

Hormones, sexual signals, and performance of green anole lizards (*Anolis carolinensis*)

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Abstract

The evolutionary processes that result in reliable links between male signals and fighting capacity have received a great deal of attention, but the proximate mechanisms underlying such connections remain understudied. We studied a large sample of male green anole lizards (*Anolis carolinensis*) to determine whether testosterone or corticosterone predicted dewlap size and/or bite-force capacity, as dewlap size is known to be a reliable predictor of bite-force capacity in territorial males. We also examined whether these relationships were consistent between previously described body size classes (“lightweights” and “heavyweights”). Heavyweights had 50% higher testosterone concentrations than lightweights during the breeding season, suggesting a mechanism for the disproportionately larger heads and dewlaps and higher bite-forces of heavyweights. Plasma testosterone concentrations were positively correlated with dewlap size and bite-force performance in lightweights (but not heavyweights) but only because of mutual intercorrelation of all three variables with body size. We suggest two possibilities for the relationship between testosterone levels and body size: (1) testosterone promotes growth in this species or (2) smaller sexually mature males are unable to compete with larger males such that the benefits of elevated testosterone do not outweigh the costs. Corticosterone levels did not differ between the male morphs, and lightweights, but not heavyweights, showed an inverse relationship between testosterone levels and corticosterone levels. Our results suggest that testosterone is important for traits related to dominance in adult male green anoles and may influence the ability to compete with rivals via fighting ability or through the use of signals.

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Introduction

Elaborate secondary sexual traits often are used during male–male contests to reliably advertise male ‘quality’ (reviewed in Maynard Smith and Harper, 1995; Searcy and Nowicki, 2005). In some species, sexual signal size is a strong predictor of fighting capacity (e.g., Berglund et al., 1996; Lappin et al., 2006; Pratt et al., 2003; Vanhooydonck et al., 2005a,b), suggesting an adaptive link between these traits. Despite recent progress in understanding how sexual signals may evolve, the proximate mechanism(s) that governs the

reliable link between sexual signal size and fighting ability remains poorly understood (Irschick and Lailvaux, 2006; Sinervo et al., 2000). A potential mechanism that may link sexual signal size and fighting ability is the endocrine system. The widespread effects of circulating levels of the androgen testosterone on aggression, secondary sexual traits, and skeletal muscle growth in males of many vertebrate species are well documented (e.g., Hau, 2007; Ketterson and Nolan, 1999; Ketterson et al., 2001; Marler and Moore, 1988; Oliveira, 2004; Sinervo et al., 2000; Wingfield et al., 1990; Wilczynski and Yang, 2002; Young et al., 1993). A classic example linking hormones, signal size, and fighting ability is the testosterone-mediated link of badge size and aggression in Harris’ sparrows (*Zonotrichia querula*), in which simultaneous manipulation of plumage coloration and testosterone levels revealed that males

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with darker plumage are more aggressive and dominant over birds with lighter plumage and lower testosterone levels (Rohwer, 1977; Rohwer and Rohwer, 1978). Despite the value of this example, in general it remains unclear what aspect of male fighting ability is altered by increased testosterone, leading to increased dominance. Androgens, however, are not the only potential endocrine regulator of reliable sexual signals. In some species, glucocorticoid stress hormone levels (corticosterone or cortisol) are negatively related to locomotor abilities (e.g., Meylan and Clobert, 2004) and sexual signal quality (e.g., Spencer et al., 2003; Leary et al., 2006a), suggesting that elevated stress hormone levels may compromise fighting ability and/or the signal that advertises it. Furthermore, male territorial behavior governed by testosterone is energetically expensive, often resulting in elevated corticosteroid concentrations for energy mobilization (Emerson, 2001; Moore and Jessop, 2003) and concomitant changes in male condition. For instance, in male Great Plains toads (*Bufo woodhousii*) increased corticosterone concentrations are associated with reduced vocalization quality (Leary et al., 2006b) and an adoption of alternative ‘satellite’ mating tactics (Leary et al., 2006a). Hence, an interaction between testosterone and corticosterone may be in part responsible for signal quality and fighting ability.

In sexual selection studies, defining male ‘quality’ has proven difficult, but whole-organism performance traits represent good measures because of their often direct link to fighting ability. Whole-organism performance can be broadly defined as how well an organism conducts an ecologically relevant task when pushed to its maximal limits (Arnold, 1983; Irschick and Garland, 2001; Lailvaux and Irschick, 2006). Key to the biological importance of performance traits is that they represent the integrated output of complex functional systems and are expected to be direct targets of natural and sexual selection (Husak et al., 2006b; Lailvaux et al., 2004; Sneddon et al., 2000), with changes in lower-level traits (e.g., morphology and physiology) occurring secondarily. We focused on lizard maximal bite-force performance, as it represents a target of intrasexual selection in polygynous, territorial male lizards and high bite-forces can inflict significant costs upon rivals during agonistic interactions (Husak et al., 2006a; Huyghe et al., 2005; Lailvaux et al., 2004; Lappin and Husak, 2005).

We investigated the roles of testosterone and corticosterone as predictors of sexual signal size and bite-force performance in a large sample of free-ranging adult male green anole lizards (*Anolis carolinensis*). This is a good species for linking the endocrine system and sexual traits because of decades of neuroendocrine research on proximate mediators of courtship morphology and behavior (reviewed in Lovern et al., 2004a; Wade, 2005) and aggression (Wilczynski and Yang, 2002), and much is known about its behavioral ecology (Irschick et al., 2005; Jenssen et al., 1995, 2001, 2005). Recent work showed that the size of the extendable throatfan (dewlap) in adult males predicts bite-force performance (Irschick et al., 2006a; Vanhooydonck et al., 2005a), with the latter trait being important to contest outcome (Lailvaux et al., 2004). Several lines of evidence suggest that testosterone could mediate the relation-

ship between dewlap size and bite-force performance in this species. First, dewlap size and bite-force performance change seasonally (Irschick et al., 2006a), as do circulating plasma testosterone concentrations (Jenssen et al., 2001). Second, the relationship between dewlap size and bite-force performance disappears after the breeding season when males are not competing for mates (Irschick et al., 2006a). Third, the size class (and presumably age) of sexually mature males determines the magnitude of relative dewlap size, head shape, and bite-force performance (Vanhooydonck et al., 2005a), with smaller ‘lightweight’ males having relatively smaller dewlaps, smaller heads, and lower bite-forces than larger ‘heavyweight’ males (see Lailvaux et al., 2004; Vanhooydonck et al., 2005a). These differences within sexually mature males are intriguing, as they represent a major ontogenetic transition after maturation in body shape, with heavyweights having disproportionately wider and deeper heads and higher bite-force capacities.

We tested several predictions concerning whether circulating plasma testosterone or corticosterone concentrations predict dewlap size or bite-force performance within green anoles. (1) Because of the striking head shape and bite-force performance differences between male morphs, we predicted that heavyweight males would have higher testosterone than lightweight males. (2) We predicted that testosterone levels would positively correlate with dewlap size and bite-force performance within each age class, whereas corticosterone would negatively correlate with those traits within each male morph. (3) We predicted a negative relationship between testosterone and corticosterone within each male morph (e.g., Knapp and Moore, 1997). (4) We predicted that testosterone and corticosterone concentrations would decrease after the breeding season.

Methods

Study site and field methods

We studied a population of green anoles on the Tulane University campus in Orleans Parish, Louisiana, USA (see Irschick et al., 2005 for details of the site). The population has been well studied and exhibits seemingly amplified sexual selection due to high population density and a lack of predators (Bloch and Irschick, 2006). However, our population is similar to a nearby non-urban population in its pattern of morphological and performance changes across seasons and in the presence of male morphs (Bloch and Irschick, 2006; Irschick et al., 2005; D. J. Irschick, unpubl. data). Intensive sampling ($n > 200$ males) revealed a large gap in the frequency distribution of sexually mature male body sizes at approximately 64 mm snout–vent length (SVL), as well as an exponential increase in head size and bite-force performance at that same size (see Fig. 2 in Lailvaux et al., 2004). Further, Vanhooydonck et al. (2005a) conducted detailed statistical analyses of this bimodality, and also concluded that the gap occurred around 64 mm SVL. We sampled the population during the peak of the breeding season (May 2006) and after the breeding season (October 2006). Lizards were captured from clumps of vegetation by hand or noose. Because anoles are less common in the fall and male mortality within a season is high (Irschick et al., 2006b), we had unequal sample sizes (85 lizards in May, 26 in October; see Table 1 for details) in the breeding versus post-breeding seasons despite similar sampling effort. Within 2 min of capture, we collected whole blood from the suborbital sinus with a heparinized microhematocrit capillary tube to assess baseline hormone levels. Blood was collected between 10:00 and 13:00 h each day to minimize diel variation in circulating hormone concentrations. If a lizard moved more than 1 m prior to capture, we did not collect blood from that lizard for that day. Once a sample was collected it was transferred to a 0.75-ml

Table 1
Patterns of circulating plasma testosterone and corticosterone in lightweight and heavyweight male green anoles during the breeding and post-breeding seasons

Hormone	Breeding season		Post-breeding season	
	Lightweights	Heavyweights	Lightweights	Heavyweights
<i>n</i>	44	38	20	6
Testosterone	9.31±1.45	13.78±1.22	1.78±0.30	2.23±0.68
Corticosterone	5.39±0.69	5.42±0.66	7.47±0.73	12.43±3.28

Numbers represent means±1 standard error of the mean.

microcentrifuge tube and placed on ice until it was returned ≤5 h later to the laboratory, where the plasma fraction (mean volume±SEM=12.4±0.59 µl) was separated by centrifugation and stored at −70 °C until assays were conducted. We took lizards to the laboratory where we measured morphology and bite-force performance of each lizard (see below) on the day of capture. After all measurements were taken, lizards were individually marked with a subcutaneous injection of biocompatible elastomer (Northwest Marine Technology, Shaw Island, WA 98286; see Nauwelaerts et al., 2000; Irschick et al., 2006b for details) into the ventral portion of their limbs (in different places depending on the unique code, see Irschick et al., 2006b) so that fall recaptures could be identified. Breeding season data for individuals that were recaptured in the Fall were discarded so that only their postbreeding season data were used. All lizards were returned to their exact point of capture within 48 h. All work was done in accordance with an approved Virginia Tech animal care and use protocol.

Morphology and bite-force performance

For each lizard we measured the following morphological traits (following Irschick et al., 2006a) with digital calipers: SVL, head height, head width, and dewlap area (all by the same person). Males with SVL exceeding 64 mm were considered heavyweights, whereas those with SVL less than 64 mm were considered lightweights (following Lailvaux et al., 2004). We measured dewlap area for each individual by positioning each lizard on its left side under a digital camera mounted to a tripod and taking a picture using a Canon 10-D digital camera. The base of the second ceratobranchial was gently pulled with a pair of forceps completely anterior at an angle parallel to the lens of the camera, maximally extending the dewlap. The area of each dewlap (the entire structure, see Vanhooydonck et al., 2005b) was quantified with the program TPSDIG (v. 1.39; J. Rohlf, SUNY Stony Brook). This technique is highly repeatable for anoles (Vanhooydonck et al., 2005b). To avoid measurement error, one person photographed and measured all dewlaps.

We measured bite-force performance following standard procedures (see Herrel et al., 2001), using an isometric Kistler force transducer (type 9023, Kistler Inc. Wintherthur, Switzerland) mounted on a retort stand and connected to a Kistler charge amplifier (type 5058a, Kistler Inc.). The free ends of the plates were placed between the jaws of the animal and the lizard induced to bite forcefully. Lizards were induced to bite with a light tap on the snout. Bite measurements were repeated five times for each animal with several minutes of rest between measures. The highest bite-force value obtained from all five sessions was used as maximal bite-force performance for that individual. All individuals were placed inside an incubator at 32 °C (similar to *A. carolinensis* field temperatures in New Orleans; Lailvaux and Irschick, 2007) for at least 1 h prior to bite-force measurement, and between measures. We only included males in our analyses for which we obtained good maximal bite-force measurements (i.e., an adequate number of trials and no obvious problems with willingness to bite). In total, six lightweights and four heavyweights were excluded for these reasons.

Hormone assays

Concentrations of testosterone and corticosterone were measured by standard radioimmunoassay techniques following extraction and chromatographic separation (Moore et al., 2000; Wingfield and Famer, 1975). For individual extraction efficiency determination, we equilibrated each sample overnight with 2000 cpm of tritiated steroid. Each sample was extracted with 5 ml of distilled dichloromethane with the dichloromethane phase removed and

dried in a warm water bath, under a stream of nitrogen gas, and resuspended in 10% ethyl acetate in iso-octane. To remove neutral lipids and to isolate 5 α -dihydrotestosterone (DHT), testosterone, and corticosterone, all samples were transferred to diatomaceous earth (Celite, Sigma) columns for chromatographic separation. Neutral lipids and other steroids were eluted with 2 ml of iso-octane and discarded, and DHT was eluted with 1.5 ml of 10% ethyl acetate in iso-octane. DHT levels were generally low and paralleled testosterone and thus will not be discussed further. Testosterone and corticosterone were eluted with 2 ml of 20% and 2.5 ml of 52% ethyl acetate in iso-octane, respectively, and saved. After this, samples were dried in a 40 °C water bath under nitrogen gas, resuspended in 600 µl phosphate buffered saline, and maintained overnight at 4 °C. Individual extraction efficiency for each steroid (mean recoveries were 77.4% for testosterone and 71% for corticosterone) was determined from 100 µl of the sample while 200 µl of the sample was allocated to each of two duplicates for the assay. Serial dilutions for the standard curves were performed in triplicate (range of curves: testosterone, 500–1 pg; corticosterone, 2000–4 pg). All samples were then incubated overnight with 100 µl of antiserum (testosterone: T-3003, Wien Laboratories, Succasunna, NJ 07876; corticosterone: Esoterix Endocrinology, Calabasas Hills, CA 91301) and 100 µl of tritiated steroid. Unbound steroid was separated using dextran-coated charcoal and the bound steroid decanted into scintillation vials. Samples were counted on a liquid scintillation counter and final concentrations were corrected for individual extraction efficiency. Spring samples were run in two assays, and fall samples were run in a separate assay. Average intra-assay coefficients of variation (CV) were 8% for testosterone and 18% for corticosterone. Inter-assay CVs were 17% for testosterone and 20% for corticosterone.

Statistical analysis

All data were log₁₀-transformed before analyses to ensure normality. We first tested for differences in hormone concentrations between lightweight and heavyweight morphs and seasons using two-factor ANOVAs (morph and season as factors). Because sample sizes differed dramatically between seasons, and because we were interested in *a priori* comparisons within factors, we examined simple effects (using the SLICE option in SAS v. 9.1) to compare levels of one factor within each level of the other factor (e.g., lightweights versus heavyweights in the breeding season or breeding versus post-breeding season within lightweights). We feel that our analyses make the most biological sense and do not violate any statistical assumptions. Pairwise comparisons were conducted with Tukey's adjustment. We then used Pearson's correlation analyses to determine whether hormone levels were correlated with SVL, dewlap size, or bite-force performance. We used stepwise multiple linear least-squares regression to determine which set of variables best predicted dewlap size and bite-force performance during the breeding season, including SVL, head width, head depth, testosterone concentration, and corticosterone concentration. We conducted regression analyses within lightweights and heavyweights separately. Finally, we used Pearson correlation analysis to determine whether testosterone levels correlated with corticosterone levels within each morph separately. Correlation analyses were used instead of linear regression, because the direction of causality for the relationships that we examined is unclear (see Discussion). However, for visualization purposes, figures have regression lines added to emphasize directionality of significant correlations.

Results

Morph and season differences in hormone concentrations

Because of our *a priori* interest in specific comparisons, we examined simple effects for the season × morph interaction in testosterone concentrations ($F_{1,104}=1.22$, $P=0.27$). Testosterone declined significantly after the breeding season (Table 1) for both heavyweight (84% decline on average, $P=0.0002$) and lightweight males (81% decline on average, $P<0.0001$). During the breeding season, heavyweight males had 50% higher testosterone on average than lightweights ($P=0.01$), but this

difference disappeared after the breeding season ($P=0.99$). For corticosterone, examination of simple effects for the season \times morph interaction ($F_{1,104}=0.79$, $P=0.38$) revealed that corticosterone increased by 125% for heavyweights ($P=0.007$) and 39% for lightweights ($P=0.002$), but morphs did not differ from each other during the breeding ($P=0.99$) or postbreeding ($P=0.70$) seasons (Table 1). Because samples from the different seasons were analyzed in separate assays, a bit of caution must be taken when interpreting seasonal differences.

Phenotypic correlates of testosterone levels

For lightweights, testosterone levels were positively correlated with dewlap size ($r=0.42$, $P=0.01$; Fig. 1A), bite-force performance ($r=0.39$, $P=0.015$; Fig. 1B), and SVL ($r=0.42$, $P=0.01$; Fig. 2). For heavyweights, testosterone levels were not correlated with SVL ($r=0.059$, $P=0.74$; Fig. 2), dewlap size ($r=0.088$, $P=0.61$), or bite-force performance ($r=0.006$, $P=0.97$). Because testosterone correlated with SVL in lightweights, we calculated residuals for testosterone, dewlap size, and bite-force performance (the latter of which also correlate with body size: Irschick et al., 2006a) by regressing each (log-

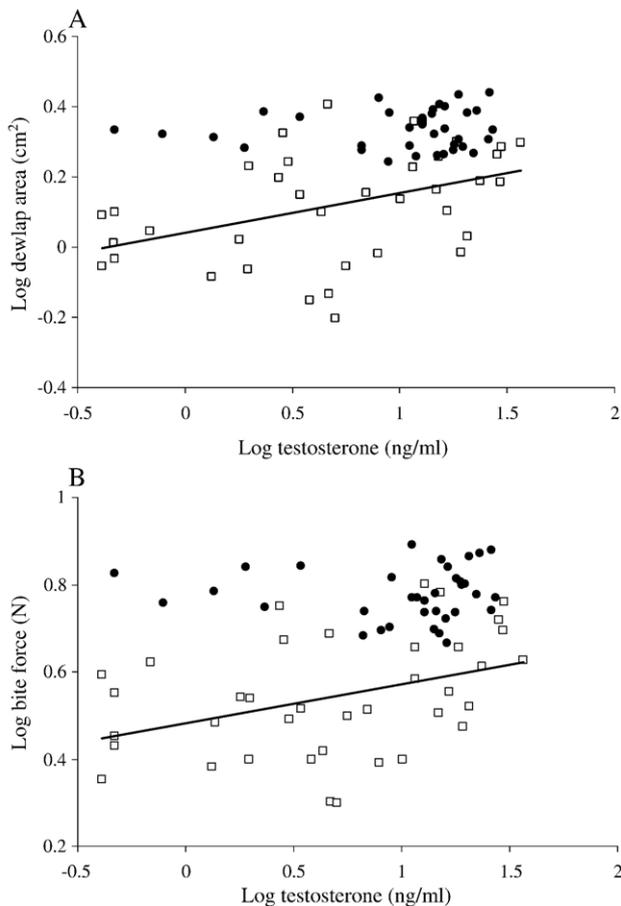


Fig. 1. Relationship between circulating plasma testosterone concentrations and (A) dewlap size and (B) maximal bite-force performance for lightweight (open squares) and heavyweight (closed circles) male green anoles. There were significant positive correlations for lightweights but not heavyweights. Regression lines have been added for visualization. Data are \log_{10} -transformed.

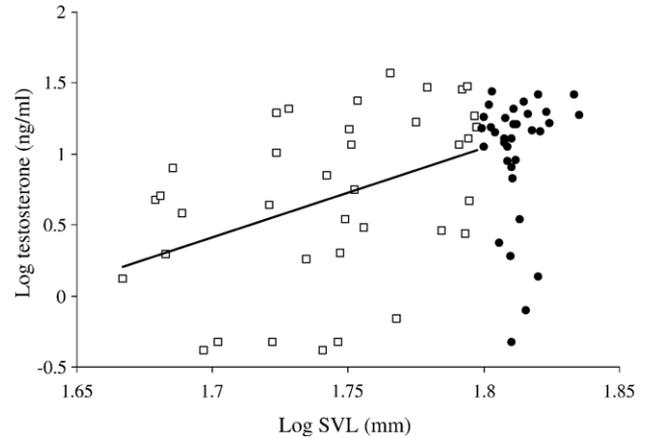


Fig. 2. Relationship between circulating plasma testosterone concentrations and body size (SVL) for lightweight (open squares) and heavyweight (closed circles) male green anoles. There was a significant positive correlation for lightweights but not heavyweights. A regression line has been added for visualization. Data are \log_{10} -transformed.

transformed) variable against (log-transformed) SVL. Residual testosterone was not correlated with residual dewlap size ($r=0.13$, $P=0.46$) or residual bite-force performance ($r=0.016$, $P=0.93$).

Phenotypic correlates of corticosterone levels

For lightweights, corticosterone levels were not correlated with SVL ($r=0.19$, $P=0.27$), dewlap size ($r=0.16$, $P=0.34$), or bite-force performance ($r=0.23$, $P=0.17$). Similarly, for heavyweights, corticosterone levels were not correlated with dewlap size ($r=0.025$, $P=0.89$); however, there was a trend for lizards with higher corticosterone to be of larger SVL ($r=0.28$, $P=0.10$) and have higher bite-force performance ($r=0.30$, $P=0.09$).

Predictors of dewlap size and bite-force performance

Stepwise multiple regressions with dewlap size as the dependent variable and SVL, head width, head depth, testosterone concentration, and corticosterone concentration as independent variables resulted in different models for lightweights, heavyweights, and pooled males. For lightweights, there was a significant model ($F_{2,34}=59.73$, $r^2=0.78$, $P<0.001$), with head width ($\beta=1.36$, $P=0.03$) and head depth ($\beta=1.49$, $P=0.008$) significantly predicting dewlap size. For heavyweights, there was a significant model ($F_{1,33}=6.31$, $r^2=0.16$, $P=0.02$), with only SVL predicting dewlap size ($\beta=2.62$). For pooled males, there was a significant model ($F_{2,69}=183.25$, $r^2=0.84$, $P<0.001$), with SVL ($\beta=2.19$, $P<0.001$) and head depth ($\beta=0.94$, $P=0.01$) significantly predicting dewlap size. Although there were differences in model parameters, a commonality among the three is that neither testosterone nor corticosterone levels were significant predictors of dewlap size given variation explained by other variables.

Stepwise multiple regressions with bite-force performance as the dependent variable and SVL, head width, head depth,

testosterone concentration, and corticosterone concentration as independent variables also resulted in different models for lightweights, heavyweights, and pooled males. For lightweights, there was a significant model ($F_{2,34}=196.14$, $r^2=0.91$, $P<0.001$), with only SVL ($\beta=1.79$, $P<0.001$) and head depth ($\beta=1.18$, $P=0.001$) significantly predicting bite-force performance. For heavyweights, there was a significant model ($F_{2,33}=11.40$, $r^2=0.42$, $P<0.001$), with head width ($\beta=1.45$, $P=0.006$) and head depth ($\beta=1.52$, $P=0.002$) predicting bite-force performance. For pooled males, there was a significant model ($F_{3,69}=253.17$, $r^2=0.92$, $P<0.001$), with SVL ($\beta=1.21$, $P=0.006$), head width ($\beta=0.70$, $P=0.04$), and head depth ($\beta=1.18$, $P<0.001$) significantly predicting bite-force performance. As in the regressions for dewlap size, a commonality among the three models of bite-force performance is that neither testosterone nor corticosterone levels were significant predictors of bite-force performance given variation explained by other variables.

Corticosterone–testosterone relationships

For lightweight males, testosterone levels were negatively correlated with corticosterone levels ($r=-0.37$, $P=0.015$; Fig.

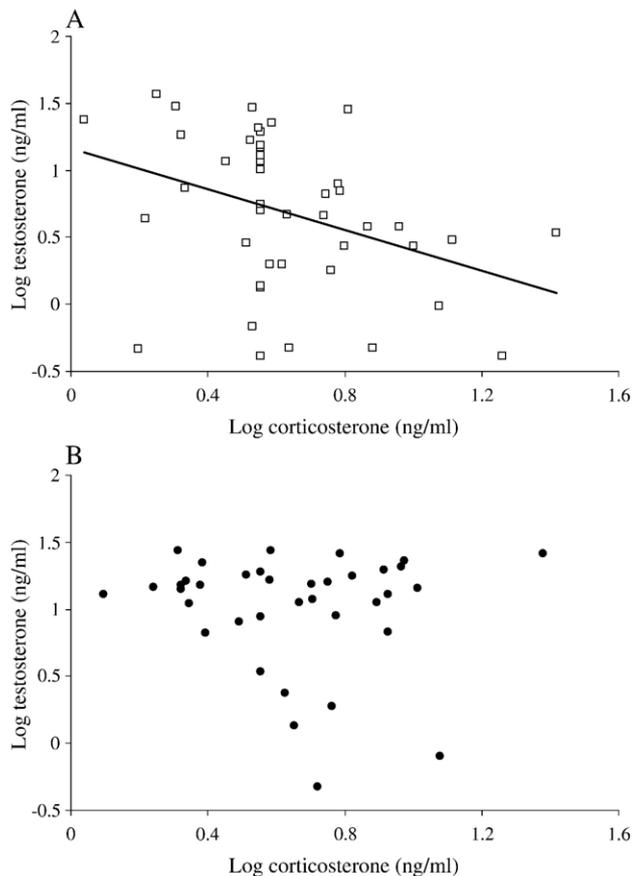


Fig. 3. Relationship between circulating plasma testosterone and corticosterone concentrations for lightweight (A) and heavyweight (B) male green anoles. There was a significant negative correlation for lightweights but not heavyweights. A regression line has been added for visualization. Data are \log_{10} -transformed.

3A). However, there was no significant relationship between the two variables for heavyweight males ($r=0.074$, $P=0.66$; Fig. 3B).

Discussion

Our results revealed several key findings. First, during the breeding season, heavyweight males had 50% higher testosterone on average than lightweight males. This result is consistent with previously described morphological and performance differences between the morphs (Lailvaux et al., 2004; Vanhooydonck et al., 2005a), though the difference in testosterone seems to be influenced by body size. Second, plasma testosterone concentrations increased by nearly two orders of magnitude with body size within lightweight males. The relationship was not statistically significant within heavyweight males. Third, there was a negative relationship between plasma corticosterone concentrations and testosterone concentrations within lightweights but not within heavyweights. Finally, within heavyweights, corticosterone levels on average were over two times higher after the breeding season, perhaps due to the detrimental effects of male–male interactions over the course of the breeding season (Knapp and Moore, 1995, 1996; Woodley et al., 2000). However, corticosterone levels did not significantly correlate with any measure of morphology or performance that we measured.

The most striking results from our study were the overall differences between lightweight and heavyweight males. Not only were testosterone levels significantly different between the morphs during the breeding season, but correlations between testosterone levels and other traits differed between the morphs as well. The differences between morphs in head shape and relative bite-force performance may be due to the dramatic differences in circulating testosterone levels, a hypothesis that is best tested by manipulating testosterone levels. Indeed, it may not be surprising that heavyweights have higher testosterone levels, since they are most likely to display aggression toward similarly large rival males (Smith and John-Alder, 1999). There would be few benefits to outweigh the costs for lightweight males to maintain high testosterone levels if they are not large enough to compete with heavyweights (see below). On the other hand, higher testosterone may be advantageous to heavyweights if it gives a competitive advantage during male–male interactions (Wingfield et al., 1990). Although testosterone levels were relatively high in heavyweight males (Fig. 2), we noted six individual heavyweights with a wide range of unusually low testosterone levels that seemed to be separate from a distinct cluster of the other uniformly high-testosterone males. If the six low-testosterone males are removed, bite-force performance positively correlates with testosterone ($r=0.40$, $p=0.03$) even though neither correlates with SVL (bite-force: $r=0.29$, $p=0.09$; testosterone: $r=0.27$, $p=0.16$). This finding is consistent with the hypothesis that high testosterone in heavyweights results in proportionally larger heads and higher bite-forces. The reason for why some heavyweights exhibit low testosterone levels deserves future attention, as having a higher bite-force capacity, and presumably high testosterone, is ad-

vantageous for heavyweights during male combat (Lailvaux et al., 2004).

Dewlap size and bite-force performance were significantly correlated with circulating levels of testosterone within lightweights, but this was because all three variables were correlated with body size, meaning that larger males have higher circulating testosterone levels and relatively larger dewlaps, and higher bite-forces than smaller males. The question then is: what is the causative relationship between testosterone and body size? One possibility is that having higher testosterone promotes growth, leading to larger body size (e.g., Borski et al., 1996; Fennell and Scanes, 1992; Gatford et al., 1998; Holloway and Leatherland, 1998; Huggard et al., 1996; Wehrenberg and Giustina, 1992). Cox and John-Alder (2005) found a positive relationship between testosterone and SVL in free-living, yearling male *Sceloporus jarrovi* lizards. Moreover, they experimentally showed in *S. jarrovi* that increased testosterone leads to increased growth rates (but see Cox and John-Alder, 2005; Cox et al., 2005). A testable hypothesis is that testosterone promotes body size growth in male green anoles, with a corresponding increase in bite-force performance and the signal that advertises it (the dewlap). Experimental manipulations of testosterone in our study species will help determine if increased testosterone promotes growth, as well as determine whether increased testosterone at a certain age or body size affects jaw musculature and dewlap morphology (see Lovern et al., 2004b; O'Bryant and Wade, 1999, 2001, 2002) and leads to the disproportionately larger heads and dewlaps in heavyweights. The major question is: at what point during ontogeny would testosterone promote growth? Presumably, effects would be seen after the onset of sexual maturation and testosterone production (i.e., a small lightweight), but how testosterone concentrations change during ontogeny is unknown.

Another possibility is that causation is in the opposite direction. That is, large males do not have high testosterone levels per se, but small males have lower testosterone levels. In this scenario, testosterone should be highest among the largest size class of territorial males (heavyweights), with testosterone increasing in smaller males, thus supporting higher levels of aggression (see also Wikelski et al., 2004), as they approach a body size that is competitive with larger males. This is consistent with our results and what we know about green anole social behavior. We found no relationship between SVL and testosterone in heavyweights, suggesting that these territorial males are maintaining testosterone at levels near their physiological maximum (Goymann et al., 2007; Greenberg and Crews, 1990). Further, many lightweight males that were just below the heavyweight cutoff in SVL had testosterone concentrations at levels approaching or exceeding those of heavyweights (Fig. 2). A hypothesis for this direction of causality is that as lightweights approach heavyweight size they begin to compete aggressively with territorial males for access to territories, simultaneously elevating testosterone levels to achieve the necessary levels of agonistic behavior and the bite-force capacity to compete. Prior to that body size, the

detrimental effects of testosterone (increased parasite loads: Folstad and Karter, 1992; Klukowski and Nelson, 2001; Salvador et al., 1996; reduced immunocompetence: Peters, 2000; decreased body condition: Moore et al., 2000; increased energy use: Marler and Moore, 1988; Wikelski et al., 1999, 2004; reduced survival: Marler and Moore, 1988) may explain why small lightweights do not have high testosterone: they cannot competitively exclude larger males from access to females, so there are no fitness benefits to outweigh the costs. Thus, our results suggest a complex picture of how testosterone may mediate morphology and performance in male green anoles. Testosterone levels are likely suppressed until a body size competitive with larger males; then, elevated testosterone levels may promote disproportionate increases in head shape and bite-force performance.

Even though corticosterone levels did not predict morphology or performance in either lightweights or heavyweights, there is still potential for it to affect the phenotype of males. We found a negative relationship between testosterone and corticosterone concentrations in lightweights but not heavyweights. This result is similar to research on alternative reproductive morphs in tree lizards (*Urosaurus ornatus*; Knapp and Moore, 1997; Knapp et al., 2003) where the subordinate orange-throated male morphs had an inverse corticosterone–testosterone relationship but the dominant orange–blue-throated morphs did not. Perhaps the presence of a negative corticosterone–testosterone relationship in subordinate social classes, regardless of the ontogenetic origin of that class, is more widespread than heretofore considered. The ambiguity in how corticosterone affects male green anole phenotypes, however, warrants further investigation into the role of corticosterone in governing male morphology, performance, sexual signals, and social behavior in this species.

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