Sculpting reproductive circuits: Relationships among hormones, morphology and behavior in anole lizards

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ABSTRACT

Morphology parallels function on a variety of levels in reproductive circuits in anole lizards, as in many vertebrate groups. For example, across species within the anole genus the muscle fibers regulating extension of a throat fan used in courtship are larger in males than females. Endocrine factors controlling behavior and morphology have been studied in detail in one species, the green anole (Anolis carolinensis). This review briefly describes the results that have been obtained and highlights key areas for future investigation that will provide insights on mechanisms from a comparative perspective.

1. Introduction

Relationships between structure and function have long been investigated across diverse areas of biology. Some of the most intriguing examples exist in the field of reproduction. Specific regions of the brain and spinal cord, as well as particular muscles associated with sexual behaviors, are often enlarged in animals that display sexual behaviors more frequently. These types of parallels between morphology and behavior exist at multiple levels, including sex differences that develop permanently during ontogeny and plasticity that occurs across breeding seasons in adulthood.

Structures regulating male courtship behaviors are often larger in males than females. For example, species of frogs, fish and songbirds all produce vocalizations to attract females, and in each case the muscles and motoneurons are larger in males compared to females (reviewed in [45]). Areas of the forebrain involved in the learning and production of birdsong are also enhanced in males [47]; the neurons in these areas are increased in size and number. These types of sex differences in both structure and function are commonly regulated by steroid hormones. During a critical period in development, testosterone (T) can permanently masculinize components of the nervous system and muscles, as well as the capacity to display adult sexual behaviors. This process is referred to as “organization”. In adulthood, the same hormone typically acts on these structures to permit or facilitate the production of specific masculine behaviors, which is known as “activation” [2]. In many cases, it is not just the behaviors that are affected, but also morphology. Muscle fibers and motoneurons associated with courtship and copulation grow with seasonal increases in T (see [48] and references therein). In parallel, forebrain areas regulating the production of song can grow in some species via the incorporation of new neurons on a seasonal basis; the survival of these cells is modulated by T as well as singing itself [28,41].

These diverse models have provided a wealth of information regarding relationships between anatomy and behavior, as well as the endocrine and in some cases molecular mechanisms regulating both structure and function within species. However, it has been challenging to draw conclusions about mechanisms on a broad scale. It has also been difficult to evaluate their evolution. At least two main factors contribute to these issues. First, courtship and copulatory systems have largely been investigated in different vertebrate groups – the former in fish, frogs and birds, and the latter primarily in mammals. Second, even within courtship systems, the structures are diverse. Many involve the generation and perception of acoustic cues, but the motor systems and end-organs differ dramatically. For example, frogs generate sounds via the larynx, birds through the syrinx, and some of the fish most thoroughly investigated (plainfin midshipman; Porichthys notatus) use their swim bladders for these signals.

2. Anole lizards

Anoles offer some advantages over these other model systems. A long history of research into the hormones, brain and behavior exists for one species, the green anole (Anolis carolinensis), and a substantial amount of data is also available for the brown anole (A. sagrei). These studies indicate that the hormonal regulation of behavior appears quite similar in these two species of anoles.
then insert one of two bilateral, independently controlled hemipe- 
maneuver his tail under hers to appose their cloacas. The male will 
mount her back, grip the skin of her neck with his teeth, and 
under non-receptive conditions. However, they will not allow the male to engage in these behaviors 
other than a slight neck bend which facilitates the male's grip. 
male of this species, damage to the basal hypothalamus inhibits 
play of sexual behaviors by male green anoles [15,17,33]. In fe-
(stained). These areas – portions of the limbic forebrain, which control higher 
level or more motivational aspects of sexual behavior, and both 
courtship and copulatory neuromuscular systems, all of which lend 
themselves to investigations in the field and laboratory. Second, 
more than 350 species of anole lizards span the Southeastern US, 
Caribbean islands and Central and South America [29]. Information 
on the behavioral ecology and phylogenetic history of many of 
these is accessible. And, while limited data on the neural and 
muscular structures regulating courtship and copulation are 
currently available, it is clear that species across the genus exhibit 
beautiful variation in the degree of sexual dimorphism in morphol- 
ology on a gross level. Anole lizards therefore represent a terrific 
opportunity for more detailed investigations from an evolutionary 
perspective.

These animals exhibit stereotypical sequences of behaviors, 
which have been studied in the most detail in A. carolinensis. The 
displays are remarkably similar in the field and laboratory, making 
this species particularly useful for the investigation of relatively 
natural functions in a controlled setting [46]. During the breeding 
season (which extends from approximately April through July for 
green anoles), males are quite aggressive with each other and 
defend territories. Females hold smaller, satellite territories, sur-
rounding those of males. In both aggressive (with another male) 
and courtship (with a female) contexts, male green anoles extend 
a red throat fan (dewlap) in conjunction with a series of head bobs 
(Fig. 1). The animal's posture is different in these two situations, 
but dewlap extension is a feature common to both aspects of the 
reproductive process – establishment and defense of a territory 
and attracting females. Females green anoles do extend their dew-
laps in aggressive encounters, but do so far less frequently than 
males. Relative size and degree of use of the dewlap varies tremen-
duously across anole species (see below), and the coloration differs 
in stunning ways across species. However, the origins and evolu-
tion of these visual patterns are not clear [36].

Once a male anole has successfully attracted a female, he will 
mount her back, grip the skin of her neck with his teeth, and 
maneuver his tail under hers to appose the cloacas. The male will 
then insert one of two bilateral, independently controlled hemipe-
nises (Fig. 2). Females do not have an obvious receptive posture, 
other than a slight neck bend which facilitates the male's grip. 
However, they will not allow the male to engage in these behaviors 
under non-receptive conditions.

2.1. Relationships between structure and function

2.1.1. Green anoles: Forebrain

At least three regions of the forebrain are involved in the display 
of reproductive behavior in lizards. Lesion and hormone implant 
udies have documented the importance of the preoptic area 
(POA) and ventromedial portion of the amygdala (AMY) in the dis-
play of sexual behaviors by male green anoles [15,17,33]. In fe-
nalas of this species, damage to the basal hypothalamus inhibits 
ceptivity [16]. In other lizards, lesions of the ventromedial hypo-
thalamus (VMH) specifically prevent the display of these female 
sexual behaviors [25]. Our research has focused on each of these 
three areas – the POA, AMY and VMH.

The basic morphology of these regions (defined with a Nissl 
ast stain) is enhanced in a variety of ways in unmanipulated animals 
that are more likely to display sexual behaviors. For example, the 
POA is larger in volume in males compared to females, and the 
POA and VMH are larger in the breeding than non-breeding season 
across the two sexes [4]. Cell bodies in the POA and AMY are larger 
in the breeding season as well [39]. One of the most direct parallels 
between morphology and function comes from the AMY in which 
the average size of cell bodies correlates positively with the rate 
at which individual males extend their dewlaps [34].

2.1.2. Green anoles: Dewlap neuromuscular system

Dewlap extension is controlled by a pair of muscles in the 
throat, the ceratohyoids. These run between bilateral cartilages 
atached to a long paired set of cartilage at the midline (2nd cerato-
branchial) that lies under the skin of the throat and chest. The 
muscles are innervated by motoneurons in the caudal brainstem, 
in nucleus ambiguus X (AmbX) and the region containing nucleus 
ambiguous IX and the ventral motor nucleus of the facial nerve (Am-
blX/VLm). When these neurons provide a signal for the muscles 
to contract, the system of cartilages acts like a lever, causing the 
midline 2nd ceratobranchials to bow out and reveal the colored 
skin of the dewlap (reviewed in [45,46]; Fig. 1).

Male biased sex differences exist at a variety of levels in this 
system. Extended dewlap size is substantially larger in males,
As indicated above, adult males have bilateral hemipenises. The IX + X nerve is thicker, and motoneuron cell bodies in Amb IX/VII im and AmbX are larger in males [37,40]. Sex differences in the cartilage length and muscle fiber size appear 2–3 months after hatching, and motoneuron soma size differentiates sometime between then and adulthood [38].

In both the laboratory and field, muscle fibers that regulate dewlap extension are larger in animals that display most frequently (top 18%) compared to those who display the least (bottom 18%). Plasma T values were similar in the two settings. However, a significant positive correlation between the size of these fibers and rate of dewlap extension was detected only in the more controlled laboratory study [23,34]. This value therefore differs in a manner parallel to function not just between the sexes, but also among males, and 2nd ceratobranchial cartilages and ceratohyoid muscle fibers were enhanced in males. However, while a general pattern between dewlap use and morphology clearly exists within species, phylogenetic comparative analyses found no association between dewlap morphology and degree of use across them, suggesting that morphological components evolved independently of rate of dewlap extension.

2.2. Endocrine regulation

As is common across vertebrates, steroid hormones are critical factors in aspects of both developmental organization and adult activation of reproductive circuits in green anoles. Estradiol and progesterone are important for the facilitating of female receptivity in this species [49,50]. In contrast to some other groups of vertebrates, the most potent activator of sexual behavior in adult male green anoles is T itself, rather than one of its metabolites (reviewed in [46]). In some model systems, including rodents and Japanese quail, T secreted by the testes is metabolized into estradiol (via aromatase enzyme) and into 5α-dihydrotestosterone (by 5α-reductase) locally within a variety of tissues. Estradiol action within particular regions of the brain is important for the display of male sexual behavior in these species [3,10,21].

The same types of steroid metabolism occur within the green anole brain. Aromatase activity is relatively high in the hypothalamus and POA, which are important for the initiation of sexual behavior. In contrast, 5α-reductase is particularly high in the brainstem, where dewlap motoneurons are located [44]. The activity of these enzymes is selectively regulated by T. Whole brain estrogen synthesis is greater in males than females during the breeding season, and is increased within males in the breeding compared to the non-breeding season. T induces an increase in this activity, but only in males and only in the breeding season. Thus, the enzyme is selectively regulated by its own substrate in adult males. T also increases 5α-reductase activity in the male brain, but this effect occurs in both seasons [13].

Patterns of aromatase and 5α-reductase expression in particular neural regions are consistent with at least supporting roles in the display of sexual behaviors. Adult males have more cells with aromatase mRNA in the preoptic area than females [14]. These data are consistent with estradiol modulating the display of male sexual behavior in green anoles [27]. While metabolites of T appear not to have a primary function in the activation of these behaviors, estradiol does appear to increase the motivation for their display.
Female-biased sex differences in the density of these cells were detected in the VMH and AMY [14]. While it is not always obvious how or whether these measures translate into variability in function, an increased number and/or density of cells synthesizing aromatase could result in greater estrogen synthesis in a specific region. The increased expression in females could support their display of sexual behavior; extra-gonadal estrogen synthesis facilitates receptivity [49]. The VMH is a region well known to be important for the regulation of female receptivity [9] also see above, and while the portion of the amygdala we have investigated is linked to male sexual behavior [17], the region could very well also play a role in the integration of visual courtship signals and motivated behaviors in female anoles e.g. [32].

Plasma T is quite a bit higher in adult male compared to female green anoles, and it is increased in the breeding compared to the non-breeding season [30]. However, hormone replacement studies in gonadectomized animals suggest that these differences in circulating levels may not be responsible for local expression of aromatase as we measured it (Cohen and Wade, unpublished). Another possibility is that the sex differences are permanently organized during development, but if they are, it is probably not by post-hatching T (Cohen and Wade, unpublished). No sex differences in aromatase mRNA containing cells were detected in the POA, AMY or VMH on the day of hatching or 50 days later. Plasma T is equivalent in males and females at the first of these developmental stages, but higher in males than females at the second age [31].

5α-reductase exists in two isoforms – I and II. 5α-reductase II is expressed in many regions of the adult brain, including the POA, VMH and AMY, whereas 5α-reductase I is only detectable in the brainstem of adult green anoles. Across these regions, the only sex difference we have detected is an increased density of cells expressing 5α-reductase II mRNA in the AMY of females compared to male [12]. As with aromatase, this dimorphism appears not to be regulated by adult T exposure (Cohen and Wade, unpublished).

Two other effects likely are mediated by adult T, however. This steroid increases the density of cells expressing 5α-reductase II mRNA in the VMH of females and decreases this measure in males during the breeding season specifically (Cohen and Wade, unpublished). The functional consequences of these effects are not known, but one possibility is that increased enzyme activity serves to regulate the amount of T in the VMH in adaptive ways. For example, while this region certainly has other functions, it is traditionally associated with regulation of female sexual behavior (see above). Increased 5α-reductase II in females in response to T should result in decreasing local availability of this androgen, which might be important for reducing the likelihood of undesirable T-dependent functions. In parallel, a decrease in 5α-reductase II in breeding season males with high T would increase availability of T itself, which is the most potent activator of male sexual behavior in green anoles.

Unlike in adult anoles, both isozymes of 5α-reductase are expressed in the developing forebrain. Both the number of cells expressing 5α-reductase I in the POA and the density of these cells in the AMY decrease between the day of hatching and day 50 (Cohen and Wade, unpublished). These changes across ages appear to reflect a decrease in expression within existing cells rather than an overall change in cell number or density at these stages. The decreases are consistent with the lack of detection of 5α-reductase I in the adult forebrain, and suggest that differentiation of the enzyme expression is underway by 50 days post-hatching.

Similar to its effects on male sexual behavior, T can induce changes in the morphology of a variety of structures important for these functions. For example, in the forebrain, T treatment of gonadectomized adult males increases the size of cell bodies in the POA and AMY, and in the AMY this effect is magnified in the breeding compared to the non-breeding season [35]. This manipulation does not affect the sizes of dewlap or copulatory motoneurons or cross-sectional area of ceratohyoid muscle fibers. However, parallel to the results on AMY cells, T enhances the size of copulatory muscle fibers, and does so to a greater extent in the breeding compared to non-breeding season [35]. Thus, differential responsiveness to the same dose of T exists on a variety of levels in adult male green anoles – across tissues, as well as between seasons.

It is also worth noting that gonadectomy itself appears to reveal some sex and seasonal differences in morphology in the forebrain that are not detected in intact lizards [6]. The reasons behind this are not clear, but could involve a change due to physical removal of the gonads or effects due to steroid hormones other than estradiol, which was replaced in some animals with only limited effect. We have not yet tested the effects of adult estradiol on morphology of dewlap or copulatory neuromuscular systems.

In the cases of differential responsiveness across seasons and tissue, perhaps the most logical potential mechanism involves variable expression of steroid receptors. We have investigated them in the forebrain, as well as the dewlap and copulatory neuromuscular systems. During the breeding season, the POA, VMH and AMY all express substantial levels of androgen receptor mRNA and protein in adults of both sexes [42]. To more fully understand androgen action in these limbic regions, we will need to evaluate their expression across seasons and developmental stages. We know more about the two neuromuscular systems. Androgen receptor protein is widely expressed in the dewlap and copulatory muscles, as well as their peripheral targets (cartilage and hemipenes). Androgen receptor is also expressed in AmbX motoneurons and spinal segments T7-S1 [20,42]. The expression appears relatively stable across the breeding and non-breeding season in intact animals, but T treatment increases the percentage of androgen-receptor positive nuclei in the RPM [20]. The fact that this effect was not seen in the ceratohyoid muscle, despite the clear presence of AR, suggests one mechanism through which T might modulate the size of RPM muscle fibers without affecting the dewlap muscle.

Estrogen receptor α has been investigated in the brains of adult animals, as well as around the day of hatching. In the forebrain, the mRNA for this receptor is expressed more heavily in the VMH than the AMY and POA at both life stages. In adults, this expression is also increased in both the POA and VMH of females compared to males [7,8]. We also recently cloned estrogen receptor β. It is expressed in reproductive nuclei, and in the VMH and AMY the density of cells expressing this mRNA was increased in adult females compared to males in the breeding season [11]. All of these data are consistent with estradiol’s importance in female anole sexual behavior.

Sexual differentiation of the peripheral copulatory system may be directly influenced by relative levels of steroid receptor expression. In embryonic development, prior to the regression of the hemipenes and associated muscles in females, androgen receptors are increased in males in these tissues and estrogen receptors α tend to be higher in females [5]. These data parallel effects of steroid hormone manipulation, in which estradiol treatment results in development of feminine copulatory systems and androgen supports and enhances masculine differentiation [19]. Steroid receptors have not been investigated in the dewlap system during development. However, T administration to juvenile females increases (masculinizes) the length of the ceratobranchial cartilage and the size of ceratohyoid muscle fibers. Castration of males at the same stage reduces cartilage length, but may not sufficiently eliminate circulating androgens to affect muscle fiber size [30]. Thus, steroid hormones mediate sexual differentiation of peripheral structures in both the dewlap and copulatory neuromuscular systems, but do so at very different stages of the life cycle.
3. Conclusions and future directions

The reproductive circuity of anole lizards has emerged as an exciting system for elucidating mechanisms that regulate structure and function. The studies have revealed roles of androgens and estrogens in developmental organization, as well as adult behavioral activation and neural plasticity. We have become particularly interested in selective responsiveness to T, which exists on a variety of levels between the sexes and seasons, as well as across tissues and developmental stages. Several types of factors may influence this differential responsiveness in addition to the expression of steroid receptors, including their receptor co-activators, or perhaps epigenetic mechanisms. Recent sequencing of the green anole genome provides new access to tools necessary to address molecular mechanisms in neural and muscular tissues associated with the hormonal regulation of behavior in reptiles. Considering these factors across anole species and in relation to other vertebrate reproductive models will provide important information on evolutionary processes, as well as the ubiquity and uniqueness of particular mechanisms.

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References