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The Effects of Ecological Context and Individual Characteristics on Stereotyped Displays in Male *Anolis carolinensis*

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Table of Contents

List of Figures and Tables

Abstract

Displays are ubiquitous throughout the animal kingdom. While many have been thoroughly documented, the factors affecting the expression of such displays are still not fully understood. We tested the hypotheses that display production would be affected by ecological context (i.e. the identity of the receiver) and intrinsic qualities of the signaler (i.e. heavyweight and lightweight size class) in the green anole lizard, *Anolis carolinensis*. Our results supported these predictions and show that a) ecological context, specifically displaying to conspecifics, had the greatest impact on display production; b) size class influenced display rate with heavyweight males displaying more to green females and lightweight males displaying more to green males in similar frequency between the two size classes to their respective target stimuli. Furthermore, our results provide empirical support for differential use of the three major display types (A, B and C displays), and uncover unexpected complexity in green anole display production.

Anolis carolinensis, stereotyped displays, generalized linear mixed models

Chapter 1

Introduction

Displays are common throughout the animal kingdom and have developed under a variety of influences including physiology of the signal receiver, morphology of the producer of the display, habitat constraints, costs of display production and the nature of the information being transferred (Martins *et al.* 2004). Displays can have important effects on individual fitness because of the range of selective contexts within which they operate. For example, birds have a wide variety of stereotyped threat displays which are produced during multiple ecological settings including access to food, conflicts over preferred perching locations, and proximity to a breeding colony by juvenile males, amongst others (Hurd and Enquist 2001). However, despite this variety, the precise ecological functions of the various displays are not always fully understood. Furthermore, whereas some aspects of signal design are clearly prompted by environmental conditions that affect signal propagation and perception (Endler 1992; Ord *et al.* 2007), variation in signal use and production are not always explicable based on these considerations alone. For example, the male goldeneye duck, *Bucephala clangula*, produces a variety of precopulatory display patterns which females do not seem to discriminate amongst (Dane, Harris and Reed 2013), whereas the aggressive displays of male Siamese fighting fish (*Beta splendens*) in intra-sexual interactions are not fixed, but is influenced by multiple factors, including the gender of the audience present during aggressive encounters (Matos and McGregor 2002).

Lizards of the genus *Anolis* exhibit a wide variety of well-studied displays that nonetheless lack a detailed explanation of their utility. These displays include distinct patterns of headbobs which have been shown to be highly stereotyped within different anole species (Jenssen 1977; Losos

2009), and are displayed in a variety of ecological contexts, including male-male conflicts, courtship, and displays towards conspecifics (Jenssen, Decourcy and Congdon 2005; Orrell and Jenssen 2003). The complex displays of the green anole, *Anolis carolinensis*, have been especially well-studied. Decourcy and Jenssen (1994) documented three stereotyped headbob display variants that *A. carolinensis* perform when alone or confronted with another male. These displays are called type A, B and C based on the display action patterns created from the cadence of the headbob. Type A display consists of three short headbobs; type B is one short headbob followed by a single extended headbob; and type C display is a single extended headbob. Each display type can be modified through the use, or exclusion, of the dewlap (a colorful throat fan possessed by most anole species), as well as a shudderbob (i.e. a quick shaking motion of the head up and down) at the end of the display sequence (additionally, Bloch and Irschick (2006) described a variant of the type C headbob, called "Y type", in a population of adult green anoles, although this variant has not been documented elsewhere). Previous studies have noted some important differences between the type A, B and C displays. For instance, juvenile green anoles produce the C display upon hatching, but develop the type A and B displays over time. The study of juvenile green anole displays further documented the "X display" which has two variants (Lovern and Jenssen 2003). Orrell and Jenssen (2003) also showed that while both sexes of adult *Anolis carolinensis* utilize the A, B and C display types, signaling is none-the-less sexually dimorphic, although they note that there is likely no courtship-specific display for the green anole. Jenssen *et al.* (2012) also documented seven display tendencies across the three display types for male *A. carolinensis* in nature during breeding and post breeding seasons, and showed that the same display tendencies were maintained post-breeding season, albeit in reduced frequencies. Finally, Jenssen *et al.* (2012) suggested that displays were directed primarily towards other males, as opposed to being directed towards females.

In addition to intraspecific functions for anole headbob displays, studies have noted the potential for such displays to be directed towards heterospecifics as well (Losos 2009, Ord and Martins 2006). For example, Edwards and Lailvaux (2012) showed that *A. carolinensis* produce more type C displays when the habitat contains individuals of *Anolis sagrei*, and suggested interspecific signaling as one of several potential explanations. Overall, anole headbob displays have received little attention in terms of species recognition (Jenssen 1970; Macedonia, Evans and Losos 1994; Macedonia and Stamps 1994) and a recent study on headbob displays in the brown anole, *Anolis sagrei*, yielded limited conclusions with regard to species identification, in part to the high variability of the brown anole display (Partan *et al.* 2011). In addition to other lizard species, a further potential target of these stereotyped displays could be predators. Leal (1999) suggested that a display modifier push-ups that raise the body in an up and down motion is used by *Anolis cristatellus* for communicating information on evasion ability to potential predators, in a manner similar to "stotting" in gazelles (FitzGibbon and Fanshawe 1988). However, despite the potential ecological utility of these common displays in various contexts, researchers have been reluctant to apply specific associations with display type based on ecological context (e.g. in the presence of heterospecifics, or during aggressive interactions) (Decourcy and Jenssen 1994). Indeed, Leal and Rodríguez-Robles (1995) suggested that behaviors that might be observed most readily in a specific context, such as interaction with a predator, might in fact be multipurpose as opposed to context-specific. A further potential problem with assigning "labels" to displays or behaviors is that the exact context, in which an individual might be displaying, and indeed the targets of such displays in nature, is not always known to the investigator. Nonetheless, evidence suggests that there may be context-specific trends associated with display type in *A. carolinensis* specifically (Jenssen *et al.* 1995; Jenssen *et al.* 2000) that warrant further investigation.

In addition to ecological context that could influence displays in green anoles, individual characteristics such as size (i.e. snout-vent length) or whole-organism performance capacities (i.e. any quantitative measure of an individual performance dynamic, ecologically relevant task such as jumping, running or biting; Lailvaux and Irschick 2007) might also affect display type or production. Some natural populations of *A. carolinensis* in Louisiana have a bimodal population distribution based on their morphology. In these populations larger 'heavyweight' males have bigger head morphology and stronger bite force while smaller 'lightweight' males have smaller head morphology and weaker bite force (Lailvaux *et al.* 2004; Vanhooydonck *et al.* 2005). This size distribution has potential implications for individual behavior in heavyweights and lightweights, given that large males tend to hold territories within a population to ensure direct access to females and will defend their territory through combinations of stereotyped displays whereas smaller males are unlikely to hold territories (Losos 2009) and might be expected to modulate their display behavior accordingly. Indeed, although bite force has been linked to the area of the extended male dewlap, independent of body size, in a variety of territorial anole species, including *Anolis carolinensis* (Vanhooydonck *et al.* 2005; Lailvaux and Irschick 2005), associations between display type and bite force have not previously been considered.

The aim of this study is to test the ecological function and importance of the A, B and C stereotyped displays for adult male *Anolis carolinensis* under a variety of social stimuli in the laboratory. We selected four stimuli (male green anole, female green anole, male *Anolis sagrei* and a predator) to be tested against a group of testing males. We isolated display behavior within specific ecological contexts by manipulating both the identity of the display target (conspecific male, conspecific female, heterospecifics male and a snake predator), and the heavyweight/ lightweight status of the display animal. From these trials we first examined overall counts of pooled display types produced by the test animals as a function of four different factors, namely bite force, size class (i.e. heavyweight or lightweight), ecological context and display type. Next, we examined which of these factors affected the production of the distinct display by types (i.e. A, B or C). We tested the following specific predictions: 1) Ecological context (i.e. interacting with conspecific male, conspecific female, heterospecifics male or a snake predator) will affect the number and type of display used; and 2) Morphology (i.e. size class or bite force) of individual males will affect the number of displays produced based on the morphology of the displaying male.

Methods

Acquisition of Animals and Housing

All experimental procedures were approved under IACUC #09-004. Forty-one *Anolis carolinensis* were collected on the University Of New Orleans campus in New Orleans, Louisiana during spring 2012. Four *Anolis sagrei* were collected from another population elsewhere in the city. The anoles used for the experiment were maintained in a 12:12 light dark cycle and at approximately 24°C at night and around 32°C during the day. The male *Anolis* *carolinensis* and *Anolis sagrei* were housed in individual cages (11.75"Lx7.6"Wx8"H) with mulch substrate, a dowel perch and access to heat lamps. The female *A. carolinensis* were housed in a glass tank (12"Lx7"Wx7.5"H) allowing for close proximity of the females; as they would experience naturally in the wild. The female tank contained mulch substrate, a dowel perch and access to a heat lamp. For the testing arena we used the largest tank we had access to (45"Lx22"Wx21"H) for the purpose of limiting any potential influence being constrained within the arena, in the laboratory, could have on the behavior of the testing males. The testing arena was divided into two even parts by a mesh wall which allowed for visual, auditory and any possible scent stimulus to be perceived by the testing males but preventing physical interaction during the trial. Both sides of the tank were supplied with mulch substrate, perches and a heat lamp over the middle of the arena. The perches were arranged with a single dowel placed diagonally across each half of the arena as well as a brick in the bottom of each side. Anoles were fed every other day with crickets dusted with powdered calcium lactate while water was provided to the lizards as droplets from daily misting. The predator used in the experiment was a *Lampropeltis alterna*, a snake that had been reared in captivity. The snake was housed in the laboratory in a glass tank (12"Lx7"Wx7.5"H) with shredded paper substrate and had access to fresh water. During the duration of the trial the snake was fed once and was not returned to the testing arena for twenty four hours after its meal. The snake was not used for trials during molting.

Experimental Design

The thirty primary testing males were exposed to four different stimuli in the laboratory. Each male was exposed to a male *Anolis carolinensis*, a female *A. carolinensis*, a male *Anolis sagrei*, and finally a predator during four separate trials. We did not randomize the order of trials, and consequently male-male interactions were conducted first and male-female trials second for each individual. We did this deliberately, as evidence suggests that testosterone, and hence aggressive behavior, “spikes” during the early part of the breeding season (i.e. early spring), and rapidly declines thereafter (Husak *et al.* 2007; Neal and Wade 2007). We therefore ensured that we conducted the breeding season-specific trials (namely male-male and male-female trials) as early in the season as possible. However, we do not believe that our results are confounded by this lack of randomization, as a recent study showed that there is no long term influence of previous encounters on future interactions in male green anoles (Forster *et al.* 2005). Prior to each trial a solid divider was installed into the testing arena preventing the primary testing males from observing the other half of the arena. Once the male and its stimulus were introduced into the arena, with the barrier in place, the anoles were given a 24 hour acclimation period. We selected 24 hours as the acclimation period to reduce the impact that the movement between housings could have on display production, as well as to provide the males with time to adjust to their new territory. After 24 hours the barrier was removed and the lizards received a 30 minute acclimation period, due to disturbance of the arena by removing the barrier, before data collection started. For each test a Sony Handycam (DCR-SR88) was used to record the behavior of the primary testing male when faced with each stimulus. For every trial the camera was setup to record as much of the arena as possible while ensuring that the testing male could be observed at all times.

The *Anolis carolinensis* stimuli comprised two separate trials. The first paired the testing males with another male *A. carolinensis*. For this trial the stimulus male was at least 8mm smaller than the primary testing male. We deliberately established a size asymmetry to solicit displays from the testing males due to size matched males tending to skip displays and escalate directly to physical combat (Jenssen *et al.* 2005; Lailvaux and Irschick 2007). This increased aggression by the smaller male would encourage the larger, primary testing male to display back without the potential of physical attacks due to the mesh barrier. The second context, of a female *A. carolinensis*, randomly paired the testing male with one of the four females. The mixed species trial randomly paired the testing male with one of four male *Anolis sagrei* following the same general protocol previously outlined. Finally, the testing males were placed in the arena with a predator stimulus a *Lampropeltis alterna* snake. The difference in procedure for this trial was that only the testing male, and not the snake, received the initial 24 hour acclimation period, as the snake did not require an acclimation period to the tank to associate the tank as its new territory. After the acclimation period the snake was introduced to the tank and the barrier removed. This was followed by the 30 minute acclimation period before video data was collected.

Video Analysis

We extracted data on frequency and type of headbob displays from videos of testing males during the four different trials. Displays were identified as type A, B or C by slowing down the video in the AVS Video Editor (Online Media Technologies, Ltd. 2011) to half speed to clearly see differences between the displays produced by the testing males.

Morphology Data

Data were gathered on snout-vent length (SVL) by measuring from the tip of the snout to the cloaca. From the SVL lengths the primary testing males were grouped into different classes, heavyweight and lightweight, based on previous research on life-stage morphs in male green anoles in New Orleans (Lailvaux *et al.* 2004, Husak *et al.* 2007; Husak *et al.* 2009). We used a cutoff of 63 mm SVL to classify individuals as either lightweight or heavyweight (c.a. Lailvaux *et al.* 2004; Vanhooydonck *et al.* 2005). The same SVL measurements, greater or less than 64 mm were utilized in this study for the factor “size class”.

Bite force was measured on all primary testing males by using an isometric Kistler force transducer and Kistler charge amplifier (Herrel *et al.* 1999). Before collecting bite force readings all testing lizards were placed in an incubator set at 32°C for an hour before bite force measurements and between each reading (Lailvaux *et al.* 2004). Five trials were collected for each individual. From the five data points collected the maximum bite force was used as the maximal bite force for the individual (Herrel *et al.* 1999; Herrel 2001; Lailvaux *et al.* 2004; Vanhooydonck *et al.* 2005) for the factor “bite force”.

Statistical Analysis

<u>Pooled Display Count Data</u>

The data were analyzed using R version 3.0. Due to the repeated measure nature of the study, we used the “lmer” function of the lme4 package for R to fit saturated linear mixed-effects models with “individual” as a random factor and accounted for all possible effects on the number of displays produced (Bates *et al.* 2013). The saturated models contained the factors “size class” (heavyweight or lightweight), “bite force”, “context” (testing males encountering a green male, green female, brown male anole or a predator), “display type” (A, B and C) as well as all possible interactions between factors. We ran the mixed-models with a Poisson link for the count data. We used maximum likelihood to fit the saturated model and for the model reduction.

Removal of non-significant factors from the saturated lmer model was accomplished using the MuMIn package and its “dredge” function (Bartoń 2013). This package produced sets of models with different possible combinations of the interactions for the factors from the saturated model. We accepted the model with the lowest AIC value as the model with the best fit. The model with the lowest AIC value was then re-fit using restricted maximum likelihood. This updated model was then utilized for later post-hoc analysis.

The lowest AIC models from the MuMIn “dredge” analysis were then used to perform post-hoc tests on the two way interaction of factors retained in the minimum adequate model which then allowed for comparison of significant levels of those factors. This was accomplished using the “testfactors” function in the Phia package for R (De Rosario-Martinez 2013).

Display-Specific Data

To analyze the differences based on the number of each display type produced by the testing males, we used the same mixed-model analysis and dredging procedure as before. This time we created three separate models; one for each display type (A, B and C). These three saturated models included the factors “size class” (heavyweight or lightweight), “bite force”, “context” (green male, green female, brown male anole or predator) as well as all possible interactions between them. Post-hoc analyses were based on the significant two way factor interactions, indicated by the lowest AIC models for each of the three display types, to determine which factor level differences contributed to the significance of the interaction. For the type A display, we therefore tested the interactions of “bite force” and “context”, “size class” and “context” as well as “bite force” and “size class”. For the type B display the interactions of “size class” and “context” as well as “bite force” and “context” were tested. Finally, for the type C display the interactions of “size class” and “context”, “bite force” and “size class” as well as “bite force” and “context” were tested.

Finally, we conducted chi-squared tests on the display-specific data to test whether the most commonly-used display contexts in heavyweights and lightweights differed in the ratios of observed A, B and C displays. These tests looked at the two different size classes, heavyweight and lightweight, and compared the counts of the three different stereotyped displays between contexts. The first test looked at lightweights displaying to green males compared to heavyweight displaying to green females, whereas the second test compared size classes again but this time displaying to the opposite gender of green anoles analyzed in the first test.

Results

Pooled Display Count Data

<u>Model Reduction</u>

The saturated model accounted for the factors “context”, “size class”, “display type”, “bite force” and any possible interactions of the factors. The saturated model produced an AIC value of 655, and the lowest AIC model, with a value of 640, retained several two way and three way interactions (Table 1).

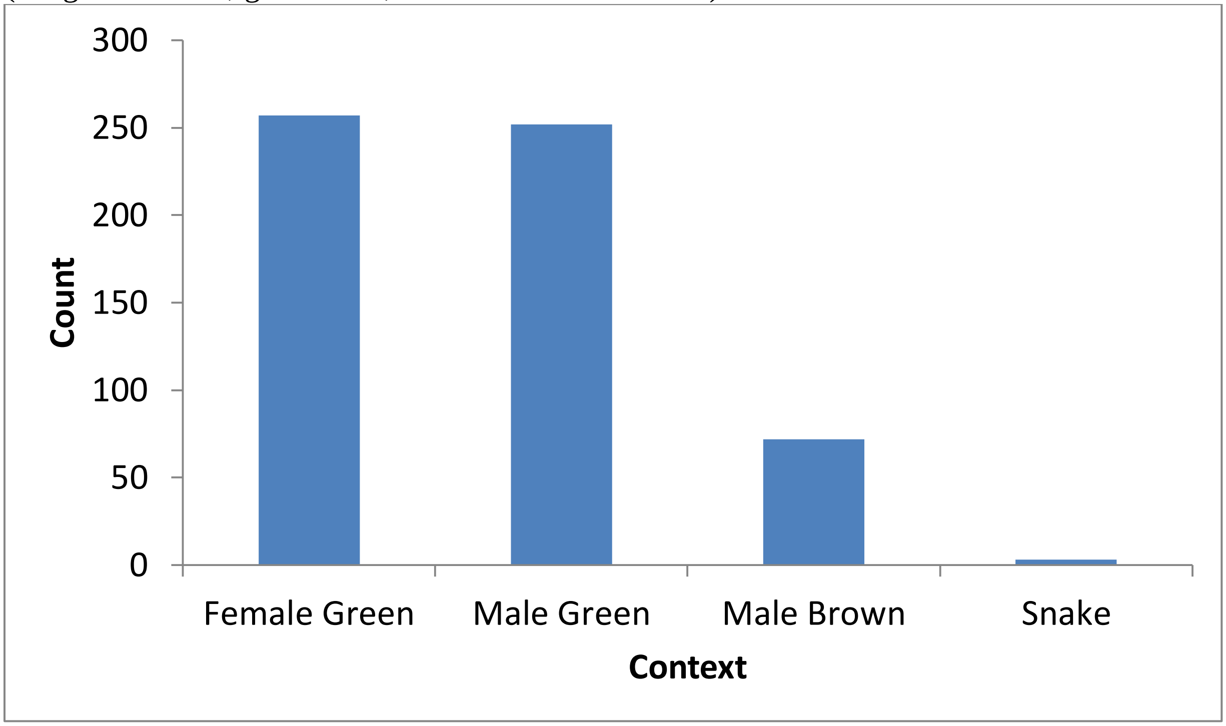
Table 1. Generalized linear mixed models for the pooled display count data. The saturated model accounted for the factors "context" (primary testing males against male green anole, female green anole, male brown anole or predator), "display type" (A, B or C display), "size class" (heavyweight or lightweight) and "bite force" and all possible interactions of these four factors. The minimum adequate model was identified based on the reduced model, where interaction of factors had been removed, with the lowest AIC value.

Models	AIC
Saturated	
Count~Context*Size Class*Display Type*Bite Force+(1|Individual)	655
Minimum Adequate Model	
Count ~ Bite Force + Context + Display Type+ Size Class + (1 | Individual) + Bite Force: Context + Bite Force: Display Type + Bite: Size Class + Context: Display Type + Context:Size Class + Display Type: Size Class + Bite Force:Context:Size Class + Bite Force: Display Type: Size Class + Context: Display Type: Size Class	640.6

Pooled Display Count Data Post-Hoc Results

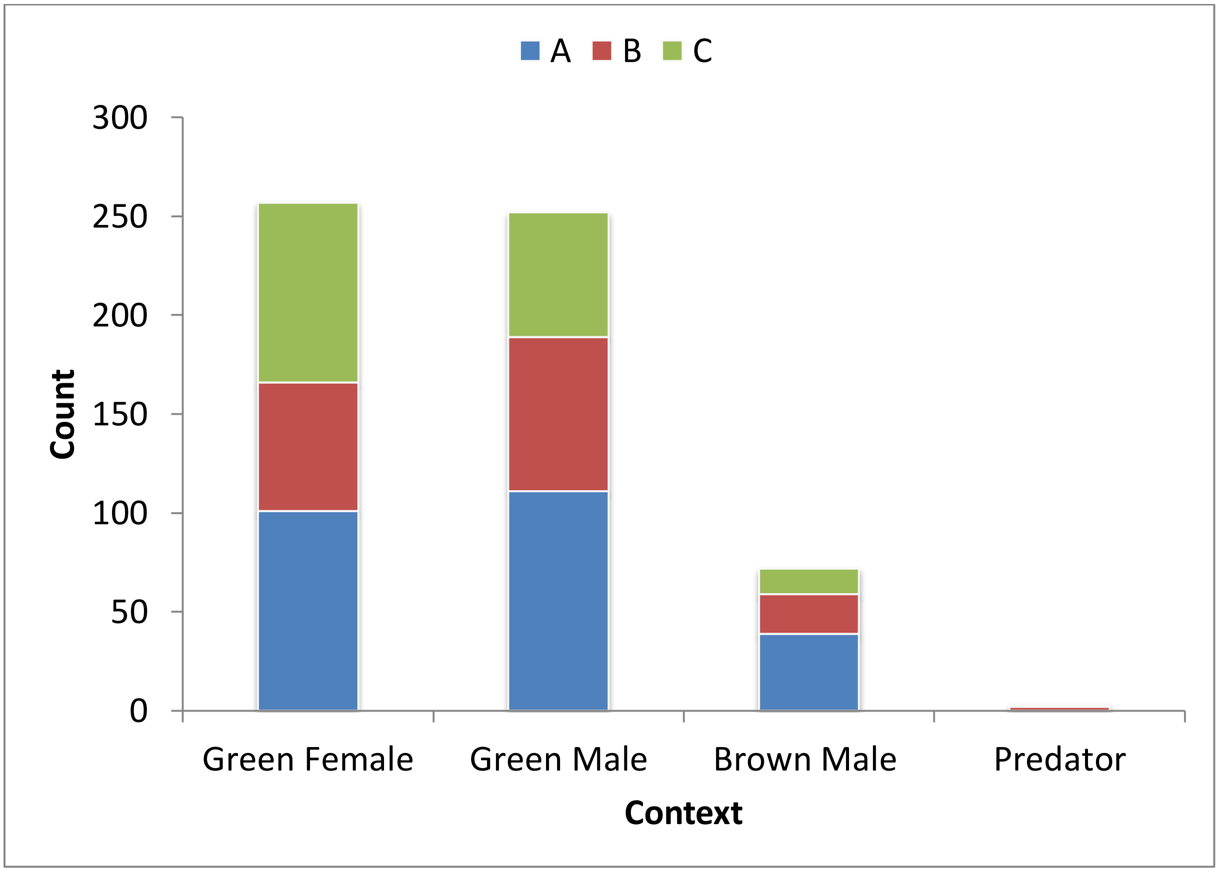
The post-hoc analysis for the interaction of "context" and "size class" factors showed that both lightweight and heavyweight males displayed overall more frequently to female and male green anoles than to a heterospecific anole, *Anolis sagrei* (Table 2 and Figure 1). The male green anoles also differed in their number of overall displays male or female conspecifics based on their size class. Specifically, the lightweight males displayed more frequently to male green anoles while the heavyweight males displayed more frequently to female green anoles (Table 2 and Figure 1).

Figure 1. Pooled display counts for the four ecological contexts encountered by the testing males (i.e. green female, green male, male brown and a snake).



The post-hoc results for interaction of "context" and "display type" factors showed that green anole males overall displayed significantly less to brown males compared to lizards of their own species, whether male or female. The male green anoles displayed less frequently to brown males for both type A and C displays in comparison to the production of displays towards green males, while the green males displayed less frequently, for all three stereotyped displays, to brown males compared to the production of displays towards a green female (Figure 2 and Table 1). Interactions accounting for the predator stimulus were also tested through post-hoc analysis but were never retained as a significant factor, and indeed green anole males overall displayed very little to the snake predator.

Figure 2. Pooled display counts broken up by display type (i.e. A, B and C).



Post-hoc analysis retained significant levels for the factors "context" and "bite force". From these tests, three contexts were retained as interacting with "bite force". There was a difference in overall display production when the testing males encountered brown males compared to green females based on the male's bite force. The difference in display production was also shown when males encountered brown males compared to green males, again in association with the male's bite force. The final level of factors retained were based on male bite force when encountering green females compared to encountering green males (Table 2).

Table 2. Post-hoc analysis for two way interactions of factors ("context", "display type", "size class" and "bite force") retained in the pooled display count minimum adequate model.

Interaction	DF	Chisq	Pr(> Chisq)
Brown Male- Green Female : A-C	1	15.65	0.001
Brown Male- Green Male : A-C	1	11.20	0.014
Brown Male- Green Female : B-C	1	9.32	0.036
Brown Male- Green Female: Heavyweight- Lightweight	1	11.47	0.004
Brown Male- Green Male : Heavyweight- Lightweight	1	10.17	0.006
Green Female- Green Male : Heavyweight- Lightweight	1	76.79	<0.001
Brown Male- Green Female : Bite	1	83.05	<0.001
Brown Male- Green Male : Bite	1	31.89	<0.001
Green Female- Green Male : Bite	1	14.58	0.001

Display-Specific

Model Reduction

The saturated models, for all three display types, accounted for all possible interaction of factors including "context", "size class" and "bite force". The type A saturated model had an AIC value of 280.4. The type B saturated model had an AIC value of 227.8. The type C saturated model had an AIC value of 242.7. For the A display the model with the lowest AIC value (276), accounted for all possible two way interactions. The model with the lowest AIC value for type B display (225.3) accounted for two possible two way interactions. Finally, the model with the lowest AIC value for type C display was the saturated model (Table 3).

Table 3. Generalized linear mixed models for the display-specific (type A, B and C) data. The saturated model accounted for the factors "context" (primary testing males against male green anole, female green anole, male brown anole or predator), "size class" (heavyweight or lightweight), "bite force" and all possible interactions of these three factors. The minimum adequate model was identified based on the reduced model, where interactions of factors had been removed, with the lowest AIC value.

Models	AIC
Display A	
Saturated	
A~Context*Size Class*Bite Force+(1|Individual)	280.4
Minimum Adequate Model	
A ~ Bite Force + Context + Size Class + (1 | Individual) + Bite Force: Context + Bite Force: Size Class + Context:Size Class	276
Display B	
Saturated	
B~Context*Size Class*Bite Force+(1|Individual)	227.8
Minimum Adequate Model	
B ~ Bite Force + Context + Size Class + (1 | Individual) + Bite Force: Context + Context:Size Class	225.3
Display C	
Saturated	
C~Context*Size Class*Bite+(1|Individual)	242.7
Minimum Adequate Model	
C ~ Bite Force + Context + Size Class + (1 | Individual) + Bite Force: Context + Bite Force: Size Class + Context:Size Class + Bite Force:Context:Size Class	242.7

Post-Hoc Analysis of Display A

The post-hoc test returned significance for the interaction of the factor "context" and "size class". Indeed, both lightweights and heavyweights produced more type A displays to green females than to brown males (Figure 3 and 4, Table 4). Conversely, green male anoles produced type A displays in different frequencies to conspecifics based on the testing males size class (Table 4). The lightweights produced more type A displays to green males while heavyweight males produced more type A displays to green females (Figure 3 and 4).

Figure 3. Display-specific counts (type A, B and C) produced by lightweight testing males during the four ecological contexts.

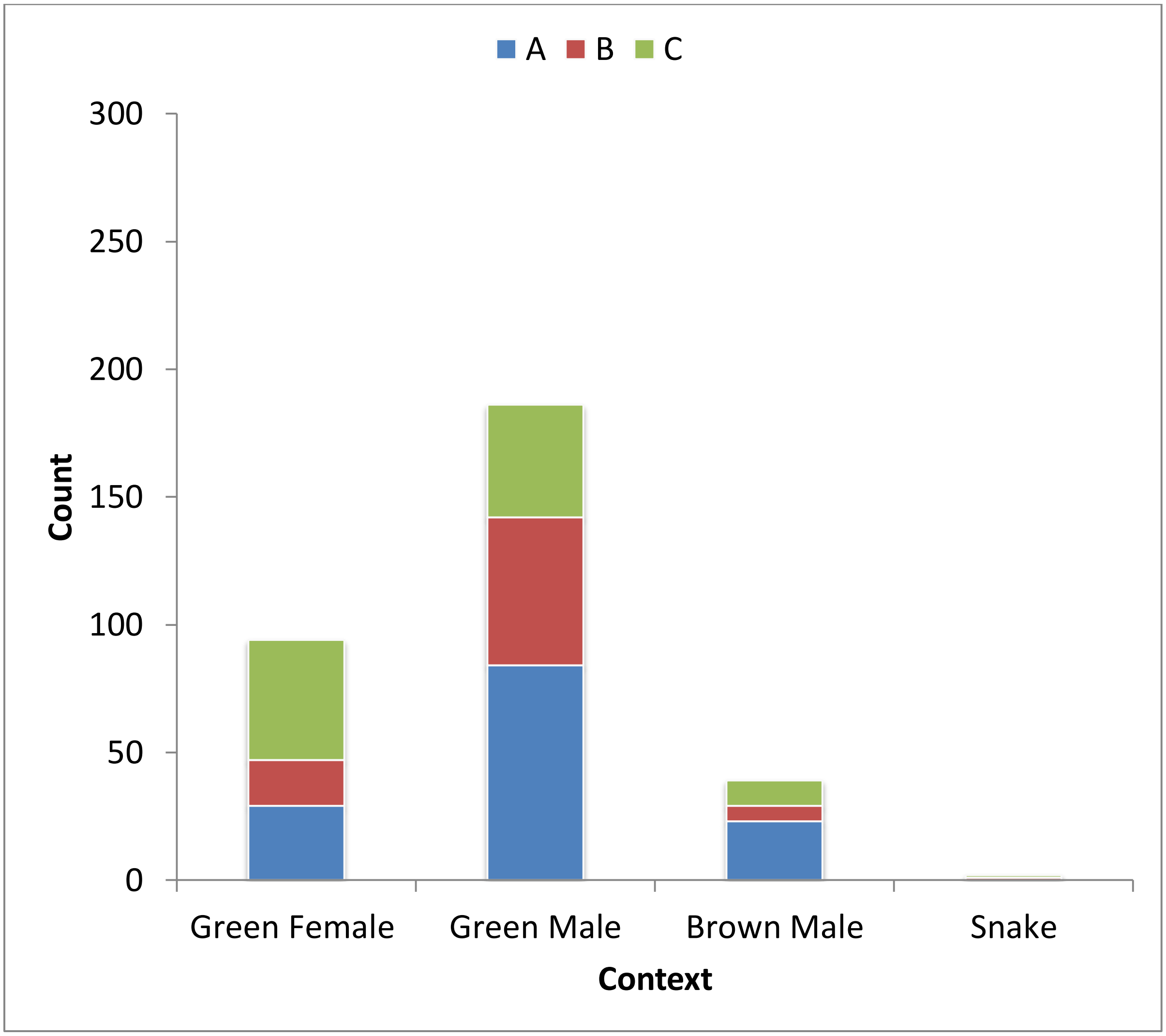
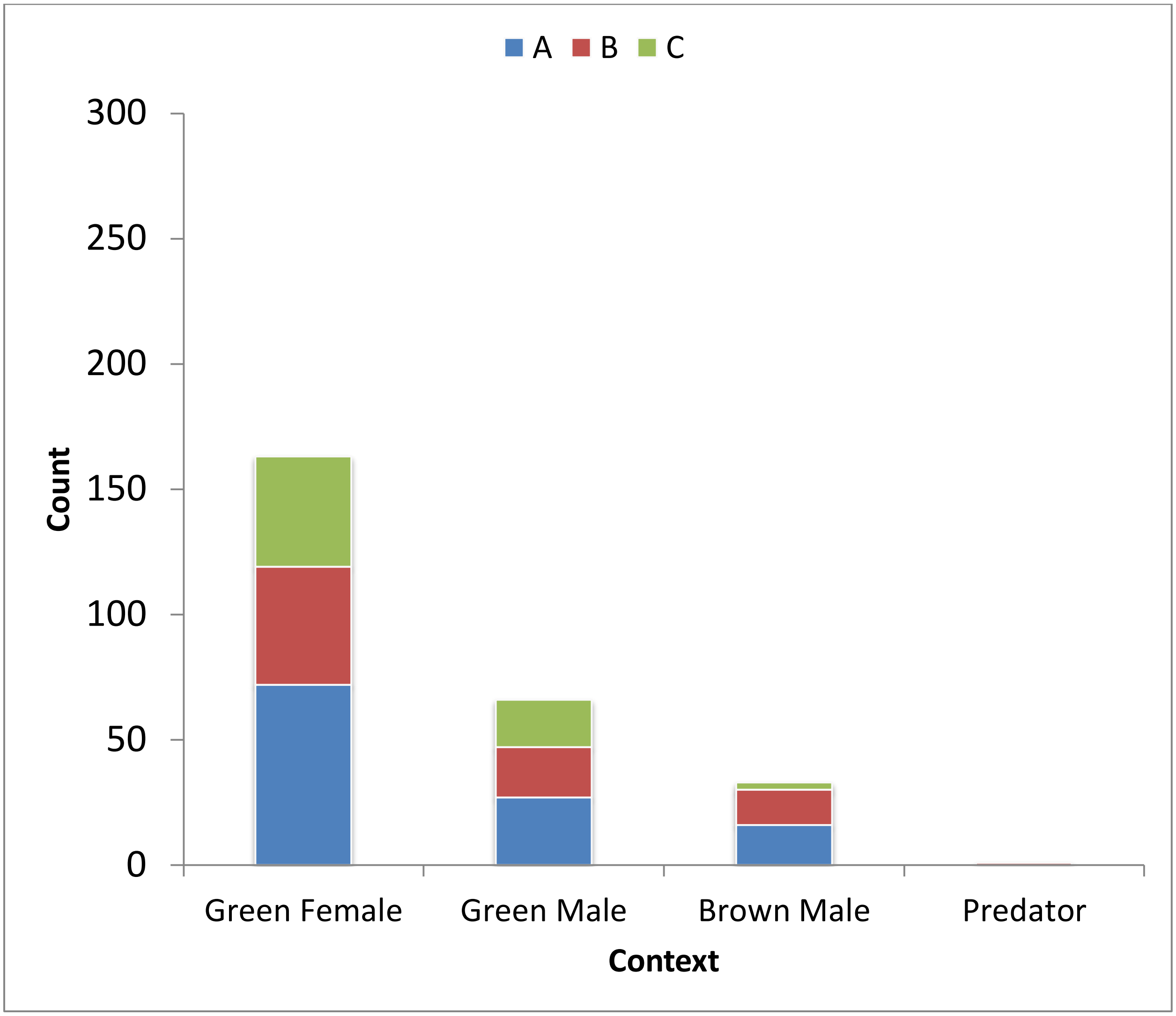


Figure 4. Display-specific counts (type A, B and C) produced by heavyweight males during the four ecological contexts.



The post-hoc test also retained significance for the interaction of the factor “context” and “bite force”. There was a significant effects on the number of type A displays produced when males encountered brown males compared to green females based on the testing male’s “bite force”. There was also a significant effect on the number of type A displays produced when males encountered brown males compared to encountering green males based on the testing male’s bite force (Table 4).

Table 4. Post-hoc analysis for two way interactions of factors ("context", "size class" and "bite force") retained in the type A, B and C display-specific minimum adequate model.

Interaction	DF	Chisq	Pr(> Chisq)
Display A			
Brown Male- Green Female : Heavyweight- Lightweight	1	8.40	0.019
Green Female- Green Male : Heavyweight- Lightweight	1	44.89	<0.001
Brown Male- Green Female : Bite	1	19.45	<0.001
Brown Male- Green Male : Bite	1	12.56	0.002
Display B			
Brown Male- Green Male : Heavyweight- Lightweight	1	12.14	0.003
Green Female- Green Male : Heavyweight- Lightweight	1	27.50	<0.001
Brown Male- Green Female : Bite	1	16.80	<0.001
Brown Male- Green Male : Bite	1	13.74	<0.001
Brow Male- Predator : Bite	1	8.04	0.009
Green Female- Predator : Bite	1	21.05	<0.001
Green Male- Predator : Bite	1	19.89	<0.001
Display C			
Brown Male- Green Female : Bite	1	31.01	<0.001
Brown Male- Green Male : Bite	1	14.77	<0.001
Green Female- Green Male : Bite	1	9.45	0.008

Post-Hoc Analysis of Display B

The post-hoc test retained significance for the interaction of the factor "context" and "size class" and had similar conclusions to the type A findings. Both size classes produce more type B displays to conspecifics, regardless of gender, over heterospecifics but differ in their production of type B displays to conspecifics based on the testing males weight class (Figure 3 and 4, Table 4).

The post-hoc test also retained significant interaction for the factor "context" and "bite force" but had more significant levels retained than type A did. Similar to the type A display post-hoc analysis the testing males produced more type B displays when in the presence of conspecifics then when encountering heterospecifics based on their bite force. In addition the testing males produced more type B displays when encountering both heterospecifics (brown males) and conspecific (green male and green female) over a predator based on their bite force (Table 4).

Post-Hoc Analysis of Display C

The only significant post-hoc test retained an interaction for the factors "context" and "bite force". The testing males produced more type C displays when in the presence of a conspecific (green male and green female) compared to heterospecifics based on the testing male's bite force. The post-hoc analysis of the type C display was the only instance of an effect on display production between the conspecifics (male or female green anole) based on the testing males bite force (Table 4).

Chi-squared

The chi-squared test used the display counts, broken down by type (i.e. A, B and C), to compare the display production of the two size classes between different contexts. The first compared display production of the size classes to their target stimuli; lightweight males displaying to green male stimulus and heavyweight males displaying to green female stimulus. This test showed that display production was similar for the two size classes displaying to their target stimuli (Figure 3 and 4, Table 5). The second test compared display production of the size classes to their non-target stimuli; lightweight males displaying to a female stimulus and heavyweight males displaying to a green male stimulus. This test showed that there was a significant difference in display production between the size classes displaying to their non-target stimuli (Figure 3 and 4, Table 5).

Table 5. Chi-square analysis analyzed how the two “size classes”, displaying to their target (lightweight/males and heavyweight/females) or non-target audience (lightweight/females and heavyweight/ males), compared in there production of all three display types.

Stimulus Comparison	x-squared	df	p-value
Lightweight to Male Stimulus and Heavyweight to Female Stimulus	0.5621	2	0.755
Lightweight to Female Stimulus and Heavyweight to Male stimulus	7.3815	2	0.02495

Discussion

Anolis lizards use repeated and sometimes stereotyped displays in a variety of ecological and selective contexts, yet the ecological utility of these displays is little understood. We predicted that the ecological context of an interaction would be a key factor influencing the types of displays produced by green anoles. We also predicted that frequencies of different display types would differ based on individual morphology (specifically heavyweight/ lightweight status) and, potentially, individual bite force capacity.

Display Type and Context

Our results show significant variation both in frequency and number of displays across measured behavioral contexts, supporting our first prediction. The majority of overall displays were observed in the green male and green female contexts. The heterospecific (i.e. brown anole) context elicited significantly fewer displays than the conspecific context, and the predator context elicited the fewest displays of all (Figure 1). However, the total number of displays produced by males when encountering a green male or a green female stimulus is similar (Figure

1). Even when breaking down the counts based on the three possible display types, for all adult males, we observed this same trend of similarities between the displays produced to green female and green male context (Figure 2). This suggests that adult green anole males alter their displays based on the ecological context, and display in different frequencies to conspecifics than to heterospecifics.

Previous studies have suggested that certain displays might be directed specifically towards predators; for example, Leal (1999) suggested that *Anolis cristatellus* pushup displays are honest signals to predators of the prey's ability to escape. In contrast to Simon (2007) who showed that *A. sagrei* only regulated dewlap extensions and not the frequency of headbobs in the presence of a predator, our data indicates that *A. carolinensis* elect to very seldom use headbob displays to predators (Figure 1). An explanation for this finding may lie in our experimental design, which may have forced individual green anoles into closer proximity with predators than they are willing to display in. Research by Leal and Rodríquez- Robles (1995) supports this conclusion with their documentation of *Anolis cristatellus* immobility, where no displays are produced, during close proximity with a predator as well.

In our results, the heterospecific (i.e. *Anolis sagrei* male) context was retained in the post-hoc interaction tests with "size class" and "display type", but display count to the brown male stimulus was always lower than the number of displays produced towards other green anoles (Figure 3 and 4, Table 2). This suggests that green anoles are not using the type A, B or C display specifically for species identification within mixed populations. This explanation is supported by Ord and Martins (2006), who suggest that the dewlap modifier is utilized for species identification, rather than the display itself. Indeed, previous studies have suggested that dewlap color is potentially more important than behavior in terms of species recognition in *Anolis* (Losos 1985, Ord and Martins 2006).

Display Differences between Heavyweights and Lightweights

Our second prediction, that displays differ depending upon individual characteristics, is also supported once the display data are analyzed according to display type (i.e. A, B or C). The interaction between "size class" and "context" in the pooled display count post-hoc analysis shows that lightweight males display most frequently to other male green anoles, whereas heavyweight males display most frequently to green female anoles (Figure 1 and Table 2). Furthermore, lightweight males used A and B displays more frequently to other green males, whereas heavyweight males instead used A and B displays more frequently to other green females (Figure 3 and 4, Table 4). This finding is consistent with previous studies which show that display use in green anoles is affected both by the ecological milieu and by age/size class (Lovern and Jenssen 2003; Edwards and Lailvaux 2012; Bloch and Irschick 2006). This differential display use most likely reflects the different priorities for heavyweights and lightweights. Male territories increase with body size (Losos 2009) where the biggest males (i.e. heavyweights) consistently maintain a breeding territory over smaller males. Decourcy and Congdon (2005) showed that in interactions between size-mismatched green anoles the smaller males were more likely to be the aggressor toward the larger males and suggested that this aggressive behavior in small males was due to the potential benefit they could gain from fighting for access to breeding territories. Even though green males direct their displays to a specific target audience, based on their size class, chi-squared tests revealed that the production of the stereotyped displays (i.e. ratios of A, B and C displays) to their target audience (lightweights/ males and heavyweights/ females) are similar in both size classes, whereas production of displays to the non-target audience (lightweights/ females and heavyweights/ males) clearly differ between the size classes (Table 5). One possible explanation for this finding is that males may be displaying randomly to non-target stimuli, but maintain similar A, B and C display ratios when displays are targets of interest. The similarity in display ratios to target stimuli could possibly be caused by both size classes conveying the same information to their respective target audience.

Our consideration of the size of the signaling male reveals important insight into male green anole display behavior that is not apparent from previous studies. For example, Jenssen and his team (2000) documented green anole males increasing their use of type A and B displays when encountering a green female stimulus. Our data, at the pooled display count level, did not reflect this trend (Table 2); however, analyzing the number of displays based on size class shows that in fact only heavyweight males increase their usage of type A and B displays compared to other contexts (Figure 4, Table 4). Our pooled display count results also differ from those of previous laboratory tests which stated that males facing off against other males had a 10 times greater rate of display compared to other stimuli (Orrell and Jenssen 2003). Again, breaking down the display counts by "size class" and "context" shows that other green males are not the sole targets of displays (Figure 3 and 4, Table 4) and that both the target audience and the displays produced are heavily influenced by the size of the signaler (i.e. heavyweight/ females and lightweight/ males) (Table 5). Our findings clearly suggest that male green anoles modulate their displays based on individual characteristics such as size, and the displays thus produced are therefore likely to be far more complex than previously understood.

Display Type and Bite Force

Although our results show that the A and B displays are used most often by male green anoles, the type C display is of special interest given the prominence afforded to it in the green anole literature (Jenssen *et al.* 2012; Orrell and Jenssen 2003). Type C displays have been primarily associated with males patrolling a territory, and are thought to be directed towards other males and not towards females (Jenssen *et al.* 2012). More recently, Edwards and Lailvaux (2012) suggested that variation in C display production is associated with variation in body size within a natural population of green anoles, with smaller males producing more type C displays compared with larger males. Our data indicates that type C displays are used broadly in a variety of behavioral situations based on the greater number of interactions retained in the type C minimum adequate model compared to type A and B. The lowest AIC model for type C retains the individual factors (i.e. “bite force”, “context” and “size class”), two way interactions (bite force/ context, bite/ size class and context/ size class) and one three way interaction (bite force/ context / size class) (Table 3). By contrast, display type B is the most specific display, identified by retaining the factors “context”, “size class” and “bite force” as well as the fewest two way interactions (bite force/ context and context/ size class) (Table 3). Type A displays fall somewhere in between the other two types, based on its retention of the individual factors (i.e. “bite force”, “context” and “size class”) as well as three two way interactions (bite force/ context, bite force/ size class and context/ size class) (Table 3). The finding that type C display is broadly utilized has been indicated numerous times through observations of individual green anoles, in their natural settings and in laboratories (Jenssen *et al.* 2012 and; Orrell and Jenssen 2003; Jenssen, Decourcy and Congdon 2005), but now has statistical support that has not previously been available. The findings that display types A and B have different levels of complexity, as identified by the minimum adequate models, are also novel.

Interactions among “bite force”, “context” and “size class” were retained in the minimum adequate models for overall display count and for the individual A, B and C display types indicating that bite force capacity is influencing display production in some manner. Many of the interactions that retained significance in association with “bite force” for display type A and B reflected the low display production to both predators and conspecifics by the testing males (Figure 3 and 4, Table 4). Post-hoc analysis of the interaction of “context” and “bite force” for display type C was the only test to show a difference between display production for the stimulus of a green female and a green male based on the displaying male’s bite force (Figure 2, Table 4). Since greater bite force is linked to larger size in *A. carolinensis* (Lailvaux *et al.* 2004) we would expect the factor “size class” would be significant interaction with “bite force” as well. However, interactions between “size class” and “bite force” as well as “size class” and “context” were not retained as significant even though the minimum adequate model, for the pooled display count data, suggested that some level of these factors would be (Table 1). Since the data suggest that display type C is potentially linked to bite force independent of body size this warrants further research to better explain the complexity of the factors influencing type C displays.

Conclusions

Our findings support the predictions that ecological or social context, and both size and performance abilities of the signaler have significant influence on display production. Our results show that green males are displaying to other green anoles more frequently over other stimuli. Our data also indicates that size class influences display production with lightweight males displaying more frequently to other green males and heavyweight males displaying more frequently to green females. Finally, our results indicate that the type C display is the most complex and generally used, and that questions still remain on the utility of this display and its association with bite force.

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Appendix

Appendix A: IACUC Approval Form

Institutional Animal Care and Use Committee

UNIVERSITY OF NEW ORLEANS

DATE: April 9, 2009

TO: Dr. Simon Lailvaux

FROM: Steven G. Johnson, Ph.D.
Chairman

RE: *IACUC Protocol* # **UNO-09-004**
Entitled: Competitive interactions and whole-organism performance in Anolis lizards

Your application for the use of animals in research (referenced above) has been approved beginning April 9, 2009 and expiring April 9, 2012.

❖UNIVERSITY OF NEW ORLEANS Institutional Animal Care and Use Committee❖

Vita

The author was born in Willimantic, Connecticut. She obtained her Bachelor's degree in biology from Eastern Connecticut State University in 2010. She joined the University of New Orleans biology graduate program in pursue of a M. S. in biology, and became a member of Professor Simon Lailvaux's research group in 2011.