Visual Communication in *Anolis* Lizards: Investigating the Visibility of Signals of Differing Color Against Complex Backgrounds

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ABSTRACT

PEREZ, CARLEY Visual Communication in *Anolis* Lizards: Investigating the Visibility of Signals of Differing Color Against Complex Backgrounds

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Lizards of the genus *Anolis* communicate through visual displays involving the opening and closing of an expandable flap of skin, called a dewlap, located on their throat. In some habitats up to ten species of *Anolis* are found in close proximity to one another. For a dewlap color to be effective an individual of the same species should be able to easily distinguish it from the dewlap color of other species located in the same habitat. We wanted to determine how different two colors must be in order to be reliably discriminated by an *Anolis* lizard.

Lizards (*Anolis sagrei*) placed in small cages were presented with a flash of a small square of color, viewed against a background consisting of a gray scale checkerboard*.* We recorded whether or not lizards shifted their gaze toward the novel color as function of the distance in perceptual space between the stimulus color and the gray background.

We found that lizard color discrimination increased as the distance in perceptual color space between the two stimuli increased. This suggests that selection pressures on dewlaps are in the direction of greater perceptual color difference between species.

Introduction

Animals communicate in many different ways. No matter what sensory modality is employed, any effective animal signal must be successful at stimulating the sensory system of its intended receiver. Differences in signal design amongst closely related species are often observed, and these differences are believed to be involved in the process of species divergence. The signal's function, the habitat the animal is signaling in, and the sensory system of the receiver, all influence the efficacy of a signal stimulus. Differences among species in any of the factors might lead to the evolution of different signal designs in closely related species (Fleishman, 2000). Two different species living in different habitat conditions may evolve different signal designs that increase their detectability. However in order to test this hypothesis one must first determine what makes a signal most effective in its respective habitat.

The members of the genus *Anolis*, with its approximately 400 species, are spread throughout many different habitats in North, South and Central America and islands of the Caribbean. Each possesses an expandable flap of skin, called a dewlap, located on their throat, which varies in color and pattern amongst different species. Anoles open and close their dewlap as part of a visual display (Losos, 2009). Although not all the functions of the displays are clear, it is believed that they are used during courtship, and the marking of territory. An important role of dewlap color during these interactions is to signal species identity so that individuals do not waste time or energy trying to chase off, or mate with, anoline lizards of a different species. In some habitats up to ten different species of *Anolis* live in close proximity to one another making species recognition a necessity (Losos 2009). Vanhooydonck et al. (2008) found support for the hypothesis that dewlaps are used for species recognition. They showed that the dewlap patterns of male *Anolis sagrei* populations located on different islands in the Bahamas increased in complexity when living among other species of *Anolis*. Other studies using a larger sampling scheme have not been able to support the species recognition hypothesis statistically (Nicholson et al., 2007). They concluded that species living in sympatry do tend to have different patterned dewlaps but when considering the large variety of dewlap patterns in existence there findings indicate that it could be due to chance and evidence for the Species Recognition hypothesis.

While courtship displays occur at close range, territorial displays are performed at long distances from the receiver and thus need to be highly detectable (Fleishman, et al., 2009). Previous research determined that dewlap color increases the likelihood that an anole will be seen by the intended receiver while displaying (Fleishman, 2000). This corresponds with the fact that anoles have excellent color vision.

The eyes of an anole are designed for “high-acuity diurnal vision” (Fleishmans & Persons, 2001). Anoles are tetrachromats: they have four types of cones in their retina, allowing them to see a full range of colors, including the ultraviolet. They have a high density of photoreceptors with an increased density located in their two foveae, one central and one temporal (Losos, 2009). A fovea is a depression in the surface of the retina where acuity is the highest because the light is bent in a way that enlarges the image. (Bradbury & Vehrencamp, 2011). Despite their good daytime vision, night vision may be difficult for anoles because they lack rods. Their small cones, packed into a high-density array provides them with excellent visual resolution. However, their small eye size, and resulting small retinal image may reduce this resolving power (Fleishman 1992).

Previous research has determined that brightness and chromaticity contrast between a stimulus and its background are important factors in signal detectability. Here, the term "brightness" refers to perceived intensity, independent of color. The experiments involved moving a dewlap-like stimulus flag across a monochromatic background on the edge of a lizard's field of vision. The brightness and color of the stimulus flag and background were changed. Researchers observed whether or not a lizard would notice the moving flag and shift its gaze toward the stimulus. The results determined what contrast combinations between a stimulus and background made a dewlap-like stimulus visible (Fleishman and Persons 2001). There has been some research testing the hypothesis that differences in habitat light conditions result in species divergence because of evolution in the direction of higher signal detectability (Leal & Fleishman, 2004). The habitat light, and dewlap characteristics were quantified in four populations of the same species of anole, some living in xeric conditions and some living in mesic conditions. The results showed that both the habitat light and dewlap design differed between the xeric and mesic populations. The dewlap design had changed to make the signal more detectable in their respective habitats (Leal & Fleishman, 2004).

The experiments described above provide insight into how background coloration can influence the detectability of a visual signal and provide an example of how this might be reflected in nature. However, in nature the backgrounds against which such signals are seen are far more complex than those utilized in the first experiment. In the wild, lizards view dewlaps against background conditions that consist of many different patches of brightness and color; not a uniform background. This adds to the difficulty of a dewlap being seen by its intended receiver. The experiments described in this paper represent an attempt to determine the factors that influence dewlap visibility under conditions that are much closer to the natural conditions in which these signals occur.

We focused on chromaticity and its effect on signal detectability. In habitats where there are sympatric populations of *Anolis* an individual of one species needs to be able to distinguish a dewlap color from that of another species. We wanted to determine how different two colors had to be in order to be reliably discriminated by an *Anolis* lizard. To better imitate the complexity of a natural background, checkered boards made up of gray squares of differing brightness were used. A moveable square in the middle of the boards served as our stimulus. Each different stimulus increased in chromaticity from the initial gray square. We predicted that as the distance in perceptual color space increased, so would detectability.

The aim of this study is to determine the quantitative relationship between differences in perceptual space and the probability of detection of signal color differences under complex natural viewing conditions. In this way we hope to understand how selection for unambiguous species recognition has impacted the evolution of differences in colors of dewlap of species living in sympatry. Ultimately, this data will be used to determine what dewlap colors are maximally visible in certain habitats, where natural background colors and brightnesses as well as the congenerics living in the area have been quantified previously, and to predict the dewlap colors of wild anole species. Comparing the predicted dewlap colors with the actual dewlap colors will test the effect habitats have on the evolution of visual signals.

Methods

Anolis sagrei were used for all experiments with 10 individuals being used per experiment. They were kept in ~86°C and 50% humidity, and were watered and fed 4-5 Phoenix worms (soldier fly larvae) every other day. The lizards were isolated in identical cages that had a screen at the top, Plexiglas on one side and a perch inside that stretched across the width of the structure, as shown in Figure 1. All the cages were placed next to, but not facing each other. Each cage was illuminated by a Solux 50 W halogen light located above and toward the front of each cage. The lights were maintained on a 12-hour light 12-hour dark cycle. The Solux bulbs approximate sunlight, but lack ultraviolet wavelengths. Before experiments began the lizards were allowed to adjust to the lab for approximately a week. The Solux bulbs were

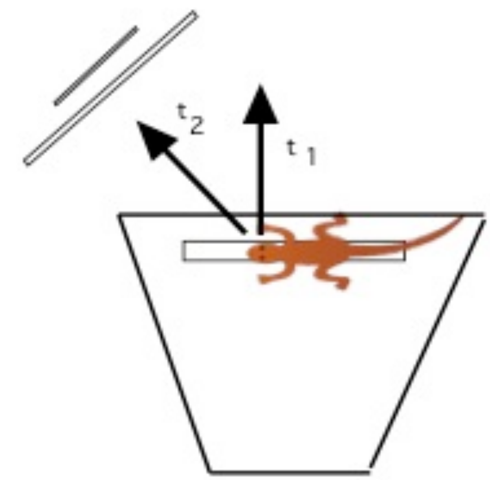

Figure 1. The experimental arrangement for all trials, showing the lizard on the perch inside a cage with a transparent Plexiglas window located on one side. Before each trial began the lizard was faced towards the wall of its cage with its line of sight pointed out at T_1. When the stimulus was moved a positive response would involve the

covered with a glass diffuser. They were positioned in such a way that they diffusely illuminated the front of the cage and the stimulus squares in front of the cage.

A movable cart was placed in front of the cage during the beginning of each trial, which held a stand containing a camera aimed towards the Plexiglas side of the cage and a checkerboard background. The camera was connected to a computer outside the room so the experimenter could observe lizards without being visible to them. The gray scale checkerboard consisted of multiple squares of varying brightness and was placed outside the cage, facing towards the lizard at an angle. A square in the middle was removed and a moveable card was placed behind it so the color of the square could be changed during trials, as shown in Figure 2. This served as the stimulus.

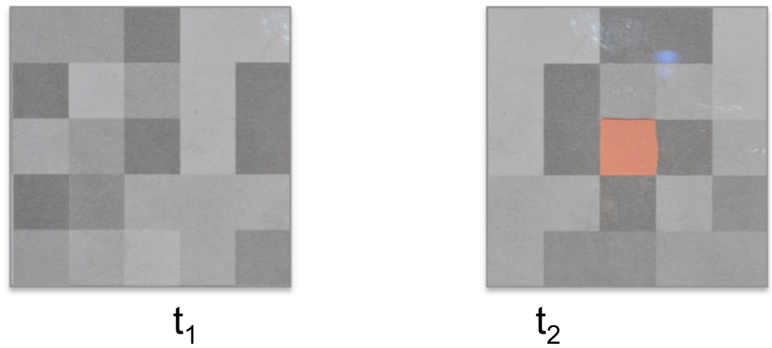

Figure 2. Lizard's eye view of the stimulus. T_1 shows the stimulus in its control position and T_2 is the stimulus after it was moved. This was held for 3 seconds and then it was returned to position T_1

Each trial began with the checkerboard background placed parallel to the transparent side of the cage. When the lizard was on the perch and faced towards one of the walls on the left or right side of its cage, with its monocular gaze directed out of the cage, the moveable box in the middle of the background was switched for 3 seconds. A positive response was recorded if the lizard shifted its gaze from the wall opposite the cage to the checkerboard background. A negative response was recorded if this did not happen. Trials were run every other day during weekdays. The order of treatments given to a lizard was randomized.

Five stimulus cards were created, as shown in Figure 3. The gray color used in all the cards was Munsell color N8. Stimulus card B was Munsell color 10R 8/1, stimulus card C was 10R 8/3, stimulus D was 10R 8/6 and stimulus E was 10R 8/8. All of the stimulus cards had a matte finish. The reflectance spectra for each stimulus card, which shows what spectra the lizard sees, are shown in Figure 4.

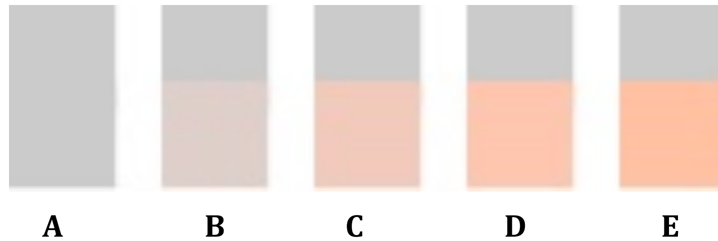

Figure 3. The five stimulus cards used in the experiment. Stimulus A served as control. Each lizard viewed the stimuli in a random order for a total of 25 trials. In each trial the color of the central square changed from gray to the lower color.

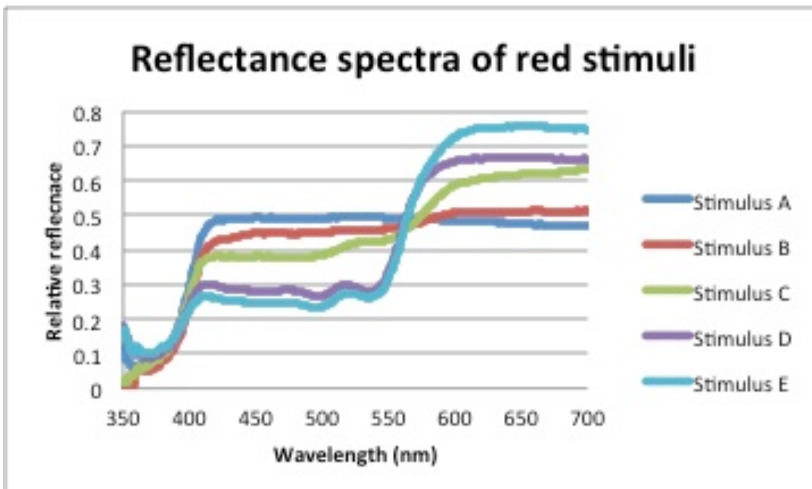

Figure 4. The reflectance spectra for each of the five stimulus cards. As chromaticity increases from Stimulus A to Stimulus E so does relative reflectance in the upper wavelength zone.

Results

We found that lizard color discrimination increased as the distance in perceptual color space between the two stimuli increased, as shown in Figure 5.

Color discrimination reached its highest level at Stimulus E. Friedman's Analysis of Variance by Ranks was used to analyze all data (Zar, 1999). Mean positive response at Stimulus B and Stimulus C was not significantly different than the mean positive response at the control, Stimulus A ($p>0.05$). Mean positive response at Stimulus D and Stimulus E was significantly different than mean positive response at the control, Stimulus A ($p <0.05$).

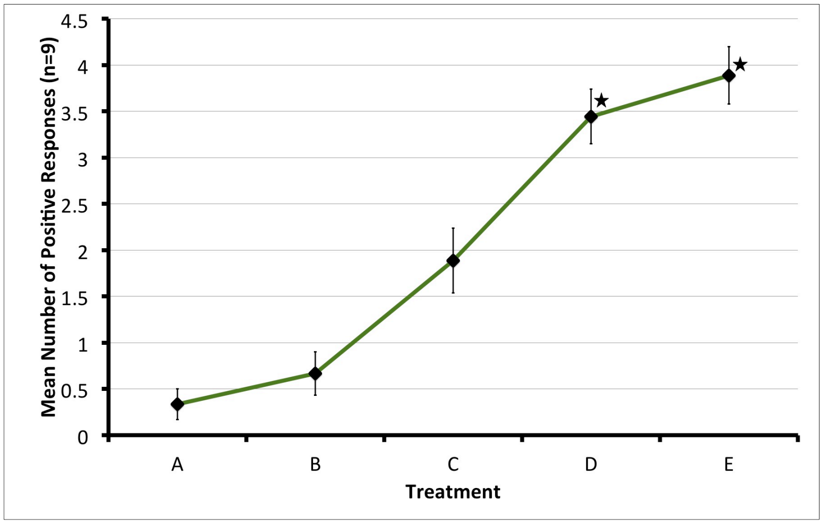

Figure 5. Mean number of positive responses for each treatment. Positive response increased to Stimulus E. The error bars indicate standard error. Asterisk indicates a significant difference from control ($p<0.05$).

Discussion

Positive response at Stimulus D and Stimulus E was significantly different than positive response at the control, indicating the anoles could discriminate color at these two points. The results indicate that detectability increased as the distance in perceptual color space between the two colors in the stimuli increased. This suggests that in the wild selection pressures on dewlaps will be in the direction of greater perceptual color difference between species. However, for perceptual distances greater than Stimulus D positive response does not seem to increase.

Species recognition has been shown to be vital for individuals living in sympatric populations as to avoid costly heterospecific matings. Several past studies have supported the hypothesis that dewlaps serve as a means of species recognition amongst *Anolis* starting with Losos who found that, in combination with head bobbing, male *Anolis marcanoi* became more aggressive around *Anolis cybotes* with dewlaps altered to be the same color as a conspecific (Losos, 1985). Vanhooydonck et al. found similar results when studying dewlap patterns in populations of *Anolis sagrei* that inhabit seven different islands in the Bahamas. On islands with more congeneric species dewlaps tended to have a more complex pattern (Vanhooydonck et al., 2008). However, when dewlap color was compared across a broad sampling of *Anolis* the pattern of different dewlap colors amongst congenerics living in sympatric populations was observed but when considering the huge variety of dewlap colors found in their experimental area this pattern was not statistically significant and thus could be due to chance (Nicholson et al., 2007). The results here suggest why this might be the case. It may not be necessary for colors of sympatric species to differ by a dramatic amount. They only need to be "different enough" to reach the discrimination threshold. This even rather modest among species differences might be sufficient to allow species discrimination to occur.

We believe quantifying an anole's ability to discriminate colors is an alternative approach to studying how dewlaps are involved in species recognition that will avoid the issues discovered by Nicolson et al. in their study. This method can be used in the future to quantify the relationship between detectability and perceptual color space using many different color combinations. Light intensity can also be varied to determine the effect different habitat light conditions have on the detectability/perceptual color space relationship. For example, theory suggests that under low light conditions greater distances in color space would be required to achieve reliable species discrimination. This suggests that species from well-shaded habitats might have to diverge more in color than those from more open habitats. Using this model for continued experimentation, the ultimate goal is to determine what makes a dewlap maximally visible in a certain habitat. Comparing the predict dewlap colors to dewlap colors scene in the wild will determine how much of an effect habitat has on dewlap evolution.

References

Bradbury, J. W., and Sandra Lee Vehrencamp. *Principles of Animal Communication*. Sunderland, MA: Sinauer Associates, 2011. Pg.179.

Fleishman, L.J. (1992). The influence of the sensory system and the environment on motion patterns in the visual displays of anoline lizards and other vertebrates. American Naturalist 139:S36-S61.

Fleishman, L.J. (2000). Signal function, signal efficiency and the evolution of anoline lizard dewlap color. *Animal Signals*. 209-236.

Fleishman, L.J. & Persons, M.H. (2001). The influence of stimulus and background colour on signal visibility in the lizard *anolis cristatelliu. Journal of Experimental Biology.* 204.

Fleishman, L.J., et al. (2009). Habitat light and dewlap color diversity in four species of Puerto Rican anoline lizards. *Journal of Comparative Physiology.* 1044-1060.

Leal, M & Fleishman, L.J. (2004). Differences in visual signal design and detectability between allopatric populations of *Anolis* lizards. *The American Naturalist*. 26-39.

Losos, J.B.L. (1985). An Experimental Demonstration of the Species-Recognition Role of Anolis Dewlap Color. *Copeia* 4:905-910.

Losos, J.B.L. (2009). *Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles.* University of California Press, Los Angeles. 507 pages.

Nicholson, et al. (2007). Evolution of *Anolis* lizard dewlap display. *PLoS ONE.*

Vanhooydonck, et al. (2008). What determines dewlap diversity in *Anolis* lizards? An among-island comparison. *Journal of Evolutionary Biology*. 293-305.

Zar, J.H. 1999. Biostatistical Analysis 4th Edition. Prentice Hall. Saddle River, New Jersey.