The Use of Color Vision
& Color Communication in Lizards

By

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Submitted in partial fulfillment
of the requirement for
Honors in the Department of Biological Sciences

UNION COLLEGE
June, 2012

CHAPTER TITLE**:** The Influence of Signal Function on the Evolution of Dewlap Color in Puerto Rican Anoles

ABSTRACT

HEREHA, VASYL The use of color vision and color communication in lizards. Department of
Biological sciences, June 2012.

ADVISOR: LEO FLEISHMAN

The reptile genus *Anolis* is a widespread and diverse group in the Caribbean region of North and South America. Most anoles have a colorful throat flap known as the dewlap, which they likely use for social behavior and communication. Dewlaps vary between anole species in terms of color and thickness (related to brightness). It has been experimentally demonstrated that anoles can detect a stimulus based on chromatic contrast and brightness contrast of the stimulus to the background. Therefore, we hypothesized that anole color diversity in the Puerto Rican anoles evolved to either (1) contrast the habitat background to appear detectable or (2) appear different than other dewlap colors which function for species recognition. We sampled light spectra at display sites for four anole species and gathered dewlap reflectance and transmission data for six dewlap species (seven dewlap colors). We also generated 82 random dewlap colors. We compared the detectability and chromatic contrast to the background for actual dewlaps and random dewlaps, in each environment. The actual Puerto Rican colors were all consistently more similar with each other, than random colors were with the Puerto Rican dewlaps. This observation does not support our species recognition hypothesis. Three of the five dewlaps were more detectable in their home habitat than 50% of the random

dewlaps. It is unclear whether dewlaps are evolving towards being more detectable than random based on this small sample.

INTRODUCTION

The use of colors in animal communication is widespread because of the many ways that animals can manipulate ambient light rays to either appear visible or discrete in their environment. Visual signals are created through methods such as: reflection of light off visual pigments on the animal's body, the animal's body movements, or self-generated luminescence. The reflection of light is one of the more common signaling techniques that an animal can utilize. In such a case, chemical pigments or structural features can help the animal blend-in with, or contrast against, the background environment, through color, brightness, pattern and/or texture contrast (Bradbury & Vehrencamp 1998). In some cases, closely related species vary greatly in visual signal design, a phenomenon that leads one to ask about the evolutionary pressures driving this divergence. The sensory drive hypothesis has been proposed as a possible factor causing divergent signal evolution. The hypothesis states that natural selection will favor the evolution of signals that effectively elicit the receiver's visual system and under the specific habitat conditions where the signal occurs (Leal & Fleishman 2004). In addition to habitat conditions and the receiver's sensory system, factors such as signal function, signal cost and the organism's evolutionary history must be considered to understand all of the forces driving the evolution of a visual communication.

The genus *Anolis* is a large group of lizards that has become a model for evolutionary biologists studying many adaptations, including visual signal radiation. Over 360 species of anoles have been discovered, and new species are observed each year. Their range extends through Central American (including many

Caribbean island) and parts of North and South America. Anoles are visually reliant reptiles that use motion and color cues for communication. Almost all species of anoles have a colorful and extendible structure on their throats, called a dewlap. The dewlap is usually more pronounced in males than females and females of some species lack a dewlap altogether. In addition to variation between sexes, the dewlap varies greatly in color and pattern between different species (Losos 2009, and references within). Dewlap variation may play a significant role in speciation but the forces responsible for this richness of diversity are not fully understood.

First let us consider the driving force of signal function as it relates to signal design. Male anoles display frequently, without an obvious signal target, but the territorial nature of anoles leads some biologists to hypothesize that the signal is directed at other adult lizards in adjacent territories (Fleishman 1992). The signal may be targeting: (1) other male lizards to warn them against entering the territory of the signaling male, (2) female lizards to entice them to enter the territory and mate with the signaling male. It is also possible that the displays are directed at potential predators to prevent a pursuit of the signaling male (Losos 2009). In the case of signaling to other members of the same species, signal function may be reduced to three main hypotheses: [1] the signal is used to increase the *detection* probability of the signaling male, [2] the signal is used to convey content and the receiver can make *discriminations* about the quality of the signaling individual, and/or [3] the signal is used for species *recognition* (Fleishman 2000). Little or no evidence exists to support the theory of *discrimination* between individuals because

dewlaps do not very greatly between individuals in a population and it is unclear whether anoles have the capacity to discriminate between small differences in color.

The hypothesis that *detection* probability drives signal evolution is related to the sensory drive hypothesis, and does have reasonable support. To build a case for the *detection* hypothesis one must argue that (1) natural selection has lead to the evolution of the visual system to be suited for specific habitat light conditions, and/or (2) that the individual elements of the dewlap have been selected to be most detectable in specific light conditions. Both of these cases depend on the existence of varying habitat light conditions between species. When observing the different Puerto Rican anoles in the wild, one may anecdotally say that the differences are evident, but one must provide actual differences in habitat light intensity or spectral composition. To quantify these properties, one needs to make two types of light measurements: (1) radiance, which can be defined as the light incident on a surface, emitted from a solid angle of the habitat background and (2) irradiance, which is the light incident on a surface from a full hemisphere of light emanating from the background (Bradbury and Vehrencamp 1998). Both of these values carry information about the habitat light spectra or brightness, but they differ in the angle of the light that they examine. Fleishman (2009) experimentally demonstrated that some differences did exist among the habitats of four Puerto Rican species (*A. gundlachi, A. pullchellus, A. krugi,* and *A. christatellus*). The light intensity varied significantly between all of the species, except between *A. krugi,* and *A. christatellus*. Habitat brightness equaled the product of the background radiance and spectral sensitivity of the lizard, summed across all wavelengths. Background spectral

radiance varied only slightly, with all environments having a predominantly green background color. Our study will look further at this question of varying habitat light conditions.

Assuming that varying light conditions do exist between species, one can then look for differences in visual system physiology. The retina of the anole eye contains cone photoreceptor and the cones contain two major components: visual pigments and oil droplets. Visual pigments are vitamin derived molecules that have a peak absorbance wavelength of light incident on the eye, while oil droplets act as filters and remove certain wavelengths of light and in turn narrow the spectra that reaches the pigments. Loew et al. (2002) sampled 16 species of anoles and one likely ancestral relative of the anoles, finding that the species did not very significantly in type of visual pigments. All tested species had four photoreceptors and all except one species, *A. carolinenses*, had vitamin A_1 (retinal) derived photoreceptors, while the exception had Vitamin A_2 derived visual pigments. The average max absorbances of the species with retinal pigments were 564nm for the long-wavelength-sensitive (LWS) pigment, 495nm for the medium-wavelength-sensitive (MWS) pigment, 455nm for the short-wavelength-sensitive (SWS) pigment, and 365nm for the ultraviolet-sensitive (UVS) pigment. The oil droplets, on the other hand did have some interspecies variation. The spectral sensitivity of each type of cone of a lizard can be calculated by multiplying the normalized visual pigment absorbance by the normalized oil droplet transmission, so oil droplet variation contributed more to differences in visual system composition. Nevertheless, one may wonder why anoles have such a conservative visual system, when it comes to

visual pigments, despite occupying different photic environments. One explanation may be that different species have evolved relatively recently and have not yet diverged. One may also claim that visual systems have evolved because of a different selective pressure than habitat irradiance, such as habitat radiance, which is dominated by green light (Loew et al 2002). The identification of the four classes of photoreceptors suggests that anoles do have the anatomy necessary for color vision, but they must also have the neural pathways to analyze inputs on the photoreceptors.

Since there is little evidence that the visual system has undergone evolutionary divergence to aid with signal detection, one may then search for evolution of dewlap components. One can hypothesize that the dewlap evolved in a manner to appear most visible (and in turn most detectable) in specific habitat light conditions. It has been experimentally demonstrated that signal detectability depends on two main factors in anolid lizards: (1) brightness contrast between the stimulus and the background and (2) chromatic contrast between the stimulus and the background (Fleishman and Persons 2001). The perception of brightness (intensity) is achieved when the nervous system sums the excitation of different classes of cones, which is known as the achromatic neural channel. The perception of color is achieved when the nervous system compares the excitation of different classes of cones, which is known as the chromatic neural channel (Fleishman and Persons 2001). It is also important to note that all cone types do not contribute equally to the achromatic pathway/ The S (short wave sensitive), UV (ultraviolet sensitive) and M (middle wavelength sensitive) cones in anoles are much less

common than the L (long wavelength sensitive) cones. As a result the lizards' spectral sensitivity is largely dependent on inputs on the long-wavelength cones. Brightness contrast and chromatic contrast made a similar contribution to positive detection (Persons et al 1999, Fleishman and Persons 2001). Chromatic contrast was shown to be especially useful in detecting blues and ultraviolets against a green background because the short and UV cones do not contribute heavily to brightness perception (Fleishman and Persons 2001).

The Fleishman and Persons (2001) study was significant not only because it identified the two most important factors impacting signal detection, but the study also created a multi-linear regression function to predict detection probability based on the independent variables of brightness contrast and chromatic contrast. The equation can be expressed as $p = 0.400C\text{b} + 0.429C\text{c} + 0.156$, where the p is the detection probability, Cb is brightness contrast and Cc is chromatic contrast. Brightness contrast is simply a comparison of habitat background brightness (Bb) and stimulus (dewlap) brightness (Bs), as defined by the formula: $C\text{b} = (B\text{s}-B\text{b})/(B\text{s}+B\text{b})$. Chromatic contrast is a more complicated calculation because the chromatic channel is dependent on a comparison between cones. Therefore, the color of the dewlap or background can be calculated using the relative stimulation of cones on a scale of 0 to 1, where 0 indicates no stimulation of the given cone and 1 indicates 100% stimulation of the given cone. Different colors can be reduced to four relative cone stimulation values (0 to 1) and can then be plotted in a tetrahedral color space for comparison. Chromatic contrast would be the distance between two colors, each represent by a point, in the tetrahedral space. One could

also arrive at the chromatic contrast mathematically, by using the formula: Cc = square root $[(X_{UV1}-X_{UV2})^2 + (X_{S1}-X_{S2})^2 + (X_{M1}-X_{M2})^2 + (X_{L1}-X_{L2})^2]$, where X is the relative stimulation of each cone (UV,S,M,L = cone type, 1=background, 2=stimulus).

The model created by Fleishman and Persons can be used to test whether dewlaps brightness and color have evolved to effectively signal a receiver in specific light environments. This model of describing the difference in appearance between two colors has been used with many species.

One past study used this model to analyze two different populations of *A. christatellus*, occupying distinct habitats by Leal and Fleishman (2004). The study looked at *A. christatellus* inhabiting a xeric habitat, which was dry and only slightly vegetated, and a mesic habitat, which was wetter and more vegetated. The xeric habitat had higher light intensity, because of less tree cover, and the spectrum shifted towards the short wavelength, because of a contribution of blue sky to the ambient light. Using the detection model, it was determined that xeric dewlaps were more detectable in xeric habitats and mesic dewlaps were more detectable in mesic habitats. Interestingly, xeric dewlaps achieved high brightness contrast by being darker than the background, while mesic dewlaps were lighter than the background (Leal and Fleishman 2004). This experiment shows an example of microevolution within a species, but no evidence of divergent evolution within the habitats. This finding leads one to believe that varying light conditions drove the evolution of varying dewlaps and gives support to the detectability hypothesis.

Although the detectability hypothesis was supported in the intraspecies analysis of *A. christatellus*, a later analysis of four Puerto Rican species (*A. gundlachi,*

A. pullchellus, A. krugi, and *A. christatellus*), failed to yield the same result. The habitat light varied between all species, except *A. krugi,* and *A. christatellus*, but the rank of detectability was nearly the same across all habitats. *A. pullchellus* and *A.* krugi were predicted as the most detectable species across all habitats, when comparing their dewlap radiance to background radiance. The red dewlap of *A. pullchellus*, also had the highest chromatic contrast with the background (Fleishman et al 2009). This result suggests that signal detectability cannot be the sole reason for evolution of Puerto Rican dewlap diversity.

What other factors can be driving signal design, besides detectability? Looking back at our signal function hypotheses, it's possible that signal is used for species *recognition.* Our study aims to explore this aspect further, but let us first ask whether signal cost or anolid evolutionary history has limited the dewlaps from attaining ideal detectability. Anoles have several avian predators, as well as other reptilian predators, such as snakes. Displaying the dewlap would increase their detectability to predators and put them at a greater predation risk, but it does not appear that anoles modify their behavior to account for this cost. Much of the anoles time budget is devoted to displaying the dewlap (Losos 2009) and the signal appears to have a non-direct target. A closer investigation of anole predation patterns may be warranted, but it is unlikely that the signal cost of detection outweighs the benefits of detection. In fact, there is evidence that the dewlap can be used to deter a predator from pursuing the anole, because the anole has seen the predator and displays an honest signal to deter a chase. Furthermore, the evolutional history also does not appear to be a hindrance on signal detectability.

The species on the same island are usually more closely related than species of different islands, yet dewlap spectral components can vary greatly between species on one island, which is the case for Puerto Rican anoles. In contrast, lizards on different island but occupying similar light habitats can have very similar dewlap appearances (Loew et al 2002). Therefore it seems likely that signal color has overcome the pressures of evolutionary history.

There were a number of goals of our research. First, we wished to collect and analyze the light conditions of the habitats of four Puerto Rican species (*A. gundlachi, A. pullchellus, A. krugi,* and *A. christatellus*), in terms of light intensity and spectral composition. For this analysis we aimed to identify components that contribute to habitat light, such as blue sky or green vegetation, and how the level of those individual components vary between habitats. Next, we aimed to analyze dewlap spectral data and, by using the model developed by Fleishman and Persons (2001), to quantify the detection probability of the various species dewlaps across all of the environments. These procedures are very similar to those described in Fleishman et al. (2009) and even use the same species; therefore we expect to obtain similar results.

If our results are indeed similar to Fleishman et al. (2009), we must still explain why rank of detectability was not highest for each species in their actual habitat. We hypothesize that Puerto Rican dewlaps evolved because of two distinct pressures: (1) detection and (2) species recognition. We will no longer assume that evolution is driving dewlap design to maximum detectability, but will rather attempt to demonstrate that actual dewlap designs are significantly more detectable

than random dewlap colors. Similarly, to make a case for the influence of species recognition we wish to demonstrate that the actual dewlap colors observed in these four Puerto Rican species vary between each other significantly more than would four random dewlap colors. If the observed dewlaps are in fact more detectable than random and/or more distant in color space than random, one can logically attribute this variation to the role of natural selection.

The difficulty in doing such an analysis is accurately modeling random dewlap colors. It may be deceptive to simply assume that a random anole dewlap colors have no biological restrictions. To illustrate this point, we can look to bird plumage coloration, especially because birds are close relatives of reptiles. A study by Stoddard and Prum (2011) analyzed the bird plumage diversity in avian color space. Avian color space can be represented with tetrahedral geometry where each corner represents one of the four avian visual pigments. Any color can be plotted in the tetrahedral space based on the relative stimulation of each visual pigment. Therefore a color that stimulates all receptors to an equal proportion will be directly in the middle of the color tetrahedron, a color that stimulates only one cone will fall at one of the corners, and one can imagine all the possibilities in between. Their analysis looked at 965 plumage sections on 111 different bird species and found that avian plumage only occupies 26-30% of the total avian color space. Structural colors, which refract light through (microscopic) physical structures, accounted for most of this color diversity, but structural colors are relatively rare, only being found in about 25 percent of the sampled species. Actual plumage pigments only occupied 6.9% of the avian color space (Stoddard and Prum, 2011). This disparity in

color diversity could be attributed to primitive color mechanism contributing to bird coloration. For example, pigments were restricted to melanins, caratinoids, porphryins, psittacofulvins, or the reflection of all colors, appearing as white. More variation can only achieved through structural colors or combined mechanisms. There also appeared to be an additional factors restricting avian coloration, because plumage color diverged from browns (bark) and greens (foliage) and even attained colors not seen in plants (Stoddard and Prum, 2011).

The next question we set out to ask is whether anoles are restricted in the pigments that they can produce. If the answer is "yes" then we should ideally restrict our theoretical dewlap model to include a smaller portion of color space. The dermis of anoles and other lizards contains three chromatophore layers: (1) the most superficial layer contains the xanthophones, containing pteridines and caratinoids; (2) the middle layer contains iridophores, which reflect and scatter light; (3) the bottom layer contains melanin (e.g. Alexander and Fahrehbach, 1969; Taylor and Hadley, 1970; Morrison et al., 1995; as sited in Macedonia et al., 2000). Of these pigments, pteridines have peak reflection largely in the UV range, except sepiapterin and drosopterin, which appear yellow and orange, respectively. Catatinoids most commonly reflect light in the red, orange and yellow range, but could theoretically create almost every color (e.g. Lee, 1977; as sited in Macedonia et al., 2000). Additionally carotanoids cannot be synthesized and are therefore obtained through the animal's diet (Olson and Owens, 1998; as sited in Macedonia et al., 2000). Irodophores are responsible for structural color, through light scattering off sheets of guanine crystals (Alexander and Fahrenbach, 1969; as sited

in Macedonia et al., 2000). In Puerto Rican anoles, orange, red and some yellow colors originate from pteridines, while most yellows originate from caratinoids (Ortiz et al., 1963; as sited in Macedonia et al., 2000). Knowing these characteristics could be useful in restricting the possibilities for random dewlap colors, but the presence of structural colors and caratinoids could provide more considerable variation. Ideally, one might like to know the total color space occupied by dewlaps from all anolid species, but for our study we only had data for 7 different dewlap colors from Puerto Rico and about 7 from the Jamaica study. We know that more diverse colors exist on different island but we did not have that dewlap data, so we did not set many restrictions to our random.

METHODS

Field Sites

The field data was collected over 10 days during July 2011. Each sampling habitat was selected carefully so that it almost excursively contained only one species of anoles and in high frequency. *A. pulchellus* were sampled in two different open and mostly unshaded locations: (1) a small field near the El Verde field station, in the Luquillo National Forest of northeastern Puerto Rico, and (2) the outskirts of a golf course, north of Rio Grande, PR. *A. gundlachi* were sampled in the under-story of the closed-canopy rain forest of the Luqillo National Forest, adjacent to the El Verde field station. *Anolis krugi* data was gathered from partially shaded forest edges with some light gaps, near Palmer and on a plot of private land with an adjacent path. *A. cristatellus* were sampled at a less densely vegetated (compared to

El Yunque) dry forest, in the Cambalache Forest Reserve, Barrio Garrochales, Arecibo, of northwestern Puerto Rico.

Sampling Habitat Light

The above field sites were our four habitat conditions, with *A. pulchellus* being sampled in two different sites, but because of a lack of light variation they are regarded as one habitat. We sampled light conditions throughout the day, but for *A. gundlachi* we started sampling at the earliest time of 0600 and ended at the latest time of 1800. For the other species we sampled between 0800 and 1745, with exception of *A. pulchellus*, which we did not sample between 1100-1400 hours because of prior knowledge of low activity during this time (Gorman and Harwood, 1977; as sited in Fleishman et al. 2009). We collected data under a range of different weather conditions, such as sun or overcast, but did not collect data during the rain. We were mindful of changing cloud cover when sampling different readings for the same individual, in an attempt to sample under a constant light intensity. We did not obtain equal numbers of samples for each species because of varying ease in finding the species and uncooperative weather circumstances. As a result we gathered the most samples for *A. gundlachi*.

Two investigators worked together when collecting data and we had two pairs for this task. A group walked through the habitat until a lizard was spotted. Once we spotted the lizard, we kept our distance and remained still, so not to disturb it. A lizard was observed for a maximum of 10 minutes or until a dewlap display was seen. If the lizard did display, we immediately moved to the location of the display to gather light data. If a lizard did not display in 10 minutes, we gathered

the data at the end of the 10 minutes from the location the lizard was last seen. When the lizard did not display, we recorded this location as a "non-display" in our field notebook, but the data was still useful to use because previous studies (Fleishman et al. 2009) showed no difference between display and non-display locations. We therefore used both locations for habitat light analysis.

We collected light data with an Ocean Optics Jaz fiber optic spectrophotometer. Five habitat light measurements were collected for each individual: radiance right, radiance left, irradiance right, irradiance left and irradiance up. The irradiance-up reading was later neglected in habitat light analysis. To gather radiance data, we pointed the fiber optic cable toward the environmental background perpendicular to the extended dewlap or where the dewlap would have been extended if it were a non-display site. For radiance readings, the spectral quality was collected for a solid angle of 4 degrees relative to the plane of the dewlap. To gather irradiance data, we also pointed the fiber optic cable to the right and left directions, perpendicular to the plane of the dewlap, but we now pointed the fiber optic cable at an Ocean Optics diffuse reflectance standard. This white standard reflects all light hitting it from 180 degrees around the plane its surface. Therefore, when calculating irradiance left, we actually measured the spectral quality of light at 180 degrees to the right of the dewlap, while irradiance right measured light at 180 degrees to the left of the dewlap. We always tried to obtain the radiance and irradiance measurements from the exact location of the lizard, but if this happened to higher than we could reach, then we gathered the data

from the highest point that we could reach. Irradiance was equal to radiance measured from a diffuse white surface multiplied by pi (Fleishman et al. 2006).

The radiance and irradiance readings were later calibrated to units of micromoles of photons per nm per second per steridian of solid angle using a standard radiance calibration lamp (Licor Li-1800).

Sampling Dewlaps

We caught lizards at our various field sites and brought them back to the El Verde field station to measure reflectance and transmission characteristics of their dewlaps. We attempted to catch 5 individuals from each species, but some individuals were more difficult to catch and we ended up having a smaller sample size. In addition to *A. gundlachi, A. pullchellus, A. krugi,* and *A. christatellus,* we also captured *A. evermanni and A. stratulus*. Even though these two species, who live in the higher canopy layers of the rainforest, did not contribute to our analysis of habitat light, their dewlap data is useful. These two species likely come into contact with the other species, especially *A. gundlachi*, so these two dewlap colors may also contribute to divergent color evolution for species detectability.

To collect reflectance and transmission data, the lizards were mounted in custom designed holders, which kept the head and body from moving. The dewlap was manually extended by gently pinching the front of the hyoid bone with forceps mounted on a bi-axial microscope stage and regulated by a set screw. A light originating from a Ocean Optics Jaz fiber optic spectrophotometer was directed at the anole's dewlap, with a detector at the opposite side of the dewlap. The light source directs all wavelengths of the visible light spectrum at the dewlap and after

its interaction with the dewlap, reflection, transmission and absorbance can be quantified.

Generating Random Dewlap Colors:

The reflectance data for the dewlaps was gathered by taking radiance data of color samples in the Munsell color standards matte collection, with the Ocean Optics Jaz fiber optic spectrophotometer. The book had 41 pages and two color chips were sampled form each page, which had a wide range of brighnesses. Going through all of the also gave us samples from each part of the visible light spectrum, other than ultraviolet (UV). We know that many dewlaps do contain reflection in the ultraviolet range, so to account for this we added a UV peak between 340 nm and 360 nm scaled to a relative UV cone stimulation of 0.00-0.20, chosen at random within this range. Since the color chips did not have the same properties as a dewlap, we also could not get random transmission data. Nevertheless, by looking at the trends from the actual dewlaps, we noticed that the transmission spectra closely resembles the reflection spectra until about 580 nm, at which point it increases sharply, allowing the transmission of long wavelength. To account for this observation when generating random transmissions, we mirrored the reflectance curve until 580 nm and then increased the relative transmission past 580 nm at a rate similar to the observed dewlaps.

Ranking Puerto Rico Dewlaps and Random Dewlaps:

In order to test our hypotheses of (1) detectability and (2) species recognition, we must rank the dewlap chromatic contrast and brightness contrast to the background, as described in Fleishman and Persons (2001) and in the

introduction of this paper. These formulas also give us an estimated rate of detectability based on chromatic contrast and brightness contrast. In order to rank these dewlaps we had to determine the visual system that we would use. Since previous studies have shown that the visual systems of these anoles do not vary greatly (Loew et al, 2002), so we used the *A. evermanni* visual system for all of the analyses. All of the dewlaps (actual and random) were ranked in all four of the habitats that we sampled (*A. gundlachi, A. pullchellus, A. krugi,* and *A. christatellus*).

The von Kries chromatic adaptation correction was used to account for something known as color constancy, which is a property of the visual system to correct for disproportionate stimulation of any of the photoreceptors. For example if a white object is viewed in a forest, where ambient light has a pronounced peak in the greens or medium wavelengths, it will still appear white instead of green because of chromatic adaptation. Because of chromatic adaptation, the dewlaps will appear similar in each of the environments, but dewlaps must be sampled in all environments because chromatic contrast and brightness contrast from the background will vary.

To rank each actual dewlap color in relation to random colors, each dewlap was ranked in its home habitat (the habitat that the anole actually inhabited). For example, *A. gundlachi* and all random colors received a chromatic contrast score and probability of detection based of the Fleishman and Persons model, in the *A. gundlachi* habitat. Then the *A. gundlachi* scores were ranked in relation to the 82 random scores. To test the hypothesis of species recognition, we assumed that animals could discriminate amongst each other if the chromatic contrast of their

dewlaps varied greatly from the dewlaps of other species on the island. Specifically, the home lizard had to be more chromatically divergent from the other Puerto Rican dewlaps than random dewlaps. To calculate this all dewlaps were ranked in one of the habitats. The home lizard was then analyzed against the non-home lizards and a minimum chromatic distance was calculated (how far was the most similar dewlap from the home lizard dewlap, in color space), as well as an average chromatic distance (what was the average distance of all non-home dewlaps from the home dewlap, in color space). The home lizard was then replaced in the analysis with a random dewlap color. The minimum chromatic distance and average chromatic distance were likewise calculated, still in the home lizards habitat. Then the home lizards minimum and average distance (from non-home species) was then ranked in relation to the minimum and average distances of the random dewlaps (from non-home species).

Please note that the two segments of the *A. pulchellus* dewlap (front UV and back red) were analyzed as separate dewlaps. When determining color distances, the *A. pulchellus* habitat was considered as the home habitat for both of the segments and the dewlaps were not compared to each other. Nevertheless, in non-home habitats both were treated as separate dewlaps and the home dewlap at that habitat was compared to both of the segments.

RESULTS

Comparison between habitats

Table 1 lists the chromatic contrast, brightness contrast and detection probabilities for each species dewlap (or two segments in the case of *A. pulchellus*),

in each of the four measured habitats. Notice that the chromatic contrast, brightness contrast and probability of detection was similar for the same species (same dewlap color) in the *A. cristatellus, A. gundlachi* and *A. krugi* environments. These three habitats have similar radiance curves, dominated by green light. The chromatic contrast, brightness contrast and probability of detection scores vary more when comparing the *A. cristatellus, A. gundlachi* and *A. krugi* environments to the *A. pulchellus* environment. The spectra of the *A. pulchellus* environment had a broader peak caused by a more open environment with a contribution from blue sky.

All of the dewlap colors, which the exception of *A. pulchellus'* UV patches, had the greatest chromatic contrast within the *A. gundlachi* habitat. This increase in chromatic contrast caused the highest probability of detection for *A. evermanni, A. krugi, A. christatellus* and *A. stratulus,* at this habitat. The brightness contrast for *A. evermanni, A. krugi, A. christatellus* and *A. stratulus,* was actual slightly higher in the *A. krugi* habitat, but this did not lead to a big enough contribution for detectability, since the chromatic contrast was lower at this environment.

Some interesting results can be seen when looking at the *A. pulchellus* environment, since it was much different than the other habitats. The two patches of the *A. pulchellus* dewlap were most detectable in the *A. pulchellus* habitat, according to our model. Their increased detectability can be attributed mostly to an increase in brightness contrast, where a large negative number indicates that the two segments of the dewlap were darker than the background. The chromatic contrast was also higher for the two color patches of *A. pulchellus,* than the other five dewlap colors. The UV patch of the *A. pulchellus* dewlap had its greatest chromatic contrast

in its home habitat and the red patch had a chromatic contrast of 0.31, which was not much lower than in the other habitats (highest in *A. gundlachi* habitat at 0.40). A few other dewlaps like *A. evermanni* and *A. stratulus* experienced a decrease in chromatic contrast in this habitat, compared to the other three habitats. The chromatic contrasts of *A. cristatellus, A. gundlachi* and *A. krugi* dewlaps were likewise low in the *A. pulchellus* habitat, but generally in the same range as in the *A. krugi* habitat. The decreases in probability of detection for *A. cristatellus* and *A. krugi* were more contingent on decreases in brightness contrast. *A. cristatellus* is modeled to appear darker than the environment in these conditions. While *A. krugi* remains brighter that the environment, it has lost the large positive brightness contrast, which made it the most detectable species in the other environments. Finally, the model predicts that *A. gundlachi* would be most detectable in this environment, thanks to a high negative brightness contrast, which would make it appear darker than the background, like the *A. pulchellus.*

Table 1. The predicted chromatic contrast, brightness contrast and detectability of Puerto Rican dewlaps at four different habitats. The values shown are relative values, so chromatic contrast can vary from 0 to 1, brightness can vary from -1 to 1, and probability of detection varies from 0 to1. The values for brightness contrast were gathered by a comparison of habitat background brightness (Bb) and stimulus (dewlap) brightness(Bs), as defined by the formula: Cb = (Bs-Bb)/(Bs+Bb). The brightness can be darker than the background (negative number) of brighter than the background (positive color). Chromatic contrast was calculated by using the formula: Cc = $(X_{UV1}\text{-}X_{UV2})^2 + (X_{S1}\text{-}X_{S2})^2 + (X_{M1}\text{-}X_{M2})^2 + (X_{L1}\text{-}$

$X_{L2})^2$, where X is the relative stimulation of each cone from 0 (0% stimulation) to 1(100% stimulation) and UV,S,M,L = cone type, 1=background, 2=stimulus. The formula p = 0.400*C*b + 0.429*C*c + 0.156 was used to calculate probabilities of detection, *C*b is the brightness contrast and *C*c is the color contrast.

Habitat of analysis	Dewlap	Chromatic Cont.	Brightness Cont.	Prob. of detection
A. cristatellus	*A. evermanni*	0.31	0.47	0.46
	A. pulchellus (front)	0.12	0.00	0.20
	A. pulchellus (back)	0.35	-0.13	0.34
	A. gundlachi	0.20	0.07	0.26
	A. krugi	0.22	0.69	0.51
	A. cristatellus	0.18	0.36	0.36
	A. stratulus	0.33	0.33	0.41
A. gundlachi	*A. evermanni*	0.38	0.47	0.49
	A. pulchellus (front)	0.11	0.00	0.19
	A. pulchellus (back)	0.40	-0.12	0.36
	A. gundlachi	0.26	0.07	0.28
	A. krugi	0.28	0.69	0.54
	A. cristatellus	0.23	0.35	0.38
	A. stratulus	0.39	0.33	0.44
A. krugi	*A. evermanni*	0.22	0.48	0.43
	A. pulchellus (front)	0.17	0.02	0.23
	A. pulchellus (back)	0.32	-0.11	0.32
	A. gundlachi	0.13	0.09	0.24
	A. krugi	0.15	0.70	0.49
	A. cristatellus	0.10	0.37	0.34
	A. stratulus	0.26	0.34	0.39
A. pulchellus	*A. evermanni*	0.16	-0.03	0.23
	A. pulchellus (front)	0.24	-0.48	0.44
	A. pulchellus (back)	0.31	-0.56	0.50
	A. gundlachi	0.13	-0.44	0.38
	A. krugi	0.13	0.29	0.32
	A. cristatellus	0.12	-0.17	0.27
	A. stratulus	0.21	-0.19	0.31

Comparison to Random Dewlaps

Refer to Table 2 for a breakdown of *A. cristatellus, A. gundlachi, A. krugi* dewlap colors and two color patches from the *A. pulchellus* dewlap, compared to 82 randomly generated dewlap colors. None of the dewlap colors ranked above 50% in minimum distance from non-home species or above 50% in average distance from non-home species. The non-home species can be considered as all the Puerto Rican anole species that do not inhabit the habitat of the anole that is being analyzed. For example the average distance of *A.cristatellus* considers *A. evermanni, A. gundlachi, A. krugi, A. pulchellus* (both patches) and *A. stratulus* as the non-home lizards. *A. cristatellus, A. gundlachi, A. krugi* amongst the 5 lowest ranks for distance from other species and their dewlap colors almost overlapped in color space according to predictions by our model. The two patches on the *A. pulchellus* dewlap theoretically differed more than these three dewlaps.

Chromatic contrast against the average background was in the top 50%, compared to random dewlaps, for all of species that we analyzed, other than *A. krugi*. Nevertheless, *A. krugi* had the highest brightness contrast of all the species, ranking #9 among random colors, which also contributed to a high probability of detection (rank:15). The *A. gundlachi* dewlap and the UV patch of the *A. pulchellus* dewlap were the only two dewlap colors that ranked below 50% compared to the random dewlaps for brightness contrast. The *A. gundlachi* color ranks very poorly for brightness contrast (rank:74), which causes the probability of detection to also decrease well below 50% (rank:70). The score value for the UV patch of the pulchellus dewlap actually looks high and one would expect it to be in the top 50%,

but the random colors seemed to be much more visible in this habitat and the 50% cut-off was harder to reach.

Table 2. Rank of dewlap properties of Puerto Rican anoles compared to random dewlaps.

Four Puerto Rican anoles species (one with two color patches) were compared to 82 random dewlap colors (see methods section for detail on how the random dewlaps were generated). Each dewlap was analyzed in its home habitat and compared to random colors as they would appear in the same habitat. *A. evermanni* and *A. stratulus* were not included in this analysis because we did not have data for their home habitat. Minimum distance indicates the smallest distance in tetrahedral color space between the home lizard and any of the Puerto Rican lizards not from that habitat (non-home lizard - including *A. evermanni* and *A. stratulus*). Because each point of the tetrahedron has a value of 1, which indicates 100% excitation of the visual cone, a minimum value of 0 and a maximum value of 1 can be obtained for the distances between points in the tetrahedron. The smallest distance was also determined from each random color to any Puerto Rican lizards, which do not occupy that habitat. For the average distance the home dewlaps and random dewlaps were compared to all of the non-home lizards and an average distance was calculated. For chromatic contrast, brightness contrast and probability of detection, each dewlap was analyzed in its home habitat and compared to how random colors were modeled to appear in the same home habitat. The rows labeled score indicate a relative value for these characteristics, so chromatic contrast can vary from 0 to 1, brightness can vary from -1 to 1, and probability of detection varies from 0 to1. A

brightness contrast can be negative if the dewlap is darker than the background. The rank rows indicate the comparison to the random dewlaps. A high score indicates that the dewlap of interest has a smaller distance to non-home lizards (ave. or min.), lower chromatic contrast to the habitat background, lower brightness contrast to the habitat background, or a lower probability of detection in the habitat, than would random dewlaps. The green numbers indicate when the rank was higher than 50% of the random dewlaps and a red number indicates when the rank was in the bottom 50%.

	Dewlap	Minimum Distance	Average Distance	Chromatic Contrast	Brightness Contrast	Probability of Detection
Score	*Anolis cristatellus*	0.0399	0.1417	0.1767	0.3582	0.3639
Rank		**82**	**80**	**41**	**34**	**36**
Score	*Anolis gundlachi*	0.0281	0.1228	0.2558	0.0706	0.2806
Rank		**82**	**82**	**34**	**74**	**70**
Score	*Anolis krugi*	0.0280	0.1209	0.1456	0.6960	0.4867
Rank		**83**	**83**	**49**	**9**	**15**
Score	*A. pulchellus (front)*	0.1786	0.2487	0.2450	-0.4793	0.4397
Rank		**59**	**50**	**36**	**50**	**47**
Score	*A. pulchellus (back)*	0.1429	0.2018	0.3099	-0.5647	0.4999
Rank		**73**	**67**	**24**	**41**	**32**

DISCUSSION

Let us consider are two hypotheses for the factors influencing signal design. We proposed that (1) dewlaps evolve to be more visible than random colors, or (2) dewlaps were more divergent from each other than random colors, which aids with species recognition. If we consider the results presented in Table 2, the Puerto Rican dewlap colors do not seem to be diverging significantly. In fact, *A. cristatellus, A.*

gundlachi, A. krugi occupy a very small area of color space and are chromatically similar. The two sections of the *A. pulchellus* dewlap are slightly more unique, but they are not more divergent than random. Therefore, this data does not support the hypothesis that the signals are used for species recognition. Based on chromatic contrast alone, the Puerto Rican anoles would need to have a very precise visual system to discriminate between neighboring lizards and lizards of the same species.

The theory that dewlaps evolve to be more detectable than random does have greater support according to our results, as three of the five dewlap colors were more detectable than random. Nevertheless, the only dewlap that was well above average was the *A. krugi* dewlap, while *A. cristatellus* and the red segment of the *A. pulchellus* dewlap were only slightly above random. Based on a sample of only 5 different lizards, it is difficult to make a concrete argument. It would be beneficial to get habitat data for *A. evermanni* and *A. stratulus* to see a clearer picture. This type of analysis has great potential but future endeavors must put some more phylogenetic constraints on the random colors. Gathering dewlap data from other anoles in the Caribbean could give us a better idea of the colors that anoles are capable of producing.

The brightness contrast was not a factor that we considered as a heavy driving force of evolution, but it may be that dewlap brightness is evolving to suit the lizard's habitats. If we look at *A. krugi*, it has an extremely similar color to *A. gundlachi* and *A. cristatellus*, but the thickness of the dewlap differs and it appears much brighter than the background because of its thin quality. As shown in Table 1,

A. pulchellus also has a very effective dewlap thickness for its environment and appears much darker than the environment, which should increase detection.

Another interesting observation may be that *A. pulchellus* is much different than *A. cristatellus, A. gundlachi, A. krugi* in terms of both dewlap color and habitat. The latter three occupy environments with a predominantly green radiance and have similar yellow/orange dewlaps. *A. pulchellus* on the other hand is a grass anole where blue sky often contributes to the background radiance and it has a UV and a red segment to its dewlap. Table 1 shows that these two chroma were quite effective in the *A. pulchellus* habitat, compared to some of the yellow dewlaps. The UV patch actually has a low chromatic contrast and brightness contrast in each of the other habitats. When we consider the *A. cristatellus, A. gundlachi,* and *A. krugi* habitats we will notice that the home lizard usually ranked well, especially compared to the poor ranks in the *A. pulchellus* habitat. The only exception seems to be *A. gundlachi,* which ranks highest in the *A. pulchellus* habitat, because of a negative brightness contrast. The chromatic contrast is still low in this environment but the brightness contrast makes up for it.

Another difficulty with this analysis may be that we are not calculating brightness and chromatic contrast against the proper background. Currently our methods sum the different component of the habitat like brown bark, green foliage or blue sky, into one average spectrum and the dewlap color is compared to this artificial light. Instead the lizards may display in such a way that they contrast a certain element of the background, like the bark for example. On the other hand, if the anoles are actually displaying with a multi-element and multi-colored

background, our model may be lacking. The Fleishman and Persons model was based on behavioral response to solid colored flags on a solid colored background. A reevaluation of the methods could be beneficial for the progress of this study. Along with restriction to the random colors, as mentioned earlier, a more accurate model for recognition can be created by using a background that corresponds better with the biological habitat.

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CHAPTER TITLE: The Color Discrimination Abilities of Australian Bearded Dragons (*Pogona vitticeps*)

ABSTRACT

HEREHA, VASYL The use of color vision and color communication in lizards. Department of
Biological sciences, June 2012.

ADVISOR: LEO FLEISHMAN

Many animals are believed to use color in their communication systems. While, the anatomical composition of an animal's retina can suggest the possibility that an animal has color vision, behavioral experiments are required to demonstrate that an animal can actually utilize it. We designed a behavioral experiment to test the color discrimination ability of lizards. Australian bearded dragons (*Pogona vitticeps)* were trained to find food under colored chips with peak reflectance at a specific wavelength (green chips, $\lambda \sim 550$) but varying in intensity. They were then given a choice between green chips and chips of varying colors, and the frequency of incorrect choices and time needed to complete the task were recorded. The experiment was then repeated using lower ambient light intensity. Our results showed that bearded dragons posses the ability to reliably discriminate objects based on their spectral quality (color), if the difference in color exceeds a certain threshold. Lowering light intensity did not reduce the lizards' success. This is the first study to demonstrate color vision in any lizard of the family Agamidae. The same techniques will be used to test the color discrimination abilities in other lizard families, in which color plays an important role in communication.

INTRODUCTION

Color vision is a characteristic that is not common to the visual system of all organisms. Such a trait would give an adaptive advantage to organisms that can utilize the color vision to discriminate between objects in their habitats. To possess color vision an organism must have different classes of photoreceptors in the retina, as well as the neural mechanisms to analyze the outputs from these photoreceptors. Achromatic neural mechanisms involve a summation of photoreceptor outputs, giving the organism a perception of object brightness, while chromatic mechanisms compare the outputs to give the perception of object color. Achromatic contrast can be useful for tasks like motion detection, but the chromatic contrast would be more reliable for discrimination task such as the recognition of a food source. For example, color can be directly related to quality and identity of food for herbivorous organisms such as bees, or frugivorous birds or mammals (Kelber et al., 2003).

Karl von Frisch (1914) first demonstrated that bees have color vision using his “grey-card” experiment. Von Frisch performed his experiment using conditioning, by training honeybees to associate a reward (sugar water) with a color (blue card which the reward rested on). He then placed the colored chip among 30 shades of gray and observed that the bees could still identify the colored chip. If the bees were using the achromatic mechanism, they would regularly mix up the colored chip with at least one of the intensities of gray, because the colored chip would match that intensity (Kelber et al., 2003).

Von Frisch's demonstration of chromatic vision inspired other behavioral experiments in many different kinds of organism. Fleishman and Persons (2001)

used a method of displaying colored flag stimuli against backgrounds of varying colors to elicit visual fixation from anolid lizards (*Anolis cristatellus)*. They founds that the major factor that influenced stimulus detection was brightness contrast between the flag and the background, which is mediated by the achromatic mechanism. Chromatic contrast also increased detection across all contrasts of brightness and especially when no brightness contrast was present. Anolid lizards have an elaborate visual communication system that relies on the extension of a throat fan structure known as the dewlap. The dewlaps of different species of anoles vary in color and thickness. It has been argued that anoles rely on differences in color among different species to recognize their own species, and avoid fighting or mating with members of other species (Losos 2009). Dewlap colors could, in theory, be discriminated using the achromatic and chromatic mechanisms. However, since brightness levels change with lighting conditions, chromatic mechanisms, which tend to produce the same stimulus under a wide variety of light conditions, are likely to be most important in species identification tasks.

To further explore the use of chromatic mechanisms by lizards, we designed a behavior experiment that conditioned lizards to associate a food reward with a color (green) and discriminate between several colors, regardless of differences in color intensities. We decided to pilot the experiment on Australian bearded dragons, specifically the central bearded dragon (*Pogona vitticeps)* because this species is easy to obtain through most pet stores and their tame nature is helpful for training behavioral experiments. Little previous research had been conducted on the visual system, the color morphology, or the significance of color vision for this organism.

Observational analysis of the eastern bearded dragon (*Pogona barbata)*, a close relative of *P. vitticeps,* indicated that dermal color changes occurred during fights. During these encounters between males, the skin color lightened during the peak of the fight and darkened by the end of the fight, especially by the defeated male (Brattstrom, 1971). It is important to point out that *P. barbata* does have a different coloration than *P. vitticeps,* with a bright blue-black beard and a yellow mouth, while *P. vitticeps* have mostly earth toned pigmentation (yellow, red, orange and brown) and a pink mouth. A study by de Velasco and Tettershall (2008) showed that *P. vitticeps* also had the ability to modify skin coloration, with a darkening in response to lower temperatures and a lightening in response to lower oxygen levels. Nevertheless, both of these examples of bearded dragon color changes are actually a change in brightness (darkening and lightening), which can theoretically be interpreted using the achromatic neural mechanisms. Therefore, a demonstration of chromatic mechanism for these lizards would be novel. In addition, it is of interest to determine whether or not bearded dragons use color vision, because color seems to play relatively little role in their social system. This contrasts the anoles, which seem to frequently use colored dewlaps for communication, but we wish to use the same procedures to test the anolid chromatic mechanism in the future. Here, we were interested in trying to determine if color vision was a widespread trait of lizards, in general, or whether it was limited to groups and species that rely heavily on color vision in their social behavior.

In addition to the behavioral experiments, we analyzed the photoreceptors of *P. vitticeps.* One of the few experiments to analyze the agamid photoreceptors was

done on a distant relative of the central bearded dragon, the ornate dragon lizard (*Ctenophorus ornatus)* This analysis revealed the presence of yellow oil droplets with an absorption of wavelengths shorter that 520 nm and a colorless oil droplet with no absorption between 350 and 700 nm. Three photoreceptors were identified with average peak absorptions at 440, 493 and 571 nm (Barbour et al., 2002) This study did not identify the presence of a UV photoreceptor, but the researchers may not have been looking for its presence. LeBas and Marshall (2000) argued that *Ctenophorus ornatus*, may use spectral reflectance in the UV range (370 nm – 400 nm), located on the female throat for mate choice, which would require the presence of a UV cone. We were therefore interested in trying to determine whether or not *P. vitticeps*, possessed a UV-sensitive cone.

METHODS

Animals/Living Conditions

Four captive bred central bearded dragons (*Pogona vitticeps)* acquired from a local commercial supplier (Petsmart) were used for the behavior study. One of the bearded dragons was obtained in January 2011 and learned the color discrimination behavior, which motivated the purchase of more individuals. Of the three younger individuals, two ended up getting through the final stage of training, but one of them lost the behavior half-way through experimental testing. More captive bred lizards were obtained for the photoreceptor analysis. I will refer to the older trained dragon as BD#1 (sex: female) and the younger trained dragon as BD#2 (sex: unsexed juvenile).

The lizards were kept in a 12 x 12 x 12 inch cage with a plexiglass front wall and a screen top, with all other walls being made of white plastic. The oldest bearded dragon would outgrow this cage and was transferred to a 12(width) x 12.5(height) x 21.5(length) inch aquarium tank, which was fitted with semi-opaque black blinders for the side walls and the top of the tank was a metal screen. A Solux Natural Daylight MR16 halogen light bulb was used as the light source for the lizards and was mounted about 6 inches above the tops of the cages. The lighting was set to a 12:12 hour light:dark timer and the temperature was kept constant, at around 80 degrees Fahrenheit (26.7 degrees Celsius), in our animal care room at Union College (Schenectady,NY). Lizards were fed and watered every weekday and one day during the weekends. The food source was Phoenix™ high calcium worms, but the dragons were also fed chopped lettuce dusted with calcium powder about once per week. The Phoenix worms were used as the reward for the behavior experiments.

Behavioral Experiment Training

The lizards were trained to a green stimulus, which was in a form of a colored paper chip, with a diameter of 1 inch, glued onto a hollow plastic hemisphere. The oldest lizard was trained with 1 inch chips, but eventually switched to chips with a diameter of 1.5 inches as he became larger. The colored chips were obtained from the Munsell, color standards matte collection. The trained stimulus came in three different brightnesses, which Munsell based on human perception. Still, we expected bearded dragon spatial sensitivity to be similar to that of other reptiles, which is similar to humans in most species that have been examined. When performing the training trails, the chips of different brightnesses were chosen at

random for each trail. The oldest lizard was initially trained to a single brightness of green, which was not from the Munsell collection, but transitioned to using three brightnesses of green for the final experiments.

Lizards were fed out of a 5(width) x 1.5(length) x 0.75(height) inch grey plastic block with round holes (wells) on opposite sides of the block, with a diameter of 0.625 inches. The reward was located on one side of the block, chosen at random with the flip of a coin (heads = experimenter's right, tails = experimenters left). The first phase of the training placed a live worm in one of the wells and the trained stimulus (green chip) was attached (with a small amount of sticky tack) behind the well, leaving the well fully uncovered. The bearded dragons were then given up to 8 minutes to find the worm. The completion of this task, in the allotted time, for 6 consecutive trails warranted proceeding to the next phase of training. About 2-3 trails were conducted each day for each individual; we started with 2 trails per day when the lizards were very young and small and did not need much food.

The next phase of the training placed a dead worm in a well, which was killed by tearing off the head. This phase of the experiment tried to discourage the lizards from responding to motion. The wells were still fully uncovered with the green chip located behind the well containing the worm. Once again, 6 consecutive suspenseful trials, in the allotted time of 8 minuted, allowed the lizard to proceed to the next stage. The next phase was a dead worm in a partly covered well, where the chip was attached with sticky tack in a manor where it covered less than 50% of the well. The next phase was a dead worm in a mostly covered well, where the chip covered more

than 50% of the well. The last phase was a fully covered well, where the lizard had to somehow move the green chip to expose the well and get the worm reward. Each stage required 6 consecutive suspenseful trails and if an individual was struggling with a certain phase of the training, the researcher had to use his/her discretion to return to a previous phase.

Once the individual made it through the stages of: live worm uncovered, dead worm uncovered, dead worm partly covered, dead worm mostly covered, and dead worm fully covered, we introduced a different colored chips to fully cover the well on the opposite side of the block. These test stimuli had either shorter or longer wavelengths than the trained stimulus. To start we placed colors that looked most contrasting with green on the other side of the block, such as reds (#5 & #6 in our classification of colors) and blues (#10 and #11 in our classification colors). Once the bearded dragons were easily discriminating between these colors and consistently flipping the green chip, we could move to the official testing phase and introduce more similar colors.

Official Behavioral Experiment Set-up:

1) Normal Light & Long Wavelength Test Stimuli

In this set of experiments lizards were given a choice between a green trained stimulus of varying brightnesses (1A, 1B, or 1C) and test stimuli distinguishing away from green toward the long wavelength side of the visible spectrum. The brightness of all of the test stimuli was approximately the same as trained stimulus 1B, based on Munsell's estimations of human perception. The trained stimuli had a Munsell classification number of 10Y 7/10, 10Y 8/10, and 10Y

9/10 for 1A, 1B, and 1C, respectively. The test stimuli had a Munsell classification number of [insert here], which we referred to as 2B, 4, 5, and 6, respectively.

The trials were randomized for non-patterned occurrence of side of the trained stimulus or brightness of trained stimulus. Randomizing combinations of 1A or 1B or 1C against (vs.) 2B or 4 or 5 or 6 was done by creating an equal quantity of occurrence for each combination and then randomizing them, all in a Microsoft Excel spreadsheet. Side of the trained stimulus on the block was randomized through a coin flip (heads = experimenter's right, tails = experimenters left). We also incorporated control trails where the worm reward was located under the test stimulus instead of the trained stimulus. This control tested whether lizards actually respond to color or qualities of the worm like smell or residual movements after being killed. A maximum of 8 minutes was given for the lizard to complete the task of knocking off the trained stimulus from atop the well. If the lizard did not complete a task it was moved to the end of the list and world be repeated later. Three to four successful trials were conducted per day, depending on motivation and size of the dragons.

The light levels of the two test dragons were very comparable. BD#1 had a tank with a light intensity of 20 μmol of photons per $m^2 \cdot s^1$, with a diffuser over the halogen lamp. BD#2 had a tank with a light intensity of 20 μmol of photons per $m^2 \cdot s^1$, without a diffuser over the lamp.

2) Low Light & Long Wavelength Test Stimuli

In this section of the experiment the lizards were given the same trained and test stimuli and time restrictions as the normal light experiment, but now the trials

were re-randomized and the light level were lowered. BD#1's light level was reduced to 4.6 μmol of photons per $m^2 \cdot s^1$, without a diffuser over the lamp. BD#2's light level was reduced to 1.8 μmol of photons per $m^2 \cdot s^1$, still without a diffuser. Placing a filter over the top screens of the dragons' cages at least 10 minutes before the beginning of testing, lowered the light to the desired level and allowed the eyes get acclimated.

3) Normal Light & Short Wavelength Test Stimuli

BD#1 lost the trained behavior during the low light experiment and had to be retrained prior to the start of this part for testing. Here, the light level of BD#2 returned to that of the other normal light experiment, at 20 μmol of photons per $m^2 \cdot s^1$. After struggles at low light, BD#1's light level was increased to 59 μmol of photons per $m^2 \cdot s^1$ by removing the diffuser. The only difference between this subset of trials is that the test stimuli were switched from diverging toward the short wavelength side of the visible light spectrum, like the blues. The brightness of all of the test stimuli was approximately the same as trained stimulus 1B. The test stimuli had a Munsell classification numbers of 5GY 8/10, 10GY 8.5/6, 5G 8/6, 5BG 8/4, which we referred to as 8, 9, 10, and 11, respectively.

Photoreceptor Analysis

For the photoreceptor analysis, the retinas of 3 juvenile individuals were analyzed through the processes of microspectrophotometry (MSP) and mRNA analysis for the expression of visual pigments and oil droplets (only MSP). Refer to Loew et al. (2002) for a detailed explanation of MSP procedures. The MSP was carried out with the kind help of Dr. Ellis Loew of Cornel University on the retina of

two of the dragons. The mRNA analysis was conducted through the efforts of [insert professor and University name] by using the retinas from one juvenile. Lizards were kept in the dark for 30 minutes prior to MSP analysis and were euthanized using [insert chemical name] applied on a rag and CO_2 pumped into a closed container.

RESULTS

1) Normal Light & Long Wavelength Test Stimuli

The results for BD#1 are summarized in figures 1&3, and results for BD#2 are summarized in figures 2 & 4. In theses figures, the color combination (condition) is plotted on the x-axis and rate correct is on the y-axis. No difference was seen between the dragons' performance on the control trials compared to the normal trials so the data from both was combined, with 24 control trials per 144 total trials for BD#1 and 12 control trials per 132 total trials for BD#2.

A binomial test on the rate of successful choice at different color conditions showed that BD #1 was significantly above average for discriminating between the green trained stimuli 1 (1A, 1B and 1C combined) and test stimuli 4, 5 and 6 (p values of $p<0.001$,$p<0.001$, and $p<0.001$, respectively, assuming a 50% success rate without color vision). BD#1 was not above random in selecting the trained stimulus when deciding between 1's vs 2B ($p=0.309$) (Figure 1).

Likewise, BD #2 was significantly above random for discriminating between the green trained stimuli 1 (1A, 1B and 1C combined) and test stimuli 4, 5 and 6 (p values of $p<0.001$,$p<0.001$, and $p<0.001$, respectively) but not above random for 1's vs 2A ($p=0.148$) (Figure 2).

We also analyzed the data by separating the conditions into performance among the different brightnesses of green. Refer to Figure 3 for a breakdown of BD#1's results. For BD#1, a binomial test indicated that the choice of trained stimuli 1A, 1B and 1C were all above random when compared to test stimulus 4 ($p=0.003$, $p=0.019$ and $p<0.001$, respectively), when compared to test stimulus 5 ($p=0.003$, $p<0.001$ and $p<0.001$, respectively) and when compared test stimulus to 6 ($p<0.001$, $p<0.001$ and $p<0.001$, respectively). BD#1's discrimination was statistically higher than random for 1C vs 2B ($p=0.019$), achieved by choosing the 1C trained stimulus in 10 out of 12 trails. BD#1 was not above random chance for choosing the trained stimulus in the condition of 1B vs 2B ($p=0.387$) or 1C vs 2B($p=0.981$). In fact, BD#1 favored test stimulus 2B instead of trained stimulus 1C in 9 out of 12 trails, the probability of such a combination is $p=0.073$, just above the 5% cut-off value.

Refer to figure 4 for an analysis of BD#2's performance across different brightnesses. According to the binomial test, BD#2 chose trained stimuli 1A, 1B and 1C at an above random proportion when compared to test stimulus 4 ($p<0.001$, $p=0.033$ and $p=0.033$, respectively), when compared to test stimulus 5 ($p<0.001$, $p=0.000$ and $p=0.033$, respectively) and when compared to test stimulus 6 ($p<0.001$, $p<0.001$ and $p<0.001$, respectively). Contrastingly, BD#2 was significantly above random on the binomial test when choosing between 1A vs 2B ($p=0.033$), but at chance level for 1B vs 2B($p=0.500$) and 1C vs 2B($p=0.7256$).

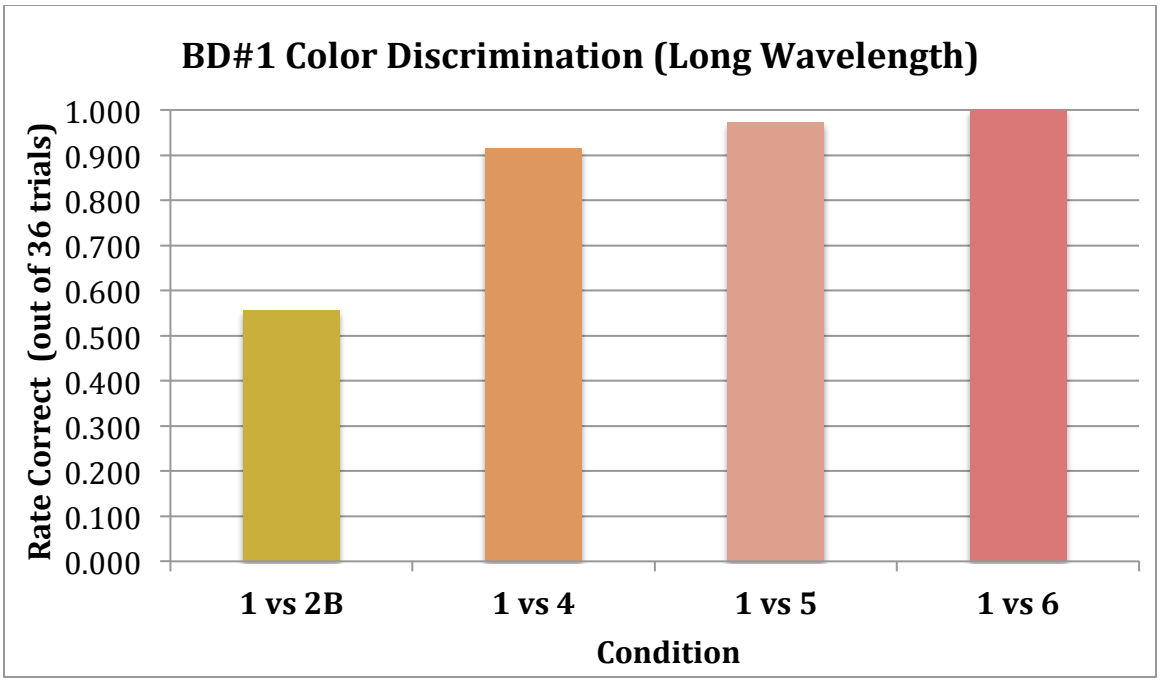

Figure 1. Color Discrimination of Bearded Dragon #1 Under Normal Light & With Long Wavelength Test Stimuli. The x-axis of the graph represents the condition of trained stimuli vs test stimuli that the dragon was presented. Trained stimulus 1 is a sum of trials across the three brightness of green (1A, 1B, and 1C). Each condition is comprised from 36 trials, divided equally among the three brightnesses (36 trails of 1 vs 2B = 12 trails of 1A vs 2B + 12 trails of 1B vs 2B + 12 trails of 1C vs 2B). The y-axis represents the lizard's success rate of choosing the trained stimulus out of 36 trails for each condition. The bar colors are approximations of the colors of the test stimuli 2B, 4, 5 and 6. As the test stimuli wavelength diverged further from the green trained stimuli, the rate of correct choice increased.

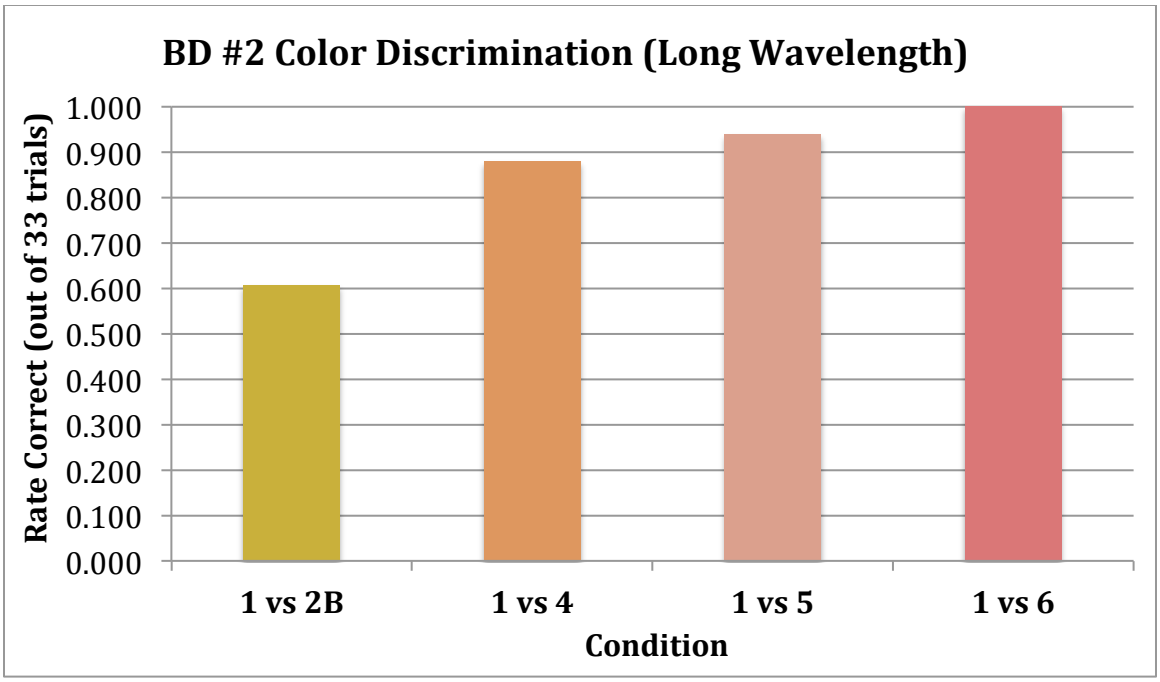

Figure 2. Color Discrimination of Bearded Dragon #2 Under Normal Light & With Long Wavelength Test Stimuli. The x-axis of the graph represents the condition of trained stimuli vs test stimuli that the dragon was presented. Trained stimulus 1 is a sum of trials across the three brightness of green (1A, 1B, and 1C). Each condition is comprised from 33 trials, divided equally among the three brightnesses (33 trails of 1 vs 2B = 11 trails of 1A vs 2B + 11 trails of 1B vs 2B + 11 trails of 1C vs 2B). The y-axis represents the lizard's success rate of choosing the trained stimulus out of 33 trails for each condition. The bar colors are approximations of the colors of the test stimuli 2B, 4, 5 and 6. As the test stimuli wavelength diverged further from the green trained stimuli, the rate of correct choice increased.

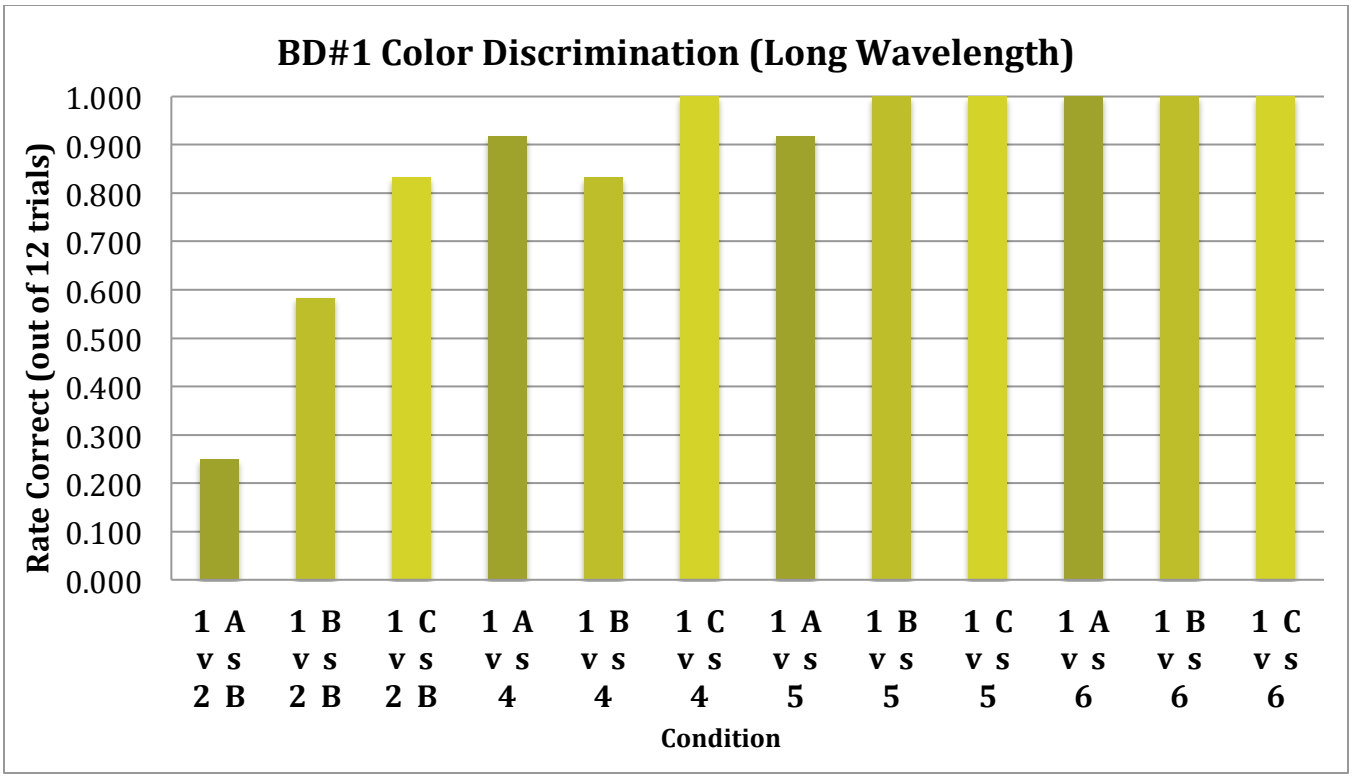

Figure 3. Color Discrimination of Bearded Dragon #1 Under Normal Light & With Long Wavelength Test Stimuli. The x-axis of the graph represents the condition of trained stimuli vs test stimuli that the dragon was presented. This figure shows the conditions from figure 1 broken down by brightnesses of the trained stimulus, instead of summing them. The y-axis represents the lizard's success rate of choosing the trained stimulus, out of 12 trails for each condition. The bar colors are approximations of the colors of the trained stimuli 1A, 1B, and 1C. This figures shows that when the test stimulus was 4, 5 and 6, the lizard was over 80% successful for choosing the trained stimulus, regardless of brightness. When the test stimulus was 2B, the lizard was most successful in choosing trained stimulus 1C, less successful in choosing 1B and least successful in choosing 1A.

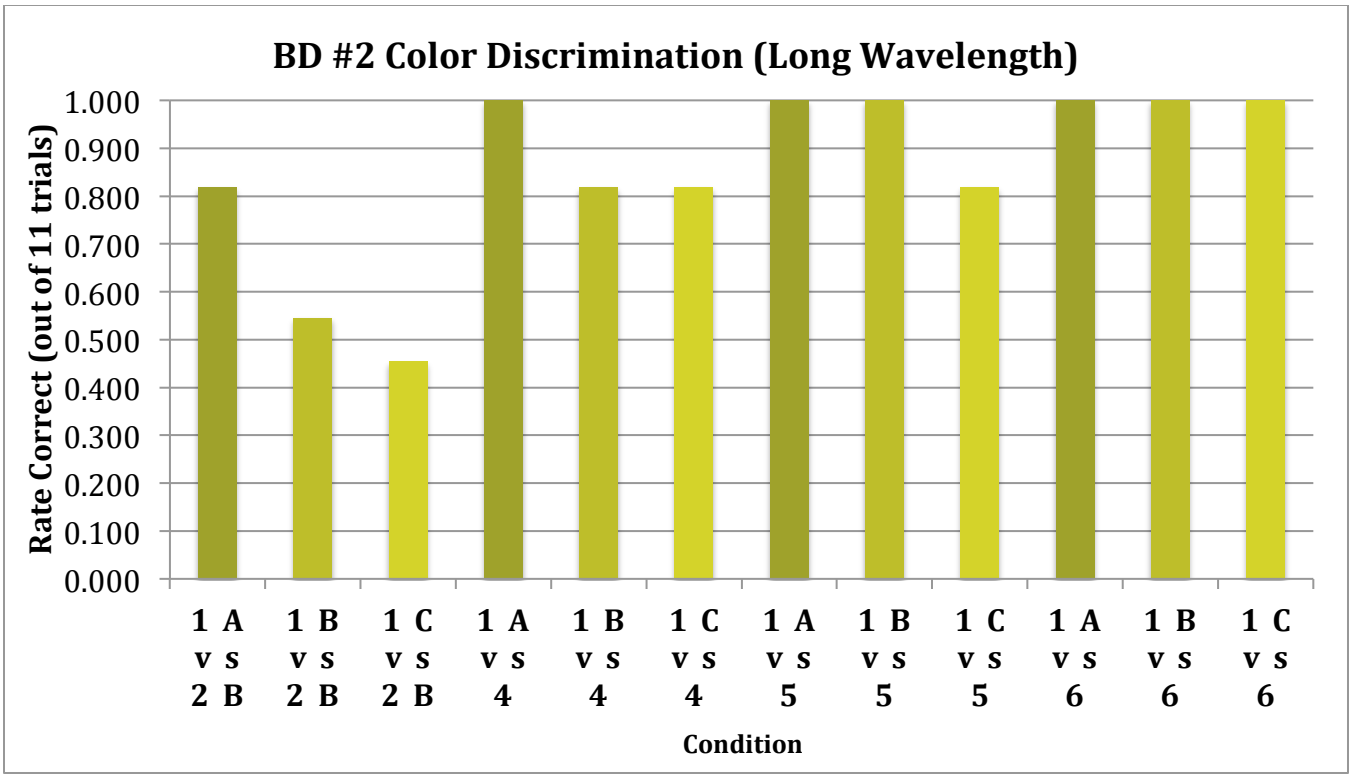

Figure 4. Color Discrimination of Bearded Dragon #2 Under Normal Light & With Long Wavelength Test Stimuli. The x-axis of the graph represents the condition of trained stimuli vs test stimuli that the dragon was presented. This figure shows the conditions from figure 2 broken down by brightnesses of the trained stimulus, instead of summing them. The y-axis represents the lizard's success rate of choosing the trained stimulus, out of 11 trails for each condition. The bar colors are approximations of the colors of the trained stimuli 1A, 1B, and 1C. This figures shows that when the test stimulus was 4, 5 and 6, the lizard was over 80% successful for choosing the trained stimulus, regardless of brightness. When the test stimulus was 2B, the lizard was most successful in choosing trained stimulus 1A, less successful in choosing 1B and least successful in choosing 1C.

2) Low Light & Long Wavelength Test Stimuli

The results on BD#2 at low light are shown in figures 5 & 6, where the color combination (condition) is plotted on the x-axis and rate correct is on the y-axis. Previous control trials indicted that the smell or residual movements of the dead worm did not influence the lizards' choice, so control trials were not conducted for this section of the experiment and total number of trials was reduced to 120.

A binomial test on the rate of successful choice at different color conditions showed that BD #2 was significantly above average for discriminating between the green trained stimuli 1 (1A, 1B and 1C combined) and test stimuli 4, 5 and 6 (p values of $p=0.001$, $p<0.001$, and $p<0.001$, respectively). BD#2 was not above random in selecting the trained stimulus when deciding between 1's vs 2B ($p=0.309$) (Figure 5).

Refer to Figure 6 for a breakdown of BD#2's results by brightness of the trained stimulus. A binomial test indicated that the choice of trained stimulus 1A was significantly above random when choosing between 1A vs 4 ($p<0.001$), 1A vs 5 ($p<0.001$) and 1A vs 6 ($p=0.011$), but not significant for 1A vs 2B ($p=0.172$). The choice of trained stimulus 1B was significantly more probable than random when choosing between 1B vs 6($p=0.011$), just slightly above the 0.05 critical value in the condition of 1B vs 5 ($p=0.055$), and even further above the cut-off for 1B vs 4 ($p=0.172$) and 1B vs 2B ($p=0.623$). The successful choice of 1C was at an above average ratio when compared to test stimulus 5 ($p<0.001$), but not statistically above random for 1C vs 6 ($p=0.055$), 1C vs 4 ($p=0.172$), and 1C vs 2B ($p=0.945$). Despite not reaching significance under several conditions, BD#1 still had a 28/40

rate in choosing 1C over all the test stimuli combined and a 29/40 ratio in choosing 1B over all the test stimuli. Both of these ratios are above random for number of correct choices, on the binomial test (p=0.008 and p=0.003).

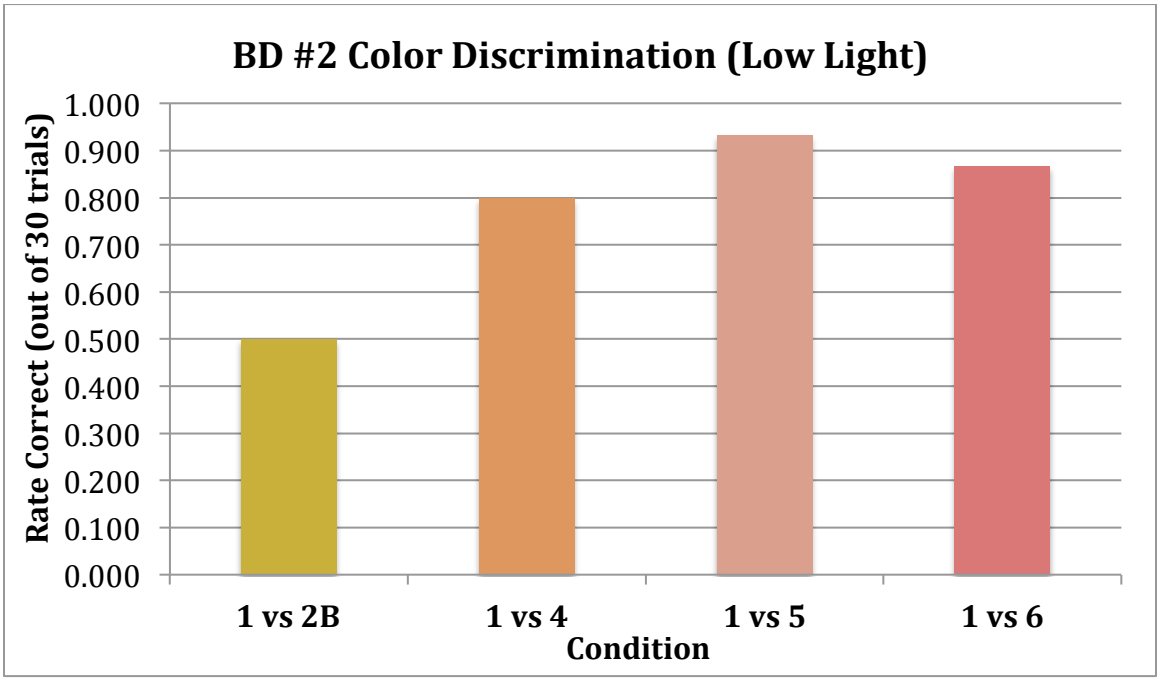

Figure 5. Color Discrimination of Bearded Dragon #2 Under Low Light & With Long Wavelength Test Stimuli. The x-axis of the graph represents the condition of trained stimuli vs test stimuli that the dragon was presented. Trained stimulus 1 is a sum of trials across the three brightness of green (1A, 1B, and 1C). Each condition is comprised from 30 trials, divided equally among the the brightnesses (30 trails of 1 vs 2B = 10 trails of 1A vs 2B + 10 trails of 1B vs 2B + 10 trails of 1C vs 2B). The y-axis represents the lizard's success rate of choosing the trained stimulus out of 30 trails for each condition. The bar colors are approximations of the colors of the test stimuli 2B, 4, 5 and 6. As the test stimuli wavelength diverged further from the green trained stimuli, the rate of correct choice increased, but decreased slightly at condition 1 vs 6.

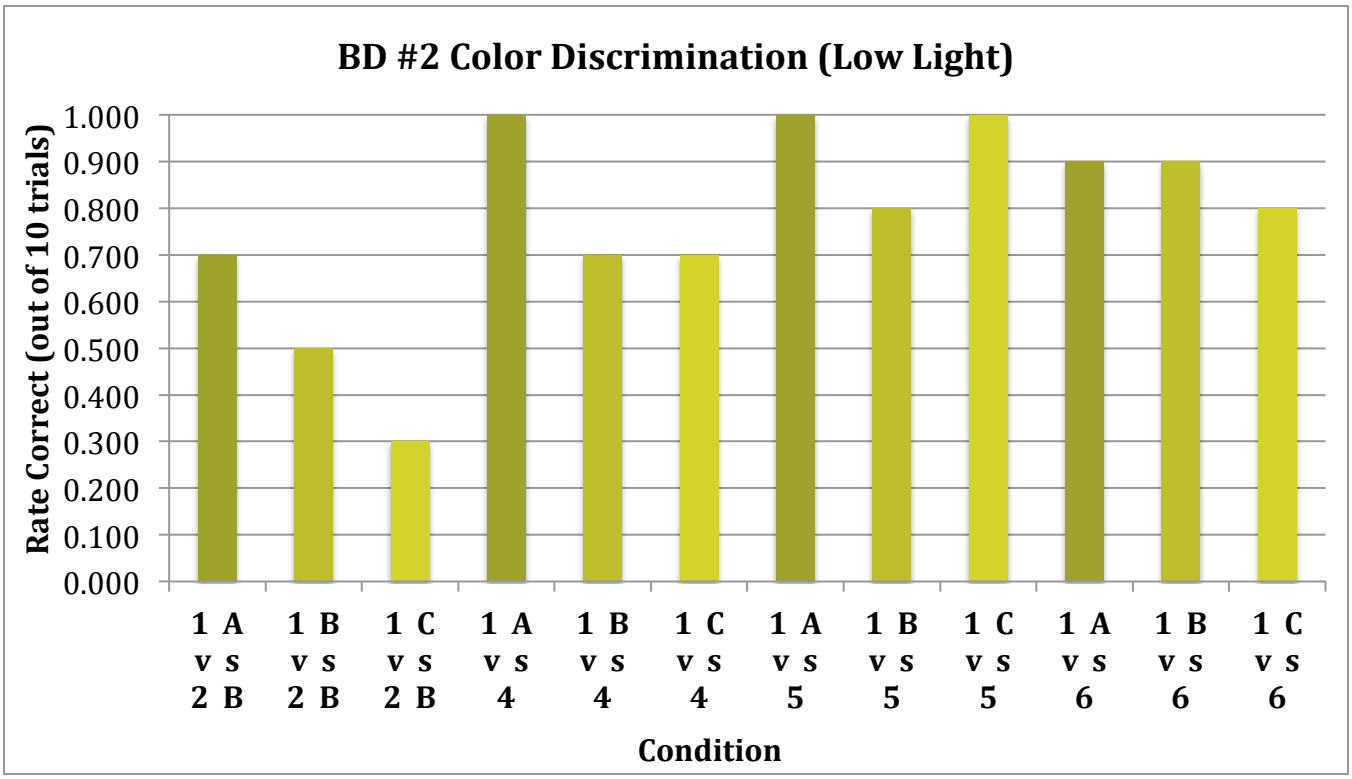

Figure 6. Color Discrimination of Bearded Dragon #2 Under Low Light & With Long Wavelength Test Stimuli. The x-axis of the graph represents the condition of trained stimuli vs test stimuli that the dragon was presented. This figure shows the conditions from figure 5 broken down by brightnesses of the trained stimulus, instead of summing them. The y-axis represents the lizard's success rate of choosing the trained stimulus, out of 10 trails for each condition. The bar colors are approximations of the colors of the trained stimuli 1A, 1B, and 1C. This figures indicates the possibility of brightness preferences when the test stimulus was 2B or 4. See discussion for detail.

3) Normal Light & Short Wavelength Test Stimuli

Refer to figure 7 & 9 for summary of the results on BD#1 and figure 8 & 10 for summary on BD#2. No control trials were used for this part of testing.

A binomial test on the rate of successful choice at different color conditions showed that BD #1 was significantly above average for discriminating between the green trained stimuli 1 (1A, 1B and 1C combined) and test stimuli 9, 10 and 11 (p values of $p<0.001$,$p<0.001$, and $p<0.001$). BD#1 was not above random in selecting the trained stimulus in the condition of 1's vs 8 ($p=0.708$) (Fig. 7).

BD #2 was significantly above random in choosing the green trained stimulus (1A+1B+1C) against all the test stimuli 8, 9, 10 and 11 (p values of $p<0.001$,$p<0.001$, $p<0.001$ and $p<0.001$, respectively). Out of 30 trails at each condition, BD#2 only made 4 mistakes on 1 vs 8 and was a perfect 30 for 30 when discriminating against the other test stimuli of 9, 10, and 11 (Fig. 8).

Refer to Figure 9 for a breakdown of BD#1's data after it has been separated by different brightnesses of the trained stimulus. A binomial test indicated that the choice of trained stimuli 1A was above random when compared to test stimulus 11 ($p=0.001$), but not above random when discriminating between 1A vs 10($p=0.054$), 1A vs 9 ($p=0.172$) and 1A vs 8 ($p=0.999$) In fact, a rate of 1 correct choice out of 10 trials for condition 1A vs 8 also does not seem random. The probability of picking 1A once or fewer at random equals 0.011 or 1.1%, according to the binomial test. When given the trained stimuli of 1B and 1C, BD#1 was above average in picking the correct chip in the conditions of 1B vs 11, 1B vs 10, and 1B vs 9 ($p=0.001$, $p=0.001$, and $p=0.001$, respectively) as well as 1C vs 11, 1C vs 10 and 1C vs 9 ($p=0.001$,

p=0.001, and p=0.001, respectively). BD#1 did not perform higher than random in the condition of 1B vs 8 (p=0.377) and 1C vs 8 (p=0.377)

Refer to figure 10 for an analysis of BD#2's performance across different trained stimuli brighnesses. According to the binomial test, BD#2 chose trained stimuli 1A, 1B and 1C at an above random proportion when discriminating between all conditions except 1B vs 8. For this condition BD#2 had a ratio of 8 correct choices out of 10 attempts, which yields a p value of 0.054 on the binomial test, a value just above the 0.05 cut-off value.

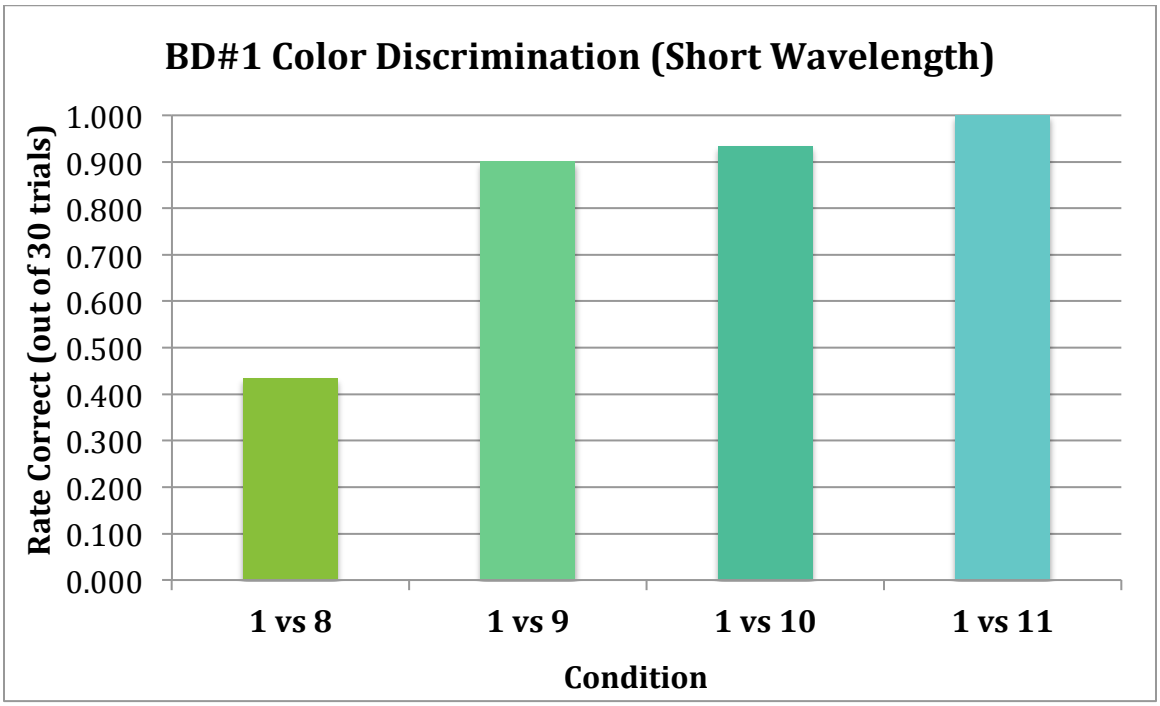

Figure 7. Color Discrimination of Bearded Dragon #1 Under Normal Light & With Short Wavelength Test Stimuli. The x-axis of the graph represents the condition of trained stimuli vs test stimuli that the dragon was presented. Trained stimulus 1 is a sum of trials across the the three brightness of green (1A, 1B, and 1C). Each condition is comprised from 30 trials, divided equally among the

brightnesses (30 trails of 1 vs 8 = 10 trails of 1A vs 8 + 10 trails of 1B vs 8 + 10 trails of 1C vs 8). The y-axis represents the lizard's success rate of choosing the trained stimulus out of 30 trails for each condition. The bar colors are approximations of the colors of the test stimuli 8, 9, 10 and 11. As the test stimuli wavelength diverged further from the green trained stimuli, the rate of correct choice increased.

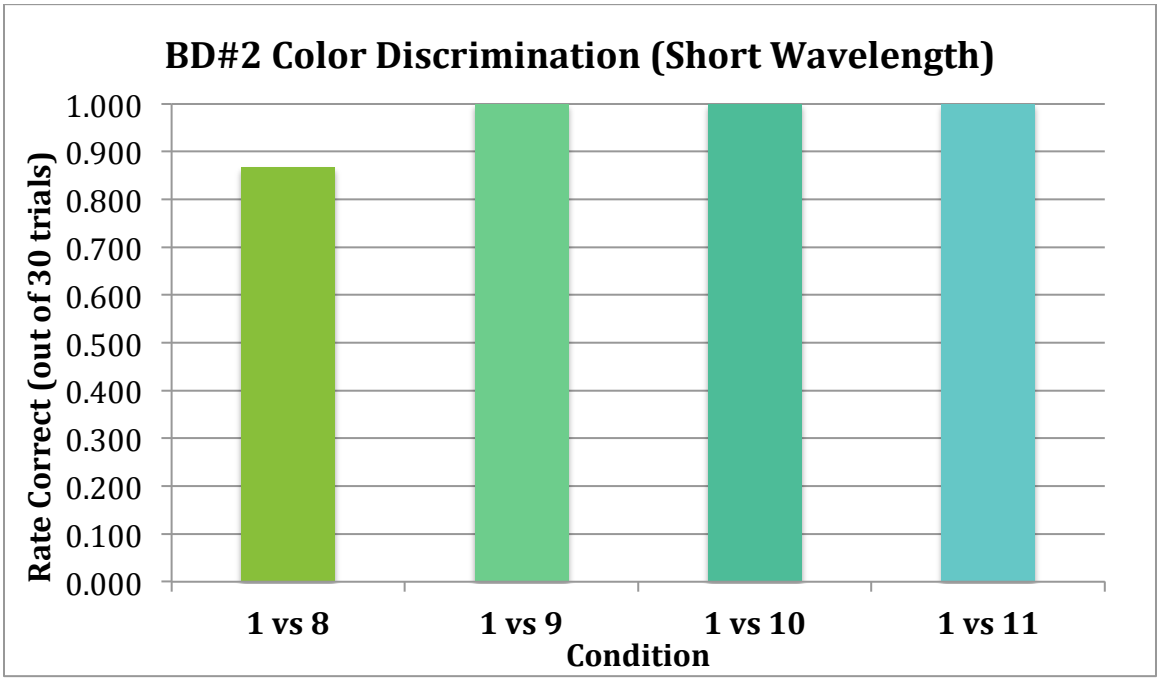

Figure 8. Color Discrimination of Bearded Dragon #2 Under Normal Light & With Short Wavelength Test Stimuli. The x-axis of the graph represents the condition of trained stimuli vs test stimuli that the dragon was presented. Trained stimulus 1 is a sum of trials across the the three brightness of green (1A, 1B, and 1C). Each condition is comprised from 30 trials, divided equally among the brightnesses (30 trails of 1 vs 8 = 10 trails of 1A vs 8 + 10 trails of 1B vs 8 + 10 trails of 1C vs 8). The y-axis represents the lizard's success rate of choosing the trained stimulus out of 30 trails for each condition. The bar colors are approximations of the

colors of the test stimuli 8, 9, 10 and 11. The success rate for all of the conditions, including 1 vs 8, was significantly higher than random choice.

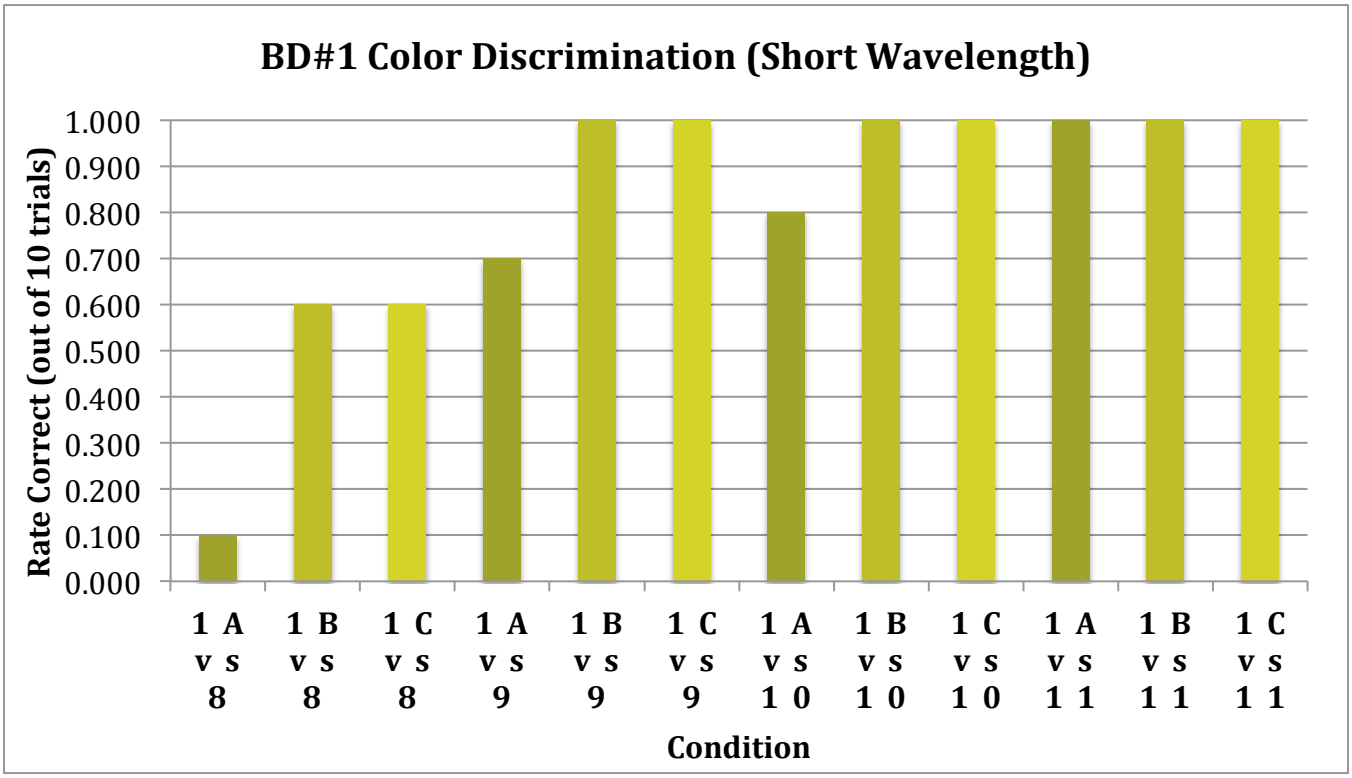

Figure 9. Color Discrimination of Bearded Dragon #1 Under Normal Light & With Short Wavelength Test Stimuli. The x-axis of the graph represents the condition of trained stimuli vs test stimuli that the dragon was presented. This figure shows the conditions from figure 7 broken down by brightnesses of the trained stimulus, instead of summing them. The y-axis represents the lizard's success rate of choosing the trained stimulus, out of 10 trails for each condition. The bar colors are approximations of the colors of the trained stimuli 1A, 1B, and 1C. The bearded dragon was less successful in identifying trained stimulus 1A in several conditions.

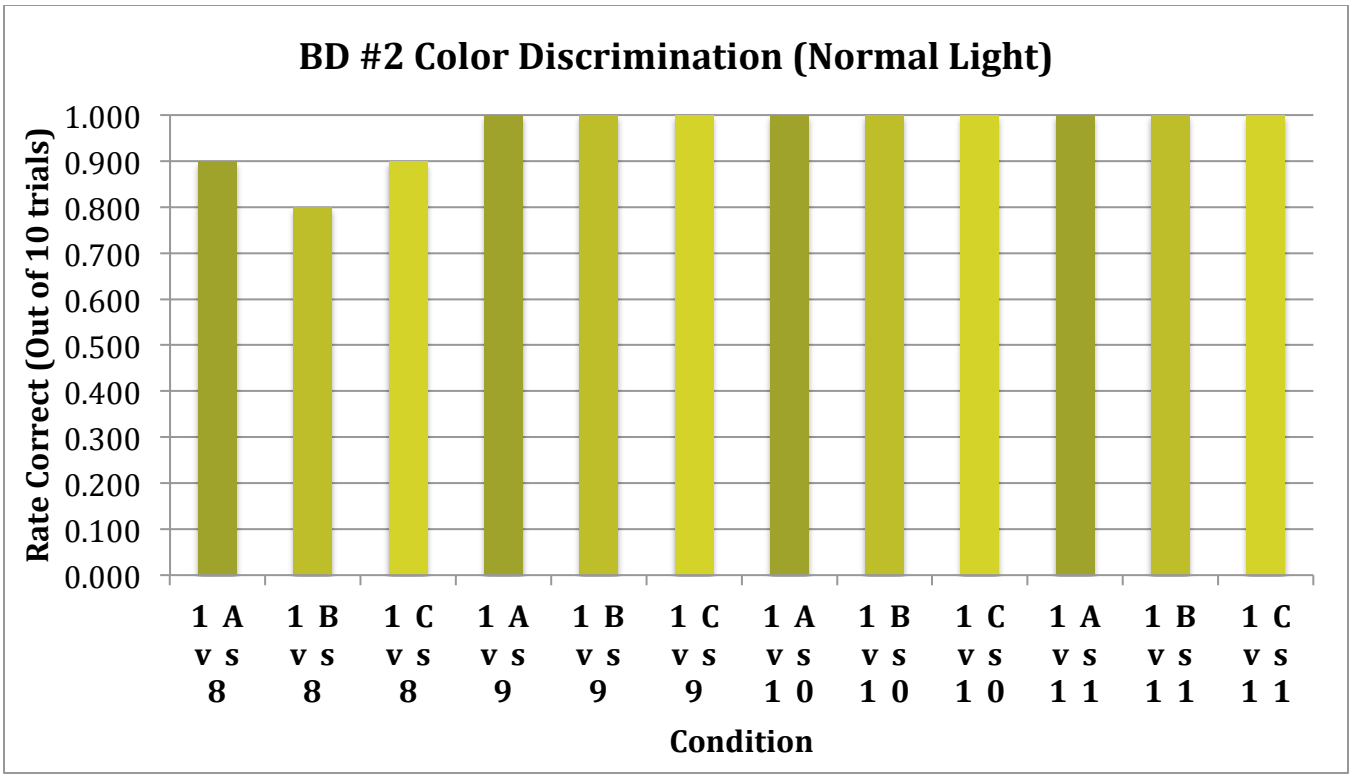

Figure 10. Color Discrimination of Bearded Dragon #2 Under Normal Light & With Short Wavelength Test Stimuli. The x-axis of the graph represents the condition of trained stimuli vs test stimuli that the dragon was presented. This figure shows the conditions from figure 8 broken down by brightnesses of the trained stimulus, instead of summing them. The y-axis represents the lizard's success rate of choosing the trained stimulus, out of 10 trails for each condition. The bar colors are approximations of the colors of the trained stimuli 1A, 1B, and 1C. The bearded dragon was successful across all conditions, regardless of brightness.

DISCUSSION

Based on numerous trials on these two individuals, we stipulate that this behavior experiment has demonstrated the use of color vision in Australian bearded dragons (*Pogona vitticeps).* We believe that these results could only have been achieved if the bearded dragons have the neural ability to use the chromatic

mechanism. Because of the design of our experiment, if the dragons were making a choice based on brightness, they would get about half of their trials wrong. All of the test stimuli were matched to the brightness of 1B, so use of the achromatic pathways would cause brightness irregularities to be seen throughout the data, even at conditions where the chromatic contrast between trained and test stimulus was high. For the most part, instances of possible brightness preferences occur where the chromatic contrast is fairly small (test stimuli 2B and 8). Figures 3 and 9 indicate that BD#1 may have developed a preference for choosing the brighter chip when chromatic contrast between the chips was small. This is very apparent in Figure 9, where the dragon made more mistakes with the 1A trained stimulus, but when chromatic contrast was high (green trained stimuli vs blue test stimulus 11), the dragon was 100% successful across all brightnesses of the trained stimulus (including 1A). Contrastingly, BD#2 may have developed a preference for the darker chip with these chromatically similar conditions (Figure 4 and 6). When we saw instances of non-significance for other conditions, it may have been caused by low sample sizes, where at least 7 or 8 correct choices out of 10 trials will fall outside of the 0.05 critical value on the binomial test. We generally observed ratios that were above random for chromatically distant choices (1 vs 4, 5, 6, 9, 10 and 11) but not significantly higher that random for chromatically similar conditions (1 vs 2B and 8). Even thought we saw evidence of brightness deviations at these similar colors, it did not affect our results because our experimental design ensured that brightness preferences would average out to approximately a 50% success rate.

The performance of BD#2 at low light (Figure 5 and 6) yielded more errors than other parts of the testing, but the results were still above random for choosing the trained stimuli against test stimuli 4, 5 and 6. This was the only time during testing that mistakes occurred at the furthest contrast in chroma (1 vs 6). We expected color discrimination thresholds to increase at low light, but we had difficulty finding a light level where the lizards would continue to do the behavior and be more challenged. BD#2 had its light levels reduced from 20 μmol of photons per $m^2 \cdot s^1$ to 4.6 μmol of photons per $m^2 \cdot s^1$, which did reduce success modestly. Such a small decrease in light can theoretically be handled quite easily by the visual system, so only a modest reduction in success is not surprising. Lowering the light levels further may have caused more errors, but unfortunately the lizards stopped performing the action and would sometimes hide or sleep when a dark filter was put over their cage. Lowering the light levels actually caused BD#1 to lose this trained behavior about halfway through dark light testing and she needed to be retrained. Lowering the light also caused the dragons difficulty in finding their worm reward after knocking off the correct chip. They would often miss the hollowed out holes (wells) in the plastic block or would not even attempt to find the worm. It is possible that because of their evolutionary history as desert lizards, bearded dragons require a high light intensity to remain active and have optimal vision.

The test stimulus with the closest chromatic contrast to the trained stimuli presented difficulty for both BD#1 and BD#2, in most cases. The scenario of bearded dragon #2 under normal light and with short wavelength test stimuli turned out to

be the only case where the lizards was able to discriminate between the closest choice (1 vs 8) at a statistically significant proportion (p=0.000 on the binomial test). BD#1 was not able to achieve the same result on the 1 vs 8 condition, achieving a 13/30 ratio of correct choice, which can be attributed to random choice. This result may simply indicate that BD#2 became more proficient at the behavior than BD#1, who had to be retrained after the low light experiments. Generally, our results demonstrated that bearded dragons posses the ability to reliably discriminate objects based on color, if the difference in color exceeds a certain threshold. The way our experiment was set up, made it difficult to quantify the threshold at which two colors become chromatically distinguishable for the bearded dragons. This experiment was a pilot for future experiments on anolis lizards and has given us the information on how to modify the set up in order to observe this threshold. In the future we wish to train lizards to a grey stimulus and present test stimuli with gradual saturations of color, until the lizards are able to identify the trained stimulus.

Our demonstration of color vision through behavior is novel to in the family Agamidae. It is additionally interesting because the bearded dragons to not seem to possess much dermal coloration or to use color in social interactions. They do occasionally eat green foliage but the colors of their diet may likewise be restricted, and movement likely serves as a better indicator for the presence of food. One may claim that color vision may be a residual remnant of the bearded dragon visual system because of phylogeny. It is true that the Agamidae family has some colorful species which could used color vision for sexual selection, and it is possible that the

evolutionary ancestors of this family had color vision. Nevertheless, evolutionary theory would argue that an energetically expensive trait, which does not have a contribution to fitness through reproduction or survival, would be lost over time. This leads one to believe that bearded dragon color vision either has a low energy cost or that color vision has survival functions that are not initially apparent. There are several theories about the adaptive advantage of color vision, including the possible use of color vision for food scavenging and general navigation.

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