its surrounding "hybrid zone": Christman, 1980), in addition to a molecular phylogenetic study of the *carolinensis* complex, might ultimately lead to a better understanding of the origins and persistence of gray throated *A. carolinensis* in southwest Florida.

LITERATURE CITED

BUTH, D. G., G. C. GORMAN, AND C. S. LIEB. 1980. Genetic divergence between *Anolis carolinensis* and its Cuban progenitor, *Anolis porcatus*. Journal of Herpetology 14:279–284.

CHRISTMAN, S. P. 1980. Preliminary observations on the gray-throated form of *Anolis carolinensis* (Reptilia: Iguanidae). Florida Field Naturalist 8:11–16.

COOPER, W. E., AND N. GREENBERG. 1992. Reptilian coloration and behavior. *In* C. Gans and D. Crews (eds.), Biology of the Reptilia. Vol. 18. Physiology E, Hormones, Brain and Behavior, pp. 298–422. Univ. of Chicago Press, Chicago.

CUTHILL, I. C., A. T. D. BENNETT, J. C. PARTRIDGE, AND E. J. MAIER. 1999. Plumage reflectance and the objective assessment of avian sexual dichromatism. American Naturalist 153:183–200.

DUELLMAN, W. E., AND A. SCHWARTZ. 1958. Amphibians and reptiles of southern Florida. Bulletin of the Florida State Museum 3:181–324.

ENDLER, J. A. 1990. On the measurement and classification of colour in studies of animal colour patterns. Biological Journal of the Linnaen Society 41: 315–352.

———. 1992. Signals, signal conditions, and the direction of evolution. American Naturalist 139:S125–153.

———. 1993. The color of light in forests and its implications. Ecological Mongraphs 63:1–27.

———. 1997. Light, behavior, and conservation of forest-dwelling organisms. *In* J. R. Clemmons and R. Buchholz (eds.), Behavioral Approaches to Conservation in the Wild, pp. 329–355. Cambridge Univ. Press, Cambridge.

FLEISHMAN, L. J. 1992. The influence of the sensory system and the environment on motion patterns in the visual displays of anoline lizards and other vertebrates. American Naturalist 139:S36–61.

———. 2000. Signal function, signal efficiency, and the evolution of anoline lizard dewlap color. *In* Y. Espmark, T. Amundsen, and G. Rosenqvist (eds.), Signalling and Signal Design in Animal Communication, pp. 209–236. Tapir Academic Press, Trondheim, Norway.

FLEISHMAN, L. J., AND M. PERSONS. 2001. The influence of stimulus and background colour on signal visibility in the lizard *Anolis cristatellus*. Journal of Experimental Biology 204:1559–1575.

FLEISHMAN, L. J., E. R. LOEW, AND M. LEAL. 1993. Ultraviolet vision in lizards. Nature 365:397.

FLEISHMAN, L. J., M. BOWMAN, D. SAUNDERS, W. E. MILLER, M. J. RURY, AND E. R. LOEW. 1997. The visual ecology of Puerto Rican anoline lizards: habitat light and spectral sensitivity. Journal of Comparative Physiology, A 181:446–460.

GRILL, C. P., AND V. N. RUSH. 1999. Analysing spectral data: comparison and application of two techniques. Biological Journal of the Linnaen Society 69:121–138.

JACKMAN, T. R., A. LARSON, K. DE QUEIROZ, AND J. B. LOSOS. 1999. Phylogenetic relationships and tempo of early diversification in *Anolis* lizards. Systematic Biology 48:254–285.

LEAL, M., AND L. J. FLEISHMAN. 2001. Evidence for habitat partitioning based on adaptation to environmental light in a pair of sympatric lizard species. Proceedings of the Royal Society of London B Biological Sciences 269:351–359.

LOEW, E. R., L. J. FLEISHMAN, R. G. FOSTER, AND I. PROVENCIO. 2002. Visual pigments and oil droplets in diurnal lizards: a comparative study of Caribbean anoles. Journal of Experimental Biology 205:927–938.

LOSOS, J. B., AND L.-R. CHU. 1998. Examination of factors potentially affecting dewlap size in Caribbean anoles. Copeia 1998:430–438.

MACEDONIA, J. M. 2001. Habitat light, colour variation, and ultraviolet reflectance in the Grand Cayman anole, *Anolis conspersus*. Biological Journal of the Linnaen Society 73:299–320.

MEDVIN, M. 1990. Sex differences in coloration and optical signalling in the lizard, *Anolis carolinensis* (Reptilia, Lacertilia, Iguanidae). Animal Behaviour 39:192–193.

PERSONS, M. H., L. J. FLEISHMAN, M. A. FRYE, AND M. E. STIMPHILL. 1999. Sensory response patterns and the evolution of visual signal design in anoline lizards. Journal of Comparative Physiology A 184: 585–607.

PROVENCIO, I., E. R. LOEW, AND R. G. FOSTER. 1992. Vitamin A$_2$-based visual pigments in fully terrestrial vertebrates. Vision Research 32:2201–2208.

RAND, A. S., AND E. E. WILLIAMS. 1969. The anoles of La Palma: aspects of their ecological relationships. Breviora 327:1–19.

SCHWARTZ, A., AND R. W. HENDERSON. 1985. A Guide to the Identification of the Amphibians and Reptiles of the West Indies Exclusive of Hispaniola. Milwaukee Public Museum, Milwaukee, WI.

WADE, J., A. C. ECHTERNACHT, AND G. F. MCCRACKEN. 1983. Genetic variation and similarity in *Anolis carolinensis* (Sauria: Iguanidae). Copeia 1983:523–529.

WILLIAMS, E. E. 1969. The ecology of colonization as seen in the zoogeography of anoline lizards of small islands. Quarterly Review of Biology 44:345–389.

WILSON, M. A., AND A. C. ECHTERNACHT. 1989. A comparison of heat and cold tolerance of two morphs of *Anolis carolinensis* (Iguanidae) from Southern Florida. Journal of Herpetology 24:330–333.