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Relationships among reproductive morphology, behavior, and testosterone in a natural population of green anole lizards

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ABSTRACT

Laboratory studies of reproductive systems have long supported the idea that neural and/or muscular structures used frequently are often enhanced in size. However, field studies integrating behavioral, morphological, and hormonal data are needed to better understand relationships in natural environments. We examined a natural population of green anole lizards (*Anolis carolinensis*) to determine whether variation in reproductive morphology both within and between the sexes paralleled differences in courtship and copulatory behaviors and circulating testosterone levels. Display rate in males was positively correlated with the sizes of the cartilage supporting the dewlap (a throat fan used in courtship and aggression) and renal sex segments (portions of the kidney that function similarly to the mammalian prostate), but correlated negatively with seminiferous tubule size. Plasma testosterone in males was negatively correlated with display behavior and was not correlated with any measures of morphology. Females, which display rarely, exhibited no relationships between morphology and frequency of behavior. Comparisons between the sexes show that males have consistently larger courtship and copulation morphologies than females, even when accounting for sex differences in body size. The results not only support the idea of relationships between increased function and enhanced structures, but also show the complexity of mechanistic interactions associated with reproductive behavior in wild animals.

1. Introduction

Successful reproduction requires a suite of specialized morphological and physiological traits, as well as the performance of stereotyped behaviors demonstrating an animal's motivation to copulate [1]. Thus, examinations of reproductive morphology and behavior offer excellent opportunities to investigate relationships between structure and function (reviewed in [2]). Studies across diverse taxa document that frequently-used reproductive structures, particularly required muscles and components of the nervous system, are often larger than those used more rarely. This relationship is clearly observed in comparisons between the sexes. For example, in species where males perform complex vocal courtship displays but females do not [e.g., zebra finches (*Taeniopygia guttata*), African clawed frogs (*Xenopus laevis*), and midshipmen fish (*Poricthys notatus*)], the neuromuscular structures supporting these behaviors are larger in males than females [3–7]. Both developmental organization and adult maintenance of these sexual dimorphisms are often facilitated by steroid hormones (reviewed in [8]).

Many studies have examined the relationships between reproductive behavior and morphology in the lab (reviewed in multiple chapters of [9]), and many others have investigated associations between sex steroid hormone levels and behavior in the lab [e.g., 10] or field [e.g., 11–13]. However, relatively little work in reproductive biology has focused on the associations among neuromuscular, endocrine, and behavioral traits within individual animals in their natural habitats.

The green anole (*Anolis carolinensis*) is an arboreal lizard that reproduces seasonally, from approximately April to July in the southeastern US [14–16]. Decades of field research on this species has provided extensive information regarding its behavior and reproductive ecology [e.g., 17,18]. During the breeding season, males court females and defend territories with displays that include head-bobs, push-ups, and extensions of a reddish-pink throat fan called a dewlap [19] (Fig. 1). Females have much smaller dewlaps than males, and they also perform these displays to defend territories, but at much lower rates than males [20,21]. Dewlap displays are

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Fig. 1. Male *A. carolinensis* with extended dewlap (Jean Lafitte National Park, Barataria, Louisiana, USA).

important for courtship and mate selection, as well as territory defense [22,23]. Male courtship generally (and perhaps necessarily) precedes copulation, which occurs when a male mounts a female, maneuvers his tail under hers, everts one of two bilateral copulatory organs called hemipenes, intromits into her cloacal vent, and ejaculates [16]. These male reproductive behaviors are facilitated by increased androgen, primarily T (rather than its metabolites), during the breeding season [24–27]. Receptivity is activated by ovarian hormones, and while neural metabolism of T into estradiol may facilitate this behavior [25], specific roles of circulating T itself in females are not clear [28].

The neuromuscular structures supporting the movements of the dewlap and hemipenes have been well-characterized in green anoles (reviewed in [28]). The dewlap extends when the ceratohyoid (CH) muscles on each side of the throat contract, causing the second ceratobranchial cartilage to bow out, revealing the flap of red skin of the dewlap [29,30]. Movements of the hemipenes are controlled by two muscles, the transversus penis (TPN) and the retractor penis magnus (RPM). The former wraps over the hemipenis and facilitates its eversion, and the latter is attached to the caudal end of each hemipenis as it lies in the tail and causes its retraction [31].

The goal of the present study was to build on and begin to merge existing literatures on the behavioral ecology (from the field) and relationships between behavior and morphology (from the lab) in green anoles. While field studies examine animals in their natural environments, the complexity of these environments may limit our ability to identify causal relationships among traits; yet, the simpler environments of laboratory studies may constrain our ability to extrapolate findings to the natural world. Thus, these two approaches are complementary. Although it was not the primary focus of our experiment, we evaluated circulating T in males to begin to assess whether relationships between this hormone and behavior, and particularly T and morphology, detected in the lab were observed in unmanipulated wild animals.

2. Materials and methods

2.1. Field data collection

We conducted behavioral observations on adult green anole lizards (18 males, snout-vent-length [SVL] = 58–68 mm, and 20 females, SVL = 42–59 mm) in Jean Lafitte National Park, Barataria Preserve in Marrero, Louisiana (N 29°47.22, W 90° 06.53) in May 2008. We located animals between 08:30 and 18:00 by walking through the forest until finding an apparently undisturbed lizard. During each observation (males: 64–178 min, average 131 min; females: 22–180 min; average 113 min), we recorded all display behaviors (dewlap extensions, head-bobs, and push-ups) and determined the proportion of time the lizard displayed during the observation period. Because these displays do not obviously differ with social context (territorial or courtship displays), any dewlap extension was counted as a display bout. We also recorded all locomotor behaviors (crawls, runs, and jumps), prey captures, and copulations. As copulation was observed rarely, we were unable to include it as a variable in subsequent statistical analyses. Immediately following each observation, we captured the lizard with a noose. Because the primary focus of this study was to elucidate naturally occurring relationships between behavior and morphology, if observations of other lizards were in progress, we kept the captured lizard in an air-filled clear plastic bag until we could process it. Lizards were held for an average of 56 min (maximum of 3 h).

For each lizard, the same person took the following external measurements: SVL (measured with a ruler), mass (using a Pesola spring scale), and the length of the second ceratobranchial cartilage (hereafter, 'cartilage') as seen under the skin (measured with digital calipers). Animals were then rapidly decapitated and the brain, kidneys, and gonads harvested. We also collected the portion of the throat that contains CH muscles and the portion of the tail with the hemipenes and RPM. All tissues were immediately frozen on dry ice until transported to Michigan State University where they were stored at −80 °C. We collected blood from the head and trunk of males, and stored it on ice in the field. Within 8 h, we centrifuged the samples and froze the plasma on dry ice until it was transported to Michigan State University and stored at −80 °C.

All procedures were performed in accordance with the guidelines of the Michigan State University Institutional Animal Care and Use Committee, with permits from the National Park Service (permit # JELA-2008-SCI-003) and the Louisiana Department of Wildlife and Fisheries (permit # LNGP-08-059).

2.2. Histology

Frozen tissues were sectioned at 20 μm, stained with hematoxylin and eosin, and measured using Scion (NIH) Image software. For each tissue measured, we calculated an average per individual for use in statistical analyses.

In the throat, we measured cross-sectional areas of 25 arbitrarily selected fibers in the CH and GG muscles (as in [21,32,33]). The GG is a muscle involved in tongue extension, located in the throat near the CH. Values were obtained from one section for both the left and right sides within the middle third of the rostro-caudal extent of each muscle. We also measured the cross-sectional area of the cartilage and trachea in 5 tissue sections in the middle third of the muscle [21]. Because they are in the same sections of tissue but are not involved in dewlap extension, we used the cross-sectional areas of GG fibers and the trachea as controls for general differences in body size (as in [21,33]).

In the tails, we measured the cross-sectional areas of 25 arbitrarily selected muscle fibers of the RPM and, as a control, the caudofemoralis (CF), a muscle involved in leg movement that lies near the hemipenes on each side of the tail. Values for these muscles were obtained from