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The Ecology of Dynamic Body Color in the Green Anole, *Anolis carolinensis*

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THE ECOLOGY OF DYNAMIC BODY COLOR IN THE GREEN ANOLE, *ANOLIS CAROLINENSIS*
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A DEPARTMENT HONORS THESIS SUBMITTED TO THE
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Abstract

Organisms capable of physiological body color change can respond rapidly to changes in their social or external environment. Given that color change is often context-dependent, studying the role of color change can provide insights into how different organisms respond to and interact with their immediate environment. Currently there are three main hypotheses which explain the adaptive significance of rapid color change, including camouflage, social signaling, and thermoregulation. Green anoles, *Anolis carolinensis*, are one such species that use physiological color change to rapidly shift their dorsal body color from bright green to dark brown within seconds. Thus, they may use darkening and lightening of body color to either regulate their body temperature throughout the day, or as a visual signal during intraspecific communication. Previous studies have determined the physiological mechanisms by which color change occurs in green anoles, but few naturalistic studies have determined the ecological role of dynamic color change in anoles, and fewer still have examined how males and female green anoles may differentially use color change.

In this thesis, I performed two studies testing two major hypotheses for the evolution of physiological color change. In my first study I examined the relationship between body color and body temperature of green anoles, and whether this varies between the sexes, or among different substrate types. My data showed that while males choose marginally warmer substrates and more exposed perch sites than females, there was no association between body color and body temperature in either sex. In the second study I tested whether body color is used in conjunction with behavioral displays of green anoles, and if this differs between the sexes. My results showed that overall, males are far more likely to be green than females. Further, males and females differ in how they use body color during social displays. In sum, my studies found that

body color change is predominantly used in behavioral displays of green anoles, and males and females differ in how they use color during social signaling. My findings also indicate that thermoregulation is likely not a primary reason for body color change in either sex, and color likely plays less of a role in thermoregulation in anoles than previously believed.

Acknowledgments

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likely had some influence on my path towards biological field work.

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Chapter 1: The Biology of Color Change

Introduction

Many species rely on color vision to interact and engage with the environment around them (Kelber *et al.* 2003). The maintenance of color vision in a diversity of animal species (e.g., Chittka & Menzel 1992, Osorio & Vorobyev 1996) suggests that color has continually played an important ecological role in their evolution. For example, body color is often an adaptive phenotype with a number of potential functions. An organism with a body color similar to its environment can use camouflage to remain hidden from predators (Kaufman 1975) or prey (Théry & Casas 2002). Alternatively, elaborate body colors and patterns can be used to advertise toxicity (Maan & Cummings 2012) or can be used as an honest signal in mate choice (Griggio *et al.* 2010) or aggressive interactions. Yet while most studies of body color have analyzed static body color expression, many organisms also have the ability to individually modify their body color in response to changes in their environment, across a variety of timescales (Duarte *et al.* 2017).

Organisms may modify their outward appearance in two primary ways: morphological and physiological color change. Morphological color change is a relatively slow, progressive change, which takes place over a period of days or weeks (Leclercq *et al.* 2010). These changes require a structural change in the number of chromatophores (Ohta *et al.* 2008), cells that contain pigments within the animal's skin (Sugimoto 2002). Morphological color changes increase or decrease the number of chromatophores, the pigment concentration within the chromatophores, or a combination of the two (Green 1964). These slower shifts in color generally depend on ontogenetic, dietary, or seasonal factors (Nery & Castrucci 1997). In contrast, physiological color change is much more dynamic. It involves the rapid movement of melanophores within chromatophores, allowing color change to occur anywhere within milliseconds to hours (Stuart-

Fox & Moussalli 2009). This rapid change in color allows organisms to appropriately adjust their

appearance quickly to any shifts in environmental or social situations, giving organisms

increased behavioral flexibility (Sköld *et al.* 2013).

A number of organisms are capable of physiological color change (amphibians: Novales

& Davis 1969; insects: Raabe 1982; cephalopods: Hanlon 2007; Auerswald *et al.* 2008; fish:

Sköld *et al.* 2008; and crustaceans: Stephens 2016). One of the more notable groups in which

physiological color change is widely pervasive include agamid and iguanid lizards (Stuart-Fox &

Moussalli 2008). Most of the earlier work on physiological color change within these lizard

groups primarily centered on understanding the physiological mechanisms of dynamic color

change in response to ecological factors such as temperature changes or the presence or absence

of light (Taylor & Hadley 1970, Sherbrooke & Frost 1989, Greenberg & Crews 1990,

Sherbrooke *et al.* 1994, Morrison *et al.* 1996). However, more recent studies on color change

within lizard species have examined the adaptive benefits of rapid color change.

Currently there are three major hypotheses for dynamic color change within this group,

including camouflage, physiological regulation, and behavioral comunication (Caro 2005).

First, many lizard species are diurnal and may rely on color change to remain cryptic in their

natural environment to ward off visual predators (Ito *et al.* 2013, Smith *et al.* 2016). Second,

physiological color change can benefit ectothermic lizards by providing the flexibility to actively

regulate heat absorption or reflection and thereby maintain internal body temperatures despite

changes to environmental temperatures (Rosenblum 2005, Krohn & Rosenblum 2016). Finally,

many lizards use colorful displays in both intra- and intersexual communication (Robertson &

Rosenblum 2009). However, to fully understand the role that dynamic color change may play in

the evolution of lizards, it is important to consider that conflicts may arise between each of these

three hypotheses, especially when mechanisms for camouflage, communication, and thermoregulation depend on different colors (Smith *et al.* 2016). Therefore, different species of lizards may utilize color for different adaptive strengths depending on which strategy is most important for either survival or reproduction. These competing requirements may be mediated by predominantly adjusting color for one function over another, or partitioning color change to different areas of the body (Smith *et al.* 2016) and may even differ between the sexes (Marshall & Stevens 2014, Keren-Rotem *et al.* 2016). Thus, the adaptive benefits of dynamic color change are complex and may vary across populations or species due to differences in ecological constraints.

In this thesis, I analyze the ecological role of color change in the green anole lizard, *Anolis carolinensis* (Figure 1.1). Green anoles are a highly visual, diurnal species and are capable of changing their dorsal body color from green to brown, and vice versa. This color change is regulated by melanotropin, which allows the skin of anoles to darken in response to stressors (Greenberg & Crews 1990). In this study I examined how several ecological factors may affect body color change of *Anolis carolinensis* individuals, and if this differs between the two sexes. While most studies of color change prioritize males, there has been some research on how either sex may differentially use color change in similar environmental conditions. For instance, a study by Stuart-Fox & Mousalli (2007) demonstrated that display coloration differs dramatically between male and female dwarf chameleons, genus *Bradypodion*, with females generally exhibiting cryptic coloration. Finally, given that green anoles both thermoregulate and demonstrate complex social display behaviors, this make green anoles an ideal study group to compare these two potentially conflicting ecological constraints of body color change.

Here, I explore two important hypotheses for dynamic color change in green anoles. In Chapter 2, I examine the Thermoregulation Hypothesis, which predicts that shifts in body temperature are associated with changes in body color. This predicts that green anoles who are green in body color should differ in temperature from anoles who are brown, given that lighter colors should reflect heat whereas darker colors should absorb heat. In Chapter 3, I examine the Social Behavioral Hypothesis, which predicts that social display behaviors are associated with body color. Therefore, green anoles who use body color as a visual signal may associate either brown or green color changes with social behavior displays.

Fig 1.1. *Anolis carolinensis* (the green anole) is able to rapidly transition from a bright green body color (A) to a dark brown body color (B) within seconds. (Photographs by Michele Johnson)

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Chapter 2: Body Color Change as a Thermoregulatory Mechanism in the Green Anole, *Anolis carolinensis*

Introduction

The ability to regulate internal body temperature is critical for organismal survival, as body temperature facilitates proper locomotion, immune function, courtship, foraging and growth under changing environmental conditions (Angilletta *et al.* 2002, Walguarnery *et al.* 2012). Endothermic animals can maintain internal temperatures through physiological mechanisms, but ectotherms must actively regulate their temperature. Lizards are one such group that regulate body temperatures through thermoregulatory behaviors such as modifying their posture to alter exposure to the sun, or by regulating the times they are most active (Huey 1974). However, these behaviors come at a cost, including expending energy to shift from sun or shaded environments or exposing oneself to predators while basking (Huey & Slatkin 1976). For example, Cadena & Tattersall (2009) found that in inland bearded dragons, *Pogona vitticeps*, under different thermal conditions, had less thermoregulatory precision in environments demanding more locomotor movements to obtain an optimal temperature. Similarly, Huey (1974) found that for the crested anole, *Anolis cristatellus*, the cost to increase temperature was much greater for forest anoles, who need to spend more time shuttling between sun and shade habitats, than anoles in open parks.

Given the behavioral costs associated with locomotion, regulation of body color changes provides an adaptive alternative for maintaining body temperature. The thermal melanism hypothesis addresses the role of body color in temperature regulation, stating that compared to lighter individuals, darker individuals are able to warm up faster at lower temperatures at a given level of solar radiation (Clusella-Trullas *et al.* 2007). Generally, melanistic and patterned individuals tend to inhabit to colder, wetter habitats, reinforcing the hypothesis that darker individuals should have increased benefits in cooler climates (Broennimann *et al.* 2014), such as

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shorter periods of thermoregulation than lighter animals. Reducing the amount of effort expended in maintaining body temperature can save energy which may be allocated to other activities (Reguera *et al.* 2014). In some melanistic snakes, individuals with darker body color are found to have longer periods of activity, resulting in higher growth rates and larger body sizes than individuals that are normal (lighter) colored (Tanaka 2009). Yet darker colors may have associated costs as well. A study by Adrén & Nilson (1981) found that although melanistic male adder vipers, *Vipera berus*, were significantly heavier than normal color morphs, they also endured more predator attacks due to their increased conspicuousness.

However, dynamic color change may give individuals a more finely-tuned adaptive response to environmental shifts in temperature. Physiological body color change allows an organism to rapidly lighten or darken their skin color, thus providing a broader spectrum of color expression, and a broader range of solar absorptivity of the skin (De Velasco & Tattersall 2008). This potentially gives organisms greater flexibility in regulating their body temperature, such that a darker color is used in a cooler environment whereas a lighter color is used in a warmer environment. For instance, bearded dragons expressed dark brown coloration when their body temperature was lower than their preferred temperature range and were lighter yellow when their body temperature was above these values (De Velasco & Tattersall 2008). Additionally, the large Psammodromus, *Psammodromus algirus*, was found to be darker at higher, colder altitudes, whereas individuals tended to be lighter at lower altitudes (Reguera *et al.* 2014). Further, some evidence suggests that ectotherms with variable body color reap large benefits for their flexibility, occupying broader geographic ranges, using broader niches, and being less at risk of being endangered (Delhey *et al.* 2013). However, one study by Herczeg *et al.* (2007) found no support for color having an effect on heating rate in the snake-eyed skink (*Ablepharus kitaibelii*),

common wall lizard (*Podarcis muralis*), and the green lizard (*Lacerta viridis*), yet they did find a significant positive relationship between body size and heating rate across these three species. These studies highlight the complex relationship between color and thermoregulation.

The green anole, *Anolis carolinensis*, is a useful study system to better understand how dynamic color change may be selected for thermoregulation. The green anole has a broad distribution which covers a wide range of photoperiods and air temperatures (Jenssen *et al.* 1996), providing the opportunity for anoles to use body color to regulate internal body temperatures across a diversity of habitats. While older studies have assumed that green anoles use body color to match to their substrates (Hadley & Goldman 1969), a more recent study clearly disproved the camouflage hypothesis (Jenssen *et al.* 1995). This may be due to the potential conflict between thermoregulation and camouflage, such that individuals may accommodate one requirement at the expense of the other or they may utilize backgrounds which optimize both camouflage and thermoregulation (i.e., light background when hot or dark background when cold; Smith *et al.* 2016). Previous studies on the thermoregulatory use of green anole body color change have been varied in their results, with some supporting the thermoregulation hypothesis (Cooper & Greenberg 1992) and others offering little to no support (Yabuta & Suzuki-Watanabe 2011).

In this study I analyzed the relationship between body temperature and body color, and determined if temperature and color are also associated with different substrate types. If body color of anoles provides the adaptive advantage of thermoregulation, then lighter green anoles should be substantially cooler than darker, browner anoles. Further if substrates do vary significantly in surface temperatures, such that artificial substrates attract more heat than natural substrates, these temperature differences should then also affect the body color of a lizard when

found on a particular substrate. Additionally, if lightening or darkening body color is associated with thermoregulation, then the prevalence of green and brown body color among lizards observed should differ across different times of the day. For instance, the desert ornate tree lizard, *Urosaurus ornatus*, darkens their dorsal skin color during cool mornings to increase the effectiveness of thermoregulation (Castrucci *et al.* 1997). Therefore, more anoles should be found brown in the cooler morning and more anoles should be found green in the warmer afternoon. Finally, I developed a mathematical model to predict whether male and female anoles are likely to be green based on either one or a combination of body and ecological measurements including SVL (snout-vent length), mass, body temperature, substrate temperature, and structural habitat features (presence of absence of sunlight, distance to nearest perch, substrate category, substrate color, and whether or not the substrate is artificial or not). This test gives me the opportunity to evaluate the relative strength of morphological measurements and ecological conditions on the expression of body color within this species.

Methods

To study the relationship between body color change and thermoregulation, I collected behavioral and temperature data on 201 adult green anole lizards, including 90 males and 95 females. (Sixteen lizards were excluded from analysis because their sex could not be confirmed without capture.) The field sites for this study including forested areas in Palmetto State Park, Gonzales County, Texas and green areas near or at Trinity University in San Antonio, Bexar County, Texas. When an undisturbed lizard was located, I then performed a 1-3-min focal observation, noting the lizard's sex, initial behavior (categorized as no activity, movement, or social interaction), initial body color (green or brown), type of substrate on which the lizard perched (leaves, trunk, branch, plastic, metal, or rock), and degree of direct sun (no shade, partial shade, or full shade). After this observation, I captured a subset of these lizards by noose (41 males and 42 females). Immediately after capture, I recorded each lizard's internal body temperature by inserting a thermocouple (Type T, Copper-Constantan) 1 cm into the cloacal vent and read the measurement on the connected automated temperature logger (HH603A, OMEGA) to the nearest 0.1°C (Muñoz *et al.* 2014). I confirmed the lizard's sex by checking for a large dewlap, hemipenes, and for the presence of enlarged post-anal scales, all of which distinguish males from females. I measured the lizard's SVL (snout-vent length) to the nearest mm using a clear plastic ruler and measured its mass to the nearest 0.1g using a Pesola spring scale. I also measured the temperature of the substrate the lizard was initially perched on by placing the thermocouple on the substrate surface. Additionally, I recorded the distance of this perch to the next closest available perch (hereafter, distance to next perch) as a proxy for the amount of habitat exposure, which may play a factor in an organism's expression of color.

I performed a linear regression to determine if lizard body temperatures were correlated with substrate temperature. Because these two measures were highly associated ($F_{1,83} = 128.5$, $R^2 = 0.61$, $p < 0.001$, Fig. 2.1.), in subsequent analyses we used substrate temperature as a proxy for body temperature of lizards to generate a larger sample size. Then I performed a two-way analysis of variance (ANOVA) to determine whether substrate temperature differed based on sex, the anole's initial body color (green or brown), or an interaction of sex and body color. I performed a series of one-way ANOVAs to determine if there was a difference between the substrate types (plastic, leafy, tree trunk, branches, metal, and rock) in substrate temperature (followed by a Tukey's HSD post hoc test), to compare the substrate temperature of artificial (plastic and metal) and natural perches (leafy, tree trunk, branches, and rock), and to compare the average distance to the next perch for both male and female anoles. I performed Wilcoxon Signed Ranks Tests to determine whether the total number of lizards observed, or the proportion of lizards who were green, differed between the sexes throughout the day. All analyses were conducted in IBM SPSS statistical software.

I generated generalized linear models in R (R Core Team 2018) to estimate which ecological and body size conditions would predict the probability that a given individual lizard's body color would be green. I included the following ecological predictors in a series of logistic regression models: lizard body temperature, substrate temperature, distance to the nearest perch, amount of sunlight, substrate category, substrate color, and artificial/non-artificial substrate. I also included SVL and mass in these models as measures of body size. These independent variables were included in my models as covariates. I first constructed a full covariates model, which included all possible predictor variables as covariates. Then I constructed additional models containing 45 possible combinations of subsets of these covariates. All the models I

created included color as the dependent variable, and I created models where each predictor variable was analyzed independently, then additional models were analyzed with combinations of variables that were morphologically or ecologically related. I also constructed a null model which includes a random variable (color) as a cofactor (see Appendix 1 for full list of predictors tested). I used the Akaike Information Criterion (AIC) to select the top models for analysis. Following statistical convention, models with a delta AIC less than two were selected as the top models, and all of these models were considered to have equal weight (Akaike 1973, Burnham & Anderson 2002).

Results

Substrate temperature and body temperature

Across all lizards captured, substrate temperature was highly correlated with body temperature (Fig 2.1.). This allowed the use of substrate temperature as a proxy for body temperature in further analyses.
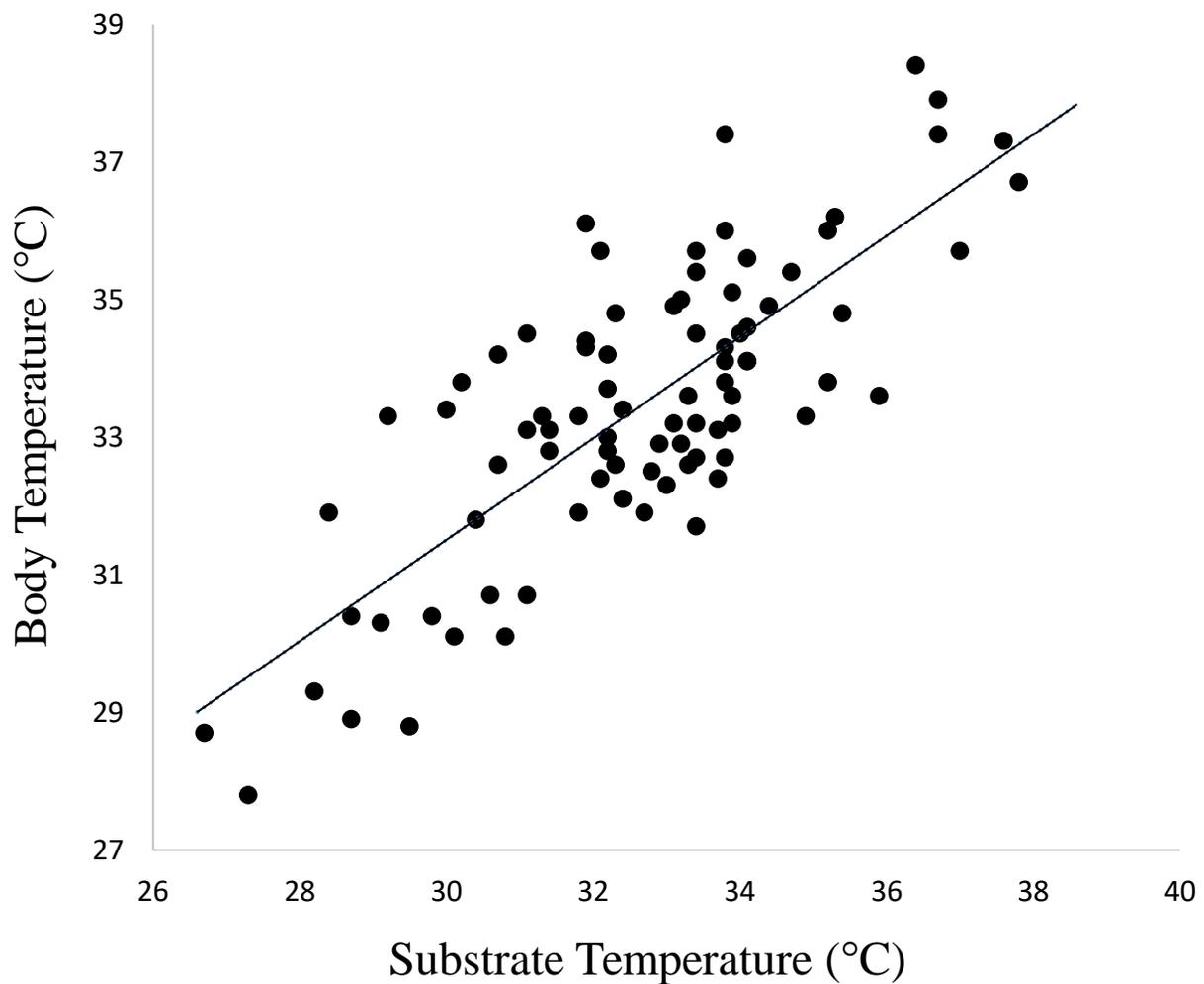
Fig. 2.1. Body temperatures of adult lizards (N = 80) as a function of the surface temperature of perched substrates.

Body color, sex, and temperature

Body color was not associated with substrate temperature for either sex. Thirty-nine of the 56 females were green upon first sighting; however, substrate temperature did not differ significantly between females who were found green or brown ($F_{1,93} = 2.147$, $p = 0.146$). Using the subset of lizards for which we obtained body temperature, we also did not find a difference in body temperature between green and brown females ($F_{1,40} = 0.669$, $p = 0.418$). Twenty-one of the 68 males observed were green, yet differences in color were not associated with differences in substrate temperature ($F_{1,87} = 0.168$, $p = 0.683$), or for the subset of lizards we captured, in body temperature ($F_{1,39} = 0.035$, $p = 0.852$).

Between males and females, there was no difference in temperature for color ($F_{1,180} = 0.45$, $p = 0.50$), but there was a significant main effect for sex for sex ($F_{1,180} = 3.52$, $p = 0.062$). However, there was no evidence of an interaction between sex and color ($F_{1,180} = 1.624$, $p = 0.204$, Fig. 2.2.). We also did these analyses including only the lizards for which we had body temperature measures, and found similar results (color: $F_{1,78} = 0.496$, $p = 0.483$; sex: $F_{1,78} = 9.502$, $p = 0.003$; color x sex: $F_{1,78} = 0.202$, $p = 0.655$).
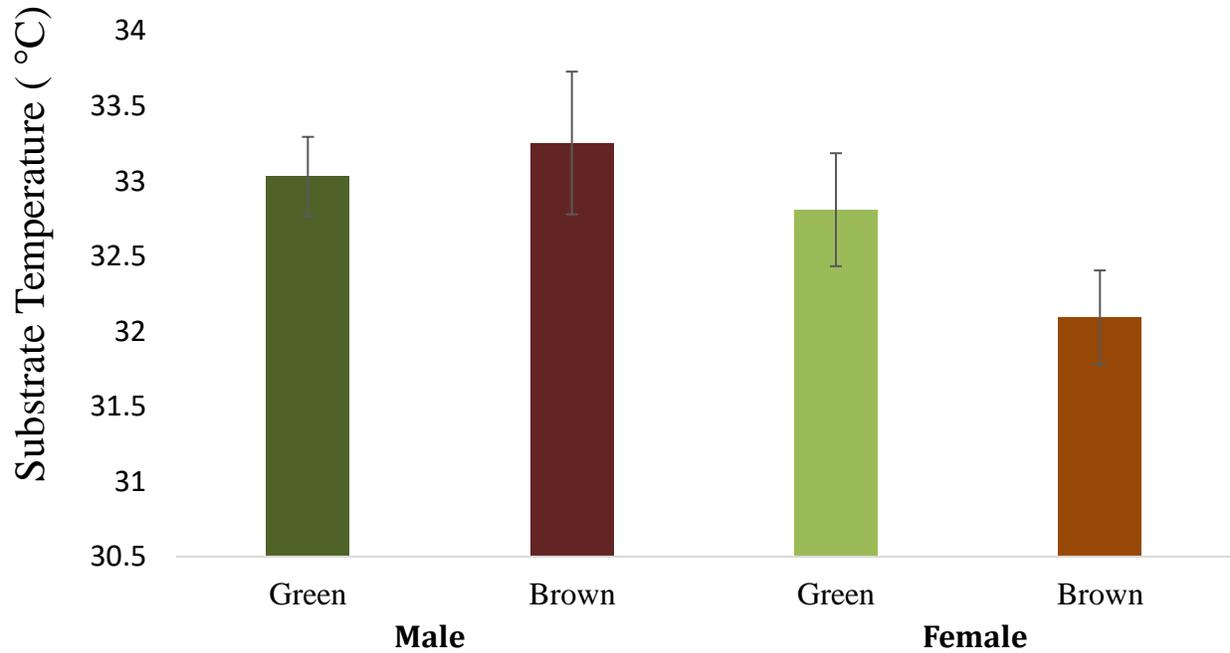
Fig. 2.2. Average substrate temperature (± SE) for green and brown males and females. Differences in body color were not related to differences in temperature for either sex.

Sex differences and habitat exposure

Males used more exposed substrates – those with larger distances from their current perch to the next – than females ($F_{1,169} = 18.408$, $p < 0.001$). Habitat exposure did not differ by body color for either sex (female: $F_{1,84} = 0.009$, $p = 0.924$; male: $F_{1,83} = 0.066$, $p = 0.798$, Fig. 2.3.).
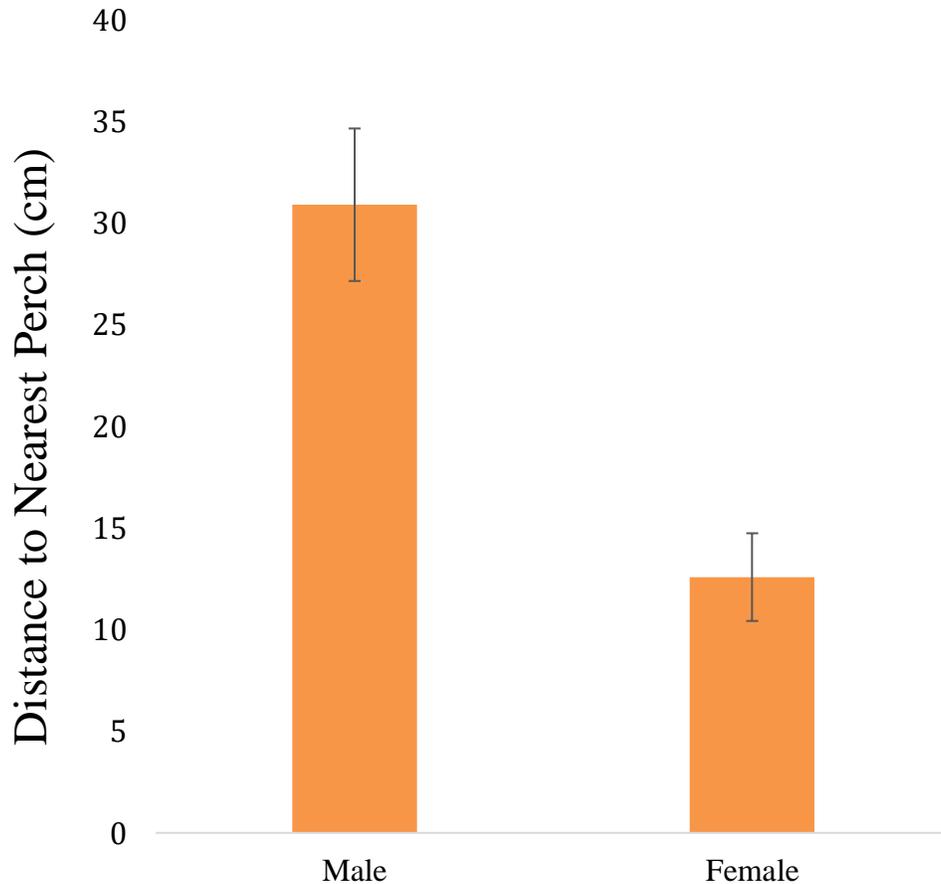
Fig. 2.3. Amount of habitat exposure defined by the average (± SE) distance from the initially occupied perch to the nearest available perching location for male and female anoles. Distance is larger for males than for females.

Differences across substrate types

Across the substrates used by lizards for perching (branches, trunk, leafy material, metal, rocks, or plastic surfaces), only leafy and plastic substrates were cooler than the other substrate types ($F_{5,194} = 3.75$, $p = 0.003$, Fig 2.4.). Body temperature of lizards did not vary with different substrate categories ($F_{4,80} = 2.355$, $p = 0.061$), or whether the substrate was natural or artificial, for either sex (female: $F_{1,40} = 0.491$, $p = 0.487$; male: $F_{1,39} = 0.195$, $p = 0.661$).
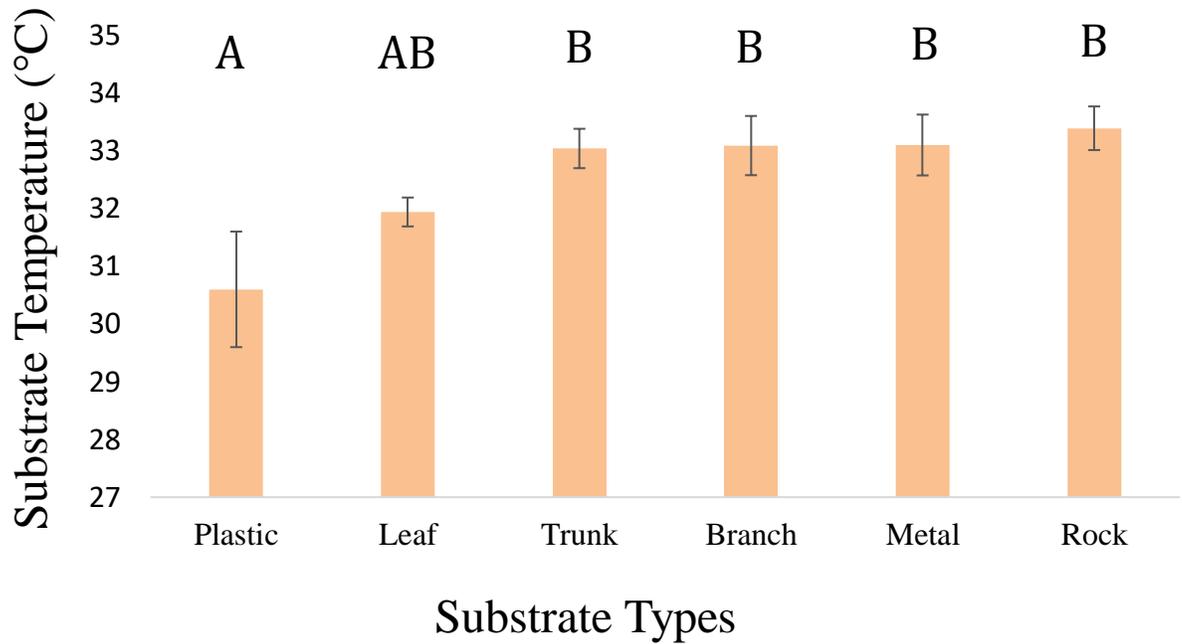
Fig. 2.4. Average (± SE) temperature for different categories of substrates perched by anoles. Columns with different superscripts were significantly different from one another. Plastic and leafy substrates were cooler than other potential categories.

Body color across time of day

The total number of individual anoles found during the day did not vary between sexes ($Z = -1.060$, $p = 0.289$). However, the proportion of individual anoles found green throughout the day differed between males and females ($Z = -2.578$, $p = 0.01$).
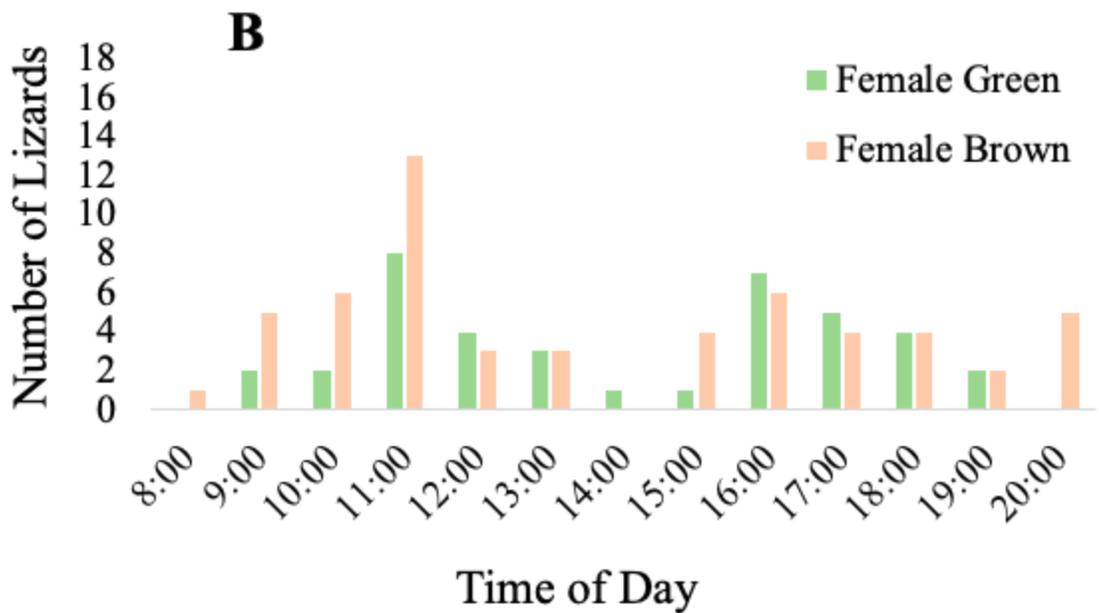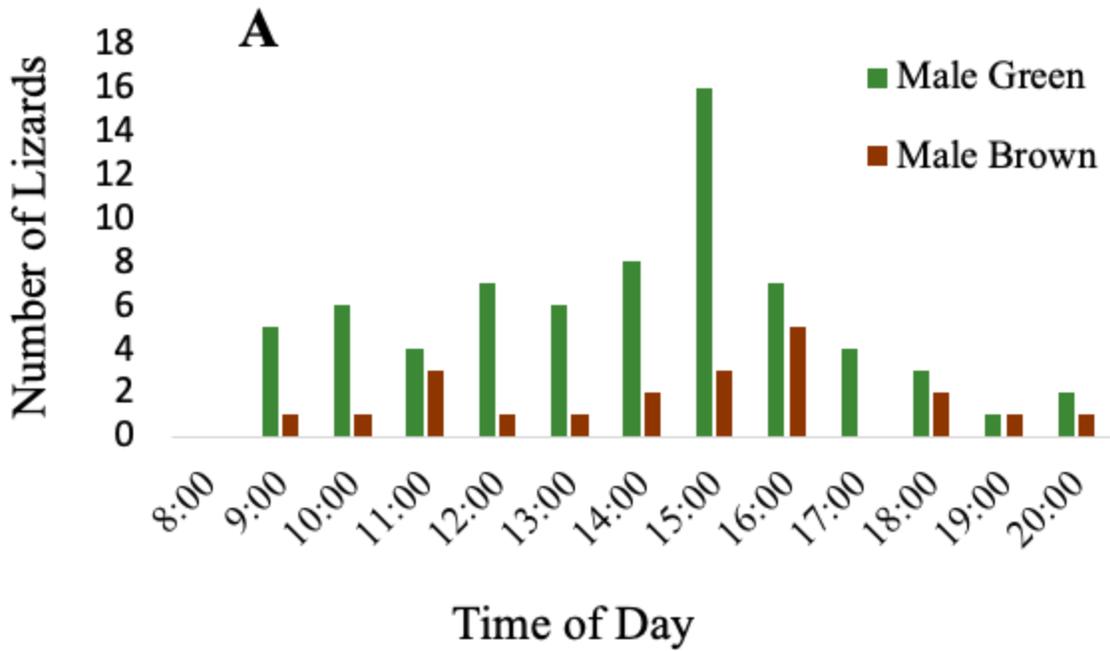
Fig. 2.5. Number of lizards observed between the hours of 8am through 8pm, divided by sex and body color.

Color and body size

Male anoles who were found green were also larger in mass ($F_{1,38} = 5.432$, $p = 0.025$) and SVL ($F_{1,39} = 5.271$, $p = 0.027$) than those found brown. However, females did not exhibit these

relationships between color and body mass ($F_{1,39} = 2.275$, $p = 0.140$) or SVL ($F_{1,40} = 0.071$, $p = 0.791$).

Body and ecological measurements as predictors of green body color

Body measurements were included as covariates in all of the top models (< 2 ΔAICc). However, none of the ecological predictors, were included in the top models. Increasing snout-vent length (SVL) increased the probability of being green (Fig. 2.6.). Sex alone did not increase the probability of being green, but both sex and SVL (Fig. 2.7.) and a combination of sex, SVL, and mass did. Mass alone was not a strong predictor, yet both variables of SVL and mass did predict color. Forty-one of the 48 total models analyzed were stronger than the null model (Appendix 1). See Table 2.1 for results of top models and models with each predictor variable included separately. See Appendix 1 for results of all models considered.
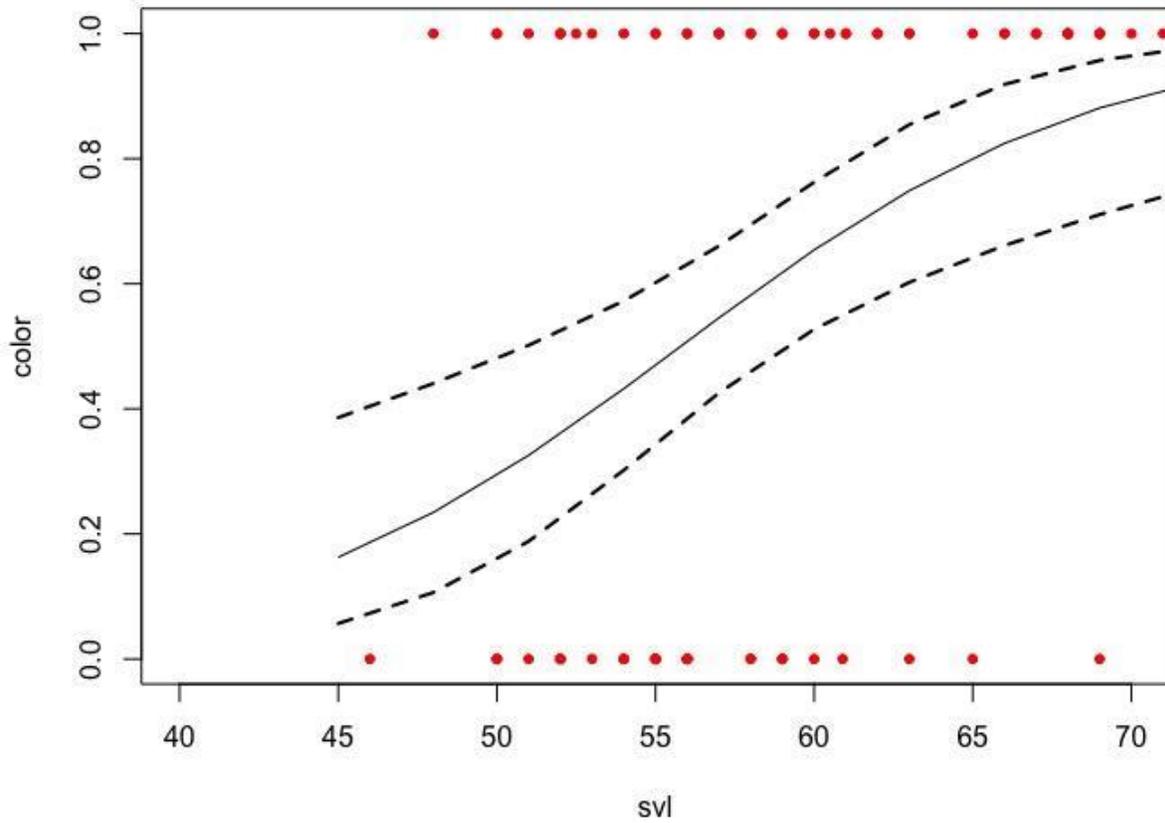
Fig 2.6. Snout-vent length (SVL) as a predictor of color change (0 = brown, 1 = green). Among adult *A. carolinensis*, larger lizards tend to be green whereas smaller lizards are more likely to be brown.
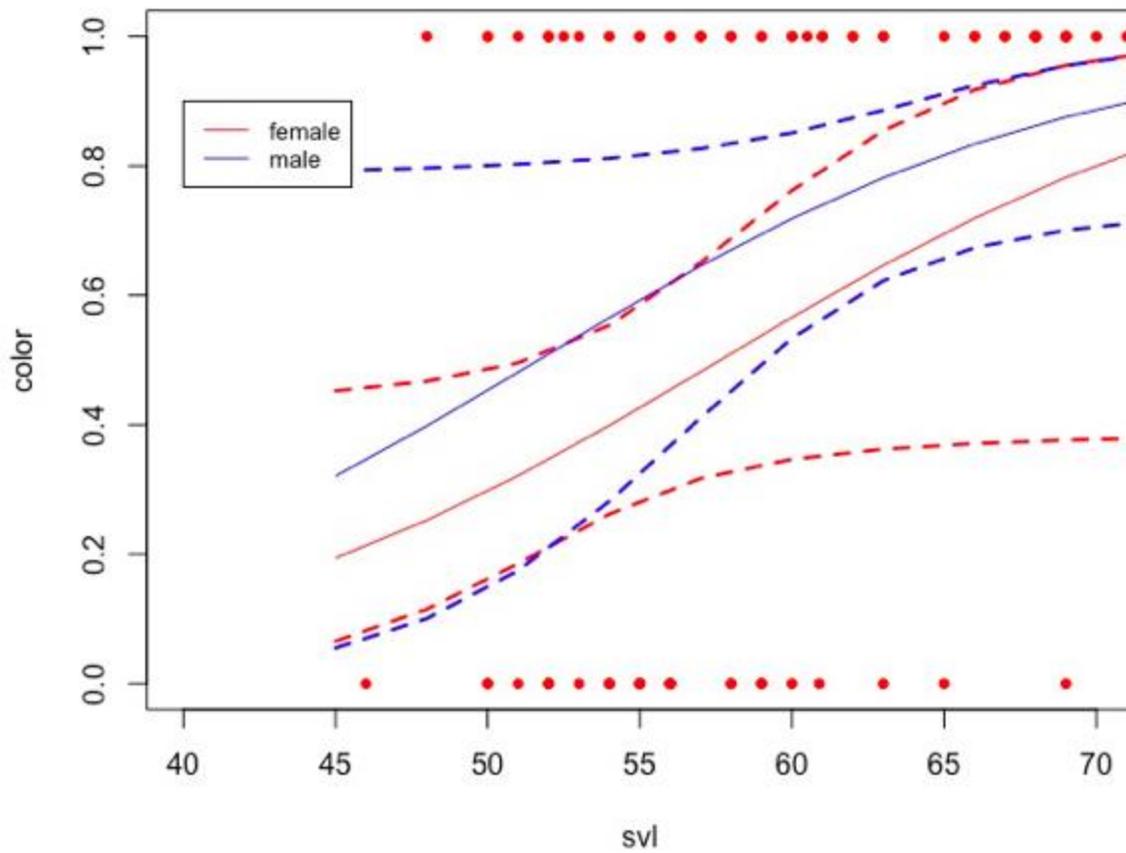
Fig 2.7. Snout-vent length (SVL) as a predictor of color (0 = brown, 1 = green) for both male (blue) and female anoles (red). Size was a stronger predictor of body color for male *A. carolinensis* than for females.

Table 2.1. General linear models predicting color (dependent variable) using individual or multiple variables within an anole population. Each column consists of variables analyzed and rows correspond to individual model tests. The top models (as defined by Δ **AICC** < 2) are bolded below.

Color	Sex	SVL	Mass	Lizard Temperature	Substrate Temperature	Closest Perch	Sun	Substrate Category	Substrate Color	Artificial	R^2	Sigma	AICc	Δ AICc	Weight
x		**x**									**0.64**	**0.60**	**100.96**	**0.00**	**0.20**
x		**x**	**x**								**0.65**	**0.60**	**101.56**	**0.60**	**0.15**
x	**x**	**x**									**0.65**	**0.60**	**102.02**	**1.06**	**0.12**
x	**x**	**x**	**x**								**0.65**	**0.60**	**102.72**	**1.77**	**0.08**
x		x	x							x	0.65	0.60	103.01	2.05	0.07
x				x							0.59	0.65	116.09	15.13	0.00
x			x								0.59	0.65	117.04	16.08	0.00
x	x										0.17	0.92	230.52	129.56	0.00
x						x					0.10	0.96	250.55	149.59	0.00
x							x				0.02	1.00	273.84	172.88	0.00
x					x						0.01	1.00	274.53	173.57	0.00
x									x		0.01	1.01	275.08	174.12	0.00
x										x	0.00	1.01	275.95	175.00	0.00
x								x			0.03	1.00	277.61	176.65	0.00

Discussion

In populations where color functions as a thermoregulatory mechanism, it is predicted that lighter hues cool an organism, whereas darker hues warm an individual. In green anoles, greener lizards should generally be cooler in body temperature as their lighter body color reflects excess heat, whereas browner lizards should be warmer as their darker color absorbs additional heat. However, my data indicate that brown and green body color did not differ in temperature, for either sex. Additionally, although anoles did vary in substrates used, these substrates differed little in temperatures and did not influence body color of anoles (Fig. 2.4.). The results shown here suggest that thermoregulation is not the primary mechanism for color change in green anoles. While some experimental studies have shown support for skin color change in *Anolis* as a function of changes in temperature (Cooper & Greenberg 1992, Goldman & Hadley 1969), the evidence from this observational study suggests that green anoles do not change color to thermoregulate during the summer breeding season.

Further, my overall findings are supported by a recent study conducted by Yabuta and Suzuki-Watanabe (2011), whose results confirmed that larger male lizards exhibited green body color more frequently than smaller lizards, and male anoles did not differ in temperature based on color. However, the authors note that air temperature may play a bigger role in color change, where anoles are green during warmer air temperatures and brown during cooler temperatures. Melanophores are known to be directly responsive to temperatures, with cold darkening and warm temperature lightening the body (Hadley and Goldman 1969). Yet, based on my own observational data, different body colors did not vary in temperature. Given that my study is limited to the breeding season where temperatures are somewhat consistently warm (high summer temperatures range from 91–95 °F in Bexar Country), temperature regulation through

color change may not prioritized. Instead, color change in *A. carolinensis* may be primarily used for visual signaling during this breeding period.

Across several lizard species, there has been increasing support for the hypothesis that there is a trade-off among the multiple functions of rapid color change. For instance, in the tree lizard, *Urosaurus ornatus,* individuals darken to regulate temperatures, and in certain populations use darkening for social signaling. While these lizards have a decreased sensitivity to Alpha-Melanocyte-Stimulating hormone (a hormone responsible for skin darkening) during the non-reproductive season, the mechanism generating the difference in skin darkening for thermoregulation (in contrast to darkening for signaling) is unknown (Castrucci *et al.* 1997). Further, lizards may switch between functions depending on ecological context. For the common chameleons, *Chamaeleo chamaeleon*, neither background nor body temperature influenced color change when a female was present. Instead of using color change for crypsis, males consistently used color change in sexual signaling (Keren-Rotem *et al.* 2016).

Lizards may vary in body temperature due to differences in thermal requirements that can be based on sex, or body size. Males green anoles are often larger in size and mass than females and may be warmer as a function of different physiological demands. In my study, males were both found warmer in body temperature, and selected warmer substrates than females. Therefore, my findings suggest that the sexual difference in temperature may be a product of sexual size dimorphism within *A. carolinensis.* Additionally, I found that males more often chose more exposed perch locations than females, however body color did not differ between those on more exposed or less exposed sites. Males who select more exposed substrates may better advertise their presence to both competing males and potential female mates during the breeding season.

Additionally, choosing more exposed substrates may allow males to have better access to a heat source and maintain warmer temperatures, potentially needed for physiological activities.

Additionally, I found that males and females did not differ in patterns of activity through the day, however the number of individuals found green at a given time of day did differ between the sexes. Males were more often found green earlier in the day, whereas females were more likely to be brown at the same time (Fig. 2.5.). My study contradicts the findings by Gordon & Fox (1960), who found that in general, lizards are green at night yet are brown and background matching during the day. Additionally, Yabuta and Suzuki-Watanabe (2011) found that anoles were brown more frequently in the morning than in the daytime yet were darker brown more frequently in the daytime than in the morning. These findings demonstrate the complexity between body color and time of day, likely due to variation in air temperature or natural conditions. However, my study exhibits support for sexual differences in body color expression during the day.

My observational data found support for larger sized males being more likely to exhibit green body color. This was further supported by my findings in the multivariate logistic regression analysis. Based on my data, larger lizards at any given time are more likely to be green, and this relationship seems to be stronger for males than for females (Fig. 2.7.). Lovern (2000) studied juvenile male and female anoles and found that with juvenile males, there was a significant relationship between SVL and body color for males, however not with females. The author hypothesized that green body color may be important during social signaling and allow males to minimize home range overlap. These results, along with my findings, suggest that during the breeding period, color may function as a potential honest signal for larger, more fit male lizards.

In sum, my findings establish that color change may not be a primary mechanism for thermoregulation in this species, at least within the breeding season when color change may be prioritized for social signaling. This study potentially demonstrates a tradeoff between two competing functions of dynamic color change, yet additional future studies looking into the use of body color change in both the breeding season and non-breeding season are needed to appropriately analyze whether or not anoles switch between color change functions.

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Appendix

Appendix 1. All models ran in the multivariate analysis testing the probability of lizards being green. Values are organized by increasing Delta AIC values.

Model	Color	Sex	SVL	Mass	Lizard Temperature	Substrate Temperature	Closest Perch	Sun	Substrate Category	Substrate Color	Artificial	R^2	Sigma	AICc	Δ AICc	Weight
1	x		x									0.64	0.60	100.96	0.00	0.20
2	x		x	x								0.65	0.60	101.56	0.60	0.15
3	x	x	x									0.65	0.60	102.02	1.06	0.12
4	x	x	x	x								0.65	0.60	102.72	1.77	0.08
5	x		x	x							x	0.65	0.60	103.01	2.05	0.07
6	x		x	x						x		0.65	0.60	103.11	2.15	0.07
7	x	x		x								0.64	0.60	103.15	2.19	0.07
8	x		x		x	x						0.65	0.60	103.49	2.54	0.06
9	x		x	x	x	x						0.66	0.59	103.88	2.92	0.05
10	x	x	x	x							x	0.66	0.60	104.19	3.24	0.04
11	x	x	x	x	x	x	x					0.67	0.58	104.27	3.31	0.04
12	x	x	x	x						x		0.65	0.60	104.39	3.43	0.04
13	x	X		X								0.64	0.61	105.35	4.40	0.02
14	x	x		x		x						0.64	0.61	107.04	6.08	0.01
15	x		x	x					x	x		0.66	0.59	108.62	7.66	0.00
16	x		x	x					x			0.65	0.60	108.76	7.81	0.00
17	x		x	x					x	x	x	0.67	0.59	110.00	9.04	0.00
18	x	x	x	x					x			0.66	0.60	110.04	9.08	0.00
19	x	x	x	x					x	x		0.67	0.59	110.10	9.14	0.00
20	x	x	x	x					x	x	x	0.67	0.59	111.57	10.61	0.00
21	x		x		x	x						0.61	0.64	115.86	14.90	0.00
22	x				x							0.59	0.65	116.09	15.13	0.00

	1	2	3	4	5	6	7	8	9	10	11					
23	x				x	x						0.60	0.64	116.40	15.44	0.00
24	x			x								0.59	0.65	117.04	16.08	0.00
25	x				x			x				0.60	0.64	117.07	16.12	0.00
26	x				x	x					x	0.60	0.64	118.11	17.15	0.00
27	x				x	x				x		0.60	0.64	118.44	17.48	0.00
28	x				x	x		x		x	x	0.63	0.62	120.32	19.37	0.00
29	x	x	x	x	x	x	x	x	x	x	x	0.63	0.62	120.32	19.37	0.00
30	x				x	x			x	x		0.61	0.64	122.74	21.78	0.00
31	x				x	x			x			0.60	0.64	123.85	22.89	0.00
32	x	x					x					0.24	0.88	212.35	111.39	0.00
33	x	x						x				0.20	0.91	226.38	125.42	0.00
34	x	x										0.17	0.92	230.52	129.56	0.00
35	x	x			x							0.17	0.92	231.22	130.26	0.00
36	x						x					0.10	0.96	250.55	149.59	0.00
37	x				x			x				0.03	1.00	273.53	172.57	0.00
38	x							x				0.02	1.00	273.84	172.88	0.00
39	x					x						0.01	1.00	274.53	173.57	0.00
40	x											0.00	1.01	274.56	173.61	0.00
41	x									x		0.01	1.01	275.08	174.12	0.00
42	x								x	x		0.04	1.00	275.30	174.35	0.00
43	x						x	x				0.04	1.00	275.30	174.35	0.00
44	x										x	0.00	1.01	275.95	175.00	0.00
45	x									x	x	0.01	1.01	277.09	176.13	0.00
46	x						x	x	x	x	x	0.01	1.01	277.09	176.13	0.00
47	x								x			0.03	1.00	277.61	176.65	0.00

Chapter 3: Sex Differences in Body Color Change during Social Interactions in the Green Anole, *Anolis carolinensis*

Introduction

Animals communicate by using signals to relay information to one another. When heritable, these signals are acted upon by natural selection, resulting in an effective and accurate transmission of information, where receivers may modify their behavior in response to the signal (Laidre & Johnstone 2013). Color is an important form of visual communication across a broad range of species. Visual signals utilizing color can be important for communicating information such as species identity (Endler 1983), sexual receptivity (Chan *et al.* 2009), aggression (Keenleyside & Yamamoto 1962), and dominance status (Korzan *et al.* 2008). According to Endler (1992), signals, receptors, and behaviors are not independent traits but may be functionally related to one another, therefore adaptive changes in one trait may be correlated with changes in another trait. As a group, lizards are well known for their complex display behaviors when interacting with conspecifics. These behaviors include a variety of behavioral components such as push-ups, head-bobs, cresting, jagged movements, and dewlap extensions (Johnson *et al.* 2019). These behaviors can often function in conjunction with visual signals such as whole-body color change, to either increase the conspicuousness of the signaler to the receiver or allow individuals to communicate specific information using a particular combination of behavioral motions and visual signals.

Many lizard groups are capable of dynamic body color change, where individuals can rapidly shift their skin color in response to environmental or social conditions. As discussed by Ligon & McGraw (2018), static color displays may often be used by organisms to signal their quality or ability, whereas dynamic color change can communicate short-term motivational information through graded color signals. Additionally, rapid color changes allow organisms to convey multi-functional signals depending on the color being expressed (Hutton *et al.* 2015).

While chameleons, notorious for their flexibility in body color, were often assumed to be using their abilities for camouflage purposes, current research has found that their dynamic color changes primarily function as social signals. For instance, within male veiled chameleons, *Chamaeleo calyptratus*, changes in color can be used to signal motivation during aggressive encounters (Ligon 2014, Ligon & McGraw 2016, Ligon & McGraw 2018). Further, visual signals can be modified by the organism through alterations in time, brightness, and color of the dorsal skin color, all of which can provide additional information to the receiver (Ligon & McGraw 2018). In sum, color change studies on chameleons have clearly shown that rapid color change can serve many uses, especially in the adaptive benefits of both crypsis and social communication. Specifically, Stuart-Fox & Moussalli (2009) concluded in their review of dynamic color change, that the evolution of rapid color change may function as a 'solution' to the competing demands of camouflage, signaling, and thermoregulation.

Some groups of lizards are limited in their dynamic color change, where individuals shift between a finite set of colors or by adjusting the brightness of their skin exhibit. Often, lizards who primarily lighten or darken their dorsal color exhibit this trait during social interactions. For example, the Western fence lizard, *Sceloporus occidentalis*, is able to shift their dorsal scales from blue to green in male-male intraspecific aggressive encounters (Cooper & Ferguson 1973). Similarly, Madsen & Loman (1987) found dominant, male rainbow lizards, *Agama agama*, exhibited brighter colors most of the time whereas subordinate males exhibited darker colors when in the presence of superior males. In the Indian rock agama, *Psammophilus dorsalis*, individuals not only shift the colors of their dorsal bands during interactions but can alter the rate of color change depending on social context (i.e. displaying to a male versus a female conspecific; Batabyal & Thaker 2017).

The green anole, *Anolis carolinensis*, is a highly visual species with all-cone (color sensing) retinas (Greenberg 2002). This group uses a number of visual signals to communicate with one another, such as darkening of the postorbital eyespot (Korzan *et al.* 2006), as well as the specific color or patterning of dewlaps (Persons *et al.* 1999). Additionally, green anoles are capable of rapidly changing their dorsal body color from bright green to dark brown, and vice versa. Within this species, body color changes are generally affected by social situations such as predator avoidance, mutual stalks, chases and retreats (Jenssen *et al.* 1995, Greenberg 2002). However, a number of studies have concentrated on anole body color changes and its influence in determining social contests. The results from these studies have overwhelmingly supported the hypothesis that body color can reliably determine future winners from future losers, as green color generally signals social dominance whereas brown color signals subordinance (Wilcynski 2015, Boyer & Swierk 2017). Further, these color visual cues are associated with dominant and submissive behaviors in perch selection and courtship rates (Greenberg & Crews 1990).

In this study I analyze how green anoles use dynamic body color change during intraspecific social interactions, and if this differs between the sexes. While most studies have placed emphasis on body color changes as an indicator of dominance/subordinance status in male-male contexts, we have a limited understanding of the role of body color change as a visual signal more generally, and how males and females may differentially utilize body color during these interactions. If body color is used as a visual signal, then males and females may differ in their use of body color based on sexual differences in behavioral responses to social conditions. I also test whether color change is associated with social display behaviors specifically, rather than with general activity, such that there is a relationship between color and display behaviors, but not between color and total number of movements.

Methods

    To determine whether *A. carolinensis* body color change is related to behavioral displays I examined 128 hours of focal behavioral data on adult green anole lizards, collected in the 2010 summer (May-July) breeding season at Palmetto State Park in Gonzales County, Texas, USA (29°35'14"N, 97°34'56"W). These data were initially collected to examine differences among three 1000-m^2 study plots, all within 1km of one another and connected via continuous forest canopy, that differed in vegetation structure (see Battles *et al.* 2013, Dill *et al.* 2013, Stehle *et al.* 2017). In each plot, all adult lizards within the plot (70 males and 100 females) were captured, measured, and marked, as follows. Upon capture, the snout-vent length (SVL) of each lizard was measured with a clear plastic ruler to the nearest 1 mm, from the tip of the snout to the vent at the base of the tail. Then each lizard was permanently marked with a unique combination of colored beads sewn into the dorsal tail musculature (Fisher & Muth 1989), and then released at its site of capture. Of the 170 marked adults, behavioral data were collected on 91 individuals. Sex was not recorded for some lizards observed, so 85 identified individuals were included in my data analysis.

    Lizards were observed a minimum of 24 h after capture by walking slowing through a plot until an undisturbed lizard was identified. Behavioral data were recorded from a minimum distance of 10 m using binoculars. Each lizard was observed for 5-60 min (average 34 min) in a single observation period, and no more than 3 h of observation was conducted per lizard, for an average of 2.6 periods and 86 min observation per lizard (Stehle *et al.* 2017). Observers recorded each behavioral event during the observation, including all social display behaviors (dewlap extensions, and head-bobs and push-ups, which were combined into one behavioral category called push-bobs), and locomotor movements (distinguished as running, crawling, or jumping).

They also recorded the initial color of the lizard prior to start of observation and noted each time the lizard changed body color between brown and green. Using these data, I calculated rates of color change (switching from one color to the next), dewlap display, and push-bobs per observation, and for lizards observed in multiple periods, I calculated the average per lizard. I also calculated the proportion of time the lizard was green in each observation and determined an average for each lizard.

To see if there was a sex difference in overall body color expression, I used ANOVA to compare males and females in the proportion of time individuals were green during an observation period. I used a series of correlation analyses, performed separately for each sex, to determine the relationships between rates of display behaviors (dewlap extensions and push-bobs per min), rates of color change, and proportion of time the lizard was green. Lastly, to see if color change is associated with social behaviors specifically, rather than overall general activity, I compared the relationship between color and display behaviors with the relationship between color and total movement, for both sexes. I used correlation analyses, separately for each sex, to determine the relationship between color and movement rates. Then I used a series of general linear models to compare the relationship between color data and both display rates, and movement rates, for both sexes. I performed all data analysis using IBM SPSS (version 25).

Results

Sex differences in body color

Overall, males exhibited green body color more frequently than females ($F_{1,83} = 14.1$, $p < 0.001$, Fig. 3.1.). Males spent approximately 73% of the time with a green body color, while females were green only 43% of the time.
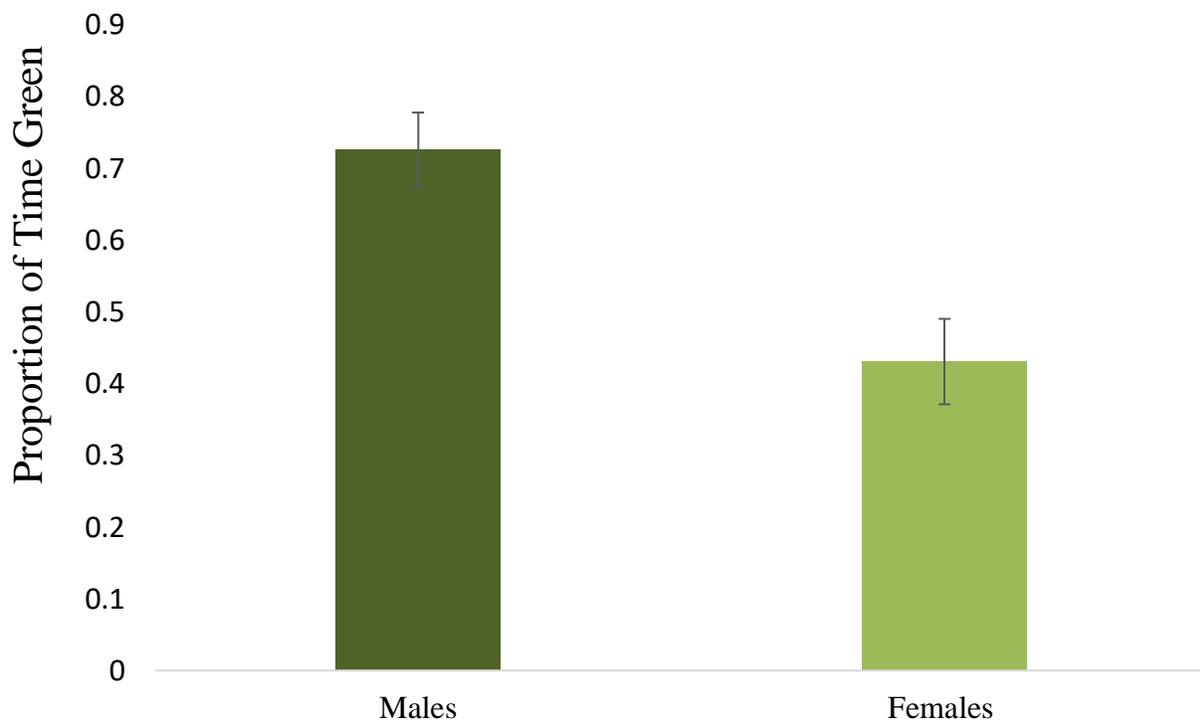
Fig. 3.1. Average time spent green for male and female anoles.

Body color and social display rates

Among males, push-bob rates were positively correlated with color change rates ($r = 0.30$, $p = 0.055$, Fig. 3.2.), such that lizards that performed more displays changed between green and brown body color more frequently. However, there was no relationship between male

dewlap displays and color changes ($r = -0.01$, $p = 0.975$). Further, female anoles did not exhibit any relationship between color change and either component of social display (push-bobs: $r = 0.04$, $p = 0.820$; dewlap: $r = -0.10$, $p = 0.512$). In female anoles, push-bob rates are positively correlated with being green overall ($r = 0.38$, $p = 0.013$, Fig. 3.3.). Yet there was no relationship between female dewlap displays and being green ($r = 0.25$, $p = 0.118$). Further, there was no relationship between male green body color and either component of social displays (push-bobs: $r = 0.23$, $p = 0.137$; dewlap: $r = 0.17$, $p = 0.263$).
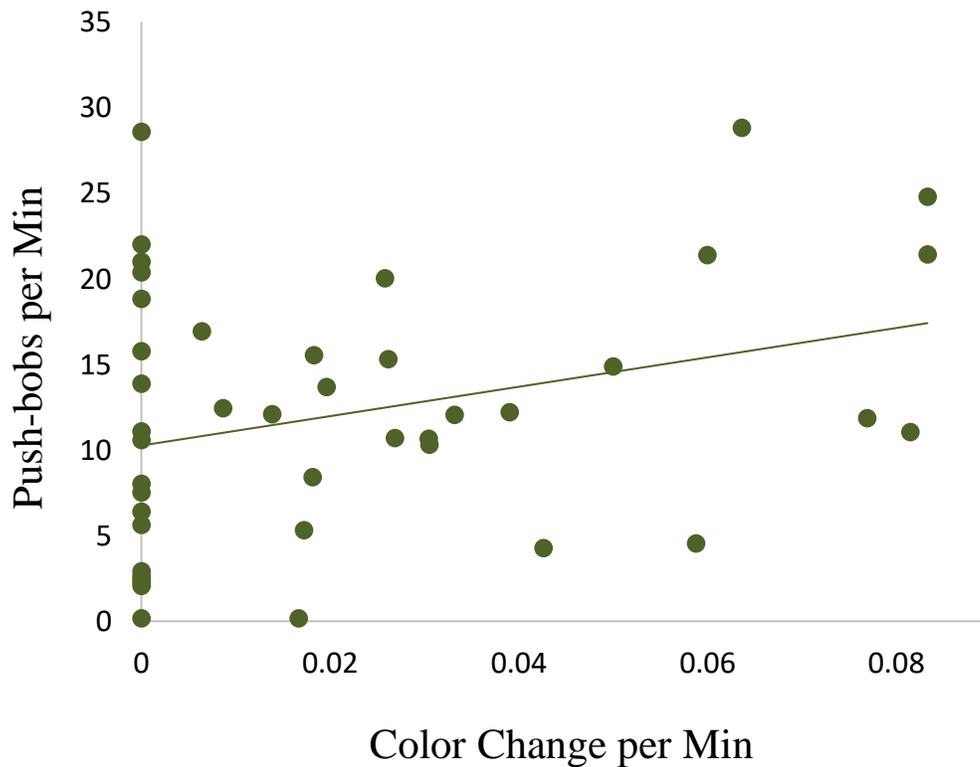
Fig. 3.2. Positive correlation between the rate of color change and push-bob rate for male green anoles.
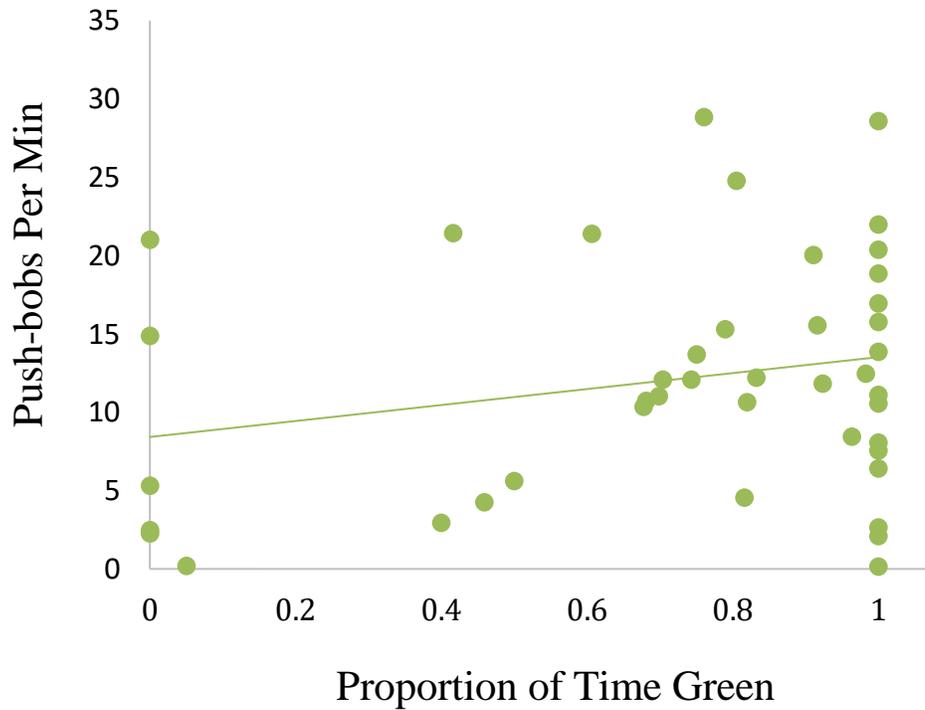
Fig. 3.3. Positive correlation between the proportion of time spent green and push-bob rate for female green anoles.

Body color and movement

For female anoles, color change was positively correlated with total movement ($r = 0.54$, $p < 0.001$), while male color change was not significantly correlated with movement ($r = 0.18$, $p = 0.255$). However, the proportion of time spent green was not correlated with any movement behavior for either sex (males: $r = 0.16$, $p = 0.306$; females: $r = 0.16$, $p = 0.299$).

Sex differences in behaviors

Male green anoles display and move more frequently than females, and in general males spend more time being green in color (Table 3.1.).

Table 3.1. Male and female display rates, movement rates, and body color measures in green anoles.

Variables	Male Average	Female Average	$F_{1,83}$	Significance
SEX				
Dewlap Rate	1.97	0.01	14.876	**< 0.001**
Push-Bob Rate	12.12	0.46	100.619	**< 0.001**
Total Move Rate	2.53	1.37	28.287	**< 0.001**
Prop Time Green	0.73	0.43	14.062	**< 0.001**
Color Change Rate	0.029	0.03	0.029	0.865

Discussion

Overall, male green anoles exhibited green body color far more frequently than females, whereas females were more often brown. Within the *Anolis* genus, visual cues are known to play an important role in influencing social interactions and status (Korzan & Summers 2004). The expression of body color in green anoles may in part be influenced by the demands of sexual selection. The prevalence of bright green body coloration among males may be due to intrasexual competition between green anoles. Among anoles, green body color signals dominance and during the breeding season males may be green at much higher frequencies as individuals compete for potential mates and defend perch site locations (Yabuta & Suzuki-Watanabe 2011). Based on my data, males also had higher display rates and movement rates than females, which suggest males are extremely active during this season and may depend on body color as a visual signal to reduce the likelihood of engaging in costly fights. As for females who generally display darker brown colors, it has been proposed that darker colors may function as a submissive social status to regulate interactions with males and may even signal reception to male courtship (Andrew & Summers 1996). It has also been hypothesized that female coloration tends to be more cryptic (Stuart-Fox *et al.* 2007), or is correlated with reproductive status, and correlated with female-female interactions or mate choice (Olsson *et al.* 2013). Further, female coloration may vary with reproductive state (Robertson & Rosenblum 2009). These results suggest that green and brown body color communicate different social signals, which may be related to the differing demands of male and female fitness (also note Chapter 2 discussion).

Additionally, my data demonstrate that both males and females use body color in conjunction with behavioral displays, which suggests physiological color change in green anoles functions as an important visual signal co-adapted with social behavioral displays during

intraspecific interactions. Based on my findings, males and females also differ in how they use color during social interactions. As males display more, they also increase the rate of body color change (Fig. 3.2.). The rapid change in color may function as a signal of dominance in anole males, as individuals who either display bolder colors, or are quicker to respond, are more likely to be dominant status individuals (Wilczynski 2015). However, Greenberg and Crews (1990) found that anoles who won fights were more likely to just remain green and show little to no color change. Further, body color and body color change, like display behaviors, may be context-dependent, such that individuals modify their color change behavior according to who they are interacting with (in chameleons: Ligon & McGraw 2018, Keren-Rotem *et al.* 2016). Given my study does not discriminate whether or not focal individuals were interacting with another male or female anole, we cannot distinguish the primary cause of color change in relation to specific social contexts. Yet, it is evident from my results that color change is important for male signaling in relation to display behaviors.

Females in contrast, spent more time being green in color as they increase their display behavior (Fig. 3.3). While males are often primarily studied for the role of visual signals in complex mating displays or agonistic interactions with competing males, my data provide evidence that females also incorporate color displays in their social behaviors. Other studies have found that female lizards capable of rapid color change, also incorporate body color during social interactions. Andrews and Summers (1996) found that female green anole aggressive display behaviors are similar to those of males, and females are more likely to exhibit darker color change when males are present. At any given point, females are more likely to have brown body color, however my data suggest that green body color may be an important visual signal, especially during interactions where social display behavior is highest.

While my study finds that males and females differ in their use in body color, I also found a sex difference in the relationship between color and different types of behaviors. Males and females did not differ in color change rate, however, both differ in their utilization of color shifts during different types of behaviors. Specifically, female color change occurs more frequently when females exhibit higher overall activity rates. In contrast, males change color more primarily during in social displays (Table 3.1.).

While many studies have investigated visual signals used by males, my study demonstrates dynamic body color change is utilized by both sexes and may potentially be associated with sex-related differences in behavior. By looking at both male and female use of body color, we can gain a wholistic understanding of color change as a visual signal. Further, body color change has been largely neglected in social signaling studies, especially within this species, however my findings establish this behavior as an important feature in intraspecific communication.

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