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Skin Pigments and Coloration in the Jamaican Radiation of *Anolis* Lizards

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ABSTRACT.—**The colorful dewlaps of *Anolis* lizards have long attracted the attention of biologists interested in the evolution of animal signals. Work on the North American green anole (*Anolis carolinensis*) and the anoles of Puerto Rico has shown that skin coloration results from the combined effects of pigments—pteridines, carotenoids, and melanin—and structural colors produced by reflecting platelet arrays in dermal iridophores. We conducted a study of skin pigments in the Jamaican radiation of anoles, known as the '*grahami* series', to examine how these anoles compare with those previously studied. We also wished to determine the histological basis for a strongly UV reflective dewlap that occurs in the only non-Jamaican member of the seven-species radiation, *Anolis conspersus* from Grand Cayman. We used thin layer chromatography to identify pteridines, spectrophotometry to detect carotenoids, and histology to reveal patterns of melanin in the skin of the study species. Our results are discussed in light of previously published work on *Anolis* coloration, and we describe a pigmentary novelty that is unique to *A. conspersus* within the *grahami* series.**

Males in many species of *Anolis* lizards exhibit colorful dewlaps that contrast conspicuously with the backgrounds against which they are displayed. Coloration of the dewlap and body skin in anoles results from a combination of pigments—carotenoids, pteridines, and melanin—and structural colors (see Cooper and Greenberg, 1992 for a review of reptilian coloration). The dermis of anoles and other reptiles contains chromatophore units comprising three primary components. Most superficially are xanthophores that contain pterinosomes (pteridines) and carotenoid vesicles (carotenoids). Beneath the xanthophores are iridophores that reflect and scatter light. Finally, under the iridophores lie melanin-containing melanophores (e.g., Alexander and Fahrenbach, 1969; Taylor and Hadley, 1970; Morrison et al., 1995).

As pigments, pteridines and carotenoids selectively absorb wavelengths of light striking them. Pteridines are hydrophylic compounds made up of nitrogenous rings that are synthesized in purine salvage pathways (e.g., Brown, 1985; Morrison et al., 1995). Pteridines can be

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identified by their chromatographic R_f values as well as by their fluorescence coloration when viewed under a UV light source. The spectra of most pteridines occur largely in the UV and thus are not considered true pigments by human visual standards. Other pteridines, such as sepiapterin and the drosopterins, are visible as yellow and orange-to-red, respectively.

Carotenoids (carotenes and xanthophylls), which are composed of hydrocarbon chains containing terminal carbon rings, constitute the largest group of natural pigments. Although most commonly observed as red, orange, and yellow pigments, carotenoids of virtually every color are known (e.g., Lee, 1977). Unlike pteridines, animals cannot synthesize carotenoids and must obtain them in the diet from plants, fungi, algae, and certain microorganisms (Olson and Owens, 1998). Carotenoid-based body coloration is common in invertebrates; in vertebrates it is prominent in the skin of fish, amphibians, and reptiles, and in the feathers of birds (Fox, 1976, 1979).

Iridophores contribute to skin coloration through the production of structural colors. Electron microscopy has shown that iridophores in the body skin of *Anolis* contain stacks of membranous sheets into which are embedded matrices of guanine crystals, termed 'reflecting platelets' (Alexander and Fahrenbach, 1969). These platelets are relatively uniform in size, shape, and distribution, and reflect and scatter short wavelength light through thin-film interference effects (Rorlich and Porter, 1972). Morrison (1995; Morrison et al., 1995) showed, however, that the wavelengths of light reflected from iridophores in *Sceloporus* vary dramatically according to reflecting platelet size and spacing, providing a mechanism for 'fine tuning' of any skin coloration. Long wavelength light that passes through the iridophores and strikes a melanosome is absorbed (Taylor and Hadley, 1970). Long wavelengths that do not strike melanosomes should continue through underlying hypodermal tissue to the fascia, a silvery-white layer of connective tissue which separates the skin and muscle. Some of the light striking this fascia is transmitted through it, but much is reflected back toward the dermis and into the external environment.

In *Anolis*, many species can change their body skin coloration rapidly from bright (usually green) to dull (typically dark brown). The green body coloration commonly seen in reptiles and amphibians is the combined result of the three chromatophore layers working in concert: ultraviolet, violet, and blue wavelengths are removed in the xanthophores, yellow, orange, and red wavelengths pass through the iridophores and are absorbed by the melanophores, leaving only green wavelengths to be scattered and reflected back (e.g., Nielsen and Dyck, 1978; Cooper and Greenberg, 1992). The lizards become brown when hormonally-stimulated melanosomes migrate through the arm-like processes of the melanophores which extend over the xanthophores (e.g., Taylor and Hadley, 1970). Although dewlap skin contains the same chromatophore layers as found in the body skin, dewlap color remains constant, or changes almost imperceptibly, with alterations in body color.

Despite the great diversity of skin color patterns witnessed in *Anolis*, very few studies of their underlying pigments have been conducted to date. Aside from von Geldern's (1921) histological investigation of *Anolis carolinensis*, only the Puerto Rican anoles have been studied with respect to integumental pigments. Using paper chromatography, Ortiz et al. (1963) and Ortiz and Maldonado (1966) found that all of the orange and red, and some of the yellow, dewlap pigmentation in Puerto Rican anoles was caused by the presence of pteridines, not carotenoids. Six types of pteridines were detected, although only two (sepiapterin and the drosopterins) were visible without the aid of a UV light source. In addition, considerable amounts of hydrophobic yellowish material, soluble both in ethanol and in petroleum ether, led Ortiz et al. (1963) to conclude that carotenoids were responsible for the majority of yellow coloration in the dewlaps of Puerto Rican anoles.

In the present study we focussed on identification of dewlap and body skin pigments in the Jamaican radiation of *Anolis* lizards, known also as the '*grahami* series' (e.g., Hedges and Burnell, 1990). This monophyletic group contains seven species, six of which evolved on Jamaica and one that is endemic to Grand Cayman. These anoles have been partitioned into three 'species groups': the '*grahami* group' (*Anolis conspersus, A. garmani, A. grahami,* and *A. opalinus*), the '*lineatopus* group' (*A. lineatopus* and *A. reconditus*), and the '*valencienni* group' (*A. valencienni*) (Hedges and Burnell, 1990). Substantial evidence exists that *Anolis conspersus* is a direct descendent of *A. grahami*, the latter having rafted on favorable currents from Jamaica to Grand Cayman (Grant, 1940; Williams, 1969) between 1 and 3 MYA (Haq et al., 1987; Jackman et al., *in press*; F. Burton, pers. comm.).

Here we report the results of a chromatographic study of pteridine pigments in the skin of the *grahami* series anoles, and of *A. sagrei*, a Cuban anole present on Jamaica and Grand Cayman but which is not part of the Jamaican radiation (Williams, 1969). We compare our results with those found by Ortiz and Maldonado (1966) for the Puerto Rican anoles (which comprise more than one taxonomic series). We also

report spectral analysis findings for carotenoids in the skin of our study species. Last, we suggest a spectral function for a distinctive melanin layer, revealed through histological studies, which occurs only in the dewlap skin of *A. conspersus*.

Materials and Methods

Thin-Layer Chromatography.—We conducted our investigation during the summers of 1997 and 1998, and tested for the presence of the same pteridines that have been examined in other studies of lizards (Ortiz and Maldonado, 1966; Morrison et al., 1995) and *Drosophila* (e.g., Wilson and Jacobson, 1977). The pteridines examined included (fluorescence colors in parentheses): drosopterins (orange and red), isoxanthopterin (purple), xanthopterin (yellowish green), sepiapterin (yellow), pterin (blue, = 2-amino-4-hydroxypteridine), and biopterin (lighter blue).

Identification of pteridines in *Anolis* skin was conducted as follows. Three to six adult males of each species/subspecies were fully anaesthetized with chloroform. The spinal cord then was cut at the base of the neck with scissors. The dewlap was excised from the body, and the cartilage was removed with forceps. Dorsal skin then was removed. These tissues were stored in Eppendorf tubes containing 70% ETOH for several weeks until used. Pteridine extractions were conducted in a ratio of 0.135 g tissue/1.5 ml fluid with each of two sequential extraction fluids: 80% ETOH/1 N HCl and 50% ETOH/1 N NH_4OH. Samples were removed from the storage vials, blotted on paper towel to absorb excess ethanol, and weighed on an analytical balance (VWR OHAUS Precision Standard model T51205). Each weighed sample first was placed on a piece of parafilm to which several drops of 80% ETOH/1 N HCl were added, and then was cut into small pieces. Samples of dewlap and back skin were maintained separately in labeled centrifuge tubes filled with the calculated amount of 80% ETOH/1 N HCl. These samples then were homogenized using the Tissue Tearor (model 985-370, Biospec Products). Next, the homogenates were centrifuged at 0 C at 4800 rpm for 15 min. The supernatants were drawn off and placed in aluminum foil-covered plastic vials and packed in ice. The second extraction fluid, 50% ETOH/1 N NH_4OH, then was added to the tissue samples. The mixtures were again homogenized and centrifuged, and the supernatants were added to the volume already collected. Last, the pH of the extractions was adjusted with NaOH from roughly 2 to about 9.5.

Pteridine standards were prepared in 80% ETOH from compounds purchased from SIGMA and ICN, and also were extracted from the eyes of *Drosophila melanogaster* strains: Oregon-R, chocolate, and sepia. *Drosophila* was used primarily to obtain standards for drosopterins. To prepare pteridine standards from files, the files first were frozen in a plastic vial at −20 C. The vial then was shaken vigorously to separate the heads from the bodies. The heads were weighed and extraction fluid (1:1 80% ETOH/1 N HCl: 50% ETOH/1 N NH_4OH) was added in 0.4 ml/10 mg of fly head proportions.

Ascending one-dimensional thin layer chromatography was carried out on microcrystalline cellulose plates in a dim and air-conditioned room maintained at 22–25 C. A 2% ammonium acetate solution was used as the solvent system. The pigment spots were examined visually in a UV light box (UVP Chromato-Vue Cabinet model CC-10 with a Mineralight Lamp MultiBand UV-254/366 nm) using the long wavelength bulb. The perimeters of fluorescing spots from skin extracts were outlined in pencil for R_f value comparisons, and the colors of the spots were recorded with respect to the pteridine standards. Sample-to-standard comparisons of pteridine concentrations were grouped into three subjective brightness categories: ++ = large to moderate amounts, + = small to trace amounts, and − = absent.

Spectrometry.—Absorbance characteristics of the skin extracts (supernatants) were made in 1 cm quartz cuvettes with a Hewlet-Packard Diode Array Spectrophotometer. Reflectance spectra of dewlap and body skin from living subjects were gathered as follows.

A subject was restrained by placing a piece of adhesive laboratory tape around the front feet, rear feet, and the end of the snout. The lizard then was laid on its side on a 'flat black' substrate (a computer mouse pad), and the apex of the outstretched dewlap was taped to the substrate. Spectral radiance measurements were obtained at an angle of approximately 70–90° to the target through a fiber optic cable (400 μm) that was fitted with a UV-VIS colimmating lens (Ocean Optics 74-UV) and connected to an Ocean Optics S2000 portable spectrometer. The data from the spectrometer were fed directly into a 100 kHz A/D card (National Instruments DAQCard-700) resident in a Compaq Armada 1130 laptop computer. The spectral data were displayed on the computer screen with OOIBASE Windows software (v 1.5, Ocean Optics, Inc.). A Whiteport Optolon 2 matte reflectance standard (>97% reflectance from 300–1100 nm, ANCAL Inc.) was used to calculate skin reflectance from the radiance measurements. A reflectance standard reading was obtained for each subject. The spectral readings were collected in the field on Jamaica and Grand Cayman during periods of clear sky conditions.

TABLE 1. Dewlap and body coloration in the study taxa. * Primary body color refers to the color of the majority of the body when in the typical 'bright' color state, not in metachrosis (dark color state).

Taxon	Dewlap color	Primary body color*
A. conspersus conspersus	royal blue	leaf green
A. conspersus lewisi	medium to sky blue	med./light brown to beige
A. garmani	golden yellow	bright 'new leaf' green
A. grahami grahami	orange with narrow yellow rim	teal
A. grahami aquarum	orange with narrow yellow rim	emerald green
A. opalinus	orange-red with a broad yellow rim	gray to brown
A. lineatopus lineatopus	off-white with sulphur yellow center	mottled tans and grays
A. lineatopus ahenobarbus	amber with narrow off-white rim	mottled browns
A. reconditus	lemon yellow	mottled browns & greens
A. valencienni	mauve	light gray with dark speckling
A. sagrei (Jamaica)	orange-red-brown	tan and brown
A. sagrei (Grand Cayman)	chocolate brown with yellowish rim	tan and brown

Histology.—Excised skin specimens were placed in Bouin's fixative overnight. After dehydration in graded concentrations of ethanol and clearing in toluene the specimens were embedded in paraplast. Sections were cut at 5 μm and mounted on poly-L-lysine coated slides. Following removal of the paraplast and rehydration the sections were stained with hematoxylin and eosin.

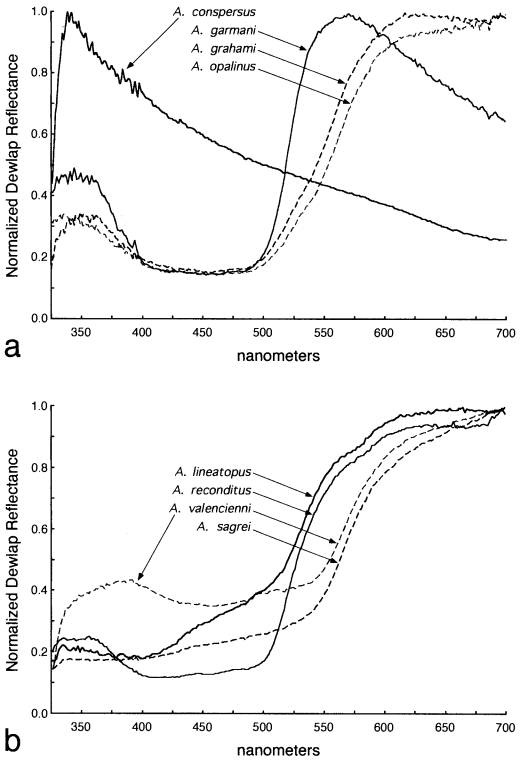

FIG. 1a, b. Representative normalized dewlap skin reflectance spectra of the study taxa. Spectral measurements were taken at dewlap centers. Subspecies shown in 'a': *A. conspersus conspersus* and *A. grahami grahami* ('Kingston' form); in 'b': *A. lineatopus lineatopus*. Species in 'a' comprise the '*grahami* group', those in 'b' are the remaining Jamaican anoles.

RESULTS

Skin Coloration.—The Jamaican radiation of anoles exhibits a diversity of skin color patterns, particularly in the dewlap (Table 1). Excluding the strongly short wavelength-biased dewlap of *A. conspersus* (Fig. 1a), however, all the dewlaps of the *grahami* series anoles (and *A. sagrei*) are primarily long-wavelength reflectors (Fig. 1a, b). Jamaican members of the *grahami* group exhibit broad, sigmoidal (i.e., step function) dewlap reflectance slopes, indicative of high chroma (strong color saturation). One can observe how the locations of the sharp spectral slopes for *A. garmani*, *A. grahami*, and *A. opalinus* correspond to the perceived yellow, orange, and orange-red hues, respectively, in these dewlaps (Fig. 1a). *Anolis lineatopus* and *A. reconditus* both possess yellow dewlaps, but the more gradually-sloping curve for *A. lineatopus* corresponds to a lower chroma dewlap in this species, as compared with the bright lemon-yellow dewlap of *A. reconditus* (Fig. 1b). The dewlap of *A. valencienni* is unique in that it exhibits a strong red and a weaker violet peak which, given its moderate chroma (Fig. 1b), results in a muted, purplish-brown coloration (mauve).

The dorsal skin surface of the *grahami* series anoles can be partitioned dichotomously into those that are green (Fig. 2a) and those that are brown and/or gray (Fig. 2b). The two taxa with shallower curves depicted in Fig. 2a exhibit blue-green (*A. grahami grahami*) and medium green (*A. c. conspersus*) dorsal coloration. *Anolis grahami aquarum* and *A. garmani*, exhibit extremely similar high chroma sigmoid curves (Fig. 2a) that we perceive as emerald green and new leaf green, respectively. The gradually rising slopes of the dorsum reflectance curves

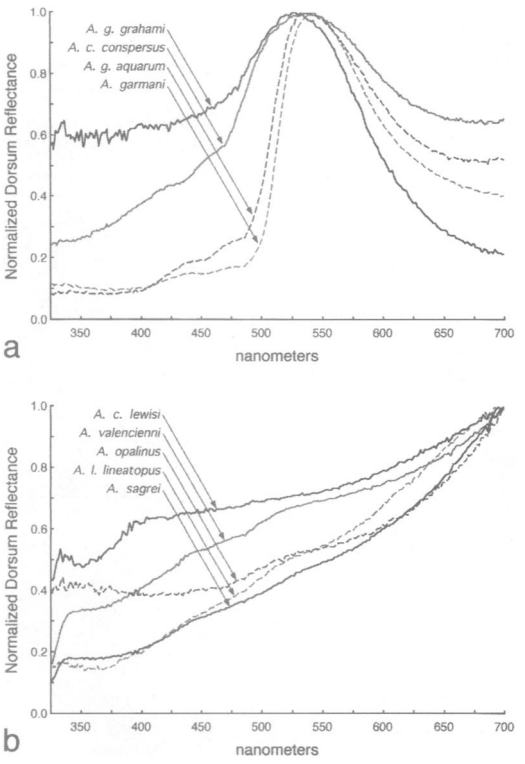

FIG. 2a, b. Representative normalized dorsum skin reflectance spectra of the study taxa. Spectral measurements taken along the spine, mid-way between the neck and tail base. Taxa are grouped by the general shape of their reflectance curves. Those in 'a' exhibit peaks in the green-yellow region of the spectrum, those in 'b' peak show red (brown) peaks.

shown in Fig. 2b, all of which peak in the red end of the spectrum, illustrate variation in brown dorsal coloration among these anoles. The shallower slopes appear tan (*A. c. lewisi*) or gray-brown (*A. opalinus*), whereas the medium browns of *A. l. lineatopus* and *A. sagrei* are seen in their very similar, slightly steeper slopes. The intermediate slope of *A. valencienni* results from the combination of black speckling on a very light gray background. We did not obtain dorsal reflectance spectra for *A. reconditus*, but their somber, muted earth tones likely would have produced curves within the range of those shown illustrated in Fig. 2b.

Pteridines.—Thin-layer chromatography revealed all of our study species except *A. lineatopus* and *A. reconditus* to possess the same four pteridines in the dewlap: drosopterins, isoxanthopterin, pterin, and biopterin (Table 2). The general lack of orange/red coloration in the dewlaps of *A. lineatopus* and *A. reconditus* appears to be due to the absence of drosopterins. Those species for which orange and/or red dewlap coloration is prominent (*A. grahami, A. opalinus, A. valencienni,* and *A. sagrei*) exhibited proportionately more drosopterins than those species lacking orange or red (*A. conspersus* and *A. garmani*, Table 2).

Sepiapterin was the most variable pteridine in its distribution among the study species. Although it is seen in high concentrations on a chromatogram as a yellow pigment, its presence or absence does not appear to be closely correlated with the amount of yellow coloration visible in a dewlap. For example, the dewlap of *A. valencienni* held large amounts of sepiapterin, but this dewlap does not have a yellow appearance (perhaps due to masking by drosopterins: Table 2). Conversely, the dewlaps of *A. garmani, A. reconditus* and *A. lineatopus* are yellow, but no sepiapterin was found in them (Table 2). Last, isoxanthopterin was present in moderate to high concentrations in the dewlaps of all the species, whereas xanthopterin was consistently absent (Table 2a).

All of our study species exhibited isoxanthopterin, pterin, and biopterin in the skin of the dorsum (Table 2b). Beyond this, the taxa fell into one of two groups: those which did not exhibit additional pteridines (*A. garmani, A. grahami aquarum, A. opalinus, A. reconditus,* and *A. sagrei*), and those which also exhibited sepiapterin (*A. conspersus, A. grahami grahami,* and *A. lineatopus*). Just as was seen for the dewlaps, isoxanthopterin was present in moderate to high concentrations in the dorsum of every species, and xanthopterin went undetected in the dorsal skin.

Carotenoids.—With two exceptions, all taxa examined returned essentially identical peak values for the diagnostically-shaped, three-peaked carotenoid absorbance spectrum (first peak = 422–426 nm, second peak = 444–446 nm, third peak = 472–474 nm; Fig. 3). The shape of the absorbance spectrum from 300–500 nm and the locations of the absorbance peaks suggest that the primary component of this yellow material may be zeaxanthin, a xanthophyll which is the pigment present in the yellow oil droplets of many bird species' retinal photoreceptors (e.g., Goldsmith et al., 1984). In contrast, neither *A. conspersus* (Fig. 3a) nor *A. valencienni* (not pictured) exhibited carotenoids in their dewlap skin.

Melanin.—Individual variation in melanophore expression within the dewlap was so extensive that no consistent among-taxon trends could be discerned. However, *A. conspersus* was unique in exhibiting a band of melanin at the base of the hypodermis, immediately overlying the fascia that separates the skin from the underlying muscle tissue (Fig. 4). This additional layer of melanin also can be observed when everting an excised dewlap: unlike the other

TABLE 2. Dewlap and dorsum skin pteridine profiles in the study species: − = absent, + = trace to small amounts, ++ = moderate to large amounts. [1] Two subspecies each of *A. conspersus* (*A. c. conspersus* and *A. c. lewisi*) and *A. lineatopus* (*A. l. lineatopus* and *A. l. ahenobarbus*) were examined and were found to exhibit the same pteridine profiles. [2] *A. sagrei* is the only *Anolis* species that is sympatric with, but not a member of, the *grahami* series radiation. Individuals from Jamaica and Grand Cayman differ in coloration but exhibited the same pteridine profiles. Dro: drosopterins, ISO: isoxanthopterin, XAN: xanthopterin, SEP: sepiapterin, TER: pterin, BIO: biopterin.

Dewlap	DRO	ISO	XAN	SEP	TER	BIO
A. conspersus[1]	+	++	—	+	+	+
A. garmani	+	++	—	—	+	+
A. grahami grahami	++	++	—	++	+	+
A. grahami aquarum	++	++	—	+	++	++
A. opalinus	++	++	—	—	+	+
A. lineatopus[1]	—	++	—	—	++	+
A. reconditus	—	++	—	—	+	+
A. valencienni	++	++	—	++	+	+
A. sagrei[2]	++	++	—	++	+	+
Body	DRO	ISO	XAN	SEP	TER	BIO
A. conspersus[1]	—	++	—	+	+	+
A. garmani	—	++	—	—	++	+
A. grahami grahami	—	++	—	+	++	+
A. grahami aquarum	—	++	—	—	++	++
A. opalinus	—	++	—	—	+	+
A. lineatopus[1]	—	++	—	+	++	+
A. reconditus	—	++	—	—	+	+
A. valencienni	—	++	—	+	+	+
A. sagrei[2]	—	++	—	—	++	+

species we examined, the inner surface of an *A. conspersus* dewlap appears black.

DISCUSSION

Pteridines.—Dewlap coloration in the Jamaican radiation of anoles is fairly diverse, and pteridines play a role in that color variation. Several similarities and differences in pteridine distribution exist between our study species and the Puerto Rican anoles examined by Ortiz and Maldonado (1966).

First, drosopterins are the only pteridines restricted to a particular body location in both groups, and in all cases these pigments are located in dewlap skin (Table 3). Given that the *Anolis* dewlap is used exclusively for signaling and is hidden at other times, the limitation of drosopterins to the dewlap skin suggests that these pigments are conspicuous to other anoles as well as to potential predators when displayed. Orange and/or red should contrast well against green vegetation in environments where the long wavelengths of direct sunlight contribute significantly to the ambient light spectrum, such as in forest light gaps, forest edges, and in open (grass-bush) areas (e.g., Endler, 1993). A few of the Jamaican and Puerto Rican species with orange (*A. grahami*) or red (*A. pulchellus*) dewlaps typically are found in direct sunlight (Rand, 1967; Schoener, 1970; Fleishman et al., 1993), although the situation is less clear for some other species (e.g., Fleishman et al., 1997).

A second similarity in pteridines between the *grahami* series and the Puerto Rican anoles is that all taxa possess isoxanthopterin in the dewlap skin, and isoxanthopterin, pterin, and biopterin in the skin of the dorsum (Table 3). This is true as well for *A. sagrei* (Table 2) and for *Anolis carolinensis* (Ortiz and Maldonado, 1966), and may be a widespread feature among anoles.

The greatest difference in pteridine distribution between the two *Anolis* radiations is that whereas two-thirds of the Puerto Rican anoles examined exhibit xanthopterin in their dorsal skin and over three-quarters exhibit this pteridine in their dewlaps, xanthopterin seems to be entirely absent in the Jamaican radiation. The apparent absence of xanthopterin in our study species should be interpreted cautiously, however. Given that isoxanthopterin was present in moderate to large amounts in all of our taxa, and xanthopterin falls immediately above isoxanthopterin on a chromatogram (as witnessed with the pteridine standards), it seems possible that small amounts of xanthopterin potentially could be obscured by upward streaking from isoxanthopterin. Nevertheless, xanthopterin should have been seen by chance on at least one chromatogram were it present.

Carotenoids.—The yellow coloration in the

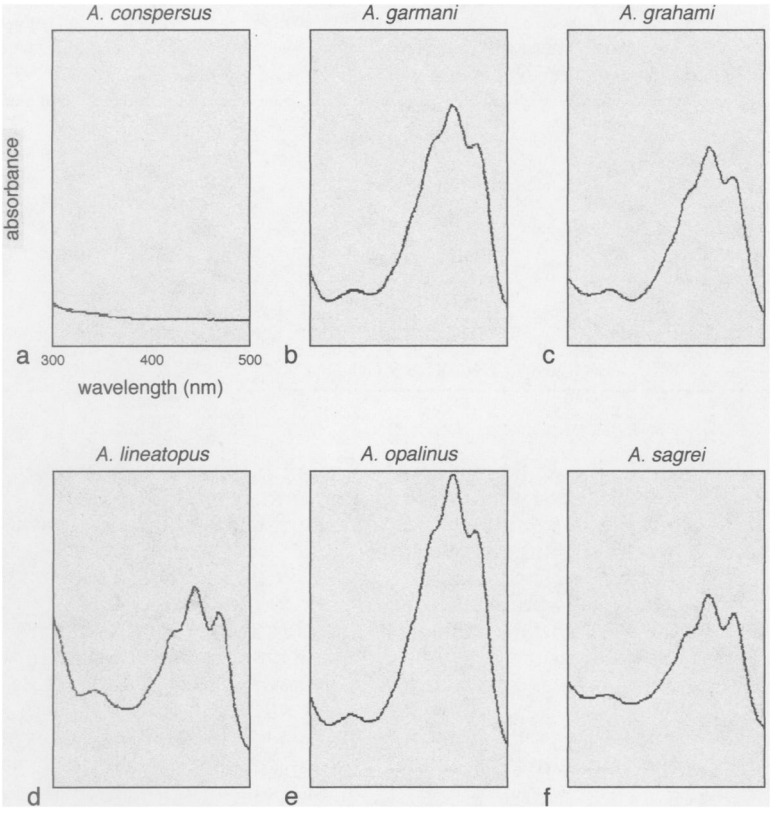

FIG. 3. Example absorbance spectra of carotenoids present in dewlap skin extracts of the study species. The plots do not depict relative abundance of carotenoids in the taxa shown; rather, they are presented to illustrate the shapes and peak locations of the carotenoid absorbance spectra.

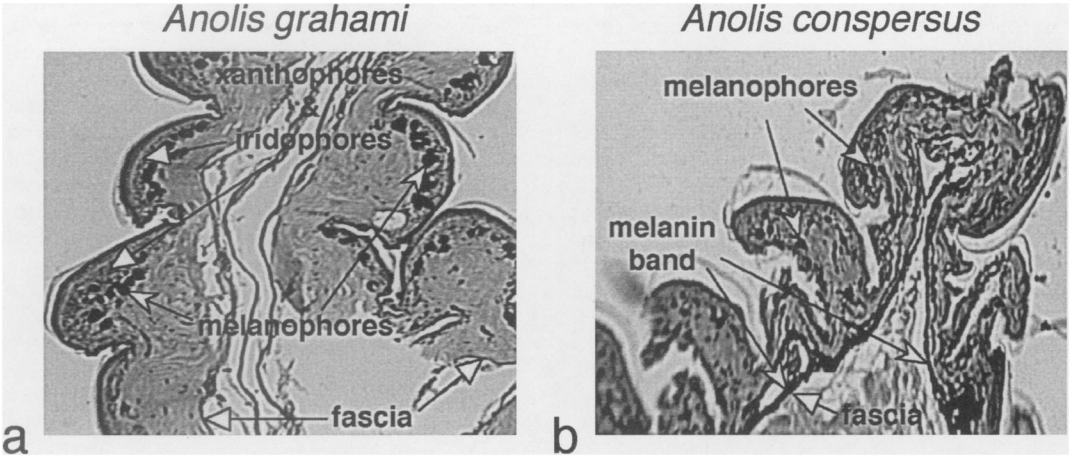

FIG. 4. 'Plan view' of dewlap sections cut in the horizontal plane. The front of each dewlap is at the top of each illustration. Skin folds seen on the left and right side of each section allow the dewlap to extend in life. Muscle tissue was displaced from the central open area present in 4a. *A. grahami* is representative of all '*grahami* series' taxa and *A. sagrei* with respect to the absence of the subdermal melanin band observed in *A. conspersus*.

TABLE 3. Comparison of dewlap and body skin pteridines in Puerto Rican and Jamaican anoles. Nine of 15 Puerto Rican *Anolis* species were examined (data from Ortiz and Maldonado, 1966); all seven *grahami* series species are represented. Legend as in Table 2.

Dewlap	DRO	ISO	XAN	SEP	TER	BIO
# Puerto Rican species	5	9	7	6	6	8
# Jamaican species	6	7	0	3	7	7
% Puerto Rican species	56%	100%	78%	67%	67%	89%
% Jamaican species	86%	100%	0%	43%	100%	100%
Body	DRO	ISO	XAN	SEP	TER	BIO
# Puerto Rican species	0	9	6	5	9	9
# Jamaican species	0	7	0	4	7	7
% Puerto Rican species	0%	100%	67%	56%	100%	100%
% Jamaican species	0%	100%	0%	57%	100%	100%

dewlaps of anoles is due largely to the presence of one or more carotenoids (Ortiz et al., 1963; Ortiz and Maldonado, 1966; this study). These pigments absorb short wavelength light and have a cutoff frequency approaching 500 nm (Fig. 3). Although sepiapterin is present in the skin of some anoles (Table 2; Ortiz and Maldonado, 1966), it is perhaps not possible to synthesize this pigment in sufficient quantities to produce green body coloration. In contrast, carotenoids are readily available to anoles from insects, which obtain them in turn from plant material.

Carotenoid-based yellow dewlaps are found predominantly in full shade ('forest shade' of Endler, 1992, 1993), where the ambient spectrum is yellowish-green from the available light being filtered through the leaves of a continuous canopy. The three species of Jamaican anoles that possess yellow dewlaps (*A. garmani, A. lineatopus,* and *A. reconditus*) are shade-dwelling (e.g., Rand, 1967; Hicks, 1973) and appear to be most abundant in closed-canopy forest (Macedonia, pers. obs.). Yellow dewlaps should be especially salient in the light of forest shade (e.g., Endler, 1992, 1993).

Cryptic and Conspicuous Body Coloration.—*Anolis* species whose body color patterns are conspicuous against a background of tree bark usually have the capacity to become less visible through metachrosis (i.e., the migration of melanin into the uppermost extensions of the melanophores). Low ambient (and thus body) temperatures as well as stress induce metachrosis (e.g., Cooper and Greenberg, 1992), which reduces contrast with the background and makes the lizards more difficult for conspecifics and predators to detect (e.g., Endler, 1992). However, because anoles figure prominently in the diets of some Caribbean birds and snakes (e.g., Grant, 1940; Cruz, 1973, 1975, 1976; Johnston, 1975; Fleishman 1985, 1992; Henderson and Sajdak, 1996), those species that spend an appreciable amount of time low on tree trunks or bushes are permanently camouflaged with color patterns in shades of browns, grays, or dull greens (e.g., *A. lineatopus, A. opalinus, A. reconditus,* and *A. sagrei*: Fig. 2; Underwood and Williams, 1959; Rand, 1967; Schoener, 1970).

Within the *grahami* series, bright green body coloration occurs in *A. garmani, A. grahami,* and *A. conspersus conspersus* (Table 1). Being similar in color to a leaf has an interesting consequence: the same individual can be cryptic or conspicuous depending upon its location and behavior. *Anolis garmani* is a particularly good example, as this species favors the understory of mature, closed-canopy forest (Rand, 1967; Macedonia, pers. obs.). Forest shade is strongest in the same range of wavelengths as those reflected from the body of *A. garmani* (Fig. 2a; Endler, 1992, 1993). When in the branches and surrounded by foliage, this species can be difficult to detect. But like many anoles, *A. garmani* spends much of its time perched in a head-down position on tree trunks and in plain view (e.g., Schoener, 1970; Trivers, 1976). The brilliant, almost-neon green body skin contrasts strongly with the dull tree bark, making *A. garmani* highly conspicuous (at least to the human eye). Nevertheless, avian and serpentine predators of anoles probably do not possess visual feature extraction capabilities similar to our own (Fleishman, 1992), and these lizards may go unnoticed by predators if they remain still, despite being in 'plain view'. Upon moving, however, their coloration should make them conspicuous to the visual systems of most terrestrial vertebrates (e.g., Endler, 1992, 1993; Fleishman, 1992). Extending the yellow dewlap (Fig. 1a) maximizes conspicuousness in *A. garmani,* as yellow reflects nearly as much of the forest shade spectrum as does green but contrasts enough with the leaves and tree bark to stand out.

The Unusual Dewlap of Anolis conspersus.—The most unusual dewlap coloration observed

in the *grahami* series anoles is the highly UV-reflective dewlap of *A. conspersus*, which humans perceive as blue (Fig. 1a: average peak reflectance >40% at 340 nm: Macedonia, unpubl. data). This dewlap is particularly intriguing given that *A. conspersus* is a direct descendent of *A. grahami*, a species possessing a bright orange dewlap (Fig. 1a). We uncovered three pigmentary factors which together may explain why the *A. conspersus* dewlap exhibits such different reflectance properties from those of its Jamaican relatives.

First, having only small to trace amounts of drosopterins in the dewlap (Table 2) attenuates the absorbance of short wavelengths. Second, the absence of carotenoids in the dewlap contributes appreciably to the strong short wavelength reflectance (Fig. 3a). Third, histology revealed the presence of an additional layer of melanin in the *A. conspersus* dewlap that was absent in the other species of Jamaican origin and in *A. sagrei*. This melanin band lies directly above the fascia that separates the hypodermis from the layer of muscle and cartilage that erects the dewlap (Fig. 4b). Virtually any light that passes through the iridophores and does not strike a melanosome should be absorbed by this melanin layer. Thus, the strong UV reflectance and visible deep blue coloration of the *A. conspersus* dewlap is a product of the underlying iridophores, plus a melanin layer that boosts chroma by absorbing wavelengths which otherwise would render the dewlap pale (e.g., light 'powder' blue).

During this decade it has been shown that UV photoreceptors (and/or UV visual sensitivity) are present in a broad diversity of vertebrate taxa (e.g., fish: Bowmaker et al., 1991; McFarland and Loew, 1994; Flamarique and Hawryshyn, 1998; amphibians: Palacios et al., 1998; reptiles: Alberts, 1989; Loew, 1994; Sillman et al., 1997; birds: Burkhard, 1989; Bennett and Cuthill, 1994; Finger and Burkhard, 1994; Bowmaker et al., 1997; Vorobyev et al., 1998; and rodents: Jacobs et al., 1991; Jacobs and Degan, 1994). In tandem, a strong interest in UV signals has emerged in the field of behavioral ecology. This is especially true for studies of avian plumage reflectance and mate choice decisions (e.g., Bennett et al., 1996, 1997; Andersson and Admundsen, 1997; Andersson et al., 1998; Hunt et al., 1998a, b; Johnsen et al., 1998).

All anoles examined to date have been found to possess a UV-absorbing visual pigment (Fleishman et al., 1993), including every taxon in the present study (E. Loew, unpubl. data). *Anolis* dewlaps vary considerably in UV reflectivity, however, and it has been suggested that this variation is correlated with the propensity of UV light in the lizards' habitats (Fleishman et al., 1993).

Considering the dewlap reflectance profile of *A. conspersus* it seems probable that ambient UV has had an impact on its color evolution. *Anolis conspersus* is found most frequently in the shade of broken canopy woodland (Macedonia and Echternacht, pers. obs.; see also Schoener, 1967; Avery, 1988; Losos et al., 1993). Both woodland and desert shade are biased toward short wavelengths because most of the down-welling light comes from skylight (Endler, 1992, 1993). Given that ultraviolet wavelengths scatter more than do visible wavelengths, but are absorbed by most green vegetation, a UV-bright dewlap should stand out conspicuously from the background in a woodland shade environment. Details of the relationship between habitat lighting and color signal evolution in *A. conspersus* are currently under investigation.

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