Social and Hormonal Effects on the Ontogeny of Sex Differences in Behavior in the Lizard,

Anolis carolinensis

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ABSTRACT

Adult green anoles, *Anolis carolinensis*, exhibit numerous sex differences resulting from divergent strategies for maximizing reproductive success. I focused on the ontogeny of sex differences in behavior in juveniles, in relation to adult sex differences, by documenting the behavior of free-ranging juveniles, examining the structure and use of headbobbing displays, and determining the role of the androgen testosterone (T) in producing behavioral sex differences. Field observations indicated that juvenile males eat and forage actively more often than juvenile females. This divergent feeding behavior may result from sexual selection, given that body size is a major factor in determining the reproductive success of males. Analyses of headbobbing displays, used by adults in aggressive and sexual interactions, revealed that juvenile males and females each give the same three A, B, and C display types described for adults. However, there may be a maturational component to display structure, as juvenile displays differ from those of adults in within-display temporal structure, and are not as stereotyped. Concerning display use, social context affects neither the types of display interactions observed nor the rates of displays and related behaviors. However, size affects nearly every aspect of display behavior. Both juvenile males and females show increased display rates and probabilities of expressing display-related behaviors with increasing body size, although in the largest juveniles, male display rates become higher than those of females. These results, like those from analyses of display structure, suggest a maturational component to display use, perhaps mediated by changes in the underlying motivational states of juveniles. Consistent with the divergence in display rates in large juveniles, males of approximately 30 d of age and older have higher plasma T concentrations than females. Furthermore, juvenile males and females that have been given T implants each respond with increased behavior levels, approaching those of breeding adult males. These analyses indicate that sexual dimorphisms in behavior in adults likely arise through underlying physiological differences between males and females that mediate the expression of behavior, rather than through fundamental sex differences in the ability to perform these behaviors.

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Background and Objectives

In many animal species, males and females express sexual dimorphism in traits not directly associated with reproduction. Such differences may be behavioral, morphological, or physiological, and are often quite dramatic. Formal scientific investigation into the reasons for sexual dimorphism began at least with Darwin (1859, 1871), although research was not widespread until the latter part of the 20th century (Andersson, 1994; Andersson and Iwasa, 1996). From previous work, two primary, but not necessarily mutually exclusive, hypotheses for the evolution of sex differences have emerged: (1) ecological niche divergence; and (2) sexual selection (e.g., Shine, 1989; Stamps et al., 1997). Ecological niche divergence occurs when the sexes differ in behavioral or morphological traits related to their habitat use or food selection. In general, this hypothesis states that sex differences evolved to reduce competition between the sexes for the same resources, or to increase the efficiency of resource use for social groups (Shine, 1989). The hypothesis does not necessarily predict the direction of divergence between males and females, however (Shine, 1989; Stamps et al., 1997). Although empirically demonstrating the selective pressures that produce sexual dimorphism remains difficult, it is likely that some dimorphisms have evolved to reduce niche overlap or increase resource use efficiency in numerous animal taxa (reviewed in Hedrick and Temeles, 1989; Shine, 1989).

The majority of research into the causes of sexual dimorphism has focused on the hypothesis of sexual selection (Hedrick and Temeles, 1989; Shine, 1989). This hypothesis states that sex differences arise through selection of traits solely concerned with increasing mating success (e.g., Searcy and Yasukawa, 1995). Unlike the evolution of sexual dimorphism by niche divergence, the direction of sex differences if they have evolved through sexual selection is predictable by the species' life history and ecology (e.g., Emlen and Oring, 1977; Davies, 1991). This may be why sexual selection is a disproportionately popular hypothesis for testing occurrences of sexual dimorphism (Shine, 1989). Below, I briefly review the mechanisms and effects of sexual selection, and suggest the green anole, *Anolis carolinensis*, as a model for examining the extent to which sexual selection can influence overall life history.

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Sexual Selection

In general, sexual selection occurs because males compete for reproductive access to females (intrasexual selection) and females preferentially mate with the winners (intersexual selection). Conceptually, it can be useful to examine intrasexual selection (competition) and intersexual selection (mate choice) as distinct components of the sexual selection hypothesis. However, rarely will a trait have evolved solely through one of these selection pressures, as mate choice partly depends on the outcome of competition, and the nature of competition depends on what traits are being chosen (Andersson, 1994; Searcy and Yasukawa, 1995; Wiley and Poston, 1996). The distinction between intra- and intersexual selection is further blurred by the fact that numerous additional mechanisms, some independent of and some incorporating competition and/or choice, have emerged as important research topics for revealing sexual selection.

Using the terminology of Cunningham and Birkhead (1998), sexual selection can arise by precopulatory mechanisms, postcopulatory, prefertilization mechanisms, and postfertilization mechanisms. Precopulatory mechanisms of sexual selection include intrasexual competition for access to mates, such as direct contests, or indirect endurance rivalry and scramble competition, in which the abilities to maintain reproductive status for an extended period of time, and to locate mates quickly, are important (Andersson, 1994; Andersson and Iwasa, 1996). They also include direct and indirect components of mate choice. Direct mate choice occurs whenever there is active discrimination, followed by preferential mating, by one sex among possible mates of the other sex (Wiley and Poston, 1996). Morphological or behavioral courtship signals, such as color patches, tail length, or song or call frequency, duration, or rate, typically serve as male traits that are chosen (reviewed in Daly and Wilson, 1983; Andersson, 1994). Indirect mate choice occurs whenever one sex restricts their set of possible mates, primarily by encouraging competition among members of the opposite sex (Wiley and Poston, 1996). For example, some females exhibit sexual swellings or give acoustic or olfactory signals when they are fertile, thereby potentially attracting several interested males who will compete for reproductive access (reviewed in Wiley and Poston, 1996). When females copulate with more than one male, postcopulatory, prefertilization mechanisms of sexual selection can become important. These involve sperm competition among the sperm of different males for fertilization of female

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gametes, as well as cryptic female choice, which can prejudice the outcome of sperm competition in favor of the sperm of particular males (Eberhard, 1996; Parker, 1998). Lastly, postfertilization mechanisms of sexual selection include selective abortion or mortality of developing embryos, biased investment into the offspring of different individuals, and infanticide (Andersson and Iwasa, 1996; Cunningham and Birkhead, 1998).

Regardless of the particular mechanisms acting in a given species, that sexual selection arises at all is largely the consequence of anisogamy (Bateman, 1948; Trivers, 1972). In species that produce by sexual reproduction, males produce small, cheap, and overwhelmingly numerous gametes, while females produce large, costly, and relatively fewer gametes. Thus, concerning mating success, males are generally limited only by the number of females with whom they can mate, but females are limited by gamete production. As a result, intersexual differences in strategies for maximizing reproductive success occur, and the outcome of these strategies is expressed in the mating system and in the relative parental effort expended by each sex (Bateman, 1948; Trivers, 1972; Emlen and Oring, 1977; Daly and Wilson, 1983; Davies, 1991).

There is an abundance of research suggesting the importance of sexual selection in producing sexual dimorphism. As of 1990, approximately 200 species, including insects and other arthropods, fishes, amphibians, reptiles, birds, and mammals, had been studied and found to show a statistically significant relationship between some sexually dimorphic trait and mating success, arising through one or more identified mechanisms of sexual selection (Andersson, 1994:124-129). Traits that are frequently modified by sexual selection include body size, body coloration, visual, vocal, and olfactory communication signals, and all the behaviors that use these traits in reproductive contexts (reviewed in Andersson, 1994; Hauser, 1996). Although sexual selection is an area of active research, there are still gaps in our understanding of the range of mechanisms that influence sexual dimorphism, and the extent to which these mechanisms affect overall life history. In particular, the development of sexually selected traits still requires considerable study, as does understanding the mechanisms of sexual selection for species in which direct mate choice (i.e., discrimination based on preferences) play little or no role (Clutton-Brock, 1988; Andersson, 1994; Andersson and Iwasa, 1996; Adkins-Regan, 1998).

Studies on the ontogeny of sex differences can provide the opportunity to assess the strength of sexual selection against opposing selection, or constraints. Numerous constraints exist on the exaggeration of sexually selected traits, including predation, energetic, and phylogenetic constraints (reviewed in Andersson, 1994). Perhaps the best known constraint is predation, in which expression of a sexually selected trait, such as conspicuous color or advertisement calling, increases mating success but also increases exposure to predators (e.g., male body color in guppies, Endler, 1983; advertisement calling in túngara frogs, Ryan et al., 1982). Compared with the extensive research documenting the constraints to expressing sexually selected traits in adults, the potential effects of sexual selection on development has received less attention. To what extent are sexually selected traits observed in adults constrained by ontogeny? Recent studies have suggested that selection pressures acting during ontogeny can directly affect future reproductive success. For example, sexual selection may affect when individuals become sexually mature (e.g., Phillips et al., 1993; Pratt et al., 1994; Bisazza et al., 1996), it may increase predation risks in juveniles through the development of morphological traits that promote reproductive success in adults but increase mortality in juveniles (e.g., Arnqvist, 1994), or it may act on traits adversely affected by environmental stressors during early development such as parasitism (e.g., Potti and Merino, 1996) or poor nutrition (e.g., Nowicki et al., 1998) via mate choice of these indicator traits in adults. Thus, sexual selection for sex differences in adults likely affects ontogeny, and the selection of traits beneficial to future reproductive success may be constrained by the selection of traits that promote juvenile survival (McLain, 1991; Clutton-Brock, 1994).

Studies of sexual selection have generally focused on the mechanism of direct mate choice, in which members of one sex discriminate among and choose particular mates of the opposite sex based on some behavioral or morpholical trait (Andersson, 1994; Wiley and Poston, 1996). In some polygynous species, however, there may be little reason for females to exert effort in direct mate choice, particularly if they do not encounter multiple males among whom they can choose, or if they do not receive material benefits to choice (e.g., Lightbody and Weatherhead, 1988; Censky, 1997). Many territorial, polygynous lizards may be good examples of species in which direct mate choice by females plays little or no role in the selection of sexual dimorphic traits (Tokarz, 1995). First, females are unlikely to encounter many males, as males

are highly territorial and attempt to spatially exclude one another while overlapping as many female territories as possible, thus preventing outside male contact (Stamps, 1983). Males can adopt this territorial strategy because females are generally sedentary. Second, males and females in polygynous lizard species typically establish territories simultaneously rather than sequentially (e.g., Moore, 1986; Jenssen et al., in review), making the applicability of choice models that assume females choose males and their controlled resources by assessing males and their territories (e.g., the Polygyny Threshold Model; Verner, 1964; Orians, 1969) unlikely. Third, male lizards do not give parental care or other direct material benefits to females, thereby reducing the reasons for females to exert direct mate choice (Tokarz, 1995). In such species, mechanisms of sexual selection other than direct female choice, such as male contests or endurance rivalries, may be more likely to produce sexual dimorphism. Thus, females avoid the costs of direct choice (e.g., searching time and energy; Kirkpatrick, 1987), but still can exert indirect choice, by preferentially mating with those males who attain territories and win inter-male contests. The apparent lack of a theoretical and practical justification for direct mate choice in polygynous lizards might help to explain the frequent inconsistencies or negative results among mate choice studies conducted with lizards (Tokarz, 1995).

In summary, while sexual selection has proven to be a widespread phenomenon, there are still opportunities for further elucidating the mechanisms and effects it has on the overall life history of a species. Studies on the development of sexually dimorphic traits, especially in species where the selection mechanism(s) have been identified for adults, are comparatively uncommon. Therefore, I have chosen to examine the ontogeny of sex differences in behavior in the green anole, *A. carolinensis*. In adulthood, this lizard shows sexual dimorphism in numerous traits arising not through direct female mate choice, but rather through male contest and endurance rivalry competition. While sex differences and their causes have been well studied in adults, little is known about the development of sexually dimorphic traits in *A. carolinensis*. Thus, this lizard offers the opportunity to examine how juveniles develop into their sexually dimorphic adult endpoints, and these ontogenetic trajectories can reveal the extent to which sexual selection is influencing life history, perhaps extending its effects to the development of individuals before they are even reproductive.

Anolis carolinensis: A Model Organism

In a genus of approximately 300 described species, *A. carolinensis* is the only one native to North America. It is a small lizard (adults are typically 45-65 mm long excluding the tail) that is especially common in the southeastern United States, although it also ranges west to Texas and can be found on the Hawaiian islands (Conant and Collins, 1998). Reproduction occurs April-July (Ruby, 1984; Jenssen et al., 1995a; Jenssen et al., in review), during which time females laying single-egg clutches at approximately weekly intervals (Andrews, 1985a). Beyond oviposition, there is no parental care exhibited by adults of either sex (Gordon, 1956). Breeding occurs in a polygynous social structure; females settle in comparatively small territories and large, competitive males establish territories that overlap on average three female territories, but range from zero to six (Ruby, 1984; Jenssen et al., 1995a; Jenssen and Nunez, 1998). Males spend the majority of their time defending their territories from intruder males, and larger males exclusively overlap with more females than do smaller males (Ruby, 1984; Jenssen et al., 1995a; Nunez et al., 1997). Whereas male reproductive success (i.e., the number of offspring produced) depends on the number of females in his territory with whom he copulates, female reproductive success depends on the number of eggs she can produce. Therefore, potential variation in reproductive success is much greater in males than in females. Given the 1:3 polygyny ratio mentioned above, approximately two-thirds of adult males appear to have little or no access to breeding females.

Consistent with life history characteristics and male and female reproductive strategies, adult *A. carolinensis* show dramatic sex differences in behavior and morphology. Males are on average one-third larger than females, they move 7-fold the distance of females per day, have territories 9-fold the size of female territories, spend 30-fold more time than females in territory defense, communicate via headbobbing displays at 8-fold the rate of females, with dewlaps (extensible red throat fans used during displays to increase conspicuousness) that are 7-fold the size (Ruby, 1984; Jenssen et al., 1995a, b; Nunez et al., 1997; Jenssen and Nunez, 1998; Jenssen et al., 2000). These sexual dimorphisms do not appear to arise through direct mate choice (Andrews, 1985b; Tokarz, 1995), but rather through male contest and endurance rivalry competition (Jenssen and Nunez, 1998; Jenssen et al., in review). Larger males are better able to

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attain breeding territories, with access to more breeding females. To be reproductively successful, males must be able to maintain these territories and stay in reproductive condition over an extended time period, because females remain receptive and continue to lay single egg clutches over the four month breeding season.

In the following chapters, I investigate the development of behavioral sex differences in *A. carolinensis*, in relation to the expression of sex differences in adults. Throughout, I take the position that understanding the ontogenetic trajectory and function of juvenile behaviors that show sexual dimorphism can be useful for understanding the selection pressure and function for sex differences in behavior observed in adults. Sexual selection for traits beneficial to future reproductive success should be constrained by natural selection for traits that promote juvenile survival (McLain, 1991; Clutton-Brock, 1994), particularly given that juveniles are highly precocial and immediately responsible for finding their own food, shelter, and predator refugia (Gordon, 1956; Stamps, 1978). In Chapter 1, I describe the behavior of free-ranging juvenile males and females to determine if and when sex differences become apparent. In Chapters 2 and 3, I examine the ontogeny of the structure and use, respectively, of the conspicuous headbobbing displays used by adults in aggressive and courtship interactions in juveniles of a variety of ages and in different social contexts. In Chapter 4, I examine the possible role of the androgen testosterone in producing sex differences in behavior during development, both by documenting its endogenous levels in developing lizards of different ages, and by artificially elevating its concentration in the plasma and testing subsequent effects on behavioral interactions.

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Chapter 1

Behavioral Ontogeny in Free-ranging Juvenile Male and Female Green Anoles, *Anolis carolinensis*, in Relation to Sexual Selection

Abstract

Anolis carolinensis is sexually dimorphic in behavior and morphology in adults, primarily due to intrasexual selection acting to produce large, conspicuous, and aggressive males. However, the extent to which selection pressures acting on adults might also produce sex differences during ontogeny, but before sexual maturity, has not been considered. During June-August 1995 and 1996, I recorded the behavior of 20 juvenile male and 17 juvenile female *A. carolinensis* for 30-60 min each at a field site near Augusta, Georgia, USA: (1) to determine if, as groups, juvenile males and females behaviorally differ, thereby suggesting the presence of sex differences from hatching; and (2) to determine if juvenile males or females show sex-specific, size-related behavioral changes, in order to describe the ontogenetic schedule by which sex differences in behavior arise. I examined potential indicators of sex differences in space use (perch height and diameter, home range volume), social behavior patterns (body color and shift rate, nearest neighbor distance, headbobbing display rate and display context), and behavior patterns affecting growth (feeding rate and foraging mode, proportion of time spent moving). Based on potential tradeoffs between behavior relevant to the immediate life history of juveniles and behavior that will lead to future reproductive success, I hypothesized that juveniles would show no sex differences in space use or social behavior patterns, but would differ in behavior patterns affecting growth. As expected, juvenile males ate and foraged actively more often than juvenile females. Furthermore, overall comparisons between juvenile males and females revealed no sex differences in space use or social interaction. However, contrary to my hypotheses, juvenile males did not decrease amounts of time spent moving with respect to juvenile females, and larger males occupied larger home ranges and spent more time green while females showed no ontogenetic shifts in these variables. My results suggest that juvenile *A. carolinensis* show both sex differences present from hatching and arising through ontogeny leading up to the adult endpoints, and that these differences may arise through the effects of sexual selection on ontogeny.

Introduction

Because of the popularity of sexual selection research (Gross, 1994), sexually dimorphic behavior and morphology and how it relates to the overall social organization of different species has been well documented (e.g., Werner, 1978; Clutton-Brock et al., 1982; Davies, 1991; Thompson et al., 1993; Searcy and Yasukawa, 1995; Baird et al., 1996). Studies that have examined variation in morphology and behavior among sex and size classes of reproductive individuals have found differences consistent with predictions from sexual selection. In particular, studies on polygynous species have documented the importance of coloration (e.g., Zucker, 1989; Carpenter, 1995; Searcy and Yasukawa, 1995), body size (e.g., Berry, 1974; Werner, 1978; Baird et al., 1996; Bisazza et al., 1996), and behavior (e.g., Gross, 1982; Pratt et al., 1994; Baird and Timanus, 1998) as indicators of sex, age class, and reproductive status. These studies have shown that even though individuals of different age classes may have the physiological ability to reproduce, they can differ substantially in their abilities to mate successfully. In general, younger or smaller males are not able to compete with older or larger males for access to females, and they may be rejected by females even if they have the opportunity to court them.

Another approach to studying sexual selection and social organization is to investigate possible sexual and ontogenetic differences among juveniles (i.e., individuals who have not yet become physiologically capable of reproduction). Compared with the extensive research documenting the effects of sexual selection on adults, the potential effects of sexual selection on juveniles have received less attention (Clutton-Brock, 1988; Andersson and Iwasa, 1996; Adkins-Regan, 1998). However, recent studies have suggested that selection pressures acting during ontogeny can directly affect future reproductive success. For example, sexual selection may affect when individuals become sexually mature (e.g., Phillips et al., 1993; Pratt et al., 1994; Bisazza et al., 1996), it may increase predation risks in juveniles through the development of morphological traits that promote reproductive success in adults but increase mortality in juveniles (e.g., Arnqvist, 1994), or it may act on traits adversely affected by environmental stressors during early development such as parasitism (e.g., Potti and Merino, 1996) or poor nutrition (e.g., Nowicki et al., 1998) via mate choice of these indicator traits in adults. As these

examples illustrate, studying juveniles can be useful for assessing the potential strength of sexual selection in producing sex differences during a developmental period when selection for traits beneficial to future reproductive success should be constrained by selection for traits that promote the survival of juveniles (McLain, 1991; Clutton-Brock, 1994).

In the present study, I examine behavioral ontogeny in the green anole, *Anolis carolinensis*. This polygynous lizard has numerous morphological and behavioral sex differences as adults that are due to intrasexual selection acting on males (Ruby, 1984; Jenssen et al., 1995; Nunez et al., 1997; Jenssen and Nunez, 1998), and that result in large asymmetries in mating opportunities among males (Ruby, 1984; Jenssen and Nunez, 1998). Juvenile *Anolis* receive no parental care (Gordon, 1956; Stamps, 1978), and are therefore immediately subjected to threats to their survival. Because juveniles suffer higher mortality than adults (Gordon, 1956), selection for behavior that reduces mortality should act most strongly on the juvenile age class (Emlen, 1970; McLain, 1991). *Anolis carolinensis* thus appears to be a good species for examining the extent to which sexual selection acting on adults might affect the behavior of juveniles. As a first step to this end, I documented the behavior of free-ranging juvenile males and females. My specific objectives were: (1) to determine if, as groups, juvenile males and females differ behaviorally, thereby suggesting the presence of sex differences from hatching; and (2) to determine if juveniles show sex-specific, size-related behavioral changes, in order to describe the ontogenetic schedule by which dimorphisms develop. I assumed that the requirements for the survival of juvenile males and females (primarily habitats containing a consistent food supply and substrata suitable for thermoregulation and retreating from predators; Stamps, 1994) were equivalent, and that natural selection should therefore produce no sex differences in behavior. This assumption is supported by the fact that adult male and female *A. carolinensis* do not differ behaviorally outside of the breeding season (Jenssen et al., 1996). When I did find sex differences, I examined them to infer if they were consistent with adult differences due to intrasexual selection acting on the males. I hypothesized that, unlike breeding adults, juvenile males and females would not differ in their space use or social behavior patterns. In contrast, I hypothesized that juvenile males and females would differ in behavior patterns that could affect growth. Because larger adult males have more mating opportunities than do smaller adult males (Ruby, 1984; Jenssen and Nunez, 1998), I expected that, in comparison to juvenile

females, juvenile males would eat more frequently and spend more time actively foraging while minimizing movements unrelated to foraging, to maximize energy available for growth.

Methods

I collected data from an *A. carolinensis* population along the Augusta Canal, 12 km northwest of Augusta, Georgia, USA (latitude 33° N). This population has previously been used to describe the behavior of adult males and females (e.g., Jenssen et al., 1995; Nunez et al., 1997). During June-August of 1995 and 1996, I recorded the activities of 20 juvenile male and 17 juvenile female *A. carolinensis* either on videotape (15 males, 10 females) using a Quasar VM547 VHS-C video camera, or on checksheets (five males, seven females). Neither year nor recording method affected my results for any variable (Wilcoxon rank sum tests; all $P > 0.20$), so all data were combined for analysis. Each individual was observed for 30-60 min (mean $= 54.0$, $SE = 2.1$ min). All observations were made while I was ≥ 3 m from the subject to reduce potential observer effects, between 0800-1330 h when it was not raining, and when the air temperature was $< 35°$ C, above which activity is depressed (Wilson and Echternacht, 1990). As potential correlates with behavior, for each observation period I recorded temperature and sun exposure. Temperature was recorded every 15 min to the nearest 0.5° C using a shaded thermometer positioned 1 m off the ground. Sun exposure (the proportion of an observation period during which the sun was unobstructed by clouds) was estimated for the whole observation period, ranked 1-5, with $1 = \leq 25\%$, $2 = 26$-50%, $3 = 51$-75%, $4 = 76$-99%, and $5 = 100\%$ sun exposure. At the end of each observation period, I captured subjects by hand and sexed them using their postanal scales, which are larger in males than in females from hatching (Greenberg and Hake, 1990). I then measured snout-vent length (SVL) to the nearest mm and placed a unique pattern of non-toxic, waterproof acrylic paint on the back of each subject for short-term identification. Lizards were released where I captured them. I also used numbered strips of colored flagging tape to indicate where each individual was captured. Because the physiological capability to reproduce is attained at 42-45 mm SVL (Hamlett, 1952; Gordon, 1956; Michaud, 1990), only individuals ≤ 40 mm SVL were included in this study.

I examined a total of 11 variables as potential indicators of overall sex differences or sex-specific ontogenetic shifts in space use, social behavior patterns, and behavior patterns affecting growth. For space use, I recorded perch height, perch diameter, and home range volume. Perch height and perch diameter were ranked variables recorded every 15 min during each observation period. Perch height was recorded using a scale of 1-4 with $1 = \leq 0.5$ m, $2 = 0.6$-1.0 m, $3 = 1.1$-2.0 m, and $4 = > 2.0$ m. Perch diameter was recorded using a scale of 1-5 with $1 = \leq 5$ mm, $2 = 6$-15 mm, $3 = > 15$ mm, $4 =$ leaf (a substrate with no appreciable diameter), and $5 =$ ground. I estimated home range volume by cubing the longest axis of movement (x, y, or z) each individual covered during the observation period. Home range volume estimates were facilitated by using a 1 m vertical pole for y reference and meter tape placed along the ground for x and z reference (e.g., Jenssen et al., 1995).

For social behavior patterns, I recorded body color and body color shift rate (shifts per hour), nearest neighbor distance, display rate (number of headbobbing displays given per hour), and display context. *Anolis carolinensis* can shift body color between bright green and dark brown, and can maintain numerous intermediate color shades, depending on social as well as environmental circumstances (review in Cooper and Greenberg, 1992). Body color was recorded every 15 min and ranked on a scale of 1-3, with $1 =$ green, $2 =$ mottled green/brown, and $3 =$ brown. I recorded nearest neighbor distance as the minimum distance between the study lizard and any other lizard during an observation period. Distance estimates were facilitated by the 1 m vertical reference pole and the meter tape as described above. For determining display context, I adopted the following criteria for recording whether or not a display was directed towards another juvenile, directed towards an adult, or given in an unknown context: (1) the other lizard had to be within 3 m of the study lizard; (2) the other lizard had to be, as far as I could tell, visible to the study lizard; and (3) the study lizard had to display while facing the other lizard. When one or more of these conditions were not met, or when I saw no other lizards, display context was labeled as unknown.

As behavior patterns potentially affecting growth, I recorded percent of observation period spent moving (determined from videotaped observations only), feeding rate (feeding events per hour), and foraging mode. Foraging mode was categorized for each feeding event as

either "active", where individuals captured prey while moving, or "sit-and-wait", where individuals captured prey from a stationary position as the prey moved by them.

All descriptive statistics are reported as mean ± 1 SE, and hypothesis tests were nonparametric and two-sided with $\alpha = 0.05$. To ensure that each of the 11 variables above could be examined in tests of separate hypotheses without introducing a bias due to correlations between variables, I checked for significant correlations among all possible variable pairs. Following sequential Bonferroni adjustments to protect against group-wide type I errors (Rice, 1989), none of the variables were significantly correlated at the group-wide 0.05 significance level. I used chi-square tests for ranked data and Wilcoxon rank sum tests for continuous data to statistically address whether or not juvenile males and females differ as groups in any of the measured variables (objective 1). I used Theil-Sen regressions (a simple linear regression technique; Hollander and Wolfe, 1973) to statistically address whether or not juveniles showed sex-specific, size-related differences (objective 2). The independent variable used in all regressions was SVL, and the null hypothesis was that there was no relationship between the dependent variable and SVL (i.e., a slope equal to zero). When I found a statistical relationship between a particular dependent variable and SVL for either males or females, I then compared the slopes of the male and female regression lines to test whether they were different using Wilcoxon signed rank tests (Hollander and Wolfe, 1973). By testing slopes, I compared juvenile male and female ontogenetic trajectories (i.e., behavioral correlates SVL) to see if they were dimorphic. To consider ontogenetic effects on juvenile activities, I assumed that, within-sex, larger juveniles would tend to be older than smaller juveniles. For A. carolinensis, this assumption should generally hold, as mark-recapture (Gordon, 1956) and laboratory studies (Michaud, 1990) show growth to be continuous and linear throughout juvenile development.

Results

General and Climatic Variables

The distributions of SVLs of the 20 males and 17 females in this study did not statistically differ ($X^2_4 = 0.29$, $P = 0.96$). Males and females averaged 28.0 ± 1.0 mm (range 23-

17

39 mm) and 28.1 ± 0.9 mm (range 23-35 mm), respectively. During my observations, males and females were visually unobstructed by the habitat for $86 \pm 2.9\%$ and $90 \pm 2.2\%$ of the time, respectively ($W = 350.5$, $P = 0.37$). Time of day and air temperature were positively correlated during observation periods (Spearman's rank correlation; $r = 0.80$, $P < 0.005$). However, neither air temperature ($W = 425.5$, $P = 0.17$) nor sun exposure values ($W = 343$, $P = 0.22$) differed between males and females.

Space Use

Juvenile *A. carolinensis* used a small fraction of the physical habitat, although a large range of perch heights and diameters were available (Jenssen and Nunez, 1998). Juvenile males and females did not differ in their perch height distributions ($X^2_3 = 0.26$; $P = 0.96$). Thirty-three and 35% of the observations were at perch heights < 0.5 m, 39% and 40% at 0.6-1.0 m, 25% and 22% at 1.0-2.0 m, and 3% and 3% at > 2.0 m for males and females, respectively. Snout-vent length and perch height were positively correlated for juvenile males ($K = 62$, $P = 0.04$), but not for females ($K = 10$, $P = 0.62$). However, the slopes of the male and female regression lines were not different ($Z = 377$, $P = 0.32$).

Juvenile males and females also did not differ in their distributions of perch diameters ($X^2_4 = 0.91$, $P = 0.82$). Males and females used perches with diameters of ≤ 15 mm in 63% and 54% of the scan samples, respectively, and in only 2% and 4% of scan samples were they on perches with diameters > 15 mm. In the remaining 35% and 42% of the scan samples, respectively, males and females used substrates without easily definable diameters, predominantly individual leaves, but sometimes woody debris. Furthermore, neither sex showed a relationship between SVL and perch diameter ($K = 38$, $P = 0.22$; $K = 1$, $P = 0.97$, for males and females, respectively).

Juveniles remained in stable home ranges that they maintained within the larger territories defended by adults. I re-sighted 86% of juveniles where I first observed them, up to several weeks later (when presumably their paintmarks wore off). Mean home range volume for juvenile males and females was 13.7 ± 5.2 m^3 and 3.4 ± 0.9 m^3, respectively, but the sexes did

not differ in median home range volume (3.4 m^3 for each sex; W = 404.5, P = 0.46). Juvenile males showed significantly more variance in home range volume than juvenile females (Jackknife test; Q = -6.9, P < 0.005); the largest female home range volume was 8 m^3 (four out of 17 females), but five out of 20 males had territory volumes of $\geq$ 16 m^3. However, male and female home range distributions did not differ (X$^2_3$ = 6.1, P = 0.11). Home range volume was positively correlated with SVL for juvenile males (Fig. 1.1; K = 70, P = 0.02) but not for females (Fig. 1.1; K = 4, P = 0.80). Furthermore, the slopes of the male and female regressions statistically differed (Z = 3694, P = 0.04).

Social Behavior Patterns

Juvenile males and females did not differ in body coloration (X$^2_3$ = 0.35, P = 0.84). Juveniles were mottled green/brown during 41% and 39% of sightings for males and females, respectively, and brown 37% and 41% for males and females, respectively. Juveniles were green in the remaining 22% (males) and 20% (females) of scan samples. Although overall body color distributions did not differ intersexually, there was a significant relationship between SVL and body color for juvenile males (Fig. 1.2; K = -106, P < 0.005) but not females (Fig. 1.2; K = 2, P = 0.93). Larger males tended to spend more time green than smaller males, and this pattern differed from the female response as the slopes of the male and female regression lines were statistically different (Z = 5672; P = 0.03). Body color shift rates were similar (W = 360, P = 0.55) in juvenile males (1.1 $\pm$ 0.2 shifts/h) and females (1.4 $\pm$ 0.3 shifts/h). Furthermore, there was no correlation between SVL and sex in shift rate (males; K = -18, P = 0.56; females; K = 41, P = 0.09).

In four of 20 male and six of 17 female observation periods I failed to see another lizard within a 5 m radius of the study lizard. However, the statistical interpretation of male and female nearest neighbor distances did not change whether or not I included these observations, so they are not included in the descriptive statistics below. The nearest neighbor was another juvenile in 70% of the observation periods, and an adult in the remaining 30% of observation periods. There was no difference between juvenile males and juvenile females in whether their nearest neighbor was another juvenile (either a male or female, because I could not sex these individuals

from a distance), adult male, or adult female ($X^2_2 = 1.0$, $P = 0.60$). Mean nearest neighbor distance was similar ($W = 199$, $P = 0.22$) for juvenile males (0.8 ± 0.1 m) and females (1.1 ± 0.3 m). Furthermore, there was no relationship between nearest neighbor distance and SVL for either males ($K = -10$, $P = 0.65$) or females ($K = 5$, $P = 0.70$).

Juvenile male and female *A. carolinensis* gave headbobbing displays, sometimes including the display modifiers given by adults (e.g., dewlap extension, sagittal expansion, and lateral orientation; Jenssen, 1978). In all contexts combined, display rates of juvenile males (2.6 ± 0.7 displays/h) and females (3.2 ± 1.2 displays/h) were similar ($W = 384$, $P = 0.91$). Furthermore, there was no relationship between display rate and SVL for either sex (males; $K = -7$, $P = 0.82$; females; $K = 30$; $P = 0.22$). Juvenile males and females did not differ in the contexts in which displays were given ($X^2_2 = 3.7$, $P = 0.16$). Both males and females gave displays to other juveniles (43% and 30% of displays, respectively) as well as to adults (17% and 34% of displays, respectively). The social context for the remaining 40% and 36% of displays could not be determined. It is most likely that these displays with no apparent recipient were directed to nearby lizards that I failed to detect, rather than used in "assertion" contexts in which displays are broadcast to no specific receiver (DeCourcy and Jenssen, 1994). Juveniles in the laboratory that were isolated from other lizards did not give a single display in over 100 h of videotaped observations, even though these same juveniles frequently displayed when in visual contact with another lizard (M. Lovern, unpubl. data).

Behavior Patterns Affecting Growth

Juvenile males and females spent similar proportions of time moving (traveling or creeping; sensu Jenssen et al., 1995), averaging $47 \pm 4.3\%$ and $44 \pm 5.3\%$, respectively ($W = 184$, $P = 0.62$). Furthermore, there was a negative correlation for both sexes between percent movement and SVL (Fig. 1.3; males; $K = -42$, $P = 0.02$; females; $K = -24$, $P = 0.03$), although the slopes of these lines were not statistically different ($Z = 421$, $P = 0.17$).

Juvenile males fed 2.5 times as often as juvenile females (Fig. 1.4; $W = 434$, $P = 0.04$). Snout-vent length was not a good predictor of feeding rate for either males ($K = 16$, $P = 0.60$) or

females (K = -7, P = 0.82). Similarly, active foraging was more frequently observed in juvenile males (84% of observations) than in juvenile females (63% of observations) (X^2_1 = 4.96, P = 0.03).

Discussion

I hypothesized that juvenile males and females would not differ in space use and social behavior patterns. Sex differences in these traits in adults result directly from sexual selection acting on males (Ruby, 1984; Jenssen et al., 1995, Jenssen and Nunez, 1998), and furthermore these dimorphisms are only expressed during the breeding season (Jenssen et al., 1995). My results support this hypothesis in that juvenile males and females did not differ overall in perch height and diameter, home range volume, body color and shift rate, nearest neighbor distance, display rate, and the contexts in which displays were given. However, contrary to my expectations, males and females differed in their ontogenetic trajectories for home range volume and body color. Males increased home range volume and time spent green with body size, but females showed no change in either variable with body size. In contrast to my expectations for space use and social behavior patterns, I predicted that juvenile males and females would differ in behavior patterns that potentially affect growth. As expected, juvenile males ate more often and foraged actively more often than did juvenile females, regardless of body size. Unexpectedly, males and females were not different in the proportion of time they spent moving. In fact, both males and females showed a significant tendency to decrease the proportion of time they spent moving with increasing body size.

The fact that juvenile males both ate more frequently and foraged actively more often than juvenile females most clearly illustrates differences in the selection pressures acting on juvenile *A. carolinensis*. At hatching, juvenile males and females do not differ in either SVL or body mass, but males grow faster than females as they age (Gordon, 1956; Michaud, 1990). My results are consistent with the idea that greater food intake by juvenile males plays a role in their faster growth rates. However, both sexes must balance energy used for growth and that stored as lipids in fat bodies to be used for maintenance during the winter (Dessauer, 1955; Michaud and Echternacht, 1995), during which time they will eat little or not at all. My results that larger

juveniles, regardless of sex, decreased time spent moving and presumably increased the amount of energy available for storage are consistent with this idea. Furthermore, censuses during February and March of emerging sex and size classes reveal a 50:50 juvenile sex ratio (T. Jenssen and J. Congdon, unpubl. data), suggesting that males and females survive the winter equally well. Thus, the comparatively high juvenile male growth rates appear to be the result of sexual selection for large adult body size.

The few sex differences that arise ontogenetically probably further reveal different selection pressures acting on juvenile male and female *A. carolinensis*. Perhaps males occupy larger home ranges and spend more time green as a reflection of increased foraging activity and defense of foraging sites through development. By foraging over a larger home range, the amount of suitable prey encountered should increase. Territorial adult males in the field are primarily green (Jenssen et al., 1995), as are dominant males in laboratory cages (Greenberg et al., 1984). Similarly, green coloration in juveniles may be an important social signal that allows them to minimize home range overlap with neighboring juveniles. By minimizing overlap, juvenile males may further increase their growth rates. Stamps (1984) has shown that juvenile *Anolis aeneus* have reduced growth rates when they have overlapping home ranges.

If increased home range volume and amount of time spent green were related to feeding opportunities for juvenile male *A. carolinensis*, then why did I fail to observe more aggressive interactions (e.g., through increased display rates) in larger males over increasing home range overlap? My observations indicate that juveniles do not interact aggressively with adults (consistent with Jenssen et al., 1998) and only rarely with other juveniles (< 10% of display performances involved multiple displays, < 3% involved aggressive display modifiers and/or chases). If juvenile home range locations are stable, as my data suggest, neighbors should also be stable, and the need for active home range defense would then be reduced as neighbors would recognize one another and established boundaries (e.g., Stamps and Krishnan, 1997). Then, mutual avoidance, co-dominance among neighbors, or other subtle interactions that I failed to detect (e.g., as in *A. aeneus*; review in Stamps and Krishnan, 1998) could allow males to increase home range volume without increasing social conflict with neighbors.

In summary, I argue that the observed differences between juvenile male and female *A. carolinensis* result not from sex differences in immediate requirements (i.e., natural selection), but from sex differences in future requirements (i.e., sexual selection). Because of the relationship between male body size and reproductive success in adulthood (Ruby, 1984; Jenssen and Nunez, 1998), there appears to be selection on juvenile males to eat more and grow faster than juvenile females. Sex differences for increased home range volume and time spent green in males may indirectly relate to selection for fast male growth as behavioral mechanisms for increasing foraging area and social status among neighboring juveniles.

Acknowledgments

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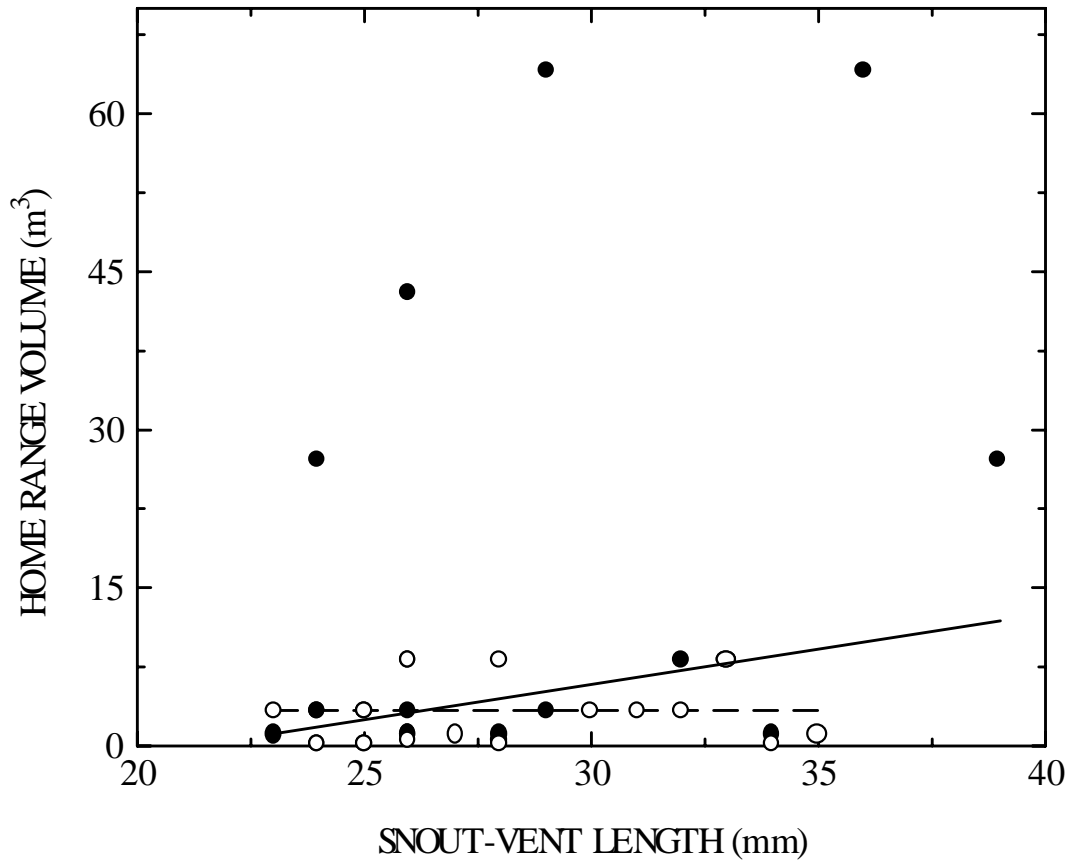
Figure 1.1. Regressions of home range volume vs. snout-vent length for 20 juvenile male (closed circles, solid line) and 17 juvenile female (open circles, dashed line) *Anolis carolinensis*. Theil-Sen regression equations: males, y = 0.67 x – 14.3; females, y = 3.38.
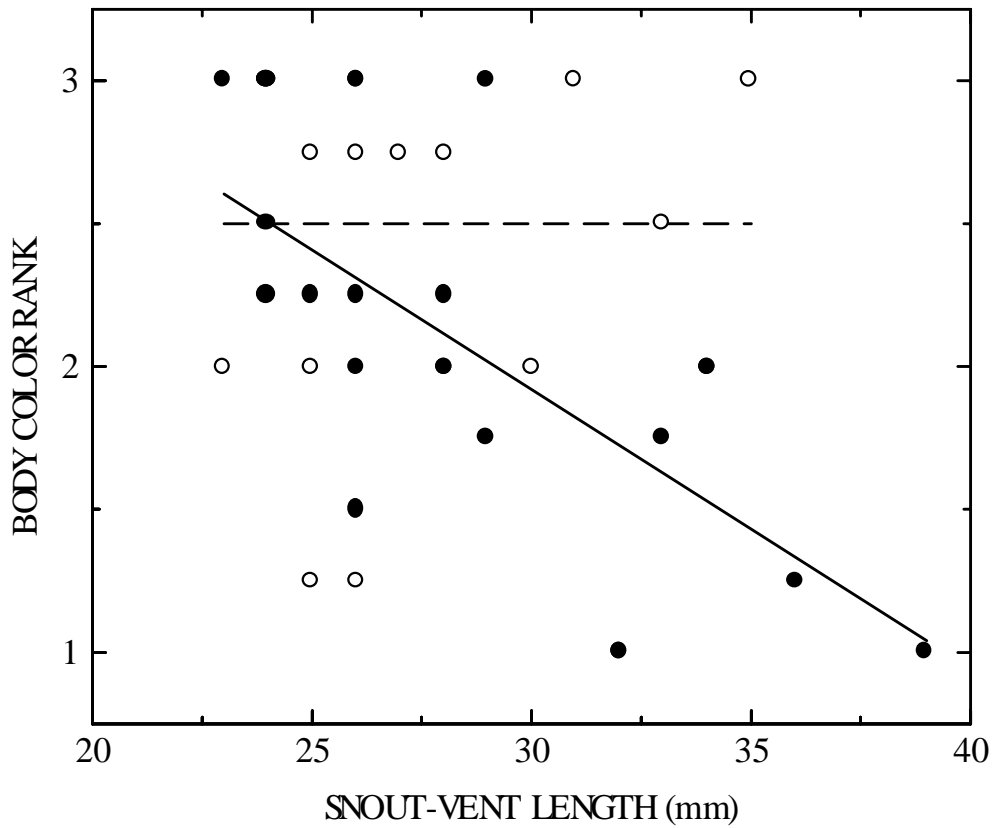
Figure 1.2. Regressions of mean ranked body color vs. snout-vent length for 20 juvenile male (closed circles, solid line) and 17 juvenile female (open circles, dashed line) *Anolis carolinensis*. Body color ranks are 1 = green, 2 = green/brown, 3 = brown. Theil-Sen regression equations: males, y = - 0.10 x + 5.0; females, y = 2.5.
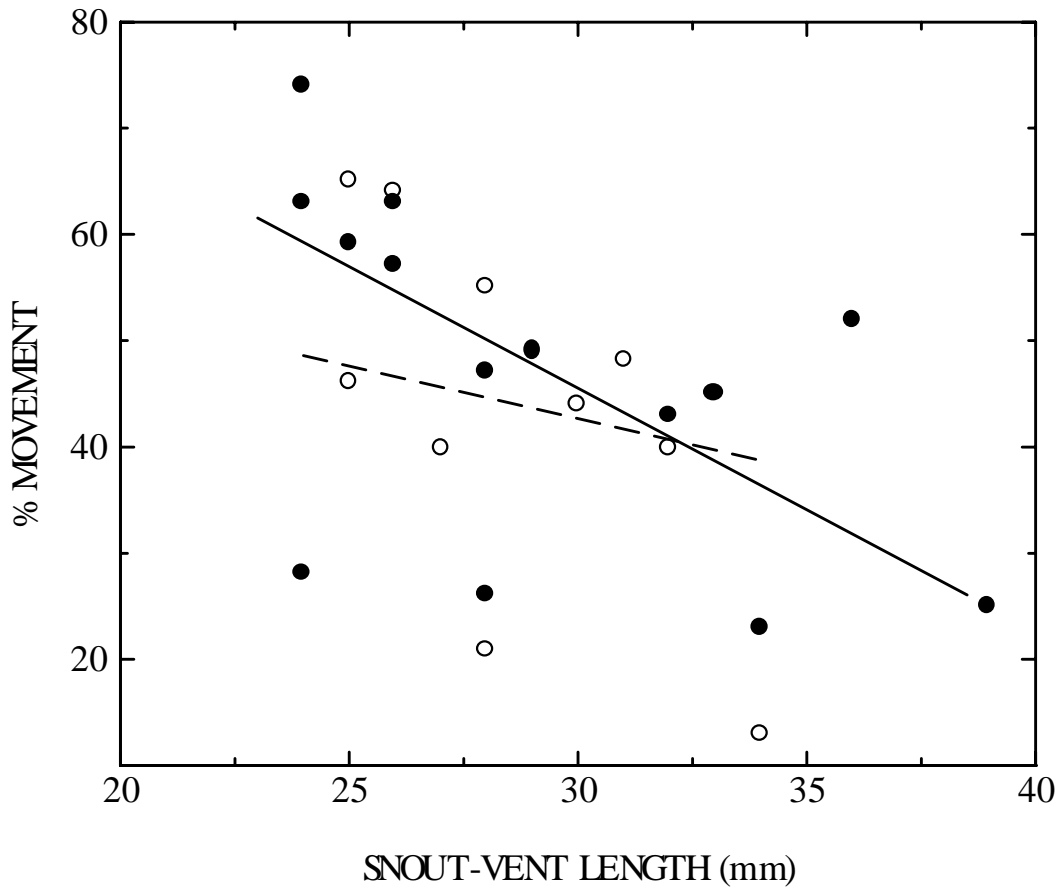
Figure 1.3. Regressions of percent of time spent moving vs. snout-vent length for 15 juvenile male (closed circles, solid line) and 10 juvenile female (open circles, dashed line) *Anolis carolinensis*. Theil-Sen regression equations: males, y = -2.4 x + 118; females, y = -3.57 x + 153.
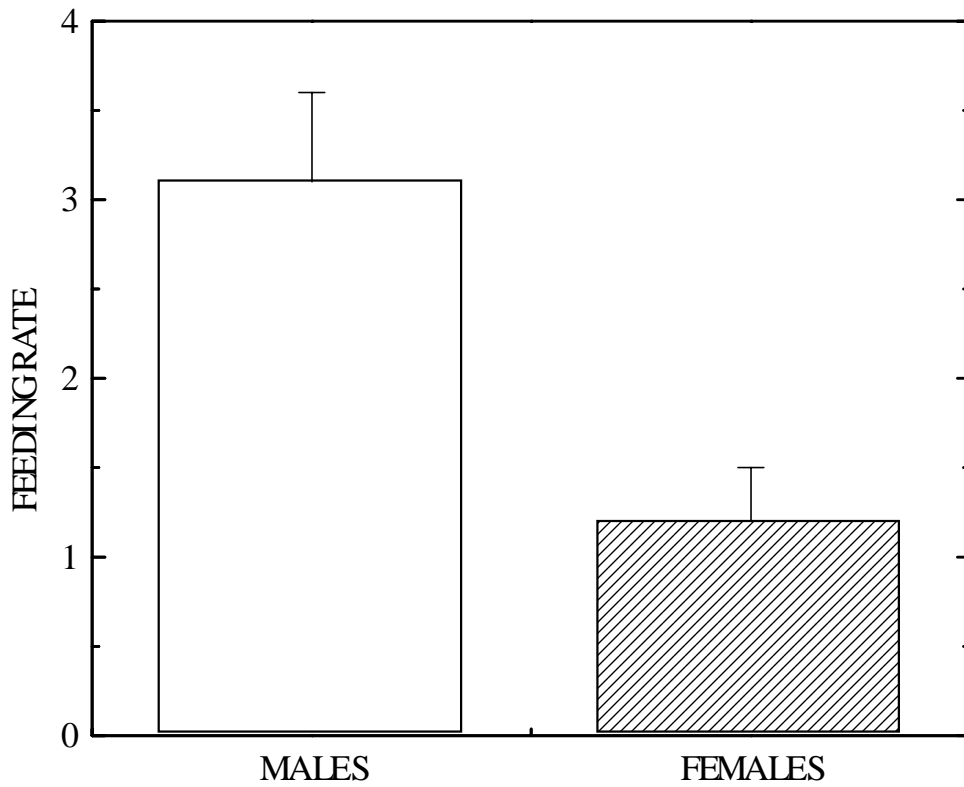
Figure 1.4. Mean (+ 1 SE) hourly feeding rate of 20 juvenile male (white bar) and 17 juvenile female (hatched bar) *Anolis carolinensis*.

Chapter 2

Form Emergence and Fixation in Social Signals: Ontogeny of Headbobbing Display Structure in Male and Female Green Anoles (*Anolis carolinensis*)

Abstract

Although the structure of adult *Anolis carolinensis* headbobbing displays has been well documented, the ontogeny of these displays has been anecdotal. Here I address the developmental issues of emergence (i.e., initial appearance), fixation (i.e., stereotypy), and sources of variation (age/size, sex, social context, and recording environment) of juvenile *A. carolinensis* headbobbing displays. From 1246 headbobbing displays analyzed from 114 juvenile lizards, I found that: (1) juveniles possess the same three A, B, and C display types found in adults; (2) C displays arise early during ontogeny while A and B displays emerge gradually; (3) A and B displays appear to differentiate from a common X display precursor that decreases in frequency as A and B displays increase in frequency; (4) sex, age or size, social context, and recording environment have no effect on display structure or stereotypy in juveniles; and (5) although juveniles and adults share the same A, B, and C display types, the temporal structure of their individual headbobs and pauses frequently significantly differed, as did their intra-individual display stereotypy, which was consistently higher in adults. Thus, my results suggest that the display types in the *A. carolinensis* repertoire emerge differentially with respect to ontogeny, show no stereotypic changes through ontogeny, but nevertheless that juvenile display type stereotypy is less than that of adults. In the Discussion, I consider the implications of these results for potential maturational processes taking place as juveniles transition into adulthood.

Introduction

The ontogeny of communication signals used in social interactions has received uneven attention across taxa. Few studies exist outside of the mammalian and avian literature, although the utility of comparative studies of signal ontogeny for understanding signal evolution and function is clear (Burghardt, 1977a, 1978, 1988; Groothuis, 1993a, b, 1994). This unevenness is partly because some taxa do not exhibit an extended period of signal development, as adult social signals (e.g., displays used in aggressive and sexual contexts) are not expressed until adulthood (e.g., anurans, Kiester, 1977; orthopterans, Otte, 1977). In contrast, studies on birds and mammals have revealed that development of their social signals is typically gradual. Songbirds go through an extended signal development period as juveniles, which includes stages for acquisition, storage, and practice of species-typical song patterns (Catchpole and Slater, 1995). Postural and vocal signals of adult gulls appear gradually during juvenile ontogeny, often arising in final adult form through the integration of independent juvenile motor patterns (Groothuis, 1989a, 1993a). Many juvenile mammals begin expressing behaviors, similar to the aggressive and sexual signals of adults, in the context of play, during which time the juvenile behaviors are variable and do not carry the functional consequences that they will in adulthood (Fagen, 1981, 1993; Walters, 1987; Thompson, 1998).

Studies conducted within the contexts of avian and mammalian life histories suggest the importance of an altricial juvenile stage, coupled with a social environment that allows for extended interactions between parents and offspring, among siblings, or among extended social groups, in the development of aggressive and sexual signals. Specifically, recurring salient factors in signal development include maturation, imitation, practice, social interaction, play, and motivation (Burghardt, 1977b; Groothuis, 1994). However, it does not necessarily follow that an avian- or mammalian-grade social organization is prerequisite for communication signals to develop via modification from environmental inputs (Burghardt, 1988; Groothuis, 1993a, b, 1994). Given the few studies on the ontogeny of aggressive and sexual signals in nonavian and nonmammalian species, the extent to which the factors listed above are generally important to signal development remains unclear (Groothuis, 1993b, 1994).

Groothuis (1993a) suggested five empirical questions related to the understanding of signal, or display, development. First, at what ontogenetic stage do adult display forms arise (i.e., form emergence)? Second, on what temporal scale do adult display forms become stereotyped (i.e., form fixation)? Third, how do juvenile motor patterns integrate into functional adult display patterns (e.g., from independent motor precursors derived from feeding or grooming, or from motor patterns used in juvenile displays)? Fourth, when does display form take on the adult display meaning? Fifth, what selective pressures shape display form and function during ontogeny? Answers to these questions will provide a framework for understanding the evolution and development of display structure and function. In particular, a comparative approach to display development will reveal whether the displays of many birds and mammals results from novel, or instead widely shared, developmental mechanisms (Groothuis, 1993b). Therefore, studies of display ontogeny in reptiles can be useful. Reptiles represent the ancestral lineage which gave rise to birds and mammals, and they express a variety of social and communication systems related to species differences in ecology and life history (reviewed in Burghardt, 1977a, 1978, 1988; Pough et al., 1998). Among reptiles, lizards appear well-suited for analyses of display ontogeny, as juveniles in numerous species express communication signals similar to those used by adults in aggressive and sexual contexts (e.g., Cooper, 1971; Burghardt et al., 1977; Stamps, 1978; Roggenbuck and Jenssen, 1986; Phillips et al., 1993).

In this study, I addressed the first two of Groothuis' (1993a) questions, display emergence and display fixation, using the green anole lizard, *Anolis carolinensis*. Adult males and females mediate all of their social interactions via a shared repertoire of three stereotyped headbobbing patterns, labeled A, B, and C (DeCourcy and Jenssen, 1994; Lovern et al., 1999; Jenssen et al., 2000). The temporal cadence of these communication displays varies neither by sex nor by the social context in which displays are given (e.g., aggressive, courtship contexts), although display rates and use across contexts can vary considerably (DeCourcy and Jenssen, 1994; Lovern et al., 1999; Jenssen et al., 2000). Juvenile *A. carolinensis* also exhibit headbobbing displays (Cooper, 1971; Greenberg and Hake, 1990; Lovern, 2000), but display structure, function, and development have not been studied. The only two studies, to my knowledge, that quantify display development in other lizards suggest that juveniles and adults have at least some display types in common, and that these display types arise and become fixed

early in ontogeny (*Anolis aeneus*, Stamps, 1978; *Sceloporus undulatus*, Roggenbuck and Jenssen, 1986).

My specific objectives were: (1) to document the temporal cadence of juvenile *A. carolinensis* headbobbing displays; (2) to determine the effects of sex, age or size, social context, and recording environment on when displays arise and when they become stereotyped; and (3) to compare juvenile display structure to the structure of previously described adult displays. These data were used to examine the possible roles of maturation, imitation, practice, and social interaction in the emergence and fixation of adult-typical displays. If maturation (e.g., neuromuscular development and motor control, somatic growth) is an important factor in display development, then several ontogenetic patterns are possible. Different display patterns could emerge at different ontogenetic stages, intra-individual stereotypy could increase through ontogeny, gradually approaching that of adults, or display characteristics affecting display duration could show trends with ontogenetic stage (e.g., as a simple physical relationship, headbobs could increase in duration with body size). Such effects should relate to age or size, regardless of sex, social context, or recording environment, if causally related to maturation. If imitation of other displaying lizards is important to development, then lizards reared socially should converge faster on common display cadences than lizards with less opportunities for social interaction. Such an effect would be manifested in inter-individual stereotypy, which should be higher for social groups than for lizards reared separately. If practice is important, then individuals with the opportunity for more social interaction (e.g., those from the field or reared socially in the laboratory) should have higher intra-individual display stereotypy than individuals reared in isolation, because juveniles reared in isolation do not give spontaneous displays (Lovern, unpubl. data). If social interaction is important, then display structure might differ depending on the sex of interactants. This pattern could arise, for example, if males or females consistently react differently to displays from other lizards. Thus, if social interaction is an important input, display structure should differ depending on the social context in which displays are given.

Methods

I analyzed 1246 headbobbing displays from 114 juvenile (65 male and 49 female) *A. carolinensis* from a population along the Augusta Canal near Augusta, Georgia, USA. Displays came from individuals under the following conditions: (1) unmanipulated; observed in the field; (2) reared from hatching in the laboratory; and (3) captured in the field and housed in the laboratory. In addition, 304 displays from 30 adult (eight male and 22 female) *A. carolinensis* was reanalyzed from Jenssen et al. (2000) for comparison to the juvenile displays in the present study.

For the field sample, free-ranging juveniles were observed on the Augusta Canal field site. I videotaped displaying individuals using a Quasar VM547 VHS-C video camera from a minimum distance of 3 m. Each individual was followed for 30-60 min, after which time they were captured and sexed by postanal scale size (males have two enlarged postanal scales, females do not), and their snout-vent length (SVL) was measured to the nearest mm. From the videotapes, nine males and six females gave 34 and 28 displays, respectively, suitable for temporal structure analysis (i.e., full display recorded on videotape, displaying lizard unobstructed by the habitat, at appropriate angle to camera, and in focus).

For the laboratory-reared sample, 16 gravid females were captured from the field and housed in the laboratory. Females were maintained in groups of four along with an adult male under conditions conducive to egg-laying. Oviposited eggs were individually placed in small plastic cups containing a 50:50 (mass) vermiculite:water mixture. All cups were covered with plastic wrap and a rubber band, and were incubated at temperatures from 24-30 C on a diel cycle. Hatching success was 89%. Upon hatching, individuals were sexed, measured for SVL to the nearest mm, and toe-clipped for permanent identification. I randomly assigned hatchlings to be reared in isolation or in groups. Those housed in isolation were placed into cages measuring 120 x 60 x 60 cm divided into fourths with opaque partitions, and those housed in groups were placed into groups of four into cages of the same size, but without the partitions. I exposed the lizards to a 14:10 h light:dark cycle using four 40W full-spectrum bulbs (Durotest Vita-Lite Plus) placed on the top of each cage. Cage temperature ranged from 27-34 C during the day and dropped to

23 C at night. Cages were equally furnished with several elevated wooden dowels for perching and numerous pieces of artificial vegetation. Lizards were watered and fed daily with vitamin-dusted crickets, mealworms, and flour beetle and waxworm larva.

Laboratory-reared lizards were videotaped at known ages while they were alone and during staged pairwise interactions with other lizards, to provide a range of stimuli for eliciting displays. At age classes of 2-5, 12-16, 28-32, and 85-95 d, each lizard was moved to observation cages set up identically to housing cages, and placed adjacent to two randomly assigned juveniles from the same rearing treatment and in the same age class. All lizards were divided by opaque, removable partitions. At 16-24 h after moving them to the observation cages, individuals were videotaped for 30 min alone and then 30 min in pairwise interactions, with one of the randomly assigned interactants, after removing a partition. I videotaped all interactions 1-7 h from the onset of the light phase from a blind using a Panasonic AG 460 video camera fitted with an Aztec video telephoto converter (2.0X). The following day this procedure was repeated with the second randomly assigned interactant. Thus, each individual was videotaped twice within each age class, but never with the same interacting lizard. After interactions, individuals were returned to their original housing cages. From the videotapes, I analyzed 180 and 123 displays from 16 males and 13 females, respectively, that were suitable for temporal structure analysis.

For the field-captured sample, juveniles were collected in the field and brought into the laboratory. All individuals were housed singly in the lab and videotaped within 14 d of capture using the same protocol as described above. Interactants were size-matched within 2 mm SVL. From the videotapes, I analyzed 105 and 82 displays from 24 males and 20 females, respectively, that were suitable for temporal structure analysis.

A total of 552 displays from 88 juveniles (49 males and 39 females) was analyzed frame-by-frame (30 frames/s) with a Panasonic AG 1950 VCR. I used the Display Action Pattern (DAP) graph method (Carpenter and Grubitz, 1961; Jenssen, 1978), which plots vertical amplitude of lizard head position and dewlap extension (y-axis) over elapsed time (x-axis) for each display. Displays were divided into naturally occurring units consisting of headbobs and

inter-bob pauses (odd- and even-numbered units, respectively). The duration of the units defined the cadence of the display, and displays of a common cadence were categorized as being of the same display type (Jenssen, 1977, 1978). For each DAP-graphed display, I recorded display type, sex, size (also age when known), social context (consexual, heterosexual, or unknown), recording condition (field observed, lab-reared in isolation, lab-reared in group, or field caught), total number of headbobs, whether dewlap extension occurred, and unit and total display durations to the nearest 0.033 s (the resolution of the VCR). There were also 694 videotaped displays that were not DAP-graphed. For these displays, I recorded the same information as above with the exception of unit and display duration. Repeatability of display typing is 100% among observers in our laboratory, and out of 12 displays sampled twice, 96% (92 out of 96) of the units were judged to be of identical durations, and the remaining 4% (4 out of 96) were within one frame (0.033 s).

I used a nearest neighbor discriminant analysis to confirm the accuracy of my visual classification of display types. Subsequent statistical comparisons were performed within displays of the same type among homologous units. I calculated unit duration, total display duration, and number of headbobs per display using intra-subject means to eliminate a sample bias due to different numbers of displays from each lizard. I also calculated the coefficient of variation, or CV (SD/mean x 100) for display unit durations for each lizard separately (intra-individual; yielding a CV for each display unit for each lizard) and for whole classes of lizards (inter-individual; yielding a single CV for each display unit) as measures of individual and population display stereotypy, respectively (Barlow, 1977). Comparatively large CVs indicate units with comparatively low stereotypy. To test for differences among groups, I used analyses of variance (ANOVAs) when the data met the assumptions of normality (Kolmogorov-Smirnov one-sample tests) and homogeneity of variance (Levene's tests), and I used non-parametric Kruskal-Wallis tests when either assumption was not satisfied. To protect against type I errors, I used sequential Bonferroni adjustments to determine whether P-values from individual statistical tests within a group (i.e., sets of P-values from tests addressing a common hypothesis, such as the set of P-values for the unit durations within a display type) indicated significant differences at the group-wide level (Rice, 1989). Minitab 10Xtra (1995) was used for all statistical tests, which were two-tailed with an overall $\alpha = 0.05$.

Results

<u>Display</u> <u>Description</u>

The 1246 displays were visually assigned to four categories. Three of these categories constituted display types identical to those described in DeCourcy and Jenssen (1994), Lovern et al. (1999) and Jenssen et al. (2000) for adult male and female *A. carolinensis*. Therefore, I followed the established convention of labeling these three display types as A, B, and C (Fig. 2.1). Overall, I DAP-graphed 79 type A, 50 type B, and 393 type C displays from the juvenile males and females observed in the field, reared in the laboratory, or captured in the field and observed in the laboratory (Table 2.1). I observed that lizards were capable of displaying on the day they hatched, as reported by Cooper (1971) and Greenberg and Hake (1990). Furthermore, each display type could be given with or without extension of the dewlap by both males and females, as has been reported for adults (DeCourcy and Jenssen, 1994; Lovern et al., 1999; Jenssen et al., 2000). Because type C displays only have six units, I used the first six units of each display type in a nearest neighbor discriminant analysis (results in a conservative analysis of discrimination, as the potential bias of comparing categories of different lengths is removed) to confirm my visual classifications of DAP-graphed displays. There was agreement on 492 of 522 (94%) displays (95, 98, and 94% for types A, B, and C, respectively).

In addition to the three display types shared by juvenile and adult *A. carolinensis*, juveniles exhibited a fourth category of displays that had considerable structural overlap with the discrete cadences of the A, B, and C display types. Because these displays lacked a discrete cadence, I have labeled them as X displays (Table 2.1). The X displays were the rarest in occurrence, accounting for only 48 (4%) of the 1246 displays observed, of which 30 were DAP-graphed (Table 2.1). Individuals of both sexes and from both the field and laboratory exhibited X displays, which occurred when either the first or second headbob within the display contained a dip in head amplitude, before rising again to complete the headbob (Fig. 2.2). As the motion of the head was continuous throughout the headbob, the head dip appeared indicative of an incipient pause. The headbob with the dip was always higher in amplitude and longer in

duration than any other headbobs within the same display. The remaining two headbobs that always occurred in X displays were intermediate in duration to the final two headbobs in display types A and B. Not surprisingly, therefore, nearest neighbor discriminant analysis showed low agreement with my classification of type X displays, agreeing with only 4 of 30 displays (13%).

Display Emergence

The emergence of the A, B, C, and X displays occurred asynchronously during ontogeny. I assigned the 114 juvenile lizards of this study to one of five size classes, from smallest to largest, and subsequently examined their relative frequencies of A, B, C, and X displays (Fig. 2.3). The type C display was the most common display type given at any size, beginning at 90% of the displays for the smallest size class (1) and steadily decreasing to 72% of the displays for the largest size class (5). This decrease in the relative frequency of the type C display corresponded with increasing use of display types A and B, which collectively accounted for 1% of displays in size class 1, but 27% of displays in size class 5. X displays were more common than display types A and B in size classes 1 and 2 (9 and 10%, respectively), but they declined in relative frequency in size classes 3-5 (3, 1, and 1%, respectively), as display types A and B were increasing in relative frequency. When considering only the laboratory-reared lizards, for whom ages were known, the relative frequencies of X displays by age class mirrored the pattern described for size class. X displays were most common in individuals < 14 d old (13% of displays), after which they dropped in frequency at 15-30 d (9%) and still more at > 30 d (3%).

The structural overlap of X displays with the discrete A, B, and C displays, coupled with the different display emergence patterns, suggest that X displays may be precursors of display types A and B. Individual display frequencies through ontogeny further support this idea. No lizard that gave an X display also gave a type A or B display during the same observation period, although nearly all of them (85%) gave type C displays. Furthermore, nine of 13 laboratory-reared lizards gave X displays early in ontogeny, but were later observed to give types A and/or B, without X. Three of the four remaining lizards continued to give X displays for the duration of the study, and one was observed to give several type B displays, but subsequently gave some additional X displays.

<u>Display</u> <u>Fixation</u>

For A, B, and X displays, I pooled across the size classes and recording conditions above to achieve a sufficient sample size for statistical analysis. For size class, individuals were scored as 1 ($\leq$ 30 mm SVL) or 2 (> 30 mm SVL). For recording condition, individuals were scored as 1 (field-hatched) or 2 (laboratory-hatched). No individual performed A, B, or X displays at more than one age or in more than one social context, so each observation used in the analyses was independent. General linear model ANOVAs (GLMs) indicated that there were no significant sex, size, context, or recording condition effects on any of the 10 unit durations for the A displays, on any of the eight unit durations for the B displays, nor on any of the eight unit durations for the X displays (all $P > 0.05$). Furthermore, total display duration as well as total number of headbobs per display were unaffected by sex, size, context, or recording condition for A, B, and X displays (GLMs; all $P > 0.05$). Intra-individual display unit stereotypy did not differ by any of the treatment effects for A, B, and X displays (GLMs; all $P > 0.05$). Similarly, inter-individual stereotypy did not differ, as there were no tendencies for display unit CVs grouped by sex, size, context, or rearing environment to be consistently high or low from one group to another.

Because C displays were much more frequent than A, B, or X displays, I was able to examine potential effects on their structure and stereotypy in more detail. Once again, however, there were no significant sex or recording condition effects on any of the six individual unit durations, on overall display duration, nor on total number of headbobs per display (GLMs; all $P > 0.05$). Inter-individual stereotypy showed no consistent effects due to sex or recording condition. Similarly, intra-individual stereotypy did not differ by sex for any unit of the type C display (GLMs; all $P > 0.05$), although it did differ by recording condition for unit 6 ($F_{3,60} = 7.75$; $P < 0.0005$). The CV for unit 6 of individuals reared in the laboratory in groups (43.5%) was significantly higher than the CVs for individuals recorded in the field (20.0%), reared in the laboratory in isolation (27.9%), and captured in the field and recorded in the laboratory (19.6%).

Numerous (N = 17) lizards gave the type C display in both consexual (male-male or female-female) and heterosexual (male-female) contexts, or at a minimum of three known ages (N = 10). Therefore, I examined potential differences due to social context or age by repeated-measures ANOVAs. I used individuals as the random effect and context or age as the fixed effect. Age for each individual was scored as 1 ($\leq$ 7 d), 2 (8-30 d), or 3 (> 30 d). For the remainder of the data set, in which individuals are represented in the sample only once, a GLM was used as above to test for context and size effects. Size was scored for each individual as 1 ($\leq$ 25 mm), 2 (26-30 mm), 3 (31-35 mm), or 4 (> 35 mm). Both analyses indicated that neither context nor age (or size) had an effect on unit duration, total display duration, or the number of headbobs in a display (all $P > 0.05$). Furthermore, intra-individual stereotypy of the type C display units did not differ between contexts or among ages or sizes (repeated measures ANOVA, GLMs; all $P > 0.05$). Finally, inter-individual stereotypy for the type C display units showed no consistent differences resulting from context, age, or size.

I used a nested ANOVA to determine the relative contributions of sex, size, social context, rearing environment, and among- and within-individual differences to the total variance in each display unit (Table 2). As would be expected from the analyses above, sex, size, context, and recording condition accounted for very little of the total variance (0.1, 5.7, 6.8, and 13.3% of the display variance, respectively). The greatest proportion of total variance came from among- and within-individual contributions, at 31.9 and 42.2%, respectively.

Comparison of Juvenile and Adult Display Structure

Juvenile display types A, B, and C were each compared to adult A, B, and C display types. Because juveniles and adults did not show homogeneity of variance for many of the unit durations (nine of the total of 24 display units exhibited heteroscedasticity; Levene's tests, $P < 0.05$), all comparisons were made using non-parametric Kruskal-Wallis tests. Unlike comparisons among juveniles, durations for three out of 10 units from the type A display, three out of eight units from the type B display, and five out of six units from the type C display were significantly different between juveniles and adults (Table 2.3). Furthermore, total display duration significantly differed between juveniles and adults for display types A and B, although

not for type C (Table 2.3). When examining the direction of difference, nine of 12 headbobs were shorter and 11 of 12 pauses were longer for juveniles than for adults (Table 2.3). The direction of difference is suggestive of a non-random deviation for headbobs (Chi-square test; $X^2_2 = 3.0$, $P = 0.08$), and fully supports it for pauses (Chi-square test; $X^2_2 = 8.3$, $P = 0.004$).

Inter-individual stereotypy, as a measure of population stereotypy, did not consistently differ between the temporal structures of juvenile and adult displays. Thus, the range of variation in display unit durations found among juveniles was not consistently greater or less than that found among adults. However, intra-individual stereotypy differed dramatically between juveniles and adults. The CVs for seven out of 10 type A units, two out of eight type B units, and one out of six type C units were significantly different between juveniles and adults (Table 2.4). The CVs for total display duration were also significantly different between juveniles and adults for display types A and B (Table 2.4). In every case where CVs were significantly different, the CV was higher for juveniles than for adults, indicating less intra-individual stereotypy for juveniles. Furthermore, for 21 out of the total 24 display units of the three display types, and for all three total display durations, the CVs were larger for juveniles than for adults, which is much more frequently than would be expected by chance (Chi-square test; $X^2_1 = 16.3$, $P < 0.0001$).

Discussion

I found no evidence that imitation, practice, and social interaction had any effects on the development of temporal display structure in *A. carolinensis*. The emergence and fixation of displays (when they arose and when they became stereotyped) followed a common trajectory for the juveniles in this study, regardless of their sex, recording condition, or the social contexts for displays. Display unit durations showed no tendency to get longer or shorter with size or age, regardless of sex, recording condition, or social context. Intra- and inter-individual stereotypy were not affected by the sex of the lizard, its recording condition, nor the social context in which displays were given. If imitation or practice were important to display development, inter- or intra-individual stereotypy, respectively, should have been higher in lizards housed socially in the laboratory, or observed in the field under natural conditions, than in lizards housed in

isolation in the laboratory. If experience from social interactions shaped display structure, then displays recorded from different social contexts should have differed in structure or stereotypy.

My data suggest that maturation (i.e., an age or size related effect) is an important factor in some aspects of the development of display structure in juveniles. Within the juvenile size class, I found no evidence suggesting that display stereotypy improves with age or size. Rather, the evidence for maturational effects comes from the relative timing of display type emergence and from comparisons of display structure between juveniles and adults. Juveniles exhibited display type C throughout ontogeny, while display types A and B gradually emerged. X displays appeared to be undifferentiated A/B precursors, or at least hybridized attempts at A or B displays, given their structural similarity to A and B displays and that their relative frequency dropped considerably as types A and B became more common. All but one lizard ceased giving X displays after giving display types A and B. However, X displays do not completely disappear from the population, but rather remain present at approximately 1 out of every 250 displays given by adults (Jenssen, unpubl. data). If the X display is a precursor to or hybrid of display types A and B, then maturation is indicated as influencing the emergence or separation of the two display types, not in the fine adjustment of a display type once it is present. Similarly, Roggenbuck and Jenssen (1986) found an undifferentiated display pattern in the very early ontogeny of *S. undulatus*, and they reported that this pattern resulted from an incomplete separation of introductory headbobs of the species-typical adult patterns. However, the undifferentiated display pattern observed for *S. undulatus* disappeared within a week of hatching, whereas the type X display that I observed for *A. carolinensis* remained relatively common in the population for at least a month after hatching, and persisted in some individuals over the entire study. Comparisons between juvenile and adult display structure also suggest maturation. Nearly half (11 out of 24) of the display units for display types A, B, and C had significantly different mean durations for juveniles and adults, and adults showed higher stereotypy for nearly every unit of each display type. Thus, the fine temporal structure of these shared display types, and display stereotypy, often differed. Furthermore, when considering the direction of difference between juveniles and adults, juveniles tended to have shorter headbobs and longer pauses than adults, indicating a relationship between body size and display unit duration.

I interpret these results as evidence for maturation, although by itself such a label explains little about underlying mechanisms (Burghardt, 1977b; Groothuis, 1994). One possible factor that may affect both when certain display types emerge and the precision of those display types is motivational state. I was unable to find any external environmental or social factors that play a role in the emergence and fixation of displays, however internal cues such as hormone status may still be important. Physiological factors can also initiate or maintain the performance of numerous behaviors by acting on the neuromuscular mechanisms already in place (e.g., regulation of sexual behaviors by sex steroids, Cooke et al., 1998), and this is no less true for communication behaviors (Groothuis, 1993b, 1994). Thus, changes in the physiology (e.g., circulating steroids) of juveniles during ontogeny might affect display type emergence. Furthermore, physiological differences between juveniles and reproductive adults, coupled with external social influences related to territoriality and courtship (present only in adults), might lead to the differences in display stereotypy that I found. In support of this possibility, relationships between sex steroid concentrations and the production and frequency of communication signals have been shown in many species (e.g., reviews in Ketterson and Nolan, 1992; Moore and Lindzey, 1992; Whittier and Tokarz, 1992), including *A. carolinensis* (Winkler and Wade, 1998).

Some potential effects on the development of headbobbing display structure in *A. carolinensis* are more likely than others, given its life history and mating system. *Anolis carolinensis* is polygynous; females settle in comparatively small territories and males attempt to establish territories that overlap those of females (Ruby, 1984; Jenssen et al., 1995; Nunez et al., 1997; Jenssen and Nunez, 1998). Females lay single-egg clutches at weekly intervals over a four month breeding season (Ruby, 1984; Andrews, 1985), thus setting up an endurance rivalry (Andersson, 1994) among males to maintain reproductive condition and to continue defending their territories against other males. Within this social system, there is no parental care by either sex, and juveniles are therefore immediately responsible for meeting their survival needs (Gordon, 1956; Stamps, 1978). Unlike some lizards (e.g., iguanas; Burghardt et al., 1977), juvenile *Anolis* do not socially aggregate, and thus must individually locate suitable habitat for foraging, predator avoidance, and shelter (e.g., Lovern, 2000). Therefore, juveniles require functional signals to mediate their social interactions, which primarily include aggressive

interactions over habitat use (e.g., Stamps, 1978, 1983; Lovern, 2000), but they are not likely to have many opportunities for learning and modifying these signals by processes such as imitation and practice. Furthermore, the social interactions themselves are not likely to result in signal modification because they are relatively infrequent and because the requirements of juvenile males and females do not differ (Lovern, 2000), making reinforcement of signal differences depending on the sex of the lizards unnecessary. However, a maturational component to display ontogeny remains possible, because the range of social contexts and fitness consequences of interactions experienced by juveniles is reduced compared to adults, as juvenile *A. carolinensis* are not reproductive and do not maintain exclusive territories (Lovern, 2000).

Overall, the development of headbobbing display structure in *A. carolinensis* is comparatively refractory to obvious environmental inputs, in contrast to the development of many social signals in birds and mammals (e.g., Groothuis 1993b). This difference in signal ontogeny primarily arises from differences in life history and the resulting requirements for survival faced by precocial juveniles and buffered for altricial juveniles. However, among the few studies thus far on signal development in reptiles, my results suggest a comparatively extended developmental period of headbobbing display emergence and fixation, which might be well-suited for several avenues of future research. First, are there motor constraints to the performance of certain display types early in ontogeny, or rather are there underlying physiological changes which adjust the thresholds of producing certain display types? Studies examining brain and peripheral neuromuscular development, especially those circuits in control of the head and dewlap, as well as studies on underlying hormone profiles during ontogeny, could help tease apart these possibilities. Second, can the pattern of display type emergence during ontogeny in *A. carolinensis* yield any information with respect to the evolution of display types? Ontogenetic trajectories may reflect phylogenetic history for particular traits when modifications to ancestral traits are terminal (Gould, 1977). Thus, evolution could occur through an increase in the number of display types within a species-specific repertoire, or perhaps through subtle changes in the cadence of ancestral display patterns. Documenting the display type structure, and its ontogeny, for anoles closely related to *A. carolinensis* could reveal such evolutionary phenomena. Similar studies have documented relationships between ontogeny and phylogeny for song in a lineage of sparrows (Irwin, 1988) and for social displays in closely

related gulls (Groothuis, 1989b). Third, does the meaning conveyed by displays differ between juveniles and adults? My results document that juvenile and adult *A. carolinensis* have three display types – A, B, and C – in common. Adults use these display types in contexts of territorial aggression (males and females), advertisement (males), and courtship (males and females), all of which are social contexts not experienced by juveniles. Therefore, at least by the time that individuals are physiologically capable of reproduction, both the intent in display production and the interpretation of displays could change. Such changes in signal meaning may arise through several inter-related factors, such as underlying physiological changes, learning through social experience, and developing sex recognition.

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Table 2.1. Sample sizes of displaying lizards and headbobbing displays (in parentheses) used to analyze temporal display structure and stereotypy in juvenile *Anolis carolinensis*, by recording condition, sex (sample size of lizards), and display pattern.

Recording condition	Display pattern			
Sex	A	B	C	X
Field				
Males (9)	1 (2)	0 (0)	7 (29)	1 (3)
Females (6)	0 (0)	1 (1)	5 (27)	0 (0)
Lab-reared				
Males (16)	7 (14)	2 (2)	16 (150)	6 (14)
Females (13)	5 (20)	3 (16)	12 (81)	2 (6)
Field-captured				
Males (24)	9 (30)	8 (22)	20 (49)	1 (4)
Females (20)	6 (13)	4 (9)	17 (57)	2 (3)

Table 2.2. Proportion of variance in intra-display unit durations from 79 type A, 50 type B, and 393 type C headbobbing displays recorded from 88 juvenile *Anolis carolinensis* attributed to sex, size, context, recording condition (RC), among-individual (A-I), and within-individual (W-I) components of a nested ANOVA.

Display type	Variance source (%)					
Unit	Sex	Size	Context	RC	A-I	W-I
Type A						
1	0.0	7.8	8.6	0.0	10.0	73.6
2	0.0	14.7	0.0	0.1	24.7	60.5
3	0.0	17.8	0.0	31.1	11.0	40.1
4	0.0	8.3	1.8	0.0	29.9	60.0
5	0.0	0.0	0.0	47.8	18.7	33.5
6	0.0	37.1	3.0	0.0	17.0	42.9
7	0.7	0.0	15.7	0.0	31.2	52.4
8	0.0	0.0	3.2	50.1	4.4	42.3
9	0.0	0.0	0.0	55.7	4.3	40.0
10	0.0	6.0	0.0	12.8	29.0	52.2
Average	0.1	9.2	3.2	19.8	18.0	49.7
Type B						
1	0.0	3.6	7.6	0.0	53.1	35.7
2	0.0	20.4	13.4	0.0	49.3	16.9
5	0.0	0.0	30.3	0.0	62.2	7.5
6	0.0	0.0	0.0	63.9	31.8	4.3
7	0.0	0.0	31.6	0.0	6.4	62.0
8	0.0	0.0	42.8	1.7	44.9	10.6
9	0.0	0.6	0.0	26.3	32.9	40.2
10	0.0	4.1	0.8	0.0	77.6	17.5
Average	0.0	3.6	15.8	11.5	44.8	24.3
Type C						
5	0.6	1.6	0.0	2.0	58.0	37.8
6	2.0	0.6	0.0	2.9	48.6	45.9
7	0.0	1.8	3.4	0.0	26.2	68.6
8	0.0	10.4	0.0	10.1	28.3	51.2
9	0.0	2.4	0.0	4.6	13.8	79.2
10	0.0	0.2	0.0	9.5	52.2	38.1
Average	0.4	2.8	0.6	4.9	37.8	53.5
Overall Average	0.1	5.7	6.8	13.3	31.9	42.2

Table 2.3. Overall mean, SE, and results from Kruskal-Wallis tests for differences between juvenile and adult *Anolis carolinensis* display type A, B, and C unit durations. Intra-subject means were used to eliminate sample biases, and sample sizes of lizards are given for each display type. Adult data are from Jenssen et al. (2000). P-values marked with an asterisk (*) indicate a significant effect following sequential Bonferroni adjustments.

Display type Unit	Juvenile mean (s)	SE	Adult mean (s)	SE	H_1	P
Type A	N = 28		N = 14			
1	0.176	0.005	0.202	0.008	8.53	0.004*
2	0.117	0.007	0.073	0.008	12.04	0.001*
3	0.148	0.006	0.143	0.003	0.10	0.970
4	0.162	0.009	0.134	0.007	5.14	0.024
5	0.216	0.012	0.176	0.009	5.05	0.025
6	0.307	0.015	0.325	0.019	0.43	0.510
7	0.129	0.003	0.139	0.008	0.54	0.460
8	0.096	0.006	0.052	0.005	22.64	< 0.0005*
9	0.139	0.005	0.143	0.005	0.56	0.450
10	0.169	0.008	0.130	0.013	5.86	0.016
1-10	1.66	0.030	1.52	0.024	8.08	0.005*
Type B	N = 18		N = 12			
1	0.156	0.007	0.182	0.006	7.29	0.007*
2	0.181	0.012	0.108	0.006	16.27	< 0.0005*
5	0.356	0.019	0.370	0.013	0.24	0.626
6	0.310	0.020	0.220	0.017	8.85	0.003*
7	0.152	0.004	0.157	0.007	0.84	0.359
8	0.075	0.007	0.051	0.007	4.49	0.034
9	0.166	0.009	0.162	0.005	0.08	0.782
10	0.238	0.013	0.214	0.014	1.62	0.203
1-10	1.64	0.041	1.46	0.022	9.43	0.002*
Type C	N = 77		N = 24			
5	0.456	0.007	0.511	0.009	15.22	< 0.0005*
6	0.211	0.009	0.175	0.014	4.19	0.041
7	0.151	0.003	0.182	0.006	14.55	< 0.0005*
8	0.191	0.004	0.130	0.005	39.32	< 0.0005*
9	0.189	0.004	0.213	0.006	9.31	0.002*
10	0.174	0.004	0.139	0.008	11.74	0.001*
5-10	1.37	0.017	1.35	0.019	0.79	0.374

Table 2.4. Comparisons of intra-individual display unit coefficients of variation (CVs) for display types A, B, and C, and results of Kruskal-Wallis tests, for juvenile and adult *Anolis carolinensis*. Sample sizes of lizards used in analyses are given for each display type. Adult data are from Jenssen et al. (2000). P-values marked with an asterisk (*) indicate a significant effect following sequential Bonferroni adjustments.

Display type	Intra-individual CV (%)		H_1	P
Unit	Juveniles	Adults		
Type A	N = 21	N = 9		
1	20.0	6.1	7.95	0.004*
2	33.9	15.1	4.73	0.030
3	22.6	8.4	7.82	0.005*
4	33.1	6.1	9.90	0.003*
5	35.0	9.5	7.91	0.004*
6	30.2	3.3	12.13	0.001*
7	13.8	12.1	0.02	0.877
8	41.6	27.7	0.86	0.353
9	22.5	12.1	6.50	0.008*
10	34.1	7.4	7.80	0.005*
1-10	11.0	1.3	7.03	0.006*
Type B	N = 10	N = 11		
1	15.1	12.8	1.44	0.230
2	23.9	14.1	1.28	0.258
5	18.8	5.5	9.17	0.003*
6	20.0	9.2	6.26	0.013
7	14.6	10.0	0.32	0.573
8	49.3	64.5	0.18	0.673
9	19.3	19.9	0.03	0.862
10	21.8	8.1	10.53	0.001*
1-10	8.0	2.1	7.38	0.007*
Type C	N = 58	N = 21		
5	14.9	10.0	0.04	0.846
6	42.6	29.4	0.47	0.495
7	23.9	14.6	1.73	0.189
8	26.6	22.9	0.93	0.334
9	24.1	28.0	0.43	0.513
10	36.9	22.5	7.31	0.007*
5-10	13.0	9.8	0.01	0.920

RELATIVE HEAD AMPLITUDE

DISPLAY DURATION (s)
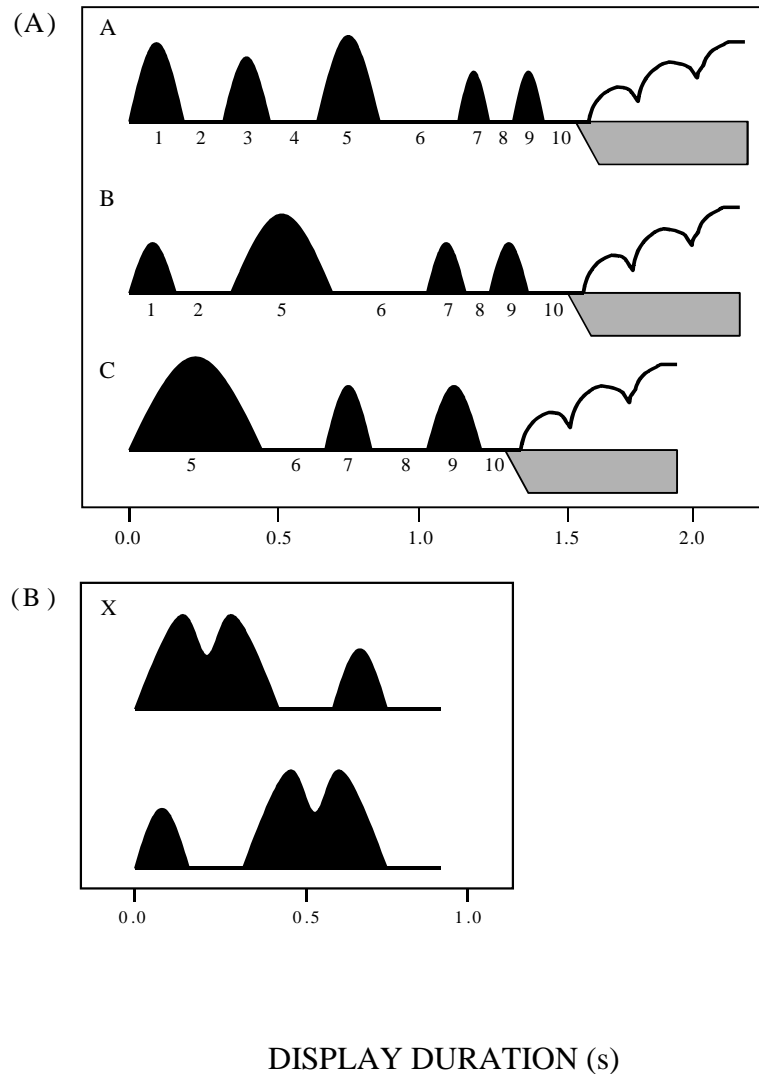
Figure 2.1. Generalized Display-Action-Pattern (DAP) graphs for (a) headbobbing display types A, B, and C, and (b) the two variants of X displays observed in juvenile male and female *Anolis carolinensis*. Graphs are based on 79 type A, 50 type B, 393 type C, and 30 X displays recorded from 88 individuals. For display types A, B, and C, head amplitude is plotted above the x-axis relative to elapsed time with stereotyped display types shown in solid black and terminal, variably produced headbobs shown by a black line. Numbers correspond to display headbob and pause units, following DeCourcy and Jenssen (1994). Dewlap extension is plotted below the x-axis by the area in gray, and may be present or absent for each display type. X displays were rare, lacked the stereotypy found in the A, B, and C display types, but could be distinguished by the unusual introductory headbobs. Consequently, X displays were not considered to be stereotyped display types, and only the introductory headbobs are shown.
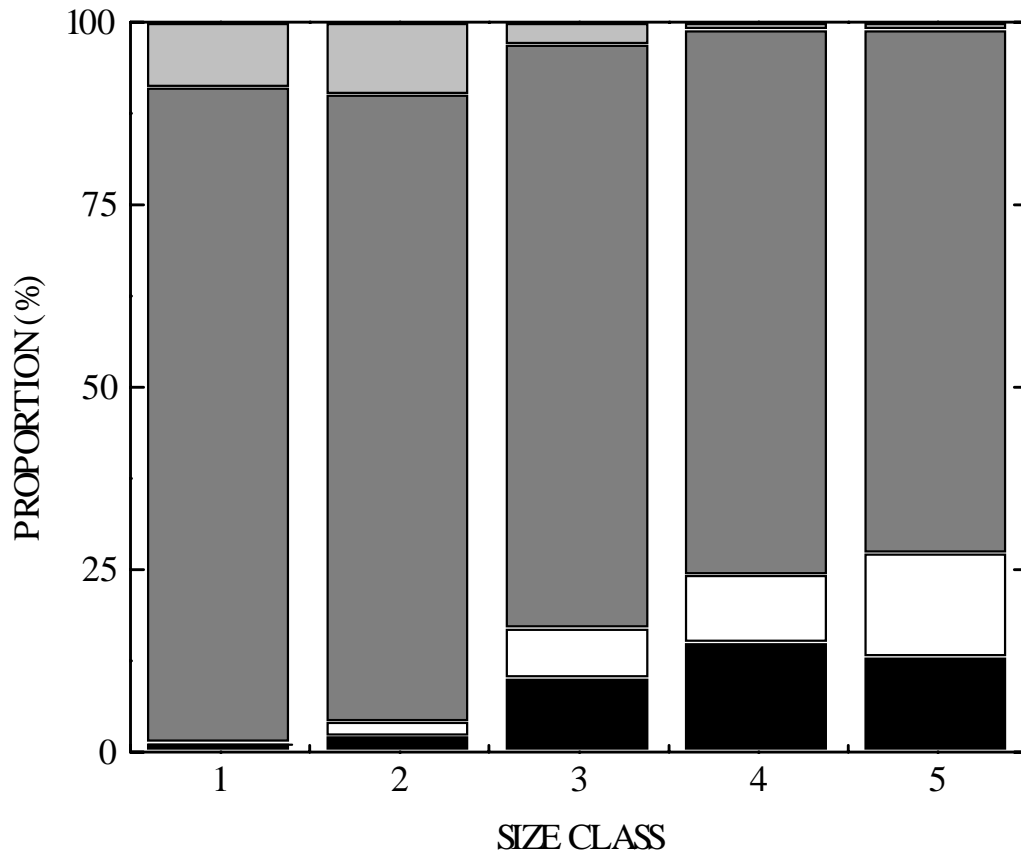
Figure 2.2. Relative proportion (%) of A (black), B (white), C (dark gray), and X (light gray) displays from 1246 displays performed by 114 juvenile *Anolis carolinensis*. Size classes are: (1) < 26 mm snout-vent length (SVL); (2) 26-30 mm SVL; (3) 31-35 mm SVL; (4) 36-40 mm SVL; and (5) > 40 mm SVL.

Chapter 3

The Effects of Context, Sex, and Body Size on Staged Social Interactions in Juvenile Male and Female Green Anoles (*Anolis carolinensis*)

Abstract

I documented the ontogeny of headbobbing display use in green anoles (*Anolis carolinensis*) by determining the effects of social context, sex, and body size on juvenile social interactions in relation to those described for adults. Sixty-eight juveniles (< 42 mm snout-vent length) captured from a field site near Augusta, Georgia, USA, were housed singly in the laboratory until behavioral testing. Within 14 d of capture, I videotaped 30 males and 30 females in trials consisting of 30 min of isolation followed by 30 min of interaction with a size-matched juvenile. The remaining four males and four females were used as controls to test the effects of partition removal on behavior in the absence of another lizard. Both juvenile males and females showed higher behavior levels during interactions than during isolation, and partition control trials confirmed that the response was due to the other lizard. Displays were only observed during interactions. Neither social context (consexual or heterosexual) nor sex affected the quality or quantity of displays and related behaviors during interactions (perch shifts, displays, display modifiers, color changes, or approaches and retreats), which always appeared to be aggressive in nature and qualitatively similar to interactions between adult females. In contrast, body size had a nearly ubiquitous effect on behavior. Juvenile males and females tended to increase overall activity as they grew, including the use of displays and display-related behaviors, and large juvenile males had higher display rates than large juvenile females. These results suggest that juvenile social interactions, like those between adult females, are agonistic in nature and that they result from a need to protect resources. Furthermore, they suggest that resource value increases as juveniles grow. Ecologically, such a shift would be expected if resource value has both a present and future benefit to juveniles. It is likely that resource protection confers the present benefits of suitable habitat for foraging, thermoregulation, and predator avoidance, and it is hypothesized that the primary future benefit is the acquisition of the eventual breeding territory that juveniles will hold as adults.

Introduction

The structure and use of communication signals have been well documented for many species, but their development has received comparatively less attention (Groothuis, 1994). In recent textbooks on animal communication, ontogeny is not covered in one (Bradbury and Vehrencamp, 1998) and, in the other, restricted to discussion of the comparatively well studied examples of song development in birds and alarm calling and social signals (e.g., those used in aggressive and/or sexual contexts) in squirrels and primates (Hauser, 1996). However, research thus far suggests that, in contrast to what might be inferred from these textbooks, the patterns and mechanisms of signal ontogeny can vary greatly across taxa. One common pattern occurs when there is no obvious signal development at all. The social signals used by adults are not expressed until adulthood, when they appear as (usually) sex-specific and fully developed signals (e.g., orthopterans: Otte, 1977; Moore et al., 1995; fishes: Brown and Colgan, 1985; anurans: Kiester, 1977; Ryan, 1985). Social organization in these species generally gives juveniles little opportunity for acquiring the signals via social processes (e.g., through extended interactions among parents and offspring or related social groups), and little need for using the signals, as interactions among juveniles are infrequent or entirely unlike those of adults.

A second pattern of signal ontogeny is characterized by an extended period of signal development. Songbirds acquire song as juveniles during discrete developmental stages for acquisition, storage, and practice of species-typical patterns (Catchpole and Slater, 1995). Many mammals begin expressing the signals used in adult social interactions during play in the juvenile stage (Fagen, 1981, 1993; Walters, 1987; Thompson, 1998). Birds and mammals share a comparatively altricial juvenile life stage, thus creating a social environment that allows for reliable interactions between parents and offspring, among siblings, or among extended social groups, in the development of social signals. During ontogeny, the expression of these signals is typically variable or incomplete, and the juvenile signals do not carry the same social consequences that they will in adulthood (Fagen, 1981; Catchpole and Slater, 1995), although the possibility that the juvenile signals have a functional meaning is not precluded (Groothuis, 1994).

A third pattern of signal ontogeny is one in which social signals appear in juveniles as structurally similar or identical to those of adults. This pattern is exhibited in the headbobbing displays of some lizards (e.g., Cooper, 1971; Stamps, 1978; Roggenbuck and Jenssen, 1986; Greenberg and Hake, 1990; Phillips et al., 1993; Lovern and Jenssen, in review). The few studies that have quantified the ontogeny of display structure suggest that experience plays a comparatively minor role in signal ontogeny; juveniles are capable of giving displays at hatching, and sex, age and social context have little effect on display structure (Stamps, 1978; Roggenbuck and Jenssen, 1986; Lovern and Jenssen, in review). This pattern of signal ontogeny also relates to social organization. Juvenile lizards receive no parental care, and because most species do not form social aggregations (but see Burghardt et al., 1977; Burghardt and Rand, 1985), juveniles are immediately and individually responsible for their own survival. Functionally, this suggests that juvenile displays would be necessary to settle conflicts over resources, which may be intense in situations where resources are limiting (e.g., Phillips et al., 1993; Stamps, 1994). However, juveniles, by definition, are not yet reproductive, and therefore they do not participate in courtship interactions and their habitat needs are not impacted by breeding considerations (e.g., exclusive overlap of female territories by males, or defended oviposition sites by females). Thus, juvenile display use, such as the frequency or contexts in which displays are given, can be expected to differ from adult use.

The green anole lizard, *Anolis carolinensis*, is a species to which this third pattern of signal ontogeny can be applied. Adult males and females share a repertoire of three headbobbing display types, labeled A, B, and C (Jenssen et al., 2000). Adults do not differ intersexually in the structure of the three display types, but there are numerous sex differences in display use (e.g., display rate) related to intrasexual selection acting on males and the resulting sex differences in reproductive strategies (Ruby, 1994; Jenssen et al., 1995; Nunez et al., 1997; Jenssen et al., 2000). *Anolis carolinensis* exhibits a polygynous social organization. Adult males display 8-fold more frequently than adult females overall, 35-fold more frequently during aggressive interactions, and only males display in a territorial advertisement (i.e., solitary) context (Nunez et al., 1997; Jenssen et al., 2000). Furthermore, male displays are more conspicuous because males can extend dewlaps (red throat fans) with 7-fold greater area than those of females (Jenssen et al., 2000). Interactions between males involve frequent use of the dewlap and display modifiers

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that can optionally be added to displays to increase apparent body size (sensu Greenberg, 1977; Jenssen, 1977). Males alter the relative proportion of display types used with interaction distance, using more A and B displays at closer distances (DeCourcy and Jenssen, 1994). Display behavior is embedded in a ritualized combat scheme (i.e., "fixed-sequence contest"; Bradbury and Vehrencamp, 1998) consisting of approaching, circling, and ultimately jaw-locking (Greenberg and Noble, 1944; DeCourcy and Jenssen, 1994; Jenssen et al., 2000). These interactions can be extremely intense, sometimes lasting over an hour and resulting in serious injury to one or both participants (Greenberg and Noble, 1944; Jenssen et al., 2000). In contrast, interactions between adult females involve low display rates, comparatively infrequent use of the dewlap and display modifiers, no shifts in the relative proportions of display types, and a total lack of the ritualized combat scheme found in males (Nunez et al., 1997; Jenssen et al., 2000). During male-female interactions, males also display at high rates and use their dewlaps, but they do not use display modifiers or show any ritualized combat (Greenberg and Noble, 1944; Jenssen and Nunez, 1998). When interacting with males, females display without any evidence of display modifiers or ritualized combat, but unlike males, they do not use their dewlaps (Greenberg and Noble, 1944; Gordon, 1956).

Juvenile male and female *A. carolinensis* each possess the same A, B, and C display types previously described for adults (Lovern and Jenssen, in review). Juveniles can begin displaying within minutes of hatching, and these displays are used in naturally occurring contexts in the field as well as in artificially manipulated contexts in the laboratory (Cooper, 1971; Greenberg and Hake, 1990; Lovern, 2000; Lovern and Jenssen, in review). Potential context-, sex-, and size-related differences in juvenile display behavior have not been quantified, however.

In this study, I examined the ontogeny of headbobbing display use in male and female *A. carolinensis*. Given the species' social organization, I expected that juvenile display use would differ from adult display use. Juvenile males and females should not differ in their basic needs (e.g., Stamps, 1994), and their social interactions should reflect competition over the resources necessary for survival (e.g., food, shelter), regardless of the sex of the lizards. I expected display behavior to reflect this commonality. First, I hypothesized that display behavior would function in agonistic encounters with other lizards, and therefore that it would not be observed while

lizards were alone, but only during interactions. Second, because juvenile requirements are not

sex-specific, I hypothesized that juveniles would not differ in display behavior during

interactions, regardless of their sex, body size (and indirectly, age), or the social context of the

interaction (consexual or heterosexual). To test these hypotheses, I staged social interactions

between juveniles of various body sizes and in different social contexts, and I compared juvenile

responses to those previously described for adults (e.g., DeCourcy and Jenssen, 1994; Jenssen et

al., 2000).

Methods

In July and August of 1997, I collected 68 juvenile *A. carolinensis* (34 males and 34

females) at a field site near Augusta, Georgia, USA, and brought them back to the laboratory.

For each individual, I recorded sex (males, but not females, have two enlarged post-anal scales)

and snout-vent length (SVL) to the nearest mm, and I applied a unique dorsal paintmark to each

for easy identification. To ensure that the collected lizards were juveniles in their summer of

hatching, I used growth rates for juvenile *A. carolinensis* from Michaud (1990) to determine the

maximum SVL a lizard in its first summer could attain by any collection date, assuming a

conservatively early hatch date of 15 May (Gordon, 1956; pers. obs.). Lizards were divided into

four size classes, all within the juvenile size range: (1) < 26 mm SVL; (2) 26-30 mm SVL; (3)

31-35 mm SVL; and (4) 36-42 mm SVL. Based on growth rates (Michaud, 1990), these size

classes approximated age classes of < 14 d, 14-37 d, 38-61 d, and 62-100 d. Juvenile *A.

carolinensis* show no sex differences in SVL or mass at hatching, although juvenile males grow

faster than juvenile females (Gordon, 1956; Michaud, 1990). However, even by 100 d, the

magnitude of difference in SVL between males and females is < 2 mm, so the size classes I

chose contained juvenile males and females of about the same range of ages.

All lizards were housed singly in cages measuring 30 x 60 x 60 cm. I exposed the lizards

to a 14:10 h light:dark cycle using four 40W full-spectrum bulbs (Durotest Vita-Lite Plus) placed

on the top of each cage. Temperatures inside each cage were 27-34 C during the day (depending

on site within the cage) and 23 C at night. Cages were identically furnished with multiple

wooden dowels for perching and numerous pieces of artificial vegetation. I watered and fed lizards daily on vitamin-dusted crickets, mealworms, and flour beetle and waxworm larva.

I observed 60 lizards (30 males and 30 females) in isolation and during pairwise interactions with consexuals and heterosexuals to investigate the potential effects of context and size on behavior (Table 3.1). After 7-14 d in the laboratory, lizards were moved to observation cages set up identically to housing cages and matched with two other lizards for subsequent trials. Prior to the trials, lizards were isolated by opaque, removable partitions. All interactants were within 2 mm SVL, and were always from the same size class. At 16-24 h after moving lizards to the observation cages, trials were conducted by videotaping pairs of lizards for 30 min alone and then 30 min together following the removal of the partition. After the 60 min trial, each lizard was again separated by replacing the partition. The following day this procedure was repeated with different pairings. Thus, each lizard was observed in two trials, but no two lizards interacted with each other more than once. An additional eight lizards (four males and four females; one of each sex from each of the size classes described above) were used once in partition control trials. Housing and trial protocols for these lizards were identical to those described above, except that following partition removal there was no adjacent lizard, which allowed me to determine whether responses by lizards were to each other or simply to partition removal.

All trials were videotaped from a darkened blind using a Panasonic AG 460 video camera fitted with an Aztec video telephoto converter (2.0X). Videotapes were subsequently analyzed using pre-printed checksheets that allowed me to describe and quantify the behaviors elicited in the trials. To examine overall differences in display behavior among trials from different social contexts and size classes, I created a behavior index (BI; modified from Ortiz and Jenssen, 1982) that represented behavioral intensity for each lizard while alone and during interactions. Each behavior listed in the BI was assigned a point value that reflected the position of the behavior in a sequence of increasingly socially-motivated behaviors (Table 3.2). Behaviors with low point values typically appear early in social interactions, or even when lizards are not interacting, whereas behaviors with high point values appear later in prolonged interactions and rarely or never when lizards are not interacting (Ortiz and Jenssen, 1982). Thus, in addition to

headbobbing displays, the BI included behaviors that might arise in the context of display interactions, allowing me to fully assess potential differences in juvenile responses among social contexts and size classes. I calculated BIs by summing the points of the observed behaviors for each lizard individually (individual BIs) before partition removal and also for each lizard (when examining characteristics of individual behavior) or for each pair of lizards (trial BIs; when examining characteristics of trials, e.g., differences in behavioral intensity among social contexts) after they were introduced.

I used Wilcoxon signed-ranks tests (test statistic = Z) to compare paired BIs for individuals when they were alone and when they were in interactions (averaged from the two trials in which each lizard participated) and Mann-Whitney U tests (test statistic = U) to compare BIs between control and interaction trials. I used Kruskal-Wallis tests (test statistic = H) to examine sex, context, and body size effects on BIs. Trial BIs were statistically independent from one another because each pair of interacting lizards was unique. When my objective was to examine specific behaviors (e.g., to examine sex or body size effects), I averaged individual responses and examined results using Fisher's exact tests (for 2 x 2 tables with small samples sizes), chi-square tests, or Kruskal-Wallis tests where appropriate. As a measure of how the interaction BI of one lizard related to the interaction BI of the other lizard, I used Spearman rank correlations. Descriptive statistics are reported as mean $\pm$ 1 SE. I used Minitab (version 10Xtra) for all statistical analyses, and hypothesis tests were two-tailed with $\alpha = 0.05$.

Results

The BIs from the partition control trials did not differ from the BIs of individuals videotaped while alone (N = 8, BI = 7.8 $\pm$ 2.9 and N = 60, BI = 12.6 $\pm$ 0.8, respectively; U = 2156; P = 0.19). Thus, behaviors elicited during interactions were due to the interaction between lizards rather than to partition removal. Furthermore, individual interaction BIs were unaffected by trial order (N = 60, Z = 1003, P = 0.52), indicating that there was no habituation or priming effect on lizard behavior from their first to their second interactions.

<u>Effects</u> <u>of</u> <u>Interaction</u> <u>on</u> <u>the</u> <u>BI</u>

Individual BIs were significantly lower for lizards when they were alone than during their interactions (N = 60, 12.6 $\pm$ 0.8 and 56.1 $\pm$ 5.3, respectively; Z = 1753, P < 0.0005). This difference in behavioral intensity held regardless of the sex or size class of the lizard, or the social context of the interaction (all P < 0.01). Of the 11 behaviors in the BI, only the four lowest in intensity (head-up posture, perch shift, tongue touch, color change; Table 3.2) were ever observed while lizards were alone; they frequently exhibited the head-up posture and perch shifting (88 and 80% of lizards, respectively), and to a lesser extent exhibited tongue touching and color changing (27 and 22%, respectively). Furthermore, BIs of isolated lizards remained relatively consistent, regardless of sex (N_{males} = 30, $N_{females}$ = 30, H_1 = 1.2, P = 0.28) or size class (N_1 = 12, $N_2 = N_3 = N_4$ = 16, H_3 = 7.1, P = 0.07).

<u>Effects</u> <u>of</u> <u>Social</u> <u>Context,</u> <u>Sex,</u> <u>and</u> <u>Body</u> <u>Size</u> <u>on</u> <u>the</u> <u>BI</u>

I compared overall trial BIs (sum of the BIs for interacting lizards) among different social contexts. Trial BIs from consexual (male-male or female-female) and heterosexual (male-female) contexts did not differ ($N_{male-male}$ = 21, $N_{female-female}$ = 18, $N_{male-female}$ = 21, H_2 = 0.04, P = 0.96; Fig. 3.1). Even if BIs did not differ by social context overall, the types or frequencies of behaviors still could have differed across contexts. Therefore, I examined trials from each social context to determine whether the proportion containing any of the behaviors in the BI differed. I found no such effect; whether a particular behavior was observed did not depend on the social context of the trial (Fisher's exact tests; all P > 0.20).

Because the social context of a trial did not affect the behavior of individual lizards, I averaged their responses from the two trials in which they participated and focused on potential sex and body size effects on individual BIs and on the particular behaviors that were expressed. Sex did not affect the interaction BI overall (H_1 = 0.1, P = 0.82) and, within each age class, males and females were equally likely to exhibit each of the behaviors in the BI (Fisher's exact tests; all P > 0.40). However, individual lizard size had a significant effect on interaction BIs (H_3 = 19.0, P < 0.0005). Individuals of different size classes did not differ in their BIs while alone,

but their interaction BIs increased, from 37.5 ± 7.0 in size class 1 to 87.3 ± 9.8 in size class 4 (Fig. 3.2). This was due to an increase in the likelihood of expression of nearly every behavior (Table 3.3). Only nuchal/dorsal crest erection and attack were never observed, and eight of the remaining nine behaviors increased in probability of expression with increasing size class. Attacks may have been eminent in some trials, but simply averted by retreating behavior by one or both interacting lizards. Nevertheless, no physical contact occurred between lizards in the trials. The increases in the number of individuals performing tongue touches, color changes, and displays were significant (Table 3.3). The head-up posture, the only behavior not to increase with size, was exhibited by every lizard in each size class.

Individual Display Behavior

Juveniles gave 515 displays during interactions that fell into one of three species-specific headbob display type categories (labeled A, B, and C), previously described for juveniles (Lovern and Jenssen, in review) and adults (DeCourcy and Jenssen, 1994; Lovern et al., 1999; Jenssen et al., 2000). Juveniles also gave an additional 18 displays that were not one of the three species display types, but rather followed the pattern previously labeled as X and inferred to represent a developmental precursor to display types A and B (Lovern and Jenssen, in review). These few X displays are not included in the present analyses.

None of the juveniles displayed when alone, although 80% (24 of 30) of the males and 73% (22 of 30) of the females did so during interactions, thus indicating no sex difference in the likelihood of displaying ($X^2_1 = 0.38$, P = 0.54). Overall, 9% of juvenile displays were type A, 11% were type B, and 80% were type C. There was no sex difference in the relative proportion of display types given ($X^2_2 = 4.6$, P = 0.21), there was no effect of interaction distance (long, > 30 cm; short, < 20 cm; $X^2_1 = 2.1$, P = 0.73), and there was no effect of social context ($X^2_2 = 3.8$, P = 0.28). Thus, males and females gave the same display types in the same proportions regardless of whether they were in consexual or heterosexual interactions. However, relative display type proportions differed by size class (Fig. 3.3), with types A and B increasing in frequency with increasing size class. Chi-square tests indicated that all pairwise comparisons

between the relative display proportions of different size classes were significant (all $P < 0.005$) except for the comparison between size classes 2 and 3 ($X^2_2 = 1.9$, $P = 0.39$).

Juvenile males and females also did not differ in the proportion of displays that were accompanied by dewlap extension (59% and 52% of displays for males and females, respectively; $X^2_1 = 2.2$, $P = 0.14$). Furthermore, males and females were each equally likely to use their dewlaps in consexual or heterosexual interactions ($X^2_1 = 0.1$, $P = 0.76$; $X^2_1 = 0.3$, $P = 0.70$; for males and females, respectively). However, size again played a role in the expression of behavior. Displays were accompanied by dewlap extension 77% of the time in size class 1, 60% and 61% of the time in size classes 2 and 3, and 46% of the time in size class 4. This trend of decreasing dewlap use with increasing body size was significant for comparisons between size classes 1 and 4, 2 and 4, and 3 and 4 (chi-square tests; $P < 0.05$), and appeared related to the increase in the relative proportions of display types A and B, and the decrease in type C. Only 9% and 5% of A and B displays, respectively, were accompanied by dewlap extension, in contrast to 74% of C displays.

Juvenile males and females were nearly identical in the behaviors expressed during interactions with other lizards. However, display rates were sexually dimorphic (Fig. 3.4). For size class 4, display rates were higher in males than in females ($N = 8$, 18.5 ± 3.2 / h and $N = 8$, 9.1 ± 2.2 / h, respectively; $U = 89$, $P = 0.03$). Even with this sex difference, when I pooled sexes I found a significant effect on display rates due to size class ($H_3 = 15.7$; $P = 0.001$). From size class 1 to 4, display rates (displays/h) were 3.0 ± 1.3, 4.1 ± 1.5, 6.5 ± 3.1, and 14.3 ± 3.1, respectively.

Individual Responses to Interaction

The behavior expressed by one lizard in an interaction could affect the behavior expressed by the other lizard. Furthermore, this relationship could change with social context or size class, if the relative impact of the behaviors differs by whether they are expressed by males or females, or by individuals of different ages. Overall, there was a very strong positive correlation between the BIs of interacting lizards ($N = 60$, $r = 0.62$, $P < 0.00005$). Furthermore,

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when examined by social context, male-male (N = 21, r = 0.74, P = 0.005), male-female (N = 21, r = 0.78, P = 0.0003), and female-female (N = 18, r = 0.49, P = 0.03) interactions all showed significant positive correlations of the BIs of interacting lizards. The correlation between the BIs of interacting lizards increased with increasing size class (Fig. 3.5a-d). The BIs in size class 1 (N = 12, r = 0.26, P = 0.39) and size class 2 (N = 16, r = 0.51, P = 0.07) were not significantly correlated, but were significantly positively correlated in size class 3 (N = 16, r = 0.73, P = 0.006) and size class 4 (N = 16, r = 0.67, P = 0.009).

Discussion

My hypothesis that display behavior would only be observed during interactions was overwhelmingly supported. None of the 60 juveniles displayed while alone, and overall individual behavior levels, as measured by the BI, were comparatively low (BI = 13). In contrast, 77% (46 of 60) of the lizards displayed during interactions, during which time individual behavior levels were comparatively high (BI = 56). I also hypothesized that juveniles would not differ in display behavior during interactions, regardless of their sex, body size (as an indicator of age), or the social context of the interaction (consexual or heterosexual). In support of this hypothesis, I found that whether lizards were in consexual or heterosexual interactions had no effect on behavior levels or the types and frequencies of behaviors observed. Sex generally had no effect on display behavior, either, with the single exception that juvenile males in the largest size class had higher display rates than similarly sized juvenile females. In contrast to my hypothesis, body size had a nearly ubiquitous effect on juvenile display behavior. Larger juveniles had higher behavior levels, involving the expression of more behaviors at greater frequencies. One notable exception to this positive trend was the negative relationship between dewlap use and size class. Finally, body size affected the extent to which interacting lizards matched behavior levels, which were not correlated in size classes 1 and 2 ($\leq$ 30 mm SVL), but were highly positively correlated in size classes 3 and 4 (31-42 mm SVL).

Juvenile interactions, regardless of sex, appeared much more similar to adult female-female agonistic interactions than to either adult male-male agonistic or male-female courtship interactions (Introduction; Table 3.4). Unlike adult males, juveniles did not display alone in an

advertisement context, nor did they show any evidence of a ritualized aggression pattern. Juvenile interactions, regardless of sex, involved comparatively low display rates. Juvenile males and females used all three species-specific A, B, and C display types regardless of social context or separation distance between lizards, and exhibited occasional close (< 30 cm) approaches and/or retreats without any ultimate physical contact. Given the similarity between juvenile and adult female-female interactions, it appears that juveniles, like adult females, display in an agonistic context as a means of protecting resources. However, the outcomes of juvenile interactions, like those between adult females, carry comparatively few consequences as the resources important to juveniles (habitat for foraging, thermoregulation, predator avoidance) and adult females (additionally habitat for oviposition) do not appear to be limiting (Nunez et al., 1997; Jenssen and Nunez, 1998; Lovern, 2000). Field observations suggest that, across all contexts, juvenile display rates are low, averaging 3 displays/h for both males and females (Lovern, 2000). Even in the present study, when interactions were forced, display rates were not much higher (10 and 9 displays/h for juvenile males and females, respectively; Table 3.4), and these interactions are likely to reflect the most intense encounters in which juveniles participate, as all individuals were size-matched to within 2 mm SVL and therefore potentially competing for the same resources with little asymmetry in resource-holding potential (Bradbury and Vehrencamp, 1998).

I have argued that the outcomes of juvenile interactions have comparatively few consequences. However, given the clear and consistent effects of body size on behavior that I observed, it is possible that the consequences become more important for juveniles as they grow. Ecologically, this shift in resource value could occur if there is not only a present, but also a future, benefit at stake. As indicated for *Anolis limifrons* (Andrews and Rand, 1983), juvenile home ranges are typically very close or actually contained within their eventual home ranges as adults, because juvenile home ranges occur in the same habitat as those of adults. In a field study with *A. carolinensis*, Lovern (2000) found that a majority of juveniles could be re-sighted, up to 4 weeks after initial sighting (when presumably paintmarks that identified lizards wore off), within 2 m from where originally observed. These observations suggest that juvenile home ranges become adult home ranges in at least some anoline species, and that the value of these home ranges becomes greater with increased residence time. Furthermore, this explanation may

also account for the sex difference in display rate observed in large juveniles. Juvenile males may become more aggressive towards other juveniles because of the comparative importance of holding a large territory to reproductive success once they are adults. Adult males have territories that are 8-fold larger than those of adult females, and their reproductive success depends on maintaining exclusive access to breeding females contained within their territories (Ruby, 1984; Nunez et al., 1997; Jenssen and Nunez, 1998). However, the protocol I used for creating social interactions clearly indicated that juvenile females were equally likely to express the behaviors observed in males, and that all interactions, regardless of context, were aggressive. Because adult male territories are defended only against other males, one might have expected that large juvenile males and females would have less aggressive heterosexual than consexual interactions. A laboratory protocol using longer observation periods, repeated observations between individuals, and/or larger sample sizes, could further test this hypothesis, as could introductions of lizards (e.g., tether tests) into naturally occurring home ranges in the field.

Overall, juvenile display use and ontogeny in *A. carolinensis* should make sense in light of the species life history traits and the resulting social organization. Previous studies strongly suggest that sex differences in adult display behavior arise from the divergent mating strategies of males and females (Ruby, 1984; Jenssen et al., 1995; Nunez et al., 1997; Jenssen and Nunez, 1998; Jenssen et al., 2000), especially because these sex differences disappear outside of the breeding season (Jenssen et al., 1995; Jenssen et al., 1996; Jenssen et al., in review). Therefore, it is not surprising to find almost no sex differences in display behavior between juvenile males and females, neither of which are reproductive. This is in contrast to typical signal ontogeny in diverse taxa such as frogs, songbirds, and mammals, in which social signals either do not emerge until adulthood or develop gradually, uncoupled from their eventual functional meaning (e.g., Burghardt, 1977; Kiester, 1977; Fagen, 1981; Groothuis, 1993; Catchpole and Slater, 1995). Because juvenile *Anolis* hatch from single-egg clutches, receive no parental care, and do not form social aggregations, they receive no help in meeting their survival needs (Gordon, 1956; Stamps, 1978; Lovern, 2000). As a result, *A. carolinensis* is equipped from hatching with communication signals, like those of adults, for resolving conflicts over resources (present study; Lovern and Jenssen, in review).

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Table 3.1. Sample sizes of lizards and social contexts of pairwise trials, by size class, used to analyze display behavior in juvenile *Anolis carolinensis*. Each lizard was used twice. Size classes are: (1) < 26 mm snout-vent length (SVL); (2) 26-30 mm SVL; (3) 31-35 mm SVL; (4) 36-42 mm SVL.

Size class	Lizards		Trial type			Total
	Male	Female	Male-male	Female-female	Male-female	
1	6	6	4	4	4	12
2	7	9	5	5	6	16
3	9	7	6	4	6	16
4	8	8	6	5	5	16
Total	30	30	21	18	21	60

Table 3.2. Behaviors, definitions, and point values used to create a behavior index for analyzing display behavior in juvenile male and female *Anolis carolinensis*.

Behavior	Definition	Point value
Head-up[1]	Posture reflecting alertness to the environment; > 60 s (consecutively) with head raised higher than body	1
Perch shift	Any movement > 1 body length (excluding tail) from one perch site to another; movements > 15 s apart were scored as separate perch shifts	2
Tongue touch[2]	Potential chemosensory behavior involving brief touch of the tongue to the substrate	3
Color change[1,2]	Change in lizard body color between green, olive, or brown as a potential indicator of social stress or arousal	4
Headbob	Series of vertical head movements in species-specific temporal cadences used for communication; noted display type (A, B, C), separation distance between displaying lizards, and whether dewlap extension also occurred	5
Eyespot[1,3]	Development of dark spot posterior to each eye indicating increased adrenergic activity	6
Nuchal/dorsal crest[1,2]	Display modifier in which a flap of skin is erected along the neck and back	7
Engorged throat[1,2]	Display modifier in which the ventral throat area remains enlarged	7
Sagittal expansion[1,2]	Display modifier in which the lateral view of the lizard becomes enlarged	7
Approach/retreat	A perch shift directly toward or away from another lizard when the separation distance is < 30 cm	8
Attack	Lunge toward another lizard, within 10 cm, with an attempt at or actual physical contact such as biting	9

[1]These behaviors were scored a maximum of once per pairwise interaction trial for each lizard.
[2]Greenberg, 1977
[3]Hadley and Goldman, 1969

Table 3.3. Proportion (%) of juvenile *Anolis carolinensis* exhibiting specific behaviors by size class (sample size in parentheses), and P-values from Fisher's exact tests between size classes 1 and 4. Size classes are: (1) < 26 mm snout-vent length (SVL); (2) 26-30 mm SVL; (3) 31-35 mm SVL; (4) 36-42 mm SVL. See Table 3.2 for definitions of the behaviors.

Behavior	Size class				P
	1 (N = 12)	2 (N = 16)	3 (N = 16)	4 (N = 16)	
Head-up	100%	100%	100%	100%	1.00
Perch shift	92	100	100	100	0.44
Tongue touch	50	63	63	88	0.04
Color change	58	75	81	94	0.03
Display	50	69	94	88	0.04
Eyespot	0	0	19	19	0.17
Nuchal/dorsal crest	0	0	0	0	1.00
Engorged throat	33	38	38	50	0.21
Sagittal expansion	33	38	38	50	0.21
Approach/retreat	33	38	44	56	0.15
Attack	0	0	0	0	1.00

Table 3.4. Comparison of display behavior observed in juvenile and adult *Anolis carolinensis*. Juvenile data are from this study, adult data are from Jenssen et al. (2000) unless otherwise noted.

Behavior	Juveniles		Adults	
	Males	Females	Males	Females
A, B, C display types	Yes	Yes	Yes	Yes
Display type proportions				
Shift with context	No	No	Yes	Yes
Shift with distance	No	No	Yes	No
Displays / h				
Solitary context	0	0	18	0
Interaction	10	9	168	17
Ritualized aggression	No	No	Yes	No
Display modifier use				
Consexual context	Yes	Yes	Yes	Yes
Heterosexual context	Yes	Yes	No[1]	No[1]
Dewlap use				
Consexual context	Yes	Yes	Yes	Yes
Heterosexual context	Yes	Yes	Yes[1]	No[1]

[1]Greenberg and Noble, 1944
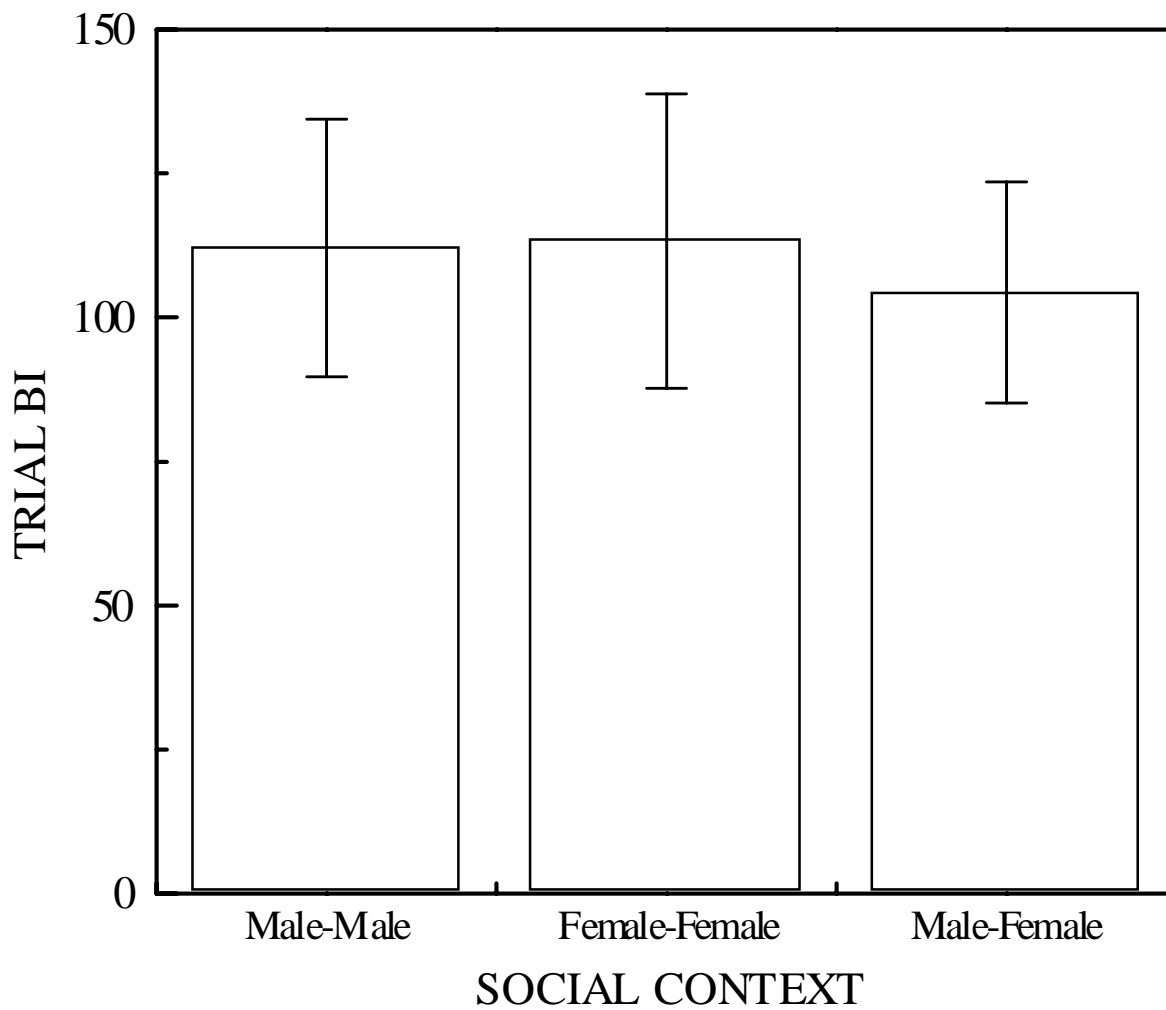
Figure 3.1. Mean ($\pm$ 1 SE) behavior index (BI) by social context (male-male N = 21, female-female N = 18, male-female N = 21) for pairwise interaction trials between juvenile *Anolis carolinensis*. See Table 3.2 for definitions of the behaviors included in the BI.
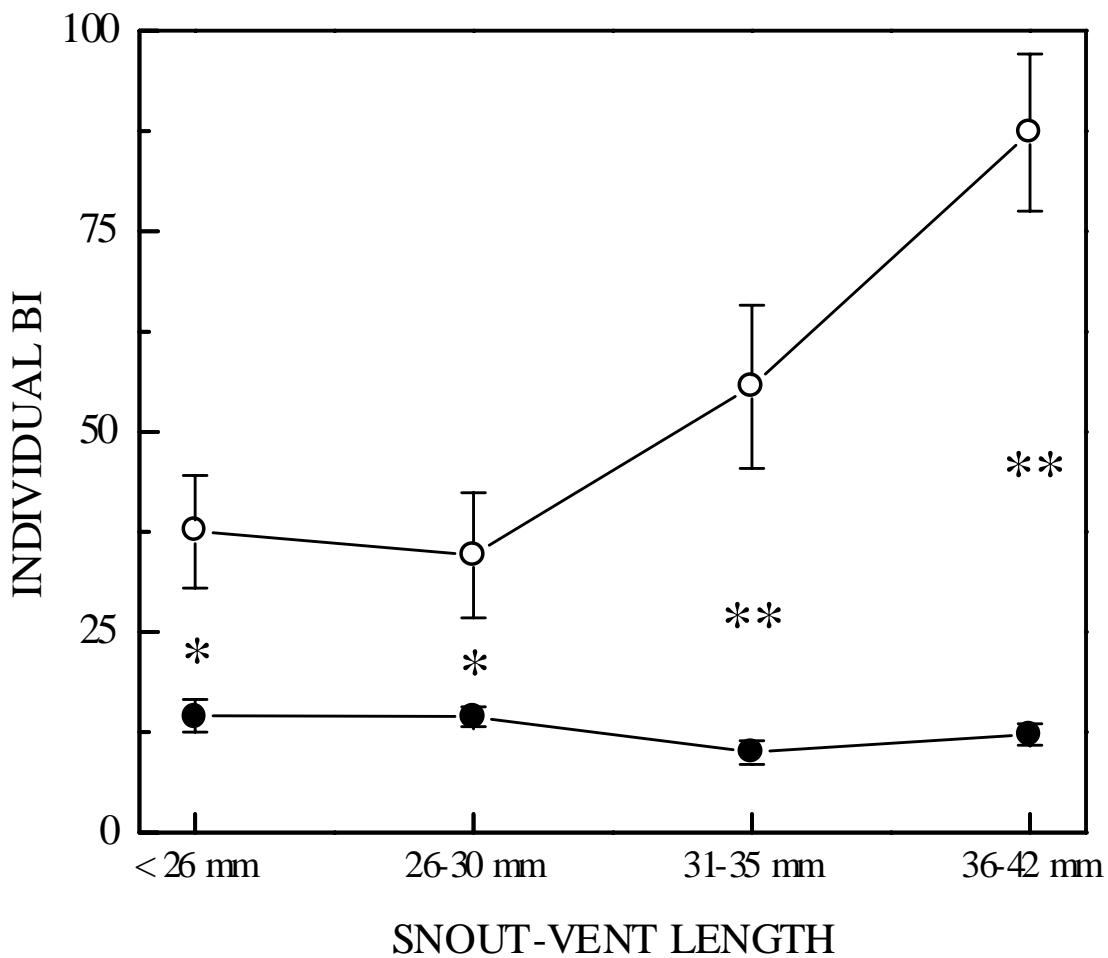
Figure 3.2. Mean ($\pm$ 1 SE) individual isolation (solid circles) and interaction (open circles) behavior indices (BI) by size class for juvenile male and female *Anolis carolinensis*. See Table 3.1 for samples sizes, and Table 3.2 for definitions of the behaviors included in the BI. The BIs from the two trials in which each lizard participated were averaged to create their individual BIs. *P < 0.05, **P < 0.005; Mann-Whitney U tests.
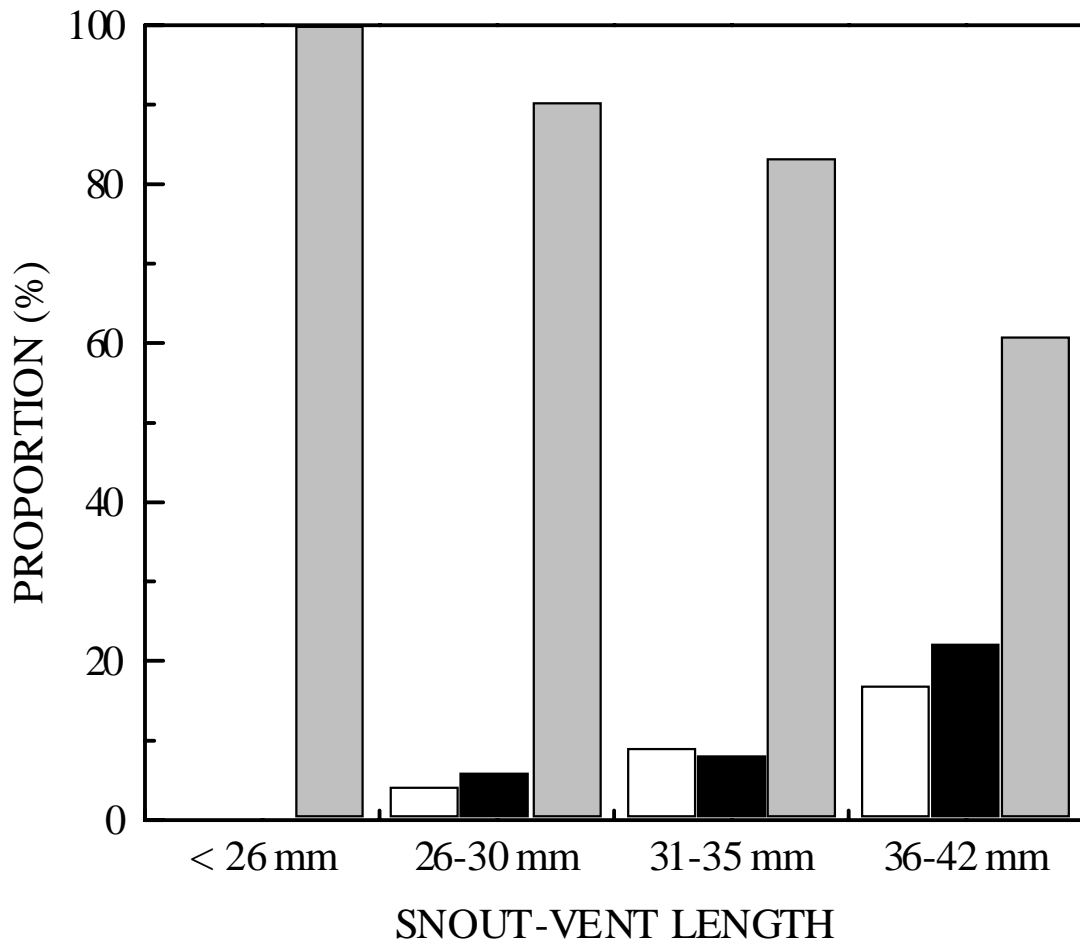
Figure 3.3. Relative proportions (%) of 515 headbobbing displays given during pairwise interactions that were type A (white bars), B (black bars), and C (gray bars) by size class by 60 juvenile *Anolis carolinensis*.
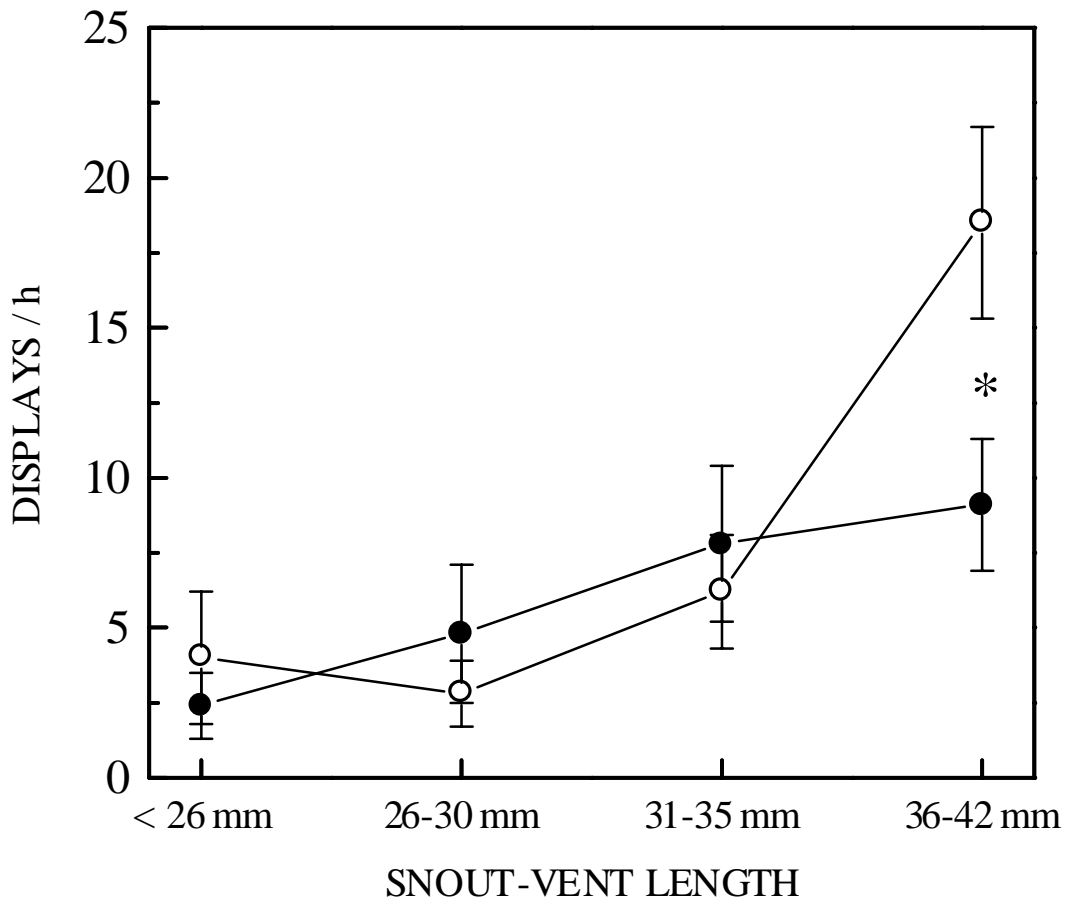
Figure 3.4. Mean ($\pm$ 1 SE) hourly display rate by size class during pairwise interactions for 30 juvenile male (open circles) and 30 juvenile female (solid circles) *Anolis carolinensis*. Kruskal-Wallis test; $H_{size} = 15.7$, P = 0.001. * P < 0.05; Mann-Whitney U test.
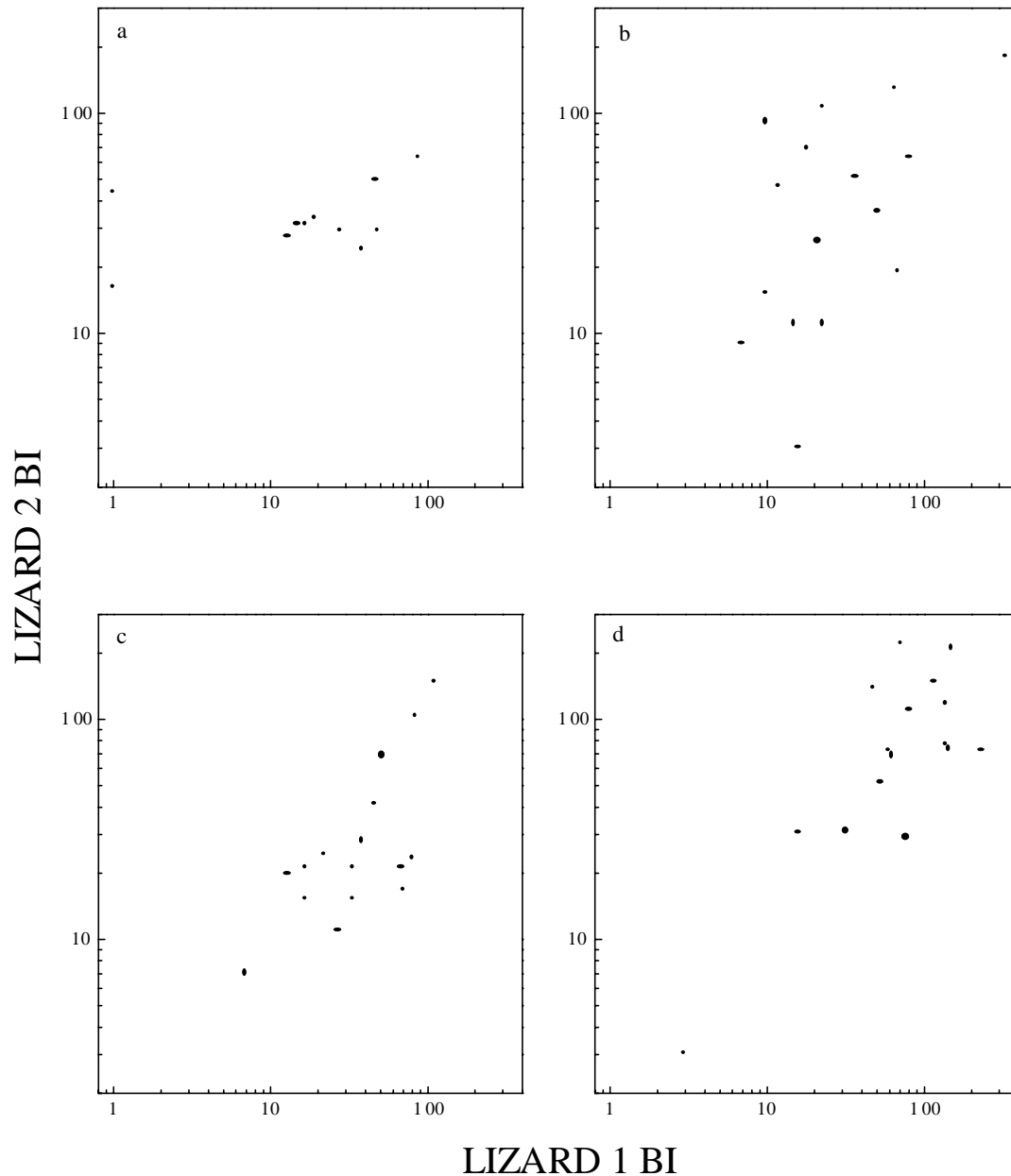
Figure 3.5. Lizard 2 behavior index (BI) vs. lizard 1 BI for (a) 12 size class 1 (< 26 mm snout-vent length, SVL), (b) 16 size class 2 (26-30 mm SVL), (c) 16 size class 3 (31-35 mm SVL), and (d) 16 size class 4 (36-42 mm SVL) interactions for juvenile *Anolis carolinensis*. Axes are log-transformed. Spearman's rank correlations: (a) r = 0.26, P = 0.39; (b) r = 0.51, P = 0.07; (c) r = 0.73, P = 0.006; (d) r = 0.67, P = 0.009.

Chapter 4:

Developmental Effects of Testosterone on Behavior in Male and Female Green Anoles (*Anolis carolinensis*)

Abstract

I documented the pattern of endogenous testosterone (T) concentrations during ontogeny, and I determined the behavioral effects of experimentally elevated T concentrations, in juvenile male and female green anoles (*Anolis carolinensis*). Endogenous T concentrations were measured in the plasma of laboratory-incubated hatchlings and field-sampled juveniles and adults, as well as in the yolks of freshly laid eggs in the laboratory. There were no sex differences in plasma T in hatchling and small juvenile (< 26 mm snout-vent length, SVL; < 14 d old) males and females; in both sexes, plasma T appeared to decline over the 14 d post-hatching period. Plasma T sharply increased in juvenile males, but not females, after approximately 14 d post-hatching (> 25 mm SVL), and it was significantly higher in juvenile males than in juvenile females after approximately 38 d post-hatching (> 30 mm SVL). Plasma T for juvenile males was within the range detected in breeding adult females, but much lower than that of breeding or post-breeding adult males. All eggs contained yolks with detectable T, but eggs that gave rise to males contained nearly twice as much yolk T as those that gave rise to females. In behavior trials conducted in the laboratory 14-21 d post-implant, juveniles with T-implants had increased activity levels compared to juveniles with blank-implants. This treatment effect was due to increased rates and probabilities of nearly every behavior monitored in the juveniles with T-implants, and it was consistent, regardless of the sex of the juvenile or the social context of the behavior trial. Varying T concentrations during ontogeny, coupled with the effects of experimental T elevation, are discussed in the context of the organization-activation theory of sexual differentiation and the particular life history of *A. carolinensis*.

Introduction

Different selection pressures acting on males and females frequently result in sexual dimorphisms in morphology and behavior (e.g., Shine, 1989; Andersson, 1994). As a guiding principle, the organization-activation theory (first formulated by Phoenix et al., 1959) has been useful for elucidating the nature of endocrine effects on morphological and behavioral sex differences. This theory states that steroid exposure can affect sexual dimorphism by two general mechanisms. First, during a critical period in early development, steroids can organize long-lasting sexual dimorphisms in the nervous system which permit morphological or behavioral trait expression in the organized sex. Organizational effects are typically considered irreversible, in that they persist in the absence of steroid exposure (e.g., Arnold and Breedlove, 1985; Moore, 1991; Wade, 1999). Second, steroid exposure later in life can activate (or facilitate) expression of sex differences by targeting the previously organized structures. Activational effects are transient, present only when steroid concentrations are above the threshold for response (e.g., Arnold and Breedlove, Moore, 1991; Wade, 1999). Research across vertebrate taxa supports the idea that gonadal steroids can be important regulators of sex differences (reviewed in Kelley, 1988; Ketterson and Nolan, 1992; Moore and Lindzey, 1992; Whittier and Tokarz, 1992; Cooke et al., 1998; Wade, 1999). However, organization and activation do not always occur as distinctly separate processes (e.g., Arnold and Breedlove, 1985). Furthermore, in addition to the traditional view of sexual dimorphism requiring both organization and activation, some traits may require only organization, only activation, or they may possibly arise by extra-steroidal mechanisms (e.g., Cooke et al., 1998; Moore et al., 1998; Wade, 1999).

There are few species for which both endogenous steroid concentrations during ontogeny and steroid effects on sexual dimorphism have been documented. The green anole lizard, *Anolis carolinensis*, offers an opportunity to conduct such a study within a life history framework in which sex differences in adult morphology, behavior, and physiology are prominent. In general, the morphological sex differences associated with courtship and reproduction appear to be insensitive to androgen manipulation in adults (e.g., the neural and muscular structures regulating dewlap extension; O'Bryant and Wade, 1999). However, androgens are necessary for

the full expression of adult male-typical behavior in both *A. carolinensis* and the congeneric *Anolis sagrei*. Gonadectomy or treatment with cyproterone acetate (an anti-androgen) can greatly reduce aggression, courtship, and copulatory behavior in males (Mason and Adkins, 1976; Crews et al., 1978; Tokarz, 1995), and exogenous testosterone (T) can reinstate or maintain these behaviors (Mason and Adkins, 1976; Adkins and Schlesinger, 1979; Winkler and Wade, 1998). Seasonal changes in T and behavior in natural populations corroborate these experimental results. Adult males have high plasma T concentrations during the breeding season when sex differences in behavior are great, and low plasma T concentrations during the nonbreeding season when sex differences in behavior are minimal or nonexistent (Jenssen et al., 1995a; Jenssen et al., 1996; Tokarz et al., 1998; Jenssen et al., in review). Furthermore, T supplementation can affect adult female behavior (Mason and Adkins, 1976; Adkins and Schlesinger, 1979), although it remains unclear whether the effect is equivalent to that seen in adult males (see Winkler and Wade, 1998).

The studies outlined above suggest that adult male-typical behavior is naturally activated by T in adult male *A. carolinensis*, and that T supplementation might also activate a male-typical response in adult females. However, our ability to fully evaluate the extent to which organization, activation, or both play a role in sex differences in behavior in this species is hindered for two reasons. First, endogenous T concentrations in juveniles and in adult females have not been documented. Second, the effects of T on the ontogeny of behavioral sex differences are unknown.

In the present study, I first documented endogenous plasma T concentrations in hatchling and juvenile males and females, and in adult females, and I compared these values to the plasma T concentrations of adult males reported in Jenssen et al. (in review). I also documented yolk T concentrations in eggs on the day of oviposition. Second, I experimentally elevated plasma T concentrations in large (i.e., old) juveniles by using T-implants and monitored their behavior in pairwise laboratory trials in comparison to the behavior of controls (juveniles with blank-implants). These data were used to examine the extent to which T plays a role in only organization, organization and activation, or only activation, of the expression of adult male-typical behavior in *A. carolinensis*. If only organization is required, then (1) during early

ontogeny, T should be higher in males than in females, and (2) associated with this hormone difference, both blank- and T-implanted juveniles should exhibit permanent sex differences in behavior. This possibility seems highly unlikely given the association of T levels with behavior in adult males, as described above. More likely is one of the following two possibilities. If both organization and activation by T are required for the expression of adult male-typical behavior, then (1) during early ontogeny, T should be higher in males than in females, and (2) T-implanted males, but not females, should respond to T elevation (assuming the threshold for response is met, which is likely higher for activation than for organization). However, if only activation is required, then: (1) T should not differ between males and females during early ontogeny; and (2) T-implanted juveniles, regardless of sex, should show a behavioral response in comparison to blank-implanted juveniles.

Methods

<u>Study</u> <u>Animal</u>

Endogenous T and the behavioral effects of elevated T during ontogeny were studied in *A. carolinensis* because the biology of adults has been well described. Unlike some reptiles which exhibit temperature-dependent sex determination (TSD; Bull, 1980), *A. carolinensis* has genotypic sex determination (GSD; Viets et al., 1994). This lizard has a polygynous mating system in which males attempt to establish territories that exclusively overlap those of several females, who lay single-egg clutches at weekly intervals over a four month (April-July) breeding season (Ruby, 1984; Andrews, 1985; Jenssen et al., 1995a; Jenssen and Nunez, 1998). Although adult males and females share a signal repertoire of headbobbing displays, their display use differs considerably (Jenssen et al., 2000). During territory patrol, adult males frequently give advertisement (i.e., undirected) displays, whereas females rarely or never do (Nunez et al., 1997; Jenssen et al., 2000). Inter-male aggression is intense, involving high display rates and the use of numerous display modifiers (aggressive postures or movements added to headbobbing displays and designed to enhance apparent body size; Greenberg, 1977; Jenssen, 1977). Furthermore, these behaviors occur within a ritualized pattern of approaching, circling, and ultimately jaw locking if one of the males does not retreat (Greenberg and Noble, 1944; Jenssen et al., 2000).

Inter-female aggression involves lower display rates, less frequent use of display modifiers, and no ritualized aggression (Jenssen et al., 2000). During courtship, both males and females display, but without using the display modifiers seen in aggressive interactions (Greenberg and Noble, 1944; Jenssen and Nunez, 1998; Winkler and Wade, 1998). Males also extend their dewlaps during courtship, but females do not (Greenberg and Noble, 1944; Winkler and Wade, 1998). Adult females, but not males, adopt a characteristic "neck-bend" posture when receptive that facilitates the male's grip during copulation (Greenberg and Noble, 1944; Jenssen and Nunez, 1998; Winkler and Wade, 1998).

Anolis carolinensis: Collection and Maintenance

Analyses of hatchling plasma T, yolk T in eggs on the day of oviposition, and the behavioral effects of elevated T were conducted in the laboratory. Lizards were collected by hand or noose from a well-studied population along the Augusta Canal near Augusta, Georgia, USA (e.g., Jenssen et al., 1995a; Nunez et al., 1997; Lovern, 2000) and maintained in the laboratory singly in cages measuring 30 x 60 x 60 cm. I exposed them to a 14:10 h light:dark cycle using four 40W full-spectrum bulbs (Durotest Vita-Lite Plus) placed on the top of each cage. Temperatures inside each cage ranged from 27-34 C during the day and dropped to 23 C at night. All cages contained wooden dowels for perching, numerous pieces of artificial vegetation, and a dish of moist potting soil for egg-laying (when housing gravid females). I watered and fed lizards daily on vitamin-dusted crickets.

Plasma and Yolk Sample Collection

For collecting plasma samples from hatchlings, 16 gravid females were housed in the laboratory. I checked for eggs daily and incubated them individually in plastic cups containing a vermiculite:water mix (50:50 by mass). A small depression was made on the surface of the mixture and eggs were placed so that they were half-buried. The cups were then covered with plastic wrap secured by a rubber band, and incubated at 24-31 C on a diel cycle. I collected plasma samples from 18 males and 18 females on the day of hatching (90% hatching success; 36 of 40 eggs). Because of the small size of hatchlings (0.2-0.3 g), blood was collected from the

trunk immediately following decapitation. Plasma (4-9 μl) was isolated from whole blood following centrifugation, transferred to 1.0 ml microcentrifuge tubes, and stored at –80 C until analysis. For each sample, I recorded mother identity and egg number, date of oviposition and hatching, hatchling sex, and plasma volume. Incubation time was 35.3 $\pm$ 0.4 d.

I also collected plasma samples from 58 juvenile ($\leq$ 42 mm snout-vent length, SVL; 32 male and 26 female) and 37 adult (> 45 mm SVL; 12 male and 25 female) *A. carolinensis* in the field. On collection dates during July and August, juveniles were captured by hand, sexed, measured for SVL to the nearest mm, and bled from the trunk. Juveniles are not yet physiologically capable of reproduction. On collection dates during April-July (breeding) and September (post-breeding), adults were captured by noose, sexed, measured, and blood was collected from the post-orbital sinus, after which they were released. For all lizards, the elapsed time from initial sighting to blood collection was always < 10 min to minimize potential stress effects on circulating steroid concentrations (e.g., Moore et al., 1991). In the field, blood samples were kept cool on ice. Within 5 h of collection, samples of 5-75 μl plasma were isolated from whole blood by centrifugation and subsequently frozen in dry ice for transportation back to the laboratory at Virginia Polytechnic Institute and State University, where they were stored at – 80 C until analysis. Plasma T data for the breeding and post-breeding adult males were initially reported in Jenssen et al. (in review; analyzed by me), and are included here for statistical comparison to adult females and juveniles.

The 94 juvenile lizards from which plasma samples were obtained were divided into five size (age) classes: (1) hatchlings (N = 36); (2) < 26 mm SVL (N = 11); (3) 26-30 mm SVL (N = 15); (4) 31-35 mm SVL (N = 14); and (5) 36-42 mm SVL (N = 18). Based on growth rates from Michaud (1990) for *A. carolinensis*, these size classes represented age classes of 0, < 14, 14-37, 38-61, and 62-100 d. Note the qualitative break between size classes 1 and 2-5; hatchlings were sampled on the day they hatched following incubation in the laboratory, and juveniles in size classes 2-5 were all sampled in the field. Adult males and females were 62.3 $\pm$ 1.2 and 52.9 $\pm$ 0.9 mm SVL, respectively.

Eight reproductive females were housed in the laboratory for analyses of yolk T concentrations. Cages were checked daily for eggs, and 15-35 mg of yolk was withdrawn from each egg on the day of oviposition through a sterile 26 G needle. Yolk samples were stored in 1.5 ml microcentrifuge tubes and identified by mother, date of oviposition, and amount of yolk collected to the nearest mg (weighed on a Mettler AE 240 balance). Immediately after weighing, yolk samples were homogenized in 0.5 ml distilled water using a vortex mixer and the addition of 2-3 small glass beads, then stored at –80 C until analysis.

After withdrawal of yolk samples, eggs were incubated as described above. Incubation time was 35.1 ± 0.3 d (mean $\pm$ 1 SE). At hatching, sex was determined by noting the presence or absence of enlarged post-anal scales; males have enlarged post-anal scales, but females do not. A hand lens was used to confirm scale size. Nineteen of the 22 eggs collected for yolk T analyses hatched (86%), of which 6 were males and 13 were females.

Hormone Assays

Yolk and plasma concentrations of T were measured by radioimmunoassay (RIA), following extraction and chromatographic separation, as described by Wingfield and Farner (1975), Moore (1986), and Schwabl (1993). Samples were equilibrated overnight at 5 C with 1000 cpm of ^{3}H-T (NET-553, Dupont NEN), and one aliquot of each sample was taken following extraction and chromatography and prior to radioimmunoassay (see below), for individual recovery determinations. Additionally, five replicate aliquots from a standard of known concentration were run in each assay, and treated identically to samples, to determine intra-assay precision and inter-assay repeatability. Yolk samples were extracted twice with 3 ml petroleum ether:diethyl ether (30:70 v:v), dried under a stream of nitrogen (N), and reconstituted in 1 ml 90% ethanol. The extracted samples were stored at –20 C overnight, then centrifuged at 2000 rpm for 5 min to precipitate neutral lipids and proteins. The supernatant was dried with N and reconstituted in 300 µl of 10% ethyl acetate in isooctane. Plasma samples were extracted twice with 2 ml diethyl ether, dried with N, and reconstituted in 300 µl of 10% ethyl acetate in isooctane. To remove additional neutral lipids and to isolate T, samples were transferred to celite (Sigma) microcolumns for chromatographic separation. Columns consisted of a

celite:ethylene glycol:propylene glycol upper phase (6:1.5:1.5 w:v:v) and a celite:distilled water (3:1 w:v) lower phase. Neutral lipids were eluted from the columns with 2 ml 100% isooctane, dihydrotestosterone was eluted with 1.5 ml 10% ethyl acetate in isooctane, and T was eluted with 2.5 ml 20% ethyl acetate in isooctane. The purified T fractions were dried with N, resuspended in sample buffer, and then placed overnight at 5 C.

Competitive binding RIA was performed with ^{3}H-T and T antiserum (T-3003, Wien Laboratories). Standards from 0.5 to 125 pg were run in triplicate; samples were run in duplicate, averaged, and corrected for individual recovery. All yolk samples were run in one assay (intra-assay coefficient of variation, CV = 7%). Plasma samples were run in four assays (intra-assay CV = 14%; inter-assay CV = 11%). Non-detectable samples were assigned the least detectable dose (0.5 pg per sample tube for all assays).

<u>Experimental T Elevation and Behavior Trials</u>

I collected 26 juvenile *A. carolinensis* (14 males, 12 females), 36-42 mm SVL, from the field and brought them back to the laboratory for use in behavior trials. Eight males and six females were randomly chosen for the T-implant group, and the remaining six males and six females were assigned to the blank-implant (control) group. Implants were made from Silastic tubing (Dow Corning; i.d. = 1.47 mm, o.d. = 1.96 mm) cut to a total length of 2-3 mm. T-implants contained approximately 0.5 mm packed crystalline T (Sigma), and blank-implants were empty. Both T- and blank-implants were closed at the ends with silicone sealant (Dow Corning). Within four days of coming to the laboratory, lizards were implanted subcutaneously, dorsolateral to the right hind leg, through a small incision in the skin that I closed with Vetbond tissue adhesive (3M). Lizards were cooled on ice for 5 min prior to surgery, and were returned to their home cages immediately following surgery.

To examine differences in behavior among sex and treatment groups (T and control), I videotaped juveniles 14-21 d post-implant. Lizards were moved to observation cages set up identically to housing cages and matched with two other lizards for subsequent behavior trials. Prior to the trials, lizards were isolated by opaque, removable partitions. All pairs were within 2

mm SVL, and were always from the same treatment group. At 16-24 h after moving lizards to the observation cages, trials were conducted by videotaping pairs of lizards for 15 min individually and then 15 min together following the removal of the partition. After the 30 min trial, the lizards were again separated by replacing the partition. The following day this procedure was repeated with different pairings. Thus, each lizard was observed in two trials in which it was exposed to a different lizard each time. I videotaped trials from a darkened blind, using a Panasonic AG 460 video camera fitted with an Aztec video telephoto converter (2.0X), and I subsequently analyzed videotapes using pre-printed checksheets. Overall, I ran 12 control trials (four male-male, four female-female, four male-female) and 14 T trials (six male-male, four female-female, four male-female). On the day of its last trial, each lizard was sacrificed and 15-25 μl of plasma was collected for confirming T concentrations in T- and blank-implanted lizards. I also confirmed that each lizard still had its implant. Plasma was handled and analyzed by RIA as described above. The samples were run in one assay (intra-assay CV = 6%).

To quantify behavior among trials from different treatment groups and social contexts, I used a behavior index (BI) following Lovern and Jenssen (in review) and modified from Ortiz and Jenssen (1982) that represented behavioral intensity. The behaviors in the BI were each assigned a point value that increased with increasingly socially-motivated behaviors (Table 4.1). I calculated BIs by summing the points of the observed behaviors for each lizard individually (individual BIs) before partition removal and also for each lizard (when examining characteristics of individual behavior) or for each pair of lizards (trial BIs; when examining characteristics of trials, e.g., differences in behavioral intensity among social contexts) following partition removal.

Statistical Analyses

Endogenous yolk T concentration was normally distributed (Kolmogorov-Smirnov test; $P > 0.15$), and was therefore analyzed by general linear model ANOVA (GLM) with hatchling sex and mother as main effects. However, endogenous plasma T concentration was not normally distributed ($P < 0.02$) and data transformation did not result in a normal distribution. Therefore, I used nonparametric Kruskal-Wallis tests to examine plasma T differences.

For behavior trials, I used Wilcoxon signed-ranks tests to compare paired BIs for individuals when they were alone and when they were in interactions (averaged from the two trials in which each lizard participated), and I used Kruskal-Wallis tests to examine main effects on BIs. Trial BIs were statistically independent from one another because each pair of interacting lizards was unique. When my objective was to examine specific behaviors, I averaged individual responses and examined results using Fisher's exact tests, chi-square tests, or Kruskal-Wallis tests. Descriptive statistics are reported as mean $\pm$ 1 SE. I used Minitab (version 10Xtra) for statistical analyses, and hypothesis tests were two-tailed with $\alpha = 0.05$.

Results

Endogenous Plasma T Concentrations

Plasma T concentrations significantly differed by class ($H_{13} = 57.8$, $P < 0.0005$; Fig. 4.1). Therefore, I used rank-based multiple comparisons (Hollander and Wolfe, 1973) to examine the relevant subset of all possible pairwise comparisons. Juvenile males generally showed increasing plasma T concentrations with size class, as lizards in size classes 1 and 2 had significantly lower T than lizards in size classes 4 and 5. Furthermore, for size classes 4 and 5, juvenile males had significantly higher plasma T concentrations than juvenile females. Breeding adult males had higher plasma T concentrations than any other class, followed by post-breeding adult males. Breeding and post-breeding adult females also had detectable plasma T, which was higher in breeding adult females.

Yolk T Concentrations

All eggs sampled contained detectable yolk T on the day of oviposition (Fig. 4.2). However, the eggs that gave rise to males had significantly higher yolk T concentrations than those that gave rise to females ($F_{1,18} = 7.9$, $P = 0.02$). There was no relationship between mother and yolk T concentration ($F_{7,18} = 0.9$, $P = 0.52$).

Lizards with T-implants had significantly higher plasma T concentrations than those with blank-implants (34.0 ± 2.3 and 0.4 ± 0.15 ng/ml, respectively; $H_3 = 19.6$, $P < 0.0005$). However, multiple comparison procedures indicated that males and females with blank-implants significantly differed in plasma T concentration (0.71 ± 0.2 and 0.02 ± 0.01 ng/ml, respectively), and exhibited similar plasma T concentrations to those of identically sized (size class 5) juvenile males (0.42 ± 0.14 ng/ml) and females (0.04 ± 0.03 ng/ml) sampled in the field ($H_1 = 0.9$, $P = 0.36$, and $H_1 = 0.1$, $P = 0.83$, respectively, for the comparisons between males and between females). The mean plasma T concentration for T-implanted juvenile lizards was 1.7 x higher than the mean, but within the range, of plasma T concentrations for breeding adult males (19.8 ± 2.7 ng/ml; min = 12.7, max = 36.7).

All lizards regardless of sex or treatment had higher behavioral intensities during interactions than when alone ($Z = 351$, $P < 0.0005$). However, trials between T-implanted juveniles were more intense than those between blank-implanted juveniles ($H_1 = 11.9$, $P = 0.001$). Trial BIs were not affected by whether encounters were male-male, female-female, or male-female, for either blank-implanted ($H_2 = 1.0$, $P = 0.62$) or T-implanted lizards ($H_2 = 2.5$, $P = 0.29$).

I examined individual responses to experimentally elevated T by averaging individual BIs across the two trials in which they participated. BIs were significantly higher in T-implanted than in blank-implanted juveniles, both for individuals while they were alone ($H_1 = 5.3$, $P = 0.02$, Fig. 4.3a) and during interactions ($H_1 = 10.8$, $P = 0.001$, Fig. 4.3b). Neither BIs from isolated lizards nor BIs from interacting lizards were affected by sex ($H_1 = 1.4$, $P = 0.23$; $H_1 = 1.1$, $P = 0.29$, respectively).

The higher BIs of T-implanted lizards, as compared to blank-implanted lizards, were the result of increases in rates or probabilities of nearly every behavior measured. During interactions, T-implanted juveniles were more likely than blank-implanted juveniles to give headbobbing displays with dewlap extension, use display modifiers, show body color changes,

and develop an eyespot (Table 4.2). Furthermore, 5 of 14 (36%) T-implanted lizards gave headbobbing displays while in isolation, whereas 0 of 12 blank-implanted juveniles did so (Table 4.2). Display rates of T-implanted juveniles were significantly higher than those of blank-implanted juveniles ($H_3 = 15.7$, $P = 0.001$; Fig. 4.4). T-implanted juveniles gave 88 ± 12 displays per hour, and blank-implanted juveniles gave 13.7 ± 3.1 displays per hour.

In addition to the display rate differences described above, the relative proportions of display types used differed between treatment groups ($X^2_2 = 7.0$, $P = 0.03$; Fig. 4.5). T-implanted juveniles gave 34% type A displays, 25% type B displays, and 41% type C displays. In contrast, blank-implanted juveniles gave 17% type A displays, 13% type B displays, and 70% type C displays. Both sexes responded to T-implants by increasing the relative proportions of display types A and B in relation to type C ($X^2_2 = 14.0$, $P = 0.001$; $X^2_2 = 50.5$, $P < 0.0001$, for males and females, respectively). However, although blank-implanted males and females did not differ in relative display proportions ($X^2_2 = 2.0$, $P = 0.37$), T-implanted males and females did differ ($X^2_2 = 39.2$, $P < 0.0001$). T-implanted males gave 19% type A, 28% type B, and 53% type C displays, and females gave 43%, 28% and 29% type A, B, and C displays, respectively.

Although T-implanted juveniles, regardless of sex, exhibited more types and higher rates of many behaviors, some behaviors were conspicuously absent. I did not observe physical contact between interacting lizards, although approaches and retreats (movements directly toward or away from the other lizard when interacting at a distance of < 30 cm) occurred in 36% (5 of 14) and 33% (4 of 12) of T and control trials, respectively. I also did not observe adult male-typical ritualized aggression (approach-circle-jaw lock), nor did I observe adult female-typical neck-bending, in any of the T or control trials.

Discussion

The present study is the first, to my knowledge, to document endogenous T concentrations during ontogeny in a natural population of lizards. I found that both juvenile male and female *A. carolinensis* had detectable plasma T and that T concentrations were the same or greater in juvenile males than in juvenile females. Plasma T concentrations in juvenile

males were in the range of those of breeding adult females (< 1 ng/ml) and considerably lower than those of breeding (20 ng/ml) or even post-breeding (11 ng/ml) adult males. Juvenile females had plasma T concentrations comparable to those of post-breeding females (< 0.1 ng/ml) and less than those of breeding females.

The variation in plasma T concentrations in juveniles during ontogeny suggests the following interpretation. For approximately the first two weeks after hatching, juveniles show declining plasma T concentrations. If hatchling T primarily comes from maternal T deposited into the yolk (see below), this decline suggests net T degradation, rather than net T production, in both sexes. Sometime after 14 d post-hatching, endogenous gonadal T production in males begins to exceed T degradation and plasma T concentrations increase. For the remainder of the juvenile growth period, male plasma T is 3- to 10-fold greater than that of females, although it remains 20- to 45-fold less than that of adult males, whether breeding or post-breeding. The comparatively high plasma T concentrations in hatchlings could be due to factors other than those suggested above. For example, they could be due to incubation conditions or stress in the laboratory (all other juveniles were sampled in the field). There are at least two reasons why this interpretation is unlikely. First, laboratory housing and other potential stressors generally cause a reduction of endogenous T, not an increase, in a wide variety of vertebrates including reptiles (e.g., Greenberg and Wingfield, 1987; Moore et al., 1991; Moore et al., 2000). Second, in the present study I found that blank-implanted juveniles housed in the laboratory for several weeks had the same plasma T concentrations as equivalently sized juveniles sampled in the field.

Epigenetic maternal effects on offspring phenotype, particularly via endocrine mechanisms, have become an exciting area of research (e.g., Birkhead et al., 2000). Maternally derived T has been documented in the egg yolks of birds (e.g., Schwabl, 1993; Gil et al., 1999; Lipar et al., 1999), turtles (with both TSD and GSD; Janzen et al., 1998) and alligators (with TSD; Conley et al., 1997). The amount of yolk T present to a developing embryo can affect post-hatching growth and behavior (Schwabl, 1993, 1996), and T may be deposited in higher concentrations when females are mated to higher quality mates (Gil et al., 1999). In the present study, I documented the presence of yolk T at oviposition in *A. carolinensis*, so maternal T also may influence development in this species. To my knowledge the present study is the first to

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report a sex difference in yolk T concentrations; specifically, T was higher in eggs containing male embryos than in those containing female embryos. I assumed that yolk T at oviposition would be of maternal origin, because embryonic gonadal tissue has not yet differentiated into morphologically distinguishable gonads in *A. carolinensis* (Forbes, 1956). That breeding females might differentially allocate T to developing males and females is surprising and difficult to explain. Anoline lizards have the unusual trait of laying single-egg clutches, alternately produced by the left and right ovary (Smith et al., 1973). Perhaps successive eggs are exposed to different steroid environments prior to oviposition, depending upon the maternal steroid profile during the yolking phase. If plasma T concentrations vary from one ovulatory cycle to the next within females, then differences in yolk T concentrations among eggs could result. While this could account for differences in yolk T concentrations among eggs, it remains unclear how maternal T concentration could correspond with embryo sex to produce higher yolk T concentrations for eggs giving rise to males.

Differential allocation of T by mothers is not the only possible explanation for the observed sex difference in yolk T. In comparison to avian embryos, reptilian embryos are well developed at oviposition. *Anolis* embryos, like those of most oviparous lizards, are at stage 30 in a 40 stage embryonic development sequence (Dufaure and Hubert, 1961; Robin Andrews, pers. commun.), approximately 20% through the time period between fertilization and hatching (the characters on which the developmental stages are based do not arise linearly by time). Thus, instead of differential T input by mothers, sex differences in yolk T at oviposition could arise by differential embryonic production of T or metabolism of maternally derived T. To resolve which of these possibilities occurs, future studies should attempt to replicate the present findings and measure the T content and steroidogenic capability of embryos of different ages.

My data for the effects of experimentally elevated T concentrations on behavior and the endogenous T data discussed above are together most consistent with the hypothesis that only activation by T is required for producing adult male-typical behavior in *A. carolinensis*. Juvenile males in this study were exposed to higher endogenous T than juvenile females. This is consistent with the possibility of an organizational effect on behavior followed by later activational effects when T varies seasonally in adults (Tokarz et al., 1998; Jenssen et al., in

review; present study). However, T-implants caused equivalent behavioral responses in juvenile males and females. Thus, comparisons of endogenous T and the effects of T implants on the two sexes argues for T affecting only activation. Juvenile males and females given T-implants, but not those given blank-implants, had markedly increased activity levels that approached those of breeding adult males tested under similar conditions (e.g., DeCourcy and Jenssen, 1994; Jenssen et al., 2000). Behaviors affected by T included headbobbing display rate (both while alone and during interactions) and the relative proportion of display types (A, B, and C) used, dewlap and display modifier use, body color change, and eyespot development. These results were consistent across social contexts (consexual and heterosexual interactions). The observation that some T-implanted juveniles displayed while isolated from other lizards was especially dramatic. None of the 12 blank-implanted juveniles displayed while alone, and in over 100 h of observations on untreated juveniles in a separate study, no displays were ever observed from lizards while they were alone, although nearly all of them displayed during interactions (Lovern and Jenssen, in review; Lovern, unpubl. data).

The observation that juveniles given T implants had higher activity levels even when they were not in interactions documents the extent of the effect of T on behavior. The stimulus of the presence of another lizard was not required to observe increased behavioral activity. Similar effects of T on general activity levels have been reported in adult males of several other species, including lizards (e.g., Marler and Moore, 1989; DeNardo and Sinervo, 1994; Klukowski et al., 1998) and birds (e.g., Chandler et al., 1994). Functionally, increased activity results in larger and more actively patrolled home ranges, and probably increased reproductive success through greater access to breeding females or greater ability to thwart intruding reproductive males (e.g., Marler and Moore, 1989; Chandler et al., 1994; DeNardo and Sinervo, 1994). However, there is also a cost, which may be especially high in juveniles and may help to explain their comparatively low plasma T levels; experimental elevation of plasma T can lead to reduced growth and higher mortality (e.g., Marler and Moore, 1988, 1989; Hews et al., 1994; Klukowski et al., 1998).

Although it clearly did not organize behavior, higher endogenous T concentrations in juvenile males than in juvenile females may affect morphological differentiation. For example,

T might initiate morphological differentiation in post-anal scale size, body length and mass, dewlap area, and underlying brain and peripheral structures associated with dewlap extension and courtship, all of which are sexually dimorphic in adults (Jenssen et al., 1995b; Wade, 1998; O'Bryant and Wade, 1999; Jenssen et al., 2000). Hatchlings develop post-anal scale dimorphism just prior to hatching (Pearson and Licht, 1974), and body length, mass, and dewlap area are not sexually dimorphic at hatching, but rather diverge during the course of post-hatching ontogeny (Gordon, 1956; Crews and Greenberg, 1981; Michaud, 1990). Thus, the timing of the differentiation of these morphological traits, coupled with the endogenous T concentrations documented in the present study, suggest that embryonic exposure to yolk T may be responsible for differentiation of post-anal scale size, and that juvenile plasma T may be responsible for differentiation of body size and dewlap area. Further work on the exact timing of morphological differentiation between males and females and how it is affected by T will be necessary to examine these possibilities.

Although juveniles showed a dramatic behavioral response to T, several behaviors that are seen in adults were not seen in juveniles. First, there was no evidence of ritualized aggression. The behaviors observed in juveniles given T implants suggest increased patrolling, territory advertisement, and heightened agonistic responses to other lizards. However, less than half of the interactions yielded close (< 30 cm) approaches, and none led to circling, jaw-locking, or indeed any physical contact. It is possible that longer interactions may have led to these behaviors, as I only observed lizard pairs for 15 min. However, responses tended to be intense but brief, typically declining prior to the end of the trial. By the 15 min point, none of the interactions that I observed appeared to be progressing in intensity, suggesting that the duration of the trial was not the main reason why ritualized aggression was not observed. Other potentially important factors could include residence time in the cage (e.g., Crews, 1980), length of exposure to T, or previous experience, each of which may have affected the probability of observing ritualized aggression. Second, copulation was never observed in juvenile interactions. Juvenile females never gave the characteristic neck-bend posture indicating receptivity. In contrast, gonadectomized adult females who are given T can exhibit either masculine courtship and mounting or feminine receptivity (e.g., Adkins and Schlesinger, 1979; Winkler and Wade, 1998). Although copulation was not observed and my sample size was small, interactions

between T-implanted juvenile male and female *A. carolinensis* did suggest at least attempted courtship by males. In three of four male-female interactions, males appeared to initiate the interaction by courting the female. Typical of courtship interactions, these males displayed towards the females, extending their dewlaps while approaching steadily. Aggressive intent appeared to be absent because no display modifiers were employed at this point. However, the females in each case immediately responded with aggressive display behavior, using their dewlaps and employing display modifiers. In response, each of the males shifted to employing aggressive behaviors as well, rapidly developing an eyespot and a change in body color to dark brown, and employing aggressive display modifiers. Thus, although the interaction clearly finished in an aggressive context, it may have begun (from the male's perspective) as a courtship interaction.

In conclusion, these data document that: (1) juvenile male and female *A. carolinensis* have detectable T in their plasma post-hatching and in the yolks of the eggs from which they hatch; (2) juvenile males have higher plasma T concentrations than juvenile females at least by the time they are > 30 mm SVL (approximately 38 d post-hatching); and (3) experimentally elevated plasma T concentrations produce increased expression and rates of behaviors in both juvenile males and females, similar to levels seen in breeding adult males. Together, these data are most consistent with the hypothesis that T activates, but does not organize, adult male-typical levels of behavior. Thus, sexual dimorphisms in behavior in adults likely arise through underlying physiological differences between males and females that mediate the expression of behavior, rather than through fundamental sex differences in the ability to perform these behaviors.

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Table 4.1. Behaviors, definitions, and point values used to score behavioral intensities for juvenile male and female *Anolis carolinensis* during isolation and pairwise interactions.

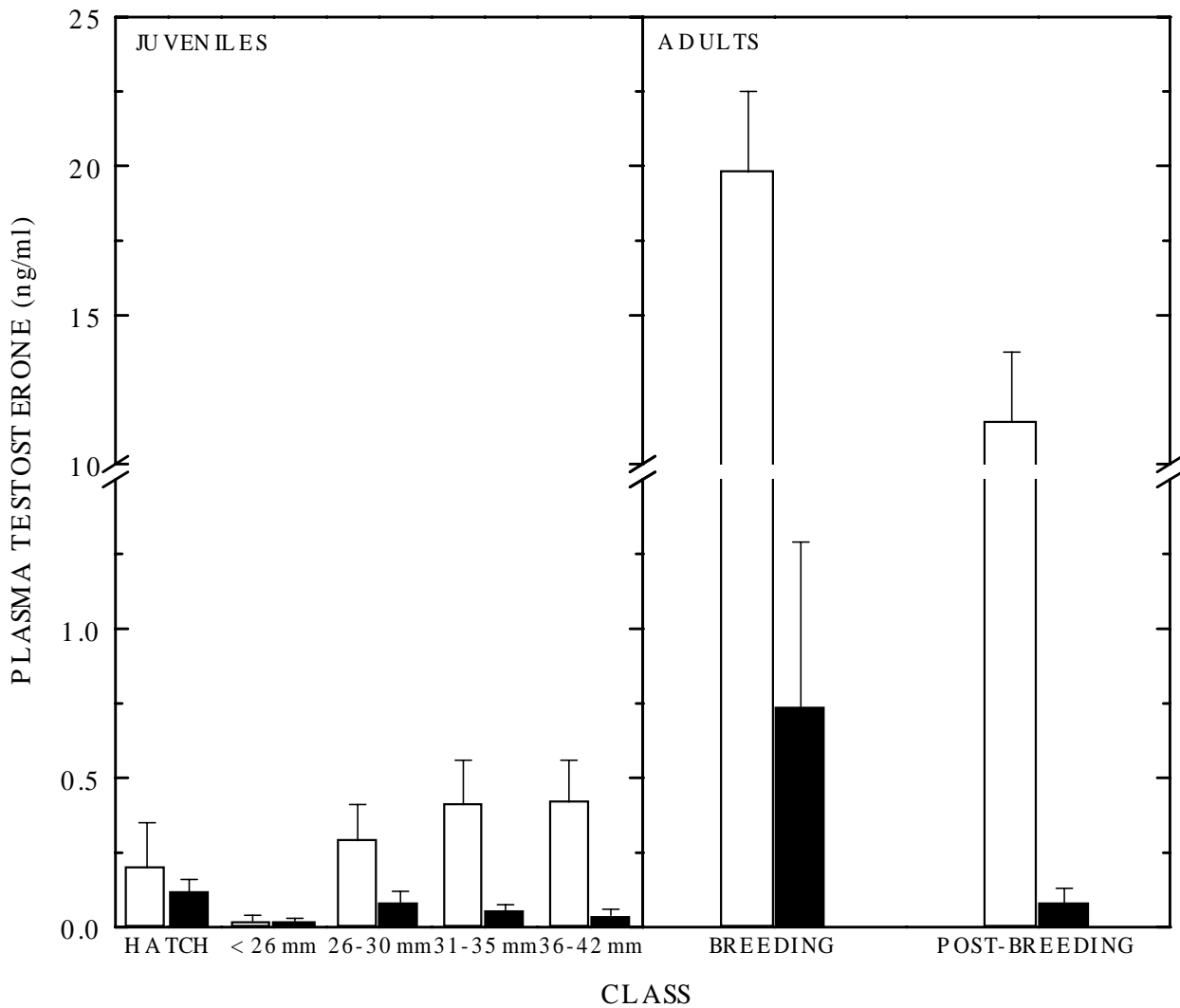| Behavior	Definition	Point value
Head-up[1]	Posture reflecting alertness to the environment; > 60 s (consecutively) with head raised higher than body	1
Perch shift	Any movement > 1 body length (excluding tail) from one perch site to another; movements > 15 s apart were scored as separate perch shifts	2
Tongue touch[2]	Potential chemosensory behavior involving brief touch of the tongue to the substrate	3
Color change[1,2]	Change in lizard body color between green, olive, or brown as a potential indicator of social stress or arousal	4
Headbob	Series of vertical head movements in species-specific temporal cadences used for communication; noted display type (A, B, C), separation distance between displaying lizards, and whether dewlap extension also occurred	5
Eyespot[1,3]	Development of dark spot posterior to each eye indicating increased adrenergic activity	6
Nuchal/dorsal crest[1,2]	Display modifier in which a flap of skin is erected along the neck and back	7
Engorged throat[1,2]	Display modifier in which the ventral throat area remains enlarged	7
Sagittal expansion[1,2]	Display modifier in which the lateral view of the lizard becomes enlarged	7
Approach/retreat	A perch shift directly toward or away from another lizard when the separation distance is < 30 cm	8
Attack	Lunge toward another lizard, within 10 cm, with an attempt at or actual physical contact such as biting	9

[1]These behaviors were scored a maximum of once per pairwise interaction trial for each lizard.
[2]Greenberg, 1977
[3]Hadley and Goldman, 1969

Table 4.2. Proportion of blank- and testosterone (T)-implanted juvenile male and female *Anolis carolinensis* expressing specific behaviors, and Fisher's exact test P-values comparing treatment effects. The number of males and females, respectively, that expressed each behavior are in parentheses.

Behavior	Blank-implant (6 males, 6 females)	T-implant (8 males, 6 females)	P
Headbobbing display	11/12 (6,5)	14/14 (8,6)	0.463
Displays with dewlap	6/11 (5,1)	13/14 (8,5)	0.017
Display modifiers			
Sagittal expansion	5/12 (3,2)	12/14 (7,5)	0.023
Engorged throat	4/12 (3,1)	12/14 (6,6)	0.009
Displays in isolation	0/12 (0,0)	5/14 (4,1)	0.030
Body color change	6/12 (4,2)	13/14 (7,6)	0.020
Eyespot	4/12 (4,0)	12/14 (6,6)	0.009

PLASMA TESTOSTERONE (ng/ml)

JUVENILES

ADULTS

25

20

15

10

1.0

0.5

0.0

HATCH   < 26 mm   26-30 mm   31-35 mm   36-42 mm   BREEDING   POST-BREEDING

CLASS

1	a	a	ab	b	b		d		c
2	a	a	a	a	a		b		a
3	ns	ns	ns	s	s		s		s
4					b a		d b		c a

Figure 4.1. Mean (+ 1 SE) plasma testosterone (T) concentrations for 50 juvenile male and 44 juvenile female *Anolis carolinensis* by size class (snout-vent length), and for 12 adult male and 25 adult female *A. carolinensis* by season. Males are represented by white bars, females by black bars. The adult male data are from Jenssen et al. (in review). The first juvenile class ("Hatch" = day of hatching) was sampled in the laboratory; all other classes were sampled in the field. Kruskal-Wallis test; $H_{13} = 57.8$, $P < 0.0005$. Results of the rank-based multiple comparisons (Hollander and Wolfe, 1973) are shown below the figure. Comparisons are only valid within rows; size and/or reproductive classes that do not share a letter designation are statistically different. 1 = comparisons of males by class; 2 = comparisons of females by class; 3 = within-class comparisons of males and females (s = significantly different; ns = not significantly different); 4 = comparisons of 36-42 mm juveniles and breeding and post-breeding adults of both sexes.

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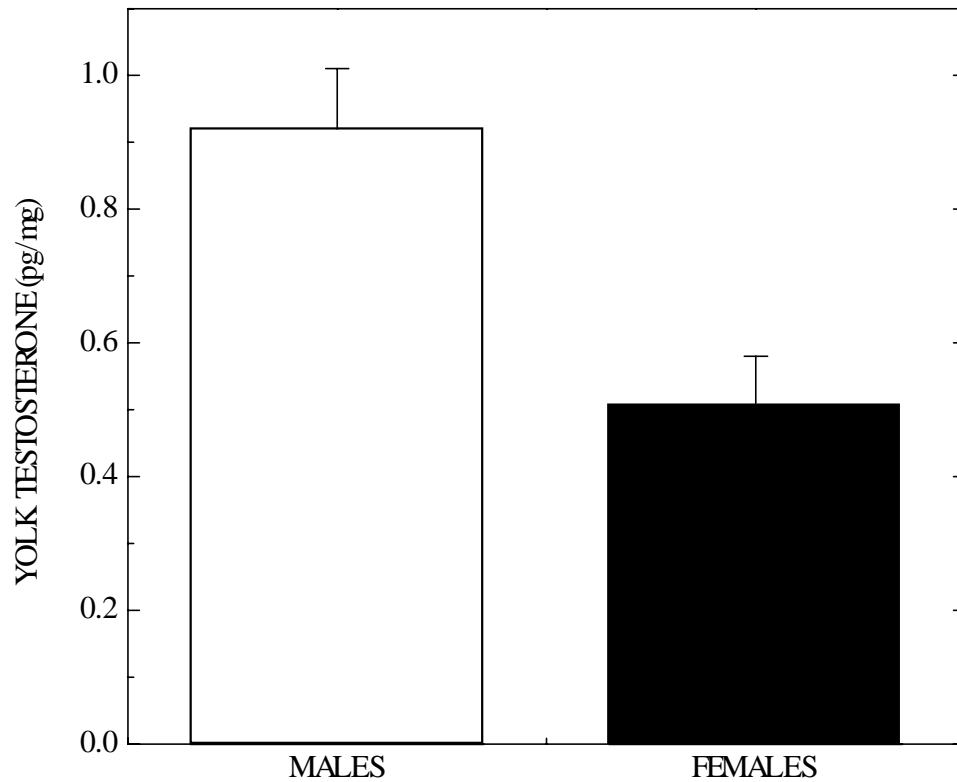
Figure 4.2. Mean (+ 1 SE) yolk testosterone concentrations from six eggs that gave rise to males (white bar) and 13 eggs that gave rise to females (black bar) for *Anolis carolinensis*. Yolk samples were collected from a total of eight mothers on the day eggs were laid. General linear model ANOVA: sex, $F_{1,18} = 7.9$, P = 0.02; mother, $F_{7,18} = 0.9$, P = 0.52.
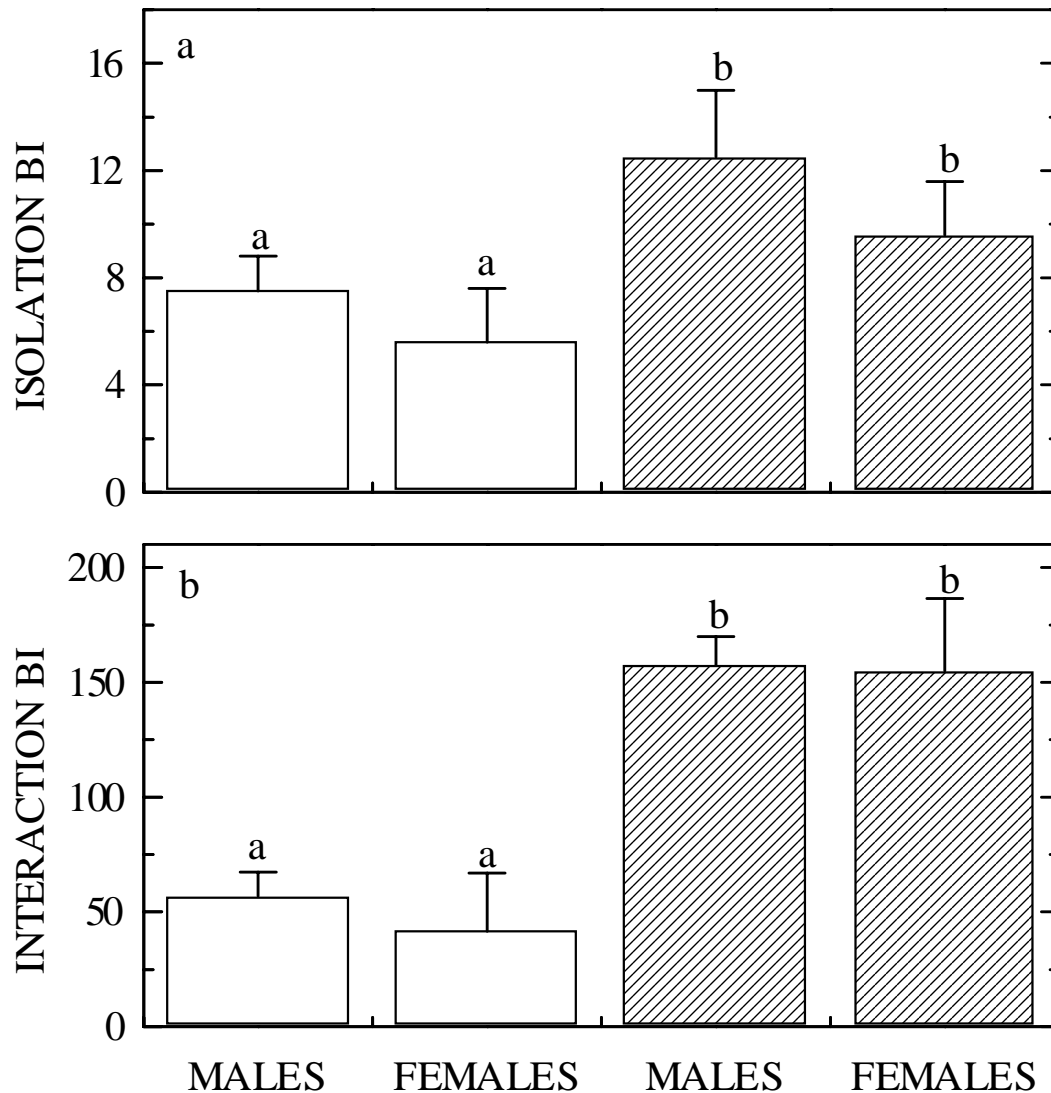
Figure 4.3. Mean (+ 1 SE) individual (a) isolation and (b) interaction behavior indices (BI) of juvenile *Anolis carolinensis* for six males and six females who received blank implants (white bars) and for eight males and six females who received testosterone implants (hatched bars). Isolation and interaction BIs represent behavior levels of individuals when alone and after introduced to another lizard, respectively. The BIs from the two trials in which each lizard participated were averaged to create their individual BIs. Bars designated with different letters are statistically different following Kruskal-Wallis tests.
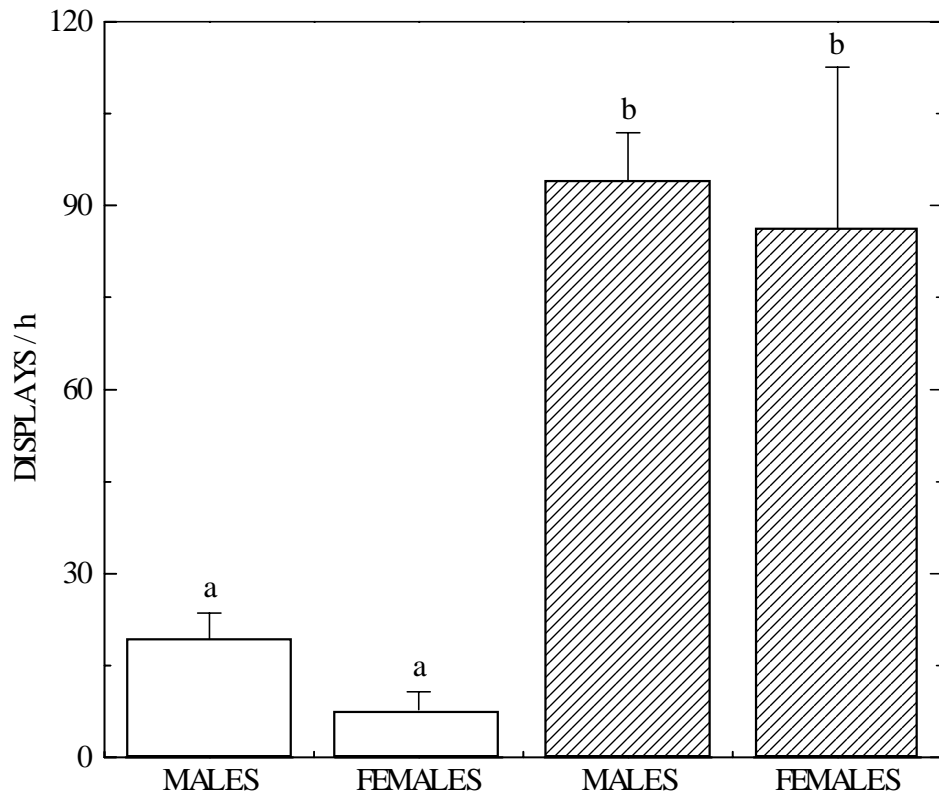
Figure 4.4. Mean (+ 1 SE) headbobbing displays per hour during pairwise interactions of juvenile *Anolis carolinensis* for six males and six females who received blank implants (white bars) and for eight males and six females who received testosterone implants (hatched bars). Kruskal-Wallis test: $H_3 = 15.7$, $P = 0.001$; bars with different letters (a or b) are statistically different based on nonparametric multiple comparisons.
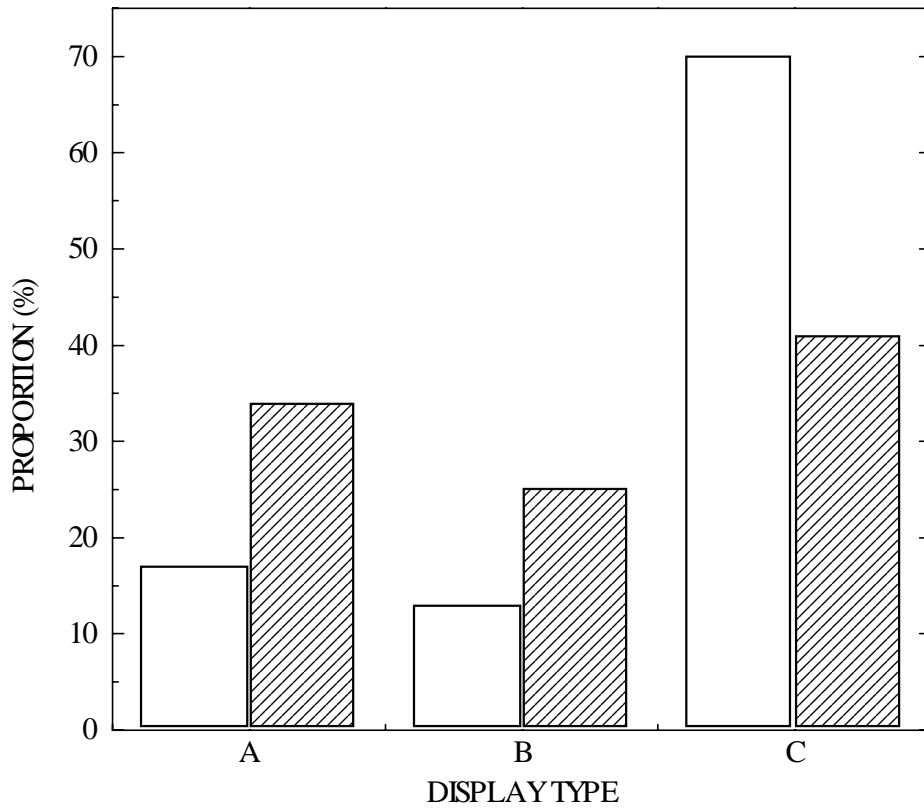
Figure 4.5. Relative proportion of headbobbing displays of juvenile *Anolis carolinensis* that were type A, B, and C for 12 juveniles (six males, six females) who received blank implants (white bars) and for 14 juveniles (eight males, six females) who received testosterone implants (hatched bars), based on 135 and 612 displays, respectively. Chi-square test; $X^2_2 = 7.0$, P = 0.03.

<div align="center">

CURRICULUM VITAE

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Birthdate, Place: 21 January 1971, Harrisonburg, Virginia, U.S.A.

Education: Ph.D., May 2000, Biology
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Professional Experience:

2000-present: Postdoctoral Research Associate, Department of Psychology, Michigan State University
1994-2000:   Graduate Teaching Assistant, Department of Biology, Virginia Polytechnic Institute and State University; Courses taught: Principles of Biology Laboratory (Majors and Non-Majors sections), Human Anatomy and Physiology Laboratory, Mammalogy Laboratory, Ornithology Laboratory
1993-1994:   Research Assistant, Department of Zoology, Duke University

Professional Activities:

2000:   Reviewer for: National Science Foundation, Journal of Herpetology
1998:   Local committee member, American Society of Mammalogists National Meeting, Blacksburg, Virginia, U.S.A.
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1995:   Chair, BGSA Seminar Committee

Principle Research Interests:

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Behavioral neuroendocrinology
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Professional Memberships:

Animal Behavior Society
Herpetologists' League
Society of Behavioral Neuroendocrinology
Society for Integrative and Comparative Biology
Society for the Study of Amphibians and Reptiles

Grants and Fellowships Received:

1995-1999:   Graduate Research Development Grant, Virginia Polytechnic Institute and State
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1998:   Theodore Roosevelt Memorial Fund Grant, American Museum of Natural History
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Publications:

Refereed:

Jenssen, T. A., Orrell, K. S., and Lovern, M. B. 2000. Sexual dimorphisms in aggressive signal
    structure and use by a polygynous lizard, *Anolis carolinensis*. Copeia 2000:140-149.
Lovern, M. B. 2000. Behavioral ontogeny in free-ranging juvenile male and female green
    anoles, *Anolis carolinensis*, in relation to sexual selection. J. Herpetol. 34:274-281.
Lovern, M. B., Jenssen, T. A., Orrell, K. S., and Tuchak, T. 1999. Comparisons of temporal
    display structure across contexts and populations in male *Anolis carolinensis*: signal
    stability or lability? Herpetologica 55:222-234.

Non-refereed:

Jenssen, T. A., Lovern, M. B., Orrell, K. S., and Passek, K. M. 1999. *Anolis carolinensis*
    unplugged: investigations into the behavioral ecology of the green anole. In: J. B. Losos
    and M. Leal (eds.), *Anolis* Newsletter V, pp. 44-54.

Presentations:

Jenssen, T. A. (presenter), Lovern, M. B., and Congdon, J. D. Social, gonadal, endocrine and
    feeding transitions during the winter/spring dispersal of *Anolis carolinensis*: a test of the
    polygyny threshold model. AES, ASIH, HL, SSAR joint meeting, State College,
    Pennsylvania, U.S.A., June 1999.
Lovern, M. B. Sex, lies, and videotape: the ontogeny of sex differences in behavior in the green
    anole lizard, *Anolis carolinensis*. Department of Biology, Virginia Polytechnic Institute
    and State University, 25 April 2000.
Lovern, M. B. Sex differences in behavior and androgen profiles during ontogeny in green
    anoles? Department of Psychology, Michigan State University, 24 September 1999.
Lovern, M. B. (presenter), and Jenssen, T. A. Sex, lies, and videotape: the ontogeny of sex
    differences in *Anolis carolinensis*. AES, ASIH, HL, SSAR joint meeting, State College,
    Pennsylvania, June 1999.
Lovern, M. B. (presenter), McNabb, F. M. A., and Jenssen, T. A. Androgen levels and their
    behavioral and morphological correlates in juvenile *Anolis carolinensis*. Society for
    Integrative and Comparative Biology annual meeting, Denver, Colorado, U.S.A., January
    1999.

Presentations – continued:

Lovern, M. B. (presenter), Jenssen, T. A., and Orrell, K. S. Comparisons of temporal display structure across contexts and populations in male *Anolis carolinensis*: signal stability or lability? AES, ASIH, HL, SSAR joint meeting, Guelph, Ontario, Canada, July 1998.

Lovern, M. B. (presenter), Jenssen, T. A., and Orrell, K. S. Comparisons of temporal display structure across contexts and populations in male *Anolis carolinensis*: signal stability or lability? Virginia Academy of Science annual meeting, Fairfax, Virginia, U.S.A., May 1998.

Lovern, M. B. A behavioral profile of free-ranging juvenile male and female green anoles. Animal Behavior Society annual meeting, College Park, Maryland, U.S.A., June 1997.

Lovern, M. B. A behavioral profile of free-ranging juvenile male and female green anoles. Virginia Academy of Science annual meeting, Blacksburg, Virginia, U.S.A., May 1997.

Lovern, M. B. A behavioral profile of free-ranging juvenile male and female green anoles. Department of Biology, Virginia Polytechnic Institute and State University, 8 April 1997.

Sherer, J. (presenter), Podos, J., Lovern, M., Peters, S., and Nowicki, S. Vocal development and the use of beak movements during song. Animal Behavior Society annual meeting, Seattle, Washington, U.S.A., July 1994.