Habitat Light and Signal Color Evolution in 5 Species of Anoles from the Dominican Republic, and The use of a Feeding Assay to Test the Effects of Visual Color Contrast on Stimulus Visibility in the Lizard *Anolis sagrei*

By

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Abstract

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Anolis lizards have excellent color vision and depend on their eyesight to detect visual signals made by other anoles. The dewlap, a colorful and expandable flap of skin, lies beneath the jaw in male anoles and is a primary signaling structure used for territorial and courtship displays. This paper focuses on dewlap color diversity and its evolutionary rudiments. With this in mind, the research team traveled to the Dominican Republic and collected natural habitat light data and dewlap and body color data from the local anoline inhabitants. We hypothesized that light environment would be the main driver behind dewlap color diversity. However we found that among the five species we sampled, which all had different dewlap colors, that the light environments were indistinguishable. We think that instead driving dewlap color diveristy, habitat light environment may set the constraints to which dewlap colors can be utilized in specific environments. In a laboratory at Union College, we ran three experiments using a feeding assay to test how anoles visually discriminate objects. In one experiment we manipulated hue, in another we manipulated hue and luminance, and in the third we manipulated hue in a low-light setting. We hypothesized that hue contrast would allow for easier detection, however, we believed that when luminance contrast was also present hue contrast would be less important for object detection. The data supported both hypotheses. Unfortunately the anoles failed to respond in the final experiment, preventing us from making any presumptive conclusions.

Introduction

Anolis is a diverse genus of small lizards in the family Iguanidae that inhabit an assortment of vegetative areas across species. Many species also differ in heat tolerance and mobile ability, and thus different species living in sympatry will occupy different

vegetative structures within that area. For instance, some forest living species strictly occupy the crown of a tree, while others occupy the crown and trunk, strictly the trunk, or the trunk and ground near trees (Losos, 2009). These differences in forest location relate to differences in the types of light environment anoles are exposed to (Fleishman, 2001). And despite the variability in light environment, anoles primarily depend on visual signals, consisting of color and motion, to communicate with one another (Fleishman, 2001).

Before delving into the details of the visual system of the anole, it should be made clear that most visual systems --including human, bee, chicken, goldfish, pigeon, primate, and of course lizard – share a number of basic anatomical and physiological properties. The neural basis of color perception for these animals is strikingly similar. This makes *Anolis* an appropriate model genus for trying to understand principles of color perception in natural environments (Kemp, et. al., 2015). Since *Anolis* encompasses a diverse group of animals we can assume basic principles and apply them broadly. Now back to the anoline visual system. Again, these lizards have excellent eyesight and color vision. Similar to the human eye, the eye of the anole contains three classes of cones that are responsible for receiving and translating color information from stimuli of short, medium, and long wavelengths. In addition, the anole eye contains a fourth type of cone that detects wavelengths in the range of ultraviolet light. However, in our experimental studies, we remained inside the boundaries of human color vision and did not explore the function of the ultra-violet receptor (Fleishman and Persons, 2001).

The perception of color, or hue, is dependent on the ratio of stimulation between the different types of cones: specifically the ultraviolet, short, medium and long wavelength receptors, which correspond to ultraviolet, blue, green/yellow, and red light respectively. For instance, an object that primarily reflects long wavelengths as opposed to short wavelengths will appear red rather than blue. Luminance, another aspect of color, is related to brightness and is determined by both the intensity, the number of photons received by the receptors per second, and the energy carried by the wavelengths emitted. The third parameter of color is chroma, the purity or saturation of a color. However we

considered this parameter less crucial when considering color, as a visual signal, and I will explain why later.

Nearly every species of *Anolis* possesses an expandable throat fan of skin called a dewlap, which is located beneath the lower jaw in male anoles. This segment of skin typically deviates from the color and or pattern of the rest of the body, and across *Anolis* species dewlap appearance is extremely variable. The dewlap is the anole's primary signaling structure, and the animal vision and communication scientists that have studied this colorful segment of skin have determined that its function may hold multiple purposes. For one, the dewlap can be used to signal territorial ownership. Most male anoles are highly territorial and one way to increase your apparent size to any encroaching males is to utilize the hyoid bone that opens your dewlap (Fleishman and Persons, 2001). Another study suggests the dewlap flash may function as a courtship display, attracting females to a male's territory, or even evoking reproductive processes in the females that detect the signal (Fleishman et al, 2009). The possible functions listed above are plausible yet they do not answer the question any evolutionary biologist would ask: Why do species distinctly differ in dewlap color and orientation, and what are the potential benefits behind such diversity? Evidence from one study suggested that dewlap patterns and colors diversified so anoles could more easily detect dewlaps in the environment as well as discern different species (Fleishman and Persons, 2001). Nonetheless, all of the possible functions are what make the dewlap such integral visual signal to anoline communication. Our research team traveled to the Dominican Republic to record the dewlap color of several species, as well as habitat light data, which we believed was at the root of dewlap color diversity.

In general, visual signals are comprised of four main characteristics: intensity of signal, spectral composition (color), spatial composition (size, shape, location/position), and the temporal variability of the three aforementioned characteristics. (Fleishman and Persons, 2001). Spectral composition is primarily determined by hue and slightly affected by chroma, and this is why we did not manipulate chroma as an independent variable in any of our experiments. In our first experiment, we sought to solely manipulate hue by

controlling for all other characteristics of color, in an effort to gather evidence that anoles can detect differences in hue. We hypothesized that the lizards would better discriminate objects that contrasted in hue rather than objects that matched the background color. We created a feeding assay, using Pheonix worms and powdered food coloring to test this effect. In another experiment we manipulated hue and luminance. Here we were interested in exploring a possible interaction between hue and luminance on object saliency, and we hypothesized that when luminance contrast was also present hue contrast would be less important to object saliency. For a third experiment we decided to replicate the first, however in a low-light setting. This time we sought to find the brightness threshold needed for color discrimination.

Methods

I. Field work

The research team traveled to the Dominican Republic to search and record data from the country's various anoline species. Our first goal was to locate habitats where anoles were abundant, as well as to identify distinct species. Our home base was in the province of Barahona, and the majority of our observations took place on trails that fell were in the vicinity of our hotel, Casa Bonita. The nearby trails as well as nearby forests provided plenty of light habitat data from five species – *Anolis brevirostris*, *A. coelestinus*, *A. cybotes*, *A. distichus*, and *A. olssoni*. Once in the field we utilized a spectroradiometer (Ocean Optics Jazz) with an attached fiber optic cable with a 2 degree acceptance angle radiance probe on the end to measure radiance and irradiance in the natural environment. We moved along the paths until we spotted an anole. We observed the anole from roughly 3 meters away for up to 10 minutes or until it displayed its dewlap. We then walked up to the perch to record radiance and irradiance from the precise location where the lizard was observed. For radiance measurements, we pointed the fiber optic left and right of the display site, perpendicular to the dewlap location of the observed lizard. For irradiance we employed a diffuse white standard oriented parallel

to the ground, and directed the fiber optic cable at the white standard. The white standard reflected all the wavelengths of light that struck it over a full hemisphere normal to its surface. For irradiance left readings the white standard faced right and for irradiance left readings the white standard was oriented to the left. We compiled both irradiance left and right readings to collect data on the light that would strike the surface of a dewlap from each side, and collected radiance readings in both direction to measure the natural spectral composition of the background against which the dewlap would be viewed. Besides radiance and irradiance, light intensity, the time of day, the presence of the sun, the color composition of the sky, and the height of the observed anole's perch were recorded.

Other times we ventured into the field to capture lizards for color measurements. During these trips, we caught anoles with our hands or with modified Cabela's Pan Fish poles, to which we tied a dental floss noose to the end. We then placed the anoles in plastic bags and brought them indoors for dewlap and body color recordings. In these instances, the lizard was placed in a small holder, which securely oriented the eyes and neck forward, allowing the hyoid bone to be pulled down by forceps mounted on an x-y microscope stage, ultimately exposing the dewlap**.** (Fleishman et al, 2009). With the spectroradiometer, fiber optics cables, and a pulsed xenon light source that mimicked the wavelengths produced by the sun, we were able to record transmission and reflectance readings from the dewlaps and reflectance readings from the body of each captured anole, relative to the light reflected from the diffuse white reflectance standard. We gave the anoles several hours to habituate after capture before making dewlap and body readings because some lizards exhibited color changes due to the stress of capture. With dewlap reflectance, dewlap transmission, and irradiance readings from each side, we were able to calculate the hue of the dewlap, as it would appear at each natural sampling site. With the reflectance readings from the body, as well as the irradiance readings we were also able to calculate the body color of the various species measure as perceived at each light sampling site. We repeated this procedure numerous times.

We plotted dewlap and body colors, as well as radiance and irradiance readings made in the field, on tetrahedrons whose vertices represented each of the four cones anoles rely on for color vision (ultraviolet sensitive (UVS), short wavelength sensitive (SWS), middle wavelength sensitive (MWS), and long wavelength sensitive (LWS). For each spectrum (habitat irradiance and radiance, dewlap and body spectrum based on field-measured irradiance values) we determined the stimulation of each cone class, and then divided by the total of all four. From this we determined a relative stimulation value (from 0-1, fro each cone class). Again, we plotted dewlap color, body color, and background color in three-dimensional space that coincided with anole color perception. A data point close to a particular vertex indicated strong stimulation of that cone, and a data point near the opposite face of a vertex indicated very little stimulation of that cone. The next morning we photographed the dewlaps of several anoles against a color pallet for a visual comparison, and hand released each subject to its respective habitat.

Vasserstats was utilized to perform t-tests and ANOVAs when comparing habitat light intensity measured in the field.

II. Laboratory Study

In a separate study, *Anolis sagrei,* commonly referred to as the brown anole, was our subject for behavioral experimentation in our laboratory at Union College. A total of twelve anoles served as participants for the experiments. Several anoles died during the experimental phase and were replaced with anoles that were accustomed to the testing environment. The anoles were stored in the experimental room, which was set at 85° Fahrenheit with roughly 50% humidity, and had a twelve-hour light cycle, from 6am to 6pm.

In preparation for the experiments, we trained our subjects to eat the stimulus material, painted Phoenix worms, by stimulating hunger and first exposing the anoles to live Phoenix worms against a clear background. After the lizards demonstrated they would eat the live worms, we proceeded with dead Phoenix worms. Once subjects demonstrated a willingness to eat the dead Phoenix worms, we began to paint dead

worms with powdered food coloring and utilized these worms as a feeding assay to determine particular aspects of anoline color perception. We used *Sweet X Dreams* powdered food coloring, small paintbrushes to color the worms, and plastic feeding dishes, approximately 4 inches in length and width. We chose pink and avocado powders for training and experimental trials. The pink powder did not adhere well to the skin or hair of the frozen Phoenix worms so instead of painting frozen worms, we removed the heads of the worms and allowed their bodily fluids to secrete and create a sticky surface that was better for powder adherence. Initially, we tried using water to increase adherence, however it changed the appearance of the colored worms. After the majority of subjects would feed on the painted worms, we manipulated the background color and exposed our subjects to painted worms on pink or green backgrounds. Each anoline subject was exposed to worms of each color and each colored background to eliminate choice based on preference. After a minimum number of eight subjects demonstrated the ability to eat the dead painted worms off the colored background, we could deploy the experimental trials.

In the first set of trials, we painted worms pink or avocado and placed them on a pink background. For the background color, we employed calibrated Munsell colored paper that closely matched the pink and avocado powders in hue and in luminance. One worm matched the background color and background luminance, and the other matched the background luminance but not color. Next we ran the same choice experiment but with the other background, so again one worm matched the background in color and luminance, while the other differed in color. In the next set of experiments, we kept the powder for coloring the worms constant, but chose new backgrounds that matched the powdered colors in hue but contrasted in luminance. Specifically we chose lighter pink and lighter avocado calibrated papers. The luminance contrast between the background and worms was +10%, respectively, as measured with a fiber optic cable and a spectroradiometer**.** Again in this second set of experiments, painted worms either matched or contrasted the backgrounds in hue but always contrasted the background color in luminance. Finally, a third experiment, a low light experiment was deployed. It

was the same as the first experiment because luminance remained constant and hue was manipulated. However this time we turned off the room's overhead lights and covered all but three heat lamps with aluminum foil, bringing the well-lit experimental room down to a very dim room. Before presenting the stimulus, we allowed the lizards to acclimate for ten minutes. We utilized the binomial test for statistical measures for the three laboratory experiments.

Results

Over the eleven days spent in Barahona we were able to obtain spectral data from *A. brevirostris*, *A. coelestinus*, *A. cybotes*, *A. distichus*, and *A. olssoni*. The background radiance spectrum for each species displays a sharp peak around 560nm.

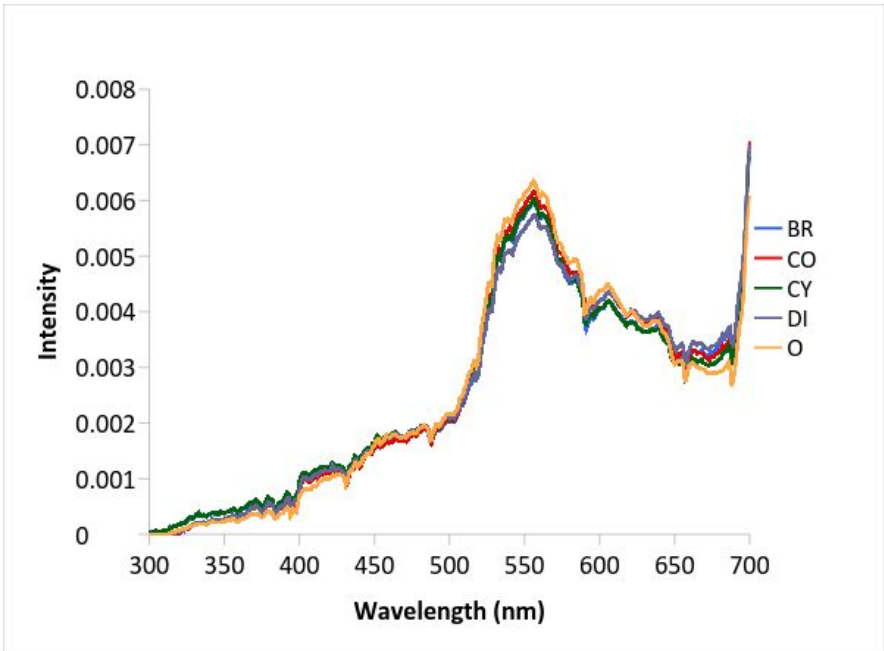

Figure 1: Intensity (units of spectral radiance) on the y-axis plotted against wavelength (on the x-axis) across *A. brevirostris* (BR), *A. coelestinus* (CO), *A. cybotes* (CY), *A. distichus* (DI), and *A. olssoni* (O)

This graph reflects strong stimulation of the middle and long wavelength cones, appearing as green to an anole or a human for that matter. There were no statistically significant differences in relative shape (spectral composition) of radiance spectrum

across the five species, however radiance light intensity differed significantly in one anoline species.

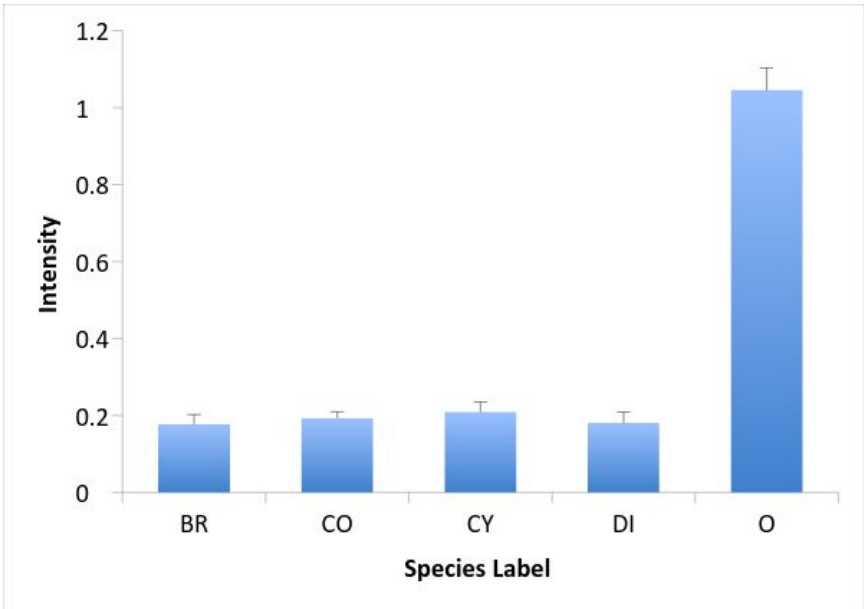

Figure 2: Radiance habitat light intensity compared across across *A. brevirostris* (BR), *A. coelestinus* (CO), *A. cybotes* (CY), *A. distichus* (DI), and *A. olssoni* (O). Radiance, a unit of light intensity, is on the y-axis and *Anolis* species is on the x-axis.

The average radiance intensity in units of Log (1 + radiance, with radiance units equal to µmol m^{-2} s^{-1} sr^{-1}) for *A. olssoni* was 1.045, while the others were 0.117**,** 0.193**,** 0.209**,** and 0.181 for *A. brevirostris*, *A. coelestinus*, *A. cybotes*, *A. distichus,* respectively. ANOVA < 0.0001, with respect to radiance intensity of *A. olssoni* compared to remaining four species.

The spectral irradiance readings were also very similar (no statistical differences) across the five species with the exception of *A. olssoni*, whose habitat demonstrates a slightly broader spectrum.

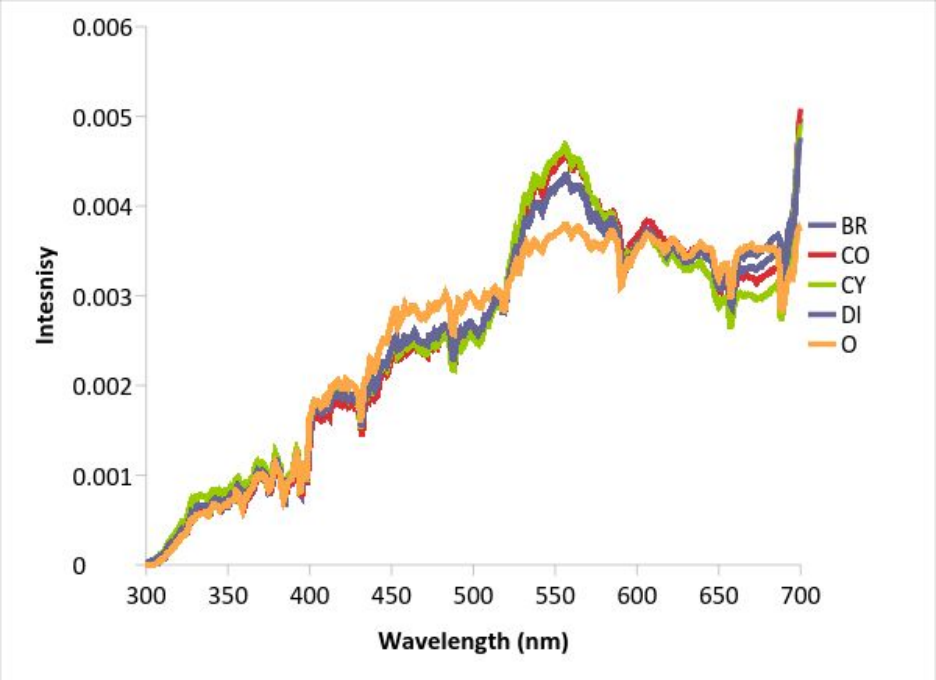

Figure 3: Light intensity (units of spectral irradiance) on the y-axis, plotted against wavelength (on the x-axis), across *A. brevirostris*, *A. coelestinus*, *A. cybotes*, *A. distichus*, and *A. olssoni*.

A. olssoni's spectrum displays peaks of relatively the same height or stimulation from 520nm to the 700nm, the terminal boundary of visible light. This spectrum resembles that of a sunlight spectrum. On the other hand, *A. brevirostris'*, *A. coelestinus'*, *A. cybotes'*, and *A. distichus'* irradiance spectra reflect that of their radiance spectrum because again we see a main peak around 560nm. The trend of *A. olssoni* as the sole outlier is consistent because yet again it differs from the other aspect of habitat light environment. This time in respect to average irradiance light intensity.

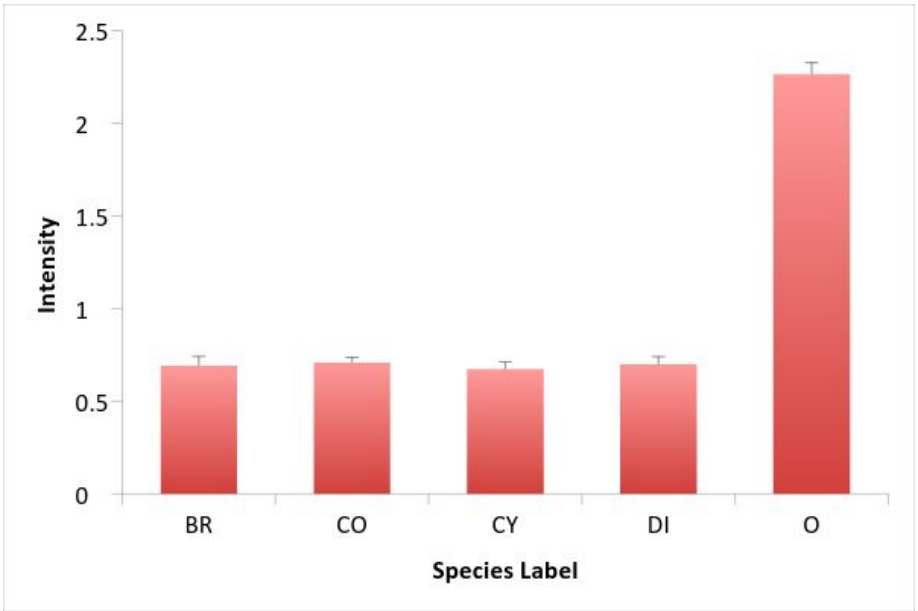

Figure 4: Habitat light intensity (log [1+irradiance]) plotted on the y-axis compared across across *A. brevirostris*, *A. coelestinus*, *A. cybotes*, *A. distichus*, and *A. olssoni*, on the x-axis.

The log (1+average irradiance intensity, in units of μmol m^{-2} s^{-1} for *A. olssoni* was 2.265**,** *A. brevirostris* 0.691**,** *A. coelestinus* 0.709, *A. cybotes* 0.673**,** and *A. distichus* 0.700. ANOVA < 0.0001, with respect to radiance intensity of *A. olssoni* compared to remaining four species.

We plotted dewlap color, body color, and background color for each field measurement location on plots of tetrahedral color space whose vertices represented short, medium, long, and ultraviolet cones. Are main focus was not the just the color of each species' dewlap, body, and background, but rather the relation or interaction between the three variables. What we found was that within each species, dewlap colors and background colors differed in color space, however body colors and background colors were relatively similar in color space or perception. For each tetrahedron presented green spheres represent the background color; light spheres represent dewlap color; darker spheres represent body color.

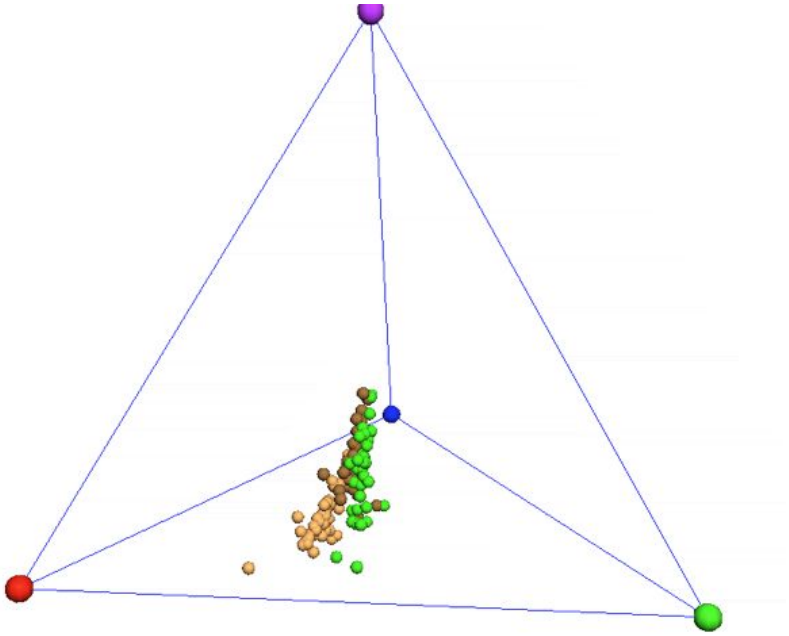

Figure 5: *A. brevirostris* Dewlap: light brown; Body: dark brown; Background: green

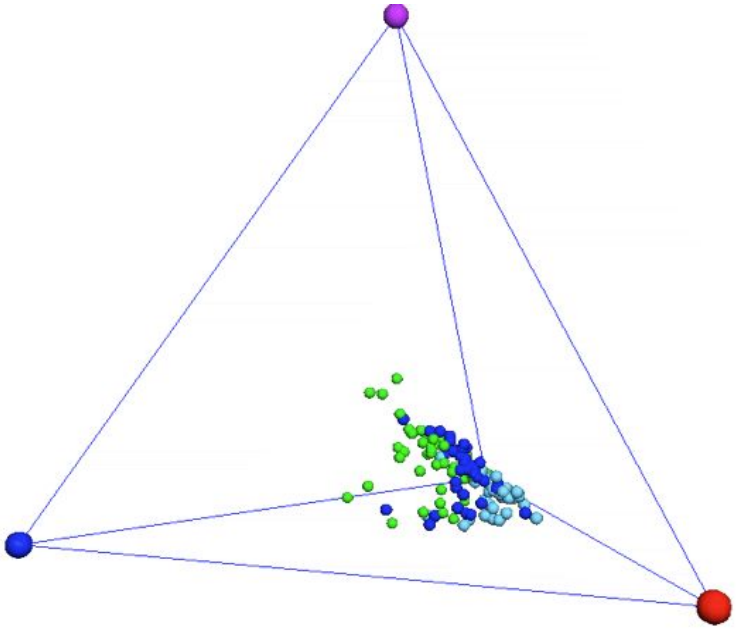

Figure 6: *A. coelestinus* Dewlap: light blue; Body: dark blue; Background: green

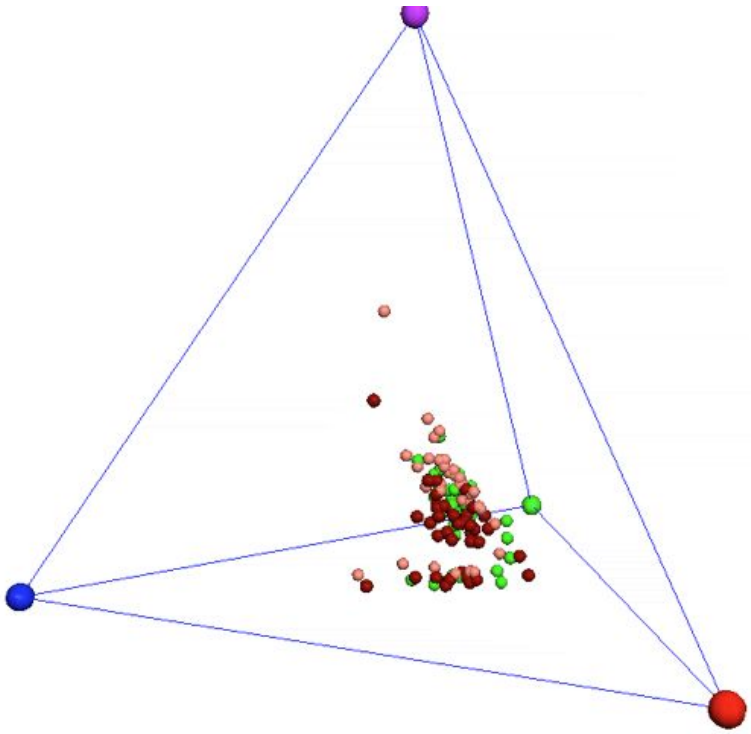

Figure 7: *A. cybotes* Dewlap: light pink; Body: dark red; Background: green

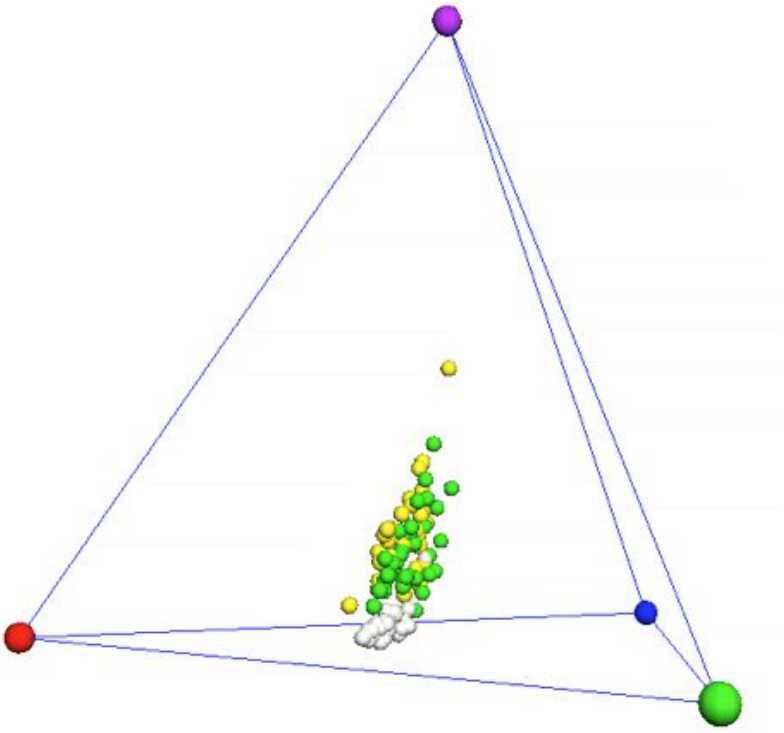

Figure 8: *A. distichus.* Dewlap: white; Body: yellow; Background: green

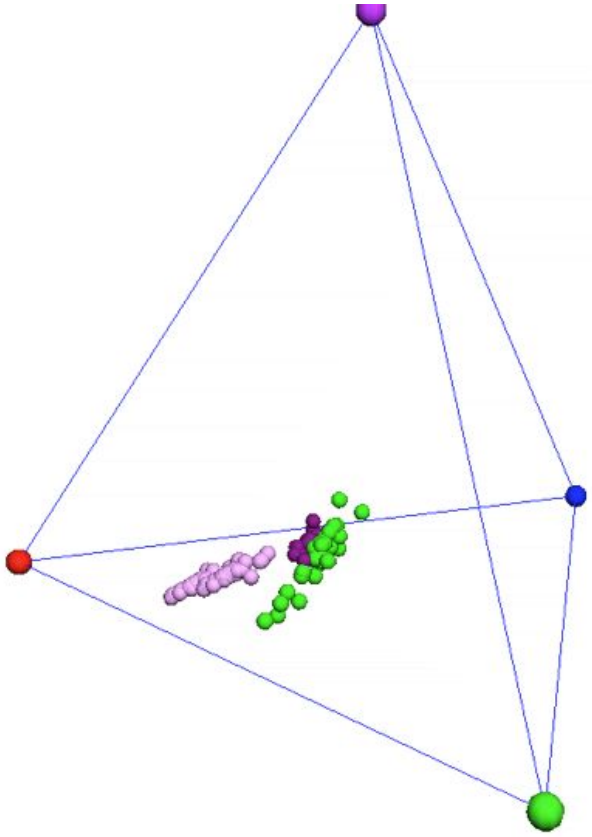

Figure 9: *A. olssoni.* Dewlap: light purple; Body: dark purple; Background: green

Then we compared dewlap color across the five measured species to understand if the anoles could in fact distinguish their cohabitants' dewlaps solely based on hue. Our color space data suggest they could.

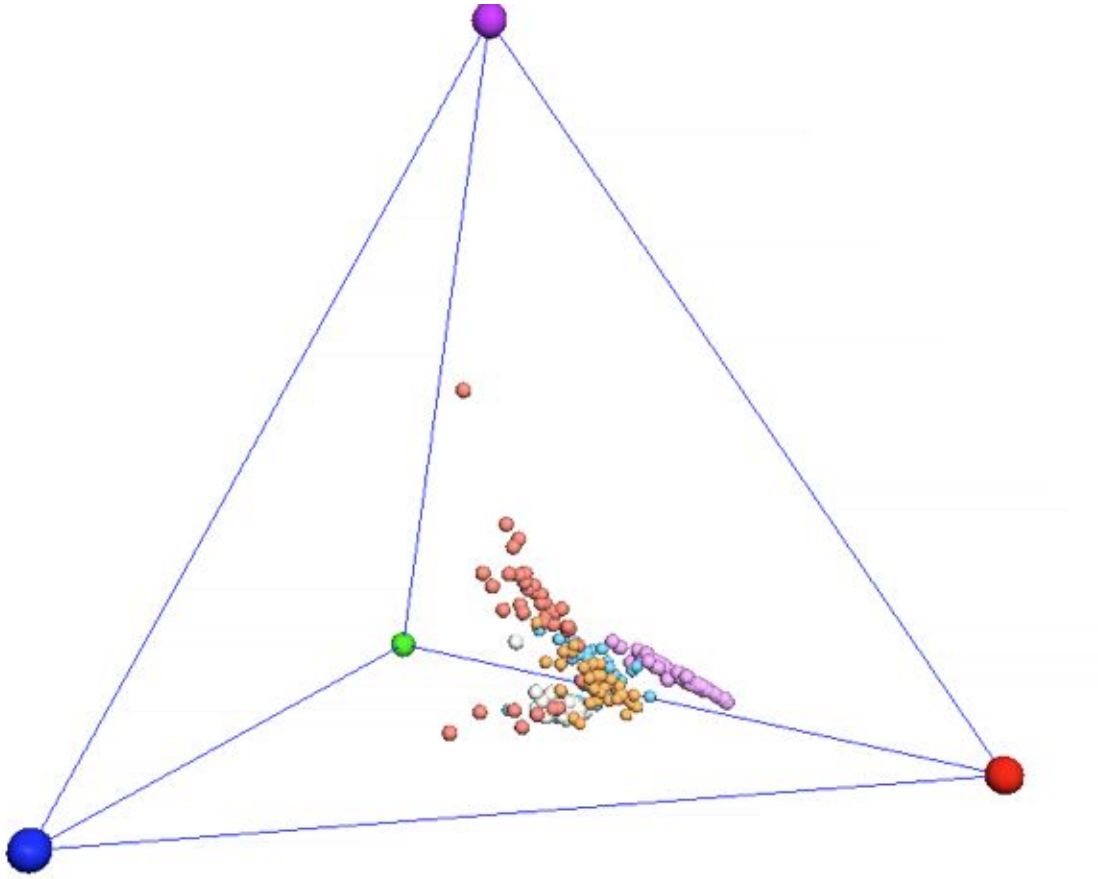

Figure 10: Dewlap color as represented by relative stimulation of short (blue), middle (green), long (red), and uv cones (purple), across *A. brevirostris* (orange) , *A. coelestinus* (blue), *A. cybotes* (pink), *A. distichus* (white), and *A. olssoni* (light purple)

Following the trip, we traveled back to the United States, and in the Fleishman laboratory, created the feeding assay to test the effect of hue and luminance contrast on color perception in *A. sagrei*. In the first experiment we controlled for luminance to solely test the effects of hue. Across the two conditions, avocado or pink background, the anoles fed on the worm that did match the background 14 out of 16 trials, leaving only two anoles that fed on the worms that matched the background in appearance. The lizards significantly shoe the non-matching food item. ($P<0.05$, binomial test).

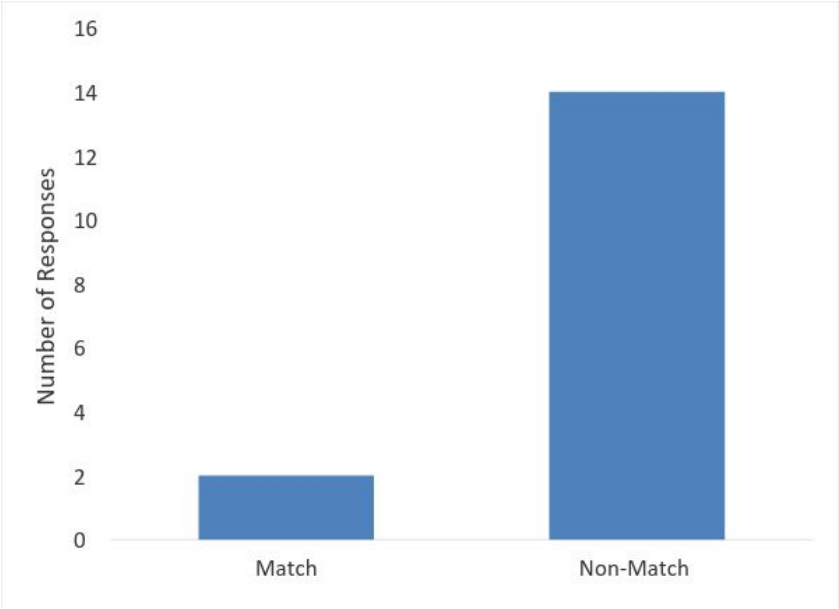

Figure 11: Object (worm) salience plotted on the x-axis, against the total number of responses as measured by feeding (on the y-axis). Hue manipulated.

The second experiment mirrored the first, however this time we manipulated luminance as well as hue. The Phoenix worms either matched or contrasted the background in terms of hue, but always were slightly darker or less luminous than the background. Lizards fed on the worms that contrasted the background hue 6 out of the 16 trials, meaning that in 10 of the trials, the participants fed on the worm that matched the background in hue. ($P>0.05$, binomial test)**.**

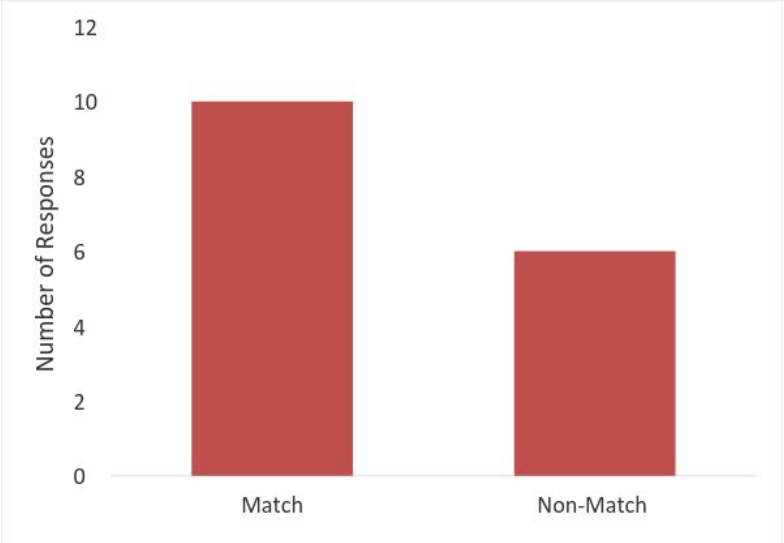

Figure 12: Object (worm) salience on the x-axis plotted against the total number of responses as measured by feeding (on the y-axis). Hue and luminance manipulated.

Our final experiment, the low light experiment, unfortunately did not yield any results because the anole subjects failed to cooperate –did not feed on either of the choices presented.

Discussion

We hypothesized that the light environment, as measured by radiance and irradiance, would influence dewlap signal design. Specifically we were interested in the diversity of dewlap hue. Our spectral data suggests that the species *A. brevirostris*, *A. coelestinus*, *A. cybotes*, *A. distichus*, and slightly *A. olssoni* inhabit regions of the forest that are indistinguishable in terms of spectral composition (Figure 1). The dewlap colors of these respective species are different, based on the tetrahedral plots of anoline perceptual space (Figure 10). Thus this finding does not support our main hypothesis, however the idea that species identification drives dewlap color diversity remains viable. Although we cannot conclude that light environment drives dewlap color diversity, perhaps spectral composition of the light environment sets the constraints to which colors can be utilized. Note that in every species, dewlap color contrasted with the respective

background color (Figures 5-9). In the future we plan to explore this notion, and look more into how light intensity influence dewlap signal design.

The hypothesis that was the basis for our first behavioral experiment was that anoles would better discriminate objects that had a greater color contrast with the background as compared to objects with less color contrast with the background. The data from this experiment supports this notion, as the subjects ate the worms that contrasted with color with more frequency than those that matched the background in color (Figure 11). Among the five species we observed in the field, and across every specimen we recorded for that matter, the respective dewlap differed from their respective background and body, in color composition. With this information on anoline color perception and aspects of their natural light environment, I argue that dewlap color has evolved to maximize chromatic contrast, but not dewlap-background contrast, since there is evidence of different dewlap colors in the same habitat light environment (Figures 5-9). For our second experiment, we hypothesized that when brightness contrast was present, color contrast would be less important, simply because differences in brightness would supply enough contrast for easy recognition. Again the data supported our hypothesis because in this setting we did not observe significant differences in the amount of feeding between worms that contrasted and matched the background (Figure 12). Once more, our third, low-light experiment did not yield any interpretable results, so unfortunately we can make any conclusions, however we plan to repeat the experiment later in the year and perhaps with a fresh group of lizards, which would inherently be subject to less stress. With the data we collected, it is evident that the dewlap was designed to stand out. Specificcally, it seems that dewlap color has evolved in response to selective pressures that favor contrast between the dewlap and the background. Simultaneously, the dewlap colors of sympatric species diverged so anoles could better discern the species of thier cohinhabitants, resulting in the dewlap color diversity many scientists have observed.

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