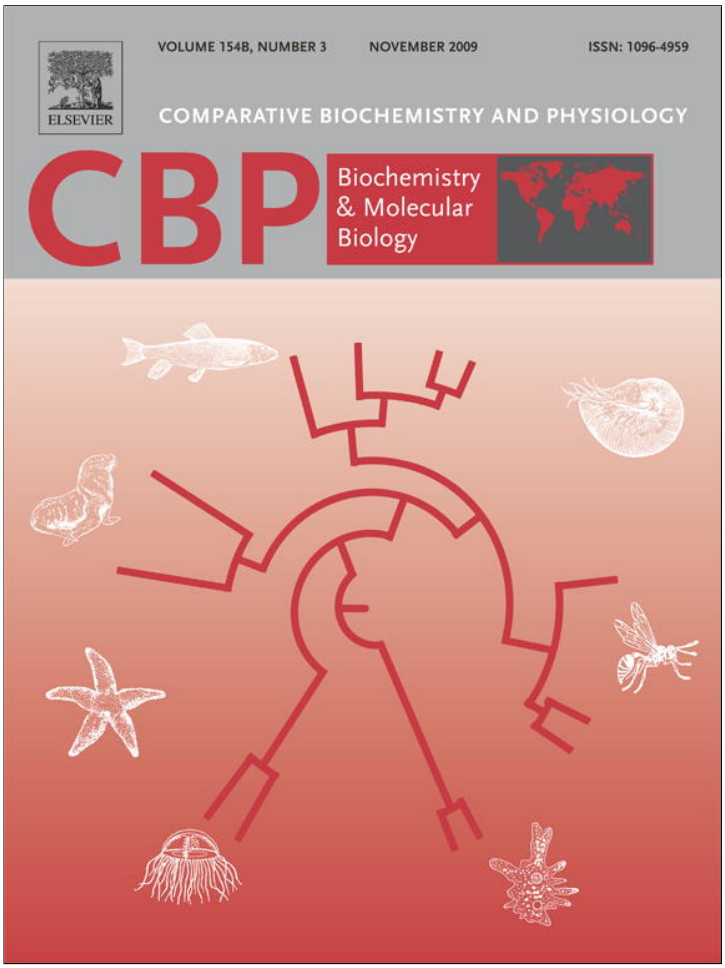

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How dewlap color reflects its carotenoid and pterin content in male and female brown anoles (*Norops sagrei*)

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ABSTRACT

Sexually selected colors in animals can be created by multiple pigments (e.g., carotenoids, melanins, pterins), but how these pigment classes interact to generate intraspecific color variation has rarely been tested, especially in reptiles. We examined full-spectrum color variation as well as pterin (i.e., drosopterin) and carotenoid (i.e., xanthophyll) pigment concentrations in the yellow and red sexually dichromatic dewlaps of male and female Brown Anoles (*Norops sagrei*) to understand their color-generating mechanisms and information content. Reflectance curves showed significant sexual differences in dewlap color that could only partially be explained by pigment composition. For example, drosopterin concentration correlated significantly with red chroma in the male's dewlap center. In females, drosopterin concentration correlated significantly with yellow and red chroma along the dewlap edge. In addition, xanthophyll concentration showed a significant inverse correlation with hue in the center of female dewlaps only. There were several other correlations between pigment concentrations and spectral variables, which hinted at ways that pigments produce color in male and female dewlaps, but these were non-significant after statistically correcting for multiple comparisons. These results demonstrate that sexes differ in how pigment classes influence dewlap spectral variation, but also that there may be other aspects of the integument not measured here that also influence dewlap color.

1. Introduction

Colorful ornaments are a well-known class of secondary sex characters in animals, and sexual dichromatism is one of the most common ways that ornaments vary. Sex differences in color, especially in lizards, are thought to play a role in sex recognition (Cooper and Greenberg, 1992; Andersson, 1994; Macedonia et al., 2003), and males are usually the more colorful sex.

The color of animal integument is caused by structural light-reflecting properties of the cells and tissues and by the light-absorbing properties of chemical pigments. Structural colors are those in which light-scattering by the integument acts alone, or in concert with melanin pigments, to produce, for example, white, iridescent, blue, and UV colors. This type of mechanism is responsible for feather color in many birds (Auber, 1957; Fox, 1976), and for blue scale color in phrynosomatid lizards (Morrison, 1995; Morrison et al., 1996). Many other vertebrate colors, especially in amphibians, reptiles, and fish, are generated by chromatophores, which may or may not interact with their integument structure (Bagnara and Hadley, 1973). These chromatophores can be xanthophores, which contain yellow pigments such as carotenoids as well as pterin pigments such as xanthopterin, sepiapterin and riboflavin. Carotenoid pigments in xanthophores are obtained through ingestion of plants or herbivores (Volker, 1938; Goodwin, 1984), and produce yellow color and permit UV reflection (Shawkey and Hill, 2005). These chromatophores can also be erythrophores, which contain red pigments (e.g. pterin), and which are endogenously synthesized from purines and produce red color (Obika and Bagnara, 1964; McGraw, 2006).

Specific to lizards, pterins have been identified as a coloring agent in the red dewlaps of male Puerto Rican anoles (e.g. Ortiz and Williams-Ashman, 1963; Ortiz and Maldonado, 1966). More recently, carotenoids, pterins, and melanins have been identified as dewlap coloring agents in males of the 'grahami' series of anoles (Macedonia et al., 2000), and it is generally believed that dewlap color in anoles is produced by variations in any of these three pigment classes and their interactions with structural mechanisms (Macedonia et al., 2000). Anole dewlaps can also reflect UV light (Fleishman et al., 1993, 1997; Leal and Fleishman, 2002, 2004; Thorpe and Stevenson, 2003) that can be perceived as a component of the visual signal (Fleishman et al., 1993). Integument coloration is at least partially the result of such structural mechanisms (Dyck, 1971a,b; Ghiradella et al., 1972), but more work is needed to understand how pigments interact with integument structure to generate color (see Shawkey and Hill, 2005; Shawkey et al., 2006 for similar work on a bird species).

Despite considerable work on the biochemical mechanisms of coloration in birds (Inouye et al., 2001; Saks et al., 2003; McGraw and

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Gregory, 2004; McGraw et al., 2005a,b), only in fish have multiple pigment types been carefully considered in how they spectrally and chemically contribute to color production and variation (Grether et al., 2001, 2005; Clotfelter et al., 2000). Virtually no work has considered how multiple pigment types contribute to color production and variation in reptiles.

We previously identified the co-occurrence of yellow carotenoids and red pterins in *Norops* dewlaps (Steffen and McGraw, 2007), and here we analyze the spectral variation in dewlaps of male and female Brown Anoles using traditional tristimulus color descriptors (hue, chroma, brightness) and measured dewlap pigment quantities, in order to investigate:

(a) intersexual and interregional differences in dewlap coloration and pigmentation.
(b) how pigment concentrations influence dewlap spectral variation measured across the range of retinal sensitivity in lizards (Fleishman et al., 1993), and
(c) relationships between dewlap color, size, and pigmentation, to evaluate the possibility that dewlap color accurately provides information about pigment concentrations.

Brown Anoles are model organisms to study these objectives because males and females differ in dewlap size and color, and males display these dewlaps in conjunction with head-bobs and push-ups to rival males (Jenssen, 1977; McMann, 2000; Paterson and McMann, 2004; Tokarz, 2002; Tokarz et al., 2003), to prospective female mates (Jenssen, 1977; Tokarz, 2002; Tokarz et al., 2003), or to predators during an attack (Leal and Rodriguez-Robles, 1997a,b; Leal, 1999). In addition, their dewlaps are comprised of two distinctly colored regions (Nicholson et al., 2007): the dewlap center, which can be viewed when the dewlap is extended; and the dewlap edge, which can be viewed when the dewlap is extended or retracted (i.e. it can be viewed during push-ups and head-bobs). Furthermore, Brown Anoles are well-known in the study of behavioral endocrinology (Tokarz, 1995; Crews and Williams 1977; Crews, 1979; Tokarz et al., 1998) and dewlap color may show an important but heretofore unstudied aspect to findings from these past studies. Finally, anoles are a classic example of an animal that has undergone an adaptive radiation (Losos and deQueiroz, 1997), and dewlap color varies widely between closely-related species (Nicholson et al., 2007). The study of how pigments interact to produce color may help to understand the physiological mechanisms that lead to closely-related species obtaining different dewlap colors and patterns that accompany the speciation process.

2. Materials and methods

Twenty two male and twelve female adult brown anoles (*Norops sagrei*) were captured from a dock and surrounding houses of Lake Carroll, in Hillsborough County, Florida, USA. Lizards were identified as adult males if they were greater than 45 mm in snout-to-vent length (SVL) and possessed a large (minimum 50 mm^2) extendable dewlap, or as adult female if they were greater than 35 mm SVL and lacked a large extendable dewlap. Lizards were housed according to approved A.U. IACUC protocol (PRN 2006-0961) in screen-topped, 38 l glass terraria that were partitioned into four separate compartments. Each lizard was placed in a separate compartment that contained a perch and a water dish on a sandy substrate. Lighting strips containing full-spectrum fluorescent bulbs (Vitalite T8, 32 W) were suspended 31 cm above each terrarium top. Natural sunlight also illuminated each terrarium through a nearby window. Lizards were fed crickets and meal worms *ad libitum*, which were dusted with multivitamin powder (repta-vite) designed for the needs of reptiles (Zoo Med laboratories, San Luis Obispo, CA, USA). Dewlap colors and size were quantified (see below for methods) one day after lizards arrived in Auburn, AL, USA. Lizards were then held in captivity for three weeks for use in behavioral experiments from a separate study.

We measured dewlap coloration of living male and female lizards using an Ocean Optics S2000 spectrometer (range 250–880 nm: Dunedin, Florida) coupled to a tungsten–deuterium light source. We used a bifurcated fiber-optic cable mounted in a metal probe that was placed at an angle of 90° to the plane of the measured dewlap surface. Following Steffen and McGraw (2007), we considered two regions of the dewlap in both males and females. The 'center' dewlap region was the area exposed when a dewlap was extended (perceived as red by the human eye). The 'edge' dewlap region was the anterior dewlap margin when extended (perceived as white or yellow by human eyes; Conant and Collins, 1998).

In each dewlap region, we took three unique, adjacent and non-overlapping measurements from males and two from females at a 90° angle from the surface of the lizard's skin. We took fewer measurements of females because the dewlap area was only large enough to measure two unique locations. The probe was kept at a 2 mm distance to the skin being measured by a small black plastic sleeve fastened to the probe tip. Color data were gathered as percent reflectance at 1 nm wavelength increments from 300 to 700 nm (which represents the lower range of photon absorption by UV-sensitive photoreceptor cones published for anoles, see Fleishman et al., 1993) and this output was smoothed and processed using CLR v 1.0 (Montgomerie copyright 2008).

From the smoothed spectral reflectance data, we calculated the 'tristimulus' colorimetric variables commonly referred to as hue, saturation (or chroma), and brightness. We used CLR v 1.0 (Montgomerie, copyright 2008) to determine mean values of chroma for UV (300–400 nm), yellow (510–549 nm) and red wavebands (550–700 nm), and we used MS Excel, along with formulas described in Montgomerie (2006), to determine UV, yellow and red brightness as well as UV and long-wavelength hue. These colorimetric variables describe different aspects of the spectral curve and have been used extensively in the literature (Endler, 1990; Hill, 1998; Montgomerie, 2006). Long-wavelength hue was calculated as location of the maximum positive slope for a portion of the spectral curve between 401 and 700 nm (i.e., $\lambda b_{\max\ pos}$, Montgomerie, 2006). We used this formulation of hue, rather than hue defined as LR max (i.e., the wavelength of maximum reflection), because the majority of variation in our reflectance curves occurs where there is a sharp reflectance increase that occurs in the middle to long wavelengths. While most of our reflection data also contain long-wavelength reflectance plateaus, LR Max does not adequately describe where the majority of variation occurs. UV hue is often an important colorimetric variable in xanthophyll-based coloration (Andersson and Prager, 2006). UV hue was calculated as the location (in nm) of the maximum negative slope for a portion of the spectral curve from 300 to 400 nm (i.e. $\lambda b_{\max\ neg}$ Montgomerie, 2006) and was compared between sexes and dewlap regions. However, because UV hue did not correlate with pigment concentrations (e.g. Female dewlap center, drosopterin concentration: $R^2 = 0.066$, $P = 0.562$; xanthophyll concentration: $R^2 = 0$, $P = 1.0$; Female dewlap edge, drosopterin concentration: $R^2 = 0$, $P = 1.0$; xanthophyll concentration: $R^2 = 0$, $P = 1.0$; Male dewlap center, drosopterin concentration: $R^2 = 0.127$, $P = 0.524$; xanthophyll concentration: $R^2 = 0.124$, $P = 0.524$; Male dewlap edge, drosopterin concentration: $R^2 = 0.002$, $P = 0.857$; xanthophyll concentration: $R^2 = 0.002$, $P = 0.884$) we did not present these data. Brightness was calculated for UV, yellow, and red wavebands as the percent total reflectance in UV, yellow, and red wavebands. UV, yellow and red chroma was calculated as proportion of light in these wavebands relative to total reflectance across the 300–700 nm spectrum. We performed a post-hoc repeatability analysis to determine how repeatable our measurements for chroma (expressed as a percentage), brightness and hue were for the UV, yellow and red wavebands. We measured centers and edges of male and female dewlaps in spring

2009 following the exact same protocol with the same sample size described in the previous paragraph. We found that all aspects of spectral variation were highly repeatable (see Table S1).

After spectrometry was performed on lizard dewlaps (see below), a digital image was taken of each dewlap (Kodak Easyshare DX4530 camera) so that dewlap area could be measured from photos. For each image, forceps were used to attain maximal extension of the dewlap (point at which further extension resulted in a change of dewlap shape without an increase in size). A plastic millimeter ruler was placed in each image for scale. Each lizard had its dewlap extended and photographed twice. Dewlap area of each male lizard was quantified with imaging software (CIAS, 1995) that converted pixel number to metric size from the ruler increments present in the digital photo. Each digital image of a male was measured twice, then the two areas were averaged, and the mean of the area obtained from two images was used as dewlap area (i.e. mean of four measurements per individual). Dewlap area was highly repeatable (Lessels and Boag, 1987) (repeatability, $r=0.99$. $F=204.03$, $N=34$; $MS_{among}=5146.87$, $MS_{within}=25.23$, $P<0.001$).

After the photographic procedure, animals were euthanized, and dewlap tissue was removed from lizards as described by Macedonia et al. (2000). The dewlap skin was excised from the body, and the hyoid cartilage underlying the skin was removed with forceps. Yellow dewlap tissue from the edge was separated from red center tissue by cutting with a sharp razor blade. The tissue was rinsed with water to rid samples of spilled blood that may contain pigments and contaminate skin samples. These tissues were then stored at −80 °C in Eppendorf tubes containing 70% ethanol for one day, and then placed on dry ice and shipped to KJM for pigment analysis and quantification.

Concentrations of carotenoid and pterin pigments were in each tissue region using the following method: 3–5 mg of tissue was removed from the yellow edge and red center tissue samples and ground using a mixer mill (McGraw et al., 2005a,b) in the presence of 1% NH_4OH. The ground material and solvent were transferred to a fresh tube, at which point tert-butyl methyl ether (TBME) was added to partition carotenoids from pterins. The solution was then shaken for 1 min, centrifuged, and the two solvents separated for pterin and carotenoid quantification. This method partitioned the carotenoids into the top (MTBE) layer and the pteridines into the bottom (NH_4OH) layer. We used absorbance spectrophotometry (*sensu* Grether et al., 2001) separately on the 2 fractions to determine carotenoid and pteridine concentrations based on standard calculations (McGraw et al., 2002). Full-spectrum spectrophotometric scans on each solvent provided λ_{max} values for calculating concentrations ($\lambda_{max}=455$ nm for the carotenoid fraction and 490 nm for the pterin fraction); λ_{max} values also provided secondary confirmation that the pigments in the two solvent fractions were in fact pterins (likely predominated by red drosopterins, see Steffen and McGraw, 2007 for evidence in other anoline lizards) and carotenoids (likely xanthophylls, such as lutein and zeaxanthin; Raila et al., 2002). Note if yellow sepiapterins, xanthopterins and riboflavins were present they were not concentrated enough to affect the λ_{max} values for drosopterin. In our calculations, we used 2550 as the extinction coefficient for xanthophyll carotenoids (Bauernfiend, 1981) and 10,000 as the extinction coefficient for drosopterins (Wilson and Jacobson, 1977).

An average of 956 μg (±246) of tissue was removed from the female dewlap center, 1061 μg (±183) was removed from the female dewlap edge; 7564 μg (±681) of tissue was removed from the male dewlap center, and 4060 μg (±235) was removed from the male dewlap edge.

2.1. Statistical analyses

We tested data for normality using the Shapiro–Wilk test. Xanthophyll and drosopterin concentrations were log-transformed to meet assumptions of parametric statistics (normality and homogeneity of variance), but figures and tables show non-transformed data. A series of one-way ANOVAs was used to assess sex differences in color descriptor variables. Two-way ANOVA was used to investigate the effects of sex, dewlap region, and a sex × dewlap region interaction on pigment concentrations. Because drosopterin concentrations covary with carotenoid concentrations in Trinidadian guppies to maintain a particular hue (*Poecilia reticulata*, see Grether et al. 2005), we used ordinary least squares (OLS) linear regression to assess the relationship between concentrations of drosopterins and xanthophylls in Brown Anoles. Pearson's Product-Moment Correlation was used to assess how drosopterin and xanthophyll pigment concentrations, and the drosopterin-and-xanthophyll ratio, related to tristimulus color scores. We also used OLS linear regression to assess the effect of dewlap area on pigment concentration. When tests were performed as multiple comparisons, the alpha level to reject the null hypothesis was subjected to sequential Bonferroni corrections (Sokal and Rohlf, 1995), to adjust for increased likelihood of Type 1 error. All the results that were statistically significant before Bonferroni correction (i.e. $P<0.05$) but that were rendered not significant after correction for multiple comparisons are described as statistical tendencies. The Bonferroni corrected alpha levels are presented in tables and supplementary information.

3. Results

3.1. Sex differences in dewlap color

The dewlap center, which appears red to the human eye, and edge, which appears pale yellow or white, were spectrally distinct in males and females (Fig. 1A and B). The center of male dewlaps had greater yellow and red wavelength chroma (and lower UV chroma) than females, but lower UV and yellow wavelength brightness than that of females (Table S2). Along the dewlap edge, males had significantly higher yellow chroma and tended to have higher red chroma than females along the dewlap edge (see Table S2). In addition, males tended to have higher yellow and red brightness than females along

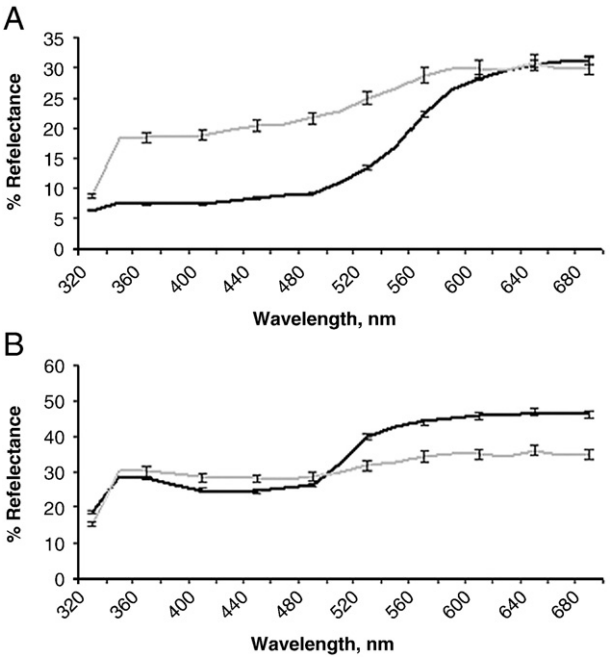

Fig. 1. A) Mean spectral curves (±S.E.) of male and female *N. sagrei* dewlap center regions; B) mean spectral curves (±S.E.) of male and female *N. sagrei* dewlap edges. Gray line represents female spectral curve, black line represents male spectral curve. $N=12$ females, 22 males.

the dewlap edge (see Table S2). Furthermore, males had significantly lower hue than females along the dewlap edge (Table S3). That is, male dewlap edges were yellow whereas female dewlap edges were red.

3.2. Pigment concentration variation and its relationship with sex and dewlap region

Dewlap xanthophyll concentrations did not correlate with drosopterin concentrations in male center or edge dewlap regions (center: $R^2=0.080$, $P=0.200$; edge: $R^2=0.003$, $P=0.829$), or in female center or edge dewlap regions (center: $R^2=0.110$, $P=0.319$; edge: $R^2=0.050$, $P=0.484$).

Xanthophyll concentrations did not differ by dewlap region (Fig. 2A; $F=0.331$, $P=0.564$) or by sex ($F=0.082$, $P=0.776$), and there was no significant interaction between region and sex ($F=0.678$, $P=0.414$; two-way ANOVA, whole model test $F_{3,61}=0.491$, $P=0.690$). Drosopterin concentrations displayed a significant main effect of dewlap region ($F=10.342$, $P=0.002$) and sex ($F=22.458$, $P<0.0001$), but there was also a significant sex-by-dewlap-region interaction (Fig. 2B; $F=6.561$, $P=0.013$; two-way ANOVA, whole model test $F_{3,61}=15.905$, $P<0.0001$). Male dewlap centers were significantly more drosopterin-enriched compared to male dewlap edge regions, and both regions were more drosopterin-enriched in males than in females (Fig. 2B).

3.3. Relationships between pigment concentrations and dewlap colorimetrics

Xanthophyll concentration tended to correlate positively with yellow and red brightness in the male dewlap edge (see Table 1). However, xanthophyll concentration tended to correlate positively only with yellow chroma in the dewlap center of females (Table 1).

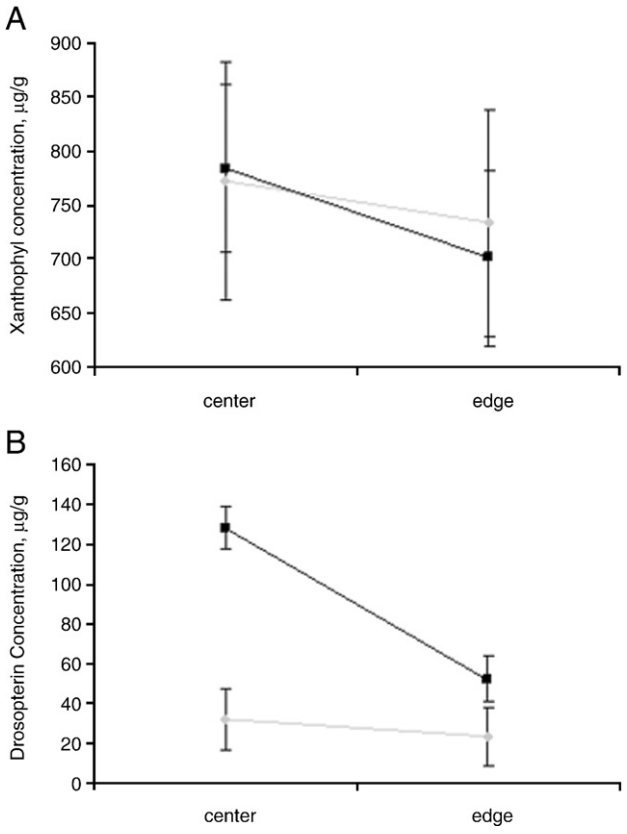

Fig. 2. A) Mean xanthophyll (±S.E.) and B) mean drosopterin (±S.E.) concentrations (µg pigment/g tissue) for males (black line) and females (gray line) by sex and dewlap region in *N. sagrei*. $N=12$ females, 22 males.

Table 1
Pearson's Product-Moment Correlations between dewlap xanthophyll concentration and brightness and chroma in female and male *N. sagrei*.

Sex	Region	Variable	UV	Y	R
F	Center	Brightness	−0.312	−0.102	−0.069
M	Center	Brightness	0.005	0.067	0.010
F	Edge	Brightness	0.252	0.158	0.148
M	Edge	Brightness	0.166	*0.483*	*0.508*
F	Center	Chroma	−0.530	*0.618*	0.552
M	Center	Chroma	−0.143	−0.006	−0.041
F	Edge	Chroma	0.254	−0.171	−0.212
M	Edge	Chroma	−0.187	0.007	0.094

Xanthophyll concentration is expressed as µg pigment/g tissue. Sex refers to female (F) or male (M). Region refers to dewlap region (center or edge). Xanthophyll concentration expressed as µg pigment/g of tissue. Brightness refers to sum of reflectance in each waveband, while chroma refers to the proportion of total reflectance in each waveband (UV = ultraviolet, Y = yellow, and R = red, see text for wavelength ranges). *Italics* = marginally significant values that were non-significant after *Bonferroni* Multiple Comparisons Test. $N=12$ females, 22 males.

Drosopterin concentration tended to correlate inversely with UV and yellow brightness (Table 2), but showed a significant positive correlation with red chroma in the male dewlap center (Table 2). Drosopterin concentration showed a significant inverse correlation with UV chroma, but a significant positive correlation with yellow and red chroma in the female dewlap edge (Table 2).

Xanthophyll concentration showed a significant, inverse relationship with hue in the center of female dewlaps (Table 3). Drosopterin concentration tended to correlate positively with hue in the male dewlap center.

Drosopterin-to-xanthophyll ratio tended to correlate inversely with UV chroma but positively with yellow and red chroma in the females' dewlap edge (Table 4).

4. Discussion

We found that variation in drosopterin concentration appeared to have the strongest contribution to dewlap color in both sexes of Brown Anoles. The red dewlap center in males was enriched with red drosopterins compared to dewlap centers of females, and to dewlap edges of both sexes, and drosopterin concentration and red chroma were correlated in male dewlap centers. The dewlap center of females revealed that drosopterin concentration showed a significant inverse correlation with UV chroma, but a significant positive correlation with yellow and red chroma. Interestingly, carotenoids were also present in both of these regions (and in both sexes) at a concentration 6–8 times higher than drosopterins. However, drosopterins and other pterins have high extinction coefficients (i.e., a measure of how spectrally absorptive a biochemical is) compared to carotenoids, and drosopterins had dominant effects on brightness and chroma when present in high concentrations in male dewlap centers.

Table 2
Pearson's Product-Moment Correlations between dewlap drosopterin concentration and dewlap brightness and chroma in *N. sagrei*.

Sex	Region	Variable	UV	Y	R
F	Center	Brightness	−0.351	−0.312	−0.242
M	Center	Brightness	*−0.459*	*−0.510*	−0.171
F	Edge	Brightness	−0.294	0.058	0.117
M	Edge	Brightness	−0.272	0.108	0.103
F	Center	Chroma	−0.412	0.220	0.342
M	Center	Chroma	−0.375	0.258	0.535*
F	Edge	Chroma	*−0.602*	0.714*	0.703*
M	Edge	Chroma	−0.424	0.352	0.275

Drosopterin concentration expressed as µg pigment/g of tissue. * = significance at 95% ($P<0.0167$) after *Bonferroni* correction. *Italics* = marginally significant values that were non-significant after Bonferroni Multiple Comparisons Test. All table headings are defined in Table 1. $N=12$ females, 22 males.

Table 3
Pearson's Product-Moment Correlations between pigment concentrations and concentration ratios with dewlap hue in *N. sagrei*.

Variable	Sex	Dewlap region	Hue
Xanthophyll concentration	F	Center	−0.683*
		Edge	−0.133
	M	Center	−0.166
		Edge	0.280
Drosopterin concentration	F	Center	0.319
		Edge	−0.292
	M	Center	*0.451*
		Edge	0.428
Xanthophyll:Drosopterin ratio	F	Center	0.350
		Edge	−0.297
	M	Center	0.172
		Edge	0.159

Concentration ratio refers to drosopterin:xanthophyll concentration ratio. Long-wavelength hue is the maximum positive slope in the spectral curve from 401 to 700 nm. * = significance at 95% ($P<0.05$). *Italics* = marginally significant values that were non-significant after Bonferroni Multiple Comparisons Test. All other table headings are defined in Table 1. $N=12$ females, 22 males.

As expected, carotenoids tended to correlate to color only in the dewlap regions where drosopterins were dilute (e.g. in yellow areas). For example, in males, xanthophyll concentration tended to correlate with yellow brightness along the dewlap edge. In females, xanthophyll concentration tended to correlate with yellow chroma in the lightly-colored female dewlap centers (also where drosopterin concentrations were relatively dilute). These results suggest that pigment ratios are important for considering the color contributions of particular pigments. Surprisingly, however, drosopterin-to-xanthophyll concentration ratios showed a tendency to influence UV, yellow and red chroma in the edge of female dewlaps only.

Grether et al. (2001) also considered how drosopterin-to-carotenoid ratios vary in color patches of male Trinidadian guppies (*P. reticulata*). In *P. reticulata*, drosopterin concentration correlates positively with carotenoid (tunaxanthin) availability, and the pterin-to-carotenoid ratio is responsible for a particular orange hue that is maintained through several streams via countergradient sexual selection, where genetic variation in drosopterin compensates for environmental variation in carotenoids (Grether et al., 2005). This hue in the color patches of males that is consistent across streams is probably maintained via female choice in *P. reticulata*, because females prefer males with more chromatic orange carotenoid-containing spots (Houde, 1987; Kodric-Brown, 1989, 1993). In *N. sagrei*, however, we found that pterin (i.e. drosopterin) concentrations did not correlate with carotenoid (i.e., xanthophyll) concentrations in either sex or dewlap region. Additionally, the ratio of pterin concentration to carotenoid concentration did not correlate with hue in male dewlaps. Finally, there is no known selective mechanism influencing dewlap hue in Brown Anoles, but this is an area of research that requires further study.

The inability of pigment concentrations to account for all spectral variation, especially brightness measurements, implies that integumentary (scale) nanostructure may differ by sex and dewlap region, and interacts with pigments in a way that influences color generation. In American Goldfinches, xanthophylls interact with feather structure to produce yellow spectral variation (Shawkey and Hill, 2005; Shawkey et al., 2006). In Eastern Bluebirds (*Sialia sialis*), sex-specific differences in UV are the result of sexual differences in bird feather nanostructure (e.g., surface area of feather barb cortices, distance between keratin rods and air spaces, and thickness of the feather barbs' spongy layer, Shawkey et al., 2006). White background, structural reflectance of feathers does not appear to be a major source of color variation in American Goldfinches (Shawkey et al., 2006), but in the context of color production structural properties have never been considered quantitatively alongside of pigment types/concentration in any lizard coloration system.

Table 4
Pearson's Product-Moment Correlations between xanthophyll-to-drosopterin concentration ratios and UV, yellow and red brightness and chroma, arranged by sex and by dewlap region (center and edge) in *N. sagrei*.

Sex	Region	Variable	UV	Y	R
F	Center	Brightness	−0.343	−0.379	−0.335
M	Center	Brightness	−0.178	−0.371	−0.186
F	Edge	Brightness	−0.299	0.065	0.114
M	Edge	Brightness	−0.049	0.081	0.046
F	Center	Chroma	−0.306	0.162	0.252
M	Center	Chroma	−0.040	−0.001	0.217
F	Edge	Chroma	*−0.607*	*0.710*	*0.668*
M	Edge	Chroma	−0.167	0.232	0.150

Concentration ratio refers to drosopterin:xanthophyll concentration ratio. All other table headings are defined in Table 1. *Italics* = marginally significant values that were non-significant after Bonferroni Multiple Comparisons Test. $N=12$ females, 22 males.

In fact, there exist only a few investigations that qualitatively describe how aspects of the integument (i.e., scales and the underlying dermis) contribute to coloration in reptiles. In ventral patches of male *Urosaurus ornatus*, transmission electron microscopy revealed that changes in spacing and osmolarity of iridophores (shiny reflective platelets found in reptile integument, Bagnara and Hadley, 1973) were responsible for changes in color from amber and light green color to rich blue (Morrison et al., 1996). In facial patches of three Sceloporine lizards (*Sceloporus jarrovi*, *S. magister*, and *S. undulatus erythrocheilus*) iridophores were present in white, blue and green patches, and presumably interacted with pterin-based chromatophores (contain drosopterin, xanthopterin, sepiapterin) to reflect yellow, in ventral scales from color patches of lizards (Morrison, 1995). An additional study on *S. undulatus erythrocheilus* (Morrison et al., 1995) revealed that iridophores likely interact with chromatophores to reflect orange in both yellow and orange morphs. While these studies did not use spectrometry to investigate the role of iridophore abundance or spacing on full wavelength spectral variation, it is clear from these studies that iridophores interact with chromatophores in complex ways in these lizards. Future research should look for the presence of iridophores, and use full wavelength spectrometry to investigate how iridophores interact with chromatophores to produce spectral variation in the skin of different animals.

The effects of xanthophyll concentrations on dewlap chroma and brightness in our study differ considerably from comparable work on plumage pigmentation in birds. In male Greenfinches and American Goldfinches, which use xanthophylls (but not drosopterins) to color feathers, xanthophyll concentrations have been shown to positively correlate with feather chroma, but not brightness (Saks et al., 2003; McGraw and Gregory, 2004). Our data show that xanthophyll concentrations correlate positively with yellow brightness along the edge of male dewlaps, but with yellow chroma and hue in the edge of female dewlaps. One possible explanation for the difference in correlation between xanthophyll concentration and spectral variation of Greenfinches, Goldfinches and Brown Anoles is that the difference is due to the presence of a dilute concentration of drosopterins in scales of the dewlap edge, which influence aspects of yellow spectral reflectance. Melanin is also present in small amounts in some carotenoid-colored bird feathers (McGraw et al., 2005a,b) and lizard dewlaps (Macedonia et al., 2000), and its light-absorbing effects should be considered in future studies. Of course, another possibility is that feather microstructure simply interacts with pigments and lightwaves differently than scale microstructure, though this possibility has not been studied.

Our results demonstrate that males devote more pigments to the dewlap than females. Given that males had larger dewlaps than females, male Brown Anoles have greater quantities of drosopterins and xanthophylls in their dewlaps compared to females (Steffen, unpubl. data). Sex differences in drosopterin concentration may represent developmental differences in the conversion of purines to drosopterins, and may reflect a need for males to have dewlaps that are more visible than those of females, especially because the red color provided by drosopterins in lizards markedly contrasts with

green backgrounds that are common in the lizards' environs (Endler, 1992). The sex differences in xanthophyll quantity, but not concentration, suggest that males simply have larger dewlaps and deposit more pigment than females to color it.

Finally, we speculate that the UV absorbing dewlap center of males (i.e. the red region) amplifies the appearance of the UV reflecting dewlap edge (the pale yellow region) via heightened spectral contrast between the two regions, and makes the dewlap edge region highly conspicuous to conspecific males and females. This idea was initially proposed by Fleishman et al. (1993), but our data describe a pigment-based mechanism that produces these spectral differences. The degree that the two dewlap regions contrasts differs in males and females, and this may relate to the relative need of males to have their dewlap seen, compared to females, in Brown Anoles.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cbpb.2009.07.009.

References

Andersson, M., 1994. Sexual Selection. Princeton University Press, New Jersey.
Andersson, S., Prager, M., 2006. Quantifying colors. In: Hill, G.E., McGraw, K.J. (Eds.), Bird Coloration I: Mechanisms and Measurements. Harvard University Press, Cambridge, pp. 41–89.
Auber, L., 1957. The distribution of structural colours and unusual pigments in the class Aves. Ibis 99, 463–476.
Bagnara, J.T., Hadley, M.E., 1973. Chromatophores and Colour Change, the Comparative Physiology of Pigmentation. Prentice Hall, Englewood Cliffs.
Bauernfiend, J.C., 1981. Carotenoids as Colorants and Vitamin A Precursors. Academic press, London.
CIAS Image Analysis, version 2.0., 1995. Jandel Scientific, CID, Inc. Vancouver, WA.
Clotfelter, E., Ardia, D., McGraw, K.J., 2000. Red fish, blue fish: trade-offs between pigmentation and immunity in *Betta splendens*. Behav. Ecol. 18, 1139–1145.
Conant, R., Collins, J.T., 1998. A Field Guide to Reptiles and Amphibians of Eastern and Central North America. Houghton Mifflin Company, Boston.
Cooper Jr., W.E., Greenberg, N., 1992. Reptilian Coloration and Behavior. University of Chicago Press, Chicago, Ill.
Crews, D., 1979. The hormonal control of behavior in a lizard. Sci. Am. 241, 242–249.
Crews, D., Williams, E.E., 1977. Hormones, reproductive behavior, and speciation. Am. Zool. 17, 271–286.
Dyck, J., 1971a. Structure and spectral reflectance of green and blue feathers of the Lovebird (*Agapornis roseicollis*). Biol. Skrift. 18, 1–67.
Dyck, J., 1971b. Structure and colour production of the blue barbs of *Agapornis roseicollis* and *Cotinga maynana*. Zeitschr. Zellforsch. 115, 17–29.
Endler, J.A., 1990. On the measurement and classification of colour in studies of animal colour patterns. Biol. J. Linn. Soc. 41, 315–352.
Endler, J.A., 1992. Signals, signal conditions, and the direction of evolution. Am. Nat. 139, 1–27.
Fleishman, L.J., Lowe, E.R., Leal, M., 1993. Ultraviolet vision in lizards. Nature 365, 397.
Fleishman, L.J., Bowman, M., Saunders, D., Miller, W.E., Rury, M.J., Loew, E.R., 1997. The visual ecology of Puerto Rican anoline lizards: habitat light and spectral sensitivity. J. Comp. Physiol. A 181, 446–460.
Fox, D.L., 1976. Animal Bichromes and Structural Colours. University of California Press, Berkeley, CA.
Ghiradella, H., Aneshansley, D., Eisner, T., Silberglied, R.E., Hinton, H.E., 1972. Ultraviolet reflection of a male butterfly: Interference color caused by thin-layer elaboration of wing scales. Science 178, 1214–1217.
Goodwin, T.W., 1984. The Biochemistry of Carotenoids. Chapman and Hall, London, U.K.
Grether, G.F., Hudon, J., Endler, J.A., 2001. Carotenoid scarcity, synthetic pteridine pigments and the evolution of sexual coloration in guppies (*Poecilia reticulata*). Proc. R. Soc. Lond. Ser. B-Bio. 268, 1245–1253.
Grether, G.F., Cummings, M.E., Hudon, J., 2005. Countergradient variation in the sexual coloration of Guppies (*Poecilia reticulata*): drosopterin synthesis balances carotenoid availability. Evolution 59, 175–188.
Hill, G.E., 1998. An easy, inexpensive means to quantify plumage coloration. J. Field. Ornitol. 69, 353–363.
Houde, A.E., 1987. Mate choice based upon naturally occurring color-pattern variation in a guppy population. Evolution 41, 1–10.
Inouye, C.Y., Hill, G.E., Stradi, R.D., Montgomerie, R., 2001. Carotenoid pigments in male house finch plumage in relation to age, subspecies, and ornamental coloration. Auk 118, 900–915.
Jenssen, T.A., 1977. Evolution of Anoline lizard display behavior. Am. Zool. 17, 203–215.
Kodric-Brown, A., 1989. Dietary carotenoids and male mating success in the guppy: an environmental component to female choice. Behav. Ecol. Sociobiol. 25, 393–401.
Kodric-Brown, A., 1993. Female choice of multiple male criteria in guppies: interacting effect of dominance, coloration and courtship. Behav. Ecol. Sociobiol. 32, 415–420.
Leal, M., 1999. Honest signalling during prey-predator interactions in the lizard *Anolis cristatellus*. Anim. Behav. 58, 521–526.
Leal, M.A., Rodriguez-Robles, J.A., 1997a. Signalling displays during predator-prey interactions in a Puerto Rican anole, *Anolis cristatellus*. Anim. Behav. 54, 1147–1154.
Leal, M., Rodriguez-Robles, J.A., 1997b. Antipredator responses to the Puerto Rican giant anole, *Anolis cuvieri* (Squamata: Polychrotidae). Biotropica 29, 372–375.
Leal, M., Fleishman, L.J., 2002. Evidence for habitat partitioning based on adaptation to environmental light in a pair of sympatric lizard species. Proc. R. Soc. Lond. Ser. B 269, 351–359.
Leal, M., Fleishman, L.J., 2004. Differences in visual signal design and detectability between allopatric populations of *Anolis* lizards. Am. Nat. 163, 26–39.
Lessels, C.M., Boag, P.T., 1987. Unrepeatable repeatabilities: a common mistake. Auk 104, 116–121.
Losos, J.B., deQueiroz, K., 1997. Evolutionary consequences of ecological release in Caribbean *Anolis* lizards. Biol. J. Linn. Soc. 61, 459–483.
Macedonia, J.M., James, S., Wittle, L.W., Clark, D.L., 2000. Skin pigments and coloration in the Jamaican radiation of *Anolis* lizards. J. Herpetol. 34, 99–109.
Macedonia, J.M., Brandt, Y., Clark, D.L., 2003. Sexual dichromatism and differential conspicuousness in two populations of the common collared lizard (*Crotaphytus collaris*) from Utah and New Mexico, USA. Biol. J. Linn. Soc. 77, 67–85.
McGraw, K.J., 2006. The mechanics of uncommon bird colors: pterins, porphyrins, and psittacofulvins. In: Hill, G.E., McGraw, K.J. (Eds.), Bird Coloration I: Mechanisms and Measurements. Harvard University Press, Cambridge, MA, pp. 354–398.
McGraw, K.J., Gregory, A.J., 2004. Carotenoid pigments in male American Goldfinches: what is the optimal biochemical strategy for becoming colourful? Biol. J. Linn. Soc. 83, 273–280.
McGraw, K.J., Hill, G.E., Stradi, R., Parker, R.S., 2002. The effect of dietary carotenoid access on sexual dichromatism and plumage pigment composition in the American goldfinch. Comp. Biochem. Physiol. B. 131, 261–269.
McGraw, K.J., Hudon, J., Hill, G.H., Parker, R.S., 2005a. A simple and inexpensive chemical test for behavioral ecologists to determine the presence of carotenoid pigments in animal tissues. Behav. Ecol. Sociobiol. 57, 391–397.
McGraw, K.J., Safran, R.J., Wakamatsu, R., 2005b. How feather colour reflects its melanin content. Funct. Ecol. 19, 816–821.
McMann, S., 2000. Effects of residence time on displays during territory establishment in a lizard. Anim. Behav. 59, 513–522.
Montgomerie, R., 2006. Analyzing colors. In: Hill, G.E., McGraw, K.J. (Eds.), Bird Coloration. I. Mechanisms and Measurements. Harvard University Press, Cambridge, Mass, pp. 90–147.
Morrison, R.L., 1995. A Transmission Electron Microscopic (TEM) method for determining structural colors reflected by lizard iridophores. Pigment Cell. Res. 8, 28–36.
Morrison, R.L., Rand, M.S., Frost, S.K., 1995. Cellular basis of color differences in three morphs of the lizard *Sceloporus undulatus erythrocheilus*. Copeia 1995, 397–408.
Morrison, R.L., Sherbrooke, W.C., Frost-Mason, S.K., 1996. Temperature-sensitive, physiologically active iridophores in the lizard *Urosaurus ornatus*: an ultrastructural analysis of color change. Copeia 1996, 804–812.
Nicholson, K.E., Harmon, L.J., Losos, J.B., 2007. Evolution of *Anolis* lizard dewlap diversity. PLOS One 2, 1–12.
Obika, M., Bagnara, J.T., 1964. Pteridines as pigments in amphibians. Science 143, 485–487.
Ortiz, E., Williams-Ashman, H.G., 1963. Identification of skin pteridines in the pasture lizard *Anolis pulchellus*. Comp. Biochem. Physiol. 10, 181–190.
Ortiz, E.E., Maldonado, A.A., 1966. Pteridine accumulation in lizards of the genus *Anolis*. Caribb. J. Sci. 6, 9–13.
Paterson, A.V., McMann, S., 2004. Differential headbob displays toward neighbors and non-neighbors in the territorial lizard *Anolis sagrei*. J. Herpetol. 38, 288–291.
Raila, J., Schuhmacher, A., Gropp, J., Schweigert, F.J., 2002. Selective absorption of carotenoids in the common green iguanas (*Iguana iguana*). Comp. Biochem. Physiol. B. 132, 513–518.
Saks, L., McGraw, K.J., Horak, P., 2003. How feather colour reflects its carotenoid content. Funct. Ecol. 17, 555–561.
Shawkey, M.D., Hill, G.E., 2005. Carotenoids need structural colours to shine. Biol. Lett. 1, 121–124.
Shawkey, M.D., Hill, G.E., McGraw, K.J., Hood, W.R., Huggins, K., 2006. An experimental test of the contributions and condition dependence of microstructure and carotenoids in yellow plumage coloration. Proc. R. Soc. Lond. Ser. B. 273, 2985–2991.
Sokal, R.R., Rohlf, F.J., 1995. Biometry. W.H.Freeman and Company, New York, NY.
Steffen, J.E., McGraw, K.J., 2007. Contributions of pterin and carotenoid pigments to dewlap coloration in two anole species. Comp. Biochem. Physiol. B. 146, 42–46.

Thorpe, R.S., Stevenson, A.G., 2003. Phylogeny, paraphyly and ecological adaptation of the colour and pattern in the *Anolis roquet* complex on Martinique. Mol. Ecol. 12, 117–132.

Tokarz, R.R., 1995. Importance of androgens in male territorial acquisition in the lizard *Anolis sagrei*: an experimental test. Anim. Behav. 49, 661–669.

Tokarz, R.R., 2002. An experimental test of the importance of the dewlap in male mating sucess in the lizard *Anolis sagrei*. Herpetologica 58, 87–94.

Tokarz, R.R., Paterson, A.V., McMann, S., 2003. Laboratory and field test of the functional significance of the male's dewlap in the lizard *Anolis sagrei*. Copeia 2003, 502–511.

Tokarz, R.R., McMann, S., Seitz, L., Alder, H.J., 1998. Plasma corticosterone and testosterone levels during the annual reproductive cycle of male brown anoles (*Anolis sagrei*). Physiol. Zool. 71, 139–146.

Volker, O., 1938. The dependence of lipochrome-formation in birds on plant carotenoids. Proc. 8th Int. Ornithol. Congress, pp. 425–426.

Wilson, T.G., Jacobson, K.B., 1977. Isolation and characterization of pteridines from heads of *Drosophila melanogaster* by a modified thin-layer chromatography procedure. Biochem. Genet. 15, 307–319.