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Relationships between hormones, physiological performance and immunocompetence in a color-polymorphic lizard species, *Podarcis melisellensis*

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ABSTRACT

Species with alternative phenotypes offer unique opportunities to investigate hormone–behavior relationships. We investigated the relationships between testosterone, corticosterone, morphology, performance, and immunity in a population of lizards (*Podarcis melisellensis*) which exhibits a color polymorphism. Males occur in three different color morphs (white, yellow, orange), providing an opportunity to test the idea of morphs being alternative solutions to the evolutionary challenges posed on the link between hormones, morphology, performance, and immunity. Morphs differed in bite force capacity, with orange males biting harder, and in corticosterone levels, with yellow males having lower levels than orange. However, morphs did not differ in testosterone levels or in the immunological parameters tested. At the individual level, across morphs, testosterone levels predicted size-corrected bite force capacity, but no relation was found between hormone levels and immunity. Our results do not support the testosterone-based polymorphism hypothesis and reject the hypothesis of a trade-off between testosterone and immunity in this species, but provide a mechanistic link between testosterone and a sexually selected performance trait.

Introduction

In the past three decades, it has become increasingly evident that reproductive tactics may vary not only between sexes, but also among individuals of the same sex (Gross, 1996, Brockmann, 2001). Alternative reproductive phenotypes exhibited by males or females of a species can be genetically fixed or plastic, and typically involve a combination of behavioral, morphological, physiological, and life history characteristics. Circulating sex hormone levels can play an important role, either through organizational or activational effects, in triggering the expression of these alternative reproductive morphs (Moore et al., 1998, reviewed in: Oliveira et al., 2008). It has been documented that in male vertebrates, high levels of circulating androgens can correlate with dominant, territorial behavior and enhance the expression of secondary sexual traits that advertise the individual's social ranking (reviews in Oliveira, 2004 and Hau, 2007; Moore and Marler, 1987). How circulating levels of testosterone result in dominant behavior is less clear. They may exert their influence primarily by affecting the brain, rendering the animal more aggressive and/or more motivated to take risks. For example, in the house sparrow, testosterone reduces responsiveness to pain stimuli, which may promote the willingness to engage in aggressive encounters (Hau et al., 2004). In all likelihood, these hormones can also affect the morphology and physiology of the individual in such a way that it may be better equipped to deal with the challenges of living a dominant life (Husak et al., 2007). For instance, it can be expected that testosterone will promote the development of morphological or physiological features that aid in fighting rivals, guarding territories, or dealing with an increased predation risk. Accordingly, testosterone is known to affect lower-level morphological and physiological traits that may contribute to performance (e.g. number of muscle units: Tobin and Joubert, 1991; enzymatic activity in muscles and neurons: Luine et al., 1980; contractile properties: Girgenrath and Marsh, 2003; muscle mass: Sidor and Blackburn, 1998; Huyghe et al., unpublished data). However, the evidence for testosterone affecting relevant whole-animal performance is scant and equivocal. In addition to testosterone, a rise in corticosterone levels, commonly occurring in response to stressors in vertebrates, may affect sexually selected traits (Husak and Moore, 2008) and reproductive behavior (Creel, 2001). However, the effects of stress and corticosterone on aggressive and territorial behavior are variable (reviewed in Moore and Jessop, 2003; Goymann and Wingfield, 2004).

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Part of the appeal of an androgen-based model of reproductive polymorphism is that the costs and benefits of androgens may help explain why different morphs can co-exist in the first place. Testosterone has been called a 'double-edged sword' that stimulates development of characteristics used in a reproductive context, driven by sexual selection, but at the same time may reduce immunocompetence (Folstad and Karter, 1992), and thus survival (natural selection) (Marler and Moore, 1988). Although it is as yet unclear whether testosterone affects immunocompetence directly (Folstad and Karter, 1992) or induces behavioral changes that increase the likelihood of infection (Owen-Ashley et al., 2004; Roberts et al., 2004), parasite loads and testosterone levels can co-vary in natural populations (Klukowski and Nelson, 2001; Saino and Moller, 1995; Mougeot et al., 2005; Perez-Orella and Schulte-Hostedde, 2005). Whereas some studies have revealed a clear positive link between experimentally elevated testosterone levels and parasite infestation (Olsson et al., 2000, Klukowski and Nelson, 2001, Cox and John-Alder, 2007), some show an opposite result where elevated testosterone levels did not result in a higher parasite load (Oppliger et al., 2004). Reproductive morphs could be seen as alternative solutions to an evolutionary trade-off between parasite load susceptibility (survival) and access to mates (reproductive success). However, as far as we know, no previous study has evaluated the prediction that male reproductive morphs should differ in parasite load.

In lizards, as in many vertebrates, testosterone is an important mediator of the expression of secondary sexual traits that differentiate males from females. In addition, testosterone levels affect the degree of trait expression among males. In male *Sceloporus* lizards seasonal color development is activated by testosterone (Rand, 1992; Cox et al., 2008). Similarly, in *Uta stansburiana*, males with orange dewlaps are ultra-territorial and have a higher testosterone level than the other two color morphs (Sinervo et al., 2000). On the other hand, organizational hormonal mechanisms in early development differentiate between territorial and non-territorial male morphs in *Urosaurus ornatus* (Moore et al., 1998). Experimental elevation of testosterone levels has been shown to improve running endurance capacity in lizards (Klukowski et al., 1998; Sinervo et al., 2000), but Husak et al. (2006a) found no relationship between sprint speed and natural variation in testosterone levels in the lizard *Crotaphytus collaris*. However, a positive relationship between plasma testosterone concentrations and bite performance was found in *Anolis carolinensis*, but only because of mutual intercorrelation with body size (Husak et al., 2007). In *Gallotia galloti*, a positive relationship was found between baseline testosterone levels and bite force capacity, but experimental elevation of circulating testosterone levels did not result in enhanced performance (Huyghe et al., unpublished data).

Color-polymorphic species provide an excellent opportunity to study the role of androgens in the mechanisms of origin and maintenance of these polymorphisms, and at the same time to test the immunocompetence handicap hypothesis. The continued existence of the different morphs in a population implies that each reflects an alternative strategy to deal with natural and sexual selection pressures. In this paper, we examine whether levels of circulating testosterone and corticosterone differ among the three male color morphs of the lacertid lizard *Podarcis melisellensis*. Males of this species can have an orange, yellow, or white ventral color, and there is circumstantial evidence that orange males are behaviorally dominant over the two other morphs (Huyghe et al., 2007). We also evaluate individual relationships between circulating hormone levels, whole-animal performance (bite force) and immunity (parasite load and T cell-response). Since orange males are more aggressive than the other morphs, we predict that orange males will have higher testosterone and higher corticosterone levels, as we hypothesize that this polymorphism is androgen mediated and might be reflected in alternative reproductive tactics. With regard to the immunocompetence handicap hypothesis, we expect that these same orange males will have suppressed immunity resulting in a larger parasite load and smaller PHA response compared to the other morphs.

Methods

Study species and field procedures

P. melisellensis is a medium-sized heliothermic, insectivorous lacertid lizard (snout-vent length up to 70 mm) that occupies a variety of habitats along the Adriatic coast and on islands in the Adriatic Sea. Males occur in three color morphs, with individuals having either a solid white, yellow or orange ventral color (Huyghe et al., 2007). In most cases, lizards could be unambiguously assigned to one of these morph classes by visual inspection. In the few cases (less than 10%) that the difference between yellow and orange was unclear, we did not use this individual.

The study site consists of an abandoned olive tree orchard (50×55 m) on the island of Lastovo (Croatia, 42°16′N, 16°54′E). Lizards reside on or near the olive trees or in small stone walls that delineate the study site. Early in their reproductive season, in June 2006 and May 2008, blood samples, and morphological, and performance measurements were obtained from adult males that were caught by noose. In May 2008, we additionally quantified parasite infestation and assessed immune activity in response to injection with a novel antigen as measures of immunocompetence. Blood samples were taken immediately after capture, after which animals were brought back to the field-based lab where the morphological, performance, and immunity traits were quantified. On all occasions, lizards were released within 48 h after capture, at the exact spot where caught.

Hormone assays

To obtain baseline hormone levels we punctured the post-orbital sinus with a needle (0.4×20 mm) within three minutes of capture and collected approximately 50–60 μL of whole blood with hematocrit microcapillary tubes. To avoid confounding effects of diel fluctuations in plasma steroid concentrations, all samples were collected between 9.00 and 12.00 h. Samples were transferred to 1.5 mL microcentrifuge tubes and kept on ice, until return (<4 h) to the laboratory, where they were centrifuged for 15 min at 7000 rpm to separate the plasma fraction (2006 mean volume ± SEM = 25.1 ± 0.92 μL used in assay; in 2008 5 μL of plasma was used in assay for all samples). Subsequently, the samples were stored at −80 °C, until assays were conducted.

Concentrations of testosterone and corticosterone were measured by standard radioimmunoassay (RIA) techniques following extraction and chromatographic separation (Wingfield and Farner, 1975; Moore et al., 2000; Husak et al., 2007). Samples from 2006 were run separately from 2008 samples. The samples from 2006 were run with chromatographic separation of steroids, whereas the 2008 samples were run in two direct assays without chromatography. Thus the 2008 samples measured total androgens while the 2006 samples separately measured two androgens (5α-dihydrotestosterone and testosterone; but see below). Samples from both years were also analyzed for corticosterone. For individual extraction efficiency determination, we equilibrated each sample overnight with 2000 cpm of tritiated steroid. Each sample was extracted twice with 4 mL of diethyl ether, dried under a stream of nitrogen gas, and resuspended in 10% ethyl acetate in isooctane. We used chromatographic column (Celite 521, Sigma) separation with increasing concentrations of ethyl acetate in isooctane to remove neutral lipids and isolate 5α-dihydrotestosterone, testosterone, and corticosterone fractions for analysis. After this, samples were dried under nitrogen gas and resuspended in 600 μL phosphate buffered saline (PBS). Individual extraction efficiency for each steroid (mean recoveries were 80.3% for 5α-dihydrotestosterone, 82.7% for testosterone, and 84.3% 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. In 2008, we conducted the same procedures, but skipped chromatographic separation of steroids and resuspended extracted samples in 1 mL of PBS. Individual extraction efficiency for testosterone (mean recoveries were 78.3% for testosterone and 73.0% for corticosterone) was determined from 200 μL of the sample while 200 μL of the sample was allocated to each of two duplicates for the assay. The remaining 400 μL of the resuspensions were maintained at 4 °C. Three days later, 53% of the samples were re-run through the assay because values were higher than the standard curve. This time, 100 μL of the resuspended sample was used. Serial dilutions for the standard curves in each assay were performed in triplicate (range of curves: 5α-dihydrotestosterone and testosterone, 500–1 pg; corticosterone, 2000–4 pg). All samples were then incubated overnight with 100 μL of antiserum (5α-dihydrotestosterone and testosterone: T-3003, Endocrine Sciences, Tarzana, CA 91356; 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 corrected for individual extraction efficiency. Intra-assay coefficients of variation (CV) were 6% for 5α-dihydrotestosterone, 7% for testosterone, and 7% for corticosterone in the 2006 assay, and 5% for testosterone and 11% for corticosterone in the 2008 assay. Inter-assay CVs were 3% for testosterone and 18% for corticosterone. The low inter-assay CVs suggest a minimal 'assay effect' that may cause differences between 2006 and 2008 samples. Samples from the 2006 assay that were off the standard curve were not used in statistical analyses. Values for 2008 samples represent total androgens (5α-dihydrotestosterone and testosterone), but we will refer to them as "testosterone" hereafter for simplicity. Similarly, the "testosterone" values reported for 2006 below actually include 5α-dihydrotestosterone and testosterone values combined to be able to compare total androgen concentrations across years.

Body size and performance measurements

Snout-vent length (SVL) was measured using digital calipers (Mitutuyo; precision 0.01 mm), and body mass was determined using a Scout Pro balance (precision 0.01 g). We only used adult males with SVL bigger than 55 mm (as in Huyghe et al., 2007). Body condition was estimated as the residuals of a linear regression of body mass on SVL (both $\log_{10}$-transformed).

Maximal bite force capacity was estimated by the highest of five recordings of a lizard biting on two metal plates connected to an isometric force transducer and a charge amplifier (see Herrel et al., 1999 for more details on the experimental set-up). Prior to the performance trials, lizards were allowed to rest for at least one hour, by keeping them individually in cloth bags, placed in half sun, half shade. In this way, they were allowed to obtain a body temperature of approximately 34 °C, which is at or near their preferred field active body temperature (Huyghe et al., unpublished data).

Immunity

We quantified the delayed cutaneous hypersensitivity response (Belliure et al., 2004, Oppliger et al., 2004) as an indicator of one aspect of immunity, at the cellular level. This response was assessed by injecting one foot of every individual with a 20 μL solution containing 50 mg of phytohaemagluttinin (PHA; Sigma-Aldrich, L-8754) in 10 mL phosphate buffered saline (PBS). Thickness of the foot was measured before injection and twenty-four hours later, using digital calipers (Mitutuyo; precision 0.01 mm). The other foot was treated in the same way, but injected with 20 μL of PBS as a control. The PHA response was calculated as the change in thickness of the PHA injected foot minus the change in the control foot. Larger localized swelling indicates a larger immune activity. As the adaptive value of the swelling response to a PHA injection is complex and largely unclear (Kennedy and Nager, 2006), we simply use it as a measure of immune activity.

The degree of ectoparasite infestation was scored by brushing the lizards for approximately three minutes with a soft paint brush over a white paper and counting the number of mites and ticks that fell on the paper.

All work was done in accordance with University of Antwerp animal welfare standards and protocol.

Statistical analyses

Morphometric, performance, immunity and hormone data were $\log_{10}$-transformed, and parasite counts were arcsine transformed prior to analysis to meet assumptions of normality. We used general linear models to investigate differences in traits among morphs and explore relationships between traits across all individuals. Models were run in a stepwise backward manner, removing one-by-one the non-significant (interaction) effects (i.e., color as fixed effect, sampling year as random effect, any covariate and all their mutual interactions), ending with a model only including significantly contributing effects. All analyses including data collected during the two years of sampling were tested for possible sampling year effects (sampling year as random factor), and when significant this effect was retained in the final model and reported as such.

Results

Differences among morphs

Means, standard errors and sample sizes for hormone, bite force, and immunity parameters for the three different morphs are shown

Table 1
Descriptive statistics (means, standard errors and sample sizes) for the morphometric, performance, immunity and hormonal variables measured on three male color morphs in the lizard *P. melisellensis*.

Trait	White			Yellow			Orange			Factor year			Factor color		
	x	Se	*n*	*x*	se	*n*	*x*	se	*n*	*F*	*df*	*P*	*F*	*df*	*P*
SVL (mm)	61.03	0.56	39	61.31	1.07	17	63.34	0.47	38						
Mass (g)	5.57	0.16	39	5.77	0.32	17	6.22	0.14	38						
Body condition	0.0041	0.0063	39	0.0031	0.0097	17	0.0015	0.0053	38	7.38	1,89	0.008	0.22	2,89	0.80
Bite force (N)	13.78	0.51	39	13.51	1.19	17	16.7	0.57	38	7.09	1,89	0.009	8.36	2,89	<0.001
Size-corrected bite force	−0.015	0.012	39	−0.012	0.018	16	0.019	0.0084	38	9.97	1,88	0.002	3.48	2,88	0.04
Parasite load	0.69	0.25	16	1.63	1.03	9	1.44	0.51	18	NA			0.48	2,42	0.62
T cell-response (mm)	1.14	0.045	16	1.2	0.057	9	1.3	0.053	18	NA			0.30	2,30	0.75
Testosterone (ng/mL)	107.15	14.96	27	128.67	22.84	12	142.76	15.14	19	56.29	1,54	<0.001	0.038	2,54	0.96
Corticosterone (ng/mL)	35.75	3.96	39	24.17	3.87	17	40.47	4.51	38	8.97	1,90	0.004	4.27	2,90	0.017

Body condition is expressed as the residual of mass over snout-vent-length. Statistical results (*F*, degrees of freedom *df*, probability *P*) of the general linear models are shown for the random factor year and the fixed factor color.

in Table 1. Color morphs did not differ in circulating testosterone levels, but they had different corticosterone levels (Fig. 1). There were no differences in body condition between morphs, but they did differ in maximal bite force capacity, and this difference persisted after correcting for SVL (Table 1). Orange males bit harder than the other two morphs, independent of any size differences between individuals.

Ectoparasite load did not differ among morphs. Morphs did not differ in ectoparasite load or in the ability to respond by T cell-proliferation to a PHA injection (Table 1).

Corticosterone

Across all individuals, the concentration of plasma corticosterone did not co-vary with plasma testosterone ($F_{1,56}=1.94$, $P=0.17$) and was independent of SVL ($F_{1,92}=0.63$, $P=0.63$, year effect: $F_{1,92}=7.22$, $P=0.009$) and body condition ($F_{1,91}=0.70$, $P=0.40$, year effect: $F_{1,91}=7.56$, $P=0.007$). Corticosterone levels did not predict size-corrected maximal bite force capacity ($F_{1,90}=1.74$, $P=0.19$, year effect: $F_{1,90}=6.26$, $P=0.014$, T cell-response ($r^2=0.0041$, $P=0.73$) or parasite load ($r^2=0.031$, $P=0.26$).

Testosterone

Circulating testosterone levels were independent from SVL ($F_{1,55}=0.54$, $P=0.47$, year effect: $F_{1,55}=62.40$, $P<0.001$), but correlated positively with body condition ($F_{1,55}=8.37$, $P=0.005$): individuals in better condition had higher testosterone levels. Testosterone levels predicted size-corrected maximal bite force capacity ($F_{1,54}=11.11$, $P=0.002$, Fig. 2): higher testosterone levels translated into better bite performance. Thus, variation in testosterone levels may help explain inter-individual differences in bite force capacities that remain unaccounted for by body size. No relation was detected between testosterone levels and either T cell-response ($r^2=0.014$, $P=0.54$) or parasite load ($r^2=0.039$, $P=0.23$).

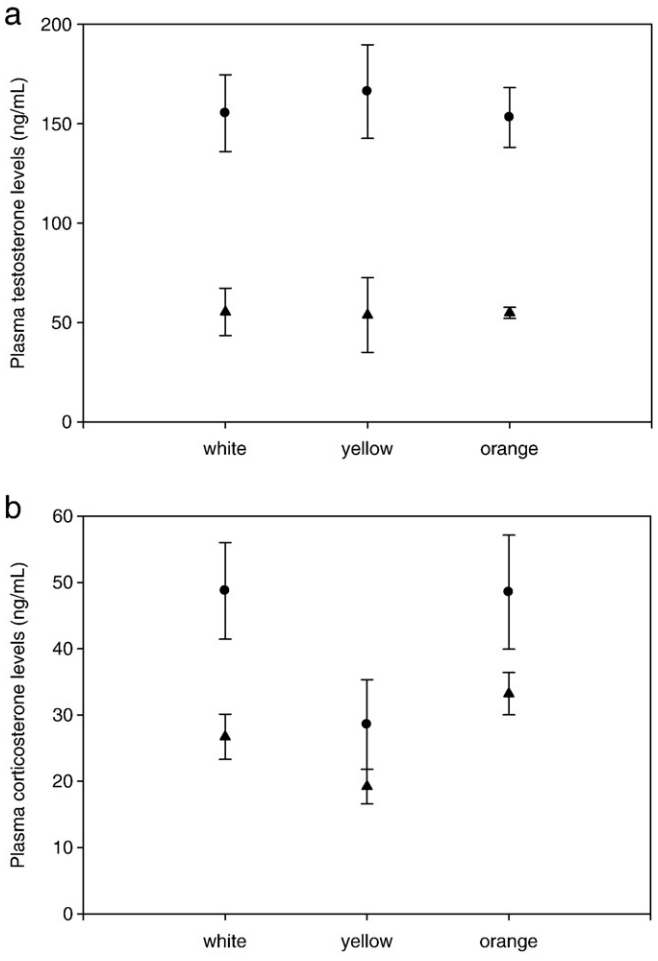

Fig. 1. Means and standard errors of plasma testosterone (a) and corticosterone (b) levels for the three color morphs (white, yellow and orange) and the different sampling years represented by triangles (2006) and circles (2008).

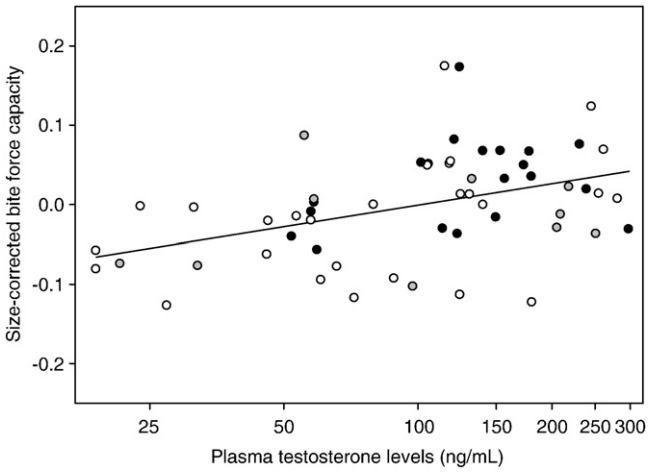

Fig. 2. Relationship between plasma testosterone concentration (*x*-axis) and size-corrected bite force capacity (*y*-axis). The different morphs are represented by differently colored symbols: white (white circles), yellow (grey circles), and orange (black circles).

Immunity

PHA response was not correlated with SVL ($r^2=0.12$, $P=0.05$) or body condition ($r^2=0.19$, $P=0.44$). The relationship between PHA response and absolute bite force capacity ($r^2=0.16$, $P=0.02$) disappears when correcting for body size ($r^2=0.066$ $P=0.15$). Parasite load was positively correlated with body size ($r^2=0.12$, $P=0.02$), but was independent from body condition ($r^2=0.058$, $P=0.12$), and (size-corrected) bite force capacity (both $r^2<0.058$, both $P>0.16$). T cell-response and parasite load were independent ($r^2=0.028$, $P=0.35$).

Discussion

In lizards, as in many other vertebrates (Wingfield et al., 1990, reviews in Rubinow and Schmidt, 1996; Oliveira et al., 2003), circulating testosterone levels often correlate with male aggression (Crews, 1979; Greenberg and Crews, 1983; Yang and Wilczynski, 2002), territorial behavior (Moore, 1987, 1988; Knapp et al., 2003; Watt et al., 2003) and the ability to acquire high-quality territories (Fox, 1983; Tokarz, 1995). For example, sexually mature male lizards with elevated testosterone levels are assumed to be better equipped to fight (Salvador et al., 1996). However, the functional basis for this relationship remains poorly understood (Perry et al., 2004; Husak et al., 2007). Our results demonstrate a positive correlation between circulating levels of testosterone and bite force capacity in adult male *P. melisellensis*. Bite force strongly influences the outcome of staged fights between males in many lizard species (Lailvaux et al., 2004; Huyghe et al., 2005; Husak et al., 2006b), and male bite capacity is a strong predictor of males fitness (Lappin and Husak, 2005, Husak et al., in press). Assuming a similar relationship between bite capacity and dominance in *P. melisellensis*, our data suggest that testosterone may be exerting its influence on dominance at least partly by affecting physiological performance. Similarly, the relationship between testosterone levels and the intensity of intra-sexual signals such as color

badges and behavioral displays (e.g. Pryke et al., 2001; Andersson et al., 2002; Wikelski et al., 2005; Blas et al., 2006; Whiting et al., 2006; Madsen et al., 2007) is easier to understand when testosterone levels also affect relevant whole-animal performance. In this way these sexually selected signals honestly reflect one aspect of the individual's quality (Vanhooydonck et al., 2001).

Testosterone may affect bite force capacity in a number of ways. Bite force in lizards depends not only on the size of the head (Herrel et al., 1999), but also on the shape of the skull (Herrel et al., 2001; Herrel et al., 2007; Lappin and Husak, 2005) and on the size and morphology of the jaw adductor musculature (Herrel et al., 2007). It seems likely that differences in jaw muscle architecture or physiology may also contribute to variation in bite force. Indeed, gonadal hormones are known to influence the size and shape of body parts, including the head (Shine and Crews, 1988; Fink et al., 2005). Testosterone levels may also affect skeletal muscle mass, morphology, physiology and performance. In humans, the administration of exogenous testosterone may affect muscle mass and performance through various pathways, especially in combination with training (reviewed in Bhasin et al., 1996; Hartgens and Kuipers, 2004; Harridge, 2007). In male mice, fiber-type distribution in the masseter muscle is influenced by castration, causing a proportional phenotype transition from faster to slower fiber-types (Eason et al., 2000). In castrated male African clawed frogs (*Xenopus laevis*), testosterone implants increased the cross-sectional area of a forelimb flexor by 84% in comparison to sham-operated individuals (Regnier and Herrera, 1993), and in the gray tree frog (*Hyla chrysoscelis*), exogenous testosterone increased mass and changed contractile properties of fast-twitch calling muscles (Girgenrath and Marsh, 2003). In the lizard *G. galloti* the administration of exogenous testosterone resulted in increased muscle mass, but there was no effect on bite performance (Huyghe et al., unpublished data). Future experimental work will help to establish exactly which functional mechanism is causing the relationship between bite force and testosterone in *P. melisellensis* and other lizards. Alternatively, the effect of testosterone may be primarily behavioral. In a wide variety of vertebrates, androgens play a prominent role in the organization, and activation of brain circuits (reviews in Rubinow and Schmidt, 1996; Oliveira et al., 2003). Perhaps these neural changes affect the motivation or willingness of the lizards to bite and engage in aggressive interactions. In this case, the increased bite performance of males with high testosterone levels in our study may reflect motivational differences rather than 'hardware' morphological or physiological changes.

In our study organism, plasma levels of testosterone were not correlated with PHA response or parasite load, and hence our data do not support the immunocompetence handicap hypothesis as stated by Folstad and Karter (1992). High testosterone levels do not seem to come at an immunological cost in *P. melisellensis*, in contrast to what has been observed for other lizard species where testosterone implanted individuals suffer an increased parasite load (Salvador et al., 1996; Olsson et al., 2000; Cox and John-Alder, 2007; Mougeot et al., 2007). The immunocompetence handicap hypothesis (Folstad and Karter, 1992) ascribes this association to a direct effect of testosterone on the immune function. However, increased testosterone levels could also increase an individual's level of exposure to parasites indirectly, e.g., by increasing its mobility, or by extending its activity time (Cox and John-Alder, 2007). Another cost of high testosterone levels could be a suppressed cell mediated immunoresponse, which is essential for healing wounds and resisting infection (Belliure, Smith and Sorci, 2004). Sacchi et al. (2007) found that yellow males of a closely related species *P. muralis*, have a lower immune responsiveness than the other two morphs (white and red). In contrast, we found no differences in parasite load or PHA response between morphs in *P. melisellensis*. It should be noted that our samples were taken early in the reproductive season. Possibly, a negative effect of high androgen levels may manifest itself later, when males have competed with each other for a prolonged period of time and the immune system becomes more and more challenged. Unpublished data for *P. melisellensis* (Huyghe et al.) indeed suggest a rise in parasite load as the reproductive season progresses, but morphs still suffered equally from ectoparasite infestation.

In contrast to earlier studies on birds (e.g. Ketterson et al., 1991; Ketterson and Nolan, 1992; Johnsen, 1998), lizards (Knapp and Moore, 1997; Husak et al., 2007), and snakes (Moore et al., 2000) we found no correlation between circulating corticosterone and testosterone levels. Thus for this species, our study does not support the proposal that increased testosterone comes with higher levels of stress hormones (Alberts et al., 1992; Creel et al., 1996; Smith and John-Alder, 1999; recently reviewed in Goymann and Wingfield, 2004). However, addressing this issue will require careful monitoring of corticosterone levels in response to specific social stimuli in males of different social status. Sexual selection can act on the stress response and thus corticosterone levels such that it is downregulated during periods when stress could negatively affect sexually selected traits (see Husak and Moore, 2008 for examples). Accordingly, levels of stress hormones are often negatively related with body condition (Moore and Jessop, 2003), which is in turn an honest signal for females looking for a high-quality male (Andersson, 1994). In addition to a lowered body condition, elevated corticosterone levels as a result of stress can suppress immune activity (Berger et al., 2005) and stress can induce an increase in parasite infection (Oppliger et al., 1998). However, evidence for the effect of corticosterone on immunocompetence varies and some studies even demonstrate an immuno-enhancing effect of corticosterone (e.g. Roberts et al., 2007) or at least varying effects depending on social environment, testosterone levels or reproductive strategy (Pryke et al., 2007, Mills et al., 2008). In *P. melisellensis*, corticosterone levels are not correlated with body condition, PHA response, or parasite load, suggesting that in this species stress does not result in altered (suppressed or enhanced) immunity.

Although inter-individual differences in bite force coincide with differences in ventral color, and across individuals testosterone levels correlate positively with bite performance, the level of circulating testosterone does not appear to be the principal proximate mechanism mediating the continued existence of polymorphism in this population. Also, high testosterone levels do not seem to result in direct immunity costs, but do aid, either directly or indirectly, in improving whole-animal performance important in a social context. Mean circulating corticosterone levels on the other hand significantly differed between morphs, possibly indicating differential effects of stress on the morphs. The finding that testosterone levels do not differ between morphs is contrary to our expectations, as orange males have relatively larger heads and can bite harder than the other morphs, and appear to be more aggressive (Huyghe et al., 2007). The assumed proximate link between high androgen levels and the differentiation between morphs of varying level of aggressiveness have been confirmed in several studies (Moore et al., 1998, Sinervo et al., 2000, Olsson et al., 2007). Yet in other studies, the relation between testosterone, corticosterone and alternative morphs is less clear (Knapp et al., 2003, Pryke et al., 2007, Baird and Hews, 2007). In collared lizards, levels of testosterone, dihydrotestosterone, and corticosterone are similar in males displaying alternative tactics and relationships between hormones differ among these types of males (Baird and Hews, 2007). Pryke et al. found that in the Gouldian finch (*Erythrura gouldiae*), there is a differential effect of hormones on aggressive behavior in the red- and black-headed morphs, with social environment playing a crucial role (Pryke et al., 2007). The non-territorial morph of the lizard *U. ornatus* exhibits behavioral plasticity, depending on environmental conditions, and levels of corticosterone and testosterone vary accordingly (Knapp et al., 2003). In our study, plasma levels of androgens in *P. melisellensis* seem to vary, but a long-term study is needed to investigate fluctuations in differing levels of hormones among morphs and

years, and to link these to social environment, morph densities and frequencies, and environmental conditions. Furthermore, we cannot rule out the possibility that organizational effects of differential testosterone concentrations among individuals early in life result in the male morphs (see Hews et al., 1994).

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References

Alberts, A.C., Pratt, N.C., Phillips, J.A., 1992. Seasonal productivity of lizard femoral glands: relationship to social dominance and androgen levels. Physiol. Behav. 51, 729–733.
Andersson, M., 1994. Sexual Selection. Princeton University Press.
Andersson, S., Pryke, S.R., Örnborg, J., Lawes, M.J., Andersson, M., 2002. Multiple receivers, multiple ornaments, and a trade-off between agonistic and epigamic signaling in a widowbird. Am. Nat. 160, 683–691.
Baird, T.A., Hews, D.K., 2007. Hormone levels in territorial and non-territorial male collared lizards. Physiol. Behav. 92, 755–763.
Belliure, J., Smith, L., Sorci, G., 2004. Effect of testosterone on T cell-mediated immunity in two species of Mediterranean lacertid lizards. J. Exp. Zool. 301A, 411–418.
Berger, S., Martin II, L.B., Wikelski, M., Romero, L.M., Kalko, E.K.V., Vitousek, M.N., Rödl, T., 2005. Corticosterone suppresses immune activity in territorial Galapagos marine iguanas during reproduction. Horm. Behav. 47, 419–429.
Bhasin, S., Storer, T.W., Berman, N., Callegari, C., Clevenger, B., Phillips, J.P., Bunnell, T.J., Tricker, R., Shirazi, A., Casaburi, R., 1996. The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. J. Med. 335, 1–7.
Blas, J., Pérez-Rodriguez, L., Bortolotti, G.R., Viñuela, J., Marchant, T.A., 2006. Testosterone increases bioavailability of carotenoids: insights into the honesty of sexual signaling. Proc. Natl. Acad. Sci. U. S. A. 103, 18633–18637.
Brockmann, H.J., 2001. The evolution of alternative strategies and tactics. Adv. Stat. Behav. 30, 1–51.
Cox, R.M., John-Alder, H.B., 2007. Increased mite parasitism as a cost of testosterone in male striped plateau lizards *Sceloporus virgatus*. Funct. Ecol. 21, 327–334.
Cox, R.M., Zilberman, V., John-Alder, H.B., 2008. Testosterone stimulates the expression of a social color signal in yarrow's spiny lizard, *Sceloporus jarrovii*. J. Exp. Zool. 309A, 505–514.
Creel, S., 2001. Social dominance ad stress hormones. Tr. Ecol. Evol. 16, 491–497.
Creel, S., Creel, N.M., Monfort, S.L., 1996. Social stress and dominance. Nature 379, 212.
Crews, D., 1979. Hormonal control of behavior in a lizard. Sci. Am. 241, 180–187.
Eason, J.M., Schwartz, G.A., Pavlath, G.K., English, A.W., 2000. Sexually dimorphic expression of myosin heavy chains in the adult mouse masseter. J. Appl. Physiol. 89, 251–258.
Fink, B., Grammer, K., Mitteroecker, P., Gunz, P., Schaefer, K., Bookstein, F.L., Manning, J.T., 2005. Second to fourth digit ratio and face shape. Proc. R. Soc. Lond. B 272, 1995–2001.
Folstad, I., Karter, A.J., 1992. Parasites, bright males, and the immunocompetence handicap. Am. Nat. 139, 604–622.
Fox, S.F., 1983. Fitness, home range quality, and social rank in *Uta stansburiana*. In: Huey, R.B., Pianka, E.R., Schoener, T.W. (Eds.), Lizard Ecology: Studies on a Model Organism. Harvard University Press, Cambridge, MA, pp. 149–168.
Girgenrath, M., Marsh, R.L., 2003. Season and testosterone affect contractile properties of fast calling muscles in the gray tree frog *Hyla chrysoscelis*. Am. J. Physiol. Integr. Comp. Physiol. 284, R1513–1520.
Goymann, W., Wingfield, J.C., 2004. Allostatic load, social status and stress hormones: the costs of social status matter. Anim. Behav. 67, 591–602.
Greenberg, N.C., Crews, D., 1983. Physiological ethology of aggression in amphibians and reptiles. In: Svare, B.B. (Ed.), Hormones and Aggressive Behavior. Plenum Press, Plenum Press, pp. 469–506.
Gross, M., 1996. Alternative reproductive strategies and tactics: diversity within sexes. Tr. Ecol. Evol. 22, 92–98.
Harridge, S.D.R., 2007. Plasticity of human skeletal muscle: gene expression to *in vivo* function. Exp. Physiol. 92, 783–797.
Hartgens, F., Kuipers, H., 2004. Effects of androgenic-anabolic steroids in athletes. Sports Med. 34, 513–554.
Hau, M., 2007. Regulation of male traits by testosterone: implications for the evolution of vertebrate life histories. BioEssays 29, 133–144.
Hau, M., Dominguez, O.A., Evrard, H.C., 2004. Testosterone reduces responsiveness to nociceptive stimuli in a wild bird. Horm. Behav. 46, 165–170.
Herrel, A., Spithoven, L., Van Damme, R., De Vree, F., 1999. Sexual dimorphism of head size in *Gallotia galloti*, testing the niche divergence hypothesis by functional analyses. Funct. Ecol. 13, 289–297.
Herrel, A., De Grauw, E., Lemos-Espinal, J.A., 2001. Head shape and bite performance in xenosaurid lizards. J. Exp. Zool. 290, 101–107.
Herrel, A., McBrayer, L.D., Larson, P.M., 2007. Functional basis for sexual differences in bite force in the lizard *Anolis carolinensis*. Biol. J. Linn. Soc. 91, 111–119.
Hews, D.K., Knapp, R., Moore, M.C., 1994. Early exposure to androgens affects adult expression of alternative male types in tree lizards. Horm. Behav. 28, 96–115.
Husak, J.F., Moore, I.T., 2008. Stress hormones and mate choice. Tr. Ecol. Evol. 23, 532–534.
Husak, J.F., Fox, S.F., Lovern, M.B., Van Den Bussche, R.A., 2006a. Faster lizards sire more offspring: sexual selection on whole-animal performance. Evolution 60, 2122–2130.
Husak, J.F., Lappin, A.K., Fox, S.F., Lemos-Espinal, J.A., 2006b. Bite-force performance predicts dominance in male Venerable Collared Lizards (*Crotaphytus antiquus*). Copeia 2006, 301–306.
Husak, J.F., Irschick, D.J., Meyers, J.J., Lailvaux, S.P., Moore, I.T., 2007. Hormones, sexual signals, and performance of green anole lizards (*Anolis carolinensis*). Horm. Behav. 52, 360–367.
Husak, J.F., Lappin, A.K., Van Den Bussche, R.A. The fitness advantage of a high performance weapon. Biol. J. Linn. Soc. in press.
Huyghe, K., Vanhooydonck, B., Scheers, H., Molina-Borja, M., Van Damme, R., 2005. Morphology, performance and fighting capacity in male lizards, *Gallotia galloti*. Funct. Ecol. 19, 800–807.
Huyghe, K., Vanhooydonck, B., Herrel, A., Tadić, Z., Van Damme, R., 2007. Morphology, performance, behaviour and ecology of three color morphs in males of the lizard *Podarcis melisellensis*. Integr. Comp. Biol. 47, 211–220.
Johnsen, T.S., 1998. Behavioral correlates of testosterone and seasonal changes of steroids in red-winged blackbirds. Anim. Behav. 55, 957–965.
Kennedy, M.W., Nager, R.G., 2006. The perils and prospects of using phytohaemagglutinin in evolutionary ecology. Tr. Ecol. Evol. 21, 653–655.
Ketterson, E.D., Nolan, V., 1992. Hormones and life histories — an integrative approach. Am. Nat. 140, S33–S62.
Ketterson, E.D., Nolan, V., Wolf, L., Ziegenfus, C., Dufty, A.M., Ball, G.F., Johnsen, T.S., 1991. Testosterone and avian life histories — the effect of experimentally elevated testosterone on corticosterone and body-mass in dark-eyed juncos. Horm. Behav. 25, 489–503.
Klukowski, M., Nelson, C.E., 2001. Ectoparasite loads in free-ranging northern fence lizards, *Sceloporus undulates hyacinthinus*: effects of testosterone and sex. Behav. Ecol. Sociobiol. 49, 289–295.
Klukowski, M., Jenkinson, N.M., Nelson, C.E., 1998. Effects of testosterone on locomotor performance and growth in field-active northern fence lizards, *Sceloporus undulates hyacinthinus*. Physiol. Zool. 71, 506–514.
Knapp, R., Moore, M.C., 1997. Male morphs in tree lizards have different testosterone responses to elevated levels of corticosterone. Gen. Comp. Endocrinol. 107, 273–279.
Knapp, R., Hews, D.K., Thompson, C.W., Ray, L.E., Moore, M.C., 2003. Environmental and endocrine correlates of tactic switching by nonterritorial male Tree lizards (*Urosaurus ornatus*). Horm. Behav. 43, 83–92.
Lailvaux, S., Herrel, A., Vanhooydonck, B., Meyers, J.J., Irschick, D.J., 2004. Performance capacity, fighting tactics and the evolution of life-stage male morphs in the green anole lizard (*Anolis carolinensis*). Proc. R. Soc. Lond. B 271, 2501–2508.
Lappin, A.K., Husak, J.F., 2005. Weapon performance, not size, determines mating success and potential reproductive output in the collared lizard (*Crotaphytus collaris*). Am. Nat. 166, 426–436.
Luine, V., Nottebohm, F., Harding, C., McEwan, B.S., 1980. Androgen affects cholinergic enzymes in syringeal motor neurons and muscle. Brain Res. 192, 89–107.
Madsen, V., Valkiunas, G., Iezhova, T.A., Mercade, C., Sanchez, M., Osorno, J.L., 2007. Testosterone levels and gular pouch coloration in courting magnificent frigatebird (*Fregata magnificens*): variation with age-class, visited status and blood parasite infection. Horm. Behav. 51, 156–163.
Marler, C.A., Moore, M.C., 1988. Evolutionary costs of aggression revealed by testosterone manipulations in free-living male lizards. Behav. Ecol. Sociobiol. 23, 21–26.
Mougeot, F., Pérez-Rodríguez, L., Martínez-Padilla, J., Leckie, F.M., Redpath, S.M., 2007. Parasites, testosterone and honest carotenoid-based signaling of health. Funct. Ecol. 21, 886–898.
Mills, S.C., Hazard, L., Lancaster, L., Mappes, T., Miles, D., Oksanen, T.A., Sinervo, B., 2008. Gonadotropin hormone modulation of testosterone, immune function, performance, and behavioral trade-offs among male morphs of the lizard *Uta stansburiana*. Am. Nat. 3, 339–357.
Moore, M.C., 1987. Circulating steroid hormones during rapid aggressive responses of territorial male mountain spiny lizards, *Sceloporus jarrovi*. Horm. Behav. 21, 511–521.
Moore, M.C., 1988. Testosterone control of territorial behavior — tonic-release implants fully restore seasonal and short term aggressive responses in free living castrated lizards. Gen. Comp. Endocrinol. 70, 450–459.
Moore, M.C., Marler, C.A., 1987. Effects of testosterone manipulations on nonbreeding season territorial aggression in free-living male lizards, *Sceloporus jarrovi*. Gen. Comp. Endocrinol. 65, 225–232.
Moore, I.T., Jessop, T.S., 2003. Stress, reproduction, and adrenocortical modulation in amphibians and reptiles. Horm. Behav. 43, 39–47.
Moore, M.C., Hews, D.K., Knapp, R., 1998. Hormonal control and evolution of alternative male phenotypes: generalizations of models for sexual differentiation. Am. Zool. 38, 133–151.
Moore, I.T., Lerner, J.P., Lerner, D.T., Mason, R.T., 2000. Relationships between annual cycles of testosterone, corticosterone, and body condition in male red-spotted garter snakes, *Thamnophis sirtalis concinnus*. Phys. Bioch. Zool. 73, 307–312.
Mougeot, F., Redpath, S.M., Piertney, S.B., 2005. Elevated spring testosterone increases parasite intensity in male red grouse. Behav. Ecol. 17, 117–125.
Olsson, M., Wapstra, E., Madsen, T., Silverin, B., 2000. Testosterone, ticks and travels: a test of the immunocompetence-handicap hypothesis in free-ranging male sand lizards. Proc. R. Soc. Lond. B 267, 2339–2343.

Olsson, M., Healey, M., Astheimer, L., 2007. Afternoon T: testosterone level is higher in red than yellow male polychromatid lizards. Physiol. Behav. 91, 531–534.
Oliveira, R.F., 2004. Social modulation of androgens in vertebrates: mechanisms and function. Adv. Study Behav. 34, 165–239.
Oliveira, R.F., Hirschenhauser, K., Canario, A.V.M., Taborsky, M., 2003. Androgen levels of reproductive competitors in a cooperatively breeding cichlid. J. Fish Biol. 63, 1615–1620.
Oppliger, A., Clobert, J., Lecomte, J., Lorenzon, P., Boudjemadi, K., John-Alder, J.B., 1998. Environmental stress increases the prevalence and intensity of blood parasite infection in the common lizard *Lacerta vivipara*. Ecol. Lett. 1, 129–138.
Oppliger, A., Giorgi, M.S., Conelli, A., Nembrini, M., John-Alder, H.B., 2004. Effect of testosterone on immunocompetence, parasite load, and metabolism in the common wall lizard (*Podarcis muralis*). Can. J. Zool. 82, 1713–1719.
Owen-Ashley, N.T., Hasselquist, D., Wingfield, J.C., 2004. Androgens and the immunocompetence handicap hypothesis: unraveling direct and indirect pathways of immunosuppression in song sparrows. Am. Nat. 164, 490–505.
Perez-Orella, C., Schulte-Hostedde, A.I., 2005. Effects of sex and body size on ectoparasite loads in the northern flying squirrel (*Glaucomys sabrinus*). Can J. Zool. 83, 1381–1385.
Perry, G., Levering, K., Girard, I., Garland Jr., T., 2004. Locomotor performance and social domninace in male *Anolis cristatellus*. Anim. Behav. 67, 37–47.
Pryke, S.R., Lawes, M.J., Andersoon, S., 2001. Agonistic carotenoid signalling in male red-collared widowbirds: aggression related to the color signal of both the territory owner and model intruder. Anim. Behav. 62, 695–704.
Pryke, S.R., Astheimer, L.B., Buttemer, W.A., Griffith, S.C., 2007. Frequency-dependent physiological trade-offs between competing colour morphs. Biol. Lett. 3, 494–497.
Rand, M.S., 1992. Hormonal control of polymorphic and sexually dimorphic coloration in the lizard, *Sceloporus undulatus erythrocheilus*. Gen. Comp. Endocrinol. 88, 461–468.
Regnier, M., Herrera, A.A., 1993. Changes in contractile properties by androgen hormones in sexually dimorphic muscles of male frogs (*Xenopus laevis*). J. Physiol. 461, 565–581.
Roberts, M.L., Buchanan, K.L., Evans, M.R., 2004. Testing the immunocompetence handicap hypothesis: a review of the evidence. Anim. Behav. 68, 227–239.
Rubinow, D.R., Schmidt, P.J., 1996. Androgens, brain, and behavior. Am. J. Psych. 153, 874–984.
Sacchi, R., Rubolini, D., Gentili, A., Pupin, F., Razzetti, E., Scali, S., Galeotti, P., Fasola, M., 2007. Morph-specific immunity in male *Podarcis muralis*. Amph.-Rept. 28, 408–412.
Saino, N., Moller, A.P., 1995. Secondary sexual characters, parasites and testosterone in the barn swallow, *Hirundo rustica*. Anim. Behav. 48, 1325–1333.
Salvador, A., Veiga, J.P., Martin, J., Abelenda, M., Puerta, M., 1996. The cost of producing a sexual signal: testosterone increases the susceptibility of male lizards to ectoparasite infection. Behav. Ecol. 7, 145–150.
Shine, R., Crews, D., 1988. Why male garter snakes have small heads: the evolution and endocrine control of sexual dimorphism. Evolution 42, 1105–1110.
Sidor, C.A., Blackburn, D.G., 1998. Effects of testosterone administration and castration on the forelimb musculature of male leopard frogs, *Rana pipiens*. J. Exp. Zool. 280, 28–37.
Sinervo, B., Miles, D.S., Frankino, W.A., Klukowski, M., DeNardo, D.F., 2000. Testosterone, endurance, and Darwinian fitness: natural and sexual selection on the physiological bases of alternative male behaviors in side-blotched lizards. Horm. Behav. 38, 222–233.
Smith, L.C., John-Alder, H.B., 1999. Seasonal specificity of hormonal, behavioral, and coloration responses to within- and between-sex encounters in male lizards (*Sceloporus undulatus*). Horm. Behav. 36, 39–52.
Tobin, C., Joubert, Y., 1991. Testosterone-induced development of the rat levator ani muscle. Dev. Biol. 146, 131–138.
Tokarz, R.R., 1995. Importance of androgens in male territorial acquisition in the lizard *Anolis sagrei*: an experimental test. Anim. Behav. 49, 661–669.
Vanhooydonck, B., Van Damme, R., Aerts, P., 2001. Speed and stamina trade-off in lacertid lizards. Evolution 55, 1040–1048.
Watt, M.G., Forster, G.L., Joss, J.M.P., 2003. Steroid correlates of territorial behavior in male Jacky dragons, *Amphibolurus muricatus*. Brain Behav. Evol. 61, 184–194.
Whiting, M.J., Stuart-Fox, D., O'Connor, D., Firth, D., Bennett, N., Blomberg, S., 2006. Ultraviolet signals ultra-aggression in a lizard. Anim. Behav. 72, 353–363.
Wikelski, M., Steiger, S.S., Gall, B., Nelson, K.N., 2005. Sex, drugs, and mating role: testosterone-induced phenotype-switching in Galapagos marine iguanas. Behav. Ecol. 16, 260–268.
Wingfield, J.C., Farner, D.S., 1975. Determination of 5 steroids in avian plasma by radioimmunoassay and competitive protein binding. Steroids 26, 311–327.
Wingfield, J.C., Hegner, R.E., Dufty, A.M., Ball, G.F., 1990. The challenge hypothesis — theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. Am. Nat. 136, 829–846.
Yang, E.J., Wilczynski, W., 2002. Relationships between hormones and aggressive behavior in green anole lizards: an analysis using structural equation modeling. Horm. Behav. 42, 192–205.