The Green Anole (*Anolis carolinensis*): A Reptilian Model for Laboratory Studies of Reproductive Morphology and Behavior

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Abstract

The green anole (*Anolis carolinensis*) is an excellent reptilian model for studying reproductive behavior and the neural and muscular morphology that supports it. This lizard has been the subject of behavioral and ecological study for more than 100 yr, and a rich literature exists on its natural history. Both courtship and copulatory behaviors reveal sex and seasonal differences, which allow for the study of mechanisms regulating naturally occurring variation in performance at multiple levels within a single animal model. Green anoles are readily obtained due to their abundance in the wild; once in the laboratory, they are easily maintained, bred, and reared. Background on the natural history and husbandry of this lizard is provided, and the authors' research program on the regulation of reproductive anatomy and behavior is reviewed. Discussion includes the similarities and differences in the mechanisms mediating both structure and function compared with more traditional animal models. This type of comparative research will make it possible to identify the fundamental principles governing reproductive biology, thus advancing both basic and applied knowledge.

Key Words: *Anolis;* behavior; lizard; neurobiology; reproduction; reptile; sexual dimorphism; steroids

Introduction

Individuals within a species can express dramatic differences in behavioral function and, in parallel, the morphology of the central and peripheral structures that support it. For example, in diverse species—including fishes, amphibians, reptiles, birds, and mammals—sex and/or seasonal differences in reproductive morphology and behavior can be pronounced, and such differences are frequently regulated by gonadal steroids (see reviews in Cooke et al. 1998; Emerson 2000; Rhen and Crews 2002). In general terms, these hormones tend to act as follows: Early in development, they permanently organize anatomy, which results in the display of specific behaviors after sexual maturity; later in adulthood, they transiently activate changes in structure and function on a short-term (e.g., seasonal) basis (Arnold and Breedlove 1985). However, dimorphisms in reproductive anatomy and behavior are not always regulated by steroids, and even when they are, species differ in which hormone is important, at what ages, and in which sex it acts (Arnold 1997; Emerson 2000; Wade 1999). Comparative research can help provide answers regarding why this variability might exist in clearly fundamental mechanisms such as those regulating reproduction. Insights will come not only from uncovering common mechanisms, but also from considering differences across vertebrate systems as they relate to factors such as the ecology and phylogenetic histories of individual species.

Successful reproduction requires a suite of behaviors that is performed using multiple neuromuscular systems, all of which are controlled by higher centers in the brain that coordinate the behaviors with appropriate external stimuli (i.e., environmental and social conditions). To understand the mechanisms controlling reproduction, a model species ideally will allow experimentation at these behavioral, neural, and muscular levels. Many reptiles present just such an opportunity. For example, in addition to often dramatic courtship displays, male lizards and snakes have two bilateral copulatory organs called hemipenes. Thus courtship and copulatory behaviors, their underlying neuromuscular systems, and associated higher brain centers may be investigated in the same species. In our laboratory, we have focused on the green anole (*Anolis carolinensis*) in an effort to understand the mechanisms regulating sex and seasonal differences in male courtship and copulatory behaviors and the neural and muscular structures controlling them.

Green anoles are excellent reptilian models for investigating the multiple components of the reproductive circuit described above. Male courtship involves the use of highly conspicuous headbobbing displays coupled with the extension of a bright red throat fan, called a dewlap. When courtship is successful, males copulate with receptive females by intromitting one of their hemipenes. The neuromuscular systems controlling dewlap use in courtship and hemipene use in copulation, as well as the limbic brain regions involved in reproduction, have been identified (see below).

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In the text below, we briefly discuss the general advantages that many reptiles offer as laboratory models, give background on the *Anolis* genus and the natural history for *A. carolinensis* in particular, and detail our husbandry techniques for laboratory maintenance. We then discuss male and female reproductive behavior and associated neural and muscular morphology, reviewing current knowledge about sex and seasonal influences. We conclude by suggesting future directions that should yield additional insight into green anole reproductive function and, more generally, the mechanisms that regulate nervous system structure and function in vertebrates.

Reptiles as Laboratory Models

Reptiles offer numerous advantages for laboratory studies of the relationship between structure and function and the factors that influence it (reviewed in Crews and Gans 1992; Greenberg et al. 1989). First, because of the ease of obtaining many reptile species from the field, colonies may be continuously replenished with wild stock to obtain field-relevant laboratory results. This replenishment may not be characteristic of animal models that have been bred in laboratories for generations because individuals in such colonies may become genetically distant (intentionally or otherwise) from the wild stock from which they originated. Thus experimental results may not reflect how the wild stock would have performed under the same conditions, and it may be difficult to draw conclusions about naturally occurring traits or mechanisms.

A second advantage of reptiles as models relates to their remarkable variety of life histories, which can be utilized for comparative study. For example, simply considering reproductive traits, some reptiles are oviparous and some are viviparous (even within the same genus, e.g., *Sceloporus* lizards; Méndez-De La Cruz et al. 1998). Some reptiles show genotypic sex determination in which the male is heterogametic (i.e., males possess two distinct sex chromosomes conventionally labeled X and Y, whereas females possess two copies of the X chromosome, like mammals), some in which the female is heterogametic (males are conventionally designated ZZ, females ZW, like birds), and some have no sex chromosomes. In this latter case, gonadal sex is determined by the temperature experienced during embryonic development (Bull 1980; Robert and Thompson 2001; Wibbels et al. 1994). Different combinations of these traits may also be found among close relatives—within families, genera, or even different populations of the same species (reviewed in Viets et al. 1994).

A third important characteristic of reptiles is their common ancestry with birds. Reptiles and birds form a monophyletic clade, from which no other extant species has descended (Pough et al. 2001). This evolutionary lineage, along with mammals, represents all extant amniotes (Pough et al. 2001). Research using reptiles thus provides an evolutionary perspective on the origin(s) and maintenance of particular mechanisms.

A fourth advantage of studying reptiles stems from a practical standpoint. Reptile maintenance in the laboratory can be simple and cost effective without sacrificing ecological relevance, an important consideration for generating results with external validity (e.g., Miller 1994). In other words, it is possible to create naturalistic habitats, perhaps with greater ease than for many avian and mammalian models. This possibility provides an opportunity to study a full range of activities, as would be seen in the field.

Description of *Anolis*

Anolis is one of the larger vertebrate genera, comprising approximately 400 species recognized to date (Pough et al. 2001). Most of these species are native to Central and South America and/or the Caribbean islands, although introductions and invasions have occurred elsewhere. The members of this genus are insectivorous and typically quite small (~5 g, <100 mm snout to vent length, SVL[1]), although not always (e.g., *Anolis equestris* can be >50 g and 200 mm SVL). Anoles occupy a variety of habitats and climates from grassland to forest, tropical, and temperate zones, filling niches ranging from primarily ground-dwelling to tree-crown dwelling (e.g., Schwartz and Henderson 1991; Williams 1969). They are perhaps best known for their dewlaps, or extensible throat fans, used in communicative contexts (e.g., territorial or sexual encounters), which can vary remarkably across species in color, size, and degree of sexual dimorphism (Jenssen 1977; Schwartz and Henderson 1991; Williams and Rand 1977).

In contrast to all other members of the genus, *A. carolinensis* is native to North America, occurring throughout the southeastern United States with introduced populations on the Hawaiian islands (Conant and Collins 1998; McKeown 1996). The green anole is also by far the most well-studied member of the genus, with studies dating well before 100 yr ago (e.g., Monks 1881). From a multitude of field and laboratory investigations into the demography, ecology, and behavior of *A. carolinensis,* a fairly complete picture of the natural history of this species exists. The overview presented below is synthesized from the following studies: Andrews (1985a,b); Crews (1980); Gordon (1956); Greenberg and Noble (1944); Jenssen and Nunez (1998); Jenssen et al. (1995, 1996, 2001); Lovern (2000); Michaud (1990); Nunez et al. (1997); and Ruby (1984).

Adult males and females average 55 to 65 mm SVL and 45 to 55 mm SVL, respectively (population SVLs tend to increase with northern latitude). Breeding occurs on a seasonal basis roughly from April through July, when gonads are fully recrudesced and levels of sex steroids and behavior are maximal ("associated reproductive pattern," Crews and

[1]Abbreviations used in this article: AmbIX/VIImv, AmbX, dewlap motoneurons; AMY, ventromedial nucleus of the amygdala; DHT, 5α-dihydrotestosterone; E2, estradiol-17β; POA, preoptic area; RPM, retractor penis magnus muscle; SVL, snout to vent length; TPN, transversus penis muscle; UVB, ultraviolet B.

Moore 1986). During the breeding season, males establish large territories, which they defend vigorously against other males, and these areas encompass multiple, smaller territories of females in an effort to maintain exclusive reproductive access. Each male territory overlaps those of approximately three females; thus the social organization is polygynous. The determining factor for the number of resident females in a male territory is body size; larger males encompass more female territories than do smaller males, many of which may be excluded from establishing a territory at all.

There is no evidence of direct female choice of males. Rather, females appear to establish their territories (primarily for availability of food and shelter), independent of male location, and then mate with the male that successfully establishes an overlapping territory (e.g., "indirect female choice," Wiley and Poston 1996). However, females may mate with more than one male if other males are present (e.g., neighboring territorial males, or nonterritorial males that have escaped detection by those with territories). Mating occurs over the course of the entire 4-mo breeding season, and females lay single-egg clutches at approximately 7- to 14-day intervals. After the eggs are buried in the substrate in a damp and concealed location, there is no additional parental care. Approximately 4 to 6 wk later, depending on the temperature and moisture conditions, a hatchling emerges and is immediately responsible for finding food and shelter and for evading potential predators (mainly birds). Hatchlings voraciously eat small insects and other invertebrates to grow as quickly as possible and to accumulate fat stores before the end of the summer, when food will become scarce. Most of these hatchlings will be reproductively active in the subsequent breeding season, although the smaller males may have difficulty competing for a territory.

During autumn and winter, green anoles (adults and juveniles alike) are relatively inactive. They do not hibernate but may spend days or weeks, sometimes clumped together in large groups, in locations with protection from the weather (e.g., in tree cavities, under fallen logs). On warm days, they may bask in the sun. They eat infrequently even if food is available. Because they are ectothermic, if they eat and the temperature drops, they will be unable to digest the food, which may then decay in their gastrointestinal tract. Thus over winter, lizards rely on their slowed metabolic activity and fat stores. Green anoles in the wild typically live through one or two breeding seasons beyond their summer of hatch, but they may live several years longer in captivity.

Laboratory Maintenance of *A. carolinensis*

Procurement

Green anoles can easily be obtained for the establishment of a laboratory research program. The most convenient option may be to purchase them from commercial suppliers (e.g., Charles Sullivan Co., Nashville, TN). Lizards are priced at less than $5.00/individual but may not be readily available year-round (e.g., they can be more difficult to find in the winter). Commercial vendors collect individuals from the field and ship them within days of capture. Alternatively, researchers themselves can collect green anoles. Most states have collection permits that can be renewed annually and cost $50 to 100/year. This source may be much more cost effective (although more time consuming), and it provides certainty regarding the collection history of each individual (i.e., where they were found and how they were handled).

Lizards can be captured by hand or by constructing a noose made of monofilament line attached to the end of a long pole (e.g., fishing rod). The noose can be slipped slowly around the lizard's head (they typically do not flee if the collector is steady and patient), allowing one to remove individuals from areas difficult to reach by hand. Lizards should be handled by the body, not by the tail because it can break off (autotomize) and allow the lizard to escape. The sex of the individuals can be determined quite easily by examining the postanal scales. Only males have two enlarged scales just caudal to the cloaca (Figure 1).

At least two issues should be considered when one purchases or collects green anoles for laboratory research. First, researchers should ensure that individuals have come from the same general collection area. Numerous population differences in life history traits have been documented that could confound results if a laboratory colony comprised individuals from different locales. Population differences exist in size at reproductive maturity, egg and hatchling size, and growth rate (Michaud 1990; Michaud and Echternacht 1995). Population differences also exist in the fine temporal structure of the species-specific headbobbing displays (Lovern et al. 1999), although it is not known whether these differences lead to interpopulation behavioral incompatibilities. Second, although *A. carolinensis* is the only anole endemic to North America, numerous congeners have been introduced and are now thriving, particularly in Florida. It is generally not difficult to distinguish among the different species; however, the recent establishment of the Cuban green anole (*Anolis porcatus)* in southern Florida (Meshaka et al. 1997) may be more problematic because this anole is extremely similar to *A. carolinensis* morphologically.

Housing Requirements

Green anoles can be housed in the laboratory under environmental conditions that mimic breeding and nonbreeding times of the year, resulting in the expression of all behaviors observed in the field including territoriality, courtship, mating, egg-laying, and hatching. The main environmental features important in regulating seasonality are photoperiod and temperature (Licht 1967, 1971, 1973). Animals can be maintained in reproductive condition in the laboratory by

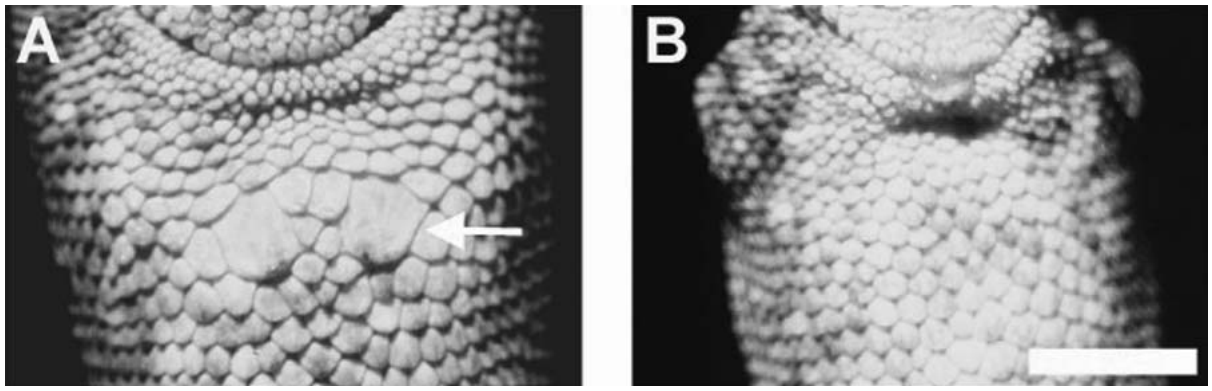

Figure 1 Photomicrograph of the cloacal regions of an adult (A) male and (B) female green anole, *Anolis carolinensis,* demonstrating the male-biased sexual dimorphism in postanal scale size (indicated by the arrow). Scale bar = 2 mm.

using long days and relatively high temperatures (we use lights on a 14:10 light:dark cycle with an ambient room temperature varying from 28°C during the day to 19°C at night). Animals can be kept reproductively inactive with short days and relatively low temperatures (we use lights on a 10:14 light:dark cycle with an ambient room temperature varying from 24°C during the day to 15°C at night). For the transition between breeding and nonbreeding conditions, we use a 2-wk intermediate condition in which lights are on a 12:12 light:dark cycle and ambient room temperature varies from 26°C to 17°C.

Across all conditions, we set relative humidity at 70%. This level ensures that animals will not be in low-humidity stress, despite their reproductive activity level (Summers 1988). A major advantage of having precise environmental control is that we are able to extend the breeding season artificially and gain more opportunities for conducting research on reproduction. We have kept animals under nonbreeding environmental conditions for as little as 12 wk (rather than the approximately 32 wk experienced in the field) and observed them initiating reproduction with the onset of longer days and higher temperatures.

Adult green anoles can be kept in the laboratory individually or in groups of one male and several females. Males housed together display considerable aggression under breeding (but not nonbreeding) conditions. Most animals are housed in 110-L glass aquaria (76 × 30 × 48 cm) that have tightly fitted metal screens (6-mm mesh size) tops (Figure 2A). These aquaria are sufficient for housing one male and up to eight females. We house individual males, females, or male-female pairs in 21-L glass aquaria (42 × 20 × 24 cm) when necessary for experimental design. All animals are toe-clipped for permanent, unambiguous identification. We have never observed this procedure to affect lizard health or locomotor activity adversely. If lizards will be housed only temporarily or never in groups, alternatives to permanent toe-clips may be more justified (e.g., marking with nontoxic paint, which comes off when lizards shed their skin; or not marking at all if the individual will be housed in isolation, in which case only the cage needs to be labeled with the relevant information). We place dividers between adjacent cages so that immediately neighboring males do not see each other.

In addition to fluorescent overhead room lights, on top and to one side of each cage we place a basking spotlight with a 60-watt incandescent bulb (40-watt for the smaller,

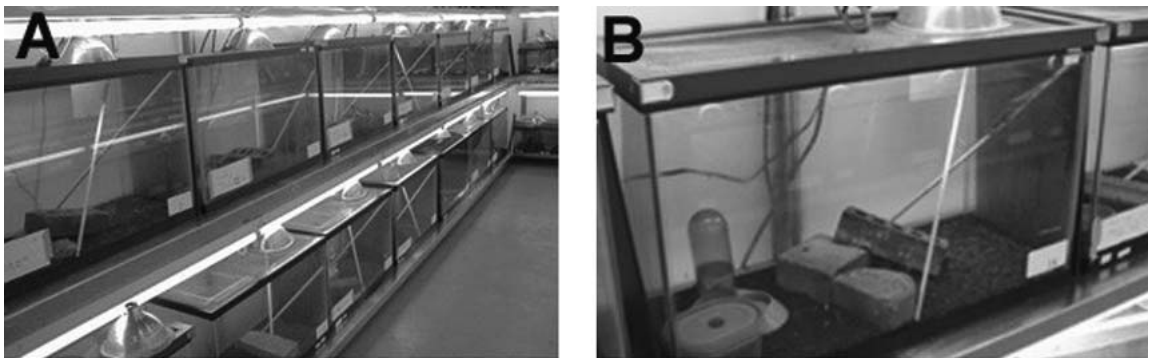

Figure 2 Photographs depicting (A) the overall organization of cages and (B) the arrangement of a typical cage for our laboratory colony of green anoles, *Anolis carolinensis*. See text for details.

individual cages). This addition provides a thermal gradient within the cage, increasing the temperature up to 10°C warmer than room temperature (as measured directly under the light). Finally, near the tops of the cages, we place lights that emit ultraviolet B (UVB[1]) in 285-320-nm wavelengths (e.g., from Duro-Test, Philadelphia, PA; or Zoo Med Laboratories, Inc., San Luis Obispo, CA). UVB is an important component of sunlight that is lacking in most sources of artificial light, and exposure to it is necessary for vitamin D_3 production, which is essential for calcium and phosphorous metabolism (e.g., Gehrmann 1994). These lights are as near the cages as is practically possible because UVB output decreases exponentially with distance. Many common materials (e.g., glass, plastics) can substantially or completely filter out UVB wavelengths, but screen allows the majority of UVB to pass through. We replace these lights annually because UVB output diminishes with time, even though visible light is still emitted.

The essential components of the cage itself are the substrate, the perching, basking, and hiding sites, an area for egg-laying (if housing reproductively active females), and a water dish (Figure 2B). The types of materials that can be used to satisfy these requirements are numerous, which is an advantage given that animal care committees from one institution to another may differ in their opinion of suitability. We have successfully used the following materials to set up naturalistic laboratory habitats at Michigan State University:

- Sphagnum peat moss substrate covers cage bottoms to a depth of approximately 4 cm.
- Wooden dowels (1-cm diameter) make excellent perches, similar to natural tree branches. Dowels can be leaned against the sides of the cage, or propped by securely arranged rock formations that allow basking and crevices into which lizards can retreat.
- Small (0.5- to 1.0-L) plastic dishes (with one hole cut in the top) serve as nestboxes and can be kept 2/3 full of peat moss dampened with distilled water. Females will retreat readily into these containers to oviposit, particularly if the cage substrate itself is not kept damp (in which case females may lay their eggs anywhere in the cage, making it difficult to find the eggs).
- Each cage contains a dish filled with fresh water. These dishes are not necessary because green anoles do not drink standing water but instead drink droplets that collect on leaves from dew or rain (which we simulate by spraying cages with water; see below). Nevertheless, many animal care committees may require dishes, which create no problem because the water is kept shallow. They provide additional structure in the cage for perching on and hiding under.

Animal Care and Husbandry

As with any animal model, maintaining green anoles in the laboratory requires considerable attention. We spray the sides of the cages with distilled water daily. Adults are fed with live crickets (*Acheta domesticus*) or darkling beetle larva (mealworms; *Tenebrio molitor*) twice per week during the nonbreeding season and three times per week during the breeding season. These insects are purchased in bulk and shipped overnight from Fluker Farms (Port Allen, LA); other vendors also exist. We purchase 2-wk-old or 3-wk-old crickets (approximately 0.75- to 1.5-cm length), and "medium" mealworms (approximately 2 cm long). Crickets are maintained in 114-L plastic bins and provided with dry food ("High Calcium Cricket Feed," Fluker Farms) and wet food (quartered apples, oranges, or potatoes). Mealworms are maintained in 5-L plastic bins and given a mix of oatmeal and cricket feed. They can be refrigerated to reduce their rate of development significantly. Each of these prey sources can also be bred with little difficulty to supplement purchases. Just before feeding the lizards, we dust crickets (but not mealworms) with a 2:1 mix of calcium powder:vitamin supplement (Rep-Cal Research Laboratories; Los Gatos, CA), which increases their nutritional value. The live prey items are subsequently placed into the cages (approximately 2-4 per lizard, depending on prey item size).

When lizards are reproductively active, we check nestboxes each day for eggs. They are incubated individually in plastic 266-mL cups, sealed at the top with plastic wrap and a rubber band, and partially buried in a mixture of 15 g of vermiculite to 15 mL of distilled water. This amount of substrate provides sufficient moisture and air within the sealed cup so that it does not require rewetting or aerating during incubation. We incubate eggs at 27 to 28°C, which produces hatchlings in approximately 34 days and typically yields a success rate of 80 to 90%. A wide range of tolerance for incubation temperature exists in this species; a majority of eggs will hatch at temperatures ranging at least from 21 to 32°C, with higher incubation temperatures producing shorter incubation durations (e.g., Viets 1993).

Although much of the care is similar, juveniles require more attention than adults. We check the incubator daily for new hatchlings and can identify sexes by postanal scale size as with adults. Because of their small size (approximately 250-300 mg, 21-24 mm SVL), we house up to 20 hatchlings per 110-L glass aquarium with fine screen (2-mm mesh size) tops. Juveniles are neither reproductive nor territorial, so multiple males can be housed together because they will not fight. Juvenile cages contain the same furnishings as adult cages, with the exception of nestboxes. They also contain artificial greenery to provide additional structure for climbing, hiding, and basking. This addition mimics conditions experienced by juveniles in the field because they tend to stay low to the ground in bushy vegetation, rather than occupying habitat space higher off the ground in trees like the adults (Lovern 2000). We spray and provide food in juvenile cages every day. Juveniles are fed baby ("pinhead") or 1-wk-old (< 3 mm long) crickets, which have been dusted with calcium powder and vitamin supplement (see above), as well as vestigial-winged (flightless) fruit flies. These food items can be purchased or bred in the laboratory.

Reproductive Behavior and Morphology

Description of Reproductive Behavior

Members of the genus *Anolis* primarily communicate using species-specific, stereotypic signals called headbobbing displays (Jenssen 1977). These displays are performed by both male and female adults and are used to mediate aggressive (typically male-male and female-female) and sexual (male-female) encounters alike. Their structure and use are well described (for *A. carolinensis,* see Crews and Greenberg 1981; DeCourcy and Jenssen 1994; Greenberg 1977; Jenssen and Nunez 1998; Jenssen et al. 2000; Lovern et al. 1999; Nunez et al. 1997; Orrell and Jenssen 2003). In this article, we focus on display use during reproductive encounters (see references above for details about display use in other social contexts).

Adult males and females possess a common display-type repertoire, but they differ dramatically in display use. The dewlaps of males (Figure 3) are approximately seven times larger than those of females, and males use dewlaps in conjunction with headbobbing displays more often than females (Jenssen et al. 2000). During courtship, both males and females perform headbobbing displays; however, male displays almost always include dewlap extension, whereas female displays rarely, if ever, use the combination (Greenberg and Noble 1944; Nunez et al. 1997). As courtship progresses, the male approaches the female, continuing to display and extend his dewlap. If she is receptive, she remains stationary, frequently bobbing her head and often adopting a characteristic "neck-bend" posture. The male then bites the female on the back of the neck as he mounts, everts one of his two bilateral, independently controlled hemipenes, and intromits (Figure 4). Copulation duration can be quite variable, lasting from less than 5 min to close to 60 min (Greenberg and Noble 1944; Jenssen and Nunez 1998).

Neuroendocrine Effects on Reproductive Behavior

Like the behaviors described above, endogenous steroid levels show sex as well as seasonal differences in a manner typical of that seen in many other vertebrates. Plasma testosterone levels are relatively high in males during the breeding season (approximately 10-fold higher than circulating levels in mammals) and are reduced by about 50% in the nonbreeding season (Lovern et al. 2001). Females ovulate a single follicle at a time, alternating between the two ovaries (Smith et al. 1973). The egg is subsequently fertilized and shelled in the ipsilateral oviduct before it is laid

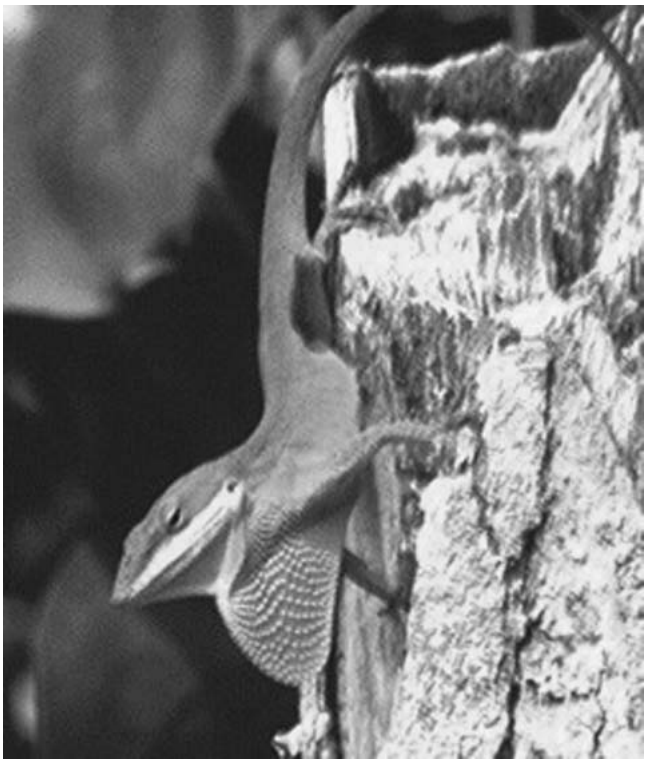

Figure 3 Adult male green anole, *Anolis carolinensis,* extending his throat fan or dewlap. Dramatic male-biased sexual dimorphism in dewlap size characterizes green anoles. Photograph courtesy of Thomas A. Jenssen.

Figure 4 Copulating adult male and female green anole, *Anolis carolinensis*. The male is biting and holding onto the back of the female's neck and wrapping his tail under hers to facilitate intromission. Photograph courtesy of Thomas A. Jenssen.

(Conner and Crews 1980). Because at any one time it is common to have a corpus luteum present, plasma progesterone levels remain consistently high in the breeding season (across multiple ovulatory cycles) compared with the nonbreeding season (Jenssen et al. 2001; Jones et al. 1983). In contrast, estradiol-17β ($E2^1$) varies during the breeding season across the ovulatory cycle (Crews 1980; Jones et al. 1983). Although plasma testosterone levels are a fraction of levels in males, they do also vary in females. Levels of testosterone are higher in breeding compared with nonbreeding females, and within breeding females, they are higher when females are likely to be receptive than at other stages in their cycle (Lovern and Wade 2001; Lovern et al. 2001). Thus, we know that both plasma E2 and testosterone fluctuate across the ovulatory cycle (Jones et al. 1983, Lovern and Wade 2001). Yet we do not know how the fluctuation patterns seen for E2 and testosterone relate to each other because these steroids have not been measured across the ovulatory cycle of female green anoles in the same study.

Experiments in which steroid levels have been manipulated have clearly demonstrated that testosterone is critical for stimulating male sexual behaviors. Castration reduces the display of male reproductive behaviors, and testosterone can prevent or reverse that effect (Adkins and Schlesinger 1979; Crews et al. 1978; Mason and Adkins 1976; Rosen and Wade 2000; Winkler and Wade 1998). In a number of vertebrate systems, testosterone acts as a pro-hormone; male sexual behaviors are facilitated only after testosterone metabolism into E2 by the aromatase enzyme and/or into 5α-dihydrotestosterone (DHT^1) by 5α-reductase (Meisel and Sachs 1994). However, aromatization is not important for the expression of male sexual behavior in green anoles. Systemic administration of E2 has little or no effect on male sexual behavior (Crews et al. 1978; Mason and Adkins 1976; Winkler and Wade 1998). Nevertheless, the data on DHT are complex. One study reports that DHT stimulates reproductive behavior in castrated males (Adkins and Schlesinger 1979), whereas another study found that this androgenic metabolite is ineffective when given alone in stimulating masculine reproductive behavior, yet in combination with E2 it can promote behavior in some individuals (Crews et al. 1978). Data from systemic inhibition of 5α-reductase indicate that testosterone metabolism into DHT is necessary for the full expression of masculine sexual behaviors, but that DHT alone is not sufficient to initiate their production (Rosen and Wade 2000). Males housed under nonbreeding conditions do not exhibit copulatory behavior, even if given androgen implants, although androgen-treated males extend their dewlaps with increased frequency (O'Bryant and Wade 2002a).

Interestingly, the level of 5α-reductase activity is approximately four times greater in the brainstem (where the dewlap motoneurons are located, see below) than in regions of the forebrain (Wade 1997). Sex and seasonal differences in 5α-reductase activity in the green anole brain have not been detected. However, similar to other vertebrate species, the level of brain aromatase activity is greater in males than in females and greater in breeding than in nonbreeding males (Rosen and Wade 2001). The conservation of this pattern is intriguing and worthy of further study, given the lack of influence of aromatase in the activation of male sexual behaviors (Winkler and Wade 1998).

During the breeding season, androgen(s) can readily facilitate masculine sexual behaviors in ovariectomized females as well as males, and the courtship displays and copulatory postures (minus intromission) are qualitatively similar in individuals of the two sexes when tested with receptive stimulus females. However, females consistently display the behaviors less frequently than males, although studies vary in terms of whether this sex difference in androgen responsiveness reaches statistical significance (Adkins and Schlesinger 1979; Mason and Adkins 1976; Winkler and Wade 1998).

Feminine receptivity is also mediated by gonadal steroids. E2 is clearly the most potent activator of the behavior (Winkler and Wade 1998), an effect that can be inhibited by administration of an estrogen receptor blocker (Tokarz and Crews 1980). However, as in a number of mammalian species, progesterone synergizes with E2 to maximize receptivity (McNicol and Crews 1979). Testosterone also facilitates receptivity in females (Adkins and Schlesinger 1979), an effect that is at least partially induced by its aromatization into E2 (Winkler and Wade 1998).

Description of Reproductive Morphology

Researchers have identified numerous central and peripheral structures critical to reproductive behaviors in *A. carolinensis*. First, in the limbic forebrain, the preoptic area (POA^1) and the ventromedial nucleus of the amygdala (AMY^1) are involved in the display of masculine behaviors (Greenberg et al. 1984; Morgantaler and Crews 1978). Second, dewlap extension is accomplished by ceratohyoid muscles on each side of the throat and bilateral sets of three pieces of cartilage, the largest of which (the paired, medially located second ceratobranchials) normally lays flat against the throat and chest and bows out to extend the skin into a fan (Bels 1990). The motoneurons innervating the ceratohyoid muscles are located in two portions of nucleus ambiguus (AmbIX/VIImv and $AmbX^1$) in the caudal brainstem (Wade 1998). Third, hemipenes are everted through the cloaca by contraction of the transversus penis (TPN^1), and are retracted by the retractor penis magnus (RPM^1) (Arnold 1984). Each of these muscles is innervated by motoneurons in the caudal spinal cord (trunk segment 17, which is comparable with the last lumbar segment in mammals, and sacral segment 1) (Holmes and Wade 2002; Ruiz and Wade 2002).

Parallel to the frequency with which particular behaviors are displayed by the two sexes, male-biased dimorphisms exist at a number of the morphological levels described above. In the dewlap neuromuscular system (see

O'Bryant and Wade 1999, 2002a,b; Wade 1998), the second ceratobranchials are longer in males than in females. Similarly, the average size of neuron cell bodies is larger in both AmbIX/VIImv and AmbX in males, as are the average cross-sectional areas of the nerve containing the axons from these cells and the muscle fibers they innervate. Males also have more fibers in the ceratohyoid muscle, and their dewlap neuromuscular junctions are larger. In the copulatory neuromuscular system of adults, the hemipenes, TPN, and RPM are present only in males (Ruiz and Wade 2002). Similarly, T17-S1 motoneurons are more numerous and larger in males compared with females (Ruiz and Wade 2002). Sex differences in neuron soma size and density were not detected in the POA or AMY in unmanipulated animals (O'Bryant and Wade 2002a).

Neuroendocrine Effects on Reproductive Morphology

Although males extend their dewlaps much more frequently when testosterone is elevated, the neuromuscular structures remain relatively stable in size when concentrations of testosterone are variable. Motoneuron soma size is not different in gonad-intact males between the breeding and nonbreeding seasons; and within the breeding season, neither motoneuron soma size nor cross-sectional areas of the nerve and muscle fibers are affected by testosterone treatment (O'Bryant and Wade 1999). Similarly, the male-biased sex difference in the size of neuromuscular junctions on the ceratohyoid muscle is present in both the breeding and nonbreeding seasons (O'Bryant and Wade 2002b).

The effects of season and androgen manipulation are currently under investigation in the copulatory neuromuscular system. However, we know that the hemipenes grow in response to androgen manipulation during the breeding season (Holmes and Wade 2003). In the forebrain, neurons in both the POA and AMY are larger in breeding than nonbreeding animals (both sexes). However, administering testosterone during the nonbreeding season does not alter the soma size, despite stimulating an increase in dewlap extension (O'Bryant and Wade 2002). This result is somewhat surprising since both the POA and AMY contain high levels of androgen receptor mRNA and/or protein (Rosen et al. 2002), although it is possible that receptor expression is diminished during the nonbreeding season. The lack of a seasonal effect on dewlap motoneuron morphology is consistent with the fact that relatively few cells contain androgen receptors in AmbX, and none were detected in AmbIX/VIImv (Rosen et al. 2002). Androgen receptors in the copulatory motoneurons and muscles of both systems have not yet been explored.

Developmental Effects on Behavior and Morphology

Like adults, juvenile males and females perform headbobbing displays, sometimes with dewlap extension, and are capable of doing so from hatching (Lovern and Jenssen 2001, 2003). However, juvenile display shows none of the dramatic sexual dimorphisms seen in adults. Reproductive behavior is not shown by juveniles but rather appears sometime after the first winter after hatching. Testosterone treatment of juveniles of both sexes stimulates increased agonistic behavior and may also stimulate sexual behavior (see Evans 1957; Lovern et al. 2001). Further work is necessary to determine precisely what environmental, physiological, and experiential factors influence the transition from nonreproductive to first-time reproductive individuals.

With respect to reproductive morphology, thus far only the dewlap neuromuscular system has been investigated during development. The length of the second ceratobranchial cartilages and the cross-sectional area of the ceratohyoid muscle fibers that extend the dewlap are not sexually dimorphic at hatching, but they become larger in juvenile males than in females before adulthood (O'Bryant and Wade 2001). These differences were observed despite a lack of sexual dimorphism in body size at the time the morphological measurements were taken, suggesting that they are developing independent of, or enhanced beyond, general somatic growth. It is presently unclear whether sexual differentiation of the dewlap system is mediated by testosterone; that work is currently under way. However, the development of sex differences follows an increase in testosterone that occurs only in males (Lovern et al. 2001), consistent with a masculinizing effect of androgen.

The potential also exists for testosterone to influence sexual differentiation of the copulatory system, which occurs primarily during embryonic development. In lizards, both male and female embryos develop hemipenes, but they regress in females before hatching (e.g., Dufaure and Hubert 1961). We have found that testosterone concentrations are higher in yolks of eggs containing male embryos than those containing female embryos from around the time of fertilization until the time the eggs are laid (Lovern and Wade 2001, 2003b). However, this consistent sex difference in maternally derived testosterone disappears during incubation (Lovern and Wade 2001) and thus may be present too early to influence masculine development of the hemipenes, although other sexual dimorphisms might be affected. Alternatively, it is possible that testosterone secretion by the gonads of embryonic males later in development, rather than yolk hormones from mothers, is important for sexual differentiation of the copulatory system. Based on the testosterone content of the embryos, it appears likely that they may become steroidogenic during the latter part of incubation, when the hemipenes are presumably differentiating sexually (Lovern and Wade, 2003a). Further work is necessary to determine precisely when steroidogenesis occurs and whether embryonic males and females show sex differences. Finally, these structures may differentiate via another mechanism entirely (hormonal or otherwise). These possibilities are currently under investigation.

Summary and Future Directions

Green anoles are well suited to research on reproductive behaviors and neural plasticity due to the ease of housing this species in the laboratory, the reliability with which natural behavior can be elicited, and the potential to utilize multiple levels of analysis simultaneously. To date, we have identified components of the model system that show plasticity across sex and season and can be experimentally modified by exogenous steroid manipulation, as well as components that are apparently stable once development is complete. Research is ongoing to identify the extent to which central nervous system structure differs by sex, when such differences develop and by what mechanisms, and ultimately how they relate to target tissue function. One exciting means by which this research can advance involves taking advantage of the novel experimental opportunities presented by the bilateral nature of the dewlap and hemipene neuromuscular systems (i.e., by allowing within animal controls for various manipulations).

Although our emphasis in this article is on structure-function relationships as they relate to reproduction, *A. carolinensis* is also a useful model for several other research areas. For example, research being conducted on aggressive behavior in males and the experiential, hormonal, and neurobiological factors that influence it may yield general structure-function principles governing multiple behavior systems within the same species (e.g., Greenberg and Crews 1990; Korzan et al. 2000, 2001; Larson and Summers 2001; Summers and Greenberg 1994; Yang et al. 2001). Relatedly, research on stress and the widespread effects it can have on other aspects of organismal biology has garnered much attention, and the green anole is a useful model for advancing such knowledge (e.g., Greenberg 2002; Greenberg et al. 1984; Summers 2001, 2002). The diversity of research programs that utilize *A. carolinensis* to address fundamental research questions is a testament to the value of this species as an animal model.

As we continue to gain an understanding of the biology of *A. carolinensis,* future research should take a comparative approach using the extraordinary number and diversity of species within the genus. The phylogenetic relationships among anoles have been studied extensively (e.g., Jackman et al. 1999) and have provided a solid evolutionary framework for constructing comparative hypotheses. Yet to date, very little work has been conducted on the relationships among nervous system, hormones, and behavior in these other species. One notable exception that may provide initial interesting hypotheses for comparative research is the congeneric—but not closely related—*Anolis sagrei,* in which behavior and underlying physiology have been studied in some detail (e.g., reproductive behavior: Tokarz 1988, 1998, 2002; Tokarz and Kirkpatrick 1991; aggressive/territorial behavior: Tokarz 1985, 1995; physiological regulation: Tokarz 1986, 1987; Tokarz et al. 1998, 2002).

Across a broader comparative framework, research with *Anolis* can of course be informed, and vice versa, by research conducted in nonreptilian taxa. Examples that may be particularly informative include the neuromuscular and forebrain regions involved in song production in birds, vocalization in frogs, and copulation in mammals (reviewed in Cooke et al. 1998). Indeed, our understanding of the fundamental principles governing reproductive biology and neural plasticity in vertebrates—including humans—will necessarily be the result of continued, synthesized comparative research.

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