CAROTENOID-BASED DEWLAP COLOR AS A VISUAL SIGNAL IN SOCIAL COMMUNICATION OF BROWN ANOLES (*Norops sagrei*)

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Date of Graduation

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John Edward Steffen, son of Fred W. Steffen and Marcia A. Haines, was born October 7, 1966, in Cleveland, Ohio. He graduated Brecksville High School in 1985. He attended Ohio University in September 1985, and graduated summa cum laude in May, 1989, with a B.S. in Zoology. After being employed as a technician for several ecology laboratories, as well as working as a teacher, social worker, and musician in Seattle, WA, he entered graduate school at Western Washington University, in Bellingham, WA, on September, 1999. He graduated with a M.S. in Ecology, and then entered Auburn University Graduate School in September, 2001 to pursue a Ph.D. While participating in an Organization for Tropical Studies graduate course in Costa Rica, he met fellow biologist Lindsay Amsberry. He married Lindsay Amsberry on August 7, 2004.

DISSERTATION ABSTRACT

CAROTENOID-BASED DEWLAP COLOR AS A VISUAL SIGNAL IN SOCIAL COMMUNICATION OF BROWN ANOLES (*Norops sagrei*)

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Carotenoids have been shown to be important integumentary coloring agents in many birds and fishes. The role of carotenoids as a prominent integumentary coloring agent of the dewlap has been noted in many Anoline lizards, but the role of carotenoids as a social signal has not been considered. Here, I investigated some proximate causes of male and female dewlap color, and found that males and females differed in pterin pigment concentrations but not in carotenoid concentrations. I also found that sexes differed in UV as well as long wavelength reflectance, and that carotenoid-based UV color correlated positively with body condition. I then used a visually-based color detection model that incorporates knowledge about UV vision to simulate and describe the conspicuousness of dewlap colors as conspecific lizards should see it, under different forest light environments. Two sets of behavior experiments were performed to test assumptions about signal spectral variability, signal honesty and signal use. One set of experiments investigated a potential role for UV (a carotenoid-based dewlap color) to be used as a visual signal in contests for females. I quantified natural dewlap coloration, and paired males into size-matched dyads that naturally differed in UV reflectance. Males that won male-male contests, and copulated with females, had lower UV reflectance than males that lost contests. I then manipulated the UV component in dewlap colors, and I investigated whether the manipulation changed the contest outcome. While manipulations had no effect on contest outcomes, the underlying natural colors still correlated with contest success. A final set of experiments investigated the dual contributions of nutritional stress and carotenoid access on male dewlap color. I found that carotenoid availability altered UV and long wavelength reflectance, and that UV amplitude decreased with nutritional stress.

These results summarily suggest that dewlap color is a signal used to communicate information about the senders' phenotypic quality to the receiver, and that dewlap color (including ultraviolet wavelengths) is highly visible in some light environments. Furthermore ultraviolet aspects of dewlap color among males correlates with contest success. Finally, dewlap color is at least partially influenced by availability of carotenoids during adulthood, and can convey information about an individuals' health.

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Computer software used: Microsoft excel, JMP, SAS, and SPSS statistical packages, Etholog v. 2.1 (event recording software), OOibase spectrometry software, and a Spectral Processing Program copyright Bob Montgomerie 2002.

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CHAPTER 1: INTRODUCTION

Darwin devised the idea of sexual selection to explain traits that seemed to defy explanation by natural selection: some traits seemed to aid in the attraction or defense of mates, at an apparent cost to survival or fecundity of the individual (Darwin, 1871). Ornaments are a well-known class of phenotypic traits that meet the criteria of a sexually selected trait. Ornamental traits are morphological features that are exaggerated and presumably physically constrain an individual by limiting its mobility and increasing its conspicuousness to predators. A well-known example is the tail length of male African long-tailed widow birds (Andersson, 1982). The tail is approximately half a meter long, and presumably hinders a male's flight ability, yet the trait is maintained in the population, despite its reduction of one's survival. Observational data show that territories of males with larger tails contained more nests than territories of shorter tailed males. Experimental manipulations of male tail length in territory holders altered female nest numbers contained in the territories, whereby males with enlarged tails contained more female nests than males with artificially reduced tail lengths.

Ornaments are often very colorful and further advertise the possessors' presence to predators. Carotenoid-based ornament color has been of interest to biologists because it reveals aspects of an individuals' health, and thus acts as an honest visual signal in intraspecific communication. Carotenoids reveal aspects of an individuals health or 'condition' because a) they cannot be synthesized de novo and must be ingested through a dietary source, b) may be limiting in the environment, and c) their expression in the integument is influenced by physiological state (Hill, 2002; Lozano, 1994b; Hill, 1992). Carotenoid-based color signals have been studied in many birds and fish, especially noteworthy are research on house finches (Hill, 2002), goldfinches (McGraw et al., 2001; McGraw, 2004; McGraw and Hill, 2001), guppies (Kodric-Brown, 1984; Kodric-Brown, 1989; Brooks, 1995; Kodric-Brown, 1985), and sticklebacks (Bakker, 1993; Baube, 1995; Frischknecht, 1993). Carotenoid color signals have not been studied extensively in any reptile.

Anoles are a group of lizards inhabiting tropical and subtropical habitats, and are the most species-rich genus of lizard in the world (Jackman et al., 1999; Nicholson, 2002). In many tropical habitats, it is common to see several species co-occurring in sympatry, and a great deal of research shows that sympatric species partition the habitat according to perch location and light in the habitat. In fact, Anoles are a model organism that helped to define the concept of an ‘ecomorph’ (Williams, 1983); that is, species of different phyletic origin with similar morphological adaptations to similar niches. In addition, in part because there are so many species and they seem to partition available habitats in relatively consistent ways, anoles have become a classic example of a group undergoing adaptive radiation (Schluter, 2000).

Anoles possess a mating system that can be described as a resource defense polygyny. Males are highly territorial, and appear to aggregate in and defend areas where there are females present, while females appear to aggregate around areas of high food availability. In most species, males and females maintain home ranges, female home ranges tend to be smaller than males home ranges, and are encompassed by one or more male territories. Anole species vary widely in sexual size dimorphism, but large body size is a major determinant of male courtship success in social contests (Tokarz, 1985). Females of most species lay 1 egg every 10 -14 days, and reproductive activity can occur seasonally or year round.

Anoles are also well known because males possess an extendable throat fan known as a ‘dewlap’. These dewlaps are extended conspicuously as a social signal to advertise one’s presence to males in male-male territorial contests – which may help establish dominance in male-male rivalry interactions, and to females in courtship interactions – and potentially used by females to aid in mate choice decisions. Furthermore, females possess a dewlap in some species, which is used in social interactions. Little is known about the communicative role of female dewlaps.

The dewlaps of male anoles are often colored so that extended dewlaps contrast maximally with the surroundings, and dewlaps can contain up to 3 human-visible ‘colors’ in one individual. Differences in dewlap color among sympatric species have been shown to communicate information about species identity (Losos, 1985), and presumably serves to reduce courtship between sympatric species (Williams and Rand, 1977). Recent research that tests predictions of dewlap color derived from the species recognition and ecomorph convergence hypotheses, has concluded that much of the dewlap color in Caribbean anoles is not adequately explained by these hypotheses (Nicholson et al., 2007).

Many different species of anoles who have had their dewlaps measured with a UV-visible spectrometer have been shown to have strong components of UV reflectance to the dewlap spectral variation (Fleishman et al., 1993). These dewlaps have also shown that dewlap color (including UV) varies with respect to light environment, and their variation in color appears to maximize dewlap detectability in some environments and not in others (Fleishman et al., 1997; Leal and Fleishman, 2004).

Recent analyses of dewlap pigments show xanthophyll carotenoids to be present in the dewlap of several caribbean anoles (Macedonia et al., 2000; Steffen and McGraw, 2007), including the Brown Anole, Norops sagrei (Steffen and McGraw, 2007). The Brown Anole is a successful invader that can occur at extremely high population densities. Brown anoles are seen to occupy a variety of habitats in areas that represent its current geographic range, and presumably communicate with conspecifics in a variety of habitats and light conditions. Brown anoles live for 1-2 years. Adult males tend to be larger than adult females.

In this dissertation, I investigate the role of carotenoid-based dewlap color as a social signal in the Brown Anole, and I rely on a few anatomical terms to describe the different colored regions of the dewlap in both males and females (males, see Figure 1a and b; females, see Figure 1 c). The lateral dewlap region is the area exposed when the dewlap is extended (perceived as red by the human eye). This region is lateral to the midline of the body, which is the plane of dewlap extension. The midline dewlap region is the anterior margin when extended (perceived as white or yellow by human eyes; (Conant and Collins, 1998). Because no anatomically-based nomenclature has been offered to describe these regions, I suggest that future authors consider this terminology.

In this dissertation, I present four chapters that examine different facets of the Brown Anole visual signal ecology. In Chapter Two, I quantify pigments in, and measure color of male and female dewlaps, in order to understand how pigments are dispersed throughout male and female dewlaps and how the dewlap colors reflect the pigment content. Furthermore, I investigate the potential for dewlap color to act as a condition dependent visual signal. Males and females were found to differ in dewlap coloration, as well as in concentrations of the pterin pigment which is responsible for red dewlap coloration. Sexes did not differ in carotenoid concentrations. In males, UV color correlates with body condition, and provides support for dewlap color acting as a condition-dependent signal.

In Chapter Three, I use an anole-based visual physiology model to predict how conspicuous dewlap colors would appear to conspecifics in common light environments, as well as against different display backgrounds. This model takes into account that Anoline lizards have tetra chromatic vision (i.e., color vision is the result of four different cones, including one that is especially sensitive to ultraviolet radiance). Dewlap color differs between the sexes, and is highly conspicuous in woodland shade, which presumably represents pre-invasion light environments, and may also represent the type of light available in some suburban environments.

In Chapter Four, I perform social experiments in which two males compete for access to a female in the lab and I determine if natural dewlap color correlates with intrasexual contest dominance and intersexual copulation success. Based on findings from these experiments, I then performed a manipulation of UV dewlap color in one of the dyadic males, to determine if an experimental switch in UV reflectance altered dominance and copulation success among the two males. This research partially supports a role for the use of UV as a badge of status between males engaged in male-male rivalry.

In Chapter Five, I perform a two factor experiment in which I manipulate carotenoid access and food provisioning rate, to determine if adult male dewlap color is dependent on carotenoid access, as well as nutritional condition. This research suggests that the UV and yellow expression of Brown Anole dewlaps is dependent on carotenoid availability, and that UV also can be affected by nutritional stress.

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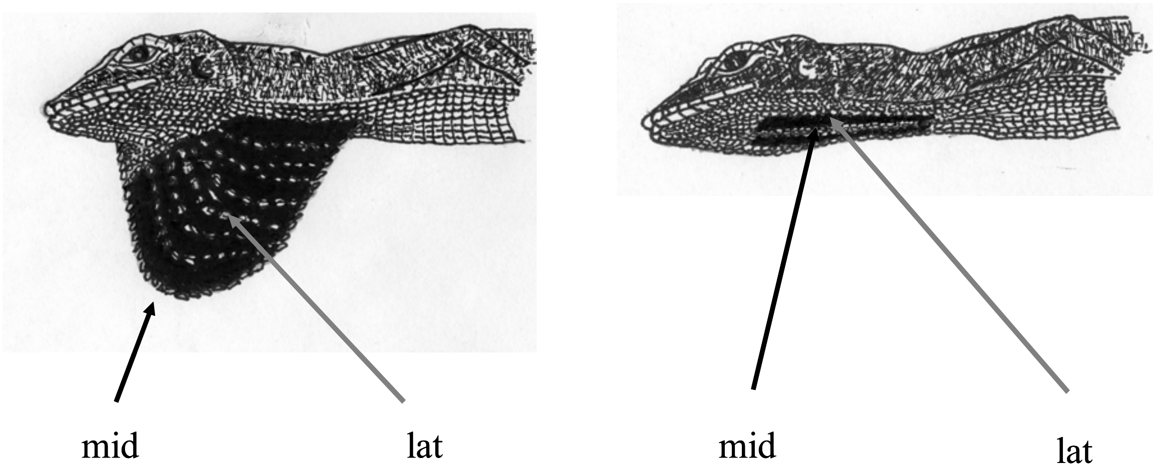

Figure 1 a & b. The dewlap regions of a male Brown anole, *Norops sagrei*. ‘mid’ refers to the midline dewlap region (i.e. dewlap edge), and ‘lat’ refers to the lateral dewlap region (i.e. dewlap side, when extended). Note: in males, the dewlaps’ midline region appears yellow or white to the human eye, but emits strong UV reflectance, and is visible when the dewlap is extended or retracted. The dewlaps’ lateral region appears red and/or yellow to the human eye, does not emit UV strongly, is relatively inconspicuous when the dewlap is retracted, and is highly visible during times when the dewlap is extended only. Drawings by J.E.Steffen

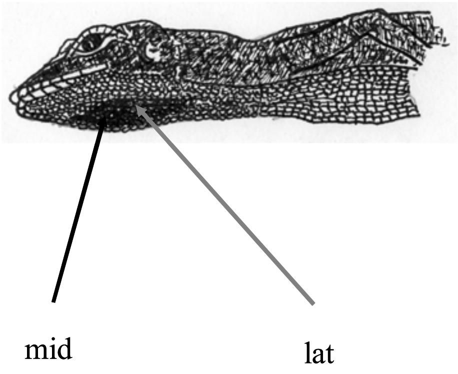

Figure 1 c. The dewlap regions of a female Brown anole, *Norops sagrei*. ‘mid’ refers to the midline dewlap region (i.e. dewlap edge), and ‘lat’ refers to the lateral dewlap region (i.e. dewlap side, when extended). Note: in females, the dewlap is reduced to a small patch of color on the throat, and which can only extend to a small degree, because of an underdeveloped (‘feminized’) hyoid apparatus. In female Norops sagrei, there is less pigmented skin in the dewlap region but more scalation, and consequently there is less color observable to the human eye. However, the midline dewlap region is white to the human eye, but emits strong UV reflectance. The lateral region of the female dewlap appears light red or white to the human eye (because of the overlying scales), and also emits UV strongly. Drawing by J.E. Steffen.

CHAPTER 2: DEWLAP SIZE AND COLORATION IN RELATION TO SEX AND PIGMENT CONTENT IN THE BROWN ANOLE, *Norops sagrei*

Abstract

Sexual selection has led to a diversity of colorful displays in animals, and the pigments responsible for sexual coloration often belong to several pigment classes. These pigment classes differ in important mechanistic and functional ways, and understanding the identity and distribution of pigments in male and female integuments can lend great insights into their roles as visual signals. We examined full-spectrum color variation as well as pterin and carotenoid pigment concentrations in the sexually dichromatic dewlaps of male and female Brown Anoles (*Norops sagrei*). To the human eye, male dewlaps are red and yellow in color, and are large and extendable, whereas female dewlaps are light red or pink, and are non-extendable. UV-VIS reflectance spectrometry revealed UV to be a major and variable component of dewlap color in *N. sagrei*. Female dewlaps had greater UV reflectance than males, but males had greater long-wavelength reflectance than females. Absorbance spectrophotometry identified the pterins bound in dewlap tissue as drosopterin, and carotenoids as lutein or zeaxanthin. Male dewlaps had greater pterin concentrations, but not carotenoid concentrations, than female dewlaps. Total pterin and carotenoid concentrations significantly increased brightness of the lateral dewlap region of males, and high carotenoid concentrations significantly increased brightness of midline dewlap regions. Pterin concentrations increased chroma of female dewlaps. Carotenoid concentrations, but not pterin concentrations, correlated significantly and positively with dewlap size in males. UV reflectance from the midline dewlap region in males correlated positively with a body condition index. These results show that sexes differ in the ways that pigment classes influence dewlap spectral variation, and we speculate that these differences relate to differences in dewlap use as a visual signal.

Introduction

Colorful ornaments are a well-known class of conspicuous secondary sex characters in animals. Sexual dichromatism, or difference in color between the sexes, is one of the most common ways that ornaments vary. Sexual dichromatism, especially in lizards, is thought to be a visual signal that is used for sex recognition (Cooper and Greenberg, 1992; Macedonia et al., 2003; Andersson, 1994), and in most cases males are the more colorful sex. Aspects of color can vary within a sex as well, and have been shown to advertise information about phenotypic quality of an individual in a number of species (Kodric-Brown and Brown, 1984; Andersson 1994).

The colors of animal integuments are caused by the interaction of pigment molecules with their integumentary structures. Structural colors refer to those in which properties of the integument act alone, or in concert with melanin pigments, to produce white, iridescent, blue, and UV colors. This type of coloration 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 vertebrate colors, however, are produced by the interaction of non-melanin pigment molecules with their integument structure. Among several reptiles studied, non-melanin-based pigments are contained in specialized integumentary cells called chromatophores. Xanthophores and erythrophores are chromatophores that include red or yellow light-filtering pigments, respectively, and these pigments include fat-soluble carotenoids, obtained in the diet, and pteridines, produced during purine synthesis (Obika and Bagnara, 1964; McGraw, 2006). Animals that possess sexual colors obtained by both of these pigment types are useful in proximate studies of integumentary color because they offer the opportunity to understand how different pigment classes interact to produce color.

Dewlaps are colorful throat patches that are displayed to conspecifics in many families of lizards. In most anoles (Family Polychrotidae), male dewlaps are large, extendable, and conspicuously colored compared to those of females. Pteridines (hereafter pterins, the most common pteridine in squamates) have been identified as a coloring agent in the dewlaps of male Puerto Rican anoles (e.g. Ortiz and Williams-Ashman, 1963; Ortiz and Maldanado, 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 (Macedonia et al., 2000).

In conjunction with head-bobs and push-ups, dewlaps are displayed to other males during territorial contests (Jenssen, 1977; Leal and Rodriguez-Robles, 1997; Leal, 1999; McMann, 2000; Paterson and McMann, 2004; Tokarz, 2003; Tokarz et al., 2002), to females in courtship interactions (Jenssen 1977; Tokarz 2002; Tokarz et al., 2003) and to predators during an attack (Leal and Rodriguez-Robles, 1997; Leal, 1999). Male dewlap color is purported to serve a role in species recognition (Losos, 1985), and may be under the influence of sensory drive (Leal and Fleishman, 2002, 2004). Some speculate a role of male dewlap color in mate choice (Greenberg and Noble, 1944; Sigmund, 1983) but results of these studies are equivocal. Females also have colored throat patches in many anole species, but they appear to be drab to the human eye, and are small and non-extendable. Females of many anoles also perform head bobs and push-ups, but few studies (Jenssen et al., 2000) consider the source or function of color in female dewlaps.

Steffen and McGraw (2007) previously quantified pterin and carotenoid concentrations in two Norops lizards [i.e. beta-Anolis, *sensu* Williams (Williams, 1976 a; Williams, 1976 b)] with red and yellow dewlaps. Although the sample sizes were small, the two species, *N. sagrei* and *N. humilis*, appeared to differ in the distributions of and relationships between carotenoids and pterins in different regions of the dewlap, despite the apparent similarities in color pattern. This led me to ask whether the two pigment classes might interact in interesting ways in each of the two species to produce the observed colors. Unfortunately, in this previous study, full-spectrum spectrometry was not performed and how pigment types or amounts relate to dewlap color could not be determined. To date, no study exists that directly relates pigment concentrations to dewlap spectral reflectance; such relationships have only been conducted in bird feathers (Saks et al., 2003; McGraw and Gregory 2004; McGraw et al., 2006).

I studied pigment and color variation in dewlaps of male and female Brown Anoles (*Norops sagrei*). Brown Anoles are native to Cuba, the Bahamas, and related islands (Schwartz and Henderson, 1991) but are successful invaders of the southeastern United States (Lee, 1992; Means, 1990; Lee, 1985; Echternacht et al., 1995), as well as Hawaii (McKeown, 1996; Goldberg and Bursey, 2000). Throughout their range, Brown Anoles occupy and display in a wide variety of habitats that range from forests (Paterson, 2002; McMann and Paterson, 2003) to disturbed environments such as commercial buildings and houses (Echternacht et al., 1995). Here I describe how pigment concentrations influence hue, chroma, and brightness in male and female lizards using two common methods for summarizing color data: Principal Components Analysis (PCA) and Tristimulus Scoring. I investigate chromatic and achromatic intersexual differences in dewlap coloration, including UV, and quantify the extent to which these color properties have a pigmentary basis and are condition-dependent.

Methods

Adult male and female Brown Anoles were obtained from a pet store (Glades Herp, Bushnell, Lee County, Florida), and shipped to Auburn, AL after one day in captivity. Lizards were identified as adult males if they possessed a large (minimum 50 mm^2) extendable dewlap, or as adult female if they lacked one. Lizards were housed in screen-topped, 37.90 liter (i.e. 10-gallon), glass terraria, and each individual terrarium was partitioned into 4 separate compartments. Each lizard was placed in a separate compartment which contained a perch and a water dish on a sandy substrate. Lighting strips containing full-spectrum fluorescent bulbs (Vitalite T8, 32 watt) were suspended 30.48 cm (i.e., 12 inches) 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 repta-vite (Zoo Med laboratories, San Luis Obispo, CA). Dewlap colors and size were quantified 1 day after lizards arrived in Auburn, AL (see below for methods). Lizards were then held in captivity for three weeks for use in behavioral experiments. After these experiments, I measured snout-to-vent length (SVL; nearest mm) of male and female lizards and body mass (nearest 0.01 g). Animals were then sacrificed for dewlap removal and pigment quantification. Estimates of body condition index (BCI) were obtained from residuals generated by regressing body mass on SVL. This is a common index of body condition in the herpetological literature (Brandt et al., 2003; Whiting et al, 2005; Kotiaho, 1999; Jakob, 1996; LeBas and Marshall, 2001) and regressions of mass on SVL for males and females were statistically significant (males: $R^2 = 0.789$, $P < 0.0001$, N = 20; females: $R^2 = 0.805$, $P = 0.0002$, N = 11).

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, 2000) that converted pixel size to metric size from the ruler increments present in the digital photo. Each digital image of a male was measured twice then averaged, and the average of the two images was used as dewlap area (i.e., four measurements per individual). Dewlap area was highly repeatable using this method ($R^2 = 0.953$, $P < 0.001$, N = 20). Dewlap area was not calculated for females because small dewlap size in this sex prevented reliable dewlap extension.

We measured dewlap coloration of living male and female lizards using an Ocean Optics S2000 spectrometer (range 250-880 nm: Dunedin, Florida), with 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 any tissue that was measured.

Following Steffen and McGraw (2007), we considered two regions of the dewlap in both males and females. The lateral dewlap region was the area exposed when a dewlap was extended (perceived as red by the human eye). The midline dewlap region was the anterior dewlap margin when extended (perceived as white or yellow by human eyes (Conant, 1998).

In each dewlap region, we took three non-overlapping measurements from males and two from females. We took fewer measurements of females because the dewlap area was only large enough to measure two unique locations. Color data were gathered as percent reflectance per wavelength (nm) of light and this output was processed using ColoR v. 1.5 software (R. Montgomerie copyright 2002). We generated a spectral reflectance curve for every measurement, and determined mean reflectance curves for the two dewlap regions in each individual lizard.

We described color from PCA. Each reflectance curve comprised 382 data points (the reflectance intervals from 320-700nm at 1-nm intervals). We reduced the curves to 19 data points per dewlap region per sex by determining the means of each 20-nm spectral increment (Cuthill et al., 1999). We then used SPSS to perform PCA (Grill, 2000; Macedonia, 2001) on the 20-nm bandwidth means of each dewlap region for each sex. PC coefficients (factor loadings) were used to transform the original variables into PC scores that represented how an individual varied in some spectral parameter, relative to the principal components derived by all the individuals analyzed together.

The main limitation to PCA is that the nature of the principal components (i.e., the transformation that relates PC scores to the original data) is dependent on the data included in the original analysis (Cuthill et al., 1999). That is, unless we perform a PCA that includes data from both dewlap regions for both sexes, we cannot compare sexes or dewlap regions from data analyzed for each dewlap region or sex separately. Thus, to overcome this limitation, we performed 6 PCA's on dewlap color data, to allow for different statistical comparisons. We performed two PCA's, one on male and female lateral dewlap region spectral variation, and a second PCA on male and female midline dewlap region spectral variation, to investigate region-specific sex differences in PC scores. We graph PC coefficients from this analysis in the results section (see below for rationale), and we use this analysis as the basis for comparing PC scores by sex within dewlap region. We then performed four separate PCA's, one on each specific dewlap region of each sex, to precisely describe spectral variation in each dewlap region of each sex, without the influence of different regions or sexes. The PC scores generated from these analyses were used in multiple regressions (see below) to understand how pterins and carotenoids contributed to PC1 and PC2.

To interpret the spectral significance of the first two PC's, we followed two methods. In one, outlined in Cuthill et al. (1999), we graphed PC coefficients across each 20-nm wavelength increment. Inspection of spectral patterns in this way described behavior of known variables in a data set, and can be used to infer biological significance of principal components (Cuthill et al. 1999; Cuthill 2000). If coefficients relating PC1 to wavelength were positive and of similar magnitude, then they represented variation in mean reflectance (Cuthill et al., 1999). This describes reflectance variation along a black-gray-white axis. If coefficients relating PC2 to wavelength had positive values associated with long wavelengths, and negative values associated with medium and short wavelengths, then PC2 represented relative contributions of short wavelengths of light to medium and high wavelengths of light. This describes reflectance variation in the UV and blue wavelengths relative to variation in the green and red wavelengths. If a plot of PC2 coefficients versus wavelength showed rounded peaks and troughs, then PC2 represented chromatic variation in addition to reflectance. If a plot of PC coefficients versus wavelength showed multiple adjacent peaks that were all positive or all negative, then PC2 would represent hue (associated with the wavelength of each peak) as well as brightness and chroma.

I described color using a second method, referred to as ‘tri-stimulus scores’, which describe spectral variation along three non-independent axes (hue, chroma, and brightness), but are standard ways of describing color in the biological literature (Hill and McGraw, 2006). For these scores, hue, the everyday meaning of color, was defined to be the location of the maximum reflectance in a spectral curve. Chroma described the relative peak height (saturation) of a spectral curve at a given wavelength or bandwidth. Brightness described differences in percent reflectance (i.e. amplitude or overall intensity) of a curve. Areas of a curve that were peaked in shape were assumed to have higher chroma (more area under the curve) than areas that were flat, assuming the minimum reflectance within the bandwidth was the same.

Dewlap tissue was removed from lizards as described by Macedonia et al., (2000) and Steffen and McGraw (2007). Concentrations of carotenoid and pterin pigments were measured in each tissue region using the methods described in Steffen and McGraw (2007).

Statistical analyses

Normality was tested using the Shapiro-Wilk test. Carotenoid and pterin concentrations were log-transformed to meet assumptions of parametric statistics (normality and homogeneity of variance), but non-transformed data are displayed in the figures and tables. We used a two-way ANOVA to investigate the effects of sex and dewlap region on pigment concentrations. 4 separate one-way ANOVA's were used to compare PC's by sex and dewlap region. Linear regression was used to investigate the relationship of pterin concentration to carotenoid concentration in each sex and dewlap region separately. We used backward step-wise multiple regression in two ways. In one set of regression models we assessed the relative contributions of tristimulus scores to PC1 and PC2 scores. In a second set of regression models we assessed the relative contributions of pterin and carotenoid pigment concentrations (independent variables) to PC1 and PC2 scores (dependent variables). In all of these regression models, P to remove was > 0.1. We chose this elevated significance level to allow investigation of marginally-significant relationships. When multiple regressions yielded independent variables that contributed significantly to the dependent variable, we performed linear regressions of each significant independent variable against the dependent variable to assess the direction (positive or negative) of the significant relationship. We also used linear regression to assess the effect of dewlap area on pigment concentration and the effect of BCI on PC1 and PC2.

To examine how carotenoids and pterins interact to produce observed reflectance spectra we created four groups of males according to the relative concentrations of pterins and carotenoids present in the midline dewlap region. The four groups were high pterin-high carotenoid, low pterin-high carotenoid, high pterin-low carotenoid, and low pterin-low carotenoid, with high and low being relative to mean pterin and carotenoid concentrations of all individuals. We compared the spectral qualities of the four groups to determine how the combination of pigments affected the shape of the spectral curve.

Results

Reflectance spectra of dewlap regions

Lateral and midline regions of the dewlap in males and females were spectrally distinct (Figure 2 a & b). Female lateral dewlap regions showed a maximum reflectance (~30%) in the orange-red portion of the spectrum (600-700 nm), but there was relatively high reflectance across all wavelengths. Male lateral dewlap regions showed a maximum reflectance (~30%) in the red portion of the spectrum (640-700 nm), but the spectral curve showed low reflectance at short and medium wavelengths of light (320-500 nm). The female midline dewlap region had a maximum reflectance (40%) in the orange-red portion of the spectral curve (600-700 nm), but reflected relatively strongly across all wavelengths. Male midline dewlap regions showed a maximum reflectance (45%) in the upper middle and long wavelength portion of spectrum (540 -700 nm, which is yellow to the human eye), with relatively low reflectance in the mid-wavelengths and a second reflectance peak in the UV.

PC interpretations of dewlap reflectance spectra by sex and region

PC1 accounted for 89.7% and 69.4% of the variance in male midline and lateral dewlaps, respectively, and for 92.4% and 96.2% of the variance in female midline and lateral dewlaps, respectively. Because correlation coefficients of PC1 were consistent in magnitude across all wavelengths of light, PC1 represented brightness independent of chroma in both dewlap regions of both sexes (Figure 2 c-f). This interpretation was supported by tri-stimulus multiple regression, which showed that total % reflectance in

UV, blue, green, and red regions all contributed significantly to PC1 variance for both sexes and in both dewlap regions (Table 2 a).

PC2 accounted for 7.1% and 22.6 % of the variance in the male midline and lateral dewlap regions respectively, and for 6.4% of the variance in female midline dewlap region; there was no significant PC2 for the lateral region of female dewlaps. Interpretation of PC2 differed between sexes and dewlap regions. In males, PC2 represented UV brightness and chroma in the midline region, and long wavelength (red, orange, and yellow) brightness and chroma in the lateral region (Figure 2 b). These interpretations were supported by tri-stimulus multiple regression models in which chroma in the UV, yellow and red portions of the spectra significantly contributed to PC2 for male midline regions and in which % reflectance and chroma in the yellow and red portions of the spectrum contributed significantly to PC2 for male lateral regions (Table 2). In the case of male midline regions, separate linear regressions showed that UV contributed positively while yellow and red contributed negatively to PC2 (UV chroma = 0.159 + 0.03172 PC2, $P < 0.0001$; Yellow chroma = 0.09159 - 0.03168 PC2, $P = 0.0104$; Red chroma = 0.43139 - 0.03056 PC2, $P = 0.0011$). In the case of male lateral regions, separate linear regressions showed that yellow and red chroma contributed positively to PC2, while UV chroma and % reflectance contributed negatively to PC2 (Yellow chroma = 0.89612 + 0.33451 PC2, $P = 0.0148$; Red chroma = 0.62674 + 0.07838 PC2, $P = 0.0132$; UV chroma = 0.09804 - 0.03403 PC2, $P = 0.0132$).

In females, PC2 represented presence of UV, blue and green wavelengths that are induced by the relative absence of yellow and red pigments in the midline dewlap region. PC2 did not explain a significant amount of variation in the lateral dewlap region (Figure

2 f). Interpretation of results for the female midline region were corroborated by tri-stimulus multiple regression analysis in which UV, blue and red chroma explained a significant proportion of variation in PC2 (Table 2 b). Separate linear regressions showed that UV and blue chroma contributed positively to PC2, while red chroma contributed negatively to PC2 (UV chroma = 0.14501 + 0.0231 PC2, $P = 0.0002$; Blue chroma = 0.24737 + 0.01341 PC2, $P = 0.0184$; Red chroma = 0.43328 - 0.0319 PC2, $P <.0001$).

When examined by ANOVA, sexes did not differ in midline brightness (i.e. PC1), but did differ in midline UV chroma and brightness (i.e., PC2, Table 3). Female dewlaps had higher PC2 values than those of males. Sexes differed in lateral dewlap brightness (PC1); females had higher PC scores than males (Table 2 c). Finally, sexes differed in long wavelength brightness and chroma (i.e., PC2) in that male dewlaps were brighter and more chromatic at yellow-red wavelengths than were those of females (Table 2 c).

Pigment concentrations & how they relate to dewlap coloration

Absorbance spectrophotometry identified pterins bound in male and female dewlap tissues as drosopterins (λ_{max} = 490 nm) as drosopterins, and carotenoids as xanthophylls, such as lutein and/or zeaxanthin (λ_{max} = 455 nm). Two-way ANOVA revealed that carotenoid concentrations did not differ by dewlap region ($P_{0.3301,\ 1} = 0.5677$) or sex ($P_{0.0818,\ 1} = 0.7758$) and there was no significant interaction between region and sex ($P_{0.4132,\ 1} = 0.4132$, see Figure 2 g). Pterin concentrations displayed a significant sex-by-dewlap-region interaction (Figure 2 h**;** $P_{6.561,\ 1} = 0.0129$). Male lateral dewlap regions were significantly more pterin-enriched compared to male midline dewlap regions and both regions were more pterin-enriched in males than in females, especially in the lateral dewlap region (Figure 2 h). Dewlap carotenoid concentrations did not correlate with pterin concentrations in male lateral or midline dewlap regions (lateral: $R^2 = 0.080$, df = 21, $P = 0.200$; midline: $R^2 = 0.003$, df = 19, $P = 0.829$), or in female lateral or midline dewlap regions (lateral: $R^2 = 0.110$, df = 9, $P = 0.319$; midline: $R^2 = 0.050$, df = 11 , $P = 0.484$).

Because sexes and dewlap regions differed in reflectance properties, we examined relationships between pigments and spectral principal components separately, according to sex and dewlap region. In males, carotenoids explained 17.9% of the variance in PC1 for the midline dewlap region (Table 2 d). This was further supported by a separate, marginally-significant, positive, linear regression between carotenoid concentration and average total percent reflectance in the male midline dewlap region **(**$R^2 = 0.179$, $P = 0.06$). Pigments did not contribute significantly to variance in UV relative to red chroma in the midline dewlap regions for males (Table 2 d). In the lateral dewlap region of male *N. sagrei*, both pterin and carotenoid concentrations contributed significantly to PC1 (Table 2 d). In separate linear regressions, however, increased pterin concentrations yielded significant increases in PC1 ($R^2 = 0.213$, df = 19, $P = 0.03$), while increased carotenoid concentration did not correlate with PC1 ($R^2 = 0.000$, df = 19, $P = 0.994$). No pigment explained significant variation in PC2 of the lateral dewlap region (Table 2 d).

In females, neither carotenoid nor pterin concentration explained a significant amount of variation in PC1 for either dewlap region (Table 2 d). Pterin concentration, however, explained a significant amount of variation in PC2 of the dewlap midline (Table 2 d). Pterin concentration was inversely proportional to PC2 ($R^2 = 0.574$, df = 9, $P =$

0.007), such that the tissue reflected more short- and medium-wavelength light when fewer pterins were present.

Dewlaps that possessed higher-than-average concentrations of pterins and carotenoids reflected less UV light and more long-wavelength (yellow) light (Figure 2 d). In contrast, dewlaps that contained lower-than-average concentrations of pterins but higher-than-average concentrations of carotenoids strongly reflected UV and yellow light. In dewlaps that had either higher-than-average pterin concentrations but low carotenoid concentrations, or lower-than-average pterin and carotenoid concentrations, the spectral curves were intermediate between the two extremes (Figure 2 i).

These data also showed that pterin concentration correlated significantly with hue (i.e., wavelength of maximum reflectance) in the lateral dewlap region ($R^2 = 0.199$, df = 19, $P = 0.055$), but carotenoid concentration did not ($R^2 = 0.003$, df = 19, $P = 0.812$). The distribution of hues in male lateral dewlap regions studied here showed a range from yellow to orange to red, and red (690-700 nm) was the most frequent hue of the lateral dewlap region (Figure 2 j). Orange hues (650-660 nm) were nearly as frequent, and probably resulted from a particular pterin: carotenoid concentration ratio.

Condition dependence

In males, pterin concentration did not correlate with dewlap area ($R^2 = 0.099$, df = 19, $P = 0.177$), but carotenoid concentration did (R2 = 0.235, N = 20, $P = 0.03$, Figure 2 k). Because male SVL was positively correlated with dewlap area ($R^2 = 0.389$, df = 19, $P = 0.002$), high carotenoid concentrations occurred in the largest males. Of all possible significant principal components with respect to male dewlap region, only PC2 of the midline regressed significantly against BCI (Figure 2 l). Hence, UV chroma increased with increased body condition. Neither pterin concentration ($R^2 = 0.05$, df = 21, $P = 0.343$) nor carotenoid concentration ($R^2 = 0.029$, df = 21, $P = 0.476$) regressed significantly against BCI.

Discussion

We detected sex differences in visible- and UV-light reflectance from the dewlaps of Brown Anoles. Female dewlaps had greater UV brightness and chroma than those of males, whereas dewlaps of males had higher long-wavelength brightness and chroma than those of females. Other studies of UV coloration between the sexes of vertebrates show that UV is sometimes sex-limited in its expression [e.g., among males in guppies (Kodric-Brown and Johnson 2002) and sticklebacks (Rick et al., 2004)], or is greater in males than females [as in butterflies (Rutowski et al., 1995), blue tits (Andersson et al., 1998), and wall lizards (Molina-Borja et al., 2006)]. In two taxa studied, however, an agamid lizard (LeBas and Marshall, 2000) and Starlings (Cuthill et al., 1999), however, females have greater UV brightness and chroma than males. In all of these taxa, UV has been shown to be an important aspect of social signals between the sexes. It is unclear if female UV reflectance in *N. sagrei* is used in signaling, though females do perform occasional head-bobs (and dewlap extensions) in social interactions in the Green Anole (Jenssen et al., 2000; Jenssen, 1970) and Brown Anole (*personal observation*).

UV reflectance (in addition to structural iridescent and blue color) is the result of short-wave scattering by nanostructural elements in integumentary tissues such as feathers and scales). In birds, such elements are found within the medullary ‘spongy layer’ of the feather barb and ramus (Dyck, 1971; Dyck, 1971; Prum, 1999; Shawkey, 2005). In butterflies, however, special ellipsoid structures called ‘laminate ridges’ in the reflecting scales cause scattering of light (Ghiradella et al., 1972). The proximate cause to UV color in lizards is unstudied, but it is thought that iridophores scatter light in a way that is analogous to feather barbs (Morrison, 1995). Pigments are also thought to play a role in shaping UV reflectance peaks, by selectively filtering non-UV wavelengths, which influences the shape of the reflectance curve in the UV region (Bradbury and Vehrencamp, 1998). Bird feathers containing xanthophyll carotenoid pigments, for example, produce spectral curves that have a peak in the UV, as well as the yellow portion of the spectrum, because these carotenoids absorb violet and blue light (MacDougall and Montgomerie, 2003). Feather keratin itself reflects strongly at all UV-VIS wavelengths, and hence is white (Shawkey and Hill, 2005), but increases in plumage carotenoid concentration causes absorption of middle wavelength of light, which in turn increases the amplitude of reflectance in the yellow and UV wavelengths (Shawkey et al., 2006). The UV and yellow reflectance peaks we observed in the midline regions of male *N. sagrei* dewlaps are consistent with this mechanism of color production, because individuals that possess lower-than-average concentrations of pterins but higher-than-average concentrations of carotenoids showed a dual-peak spectral curve, with strong reflectance at UV and yellow wavelengths.

In many fishes, amphibians, and reptiles, the yellow and red-producing pigments, carotenoids and pterins, occur together in erythrophores (Bagnara and Hadley, 1973). In a previous study, Steffen and McGraw (2007) showed that red pterins and yellow carotenoids are together in both regions of the dewlap in *N. sagrei*. In the present paper, we quantified concentrations of pigments from each of these pigment classes and found that males had greater concentrations of pterins, but not carotenoids, in the dewlaps than did females. Given that males had larger dewlaps than females, male Brown Anoles must have greater quantities of pterins and carotenoids in their dewlaps compared to females. The sex differences in pterin concentration probably represent developmental differences in the conversion of purines to pterins, and may reflect selection for males to have dewlaps that are more visible than those of females, especially because the red color provided by pterins in lizards markedly contrasts with green backgrounds that are common in the lizards' environs (Endler, 1992). The sex differences in carotenoid quantity, but not concentration, implies that males do not have a greater ability to enrich the integument than females. The dewlaps of males do have greater quantities of carotenoid than females, and this is probably a function of dewlap size, at least in part.

This study also details how pigment concentrations related to reflectance characteristics in the dewlaps of male and female Brown Anoles. In male *N. sagrei*, both pterin and carotenoid concentrations contributed positively to total brightness in the lateral dewlap region. In the midline region, however, carotenoids alone contributed significantly (and positively) to overall brightness of males. In females, however, pterin concentrations contributed significantly (as well as positively) to chroma, but not total brightness, of the lateral dewlap region only. Overall reflectance (PC1), was not related to pigments of female dewlaps, and may instead be the result of differences in scale number and/or ultrastructure in the throat region.

The effects of pigment 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 carotenoids but not pterins to color feathers, carotenoid concentrations have been shown to positively correlate with feather chroma, but not brightness (Saks et al. 2003, McGraw and Gregory, 2004). The difference in the way these two pigments influence brightness and chroma between birds and lizards may be related to the degree to which feather ultrastructure contributes to spectral variation compared to the degree to which scale ultrastructure, as well as iridophore structure and abundance contributes to spectral variation. Investigations of how scale structure contributes to aspects of color has been studied in only a few lizards (Morrison, 1995; Morrison et al., 1996) and this possibility awaits further investigation in *N. sagrei*.

Pterins and carotenoids also combine to create colorful skin patches in male Trinidadian Guppies (Grether et al., 2001). In this species, pterin (in particular drosopterin) concentration correlates positively with carotenoid (tunaxanthin) availability, and the pterin-to-carotenoid ratio is responsible for a particular orange hue that is maintained across a gradient of carotenoid availability in several streams. In *N. sagrei*, however, pterin concentrations did not correlate with carotenoid concentrations in either sex or dewlap region. Furthermore, hue did not appear to be a statistically significant source of spectral variation, because the PC coefficient analyses did not show multiple adjacent peaks in long wavelengths that would reveal yellow-orange-red hue as an important source of spectral variation (see methods for explanation). Still, pterins and carotenoids are found together, especially in male lateral dewlap regions, and basic primary color theory suggests that yellow (carotenoid) mixed with red (pterin) should produce orange. The distribution of hues in male lateral dewlaps studied here range from yellow, through orange, to red, with red (690-700 nm) and orange hues (650-660 nm) being most common.

Pterins found in dewlaps of *N. sagrei* (especially the lateral dewlap region) appear to absorb highly in the UV range, but permit strong reflectance of the long wavelengths. This is because dewlap regions (e.g., lateral dewlap regions in males) that possessed high pterin concentrations as well as high carotenoids have spectral curves low in UV and high in the long (i.e., yellow-orange-red) wavelengths of light. In contrast, midline dewlap regions of male and female *N. sagrei* are pigmented mostly by carotenoids, and have high UV reflectance. UV reflectance along the male midline dewlap region correlated positively with BCI, despite the finding that concentration of neither pigment correlated significantly with BCI. Still, the juxtaposition of a patch in males that is colored by UV absorbing pigments next to a color patch that is highly UV reflecting is curious and begs the question 'why ? A possible answer lies in understanding the placement of pterins in *Colias* butterfly wings, and the influence of this pigment on UV reflectance. Similar to *N. sagrei*, pterins found in the *Colias* absorb highly in the UV range (Rutowski et al., 2005), and the pigments are thought to amplify aspects of low wavelength iridescence, because pterins increase the spectral purity and directionality of the UV signal especially when a butterfly is in flight (Rutowski et al., 2005). We speculate that the UV absorbing lateral dewlap 'amplifies' the appearance of the UV reflecting midline dewlap via heightened spectral contrast between the two regions, and makes the midline dewlap region highly conspicuous to conspecific males and female *N. sagrei*.

The size of UV-based color patches correlates with body condition index in an agamid lizard (LeBas and Marshall, 2001). To our knowledge, however, our study is the first to show some aspect of color *per se* (and not color patch size) as a potentially condition-dependent trait in lizards. The condition dependency of UV brightness may be related to variation in the keratin-based integumentary microstructure, or in the abundance of iridophores underlying the pterin and carotenoid pigments, since neither pterin nor carotenoid concentration related to body condition index in *Norops sagrei* studied here. Keratin structure may display information about health, independent of the susceptibility of pigments to environmentally-based variation (Keyser and Hill, 1999; Keyser and Hill, 2000; McGraw et al., 2002; Doucet and Montgomerie, 2003; Siefferman and Hill, 2003). Although empirical evidence for the condition-dependence of structural color is accumulating, the proposed mechanism for an association between health and the quality of structural coloration remains a matter of debate (Prum, 2006; Hill, 2006). Investigation into the nature of UV coloration, and the condition dependence of structurally- and/or pigmentary-based colors in reptiles is an exciting area of research that awaits future investigation.

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Figure Legends

Figure 2a & b. a) Mean spectral curves (±S.E.) of male and female *N. sagrei* lateral dewlap regions; b) mean spectral curves (±S.E.) of male and female *N. sagrei* midline dewlap. Gray line represents female spectral curve, black line represents male spectral curve.

Figure 2 c - f. Top: Relationship of PC 1 coefficients (black) and PC2 coefficients (gray line) and spectral wavelength for (a) male midline, (b) male lateral, (c) female midline and (d) female lateral dewlap regions. PC 1 represents variation in mean spectra across 20-nm intervals for male midline and lateral dewlap regions, and for female midline dewlap regions. PC 2 represents variation in short wavelengths relative to medium and long wavelengths of light in male midline regions. PC2 represents variation in long wavelengths relative to short and medium wavelengths in male lateral dewlap regions. PC2 represents variation in short wavelengths and medium wavelengths relative to long wavelengths of light in female lateral dewlap regions.

Figure 2 g & h. Carotenoid (a) and pterin (b) concentrations for male (black line) and female (gray line) by sex and dewlap region in *N. sagrei* (lat = lateral; mid= midline).

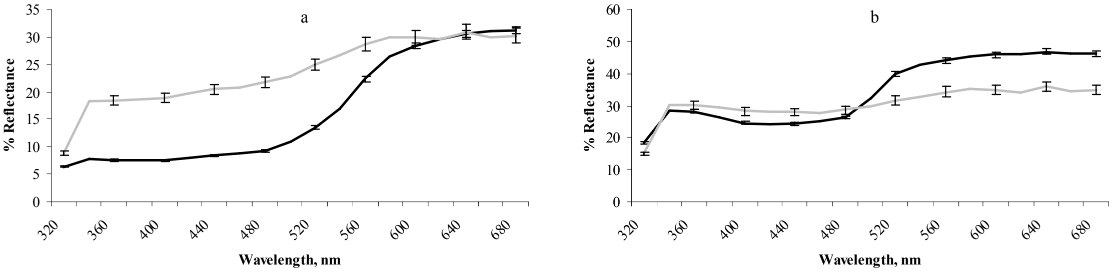
Figure 2 i. Effect of pterin absorption on carotenoid absorption in lateral dewlap region. Treatments are higher than average concentration of pterins and carotenoids (gray solid line), high pterin concentrations but low carotenoid concentrations (black short dashed line), low pterin concentrations but high carotenoid concentrations (black solid line), and low concentrations of pterins and carotenoids (black long dashed line).

Figure 2 j. Frequency histogram of hues from male lateral dewlap regions. Orange (650-660 nm) and red (690-700 nm) hues are frequent in frequency distribution of wavelength of maximum reflectance (a corollary of hue).

Figure 2 k. Regression of PC2 against BCI, midline dewlap region for male *N. sagrei*. See text for details about derivation of BCI and biological interpretation of PC2. (R^2 = 0.182, $P = 0.047$. N = 22).

Figure 2 l. Regression of carotenoid concentrations on male dewlap area (R^2 = 0.235, N = 20, $P = 0.030$).

Figures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35
30
25
20
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% Reflectance
320
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Wavelength, nm
b
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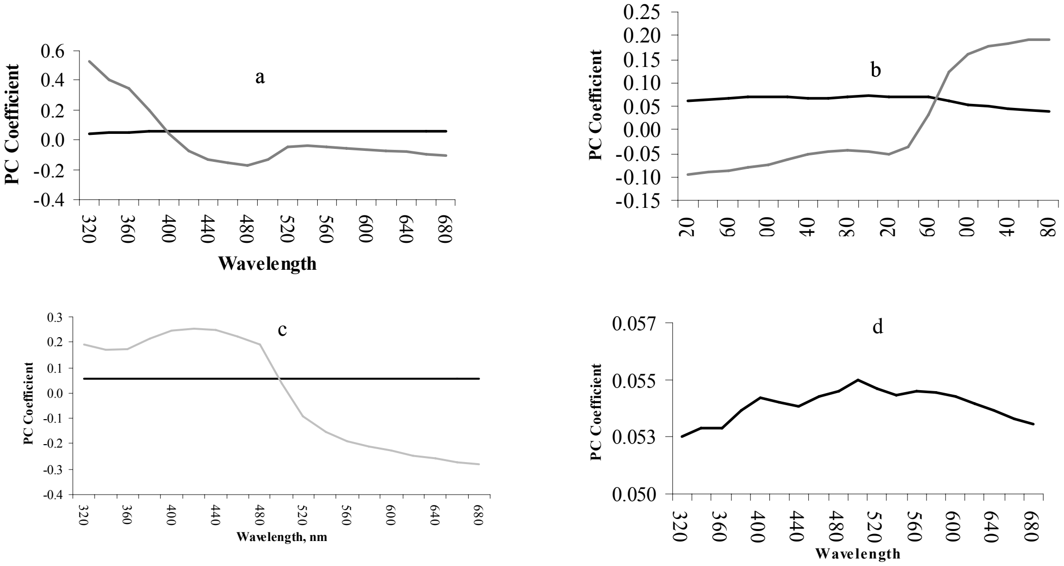
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320
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400
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560
600
640
680
Wavelength
b
PC Coefficient
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
20
60
00
40
80
20
60
00
40
80
c
PC Coefficient
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
320
360
400
440
480
520
560
600
640
680
Wavelength, nm
d
PC Coefficient
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0.055
0.053
0.050
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360
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600
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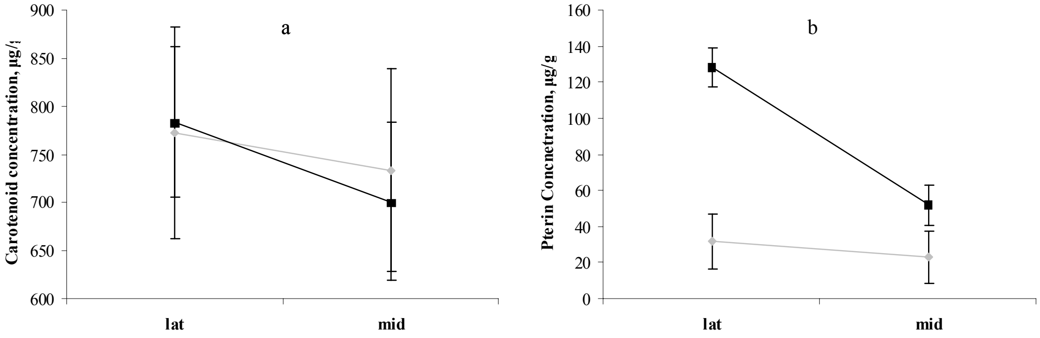
680
Wavelength


a
Carotenoid concentration, µg/g
900
850
800
750
700
650
600
lat
mid
b
Pterin Concnetration, µg/g
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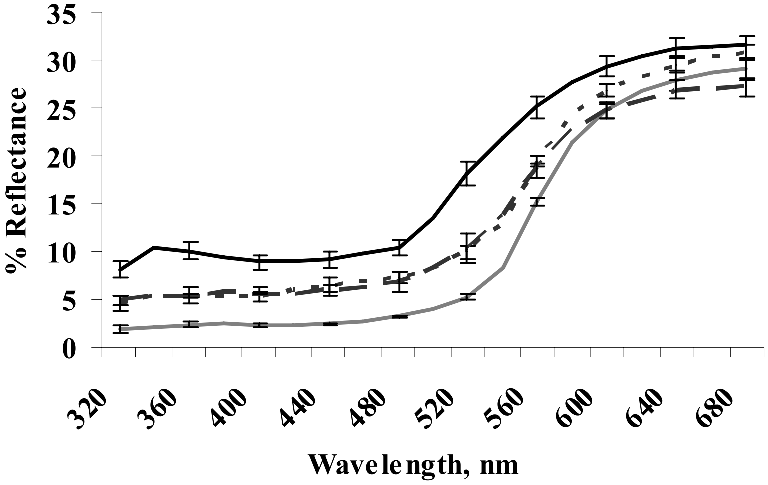
140
120
100
80
60
40
20
0
lat
mid



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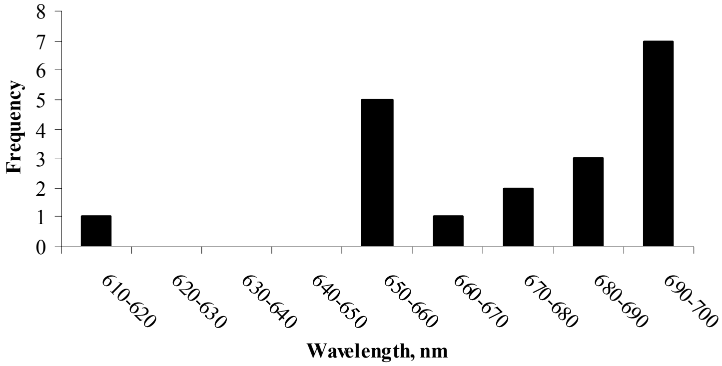
Wavelength, nm


Frequency
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7
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5
4
3
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1
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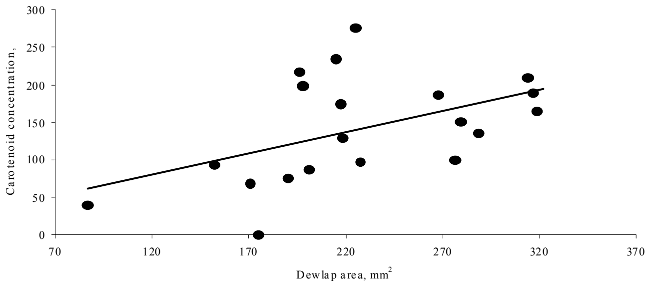
Wavelength, nm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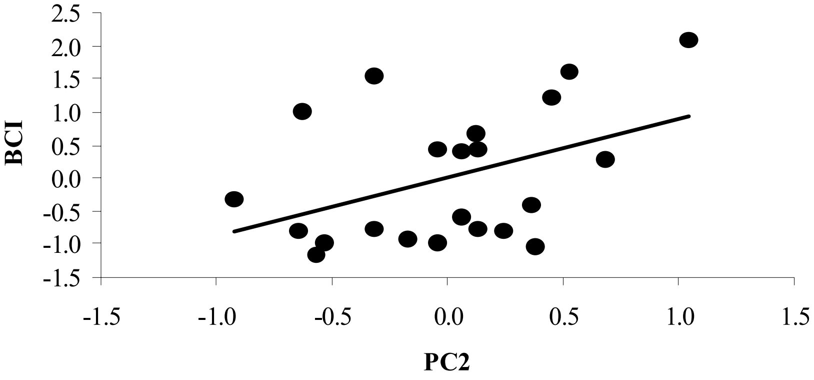
250
200
150
100
50
0
70
120
170
220
270
320
370
Dewlap area, mm²


2.5
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-1.0
-1.5
BCI
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
PC2

Tables

Table 2 a. Backwards stepwise multiple regression. Assessment of contributions of tri-stimulus concept of chroma (UV, blue, green, yellow and red) to PC1 by sex and dewlap region

Sex & Region	R2adj	F, UV	P, UV	F, Blue	P, Blue	F, Green	P, Green	F, Yellow	P, Yellow	F, Red	P, Red
Mlat	1	695.512	<0.001	1729.557	<0.001	520.194	<0.001	removed	0.6498	2346	<0.001
Mmid	1	927.094	<0.001	294.361	<0.001	37.826	<0.001	12.814	0.0038	134.8	<0.001
Flat	1	877.404	<0.001	1795.39	<0.001	251.705	<0.001	removed	0.5629	1673	<0.001
Fmid	1	320.464	<0.001	305.193	<0.001	20.025	<0.001	removed	0.7945	124.5	<0.001

Table 2 b. Backwards stepwise multiple regression. Assessment of contributions of tri-stimulus concept of chroma (UV, blue, green, yellow and red) to PC2 by sex and dewlap region

Sex & Region	R2adj	F, UV	P, UV	F, Blue	P, Blue	F, Green	P, Green	F, Yellow	P, Yellow	F, Red	P, Red
Mlat	0.264	removed	0.438	removed	0.990	removed	0.355	removed	0.220	7.457	0.014
Mmid	0.953	238.953	<0.001	removed	0.375	removed	0.383	12.113	0.004	13.544	0.002
Flat	none	none	none	none	none	none	none	removed	0.815	none	none
Fmid	0.937	8.454	0.023	36.76	0.001	removed	0.828	removed	0.892	7.663	0.028

Table 2 c. Four separate 1-Way ANOVA's that compare sex differences in Principal Component Scores by dewlap regions

	Male Mean	Male S	Female Mean	Female S	F	P
PC1 midline	0.050	0.216	-0.101	0.306	0.163	0.690
PC2 midline	-0.375	0.183	0.750	0.258	12.643	0.001
PC1 lateral	-0.416	0.174	0.833	0.246	17.262	0.000
PC2 lateral	0.381	0.181	-0.763	0.256	13.274	0.001

Table 2 d. Multiple regression of male pigment concentrations (top table) and female pigment concnetrations (bottom table) against Principal components (PC1 & PC2). For details about spectral identity of PC's. see Figs 4a & b, and consult text. Par ID = parameter identity (i.e. pigment identity). Par 1 = 1st parameter entered into multiple regression model, Par 2 = 2nd parameter entered into multiple regression model. F = F statistic, R^2 = amount of variance explained by each regression model, and P = probability of Type II error.

	R^2	R^2ad	Par 1	Par 1 ^{2}	F, Par	P, Par	Par 2	Par 2 ^{2}	F,	P, Par
PC1	0.179	0.134	⌈Ct	0.179	3.934	0.063				
PC2	0	0	non							
PC1	0.2828	0.2073	⌈Pt	0.155	5.00	0.03	⌈Ct	0.282	3.37	0.08
PC La	0	0	non							

	R^2	R^2ad	Par 1	Par 1 ^{2}	F, Par	P, Par	Par 2	Par 2 ^{2}	F,	P, Par
PC1	0	0	non							
PC2	0.5739	0.5266	P	0.57	12.1	0.006				
PC1	0	0	non							
PC2	no pc 2	no pc 2	non							

CHAPTER 3: LIGHT ENVIRONMENT INFLUENCES DEWLAP CONSPICUOUSNESS OF MALE AND FEMALE BROWN ANOLES (*Norops sagrei*)

Abstract

Signal theory hypothesizes that visual signals should evolve conspicuousness sufficient to allow for detection in some light environments at the cost of conspicuousness in other light environments. We quantified the spectral variation of male and female dewlaps from a population of Brown Anoles, *Norops sagrei* that inhabited a semi-disturbed habitat in central Florida, using a UV-sensitive spectrometer. *Norops sagrei* display dewlaps that, to the human eye, appear to have a yellow or white midline dewlap region flanked by a pinkish-red lateral region. We applied these spectral data to a visual model that integrated information about *Norops sagrei* visual physiology and coloration of background vegetation to estimate the conspicuousness of male and female dewlaps in four classic light habitats, and in dim vs. bright illumination conditions. The chromatic aspects of dewlap color of male and female *Norops sagrei* was most conspicuous in light conditions typical of woodland shade, and were slightly less conspicuous in light conditions typical of large and small gaps. Green backgrounds generally rendered dewlap color more conspicuous than brown backgrounds, as did illumination intensity (bright illumination rendered dewlap spectral variation more conspicuous than dim illumination). Sexes could be discriminated based on differences

in their dewlap color in woodland shade only. Brightness of male and female dewlaps could be used to determine the sex of an individual in woodland shade only. We discuss how these results may influence social behavior of Brown Anoles.

Introduction

Animal coloration has captured the interest of naturalists since the time of Darwin (Darwin, 1871) and this trait is used often in sex attraction, sex recognition, and mate assessment (Andersson, 1994). The effectiveness of coloration as a visual signal is dependent not only on the spectral reflectance of the color patch but also on irradiant light environment and display background. Moreover, signal effectiveness is also influenced by receiver physiology (visual system) and behavior (between signaler and receiver (Lythgoe, 1979; Endler, 1990; Endler, 1992; Endler, 1993). Forest-wide variation in at least two of these factors, irradiant light environment and display background, may influence microhabitat choice, mating success, and mortality (Ryan and Wylczyski, 1991; Endler, 1991, Endler, 1992; Endler, 1993, Endler, 1996).

Forests throughout the world contain five major irradiant light habitats, when the sun is not blocked by clouds: forest shade, woodland shade, small gaps, large gaps, and early/late light habitats (Endler, 1993, Fleishman et al., 1997, Chiao et al., 2000). The overall light spectrum of a habitat is influenced by the relative amount of direct and indirect light, which is a consequence of light reflected from sky, leaves or other substrates. The first four light habitats arise because of the way in which differences in forest structure contribute to relative differences in direct and indirect illumination, and assumes that the sun is directly overhead, while the fifth light habitat (early/late) represents differences in illumination intensity (i.e. dim vs. bright), and is a result of the sun nearing the horizon. For the purposes of this paper, we focus on the structurally-based light environment.

Recent advances in spectrometry have revealed that human-based vision has largely underestimated the spectral variation of bird coloration (Burkhardt, 1991; Burkhardt and Finger, 1989). Evidence is accruing that this is also the case in lizards (Fleishman et al., 1993; LeBas and Marshall, 2000, 2001; Whiting et al., 2006; Molina-Borja, 2006; Macedonia et al., 2001; Stuart-Fox et al., 2004; Thorpe and Stenson, 2003, Steffen and McGraw, Chapter 2). In addition, microspectrometry has allowed researchers to identify and assess the spectral sensitivity of UV-sensitive cone photoreceptors that are present in the eyes of many species from all vertebrate classes. Although ethologists have long interpreted these colorful ornaments and visual signals from the perspective of the sensory physiology of the signal receiver (Guilford and Dawkins, 1991), recent models proposed by Voroboyev and colleagues (Vorobyev and Osorio, 1998; Vorobyev et al., 1998; Vorobyev et al., 2001) improve our ability to integrate information about light environment, display background, and integument reflectance to better understand the conspicuousness of visual signals.

Male anoline lizards have large, colorful dewlaps that are displayed to conspecific males and females in social interactions (Jenssen, 1977), to predators during attacks (Leal, 1995; Leal and Rodriguez-Robles, 1997 a, b; Leal, 1999), and to congeners (Losos, 1985). Leal and Fleishman (2001, 2002, 2004) used visual cone sensitivities to model dewlap detectability in two sympatric species (*Anolis cristatellus* and *A. cooki*), as well as in one species (*A. cristatellus*) in two different habitats. The relationship of dewlap reflectance relative to detectability, based on ambient light and cone sensitivities in both of these studies, is consistent with sensory drive (selection for differential conspicuousness under different ambient light environments). For example, the spectral reflectance that characterizes dewlaps of male *Anolis cristatellus* differs between xeric and mesic habitats, and correlates with differences in irradiance between these two habitats (Leal and Fleishman, 2004).

Male Brown Anoles (*Norops sagrei*) have large dewlaps that are comprised of two spectrally-distinct, adjacent dewlap regions that vary in UV, red, orange, yellow and white colors (Chapter 2). To the human eye, the male dewlaps generally appear to have a yellowish edge (midline) flanked by a pinkish-red, central (lateral) region. Females also possess dewlaps, but these structures are reduced to a small patch on the throat possessing colors that range more extensively into UV but less extensively into red, yellow and white (Chapter 2). The extent to which these colors are perceived by conspecifics is a question that is of fundamental importance to studies that attempt to determine the role of dewlaps in social communication of anoles. To date, no research has focused on differences in male and female dewlap color conspicuousness, despite the potential for male and female dewlaps to act as an important communication device in social encounters (Jennsen et al., 2000, Leal and Fleishman, 2004).

Brown Anoles are native to Cuba, the Bahamas, and related islands (Schwartz, 1991), but are successful invaders of the southeastern United States (Lee, 1985, 1992; Means, 1990; Echternacht et al., 1995; Platt and Fontenot, 1994; Campbell and Hammontree, 1995), as well as Hawaii (McKeown, 1996; Goldberg and Bursey, 2000). Throughout their range, Brown Anoles occupy, and display in, a wide variety of habitats ranging from hammock forests (Paterson, 2002; McMann and Paterson, 2003) and tropical gardens (McMann and Paterson, 2003), to disturbed environments such as commercial buildings and houses (Echternacht et al., 1995). Brown Anoles are notorious invaders because of their propensity to inhabit disturbed areas, but substantial populations persist in hammock forests throughout Florida. Hammock forests contain the four structurally based light environments described above (Endler, 1993).

In this study, we measured dewlap reflectance for a population of Brown Anoles in central Florida. To describe the conspicuousness of male and female dewlaps, we integrate information about *N. sagrei* visual systems, background radiance, illumination intensity, and ambient light irradiation from four distinct ambient light habitat types. These light environments are associated with distinct forest and suburban habitats in which these animals reside. The goals of this study were to investigate the extent to which illumination intensity, ambient light habitat, and background reflectance influence dewlap conspicuousness, and to determine light conditions under which sexes could be distinguished based on dewlap color.

Methods

Lizard collection and care

Male and female Brown Anoles (*N. sagrei*) were purchased from a pet store (Glades Herp, Bushnell, Sumter County, Florida), where the animals had been captured from a forest surrounding a park on the west side of Lake Panasofkee in Sumter County. Individuals were identified as adult males if they possessed a large (minimum 50 mm^2) extendable dewlap. Anoles were identified as adult female if their dewlap was small and limited in its ability to be extended. Individuals were housed in an screen-topped, 37.90 liter (ie., 10 gallon), glass terrarium that contained a perch, and a water dish on a sandy floor. A UV-B fluorescent bulb provided light from 12 inches above the terrarium top. Lizards were fed crickets and meal worms *ad libitum*, which were dusted with repta-vite (Zoo Med laboratories, San Luis Obispo, CA).

Spectrometry

We measured spectral reflectance of dewlaps and background vegetation using an S2000 spectrometer (range 250-880 nm: Ocean Optics, Dunedin, Florida) with a bifurcated micron fiber-optic probe. The probe was held at 90º, 1mm from the sample surface, and shielded from stray light by a rubber sheath. The reading area was illuminated with both UV (D-2000 deuterium bulb, Ocean Optics, Dunedin, Florida) and visible (tungsten-halogen) bulbs. All data were generated relative to a white standard (WS-1, Ocean Optics, Dunedin, Florida).

Following Steffen and McGraw (2007), we measured two regions of the dewlap in both sexes: lateral (side) and midline (edge). Male dewlaps were extended with a pair of forceps to allow measurement of the lateral region; the midline region was measured with the dewlap unextended. Because female dewlaps are small and limited in the extent to which they can be extended, lateral and midline dewlap regions were measured by placing the spectrometer probe against an unextended dewlap. In each dewlap region, we took three measures per male and two measurements per female. We took fewer measurements of the dewlap regions of females because dewlap area was only large enough to measure two unique locations.

Color was expressed as percent reflectance per nm wavelength and this output was processed using ColoRv1.5 software (R. Montgomerie copyright 2002). We generated spectral curves for every measurement location, and determined mean spectral curves for the two dewlap regions in each individual lizard. To characterize background colors for the study site, we measured spectral reflectance of tree trunks and leaves where lizards were observed to perch. Specifically, we measured reflectance of eight types of wood substrate and eight types of green leaves found near perch sites used by *N. sagrei*, to represent variation in available brown and green backgrounds.

Light environments

Brown Anoles are heliophilic and thermophilic squamates (Loew et al., 2002, (Ferguson et al., 2005), that can invade shady habitats lacking a shade-tolerant competitor. Therefore, we used irradiance spectral data for forest shade, woodland shade, and small gaps (John Endler, *pers. comm.*), and similar data for large gaps (courtesy of Jarrod Hadfield; http://www.bio.ic.ac.uk/research/iowens/spec/welcome.htm) to model dewlap visibility across the spectrum of habitats used by this invasive species.

Forest structure and openness responsible for creating four of the major light habitats, described by Endler, 1993, is illustrated in Figure 1. *Forest shade* describes a light environment in which the predominant light derives from leaf reflection or transmission. This light habitat is associated with a relatively high density of trees and which contains both canopy and understory trees. In this light environment, there is no direct sunlight, and little or no light comes from the open sky. Green dominates the irradiance spectrum because of reflected light from leaves.

In w*oodland shade*, light derives from leaves, but a significant portion comes from the sky. In this category, direct sunlight (white light) contributes little to the spectrum. This light environment is associated with forests with lower density of trees and lacking an understory of woody vegetation. In this habitat, bluish or bluish-gray radiance from the sky dominates the irradiance spectrum. This light habitat is not limited to forests, instead occurring anywhere that shade is insufficient to prevent significant amount of sky light to penetrate to the substrate (e.g., canopies of emergent forest trees, shaded of boulders, buildings (Endler, 1992 and *pers. comm.*).

Large gaps are 'open areas' in forests that are created by tree falls and are often characterized by downed logs, but this definition also includes open areas created from some other cause, in an otherwise forested environment. In *large gaps*, a significant fraction of light comes from the open sky, but a lesser yet significant amount comes from the sun and vegetation. The sun, blue sky and clouds are much brighter than vegetation. This light environment is predominantly white.

Small gaps are sunlit locations in the forest in which a small canopy hole allows direct sunlight to pass to some point in the forest. In this light environment, a small portion of the available ambient light comes from the open sky and essentially all light comes directly from the sun and vegetation. These areas are characterized by yellow-red ambient light.

Visual modeling

Norops sagrei possess four types of single-cone, retinal photoreceptors: ultraviolet (UVS), short-wavelength (SWS), medium-wavelength (MWS), and long-wavelength sensitive (LWS). Brown Anoles also possess a double-cone photoreceptor that is associated with long-wavelength light but does not possess rod photoreceptors (Loew et al., 2002). In lizards, color discrimination is the result of opponent processing of pairs of different types of single cone photoreceptors (Loew et al., 2002). The chromatic component of an object such as a dewlap can be represented by a point in perceptual color space whose coordinate axes represent the quantum catches of these cone photoreceptors (Goldsmith and Bursey, 1990). For animals with four types of single-cone photoreceptors, this perceptual color space can be likened to a tetrahedron, with each of the four photoreceptor types located at a vertex of this tetrahedron (Burkhardt, 1989; Goldsmith, 1990). Variation in the detectability of different colors can be approximated by calculating Euclidian distances between two points in this tetrachromatic color space (Goldsmith, 1990).

However, distances between points in such a color space do not correspond directly to perceived differences in color because these distances must exceed a certain threshold to be distinguishable. This discrimination threshold depends on noise that originates in photoreceptors and at further stages of neural processing (Vorobyev and Osorio, 1998, Vorobyev et al., 1998). When stimuli occur below this threshold, signals will not be perceived to the extent that signals above the threshold will be perceived.

Vorobyev et al. (1998) have developed a color space model that accounts for receptor-noise limited stimulation thresholds and integrates information about the transmission of the ocular media, light environment, visual background, and receptor noise, to estimate the relative conspicuousness of a color patch. We implemented a version of this model to calculate the conspicuousness of the two different dewlap color regions of male and female Brown Anoles. All equations follow Vorobyev et al (1998) and (Hadfield, 2004). Spectral sensitivities (including effects of photoreceptor-associated oil droplets) were measured by Loew et al. (2002) and provided in its present form by Leo Fleishman (Figure 2). We used these spectral sensitivities to estimate photoreceptor quantum catch in *N. sagrei*.

In a particular light environment, a color pattern is most conspicuous when the adjacent color pattern elements vary greatly in brightness and chroma (Endler, 1992). To quantify the extent that the bi-colored dewlap was perceived by a conspecific observer, we estimated conspicuousness of the two adjacent color patches. To estimate conspicuousness, we first calculated quantum catch (Q) per photoreceptor type as a proportion of total quantum catch for each dewlap region of each individual lizard using the equation:

$$Q_i = \frac{\int_\lambda R_i(\lambda) S(\lambda) I(\lambda) O(\lambda) \, d\lambda}{\int_\lambda \mathrm{R}_{tot}(\lambda) \, d\lambda} \qquad (1)$$

where λ represents wavelength, $R_i(\lambda)$ is the spectral sensitivity of receptor type i, $S(\lambda)$ is the reflectance of the color patch, $I(\lambda)$ is the irradiance of the light environment, $O(\lambda)$ is the transmission of the ocular media, and $d\lambda$ symbolizes that integration is to be performed over the range of visual spectrum that is sensitive. When spectral files are processed so that data are expressed as percent reflectance per nanometer wavelength, this integration is simply a summation of reflectance occurring throughout the range of i. Spectral sensitivities, irradiance, and ocular transmission were normalized to a mean and standard deviation of one using equation 1, from which we calculated receptor quantum catches for each single cone type (UVS, SWS, MWS, and LWS). Photoreceptors undergo chromatic adaptation to pre-exposed or surrounding background stimuli, and we accounted for this by normalizing the photoreceptor quantum catches related to perception of spectra of male or female dewlap regions to the photoreceptor quantum catches of the adapting backgrounds using the von Kries scaling algorithm (Wyszecki and Stiles, 1982):

$$q_i = k_i\, Q_i \qquad (2)$$

where the scaling factor, k_i, is defined as:

$$k_i = 1/\frac{\int_\lambda R_i(\lambda) S(\lambda) I(\lambda) O(\lambda)\, d\lambda}{\int_\lambda R_i(\lambda)\, d\lambda} \qquad (3)$$

and where $S(\lambda)$ is the reflectance spectrum of the background (Voroboyev and Osorio, 1998). This scaling factor is implemented to incorporate the assumption that receptors independently adapt to the background, and acts as a mechanism which can account for color constancy. Spectra of green leaves and brown tree trunks were measured to serve as the background. Fechner's law states that the perceived magnitude of a visual stimulus is proportional to the physical magnitude of the stimulus (Wyszecki and Stiles, 1982). In mathematical terms, the receptor signal (f_i) is proportional to the natural log of the quantum catch of a given receptor type (q_i) and is calculated as follows:

$$f_i = \ln(q_i) \qquad (4)$$

And the difference in receptor signals (Δf_i) for two stimuli, *a* and *b*, (i.e. two dewlap regions, or a color patch against its background) is defined as

$$\Delta f_i = f_{i,a} - f_{i,b} \tag{5}$$

Using equation 5, we calculated receptor signals that result from two adjacent dewlap regions, for each of the four cone types, for each sex. Color perception is achieved by comparing receptor signals across different receptor types. By extension, perceived differences in color between two objects can be determined by comparing differences in receptor signals across different receptor types. For each receptor type, the difference in receptor signals between two color patches equal Δf_i .

For a tetrachromat, we calculated the discriminability (ΔS) between two color patches (or two dewlap regions) using the following equation:

$$\begin{aligned}(\Delta S)^2 = {} & ((e_1e_2)^2)(\Delta f_4 - \Delta f_3)^2 + (e_1e_3)^2)(\Delta f_4 - \Delta f_2)^2 \\ & + (e_1e_4)^2)(\Delta f_3 - \Delta f_2)^2 + (e_2e_4)^2)(\Delta f_3 - \Delta f_1)^2 \\ & + (e_3e_4)^2)(\Delta f_2 - \Delta f_1)^2 + (e_3e_2)^2)(\Delta f_1 - \Delta f_4)^2) \\ & / ((e_1e_2e_3)^2 + (e_1e_2e_4)^2 + (e_1e_3e_4)^2 + e_2e_3e_4)^2)\end{aligned} \tag{6}$$

As mentioned above, noise in receptor types influences the perception threshold of visual stimuli. Noise in a receptor type i, (e_i), can be modeled by the following equation:

$$e_i = \underline{\mathrm{sqrt}\ ((}(1/log(T(Q_{i,a} + Q_{i,b})/2))^2 + w_i^2)/\ n_i\ ) \tag{7}$$

where T is a scaling factor which relates Q_i (which is expressed as a proportion of maximal cone catch) to an absolute quantum catch value for a receptor (Vorobyev et al 2001), w_i = the Weber fraction for cone type i, and n_i = the relative abundance of cone type i in the retina.

The mathematical expression $1/log(T(Q_{i,a} + Q_{i,b})/2))^2$ models quantum-flux-dependent noise whereby noise increases as the average absolute quantum catch for the two stimuli decreases. This may be because colors are dark (i.e., Q_i is small), or colors are viewed under poor illumination (i.e., T is small).

We compared two settings for T, 500 and 10,000., that roughly correspond to dim and bright illumination, respectively, for each ambient light setting. Dim illumination represents irradiant light available during early/late parts of the day, and occurs when the sun is near the horizon. Bright illumination represents irradiant light available when the sun is directly over head.

The Weber fraction, w_i, describes the inherent noise-to-signal ratio in receptor cells of type i, independent of the quantum catch. This constant approximates noise-to-signal ratios in photoreceptors, and permits predictions of suprathreshold differences between saturated color. We used a default value of 0.05 for all cone types following Hadfield (2004). Since no data are available for the relative abundance of cone types in anoles (Leo Fleishman, *personal communication*), we used relative abundance of cones from Blue Tits (*Parus caeruleus*, Class Aves) (Hart et al., 2000; UVS=0.37, SWS = 0.7, M = 0.99, L = 1). Because the vertebrate eye is highly conserved between different classes, bird cone abundance should not differ greatly from squamate cone abundance, and should suffice as a valid model parameter until data on squamate cone abundance become available (Campbell, 2006).

Conspicuousness, or ΔS describes a relative stimulation threshold for which values are described in units of Just Noticeable Differences. A value of <1 indicates two colors that cannot be discriminated, and values >1 can be discriminated. Values >1 represent the incremental magnitude that a value is above the detection threshold.

Quantifying contrast

We used ΔS to quantify chromatic contrast. The degree of contrast between different body regions influences short-distance conspicuousness. From a close distance, dewlap regions with high values of ΔS will be highly conspicuous.

Brightness represents variation along the achromatic (black-grey-white) axis and is calculated as the summed light reflected across all wavelengths. Variation in brightness also influences the conspicuousness of signals. Because brightness is the most abundant source of spectral variation in animal integument (Chapter 2, and Cuthill et al., 1999), we calculated measures of brightness contrast between the two dewlap regions for males and females.

The role of double cones in squamate visual perception is unknown, but because double cones are thought to be used for achromatic signal detection in birds (Cuthill et al., 2000 a & b, Hart, 2001), we calculated receptor signals for double cones in *N. sagrei* (f_D) as a proxy for brightness using the formulae described above and spectral sensitivity of long wavelength cones alone. Loew et al., (2002) found photoreceptors with double-cone morphology to be a subset of LWS photoreceptors. Therefore f_D is equivalent to f_{LWS}, and we obtained f_D (or f_{LWS}) by calculating f_i for long wavelength cones from equation 4. Receptor noise is not known to significantly influence reception by double cones, and we estimated the perceived brightness of a dewlap region as f_D, and the perceived difference in brightness of a color patch as Δf_D (i.e. $f_{D,\text{ patch 1}} - f_{D\text{patch 2}}$). We measured achromatic contrast between the two dewlap regions (to measure short-distance conspicuousness), and against different perch backgrounds (to measure long-distance conspicuousness) in dim and bright light conditions of four different ambient light environments.

Statistical Analyses

Normality was tested using the Shapiro-Wilk test. Color variables were normally distributed in all cases and were therefore not transformed. All analyses were performed using JMP statistical software. ΔS and Δf_D values of males and females were modeled for the four different light habitats, against green and brown backgrounds, and at two levels of light intensity (T = 10,000, as a model of direct sun, and T = 500, as a model of crepuscular sun); values > 1 were considered conspicuous and values ≥ 1 but ≤ 2 were considered marginally discernable. ΔS and Δf_D values of males and females within each light habitat were compared using a 1-way ANOVA to test for significant sex differences. ΔS and Δf_D values of *N. sagrei* dewlaps against each background color within each light habitat were compared using a 2-way ANOVA to test for significant differences in background color.

Results

Under bright illumination color of the two dewlap regions was conspicuous (ΔS values >1) in woodland shade, and large and small gaps, and against brown and green backgrounds (Table 2 a). Dewlaps were most conspicuous in woodland shade against green backgrounds. Dewlaps were slightly less conspicuous in woodland shade against brown backgrounds. In small gaps, dewlaps were relatively conspicuous against green background, but marginally discernable against brown backgrounds. In large gaps dewlaps were marginally discernable against green backgrounds, as well as against brown backgrounds. In forest shade, dewlaps were not conspicuous against green or brown backgrounds.

Sexes could be discriminated based on dewlap color in woodland shade only. Against green and brown backgrounds, males were significantly more conspicuous than females ($F_{green} = 52.022$, $P < 0.0001$, $F_{brown} = 54.876$, $P < 0.0001$; Table 2 a). ΔS values did not differ between sexes in large and small gaps, although the dewlaps of both sexes were conspicuous.

Under dim illumination dewlap color was most conspicuous in woodland shade against green backgrounds and brown backgrounds (Table 2 a). Dewlaps were also relatively conspicuous under dim illumination in small gaps against green and marginally discernable against brown backgrounds. Dewlaps were marginally discernable in large gaps against brown backgrounds but not noticeable against green backgrounds. Dewlap colors were also not noticeable in forest shade against green backgrounds or brown backgrounds (Table 2 a).

Under dim illumination, sexes could be discriminated based on dewlap color in woodland shade only (Table 2 a). Against green and brown backgrounds, male were significantly more conspicuous than females ($F = 77.322$, $P < 0.0001$).

The brightness of dewlap color was highly conspicuous in woodland shade only (Table 2 b). Brightness against a green background was slightly more conspicuous than brightness against a brown background (Table 2 b).

Sexes differed significantly in brightness in woodland shade only, against green and brown backgrounds, and males had greater brightness contrast than females ($F_{green} = 139.971$, $P < 0.0001$; $F_{brown} =139.971$, $P < 0.0001$)(Table 2 b). No other light environments showed significant brightness contrast between dewlap regions.

Finally, green backgrounds render the brightness aspects and chromatic contrasts of dewlap (in bright and dim lighting) more conspicuous than brown backgrounds, in general (Table 2 c). There is a background color * light habitat interaction, however ($F_{brightness} = 8.84$, $P < 0.0001$; $F_{chromatic, bright} = 20.241$, $P < 0.0001$; $F_{chromatic, dim} = 292.429$, $P < 0.0001$. Table 2 c). In light habitats where ΔS and Δf_D are roughly one magnitude above the stimulation threshold (i.e. ranging from 1-2), brown backgrounds render the dewlap more conspicuous than green backgrounds.

Discussion

Because the conspicuousness of a visual signal is dependent on ambient light level as well as background reflectance, color patches should appear different to conspecific observers when displayed in different light habitats and against different backgrounds. The hypothesis that light habitat, as well as background color, has a significant impact on the conspicuousness of visual signal was supported by our results. We found that the chromatic aspects of dewlap spectral variation in *N. sagrei* was sufficiently conspicuous to be detected by a conspecific observer in woodland shade, and large and small gap light habitats, but was not conspicuous in forest shade.

In woodland shade, ambient light increases conspicuousness of the dewlap color 5-7 times compared to that of forest shade (depending on bright or dim conditions). Woodland shade describes light environments where predominant light derives from blue sky and leaves, but there is no direct sunlight. This describes forest structure with a canopy, but where there is a relatively low density of trees, and which lacks an understory of woody vegetation. This also describes light environments in the shade along forest edges, when the edge blocks the direct sunlight, as well as shade that is created as the result of a significant obstructions blocking direct sunlight, such as that provided by roofs of house, fences, etc. Thus, in the shade provided by human dwellings, it appears that *N. sagrei* dewlaps are more conspicuous than in other light environments.

The achromatic aspect of dewlap spectral variation (i.e., brightness, or variation in the black-gray-white axis) is roughly 22-times greater in woodland shade than in any other habitat. Brightness variation is the largest source of spectral variation in animal integument, and is the primary source of spectral variation of *N. sagrei* dewlaps (Chapter 2). It also is related to movement detection in *Anolis cristatellus* (Fleishman and Persons, 2001). Therefore, irradiant light available in woodland shade renders both the chromatic and achromatic aspects of Brown Anole dewlaps relatively conspicuous compared to other light habitats.

Male and female dewlap color is barely noticeable (i.e., $1 \leq \Delta S \leq 2$) in large and small gap light habitats. Brightness differences between the two dewlap regions could not be discerned in males or females, in small or large gaps. Gaps often provide light appropriate for visual displays of many animals (e.g., butterflies and birds), but may be a trait associated with relatively mature forests. Furthermore, gaps represent a proportionately small portion of habitat space in forests and these environments may only be useful for signaling to those organisms whose populations can persist in such small spaces.

Forest shade renders color and brightness of male and female dewlaps inconspicuous. Forest shade is created by a dense tree canopy that contains canopy and understory-midstory trees, and where there no direct sunlight, and little or no light comes from the open sky.

Sexes could be discriminated according to dewlap color and brightness in woodland shade only. Therefore if a conspecific observer is to gather information based on dewlap 'color' that is sex-dependent, it can only be done when the display is presented and observed in woodland shade. Granted, there are other ways for a conspecific observer to determine sex that are not solely based on dewlap color, such as dewlap extension, dewlap size, body shape, dorsal pattern, etc., that can provide an observer with information to help determine the sex of a nearby lizard. Arguably, these other traits and behaviors are as equally strong a force as dewlap color in providing information about sex identity to conspecifics. However, these results do show that dewlap color alone can provide information to a conspecific observer about a nearby lizard's sex.

It appears that woodland shade provides optimal ambient light for the dewlap color of *N. sagrei* from this population. If dewlap color is used to assess phenotypic quality of a potential mate or rival, or as a signal of dominance and fighting ability, then it is most easily done in woodland shade. Interestingly, sexes could not be distinguished based on dewlap color in woodland shade if dewlap color was presented under dim illumination against a brown background. This means that conspecific observers must use some other means of distinguishing sex (e.g., dewlap size, dewlap movement, body shape, dorsal patterns) when observing dewlaps against a brown substrate, that is present early or late in the day. As mentioned above, these traits and behaviors are very likely to provide reliable information about sex.

When dewlap color is distinguishable, and sexes differ significantly in conspicuousness, males are brighter than females. In some sub-optimal light habitats, however, ambient light does not reflect against the dewlap and display background in a way that stimulates the photoreceptors above the stimulation threshold, and therefore are 'inconspicuous' ($\Delta S < 1$). In a few light habitats such as these, females have higher ΔS values than males. But why aren't males always brighter than females under all light environments, even when the dewlap color is not sufficiently conspicuous? We speculate that this pattern reflects that the direction of differential sex colorfulness varies when it is freed from the strong selection associated with sensory drive in optimal visual habitats.

Green backgrounds render dewlap color more conspicuous than brown backgrounds, in general, and this is true for dewlap brightness as well as chromatic variation under dim and bright illumination. Therefore, dewlap color would be more conspicuous if a conspecific observer were to observe the dewlap against a leafy background, instead of a bark background. Whether or not Brown Anoles take advantage of the increase in background contrast against green backgrounds is not known. However, in general it is thought that conspecific Anole observers often position themselves so that dewlap extension of a nearby lizard can be viewed from the side, so that the lateral dewlap is visible from a 90° angle (Fleishman et al., 2006). Furthermore, displayers use trees to perch and extend their dewlap above the perch substrate, so that more than likely, the dewlap viewing background is not the perch substrate itself, but is instead provided by the leaves, etc., surrounding the tree from which the displayer perches (personal observation).

Our data suggest that signal senders could improve the visibility of the dewlap spectral variation by behavioral modification, for example, by displaying in woodland shade, when the sun is directly overhead, and against green backgrounds. Direct observations of social interactions involving presentation of male dewlaps in different light habitats can test this prediction and tell us whether signal senders actually display most often under optimal light conditions, and whether or not observers respond most frequently to signals under optimal conditions.

Lastly, our findings show that color of female *N. sagrei* dewlaps is nearly as noticeable to conspecifics in certain light environments and against different backgrounds as are male dewlaps. The potential for female dewlap color to perform a social role in anoles has not been studied. In a laboratory study of aggression in Green Anoles (*Anolis carolinensis*), females, which have colored throats that are non-extendable, have the same repertoire of aggressive signals (e.g., head-bobs and push-ups) as males, but they differ in the social context and frequency of display (Jennsen et al., 2000). The authors speculated that directional selection on these displays has been less intense in females than in males because the outcome of consexual contests has fewer reproductive consequences for females than males. Several authors have shown that female anoles are territorial, but less so than males, and that female territories are often contained within a male's territory (Rand, 1967, Trivers, 1976, Stamps, 1977, Schoener and Schoener, 1982). Because of this, female signal use might have important consequences for female competitor recognition and male mate suitability (Leal and Fleishman, 2004). Since our results show that female dewlaps are only visible in some light environments but not others, a color-based female signal may go undetected in suboptimal light environments. Furthermore, our data show that female dewlaps are conspicuous in the same habitats that males are conspicouous in. This pattern is consistent with sensory drive because sensory drive predicts that visual signals will not be uniformally conspicuous across all light habitats, but that male and female visual signals will be conspicuous in similar habitats, to aid in the use of visual signals for mating and reproduction. There is at least one alternative explanation however: female dewlap color conspicuousness may be a correlated response to selection for male dewlap color. If female dewlap color is not noticeable to predators because females do not extend their dewlaps, and if dewlap color has negligible production costs, then female color might become as conspicuous as we measured it to be because its expression is subject to genetic correlations with male dewlap color.

The findings presented here show that the colors of *N. sagrei* dewlaps are highly visible in some light habitats, but not in others. Moreover, green backgrounds generally render the dewlap colors more conspicuous than brown backgrounds. These model predictions await empirical testing, and this will surely be an area of exciting research in the future.

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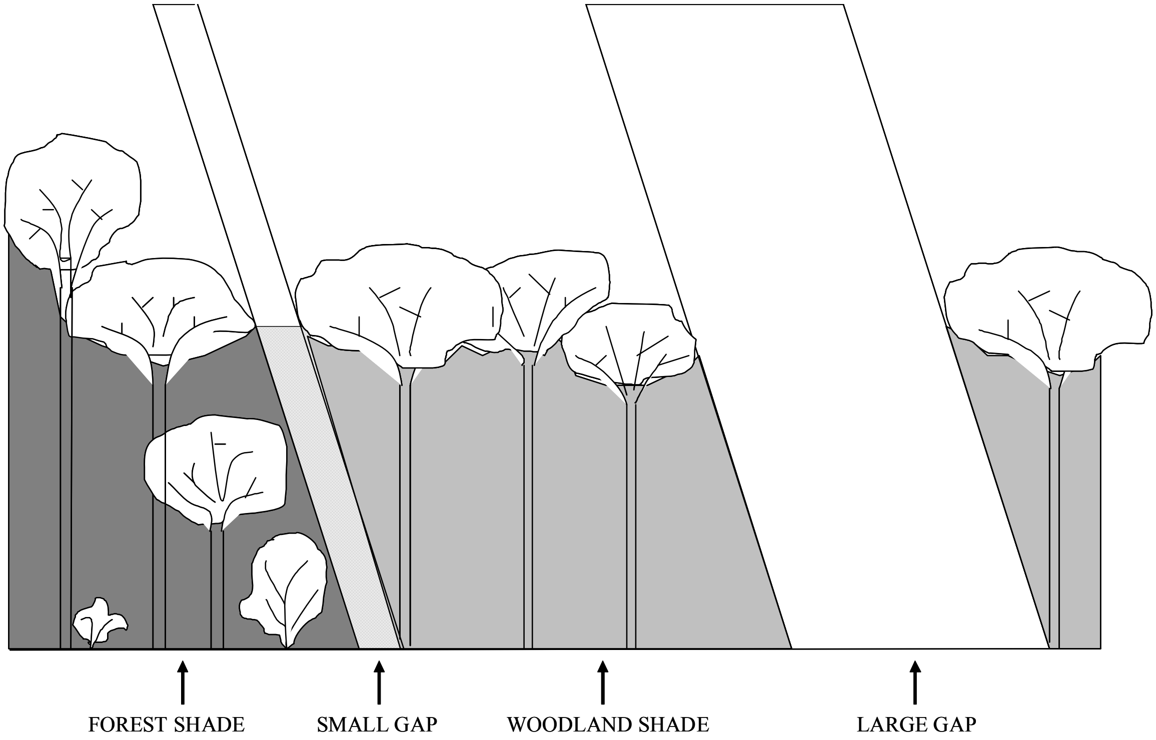
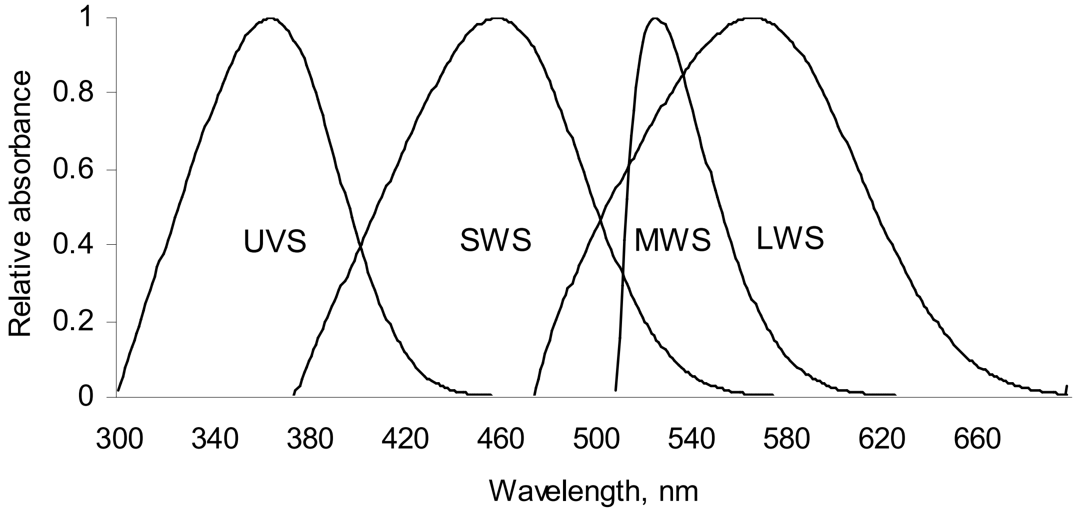
List of Figure captions

Figure 3 a. The major light habitat classes in forests when the sun is not blocked by a cloud. Shaded colors in figure represent the following: Dark grey: light from reflected leaves. Medium-gray: light from blue sky and leaf reflectance. Light gray checkers: light from blue sky and vegetation. Ligh habitats are defined as follows: Forest Shade: canopy closed, or with very small or few gaps; all or virtually all incident light is either reflected from or transmitted through the vegetation. Small Gap: skylight from a gap in the canopy, essentially no light from the direct sun and less than ~10% of the incident light is reflected or transmitted through the vegetation. Woodland Shade: canopy open, a significant fraction of the incident light comes from the sky, but none comes directly from the sun. Large Gap: direct sunlight and open (blue) sky account for most of the ambient light. Figure is redrawn from Endler, 1993.

Figure 3 b. Relative spectral sensitivities of photoreceptor cells in *Norops sagrei*. Spectral curves represent distinct single cell photoreceptor cone types, whose sensitivities are maximal in the following spectral regions, from left to right: UVS, SWS, MWS, and LWS. Norops sagrei possess single- and double- photoreceptor cell cone types. These spectral curves represent single-cell photoreceptors. The double-cell photoreceptors are used for achromatic discrimination in birds, and they are maximally sensitive in the long wavelengths, but their use in lizards is undescribed. For the purposes of this study, I assume they serve achromatic discrimination, as in birds, and are maximally sensitive in the long wavelengths. Thus, I use discrimination in LWS cones alone, to model achromatic discrimination by *N. sagrei*. Used with permission by Leo Fleishman.

Figures




1
0.8
0.6
0.4
0.2
0
Relative absorbance
UVS
SWS
MWS
LWS
300
340
380
420
460
500
540
580
620
660
Wavelength, nm

Tables

Table 2. Mean and standard error (SE) of male (M) and female (F) *Norops sagrei* dewlap chromatic conspicuousness (*ΔS*), as viewed against green (left) and brown (right) backgrounds, under bright illumination (A; T = 10,000) and dim illumination (B; T= 500 – see text for explanation). ΔS > 1 represents dewlap color contrast between dewlap regions that is conspicuous to brown anoles. ** depicts significant sexual differences in male and female dewlap conspicuousness, where P < 0.001. Light habitats are defined as in Endler (1993). N (males) = 51, N (females) = 22.

		Green Background						Brown Background					
A	Light Habitat	F Δ S	F SE	M Δ S	M SE	F	P	F Δ S	F SE	M Δ S	M SE	F	P
	Forest	0.489	0.091	0.691	0.058	3.498	0.066	0.489	0.091	0.691	0.058	3.498	0.066
	Woodland	7.156	0.071	7.767	0.046	52.022	<.0001**	4.550	0.081	5.259	0.052	54.876	<.0001**
	Large Gap	1.618	0.095	1.723	0.061	0.865	0.356	1.620	0.096	1.725	0.061	0.842	0.362
	Small Gap	4.356	0.084	4.173	0.054	3.367	0.071	1.571	0.091	1.668	0.059	0.807	0.372

		Green Background						Brown Background					
B	Light Habitat	F Δ S	F SE	M Δ S	M SE	F	P	F Δ S	F SE	M Δ S	M SE	F	P
	Forest	0.535	0.092	0.703	0.059	2.344	0.130	0.535	0.092	0.703	0.059	2.344	0.130
	Woodland	5.803	0.048	6.121	0.031	31.344	<.0001**	3.731	0.062	4.378	0.040	77.323	<.0001**
	Large Gap	0.797	0.041	0.843	0.027	0.894	0.348	1.533	0.096	1.648	0.062	1.004	0.320
	Small Gap	3.901	0.092	3.811	0.059	0.674	0.414	1.640	0.101	1.756	0.065	0.937	0.336

Table 2 c. Mean and standard error (SE) of male (M) and female (F) *Norops sagrei* dewlap brightness conspicuousness (*ΔfD*), as viewed against green (left) and brown (right) backgrounds. *ΔfD* > 1 represents dewlap color contrast between dewlap regions that is conspicuous to brown anoles. ** depicts significant sexual differences in male and female dewlap conspicuousness, where P < 0.001. Light habitats are defined as in Endler (1993). N (males) = 51, N (females) = 22.

	Green						Brown					
Light	F Δ *f*	F	M D	M	F	P	F	F	M	M	F	P
Fores	0.06	0.00	- 0.05	0.00	155.69	<.000	0.066	0.00	- 0.0	0.00	155.	<.000
Woodlan	- 23.63	0.01	- 23.80	0.00	139.97	<.0001	- 22.	0.01	- 22.2	0.00	14	<.0001
Large	0.07	0.06	0.47	0.04	29 48	<.000	0.353	0.03	0.393	0.0	1.12	0.292
Small	0.07	0.06	0.47	0.04	29.48	<.000	0.353	0.03	0.393	0.0	1.12	0.292

Table 2 d - f. Two-way ANOVA results for effect of background color on three aspects of dewlap conspicuousness. A = effect of background color on brightness of dewlap. B = effect of background color on chromatic conspicuousness under dim illumination (T = 500). C = effect of background color on chromatic conspicuousness under bright illumination (T = 10,000).

D	Light Habitat	Bckgr	Mea	S.E.	Bckgr	Mean	S.E.	F	d	P
	Forest	Brown	0.008	0.17	Green	0.008	0.171	8.88	3	<.000
	Woodland	Brown	- 21.684	- 0.1 71	Green	- 23.182	- 0.171			
	Lg Gap	Brown	0.373	0.17	Green	0.278	0.171			
	Small Gap	Brown	0.373	0.17	Green	0.278	0.171			

E	Light Habitat	Bckgr	Mean	S.E.	Bckgr	Mean	S.E.	F	d	P
	Forest	Brown	0.588	0.06	Green	0.588	0.067	245.74	3	<.00 1
	Woodland	Brown	4.803	0.06	Green	7.301	0.067			
	Lg Gap	Brown	1.670	0.06	Green	1.668	0.067			
	Small Gap	Brown	1.617	0.06	Green	4.263	0.067			

F	Light Habitat	Bckgr	Mea	S.E.	Bckgr	Mean	S.E.	F	d	P
	Forest	Brown	0.617	0.05	Green	0. 617	0.059	292.43	3	<.0001
	Woodland	Brown	3.972	0.05	Green	5.832	0.059			
	Lg Gap	Brown	1.589	0.05	Green	0.819	0.059			
	Small Gap	Brown	1.696	0.05	Green	3.866	0.059			

CHAPTER 4: UV REFLECTANCE IS NOT USED TO INFLUENCE MALE-MALE CONTEST OUTCOME IN LAB PAIRS OF *NOROPS SAGREI*

Abstract

Ultraviolet colors are an important visual signal in male-male competition in an increasing number of species of lizards. I investigated the use of UV dewlap color as a signal in male-male contests in the Brown Anole, *Norops sagrei*, by performing UV-vis spectrometry on males of the same size, and then observing which one copulated with a female made available to both males. I showed that males with less dewlap UV reflectance won contests for females significantly more than males with higher dewlap UV in a laboratory environment. Based on these findings, I experimentally reduced UV in males with higher UV from a size-matched dyad, and found that males with artificially reduced UV lost contests for females compared to males with natural dewlap UV. This suggests that dewlap UV color is correlated with some trait associated with dominance and winning access to females in the laboratory, but that manipulation of dewlap UV is not used as a basis of information about status by males in the lab.

Introduction

Animals use a diverse array of signals to communicate aspects of their social or reproductive status (Andersson, 1994). Ultraviolet color is an important visual signal in butterflies (Ghiradella et al., 1972; Rutowski, 1985; Silberglied and Taylor, 1978), fish (Losey et al., 1999), birds (Cuthill et al., 2000) and lizards (Fleishman et al., 1993;

Stapley and Whiting, 2005; Stapley and Keogh, 2006; Whiting and Stapley, 2006). Previous studies investigating UV in fish and birds have focused on its role as a signal in female mate choice (Bennett and Cuthill, 1994, Losey et al., 1999). The extent to which UV signals influence male reproductive success through their role in male-male contests remains unstudied. However, signal theory suggests that visual signals should convey information used by resident males in assessing the competitive abilities of intruding or neighboring males in contests for territorial space and mates (Rohwer, 1975). For example, a status badge is a morphological trait (often a color patch) that reliably indicates the resource-holding-potential (i.e., fighting ability) of the individual displaying the trait, and allows assessment by nearby males. Ideally, such traits are relatively inexpensive to produce but costly to maintain (Rohwer, 1981) because individuals are repeatedly tested on their dominance status through fight contests (Rohwer, 1982).

Male-male rivalry is frequently found to be an important component to lizard mating systems, and most of the color patches studied in lizards appear to conform to definitions of status badges (Cooper and Greenberg, 1992). In one example, UV has been shown to be linked to fighting ability, and acts as a status badge in *Platysaurus broadleyi* (Whiting et al., 2006). In this species males assess their opponent's UV reflectance, and asymmetries in amount of UV between males influence contest escalation (Stapley and Whiting, 2005; Whiting et al., 2006). Males that were rich in UV were better fighters than males poor in UV. Moreover, males that have UV reduced were contested more frequently than males that had natural UV colors.

UV has been found to be a frequent component to dewlap color in many Caribbean anoles (Fleishman et al., 1993; Fleishman et al., 1997). Dewlap UV varies geographically in different groups of Anolis in ways that are habitat specific (Macedonia et al., 2000; Leal and Fleishman, 2002, 2004; Thorpe and Stenson, 2003). For example, dewlap UV of four allopatric populations of *Anolis cristatellus* correlates positively with the UV light environment in a way that suggests that population abundance is highest in areas where dewlap color is most conspicuous (Leal and Fleishman, 2004). This pattern is consistent with predictions of sensory drive, namely that signal diversity evolves species or populations come to occupy different habitat conditions where selection for effective communication promotes divergence in signal designs (Leal and Fleishman, 2004). These findings further support the hypothesis that dewlap UV reflectance is an important social signal.

While several studies have focused on the role of dewlap extension in social communication of anoles (Crews, 1975; Evans, 1938 a & b; Greenberg and Noble, 1944; Leal, 1999; Tokarz, 2002; Tokarz et al., 2003; Williams and Rand, 1977), few studies have attempted to determine the role that dewlap color plays in social signaling. Adult males from two species of sympatric sibling species on Hispaniola recognize species-specific differences in dewlap color (Losos, 1985). This experiment provides evidence for dewlap color serving a role in species recognition.

The Brown Anole (*Norops sagrei*) is a small anoline lizard from Cuba, Jamaica and other Caribbean islands (Schwartz and Henderson, 1991) that is highly territorial and reaches extremely high population densities (Schoener and Shoener, 1978; Schoener, 1980). In nature, populations of anoles (including *N. sagrei*) exhibit sex-specific demographic differences, such that females aggregate around areas with high food abundance, and males aggregate in areas where females can be found (Schoener, 1980;

Guyer, 1988a). This demographic pattern creates dynamic neighborhoods in anoles (Stamps, 1994, Stamps and Krishnan, 1997). In these neighborhoods, males frequently engage in visual and physical contests for access to territorial space that includes access to one or more females, and this results in a few, large (old) males that appear to achieve dominance, and enjoy access to females and food (Stamps and Krishnan, 1994).

Several lines of evidence suggest that dewlap color may serve a social role in N. sagrei. UV color has been found to be sexually dichromatic in Brown Anoles, and correlates with body condition (Steffen and McGraw, in prep). Moreover, UV of the Brown Anole dewlap is at least partially the result of xanthophyll (i.e., carotenoid) pigmentation (Steffen and McGraw, in prep). Carotenoids have been implicated in their role as condition-dependent pigments in many animals, and results of nutritional stress experiments suggest that UV amplitude might indicate nutritional condition in N. sagrei dewlaps (Steffen and McGraw, in prep).

Here I examine whether dewlap color plays a role in determining contest outcomes by investigating pair-wise differences in natural dewlap color among dyads of size-matched males. I then investigate whether or not contest participants use UV color to convey information about dominance status by experimentally reducing UV in normally UV-bright males. I discuss these outcomes with respect to male-male contests.

Methods

Lizard Collection and Care

Lizards were collected from two sources. In 2005 and 2006, individuals were purchased from Glades Herp (Lake Thonotosassa, Hillsboro County, and Lake Withlacoochee, Marion County). In 2007, lizards were collected from Lake George, Marion County, Florida. Data from the three populations were analyzed separately, although this site effect was confounded by the fact that each site was collected in a different year. Collection sites are all within 72 miles of each other.

In all years, all lizards were immediately identified as to sex, weighed (nearest 0.001 g) and measured snout to vent (SVL; nearest mm) upon arrival at Auburn. They were then assigned a unique toe-clip. After one day in captivity, the dewlaps of all male lizards were measured with an Ocean Optics S2000 UV-visible spectrometer following the methods for midline and lateral regions described in Chapter 2. I also used all colorimetric variables (hue, brightness and chroma) related to UV, Blue, Green, and Red wavebands, as well as UV amplitude, as described in Chapter 2.

Males were size-matched to another male that differed by no more than 0.5 mm in SVL but that differed naturally in dewlap UV brightness Pairs of males were housed in 37.90 liter terraria that were partitioned with particle boards into three compartments. The largest compartment (25.40 x 30.48 cm) represented half of the terrarium divided along its width. The other half was further divided into two small compartments (25.40 x 15.24 cm) by a partition along the long axis of the aquarium. Separate males were placed into the separate small compartments. One female was placed in the large compartment of the terrarium. The outside walls of the terraria were lined with green construction paper to facilitate visual detection of male dewlap colors (Endler, 1992). The partition between males prevented them from seeing each other until an experimental trial was begun. Each compartment contained its own perch and water dish (filled with water 3 times per week). Lighting strips containing full spectrum fluorescent tubes (Vitalite T8, 32 watt) were suspended 30.48 cm above the terrarium tops and all terraria were exposed to natural light via a large window. Lizards were sprayed with water daily, fed crickets three times a week (3 per feeding) and meal worms *ad libitum*. All food items were dusted with repta-vite (Zoo Med laboratories, San Luis Obispo, CA) before being offered to a lizard. Lizards were allowed to acclimate to a terrarium for a minimum of one week before experiment manipulations were performed. Laboratory was maintained at 32.2 °C, and 60% relative humidity.

I performed natural color experiments during summers 2005 and 2007, and UV reduction experiments during the summer of 2006. Lizards were never used in trials more than once.

Natural experiments

Natural dewlap color trials were performed from May 1 -July 1, in 2005 and May 1 – June 15, in 2007. At the beginning of each experimental trial the partitions in the terraria were removed to expose the size-matched males and the female to each other. I then left the room and recorded all behaviors on an 8mm video camera. All trials were allowed to run for 1 hour.

From the video recordings all copulations were noted, and the identities of successful and unsuccessful individual males were noted. Trial winners were easily identified because females never mated with more than one male in these trials. The colormetric variables of each male contestant were placed in a file that contained each individual's contest status (won copulation or lost copulation). To determine if color differed between the two contest outcome groups, pair-wise T-tests were performed between the dependent variable, which were separate colorimetric / waveband combinations (i.e., UV chroma, Blue chroma, UV brightness, Blue brightness) and contest outcome (won copulation / lost copulation) as the independent variable. To reduce the probability of type I error (i.e., rejecting the null hypothesis when the null hypothesis is true) that arises from multiple comparisons, Bonferroni corrections were performed to the entire set of variables and only those that were significant after Bonferroni correction are reported.

UV reduction experiment

UV reduction trials were performed July 1 – August 1, in 2006. For these experiments I quantified the spectral variation of dewlaps as in natural experiments but noted which member of a size-matched dyad had higher UV reflectance. The lizards were placed into the partitioned terraria as described above, and acclimated with food and water for one week. One hour before the beginning of each experimental trial, I selected the male from the pair that had higher UV reflectance, and I applied NOAD sunscreen (spf 30) to his dewlap. This effectively reduced UV chroma in both dewlap regions, and UV reflectance in the midline dewlap region (Table 4). The lizard with the lower UV chroma received a control treatment of water rubbed onto the open and closed dewlap. Lizards were returned to their respective compartments for one hour before beginning an experiment trial by removal of the partitions.

Statistics

Pair-wise t-tests of natural color variables between size-matched males were performed using SPSS statistical software (v. 9.1). 2-way ANOVAs were performed to investigate if some natural color waveband/colormetric combination (i.e., UV reflectance) varied with respect to year and copulation status, or as an interaction between year and copulation status. Log-linear analysis was performed to determine if there was a significant relationship between experimental treatments (sunscreen and water) and contest outcome. JMP statistics was used to perform all statistics and alpha was set at 0.05.

Results

Natural color contests

Males that copulated with females, had lower dewlap UV reflectance than unsuccessful males (Figure 4 a, pairwise t-test: t = -2.494, N = 24, *P* = 0.019, after Bonferrroni correction). Unsuccessful males were an average of 13 % more UV reflectant than their successful rivals.

UV-reduced experimental contests

Log linear analysis showed that individuals with reduced UV did not copulate with females significantly more than individuals with natural (and hence higher) UV reflectance (Figure 4 b, Chi-square likelihood ratio = 4.186, N= 8, *P* = 0.0408). Out of 8 UV reduction trials, lizards with sunscreen-treated dewlaps won contests for females 75% of the time.

Discussion

Because male and female anoline dewlaps differ in color (in human visible and invisible ranges), and because these dewlaps are displayed to conspecific males and females in social interactions, it has long been speculated that they serve a role in sexual selection, especially as a signal in male-male contests (Greenberg and Noble, 1944; Noble and Bradley, 1933; Evans, 1938 a & b; Crews, 1975). My data demonstrate that, in male-male contests for access to females, male *N. sagrei* that win contests and copulate with females have dewlaps that reflect less UV (and have lower UV amplitude) than males that lose contests, and these results suggested that UV could serve as a status badge in *N. sagrei*.

In signals that serve as status badges, colors are hypothesized to be cheap to produce but expensive to maintain. Therefore, badge holders are constantly being tested through male-male disputes. Because production costs are cheap, both dominant and subordinate males can obtain sufficient amounts to be equally colorful, and the color that becomes associated with a dominant status relative to the status of a subordinate individual can be arbitrary. Thus, according to this definition, it remains possible that less colorful males are dominant to more colorful males, as long as the information behind the signal is reliable (i.e., that you are dominant and willing to fight to prove it), and that the information is repeatedly tested (Rohwer, 1977; Rohwer, 1978). Interestingly, in the house finch (*Carpodacus mexicanus*), females prefer to mate with males with more colorful plumage (Hill, 1990), but drab males appear to be more dominant, at least during the breeding season (McGraw and Hill, 2000 a & b). My data document a similar trend in anoles.

To further explore the role of UV as a status badge, I decreased UV reflectance in naturally UV-bright males to see if a contest loser could become a winner via color alone. However, males with artificially reduced UV lost contests more frequently than did males with natural color. Thus, while there appears to be a consistent pattern of coloration that associates with dominance in natural color trials, it appears that altering it does not change the direction of the outcome. That is, artificially reduced UV males (which are naturally brighter in UV) are still subordinate, despite altering them to look like dominant males.

My results beg the question ‘in naturally colored populations, why are winners less colorful than losers in *N. sagrei*?’ One possible explanation emerges from the fact that testosterone negatively influences carotenoid-based integumentary color in other vertebrates. In many vertebrates, aggressiveness is controlled largely by testosterone (Wingfield et al., 1987; Wingfield and Moore, 1987), a hormone that influences social contests for dominance (Wingfield et al., 1987). Not surprisingly, testosterone has been shown to vary seasonally in male *N. sagrei*, and correlates with breeding season behavior [March-August, (Tokarz et al., 1998)]. Testosterone alters male contest behavior (e.g. increases dewlap extension frequency, Tokarz et al., 2002), as well as increases a male’s ability to acquire high social status (Tokarz, 1995).

Testosterone directly affects carotenoid-based integument color in the house finch (Stoehr and Hill, 2001). In an experiment in which males were implanted with testosterone early or late in the breeding season, or left unmanipulated, unaltered males experienced minimum change in plumage color. Males implanted with testosterone early in the breeding season metabolized the hormone before moult, and similarly showed minimum effect on plumage color change. Males implanted with testosterone late in the spring, however, delayed moult timing and grew drab feathers. This suggests that testosterone might effect plumage color directly (by delaying the moult past the time when carotenoids are abundant (see Nolan et al., 1992), or indirectly (by affecting the ability of males to utilize carotenoids indirectly via increases in parasite numbers that result from the immunosupressing effect of testosterone (Thompson et al., 1997; Brawner et al., 2000). It is possible that testosterone affects the ability of males to utilize carotenoids via immunosupressive effect of testosterone in lizards.

Testosterone spikes, and not seasonal fluctuations in hormone levels, have been shown to be responsible for contest aggressiveness in vertebrates (Moore et al., 2007). UV color may change quickly in the Brown Anole based on xanthophyll carotenoid access (Steffen, in prep), and may be influenced by testosterone spikes at times of carotenoid acquisition (Moore et al., 2007). Thus, in light of the un-manipulated as well as experimental dewlap color findings from this lab study, it seems plausible that UV serves a communicative role in natural settings, and may also serve as a signal indicating male testosterone levels. These ideas await further investigation, and may be an exciting area of research full of new discoveries.

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Tables

Table 4. Sunscreen effectively reduces spectra in the UV wavelengths. Lateral dewlap region required no Bonferroni correction, and P of significance for the midline dewlap region (after Bonferroni correction) was P = 0.01. Ctrl = dewlap color before application of sunscreen. SS = dewlap color after sunscreen application.

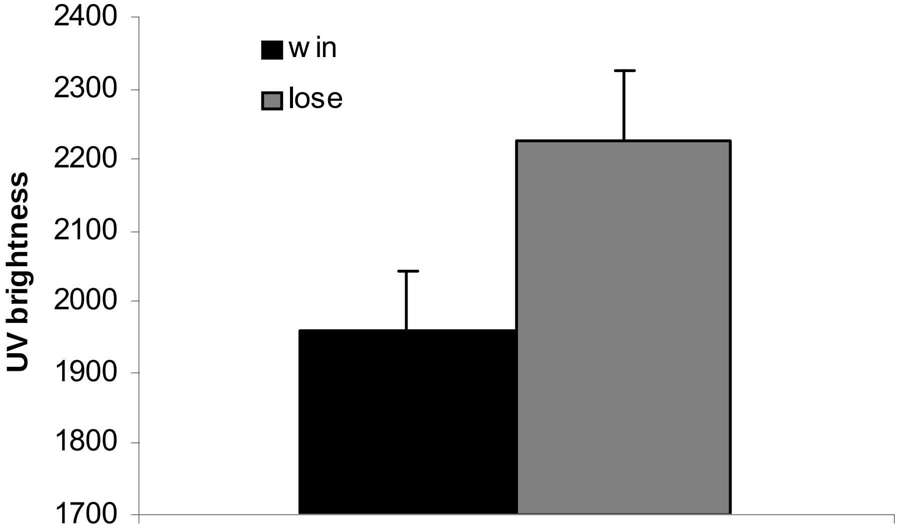
Regio	PW	Mn	SE	Mn	SE	t	d	P
La	UV	0.09	0.02	0.04	0.01	2.55	5	0.0
Mi	UV	0.15	0.02	0.02	0.00	4.17	5	0.00
Mi	UVV	0.18	0.02	0.03	0.01	6.51	5	0.00
Mi	UV	1571.1	314.0	132.0	51.83	4.17	5	0.00

Figure Legends

Figure 4 a. Significant pairwise difference in UV brightness and contest outcome in the midline dewlap region of *Norops sagrei*, after Bonferroni correction. Trials from 2005 and 2007 combined, $t = -2.494$, $df = 24$, $P = 0.019$.

Figure 4 b. Significant difference in contest win percentage by experimental group (water as control, sunscreen as treatment) in male *Norops sagrei*. Likelihood Ratio Test, Chi-square value = 4.186, $N = 8$, $P = 0.0408$.

Figures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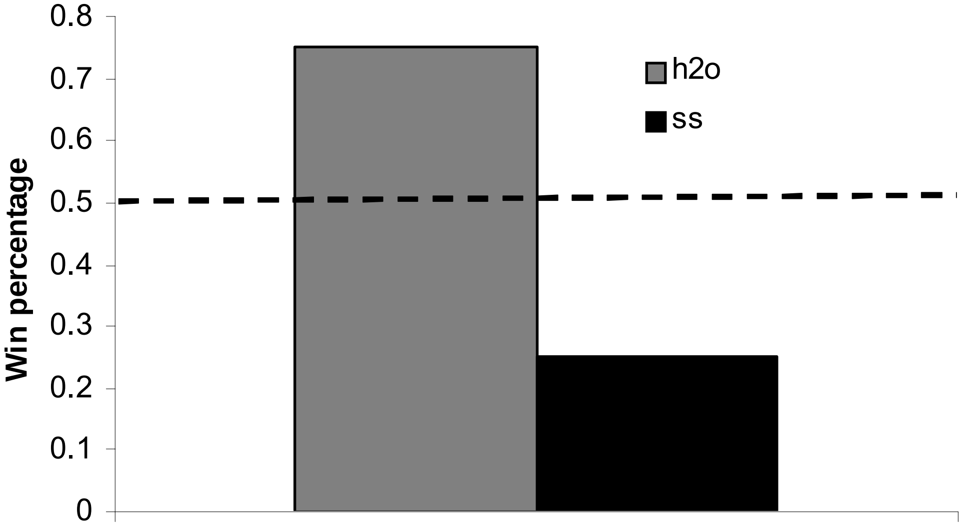
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CHAPTER 5: EFFECTS OF NUTRITIONAL STRESS AND CAROTENOID ACCESS ON THE DEWLAP COLOR OF MALE BROWN ANOLES, *Norops sagrei*

Abstract

Anolis lizard dewlaps are well known for exhibiting a diversity of colors, many of which strongly reflect in the UV spectral wavelengths. These lizards are also well known for using their dewlaps as visual displays in intra- and intersexual interactions. In previous studies, UV reflectance of the dewlap of *Norops sagrei* was found to correlate with body condition and male-male contest success. Here I perform a two-factor, repeated-measures experiment to determine the effects of access to xanthophyll, a dietary carotenoid, and food provisioning rate, on dewlap color in the Brown Anole (*Norops sagrei*). Using this design, I test the hypotheses that dewlap color is dependent on access to xanthophyll and signals male condition in anoles. Lizards fed a diet enhanced with xanthophylls had greater UV chroma on the lateral dewlap region and increased long-wavelength chroma on the midline dewlap region than lizards without access to xanthophylls. I also found that dewlap color of lizards with reduced provisioning rate, and reduced access to xanthophylls, had reduced UV amplitude. The colors that change due to access to xanthophylls are the same as those that determine male-male contest success in *Norops sagrei*. These findings have implications for understanding dewlap signal content within and among species of anoles.

Introduction

Carotenoid-based integumentary colors are well-known examples of an environmentally-determined class of ornaments that often serve in visual displays as signals of mate quality (Searcy and Nowicki, 2005). For example, xanthophylls, a common carotenoid pigment class found in feathers, are known to produce UV and yellow spectral variation in the American Goldfinch (*Carduelis tristis*) by absorbing in the blue region of reflectance in an otherwise white feather (Shawkey and Hill, 2005; Shawkey et al., 2006). Because no vertebrate can endogenously synthesize carotenoids, these chemicals must be ingested to be used as pigments in a visual display (Brush, 1978; Endler, 1980; Goodwin, 1984, Kodric-Brown, 1985; Hill, 1990; Endler, 1980). Carotenoids are important as indicator traits, because they have a high energetic cost to their obtainment and subsequent metabolism compared to other ornamental pigments (Stradi, 1996; Stradi, 1995; Stradi, 1997, Hill, 2002). Furthermore, parasites affect the expression of carotenoids. For example, ectoparasites can inhibit integumentary expression of carotenoids in guppies (Houde and Torio, 1992), intestinal parasites can affect carotenoid ingestion and color displays in domestic chickens (Ruff et al., 1974) and sticklebacks (Milinski and Bakker, 1990). Apicomplexan parasites (e.g., coccidia) can affect plumage coloration of house finches (Thompson et al., 1997; Brawner et al., 2000), and hemoparasites can reduce color expression in great tits (Horak et al., 2001).

Carotenoids also have been speculated to represent a trade-off between immuno-enhancement and ornament expression (Lozano, 1994a). Carotenoids can act as free-radical scavengers and, therefore, are potentially beneficial to an individual's health (Di

Mascio et al., 1991; Diplock, 1991; Bendich, 1993; Olson, 1993; McGraw, 2005). However, carotenoids that are placed in the integument cannot be used for reducing oxidative stress (Von Schantz et al., 1999). The logic of this trade-off hypothesis comes from the fact that carotenoids are a limited resource and are needed both for proper immune function and to create ornamental displays. This puts carotenoid use for ornamentation in direct competition with use in maintaining health. However, this is a matter of controversy (Hill, 1999; Lozano, 2001). Despite the controversy surrounding the trade-off hypothesis, a great deal of evidence shows that carotenoids are used in ornamental displays as visual signals which communicate information to signal receivers.

Despite mounting evidence of a role for carotenoids in sexual selection in many animals, squamates that use carotenoids for display might also use these pigments to facilitate species recognition (Wallace, 1889), and therefore, carotenoids might play a role in natural selection (Losos, 1985; Sigmund, 1983; Macedonia et al., 1994) within this diverse group. In particular, anoline lizards belong to a species-rich genus, comprised of 380 recognized species (Nicholson, 2002), and are becoming recognized as a classic example of a group that has undergone adaptive radiation (Schluter, 2000). Most anoles are highly territorial (Stamps, 1983), and possess an enlarged throat fan, known as a dewlap, that is used as a social signal in intrasexual and intersexual interactions (Jenssen, 1977). Dewlap spectra vary widely among species (Williams and Rand, 1977; Fitch and Hillis, 1984), and show a wide variety of patterns in which yellow is the most frequent color (Nicholson et al., 2007). Interestingly, analyses of dewlap pigments have revealed that xanthophylls are a major contributor to yellow dewlap color in Jamaican and Puerto Rican anoles (Macedonia et al., 2000), as well as one Cuban and one Central American anole (Steffen and McGraw, 2007).

Yellow carotenoid-based colors are often correlated inversely with blue, but positively with ultra-violet reflectance because carotenoids function by strongly absorbing light from the blue wavelengths of the radiant spectrum (Bauernfiend, 1981) – essentially they function like semi-transparent filters in feathers, which are structurally white in color (Shawkey and Hill, 2005). The precise proximate cause to UV reflectance in vertebrate integument (including birds) is unknown. In birds with carotenoid-based yellow colors, the pigments may interact with white feather microstructure in ways that create unexpected optical phenomenona, including UV chroma (Shawkey et al., 2006). Interestingly, they found that in the American Goldfinch (*Carduelis tri*stis), UV color variation was only indirectly related to carotenoid concentration, in the context of the change in shape of the 'peak-trough-plateau' curve, typical of carotenoid-pigmented feathers (Shawkey and Hill, 2005; MacDougall and Montgomerie, 2003).

UV is an important component to spectral variation in the dewlap of Puerto Rican anoles in that the degree to which UV is expressed is related to microhabitat-level differences in light availability (Fleishman et al., 1993; Fleishman et al., 1997). In addition, color in anole dewlaps varies geographically in *Anolis carolinensis* (Macedonia et al., 2003), as well as in *Anolis trinitatus* (Thorpe, 2002). Finally, dewlap UV of four allopatric populations of *Anolis cristatellus* correlates positively with the UV light environment in a way that suggests that population abundance is highest in areas where dewlap color is most conspicuous (Leal and Fleishman, 2004). This pattern is consistent with predictions of sensory drive, namely that signal diversity evolves species or populations come to occupy different habitat conditions where selection for effective communication promotes divergence in signal designs (Leal and Fleishman, 2004).

Given the findings of Shawkey and Hill (2005), it seems likely that xanthophylls are at least partly responsible for the UV spectral variation in anoles. Recent research by Steffen and McGraw (Chapter 2) shows that the lateral (red) portion of a dewlap in *Norops sagrei* is colored by a combination of xanthophylls and pterins (drosopterin), while the midline (yellow) region is colored predominantly by xanthophylls and has a strong UV component (Steffen and McGraw, Chapter 2). Additionally, UV plays a role in allowing *N. sagrei* dewlaps to be visible against some background environments and not others (Steffen and Sieffermen *in prep*). Finally, UV plays a role in determining which males wins contests for access to females in a lab setting (Steffen *in prep*).

The manner in which anoles color their dewlaps, including UV, has not been studied. Surprisingly, no study has traced the origin of carotenoids in dewlaps, despite the fact that scientists have recognized their presence in dewlaps for several years (Macedonia et al., 2000). In this paper I test the hypothesis that expression of dewlap color in adult male *N. sagrei* is dependent on access to dietary carotenoids, and examine whether expression of these colors is affected by nutritional condition.

Methods

Individual adult male lizards were collected on April 5th, 2007, from a forested dock on the east side of Lake George, Marion County, Florida. Upon return to Auburn, Alabama two days later, lizards were assigned a unique toe-clip identity (no more than two toes clipped per individual), and were placed into 37.90 liter terraria, each of which was separated into four compartments so that lizards could be placed singly into a compartment. Compartment walls were constructed from solid particle board which prevented lizards from seeing or physically contacting other individuals. Each compartment contained a perch, water dish, and sandy substrate. Lighting strips containing full spectrum fluorescent tubes (Vitalite T8, 32 watt) were suspended 30.48 cm above each terrarium top. Terraria, and the lizards in them, were exposed to natural light via sunlight that entered the holding room through a large window. Water was sprayed into each terrarium daily, and water bowls were refilled three times per week. The room was maintained on a 14:10 hr photoperiod, at an air temperature of 32.2 °C, with relative humidity ranging between 60-80%.

Lizards were housed for a one week, pre-trial period, during which they were fed meal worms and crickets *ad libitum*. At the beginning of the experiment, lizards were placed into one of four treatments: 1) high provision rate with carotenoid supplementation (see below), 2) reduced provision rate (see below) with carotenoid supplementation, 3) high provision rate with no carotenoid supplementation (see below), and 4) reduced provision rate with no carotenoid supplementation.

Feeding level, one main effect in the experimental design, consisted of high and low provision-rate treatments. The high provision-rate group received 3 crickets per feeding repeated 3 times per week. The reduced provision rate involved three crickets per feeding, once per week. All crickets for all treatments were 3-weeks old (Golden Crickets, Alabama; raised on a protein-rich diet that lacked carotenoids) and were maintained on a diet of dog food (lamb, rice, oat and corn-based meal, supplemented with vitamins, including A) to eliminate the possibility that lizards obtained xanthophylls from the crickets.

Carotenoid access was the second main effect in the experimental design, and consisted of a carotenoid supplement treatment and a control treatment. For the carotenoid treatment, I supplemented lizards with a xanthophyll carotenoid solution described in Navara and Hill (2003). This solution was composed of 0.28 g of powdered lutein (5% tg/p) and 0.12 g of powdered zeaxanthin (5% cws/s-tg) dissolved in 3.79 liters of tap water. The solution was shaken vigorously for two minutes. The solution was stored in a refrigerator where it was kept cool and protected from exposure to light. Carotenoids were donated by DSM, Nutritional Products, Parsippany, N.J. Lizards were fed 2 ml of this carotenoid solution via a 10 ml syringe. The blunt tip of a needle-less syringe was placed into a lizard's gaping mouth, and the syringe plunger was slowly depressed so that the lizard ingested the solution. For the control solution, a separate syringe was used to deliver water to lizards in an identical manner.

Lizards were weighed to the nearest 0.001 gram at the beginning and end of the experiment using an electronic balance. I weighed lizards three days after the last meal to minimize the possibility that weights reflected full stomachs instead of body mass. These measurements were used to document that the high and reduced food treatments represented reasonable extremes.

Color of the lateral and midline dewlap regions (Chapter 2) was measured with an Ocean Optics S2000 UV-visible spectrometer. Measurements were taken once per week, for 11 weeks (April 24, 2007 – July 17, 2007). Repeated measures of spectrometry data have notoriously high variance because the intensity of the light beam is sensitive to minute changes in electrical current (OOIbase lab manual). Furthermore, the shape of a spectral curve is strongly dependent on probe positioning and ambient light (Fleishman et al., 2006, Montgomerie, 2006). Therefore, to minimize differences in electrical current associated with time of day, I took measurements consistently at 10:00 A.M. To minimize geometric issues, I placed each lizard ventral side up on a flat black table and immobilized them with two pieces of athletic tape placed across their belly and nose. To obtain spectrometry measurements on the midline dewlap region I placed the spectrometer probe at a 90° angle to the dewlap skin. I visually inspected the probe to insure that it was placed flush against the dewlap skin and waited for a stable curve to appear on the screen before saving the spectral measurement as a spectral file. To measure the lateral dewlap region I used blunt forceps to pull the dewlap away from the body, and I fastened the dewlap margin with a small blunt clamp suspended on a string through the opening of a threaded eye screw. This allowed me to adjust the height and tension of the clamp by turning the threaded eye screw until the string holding the clamp was taut, and the dewlap appeared to be maximally extended. I then placed the spectrometer probe at a 90° angle to the extended skin of the dewlap. To prevent the probe from differing in height among consecutive measurements, I placed a small black plastic stopper on the tip of the reflectance probe, so that there was a 2mm distance between the probe tip and the tissue being measured. I took three spectral measurements per region for each lizard, and the data analyzed here represent the mean of the three spectral measurements per dewlap region.

Spectral transmission files were processed using a program by Robert Montgomerie (©2002 used with permission). This program is a series of Visual Basic macros that record each measurement as a series of readings representing the relative percent reflectance for each nanometer of wavelength reflected. This program also summarizes each spectral curve according to hue, brightness, and chroma. Total brightness was defined as the sum of reflectance for values between 300 nm and 700 nm (Andersson and Prager, 2006). Mean brightness was $\Sigma^{300}{}_{700}\, Ri \,/\, n_w$, where R$i$ refers to the sum of reflectance from 300-700 nm for an i, individual, and n_w refers to the number of specified ranges being measured. Chroma was defined as $\Sigma^{\lambda b}{}_{\lambda a}\, Ri/Bi$, where a and b define the outer limits of some specified bandwidth range, and Bi is the total brightness defined above.

I gathered data on brightness and chroma for UV, blue, green, and red using the output provided by the Montgomerie processing program. UV described the portion of the spectral bandwidth ranging from 300 to 400 nm, blue spectra ranged from 400 to 512 nm, green spectra ranged from 512 to 575 nm, and red spectra ranged from 575 to 700 nm. The red spectral region captured wavelengths appropriate for dewlap carotenoids (Steffen and McGraw, *in prep*). Thus, although xanthophyll carotenoids are known to produce yellow colors, no separation of yellow spectra was necessary. However, I used UV amplitude, a measurement that was not provided by the Montgomerie processing program, because this variable is important in describing spectral curves derived from xanthophyll carotenoids (MacDougall and Montgomerie, 2003). Xanthophylls create yellow and UV spectral peaks by absorbing maximally in the blue region. Lutein, thought to be a major xanthophyll in the dewlap of the Brown Anole (Macedonia et al., 2000, Steffen and McGraw, 2007), absorbs maximally at 450 nm (Andersson and Prager 2006). Therefore, I defined UV amplitude as the difference between short-wave reflectance minima (435-500 nm) and very short-wave reflectance maxima (UV, 320-435 nm). By this definition, UV amplitude values have a negative sign.

I first performed two-way ANOVA's to investigate effects of feeding level (high provision rate vs reduced provision rate) and carotenoid access (xanthophyll supplementation vs water) on mass loss. I then performed a two-way, repeated measures ANOVA to investigate potential change in dewlap color over time within individuals, and between main effects (food provisioning level and carotenoid access). I used this statistical tool rather than MANOVA repeated measures because it is recommended for research designs that contain a small sample size and violate assumptions of sphericity (Tabachnik and Fidell, 2001). All analyses were performed in SAS (SAS Institute, 2006) with a two-tailed alpha set at 0.05.

Results

There was a significant main effect of feeding level on mass loss in lizards during the course of this study (F = 8.56, df = 1, N = 32, *P* = 0.007), indicating that the reduced feeding rate used for this experiment was an effective form of nutritional stress. Lizards that were fed once per week lost an average of 1.22 g. of mass, from beginning to end of the study, while lizards that were fed 3 times per week gained 0.035 grams of mass (Figure 5 a). There was no significant main effect of carotenoid access on mass loss (F = 0.178, df = 1, N = 32, *P* = 0.6773), and there was no significant interaction between feeding level and carotenoid access (F = 0.0169, df = 1, N = 32, *P* = 0.8978).

Feeding level showed a nearly significant main effect on UV amplitude in the lateral dewlap region (Figure 5 b; F = 3.23, df = 1, N = 32, *P* = 0.083). Lizards in the high provision rate groups had greater negative UV amplitude (Mean = -1.584, S.E. = 0.151, N = 16) than lizards in the reduced food provision rate groups (Mean = -1.000, S.E. = 0.215, N = 16, F = 3.2, df = 1, *P* = 0.083). There was no main effect of carotenoid access on UV amplitude (F = 0.87, df = 1, N= 32, *P* = 0.36), and there was no interaction between feeding level and carotenoid access (F = 0.66, df = 1, N = 32, *P* = 0.433).

Carotenoid access had a significant effect on UV chroma in the lateral dewlap region (Figure 5 c; F = 5.89, df = 1, N = 32, *P* = 0.022). Male lizards from the carotenoid supplementation group had significantly higher UV chroma (mean = 0.113, S.E. = 0.006, N = 16) than lizards from the control group (mean = 0.086, S.E. = 0.004, N = 16). There was no main effect of feeding level on UV chroma (F = 0.16, df = 1, N = 32, *P* = 0.17), and there was no significant interaction between feeding level and carotenoid access (F = 0.62, df = 1, N = 32, *P* = 0.439).

Carotenoid access also had a significant effect on red chroma of the midline dewlap region (Figure 5 d; F = 89.15, df 1, N= 32, *P* < 0.0001). Lizards in the carotenoid supplementation treatment groups had higher levels of red chroma (mean = 0.447, SE = 0.004, N = 16) while lizards in the control group had lower levels (mean = 0.444, S.E. = 0.006, N = 16). There was no main effect of feeding level on red chroma (F = 0.06, df = 1, N = 32, *P* = 0.815), and no significant interaction between feeding level and carotenoid access (F = 0.08, df = 1, N = 32, *P* = 0.776).

Discussion

In previous studies I have demonstrated that male *Norops sagrei* differ in the degree to which UV reflectance is expressed in their dewlaps (Steffen and McGraw *in prep*), that the intensity of UV reflectance plays a role in how dewlap signals are perceived in various habitats (Steffen and Siefferman *in prep*), and that males with dewlaps that reflect more weakly in UV wavelengths win contests more often than males with dewlaps that have stronger UV reflectance (Steffen, *in prep*). In this study, I now have demonstrated that UV reflectance depends on dietary access to xanthophyll carotenoids. These findings solidify provide evidence that carotenoid-based dewlap color, especially the yellow midline region, is a condition-dependent trait in *Norops sagrei*. In this regard, *N. sagrei* possesses features of color that mirror sexually-selected color variation in House Finches. Red plumage coloration in male House Finches is maintained by a female preference for bright red plumage (Hill, 1990) created in part by dietary access to carotenoids (Hill, 2002; Hill et al., 1994). Interestingly, males with drab yellow plumage to be dominant in agonistic interactions surrounding food sources surrounding perches (McGraw and Hill, 2000 a, b). Unlike carotenoid colors in the dewlaps of *N. sagrei*, carotenoids used to pigment feathers of male and female house finches do not yield a strong UV component to color. There is some reason to expect that female choice influences male reproductive success in the Brown Anole, however, because female home ranges overlap with multiple males home ranges, and females often copulate with more than one male during the reproductive season (Tokarz et al., 1998). One anole (*Anolis carolinensis*) has special sperm storage organs, which may allow for some pattern of differential sperm allocation for egg fertilization (Fox, 1963). I have not yet tested for preferences of female *N. sagrei*, but all other aspects of color conform to the House Finch model.

Access to lutein and zeaxanthin alters UV dewlap color in adult male *N. sagrei*, and it seems likely that this alteration is created by increased absorption of blue wavelengths in lizards that ingest greater quantities of xanthophylls, as happens in bird feathers (MacDougall and Montgomerie, 2003). This effect in dewlaps of male *N. sagrei* causes a particularly striking decrease in UV amplitude in individuals with access to xanthophylls and yields stronger reflectance in the yellow wavelengths in lizards consuming xanthophylls. Previous research shows that UV color correlates positively with body condition (Chapter 2), and the results in the present paper add to evidence that carotenoid-based dewlap color may be used as a condition-dependent signal in social interactions.

In nature, anoles appear to have access to xanthophylls via two sources. Lutein and zeaxanthin are abundant in leaves of all plants. Additionally, phytophagous insects assimilate these chemicals from plant matter. Schoener (1968) presented a dietary analysis of male and female *N. sagrei* from the Bahamanian island of Bimini. Stomachs of males contained a significant volume of leaves. In adult males and females, lepidopteran larvae (which have a well-documented herbivorous life stage which sequesters carotenoids, see Czeczuga, 1986, 1990; Mummery, 1975; Rothschild and Mummery, 1985; Rothschild et al., 1986) constituted the greatest volumetric dietary item. Thus, both direct ingestion of plant material and indirect uptake through consumption of xanthophyll containing phytophagous insects seem a likely of xanthophylls in *N. sagrei* and likely for other anolines.

These results also demonstrate that dewlap UV can be increased quickly upon ingestion of xanthophylls. These experiments were run for 11 weeks, and significant increases in UV amplitude, UV chroma, and red chroma appeared after only two weeks. A typical breeding season for Brown Anoles in south Florida lasts for approximately 5 months (Tokarz et al., 1998). This rapid change in dewlap UV reflectance may be meaningful to an individual male who is attempting to send signals to receivers in order to alter its social status. Furthermore, carotenoid availability may vary geographically and differ among nearby forest locales because of differences in plant community composition, which may therefore influence breeding structure. If there is a high density of carotenoid-rich insects in a particular area, and females aggregate in these areas, then males might follow females to areas where they will turn bright, and pay a higher defensive cost compared to males from carotenoid-poor environments.

In Puerto Rico, two populations of *Anolis cristatellus* differ in habitat-based signal detectability (Leal and Fleishman, 2004). In xeric populations, dewlaps tend to be darker than dewlaps from mesic habitats. Furthermore, dewlap detection decreased significantly when a dewlap from a xeric habitat is detected under the spectral conditions of a mesic habitat, and visa versa. The major spectral difference in dewlap design between habitats was in total reflectance, but UV comprised a significant portion of this variation, and also differed between dewlaps from the different habitats. Thus, based on my findings, the differences in UV that contribute to habitat-specific differences in dewlap detectability may be proximately caused by habitat differences in carotenoid availabity.

Food availability has been shown to have an important influence on demography of at least one Caribbean, as well as two mainland anoles. Growth rates of juvenile *Dactyloa aeneus* in Grenada, West Indies, are influenced by food availability, and rainfall (Stamps, 1981). Moreover, juveniles are highly selective in their dietary habits, and prey selectivity showed a negative correlation with relative abundance for all six major prey item classes. In the lowland tropical forests of Costa Rica, experimental manipulation of food availability influenced female fecundity, male size, as well as male-male and male-female homerange overlap (Guyer, 1988a). Guyer (1994) presents demographic data that suggests that males might focus on mating opportunities and females focus on food availability, and has since experimentally confirmed a differential willingness of male and female ground anoles (*Norops humilis*) to accept food. Thus, food deprivation, in the form of reduced food availability or in the form of attention by males to other activities (like vigilance for mating opportunities), might change physiology enough to alter dewlap color in males. Moreover, research on juveniles of one species suggests that individuals might actually be able to identify particular prey items. If this is true, then anoles might actively seek (or avoid) particular prey items based on their perceived carotenoid content. Even if anoles do not discriminate carotenoid-rich prey items, it becomes apparent that dewlap color might reflect the ecological processes of food availability, and relative prey abundance, and UV dewlap color might be influenced by what and how frequently the lizard is eating.

Evidence for the use of UV-based visual signals in lizards is becoming increasingly common. UV has been documented in socially displayed color patches in several lizards studied to date (Molina-Borja et al., 2006, Lappin et al, 2006, Stapley and Whiting, 2005 Whiting et al., 2006). In these lizards, however, it appears that UV is used to establish dominance in male-male rivalry, and indicates something about ones' resource holding potential (RHP). Additionally, UV color has also been shown to be an important social signal in many other animals, especially birds (Andersson and Amundsen, 1997; Andersson et al., 1998; Siitari et al., 2002; Alonso-Alvarez et al., 2004) and fish (Kodric-Brown and Johnson, 2002; Rick et al., 2004, 2006; Siebeck et al., 2004). In all of these examples, UV shows phenotypic variation in males, which females assess and upon which they base preferences. Female choice of male UV reflectance has yet to be studied in *Norops sagrei*, and there is good reason to believe that female choice may occur through sperm competition and differential egg fertilization (Tokarz, R.R., 1998). Future research should investigate female preference for male dewlap color using behavioral experiments, and employ DNA paternity techniques to determine sire identity and dewlap color to understand if male dewlap color is used as an ornamental trait used by females to aid in mate choice decisions.

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Figure Legends

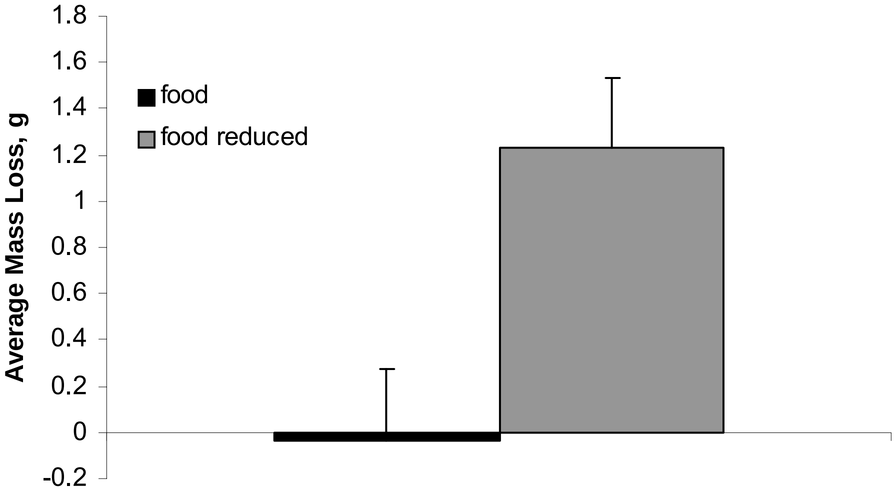
Figure 5 a. Average mass lost in high (left) and low (right) provision rate treatments. The two groups differ significantly in mass lost during the duration of the experiment (F = 8.56, df = 1, $P = 0.007$).

Figure 5 b. Effect of food deprivation on repeatedly measured values of UV amplitude in the lateral dewlap region (F = 3.23, df = 1, $P = 0.083$).

Figure 5 c. Effect of xanthophyll supplementation on repeatedly measured values of UV chroma for the lateral dewlap region (F = 5.89, df = 1, $P = 0.022$).

Figure 5 d. Effect of xanthophylls supplementation on repeatedly measured values of red chroma of the midline dewlap region (F = 89.15, df = 1, $P < 0.0001$).

Figures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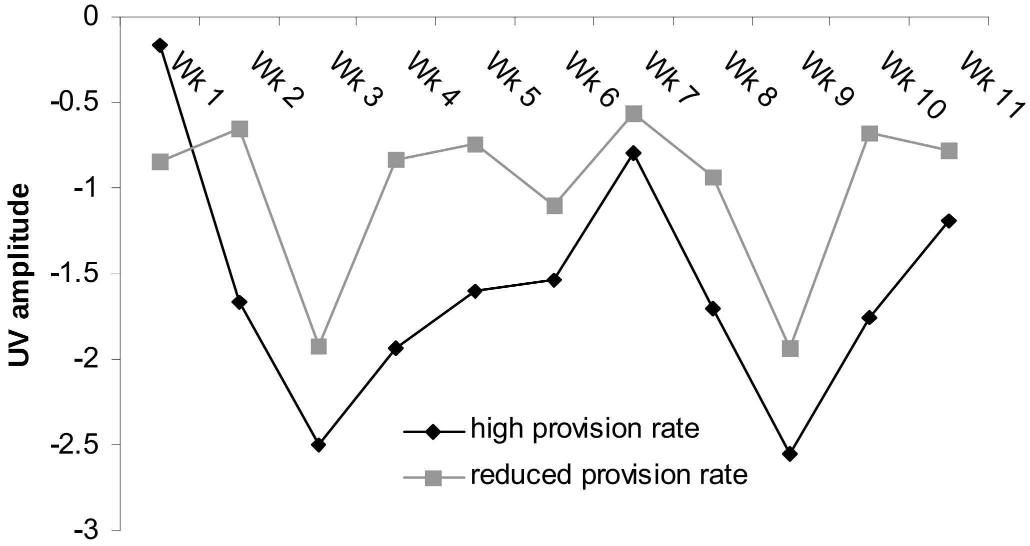
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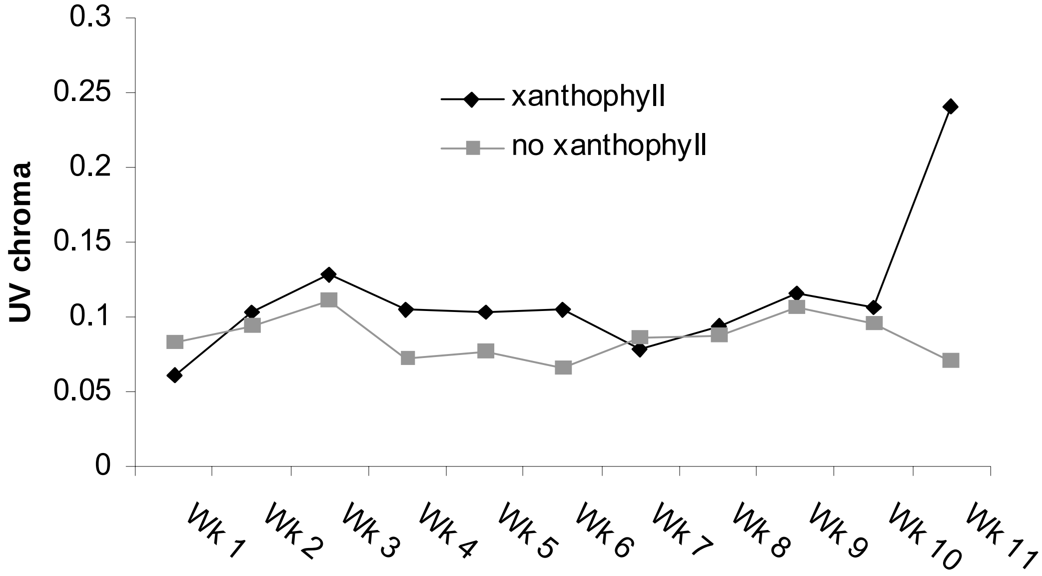
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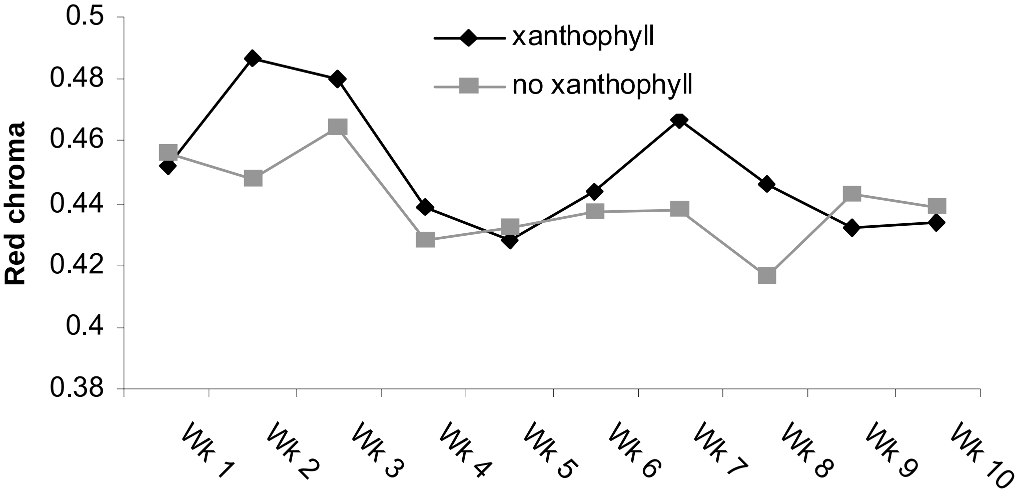

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