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Does Testosterone Facilitate Dynamic Relationships in Lizard Behavior, Morphology, and Physiology?

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DOES TESTOSTERONE FACILITATE DYNAMIC RELATIONSHIPS IN LIZARD BEHAVIOR, MORHPOLOGY, AND PHYSIOLOGY?

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Abstract

Testosterone regulates a wide variety of sexual and social behaviors, but the extent to which variation in muscle physiology facilitates these behaviors is not clear. We investigated the effects of testosterone on the morphology, physiology, and behavioral use of two muscles in green anole (*Anolis carolinensis*) and brown anole (*A. sagrei*) lizards. We examined the ceratohyoid (CH), the muscle that moves the throat fan called the dewlap in social display, and the retractor penis magnus (RPM), a muscle that moves the hemipenes during copulation. We assigned males of each species to one of three treatment groups: high T males were gonadectomied and received a testosterone implant; low T males were gonadectomied and received a blank implant; and control males underwent sham surgery, where their testes were left intact, and they received a blank implant. We found that testosterone regulates behaviors and the muscles underlying them in a complex manner. Before surgery, males assigned to these groups did not differ in morphology or behavior. Six weeks after hormone manipulation, high T males displayed their dewlaps more frequently than low T males, but testosterone did not affect dewlap or CH morphology. However, testosterone did increase the size of both the copulatory hemipenes and RPM. We also found that testosterone regulates dewlap and push-bob behaviors in a context-specific fashion (aggressive male-male contexts vs. sexual male-female contexts), and this pattern was similar across the green and brown anole. Overall, the role of testosterone in mechanisms underlying social behaviors appears to vary among muscles, yet remains consistent between species.

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Table of Contents

List of Figures

List of Tables

Chapter 1

A Review of Testosterone as a Regulator of Adult Male Social Behavior

Hormones and their mechanisms of action

How do animals regulate their behaviors? For example, how do animals know when to eat, when to sleep, when to be social, or when to reproduce? The answer is often hormone regulation. Hormones play a critical role in coordinating and synchronizing both physiological and behavioral processes throughout the body, including the brain, nervous system, organs, and muscle tissues (Adkins-Regan, 2005). Some hormones and their actions are so critical that they are highly conserved and taxonomically widespread across the animal kingdom (Hau, 2007). For example, in males, once gametes reach maturation, the gonads release gonadal hormones that can facilitate courtship behaviors, territoriality, and dominance (Hau, 2007). These behaviors may be costly if performed outside of reproductive maturity (i.e., attracting predators or wasting time and energy fighting conspecifics), so hormones help regulate these otherwise costly behaviors that may only be useful during reproductive periods (Adkins-Regan, 2005).

Steroid hormones include estrogens, progesterone, glucocorticoids, mineralocorticoids, and androgens (Carson-Jurica et al., 1990; McEwen et al., 1979; Miller, 1988; Tsai and O'Malley, 1994). Steroid hormones differ from the other classes of hormones (i.e., amino-acid derivatives, fatty-acid derivatives, and peptide hormones), in that all steroid hormones are synthesized from sterol cholesterol, giving them their classic four ring structure with three hexagons and one pentagon. Specific steroids, like the androgen testosterone, vary in the functional groups attached to the cholesterol backbone, giving them their unique functional roles as signaling molecules. These steroid hormones are synthesized and secreted by various endocrine glands as well as the brain. Once in the bloodstream, steroid hormones can travel throughout the circulatory system, targeting and binding to specific receptors in various tissues until they are eventually metabolized and excreted.

Steroid hormones are extremely effective at coordinating large scale physiological and behavioral changes. Even when only miniscule concentrations of a hormone are present in the bloodstream, the initial signals are amplified through cascade signaling within cells. Steroid hormone receptors are intracellular transcription factors that are activated when steroid hormones bind (Carson-Jurica et al., 1990; Tsai and O'Malley, 1994). These activated steroid-receptor transcription factors then bind to hormone response elements in the promoters of steroid regulated genes. And finally, in combination with co-activators and co-repressors the activated steroid-receptor transcription factors can alter the expression of different genes (Tsai and O'Malley, 1994). Steroid hormones will also occasionally regulate gene expression by affecting mRNA stability and translational efficiency (Tsai and O'Malley, 1994).

Steroid hormones can act on tissues throughout the body, including the brain, spinal cord, and peripheral motor organs. Because the main effects of steroids occur through altering gene transcription, the effects of steroids can take significant time, on the order of hours or even days. In the brain, changes in mRNA transcription can alter signaling pathways that influence behaviors (Eisenegger et al., 2011). In muscles, long-term changes in mRNA transcription and protein synthesis may result in cell multiplication and tissue growth (Kadi, 2008; Wyce et al., 2010). Hormone actions on both central and peripheral targets raise a conceptual distinction to keep in mind throughout this study – the distinction between hormone effects on (1) motivations to perform behaviors, (2) motor capabilities to perform behaviors, and (3) size and morphology of structures used to perform behaviors (Wallen, 2001).

In my study I am examining how the androgenic steroid hormone testosterone mechanistically alters behaviors in adult males. Testosterone and other androgens are typically referred to as 'masculinizing' hormones because of their role in organizing masculine morphological and physiological traits during development that are then more sensitive to androgens in adulthood (Breedlove and Arnold, 1981; Forger, 2009). In males, testosterone is primarily synthesized and secreted by the testes. Once in circulation, testosterone can bind to androgen receptors in various tissues. The overall effect of testosterone on these tissues is dependent on the concentration of androgen receptors for testosterone to bind to (e.g., androgen receptor sensitivity). In the

following sections, I will expand more on our current understanding of testosterone and its effects on behaviors and muscles.

Testosterone and behavior

As a gonadal hormone, testosterone is a key regulator of behaviors that increase mating and reproductive success across vertebrates. Testosterone improves reproductive success by promoting courtship and sexual behaviors, territorial aggression, and sperm production (Gleason et al., 2009; Hau, 2007; Wilson, 1999). There is a plethora of experimental evidence supporting this link between testosterone, aggression, and courtship behaviors.

Testosterone has been linked to aggression across many species of animals. For example, remove-and-replace experiments in mice show that testosterone regulates male aggressive behaviors (reviewed in Nelson and Trainor, 2007). In the year-long, territorial, tropical spotted antbirds (*Hylophylax n. naevioides*), testosterone implants increased song production and aggressive behaviors in staged male-male encounters (Hau et al., 2000). In another experiment, where the researchers blocked two known actions of testosterone in the spotted antbird – testosterone's binding to androgen receptors and testosterone's conversion to estradiol by aromatase enzymes – the spotted antbirds did not sing at all and showed reduced aggression (Hau et al., 2000).

Testosterone also regulates male courtship and sexual behaviors. For example, golden-collared manakins perform elaborate courtship roll-snaps with their wings, and increases in testosterone activate these behaviors at the beginning of the breeding season (reviewed in Schlinger et al., 2013). Testosterone administration also increases the unique foot-flagging displays of Bornean Rock Frogs (*Staurois parvus*), where the frogs extend and raise their feet above their heads (Mangiamele et al., 2016). And in male grey partridges, increasing testosterone towards high physiological limits, increased call length, amplitude band, and reduced the lower frequency limit of their song (Fusani et al., 1994).

The examples illustrated above represent only a small fraction of testosterone's widespread regulation of aggressive and courtship behaviors across the animal kingdom.

Future directions: *how* does testosterone regulate behavior?

It is clear that testosterone is a major regulator of adult male social behaviors. However, the question remains: how exactly does testosterone facilitate such drastic behavioral changes? Is it through (1) changing motivations to perform behaviors (brain mechanism)? Is it through (2) changing the motor capabilities to perform behaviors (neuromuscular mechanism)? Or is it through (3) changing the size and morphology of the structures used to perform the behaviors (musculoskeletal mechanism)? Thus far, evidence suggests that all three mechanisms are involved to some extent in facilitating testosterone's effects on behavior (e.g., Eisenegger et al., 2011; Tobiansky and Fuxjager, 2020; Wade, 2012).

Chapter 2

A Review of Testosterone on Muscle Morphology and Physiology

Although there is substantial evidence that neural targets play a role in regulating animal behaviors (reviewed in Cooke and Woolley, 2005; Eisenegger et al., 2011; Wade, 2005), testosterone also targets muscles directly (Bhasin et al., 2003; Feng et al., 2010; Herbst and Bhasin, 2004; Schlinger et al., 2013; Sheffield-Moore, 2000; Tobiansky and Fuxjager, 2020). Muscles tend to express higher levels of androgen receptors compared to many other peripheral tissues, which means that testosterone can directly target the musculoskeletal systems underlying different behaviors (reviewed in Adkins-Regan, 2005; Tobiansky and Fuxjager, 2020).

For example, in a species of North American gray tree frog, *Hyla chrysoscelis*, the external oblique muscles are used to power sound production (Girgenrath and Marsh, 2003). Compared to the muscles of male tree frogs in the breeding season, the muscles of male tree frogs in the non-breeding season were 50% smaller, had 60% longer twitches, and had 40% slower shortening velocities (Girgenrath and Marsh, 2003). When non-breeding season male tree frogs were treated with testosterone, the breeding season muscle characteristics were restored (Girgenrath and Marsh, 2003). In golden-collared manakins, testosterone treatment facilitated increased speed of muscle contractions without diminishing the overall force of the contraction – a typical tradeoff in the animal kingdom (Fuxjager et al., 2017). And in humans, graded doses of testosterone increased thigh muscle volume due to the hypertrophy of type 1 and type II muscle fibers (Sinha-Hikim et al., 2002).

Testosterone can also cause less outwardly visible changes in muscle morphology and physiology. For example, in the CH muscle of green anoles – a muscle responsible for controlling the extension of their colorful throat fan – testosterone-treated male green anoles had a higher percentage of fast-oxidative glycolytic fibers than males not treated with testosterone (Holmes et al., 2007). This corresponded with correlational

data, where male green anoles in the breeding season had more fast-oxidative glycolytic fibers than males in the post breeding season (Holmes et al., 2007).

On a cellular level, activated androgen receptors increase mRNA transcription of parvalbumin (PV) and IGF-I in muscle tissues (Fuxjager et al., 2012, 2013; Schlinger et al., 2013; Yin et al., 2009). Both genes are important modulators of basic muscle physiology. PV regulates calcium trafficking and IGF-I influences muscle hypertrophy. In golden-collared manakins, selectively blocking peripheral androgen receptors with bicalutamide (BICAL) decreased PV and IGF-1 expression as predicted (Fuxjager et al., 2013). BICAL also decreased the frequency of the manakins' acrobatic display maneuvers and reduced the performance quality of their displays (Fuxjager et al., 2013). Together these results appear to suggest that testosterone can indeed act through muscles to change animal behavior. The researchers further confirmed that BICAL was acting through the periphery by showing that BICAL had no measurable effects on the brain (assessed by vocalizations and persistence at their display arena, which were a proxy for overall motivation to perform behaviors). This study by Fuxjager et al. (2013) demonstrates why we should also investigate muscles directly in our aim to understand how testosterone regulates behaviors.

Variation in androgen receptor expression can also regulate behavioral display frequencies. For example, in a study comparing six different species of *Anolis* lizards, more androgen receptor expression in the bicep muscle was correlated with greater frequencies of locomotor movement and push-up displays (Johnson et al., 2018). Male blue-banded gobies (*Lythrypnus dalli*) extend and retract their pelvic and dorsal fins while defending nests and courting females, and the muscles controlling these fins contain high levels of androgen receptors that are positively associated with the rate at which the fish perform these displays (Schuppe et al., 2017). And in Bornean Rock Frogs – know for extravagant foot-flagging behaviors, where they raise their back leg above their head – androgen receptor expression in their legs was much higher than in frog species that do not foot-flag (Mangiamele et al., 2016). Overall, a higher density of androgen receptors can result in more sensitive responses to even extremely low levels of testosterone.

Just as with behavior, testosterone has widespread effects on muscle morphology and physiology across the animal kingdom as highlighted by these examples from

humans, mice, birds, amphibians, lizards, and fish. Because of this relationship between testosterone and muscles, it is important to examine whether changes in the muscles underlying behaviors help facilitate testosterone's dynamic effects on male social behaviors.

Chapter 3

Does Testosterone Facilitate Dynamic Relationships in Lizard Behavior, Morphology, and Physiology?

Introduction

It has been known for a long time that sex steroid hormones like testosterone mediate adult male social behaviors (i.e., courtship, territoriality, and aggression; Adkins-Regan, 2005; Hau, 2007). However, it remains unclear exactly how testosterone mediates these behaviors. To explore this relationship, researchers continue to probe testosterone's actions in the brain and neuromuscular systems underlying behaviors (Eisenegger et al., 2011; Tobiansky and Fuxjager, 2020; Wade, 2005). In my thesis, I am testing the hypothesis that testosterone induces muscular changes that facilitate dynamic changes in male social behaviors.

Anole (genus *Anolis*) lizards are an excellent system to study the dynamic interactions between hormones, muscle physiology, and behavior. These relatively small, arboreal, insectivorous lizards can be found across the southeastern US, the Caribbean, and Central and South America (Losos, 2009). They have clear spectacular behavioral displays (i.e., dewlap extensions and push-ups) and copulation behaviors that are readily observable and well-characterized (Johnson and Wade, 2011; Lovern et al., 2004a; Wade, 2005). Furthermore, these behaviors are each controlled by an extremely small set of muscles that have also been well-studied (Johnson and Wade, 2011; Lovern et al., 2004a; Wade, 2005). Therefore, each time a dewlap extension or copulation event is observed, we know which muscles and how many times the muscles controlling those behaviors are in use, linking muscles to behaviors and allowing us to examine hormone regulation of this relationship.

Two species of anoles are particularly well-studied: the Carolina green anole (*Anolis carolinensis*) and the Cuban brown anole (*A. sagrei*). Both species are semiarboreal. The trunk-crown *A. carolinensis* is long and slender (< 65 mm SVL) with a rose-pink to red dewlap and body colors ranging from brown to green (Collette, 1961; Lovern et al., 2004a). They can be found in both terrestrial and arboreal habitats often

perching lower to the ground in vegetation like cultivated gardens and shrubs (Collette, 1961). The trunk-ground *A. sagrei* is long-legged and short-headed (<70 mm SVL) with an orange-red dewlap and body colors ranging from a pale tan to almost black (Calsbeek, 2009; Collette, 1961). They are found mostly on or just above the ground in weeds or perched atop of woodpiles, brush, or fence posts (Collette, 1961). For my thesis, we measured the behaviors of male green and brown anoles in aggressive male-male contexts and sexual male-female contexts before and after testosterone manipulation. We then analyzed the muscles underlying these behaviors to understand better how testosterone facilitates dynamic changes in social behaviors in two distantly related species of anole lizards.

The most notable display behavior of adult male anoles is the extension of an extendable colorful throat-fan called a dewlap. The extension of the dewlap is controlled by a small set of muscles and cartilage in the throat. There is a pair of CH muscles (one on each side of the throat) that extend between a bilateral set of two pieces of cartilage – the ceratohyals and 1st ceratobranchials. A third set of cartilage at the midline (2nd ceratobranchials) is attached to the others near its rostral end. When the left and right CH muscles contract, the cartilages act as a lever, bending the 2nd ceratobranchials – which normally lay flat at the ventral surface of the throat and chest – causing them to bow out and extend the fan of colorful skin (reviewed in Wade, 2005, 2012).

The dewlap is used for communication (Rand and Williams, 1970). It is often used in combination with push-ups and head-bobs (Winkler and Wade, 1998). In my study, we combined push-ups and head-bobs into a composite measure called push-bobs. Males will dewlap and push-bob to females in sexual encounters (Crews and Greenberg, 1981; Greenberg and Noble, 1944). Males also perform these displays in aggressive encounters like when they are defending their territories against other males (Greenberg, 2003).

Another clear behavior in anoles is copulation. The copulatory system is relatively simple. Lizards unlike mammals, have two copulatory organs referred to as bilateral "hemipenes". They use one during each copulatory event, and they often alternate between the two hemipenes in subsequent copulations (Crews et al., 1978). The hemipenes are largely controlled by two muscles – the transversus penis (TPN) and the retractor penis magnus (RPM). The TPN wraps around the ventral surface of the

hemipenis in a medial to lateral orientation. Contraction of the TPN causes the hemipenis to evert through the cloaca. The RPM attaches to the caudal end of the hemipenis as it lies in the tail and facilitates its retraction following copulation. For my study we specifically examined how testosterone affects the RPM.

Testosterone has an important role in organizing the dewlap neuromuscular system during development. As juveniles the initial increase of testosterone in males increases the length of the 2nd ceratobranchial cartilages and the cross-sectional area of the CH muscle fibers (Lovern et al., 2004b; O'Bryant and Wade, 2001). Furthermore, treating juvenile females with testosterone produces male dewlap neuromuscular characteristics (Lovern et al., 2004b).

In adulthood, testosterone increases dewlap behavior in male brown anoles when they display to their reflection in a mirror and when they are displaying to other male brown anoles (Cox et al., 2009a; Tokarz et al., 2002). Testosterone also increases dewlap behavior in male green anoles when they display to females (Neal and Wade, 2007a). However, in the field and in the lab, despite large seasonal/manipulated fluctuations in testosterone that alter behavior, most components of the dewlap neuromuscular system remain stable (Holmes and Wade, 2005b; Neal and Wade, 2007a; O'Bryant and Wade, 1999).

Testosterone also organizes the copulatory system during development. Both male and female embryos begin with hemipenes, but since male embryos have higher concentrations of testosterone, they maintain their hemipenes whereas those in female embryos regress (Holmes and Wade, 2005a; Lovern and Wade, 2001).

In adulthood, testosterone regulates the neuromuscular traits of the hemipenes dramatically. For green anoles housed in breeding season like conditions, testosterone increased hemipenis and RPM size (Holmes and Wade, 2004).

As illustrated above, there is already an extensive body of research examining whether testosterone regulates dewlap and copulatory behaviors through variations in muscle morphology. However, there still remain gaps in our understanding of testosterone, behavior, and muscle morphology, and my thesis aims to address those gaps. In my study, I am exploring the hypothesis that testosterone changes social behaviors by changing the physiology of the muscles controlling social behaviors. There are three main questions my thesis will address. First, are the effects of testosterone

similar across courtship (male-female) and aggressive (male-male) social contexts? Second, does testosterone regulate behaviors by altering the structures and muscles underlying them? And thirdly, are the effects of testosterone on behavior and morphology similar across two distantly related species?

Methods

Animals and housing

All procedures involving animals were reviewed and approved by the Trinity Animal Research Committee (protocols NSF_050213_MAJ3 and 050416_MJ1).

In late April 2016, my collaborators (see acknowledgements) captured 48 males and 48 females of each species using a dental floss loop. Brown and green anoles were captured at the South Texas Botanical Gardens and several private nurseries in Corpus Christi, Texas, and additional green anoles were captured at the San Antonio Botanical Gardens and on the grounds of Trinity University in San Antonio, Texas. Within one day of capture, lizards were transported in cloth bags to the vivarium at Trinity University.

Upon their arrival at the vivarium, my collaborators measured each lizard's snout-vent length (SVL) to the nearest mm using a clear plastic ruler, and its mass to the nearest 0.05 g using a Pesola spring scale. To measure the surface area of the dewlap (hereafter, dewlap size), they photographed each male. To this end, they gently extended the dewlap using forceps to grip the base of the second ceratobranchial cartilage and pulled the cartilage gently forward. The lizards were positioned parallel to a 1 cm × 1 cm grid, and the camera was held at a consistent distance (approximately 7 cm) from each animal. This process was repeated twice for each animal, releasing the dewlap between photographs. Using ImageJ, my collaborators measured the area of the dewlap in each photograph, and the larger measure for each lizard was used in subsequent analyses. This technique for quantifying dewlap area has been highly repeatable in anoles (Lailvaux and Irschick, 2007; Lailvaux et al., 2012; Vanhooydonck et al., 2005a, 2005b).

Lizards were housed in a climate-controlled room, following the standard housing and care protocol for anole lizards established by Sanger et al. (2008). The temperature of the room ranged from 25.5-32°C, with most days ranging between 26-28°C. The humidity of the room ranged from 40-80%, with most days ranging between 55-65%. The lizards were exposed to standard reptile lighting conditions. Two T8 ReptiSun 5.0 UVB fluorescent bulbs hung directly over each cage and were set to a 13:11 light:dark cycle. Additionally, the ceiling lights in the room turned on 30 min before the cage lights and turned off 30 min after they turned off to mimic dawn and dusk. Initially, males were housed individually in large plastic Kritter Keeper cages (37.5 ×

21.0 × 28.0 cm), while females were housed in large (63 × 39 × 37 cm) mesh cages in groups of 4-6.

Each male cage contained two small PVC pipe perches, a wire mesh hammock stretching the width of the cage and was lined with R'zilla terrarium liner. Male cages were separated with plyboard sheets to prevent visual contact between lizards in different cages. Female group cages contained several perches and a small plastic plant pot filled with moist sphagnum peat moss (Fertilome Bonham, Texas) in which they could lay eggs. All lizards were fed 2-3 crickets or mealworms dusted with Fluker's calcium/phosphorus powder three times a week, and cages were misted daily to provide drinking water.

Each male was randomly assigned to one of three treatment groups (n = 16 males per treatment): castrated males receiving a blank placebo implant (low T), castrated males receiving a testosterone implant (high T), and intact control males receiving a placebo implant (control).

Baseline behavioral trials

After a minimum of 5 days after introduction to the vivarium, and before any testosterone manipulation, my collaborators conducted baseline behavioral trials for all males. Each male participated in four trials: two male-male trials, and two male-female trials. All male-male trials paired two males from the same treatment group, and all male-female trials paired a male with a novel female who was assigned to be subsequently housed with a male from the same treatment group. At the beginning of each trial, the two lizards were acclimated to the trial arena (a large 63 × 39 × 37 cm mesh cage), with each placed under an individual opaque Tupperware container on opposite sides of the arena for 10 min. After the acclimation period, my collaborators removed the containers simultaneously and observed the two lizards for 10 min from behind a blind 1.5 m away from the arena.

During the 10 min period, my collaborators recorded all social interactions between the lizards, including dewlap extensions, push-ups and head-bobs (here combined into a single measure called push-bobs), copulation attempts, and biting attempts. If one male bit another during the trial, or the lizards locked jaws, they immediately ended the trial and separated the lizards to prevent injury. At the end of

each trial, all lizards were returned to their home cage. Each lizard participated in no more than two trials per day, and at most one trial in the morning and one in the afternoon.

Testosterone manipulations

Approximately one week after the completion of the baseline behavior trials, the 48 males of each species were gonadectomized, following the procedures of Cox et al. (2009a, 2009b).

To make the implants, my collaborators cut Silastic tubing (Dow Corning, Midland, MI, USA; 1·47 mm i.d., 1·96 mm o.d.) into 4-mm segments. One end of each implant was sealed with silicone adhesive. Each piece of tubing was then filled with either 1 μL dimethyl sulphoxide (DMSO, blank) or 300 μg testosterone (T-1500, Sigma-Aldrich Inc., St. Louis, MO, USA) dissolved in 1 μL DMSO. The open end of each implant was then sealed with silicone adhesive and allowed to cure for 2 days, so the DMSO could diffuse out of the Silastic tubing, leaving either empty implants (blank) or implants filled with 100 μg crystalline T (following Cox et al., 2015).

Animals were fasted one day before surgery, and prior to surgery my collaborators anesthetized each animal with 4-5 μL of 0.25% bupivacaine HCl (Auromedics). Lizards were then placed in a -20°C freezer for 5 min so they would be immobilized for surgery. The lizards were then secured to an ice pack, on which surgery was performed. To expose the testes, they made a small vertical incision on the side of the midline where they injected the lizard with anesthetic. The low T and high T males were gonadectomied using a silk thread loop to sever the spermatic cord, which was then immediately cauterized. For control males, they made identical incisions and manipulations, but left the testes intact. After being gonadectomied or having their testes manipulated, each animal received either a testosterone implant (high T) or a placebo implant (low T and control) that was inserted into their coelomic cavity. The incisions were sealed with VetClose, and the lizard was left to rest in a plastic container overnight before being returned to its home cage (Cox et al., 2009a). The normal feeding schedule resumed one day after surgery. To allow full recovery from surgery, males remained housed individually for an additional two weeks. One brown anole male died immediately following surgery, and one green anole male died within the two-week

recovery period (nine days following surgery), for an overall mortality rate of 2%. After two weeks, a female was introduced to the home cage of each male lizard.

Post-surgery behavioral trials

Approximately six weeks post-surgery (and four weeks after the males were housed with a female), the male-male and male-female behavioral trials were repeated, exactly as described above. In male-male trials, males were again randomly paired with two other males from the same treatment, and in male-female trials, with two other novel females who were housed with males from the same treatment. After the trials, males and females were returned to their original home cages.

Tissue collection

Approximately 8.5 weeks (58-62 days) after testosterone manipulation, all lizards were euthanized. Previous studies of brown anoles indicate that circulating testosterone remains elevated following identical surgical manipulations ranging from 56 to 108 days (Cox et al., 2015, 2009a, 2009b).

To euthanize lizards, each lizard was first injected with a 2% MS-222 solution. Once the lizard was unresponsive to a firm toe pinch, they injected the lizard with a 50% MS-222 solution (Conroy et al., 2009). The lizard was then rapidly decapitated, and tissue from the jaw, including the CH muscle, and from the tail, including the hemipenes and the RPM, were collected. Trunk blood samples to measure circulating T levels were also collected. All samples were flash frozen on dry ice and stored at -80°C until further processing.

Muscle histology

To prepare the tissue samples for analysis, the frozen jaw and tail tissues were cryosectioned in ice, perpendicular to the jawbone and tail bone, respectively. The sections were cut at 20 μm, distributed into 6 series, and stored at -80°C.

One series of the jaw and tail tissue were stained using hematoxylin and eosin. Both the left and right sides of each tissue (separated by the midline) were then photographed using NIS-Elements (Nikon) and analyzed in NIS-Elements/ImageJ.

For each side of the lizard's jaw tissue I measured: 1) cross-sectional area (CSA) of the widest portion of the CH muscle, 2) number of muscle fibers in the CH cross-section, and 3) the CSA of 30 pseudo-randomly selected fibers in the CH cross-section.

I conducted the same three measures for the RPM muscle on each side of the tail tissue. I also measured the CSA of the hemipenes. Since the hemipenes are variable in both the length of its tissue and the circumference across its length, I measured the CSA in 2 to 5 sections (for an average of 4.6 sections per tissue) at approximately 300 μm intervals beginning at the distal end of the tissue and working towards the proximal end (Johnson et al., 2014). For analysis I used the largest CSA from the 2 to 5 sections that were measured.

CSA were measured by outlining the desired structure. I estimated the fiber number in each muscle by determining the number of fibers in the entire cross section of the left and right muscle. Finally, the CSA of 30 pseudo-randomly selected fibers on each side of the animal were measured.

All images for the hematoxylin and eosin stains were coded so the measurer was blind to the species and treatment of the tissue being analyzed. All measures of each morphological trait were averaged within each individual for statistical analyses.

Statistical analyses

All statistics were run in RStudio (Version 1.2.1335; RStudio Team 2018).

For all the behavioral data and non-invasive morphological data (i.e., dewlap area and mass) I ran a series of two-way mixed model ANOVAs (i.e., factorial repeated measures ANOVAs) that compared the behavioral and morphological traits by treatment and time before and after testosterone manipulation. I fit the data with a linear mixed effect model from package 'nlme' to identify main effects and interactions, and then used the emmeans() function for post hoc analyses (Pinheiro et al., 2021).

For hemipenis data and all the muscle data, I ran a series of one-way ANOVAs that compared the histological traits by treatment using the aov() function followed by post hoc analyses using the TukeyHSD() function.

In some low T males, their testes regrew, and in some high T males, my collaborators could not find their implants during autopsy. Since we could not trust that their T was actually high or low, I excluded them from all experimental analyses. I also

excluded any data from the two lizards that died following testosterone manipulations. Therefore, although we began with 48 lizards of each species, I analyzed the behaviors, dewlap area, and mass of 44 green anoles and 43 brown anoles. Furthermore, due to histological artifacts in some muscle and tissue sections, I analyzed hemipenis morphology in 38 green anoles and 37 brown anoles, RPM morphology in 39 green anoles and 41 brown anoles, and CH morphology in 43-44 green anoles and 42 brown anoles.

Results

Behavior

We measured several behaviors over the course of the 10-minute arena trials: (1) total number of movements, (2) total number of push-bobs, (3) total number of dewlap extensions, (4) total time the dewlap was extended, and finally for those lizards that extended their dewlap (Fig. 3 and 4) we (5) calculated the average time per dewlap extension by dividing the total time the dewlap was extended by the total number of dewlap extensions. We measured these aspects of behavior in staged male-male and male-female interactions. For all behaviors, there were no statistical differences between groups prior to surgery (Table 1, 2; Fig 1, 2, 5, 6, 7), but many of these behaviors increased in frequency in high T males (see below).

Movements

Testosterone did not affect how frequently green or brown anoles moved in the presence of males or females (Table 1, 2 and Fig. 1). However, in the presence of both sexes all brown anoles moved less frequently after surgery than they did before surgery (Table 2 and Fig. 1b).

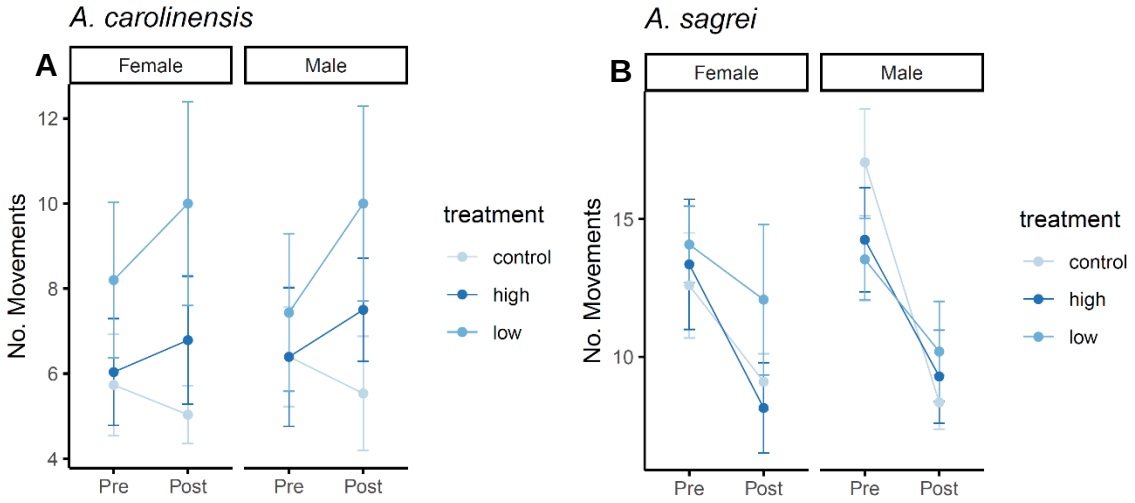

Fig. 1. Number of movements performed by male anoles treated with high T and low T in the presence females and males. (A) green anoles. (B) brown anoles. Values are means ± s.e.m.

Push-Bobs

High T green anoles push-bobbed more to both males and females than low T green anoles (Table 1 and Fig. 2a). High T brown anoles also push-bobbed more to males than low T brown anoles (Table 2 and Fig. 2b). However, testosterone did not influence the number of push-bobs brown anoles displayed to females (Table 2 and Fig. 2b).

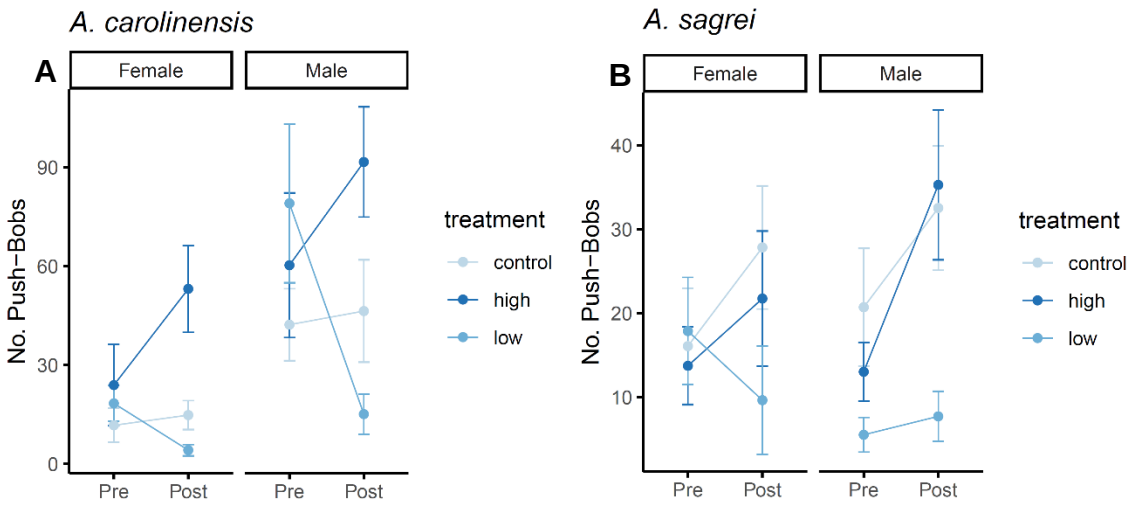

Fig. 2. Number of push-bobs performed by male anoles treated with high T and low T in the presence females and males. (A) green anoles. (B) brown anoles. Values are means ± s.e.m.

Dewlap Extensions

Prior to surgery, only about 55% of all green anoles and 67% of all brown anoles displayed their dewlaps to females (Fig. 3). Across the treatment groups within each species, the percentages of males that displayed their dewlaps to females were relatively similar (Fig. 3). After surgery, 64% of all green anoles and 65% of all brown anoles displayed their dewlaps to females (Fig. 3). However, across the treatment groups within each species, a much higher percentage of high T males displayed their dewlaps to females than low T males (Fig. 3).

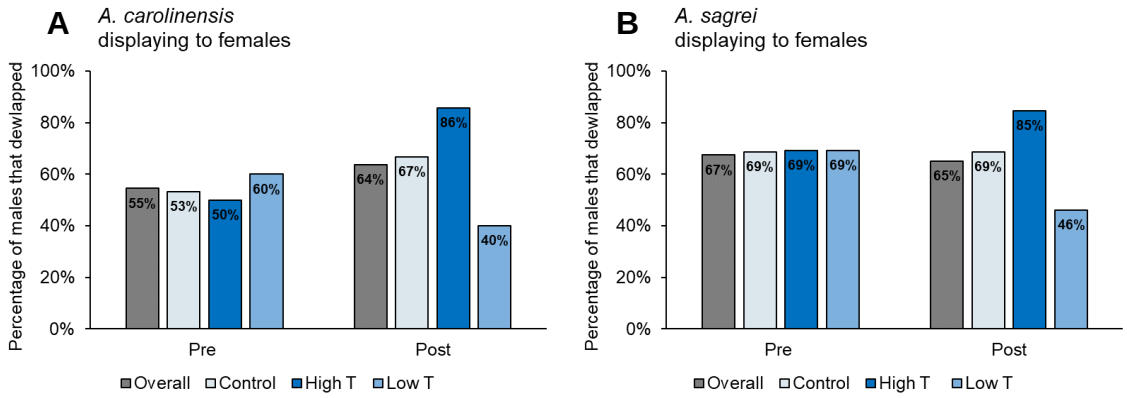

Fig. 3. Percentage of male anoles treated with high T and low T that dewlapped at least once to females during the 10-minute arena trials. (A) green anoles. (B) brown anoles. Overall = all males in a species regardless of treatment.

A similar pattern was observed when looking at green and brown anoles displaying to other males (Fig. 4). However, in this context, prior to surgery, 73% of all green anoles and 88% of all brown anoles displayed their dewlaps to males (Fig. 4). Like in the female context, across the treatment groups within each species, the percentages of males that displayed their dewlaps to other males were relatively similar prior to surgery (Fig. 4). After surgery, 80% of all green anoles and 63% of all brown anoles displayed their dewlaps to other males (Fig. 4). And again, like in the female context, after surgery, a much higher percentage of high T males displayed their dewlaps to other males than low T males (Fig. 4).

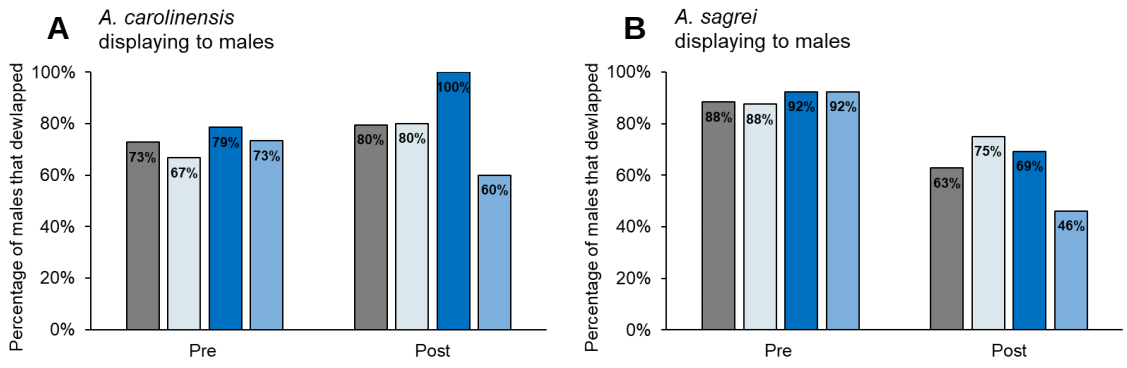

Fig. 4. Percentage of male anoles treated with high T and low T that dewlapped at least once to males during the 10-minute arena trials. (A) green anoles. (B) brown anoles. Overall = all males in a species regardless of treatment.

When in the presence of females, high T green anoles displayed their dewlap more frequently and for longer than low T green anoles (Table 1 and Fig. 5a, 6a). The same pattern was observed in brown anoles. When in presence of females, high T brown anoles also displayed their dewlap more frequently and for longer than low T brown anoles (Table 2 and Fig. 5b, 6b).

When in the presence of males, the effects of testosterone on dewlap behavior are different. Testosterone did not change how often or how long green anoles displayed their dewlap to other males (Table 1 and Fig. 5a, 6a). Yet, there was a significant reduction over time in how long low T green anoles displayed to other males (Table 1 and Fig. 6a). Like in the green anoles, testosterone did not change how often or how long brown anoles displayed their dewlap to other males (Table 2 and Fig. 5b, 6b). However, all brown anoles ended up extending their dewlaps less frequently and for a shorter duration than before surgery (Table 2 and Fig. 5b, 6b).

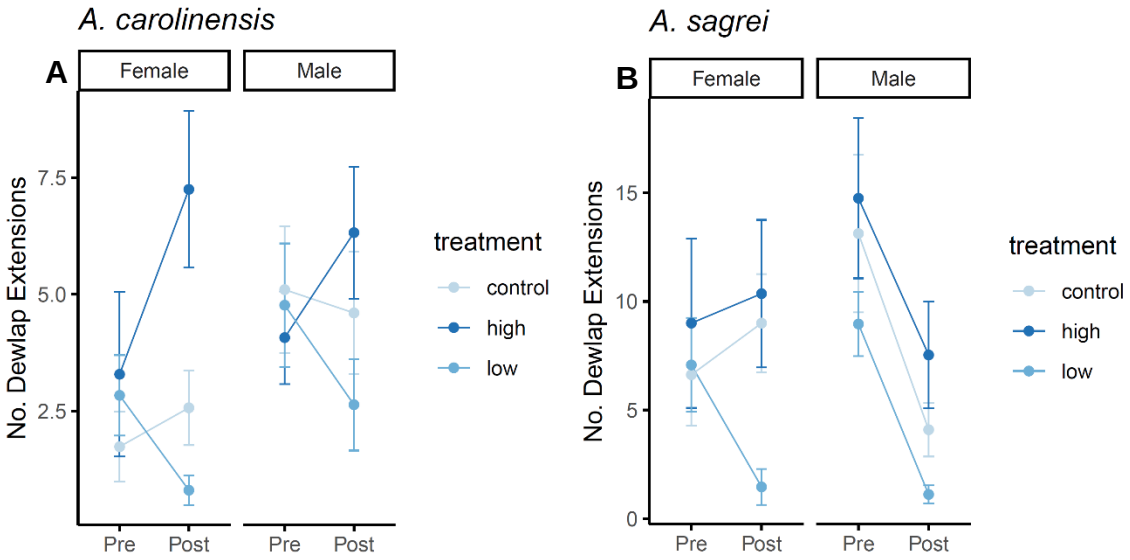

Fig. 5. Number of dewlap extensions performed by male anoles treated with high T and low T in the presence females and males. (A) green anoles. (B) brown anoles. Values are means ± s.e.m.

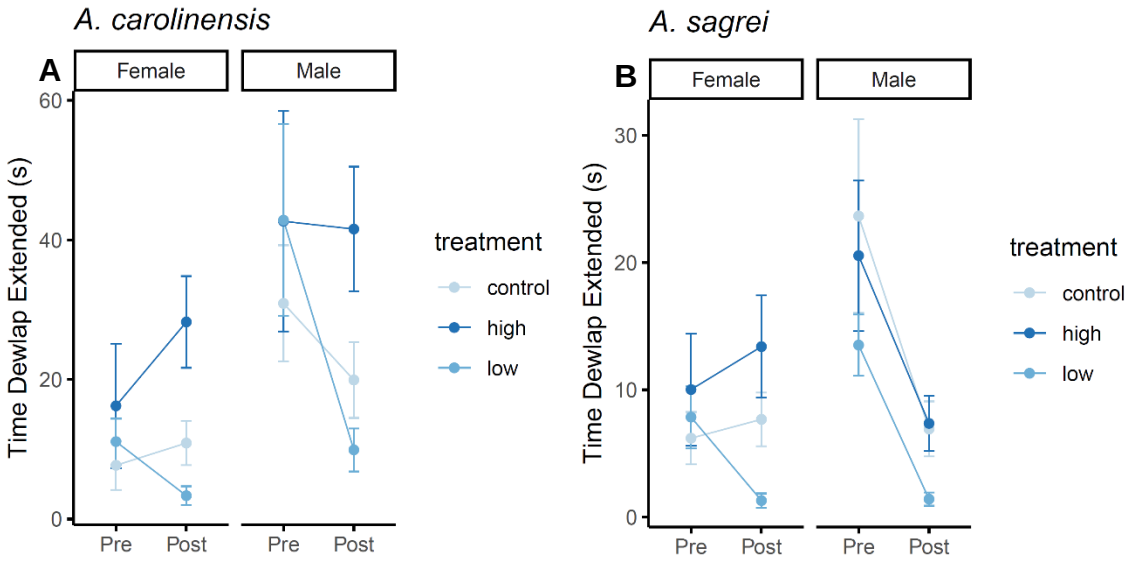

Fig. 6. Total time dewlap extensions were performed by male anoles treated with high T and low T in the presence females and males. (A) green anoles. (B) brown anoles. Values are means ± s.e.m.

To test whether testosterone altered the pattern of dewlap extensions, we calculated the average time a dewlap was extended. Testosterone did not affect the average length of green anole dewlap extensions towards females (Table 1 and Fig. 7a). However, there was an effect of treatment on the length of green anole dewlap extensions towards other males (Table 1 and Fig. 7a). In brown anoles testosterone did not affect the average length of dewlap extensions towards males or females (Table 2 and Fig. 7b).

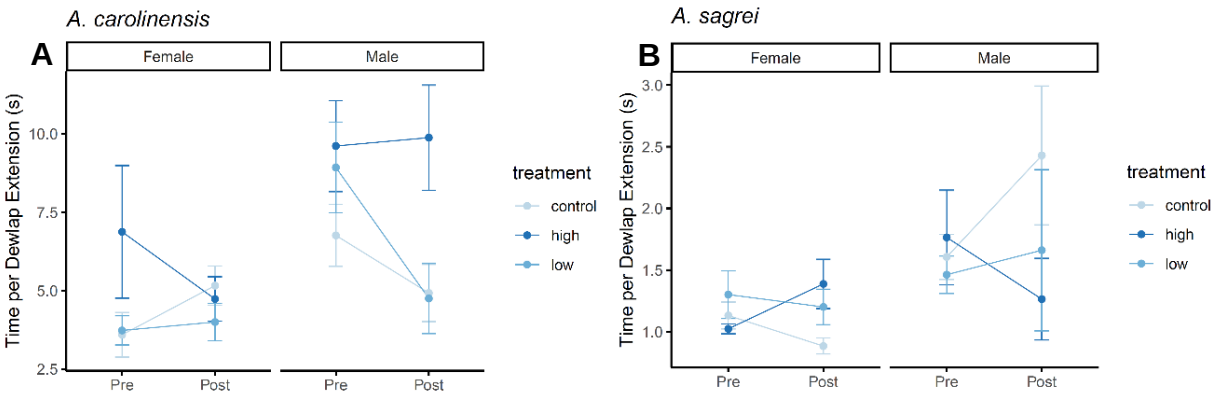

Fig. 7. Average length of a single dewlap extension performed by male anoles treated with high T and low T in the presence females and males. (A) green anoles. (B) brown anoles. Values are means ± s.e.m.

Morphology

Mass

Before surgery, all anoles within each species had similar mass (Table 1, 2 and Fig. 8). After surgery, the green anoles maintained their mass (Table 1 and Fig. 8a). However, high T brown anoles and control brown anoles weighed substantially more after surgery than they did before surgery (Table 2 and Fig. 8b).

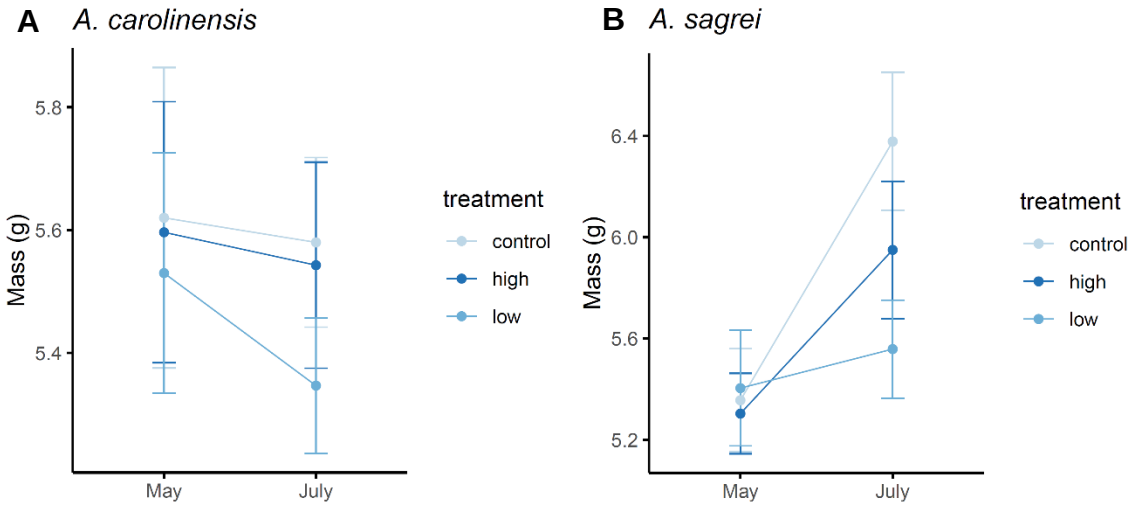

Fig. 8. Mass of male anoles treated with high T and low T. (A) green anoles. (B) brown anoles. Values are means ± s.e.m.

Dewlap and musculature

Green anoles had similar dewlap areas prior to surgery (Table 1 and Fig. 9a). After surgery, the dewlap areas of all green anoles decreased, and there were no differences in dewlap areas between the treatment groups (Table 1 and Fig. 9a).

All brown anoles also had similar dewlap areas prior to surgery (Table 2 and Fig. 9b). However, after surgery the control brown anoles showed increases in dewlap areas and had larger dewlaps than either the high T or low T brown anoles (Table 2 and Fig. 9b).

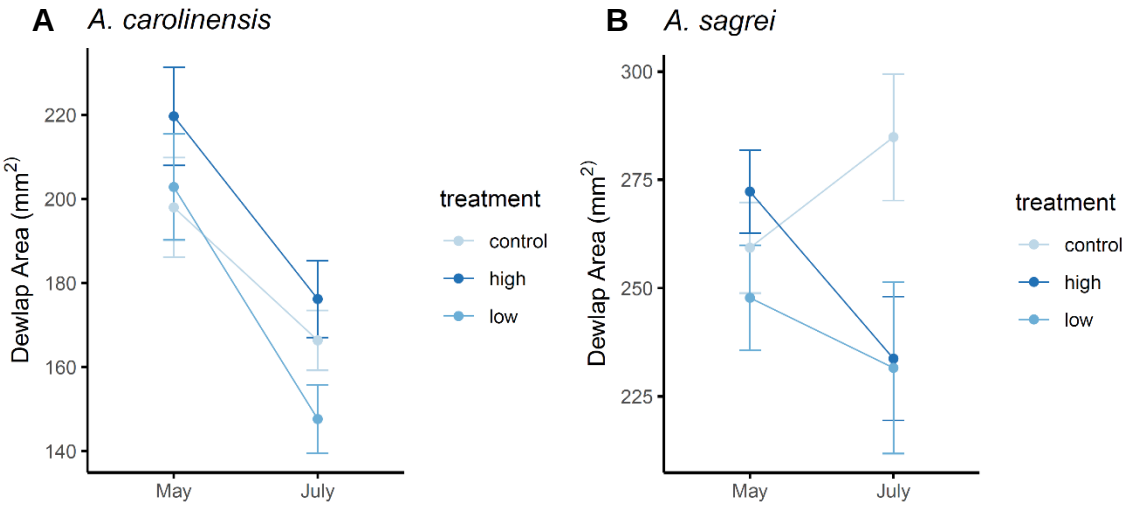

Fig. 9. Dewlap area of male anoles treated with high T and low T. (A) green anoles. (B) brown anoles. Values are means ± s.e.m.

We did not detect any changes in the musculature of the dewlap in either the green or brown anole (Table 1, 2 and Fig. 10, 11, 12). All treatment groups maintained a similar area of the CH (Table 1, 2 and Fig. 10), a similar fiber number in the CH (Table 1, 2 and Fig. 11), and a similar fiber area in the CH (Table 1, 2 and Fig. 12).

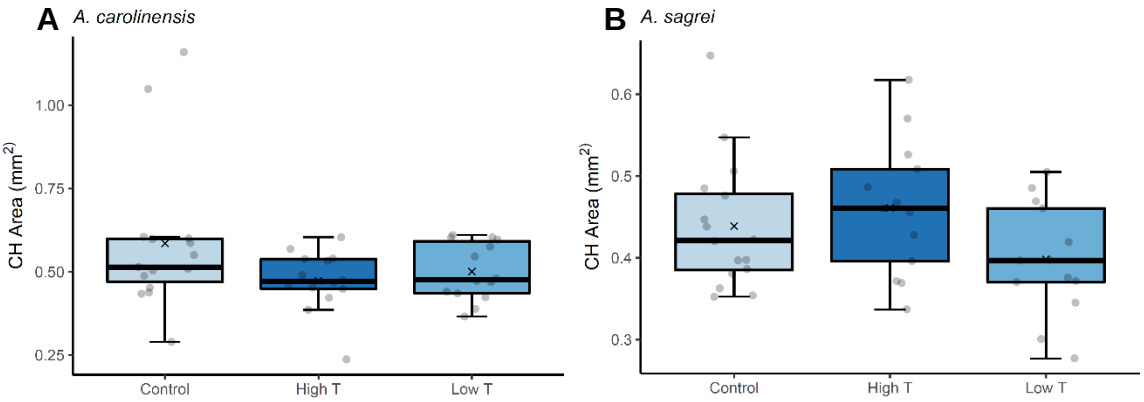

Fig. 10. Area of the ceratohyoid (CH) of male anoles treated with high T and low T. (A) green anoles. (B) brown anoles. Horizontal line = median. x = mean. Grey dots = individual data points.

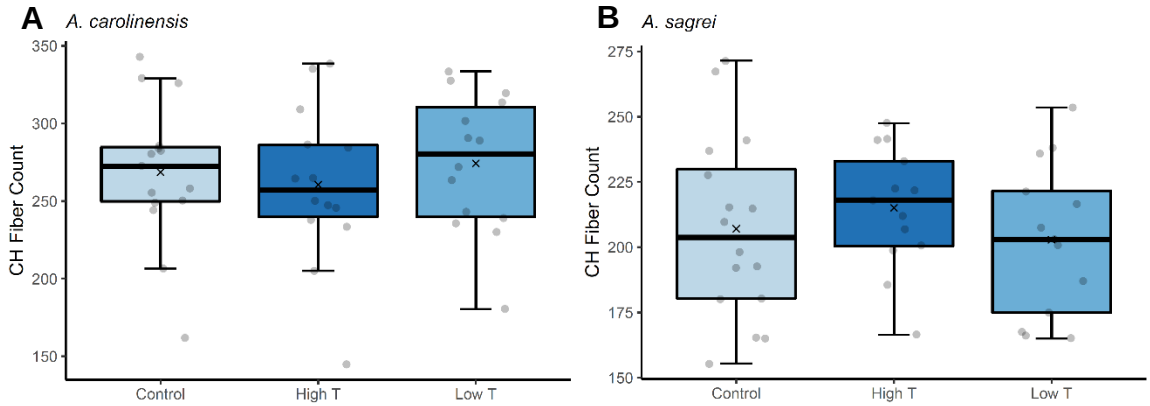

Fig. 11. Number of fibers within the ceratohyoid (CH) of male anoles treated with high T and low T. (A) green anoles. (B) brown anoles. Horizontal line = median. x = mean. Grey dots = individual data points.

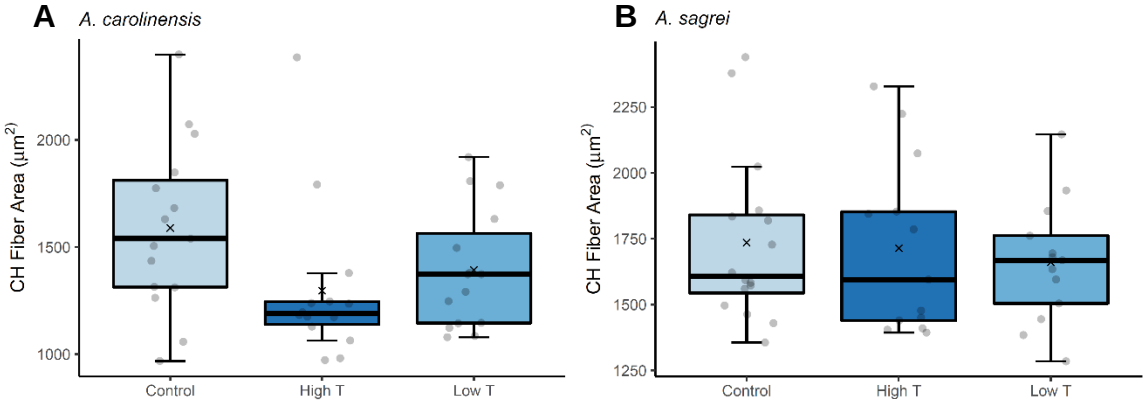

Fig. 12. Area of fibers within the ceratohyoid (CH) of male anoles treated with high T and low T. (A) green anoles. (B) brown anoles. Horizontal line = median. x = mean. Grey dots = individual data points.

Hemipenes and musculature

Both high T green anoles and high T brown anoles had much larger hemipenis areas than low T green anoles and low T brown anoles (Table 1, 2 and Fig. 13). Likewise, both high T green anoles and high T brown anoles had much larger RPM areas than low T green anoles and low T brown anoles (Table 1, 2 and Fig. 14).

Both high T green anoles and high T brown anoles also had larger individual RPM fiber areas than low T green anoles and low T brown anoles (Table 1, 2 and Fig. 15). However, the number of RPM fibers did not differ between the treatment groups in either species (Table 1, 2 and Fig. 16).

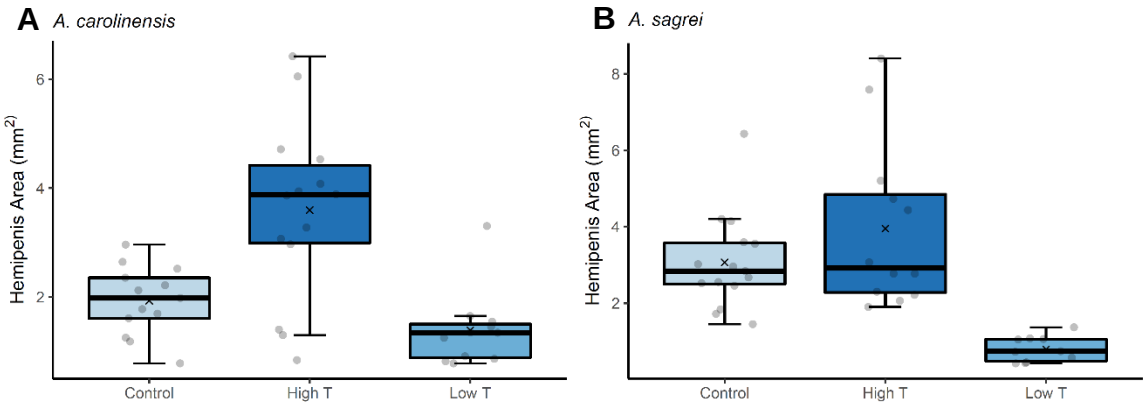

Fig. 13. Area of the hemipenis of male anoles treated with high T and low T. (A) green anoles. (B) brown anoles. Horizontal line = median. x = mean. Grey dots = individual data points.

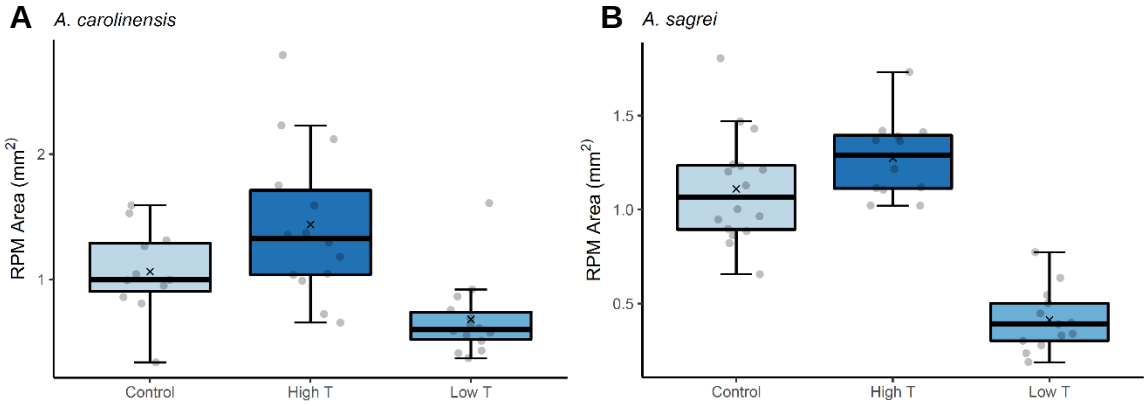

Fig. 14. Area of the retractor penis magnus (RPM) of male anoles treated with high T and low T. (A) green anoles. (B) brown anoles. Horizontal line = median. x = mean. Grey dots = individual data points.

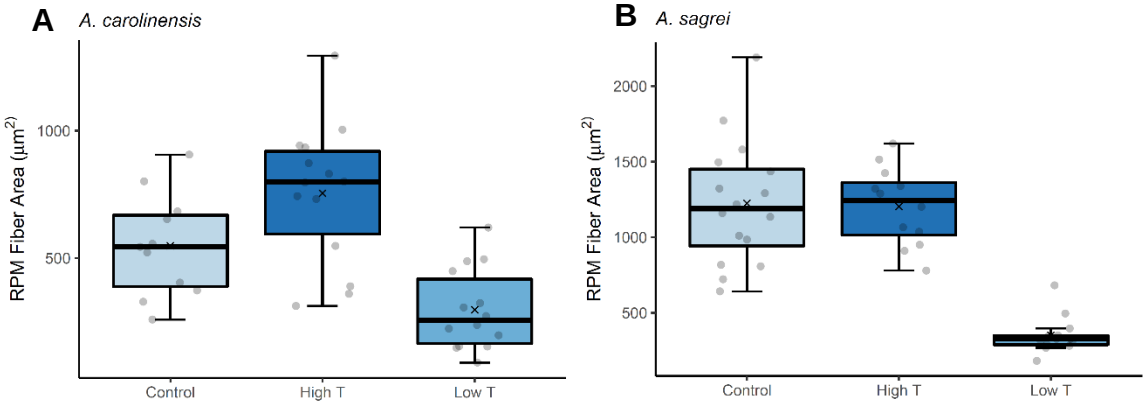

Fig. 15. Area of fibers within the retractor penis magnus (RPM) of male anoles treated with high T and low T. (A) green anoles. (B) brown anoles. Horizontal line = median. x = mean. Grey dots = individual data points.

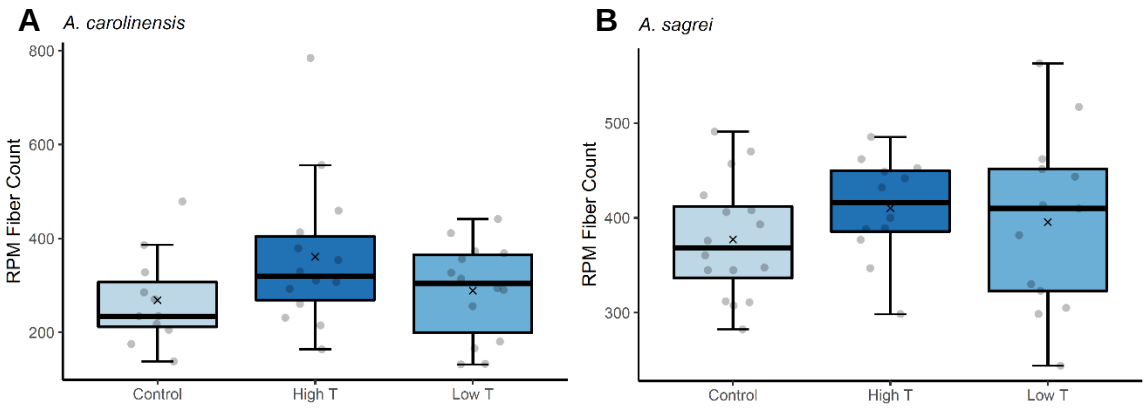

Fig. 16. Number of fibers within the retractor penis magnus (RPM) of male anoles treated with high T and low T. (A) green anoles. (B) brown anoles. Horizontal line = median. x = mean. Grey dots = individual data points.

Table 1. ANOVAs comparing behaviors and morphological traits in the green anole (*A. carolinensis*) across treatments groups and time (when applicable). Each trait was analyzed in a separate factorial repeated measures ANOVA or one-way ANOVA. Significant comparisons are highlighted in red font.

GREEN ANOLES	Treatment	Time	Treatment × Time
DISPLAYING TO FEMALES			
No. Movements	$F_{2,41}$ = 1.89, p = 0.16	$F_{1,41}$ = 0.56, p = 0.46	$F_{2,41}$ = 0.80, p = 0.46
No. Push-Bobs	*$F_{2,41}$ = 6.48, p = 0.0036	$F_{1,41}$ = 0.81, p = 0.37	*$F_{2,41}$ = 4.16, p = 0.023
No. Dewlap Extensions	*$F_{2,41}$ = 5.08, p = 0.011	$F_{1,41}$ = 0.98, p = 0.33	*$F_{2,41}$ = 3.98, p = 0.026
Time Dewlap Extended (s)	*$F_{2,41}$ = 4.50, p = 0.017	$F_{1,41}$ = 0.37, p = 0.54	$F_{2,41}$ = 2.35, p = 0.11
Time per Dewlap Extension (s)	$F_{2,29}$ = 1.57, p = 0.22	$F_{1,17}$ = 0.049, p = 0.83	$F_{2,17}$ = 2.19, p = 0.14
DISPLAYING TO MALES			
No. Movements	$F_{2,41}$ = 1.02, p = 0.37	$F_{1,41}$ = 0.86, p = 0.36	$F_{2,41}$ = 1.01, p = 0.37
No. Push-Bobs	$F_{2,41}$ = 1.77, p = 0.18	$F_{1,41}$ = 0.70, p = 0.41	*$F_{2,41}$ = 5.17, p = 0.0099
No. Dewlap Extensions	$F_{2,41}$ = 0.57, p = 0.57	$F_{1,41}$ = 0.052, p = 0.82	$F_{2,41}$ = 2.54, p = 0.092
Time Dewlap Extended (s)	$F_{2,41}$ = 1.36, p = 0.27	*$F_{1,41}$ = 4.56, p = 0.039	$F_{2,41}$ = 1.71, p = 0.19
Time per Dewlap Extension (s)	*$F_{2,36}$ = 4.58, p = 0.017	$F_{1,25}$ = 2.59, p = 0.12	$F_{2,25}$ = 1.40, p = 0.26
MORPHOLOGY			
Mass (g)	$F_{2,41}$ = 0.28, p = 0.76	$F_{1,41}$ = 0.95, p = 0.33	$F_{2,41}$ = 0.23, p = 0.79
Dewlap Area (mm^2)	$F_{2,41}$ = 1.70, p = 0.19	*$F_{1,41}$ = 50.56, p < 0.0001	$F_{2,41}$ = 1.27, p = 0.29
CH Area (mm^2)	$F_{2,40}$ = 2.15, p = 0.13	-	-
CH Fiber Area (μm^2)	$F_{2,41}$ = 2.64, p = 0.083	-	-
CH Fiber Count	$F_{2,40}$ = 0.30, p = 0.75	-	-
HP Area (mm^2)	*$F_{2,35}$ = 13.97, p < 0.0001	-	-
RPM Area (mm^2)	*$F_{2,36}$ = 9.90, p = 0.00038	-	-
RPM Fiber Area (μm^2)	*$F_{2,36}$ = 15.47, p < 0.0001	-	-
RPM Fiber Count	$F_{2,36}$ = 1.98, p = 0.152	-	-

CH = ceratohyoid, HP = hemipenis, RPM = retractor penis magus. - indicates statistics that were not computed because the morphological trait was only measured after surgery. * indicates statistical significance (p < 0.05).

Table 2. ANOVAs comparing behaviors and morphological traits in the brown anole (*A. sagrei*) across treatments groups and time (when applicable). Each trait was analyzed in a separate factorial repeated measures ANOVA or one-way ANOVA. Significant comparisons are highlighted in red font.

BROWN ANOLES	Treatment	Time	Treatment × Time
DISPLAYING TO FEMALES			
No. Movements	$F_{2,40}$ = 0.68, p = 0.51	*$F_{1,40}$ = 8.50, p = 0.0058	$F_{2,40}$ = 0.53, p = 0.59
No. Push-Bobs	$F_{2,40}$ = 0.62, p = 0.54	$F_{1,40}$ = 0.80, p = 0.37	$F_{2,40}$ = 1.45, p = 0.25
No. Dewlap Extensions	$F_{2,40}$ = 1.21, p = 0.31	$F_{1,40}$ = 0.077, p = 0.78	*$F_{2,40}$ = 3.37, p = 0.044
Time Dewlap Extended (s)	$F_{2,40}$ = 1.96, p = 0.15	$F_{1,40}$ = 0.044, p = 0.84	*$F_{2,40}$ = 3.46, p = 0.041
Time per Dewlap Extension (s)	$F_{2,31}$ = 1.97, p = 0.16	$F_{1,20}$ = 0.0023, p = 0.96	$F_{2,20}$ = 2.74, p = 0.088
DISPLAYING TO MALES			
No. Movements	$F_{2,40}$ = 0.15, p = 0.86	*$F_{1,40}$ = 29.49, p < 0.0001	$F_{2,40}$ = 2.23, p = 0.12
No. Push-Bobs	*$F_{2,40}$ = 4.40, p = 0.019	*$F_{1,40}$ = 9.27, p = 0.0041	$F_{2,40}$ = 1.94, p = 0.16
No. Dewlap Extensions	$F_{2,40}$ = 1.78, p = 0.18	*$F_{1,40}$ = 31.59, p < 0.0001	$F_{2,40}$ = 0.14, p = 0.87
Time Dewlap Extended (s)	$F_{2,40}$ = 1.25, p = 0.30	*$F_{1,40}$ = 22.45, p < 0.0001	$F_{2,40}$ = 0.23, p = 0.80
Time per Dewlap Extension (s)	$F_{2,37}$ = 0.83, p = 0.44	$F_{1,22}$ = 0.59, p = 0.45	$F_{2,22}$ = 1.90, p = 0.17
MORPHOLOGY			
Mass (g)	$F_{2,39}$ = 0.98, p = 0.38	*$F_{1,38}$ = 28.13, p < 0.0001	*$F_{2,38}$ = 4.20, p = 0.022
Dewlap Area (mm^2)	$F_{2,39}$ = 2.35, p = 0.11	$F_{1,38}$ = 0.63, p = 0.43	*$F_{2,38}$ = 4.13, p = 0.024
CH Area (mm^2)	$F_{2,39}$ = 2.239, p = 0.12	-	-
CH Fiber Area (μm^2)	$F_{2,39}$ = 0.228, p = 0.797	-	-
CH Fiber Count	$F_{2,39}$ = 0.547, p = 0.583	-	-
HP Area (mm^2)	*$F_{2,36}$ = 15.54, p < 0.0001	-	-
RPM Area (mm^2)	*$F_{2,38}$ = 49.08, p < 0.0001	-	-
RPM Fiber Area (μm^2)	*$F_{2,38}$ = 36.5, p < 0.0001	-	-
RPM Fiber Count	$F_{2,38}$ = 0.75, p = 0.479	-	-

CH = ceratohyoid, HP = hemipenis, RPM = retractor penis magus. - indicates statistics that were not computed because the morphological trait was only measured after surgery. * indicates statistical significance (p < 0.05).

Discussion

This experiment demonstrates that testosterone has complex and dynamic effects on lizard behavior and morphology. Testosterone did not affect overall activity (i.e., movements). However, testosterone had context-specific (i.e., sexual vs. aggressive) effects on social behaviors (i.e., dewlap extensions and push-bobs). Testosterone also increased copulatory structures and muscles (i.e., hemipenis area, RPM area, and RPM fiber area), but not dewlap structures and muscles (i.e., dewlap area, CH area, CH fiber area, CH fiber number). And finally, our experiment shows that testosterone regulates these behaviors and morphologies consistently between two distantly related species of anole lizards (i.e., *A. carolinensis* and *A. sagrei*).

Behavior

Testosterone did not impact overall activity of either species during our trials (Table 1, 2 and Fig. 1). We measured the number of movements during our behavioral trials because movement is a proxy for overall activity. Because testosterone did not alter how much green or brown anoles moved in either sexual or aggressive contexts, any effects of testosterone on other behaviors are not due to an underlying difference in overall activity. However, both treatments of brown anoles moved less frequently after testosterone manipulation (a main effect of time; Table 2 and Fig. 1b). Therefore, when there is only a main effect of time on any of the other behaviors for brown anoles, we need to consider the overall decreased activity in our interpretations.

Testosterone had context-specific effects on dewlap and push-bob behaviors in both species (Table 1, 2 and Fig. 2, 5). Testosterone increased dewlap extensions in sexual contexts and increased push-bobs predominantly in aggressive contexts.

Despite these overall patterns we observed across green and brown anoles, there is an interesting pattern of results when we compare different perspectives of dewlap behavior. As expected, prior to surgery, there were no differences between the treatment groups for the percentage of males that dewlapped at least once during the two 10-minute behavioral trials with novel males and novel females (Fig. 3 and 4). Not surprisingly, after testosterone manipulation, more high T males dewlapped at least once and less low T males dewlapped at least once (Fig. 3 and 4). This was true for both

species in both social contexts. An interesting pattern appears, though, when comparing the percentages between male-male and male-female contexts. Only 55-67% of male green and brown anoles dewlapped to females, while 63-88% of all male green and brown anoles dewlapped to other males (Fig. 3 and 4). This result is intriguing because, although a greater percentage of males dewlapped to other males (63-88%) than to other females (55-67%), and although testosterone ubiquitously increased these percentages in both male-male and male-female contexts, testosterone *only* appeared to regulate the frequency and time spent dewlapping when males were displaying to females, *not* to other males (Table 1, 2 and Fig 5, 6). This suggests that for both species, testosterone facilitates a change in the animals' "time-budget", where high T males spend more time courting females with their dewlap. This conclusion is consistent with results from Neal and Wade (2007a). They examined courtship and copulation behaviors in adult male green anoles, considering seasons, hormones, and the presence of females. Testosterone increased the total number of dewlap extensions and copulations in both the breeding and non-breeding season, and the greatest effect of testosterone was seen during the breeding season.

Testosterone did not change the average length of each dewlap extension in either sexual or aggressive contexts, except for the main effect of treatment we found when green anoles displayed to males (Table 1, 2 and Fig. 7). This suggests that testosterone clearly regulates the overall frequency and time anoles spend dewlapping in a context-dependent fashion, but not necessarily the pattern of dewlap display. Therefore, due to the context-dependent nature of testosterone on behavior, testosterone may be changing muscle innervations and activations rather than the overall composition of the muscle.

Even though testosterone did not regulate dewlap extensions in aggressive male-male contexts, all brown anoles dewlapped less to other males after surgery than before surgery (Fig. 5b). This may be experimental habituation since all brown anoles moved less frequently after surgery (Fig. 1b). However, the push-bob data argue against this interpretation (Fig. 2b). High T brown anoles push-bobbed substantially more to other males than low T brown anoles (Fig. 2b). If habituation was occurring, push-bob behaviors should also have decreased across the treatment groups. Additionally, testosterone had no effect on push-bobs towards females. So overall, testosterone

regulated push-bob behaviors in aggressive contexts (but not sexual contexts), and testosterone regulated dewlap behaviors in sexual contexts (but not aggressive contexts).

There are several possible explanations for this context-specific pattern of results. First, we may have demonstrated a tendency to perform certain aspects of behaviors in certain social contexts. For example, dewlap extensions may be more effective in sexual contexts, whereas push-bobs may be more effective in aggressive contexts, and testosterone regulates these behaviors in such a way that exacerbates these differences. This explanation would need further research to determine if allocating more time dewlapping to females is more effective in terms of male fitness because research thus far in the green anole suggests that there is no evidence of female choice for high performance males (Lailvaux and Irschick, 2006). However, this study only measured 'performance' in terms of dewlap area, acceleration, velocity, and bite force, not in frequency and time spent displaying the dewlap. In another study by Steffen and Guyer (2014), they pooled head-bob, push-up, and dewlap-extension frequencies for brown anole males in both male-male and male-male-female social contexts over two summers into a principal component analyses. They found that having a higher principal component of behavior meant a lizard was more likely to win contests in male-male and male-male-female contexts, which may translate to greater fitness.

Second, if there is a hierarchy in display repertoire, where dewlap extensions are the flashiest display in both sexual and aggressive contexts followed by push-bobs, we may have observed some effect of seasonality. Our post-surgery behavioral trials took place towards the end of the breeding season. Maybe the opportunity to mate with another female before the end of the breeding season necessitates dewlap extensions. While in aggressive contexts, territories have already been well established so there is not as clear of a benefit spending time and energy dewlapping over a territory. However, research in brown anoles does not support this explanation. Tokarz et al. (2002) collected male brown anoles from the field during each of 3 months in the breeding (May, June, July) and non-breeding season (Oct, Nov, Dec) and measured their behaviors towards other males in the lab. Brown anoles in the breeding season dewlapped more per minute than brown anoles in the non-breeding season, and there were no differences between the months within each season.

Lastly, maybe dewlap extensions towards males in aggressive contexts are indeed also regulated by testosterone. This would be consistent with Cox et al. (2009a), where high T brown anole males had higher mean frequencies of dewlap extensions and push-ups to other males compared to controls and low T males, and consistent with Tokarz et al. (2002), where high T males dewlapped more per minute to other males than males without T. It is possible that we observed a reduction in dewlap extensions because the aggressive bouts were so intense that the males did not want to risk damage to their dewlaps from a bite or tear from their competitors. In Cox et al. (2009a), the male brown anoles were placed in front of a mirror to emulate a similar-sized competitor, so there is no threat of damage to the dewlap. However, comparing how brown anoles display to a reflection of themselves versus a novel male competitor is tricky to tease apart. The differences between our findings and Tokarz et al. (2002) remain unexplained.

In the green anole, testosterone not only regulated push-bobs in aggressive male-male contexts but also in sexual male-female contexts (Fig. 2a). However, in a 10 min trial, on average, high T green anole males push-bobbed nearly 92 times to other males but only 53 times to females (Fig. 2a). Perhaps the difference in the magnitude of push-bob displays between the contexts also supports the explanation that push-bobs may be a more effective aspect of behavior in aggressive contexts than in sexual contexts.

Our results are fascinating with regards to teasing apart potential context-specific effects of testosterone on behaviors. However, translating these findings to natural populations of lizards is difficult. In the field, it can be challenging to discern whether males are displaying solely to females or other males, especially when the male is in a densely populated area, and his display is advertised to everyone. In this situation, it is difficult to identify the 'intended' recipient of the signal. As such, it is difficult to know if, in nature, males dewlap more frequently to females and push-bob more frequently to other males, but this would be an interesting hypothesis to explore in future studies.

Morphology

Although testosterone regulates dewlap behaviors in a context-specific manner, we did not detect any effects of testosterone on dewlap morphology. The area of all green anole dewlaps decreased over time (Table 1 and Fig. 9a). There is a similar

decrease in dewlap area in high T and low T brown anoles, but the dewlaps of the control lizards grew (Table 2 and Fig. 9b). The dewlaps in the controls likely grew because the control brown anoles grew by nearly 20% over the course of our experiment. These substantial gains in mass may be a combination of a more consistent and controlled feeding regime and the brown anoles' overall decrease in activity in the laboratory environment (Table 2 and Fig. 1b).

Because our experiment started near the beginning of the breeding season and ended towards the end of the breeding season, we propose this decrease in dewlap area may reflect the seasonal plasticity in dewlap areas found by Lailvaux et al. (2015). Due to the elasticity of the dewlap, the dewlap is larger during the breeding season when it is used more frequently and smaller during the non-breeding season when it is used less frequently (Lailvaux et al., 2015). Our results are not consistent with findings from Cox et al. (2009a), where high T brown anoles hard larger dewlaps than low T brown anoles (due to a substantial decrease in area in the low T brown anoles). However, other research examining how testosterone influences dewlap size shows mixed results. For example, when comparing lightweight and heavyweight male green anoles, there was a correlation between testosterone concentration and dewlap area in lightweights, but no such correlation among heavyweights (Husak et al., 2007).

Testosterone also did not alter the CH muscle that controls the dewlap: neither the area of the CH (Fig. 10), nor the area (Fig. 12), nor number of individual fibers (Fig. 11) within the CH. This result is consistent with Holmes and Wade (2005b) and Neal and Wade (2007a), where testosterone also did not change the number of fibers within the CH nor the area of the individual fibers within the CH. However, within a natural and unmanipulated population of male green anoles, Neal and Wade (2007b) found that 'studs' had larger CH fibers than 'duds'. In this study, 'studs' were the 8 males in the population that courted most frequently in their behavioral trials, and 'duds' were the 8 males that courted least frequently. The average number of dewlap extensions displayed to a novel female between 'studs' and 'duds' was statistically significant. Therefore, they concluded that the average number of dewlap extensions displayed to a novel female was correlated with CH fiber size. However, 'studs' did not have a significantly higher level of plasma androgens than 'duds'. Because of this, the researchers also measured a secondary sexual characteristic of androgens (renal sex segment height). They found

that ‘studs’ did have a larger renal sex segment height than ‘duds’. Although this study suggests a relationship between fiber size and behavior, the design has a small sample size and focuses on the extremes within the natural population.

Researchers have also examined whether testosterone changes other aspects of the CH muscle. Testosterone does not alter the percentage of androgen receptor positive nuclei in the CH (Holmes and Wade, 2005b). However, the CH has a heterogeneous fiber type composition (Holmes et al., 2007). During the breeding season there are more fast-oxidative glycolytic (FOG) fibers than during the non-breeding season (Holmes et al., 2007). Independent of season, testosterone treated males also had a higher percentage of FOG fibers (Holmes et al., 2007). Therefore, maybe seasonal changes in testosterone levels increase dewlap behaviors by shifting the fiber type composition of the CH muscle.

From Neal and Wade (2007a), we know that testosterone increases copulations. In my study, testosterone altered copulatory morphology in both species by increasing the area of the hemipenes (Fig. 13) and the area of the RPM (Fig. 14). The area of the RPM grew due to larger individual RPM fibers (Fig. 15), not because there were more individual fibers (Fig. 16). These results are consistent with Neal and Wade (2007a) and Holmes and Wade (2005b), where testosterone manipulation increased hemipenis size and RPM fiber size but not the number of fibers within the RPM. Holmes and Wade (2005b) also found that testosterone increased the percentage of androgen receptor positive nuclei within the RPM. Unlike the CH, the RPM contains a homogeneous fiber type of slow-oxidative fibers that do not change between the breeding and non-breeding season or with testosterone treatment (Holmes et al., 2007). Overall, these findings together suggest that testosterone may be facilitating increased copulations by enlarging copulatory structures and muscles.

Conclusions

This study is a comprehensive example of how testosterone affects behaviors and the muscles underlying them in a complex and complicated manner. Testosterone appears to regulate copulatory behaviors through changing overall muscle morphology. However, the same is not true for CH morphology and dewlap behaviors. We also found that testosterone regulates dewlap and push-bob behaviors in a context-specific fashion

that is similar across the two species of anoles we studied – the green anole *A. carolinensis* and the brown anole *A. sagrei*.

References

Adkins-Regan, E. (2005). Hormones and Animal Social Behavior (Princeton, NJ: Princeton University Press).

Bhasin, S., Taylor, W.E., Singh, R., Artaza, J., Sinha-Hikim, I., Jasuja, R., Choi, H., and Gonzalez-Cadavid, N.F. (2003). The Mechanisms of Androgen Effects on Body Composition: Mesenchymal Pluripotent Cell as the Target of Androgen Action. J. Gerontol. Ser. A *58*, M1103–M1110.

Breedlove, M.S., and Arnold, A.P. (1981). Sexually dimorphic motor nucleus in the rat lumbar spinal cord: Response to adult hormone manipulation, absence in androgen-insensitive rats. Brain Res. *225*, 297–307.

Calsbeek, R. (2009). Sex-specific adult dispersal and its selective consequences in the brown anole, *Anolis sagrei*. J. Anim. Ecol. *78*, 617–624.

Carson-Jurica, M.A., Schrader, W.T., and O'Malley, B.W. (1990). Steroid Receptor Family: Structure and Functions. Endocr. Rev. *11*, 201–220.

Collette, B.B. (1961). Correlations between ecology and morphology in anoline lizards from Havana, Cuba and southern Florida. Bull. Mus. Comp. Zool. *125*, 137–162.

Conroy, C., Papenfuss, T., Parker, J., and Hahn, N. (2009). Use of Tricaine Methanesulfonate (MS222) for Euthanasia of Reptiles. J. Am. Assoc. Lab. Anim. Sci. *48*, 5.

Cooke, B.M., and Woolley, C.S. (2005). Gonadal hormone modulation of dendrites in the mammalian CNS. J. Neurobiol. *64*, 34–46.

Cox, C., Hanninen, A., Reedy, A., and Cox, R. (2015). Female anoles retain responsiveness to testosterone despite the evolution of androgen-mediated sexual dimorphism. Funct. Ecol. *29*, 758–767.

Cox, R., Stenquist, D., Henningsen, J., and Calsbeek, R. (2009a). Manipulating Testosterone to Assess Links between Behavior, Morphology, and Performance in the Brown Anole *Anolis sagrei*. Physiol. Biochem. Zool. *82*, 686–698.

Cox, R., Stenquist, D., and Calsbeek, R. (2009b). Testosterone, growth and the evolution of sexual size dimorphism. J. Evol. Biol. *22*, 1586–1598.

Crews, D., and Greenberg, N. (1981). Function and Causation of Social Signals in Lizards. Am. Zool. *21*, 273–294.

Crews, D., Traina, V., Wetzel, F.T., and Muller, C. (1978). Hormonal control of male reproductive behavior in the lizard, *Anolis carolinensis*: role of testosterone, dihydrotestosterone, and estradiol. Endocrinology *103*, 1814–1821.

Eisenegger, C., Haushofer, J., and Fehr, E. (2011). The role of testosterone in social interaction. Trends Cogn. Sci. *15*, 263–271.

Feng, N.Y., Katz, A., Day, L.B., Barske, J., and Schlinger, B.A. (2010). Limb Muscles Are Androgen Targets in an Acrobatic Tropical Bird. Endocrinology *151*, 1042–1049.

Forger, N.G. (2009). The Organizational Hypothesis and Final Common Pathways: Sexual Differentiation of the Spinal Cord and Peripheral Nervous System. Horm. Behav. *55*, 605–610.

Fusani, L., Beani, L., and Dessì-Fulgheri, F. (1994). Testosterone Affects the Acoustic Structure of the Male Call in the Grey Partridge (*Perdix perdix*). Behaviour *128*, 301–310.

Fuxjager, M., Barske, J., Du, S., Day, L.B., and Schlinger, B.A. (2012). Androgens Regulate Gene Expression in Avian Skeletal Muscles. PLoS ONE *7*.

Fuxjager, M., Longpre, K., Chew, J., Fusani, L., and Schlinger, B. (2013). Peripheral Androgen Receptors Sustain the Acrobatics and Fine Motor Skill of Elaborate Male Courtship. Endocrinology *154*, 3168–3177.

Fuxjager, M., Miles, M., Goller, F., Petersen, J., and Yancey, J. (2017). Androgens Support Male Acrobatic Courtship Behavior by Enhancing Muscle Speed and Easing the Severity of Its Tradeoff With Force. Endocrinology *158*, 4038–4046.

Girgenrath, M., and Marsh, R.L. (2003). Season and testosterone affect contractile properties of fast calling muscles in the gray tree frog *Hyla chrysoscelis*. Am. J. Physiol.-Regul. Integr. Comp. Physiol. *284*, R1513–R1520.

Gleason, E., Fuxjager, M., Oyegbile, T., and Marler, C. (2009). Testosterone release and social context: When it occurs and why. Front. Neuroendocrinol. 460–469.

Greenberg, N. (2003). Sociality, stress, and the corpus striatum of the green anolis lizard. Physiol. Behav. *79*, 429–440.

Greenberg, B., and Noble, G.K. (1944). Social Behavior of the American Chameleon (*Anolis carolinensis Voigt*). Physiol. Zool. *17*, 392–439.

Hau, M. (2007). Regulation of male traits by testosterone: implications for the evolution of vertebrate life histories. BioEssays *29*, 133–144.

Hau, M., Wikelski, M., Soma, K.K., and Wingfield, J.C. (2000). Testosterone and Year-Round Territorial Aggression in a Tropical Bird. Gen. Comp. Endocrinol. *117*, 20–33.

Herbst, K.L., and Bhasin, S. (2004). Testosterone action on skeletal muscle. Curr. Opin. Clin. Nutr. Metab. Care *7*, 271–277.

Holmes, M.M., and Wade, J. (2004). Seasonal plasticity in the copulatory neuromuscular system of green anole lizards: A role for testosterone in muscle but not motoneuron morphology. J. Neurobiol. *60*, 1–11.

Holmes, M.M., and Wade, J. (2005b). Testosterone Regulates Androgen Receptor Immunoreactivity in the Copulatory, but not Courtship, Neuromuscular System in Adult Male Green Anoles. J. Neuroendocrinol. *17*, 560–569.

Holmes, M.M., and Wade, J. (2005a). Sexual differentiation of the copulatory neuromuscular system in green anoles (*Anolis carolinensis*): Normal ontogeny and manipulation of steroid hormones. J. Comp. Neurol. *489*, 480–490.

Holmes, M., Bartrem, C., and Wade, J. (2007). Androgen dependent seasonal changes in muscle fiber type in the dewlap neuromuscular system of green anoles. Physiol. Behav. *91*, 601–608.

Husak, J.F., Irschick, D.J., Meyers, J.J., Lailvaux, S.P., and Moore, I.T. (2007). Hormones, sexual signals, and performance of green anole lizards (*Anolis carolinensis*). Horm. Behav. *52*, 360–367.

Johnson, M.A., and Wade, J. (2011). Chapter 2 - Neuroendocrinology of Reptilian Reproductive Behavior. In Hormones and Reproduction of Vertebrates, D.O. Norris, and K.H. Lopez, eds. (Academic Press), pp. 39–61.

Johnson, M.A., Lopez, M., Whittle, T., Kircher, B., Dill, A., Varghese, D., and Wade, J. (2014). The evolution of copulation frequency and the mechanisms of reproduction in male *Anolis* lizards. Curr. Zool. *60*, 768–777.

Johnson, M.A., Kircher, B.K., and Castro, D.J. (2018). The evolution of androgen receptor expression and behavior in *Anolis* lizard forelimb muscles. J. Comp. Physiol. A *204*, 71–79.

Kadi, F. (2008). Cellular and molecular mechanisms responsible for the action of testosterone on human skeletal muscle. A basis for illegal performance enhancement. Br. J. Pharmacol. *154*, 522–528.

Lailvaux, S.P., and Irschick, D.J. (2006). No Evidence for Female Association with High-Performance males in the Green Anole Lizard, *Anolis carolinensis*. Ethology *112*, 707–715.

Lailvaux, S.P., and Irschick, D.J. (2007). The Evolution of Performance-Based Male Fighting Ability in Caribbean *Anolis* Lizards. Am. Nat. *170*, 573–586.

Lailvaux, S., Gilbert, R., and Edwards, J. (2012). A performance-based cost to honest signalling in male green anole lizards (*Anolis carolinensis*). Proc. R. Soc. B Biol. Sci. *279*, 2841–2848.

Lailvaux, S.P., Leifer, J., Kircher, B.K., and Johnson, M.A. (2015). The incredible shrinking dewlap: signal size, skin elasticity, and mechanical design in the green anole lizard (*Anolis carolinensis*). Ecol. Evol. *5*, 4400–4409.

Losos, J. (2009). Lizards in an evolutionary tree: ecology and adaptive radiation of anoles. (Berkeley, CA: Univ of California Press).

Lovern, M.B., and Wade, J. (2001). Maternal plasma and egg yolk testosterone concentrations during embryonic development in green anoles (*Anolis carolinensis*). Gen. Comp. Endocrinol. *124*, 226–235.

Lovern, M., Holmes, M., and Wade, J. (2004a). The Green Anole (*Anolis carolinensis*): A Reptilian Model for Laboratory Studies of Reproductive Morphology and Behavior. ILAR J. *45*, 54–64.

Lovern, M.B., Holmes, M.M., Fuller, C.O., and Wade, J. (2004b). Effects of testosterone on the development of neuromuscular systems and their target tissues involved in courtship and copulation in green anoles (*Anolis carolinensis*). Horm. Behav. *45*, 295–305.

Mangiamele, L., Fuxjager, M., Schuppe, E., Taylor, R., Hödl, W., and Preininger, D. (2016). Increased androgenic sensitivity in the hind limb muscular system marks the evolution of a derived gestural display. Proc. Natl. Acad. Sci. *113*, 5664–5669.

McEwen, B.S., Davis, P.G., Parsons, B., and Pfaff, D.W. (1979). The Brain as a Target for Steroid Hormone Action. Annu. Rev. Neurosci. *2*, 65–112.

Miller, W.L. (1988). Molecular Biology of Steroid Hormone Synthesis. Endocr. Rev. *9*, 295–318.

Neal, J., and Wade, J. (2007a). Courtship and copulation in the adult male green anole: Effects of season, hormone and female contact on reproductive behavior and morphology. Behav. Brain Res. *177*, 177–185.

Neal, J., and Wade, J. (2007b). Androgen receptor expression and morphology of forebrain and neuromuscular systems in male green anoles displaying individual differences in sexual behavior. Horm. Behav. *52*, 228–236.

Nelson, R.J., and Trainor, B.C. (2007). Neural mechanisms of aggression. Nat. Rev. Neurosci. *8*, 536–546.

O'Bryant, E.L., and Wade, J. (1999). Sexual dimorphisms in a neuromuscular system regulating courtship in the green anole lizard: effects of season and androgen treatment. J. Neurobiol. *40*, 202–213.

O'Bryant, E.L., and Wade, J. (2001). Development of a Sexually Dimorphic Neuromuscular System Involved in Green Anole Courtship Behavior. Brain. Behav. Evol. *58*, 362–369.

Pinheiro J., Bates D., DebRoy S., Sarkar D., R Core Team (2021). nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-152, https://CRAN.R-project.org/package=nlme

Rand, A.S., and Williams, E.E. (1970). An Estimation of Redundancy and Information Content of Anole Dewlaps. Am. Nat. *104*, 99–103.

Sanger, T., Hime, P., Johnson, M.A., Diani, J., and Losos, J. (2008). Laboratory Protocols for Husbandry and Embryo Collection of Anolis Lizards. Herpetol. Rev. *39*, 58–63.

Schlinger, B., Barske, J., Day, L., Fusani, L., and Fuxjager, M. (2013). Hormones and the neuromuscular control of courtship in the golden-collared manakin (*Manacus vitellinus*). Front. Neuroendocrinol. *34*, 143–156.

Schuppe, E.R., Pradhan, D.S., Thonkulpitak, K., Drilling, C., Black, M., and Grober, M.S. (2017). Sex differences in neuromuscular androgen receptor expression and sociosexual behavior in a sex changing fish. PLOS ONE *12*.

Sheffield-Moore, M. (2000). Androgens and the control of skeletal muscle protein synthesis. Ann. Med. *32*, 181–186.

Sinha-Hikim, I., Artaza, J., Woodhouse, L., Gonzalez-Cadavid, N., Singh, A.B., Lee, M.I., Storer, T.W., Casaburi, R., Shen, R., and Bhasin, S. (2002). Testosterone-induced increase in muscle size in healthy young men is associated with muscle fiber hypertrophy. Am. J. Physiol. Endocrinol. Metab. *283*, E154–E164.

Steffen, J.E., and Guyer, C.C. (2014). Display behaviour and dewlap colour as predictors of contest success in brown anoles. Biol. J. Linn. Soc. *111*, 646–655.

Tobiansky, D., and Fuxjager, M. (2020). Sex steroids as regulators of gestural communication. Endocrinology *161*.

Tokarz, R.R., McMann, S., Smith, L.C., and John-Alder, H. (2002). Effects of Testosterone Treatment and Season on the Frequency of Dewlap Extensions during Male–Male Interactions in the Lizard *Anolis sagrei*. Horm. Behav. *41*, 70–79.

Tsai, M.-J., and O'Malley, B.W. (1994). Molecular Mechanisms of Action of Steroid/Thyroid Receptor Superfamily Members. Annu. Rev. Biochem. *63*, 451–486.

Vanhooydonck, B., Herrel, A., Van Damme, R., and Irschick, D. (2005a). Does dewlap size predict male bite performance in Jamaican *Anolis* lizards? Funct. Ecol. *19*, 38–42.

Vanhooydonck, B., Herrel, A., Van Damme, R., Meyers, J., and Irschick, D. (2005b). The relationship between dewlap size and performance changes with age and sex in a Green Anole (*Anolis carolinensis*) lizard population. Behav. Ecol. Sociobiol. *59*, 157–165.

Wade, J. (2005). Current research on the behavioral neuroendocrinology of reptiles. Horm. Behav. *48*, 451–460.

Wade, J. (2012). Sculpting reproductive circuits: Relationships among hormones, morphology and behavior in anole lizards. Gen. Comp. Endocrinol. *176*, 456–460.

Wallen, K. (2001). Sex and Context: Hormones and Primate Sexual Motivation. Horm. Behav. *40*, 339–357.

Wilson, J.D. (1999). The Role of Androgens in Male Gender Role Behavior. Endocr. Rev. *20*, 726–737.

Winkler, S.M., and Wade, J. (1998). Aromatase Activity and Regulation of Sexual Behaviors in the Green Anole Lizard. Physiol. Behav. *64*, 723–731.

Wyce, A., Bai, Y., Nagpal, S., and Thompson, C.C. (2010). Research Resource: The Androgen Receptor Modulates Expression of Genes with Critical Roles in Muscle Development and Function. Mol. Endocrinol. *24*, 1665–1674.

Yin, H.-N., Chai, J.-K., Yu, Y.-M., Wu, C.-A.S., Yao, Y.-M., Liu, H., Liang, L.-M., Tompkins, R.G., and Sheng, Z.-Y. (2009). Regulation of signaling pathways downstream of IGF-I/insulin by androgen in skeletal muscle of glucocorticoid-treated rats. J. Trauma *66*, 1083–1090.