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Female anoles retain responsiveness to testosterone despite the evolution of androgen-mediated sexual dimorphism

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Summary

1. The evolution of sexual dimorphism presents a challenge because males and females must express two phenotypes from the same underlying genome. In vertebrates, one solution to this challenge is to link the expression of shared traits to sex steroids. However, even 'male-biased' steroids such as testosterone (T) circulate at biologically significant levels in females, raising the question of whether sexual dimorphism evolves not only through the coupling of trait expression to T in males, but also through the decoupling of trait expression from T in females.

2. We tested these alternatives by asking whether male and female brown anoles (*Anolis sagrei*) respond to exogenous T in similar fashion with respect to a suite of sexually dimorphic traits: growth, skeletal morphology, resting metabolism, fat storage, dewlap size and dewlap colour.

3. First, we established the ontogeny of sexual dimorphism in a colony raised in a laboratory common garden. Next, we treated juveniles of each sex with either T implants or empty implants at 5–8 months of age, when sexual dimorphism first began to develop for most traits.

4. T stimulated growth in both sexes and largely abolished natural sex differences in growth. This effect was associated with the stimulation of resting metabolism and the diversion of energy from fat and liver stores in both sexes. T also enlarged the dewlap in both sexes, though females never developed dewlaps equal in size to those of males. Finally, T altered the brightness and saturation of the dewlap in both sexes, inducing coloration similar to that of adult males.

5. Female brown anoles retain many of the same tissue-specific responses to T that occur in males, suggesting that the evolution of androgen-mediated sexual dimorphism has been achieved largely through the coupling of trait expression to sex differences in circulating T, without an associated decoupling of trait expression from T in females.

Key-words: *Anolis sagrei*, body size, dewlap, hormonal pleiotropy, metabolism, sexual conflict

Introduction

Males and females share an autosomal genome, which is predicted to constrain the evolution of sexual dimorphism (Lande 1980; Chenoweth, Rundle & Blows 2008; Cox & Calsbeek 2009; Mank 2009). Despite this apparent constraint, sexual dimorphism is both ubiquitous and evolutionarily labile (Fairbairn 1997; Wiens 1999; Cox, Skelly & John-Alder 2003). This genomic constraint can be overcome by linking the expression of shared loci to sex-specific regulators, such as sex steroids. In particular, the androgen testosterone (T), which is produced primarily by the gonads, has been implicated as a key regulator of sexual dimorphism in many vertebrates (Owens & Short 1995; Hau 2007; Cox, Stenquist & Calsbeek 2009). Although adult males typically exceed females in the production of T, this hormone circulates and serves important regulatory functions in both sexes (Staub & De Beer 1997; Ketterson, Nolan & Sandell 2005). Evolutionary changes in the production of T and in trait responsiveness to T can therefore influence the expression of shared traits, and their fitness consequences, in either sex (Ketterson, Nolan & Sandell 2005; McGlothlin & Ketterson 2008). Therefore, the evolution of androgen-mediated sexual dimorphism may require

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not only the coupling of T to trait expression in males, but also the decoupling of T from trait expression in females. However, the relative importance of these two processes in the evolution of sexual dimorphism is largely unknown because most studies focus on the effects of androgens in males or, conversely, on the effects of oestrogens in females.

Studies that have simultaneously explored the effects of androgens in both sexes provide support for each of the above processes. Whereas T can induce male-typical aggression, song and courtship behaviours in adult females, these masculinizing effects are often only partial in comparison to males, or absent altogether, as observed in amphibians (Kelley & Pfaff 1976), fish (Dulka & Maler 1994; Murphy & Stacey 2002), mammals (DeBold & Miczek 1981) and birds (Schlinger 1997; Lank, Coupe & Wynne-Edwards 1999; De Ridder *et al.* 2002; Clotfelter *et al.* 2004; Zysling *et al.* 2006; O'Neal *et al.* 2008). Likewise, in reptiles, T is often sufficient to induce the expression of male-typical coloration in females, though some aspects of coloration may fail to respond outside of particular developmental periods (Hews & Quinn 2003; Cox *et al.* 2005; Cox, Zilberman & John-Alder 2008). This inability of androgens to induce male-specific phenotypes in females could occur because early (organizational) effects of sex steroids during sexual differentiation render females insensitive to any later (activational) effects (Schlinger 1997; De Ridder *et al.* 2002; Hews & Quinn 2003; O'Neal *et al.* 2008). Moreover, even when the phenotypic effects of T appear to be broadly similar in males and females, analyses of tissue-wide gene-expression profiles reveal a high degree of sex specificity in responsiveness (van Nas *et al.* 2009; Peterson *et al.* 2013, 2014). Collectively, these studies support the hypothesis that androgen-mediated sexual dimorphism is often achieved not only through sex-biased production of T, but also through sex-specific responsiveness of target tissues.

To test this hypothesis, we studied the effects of T on a suite of sexually dimorphic traits in both male and female brown anole lizards (*Anolis sagrei*). Brown anoles are extremely sexually dimorphic in body size, with adult males averaging 32% longer and 2–3 times more massive than adult females (Cox & Calsbeek 2010). Sexual dimorphism in body size develops due to sex differences in juvenile growth (Cox, Stenquist & Calsbeek 2009) and is reinforced by sex-specific natural selection on adult size, with females experiencing stabilizing selection for intermediate body size and males experiencing directional selection for larger body size (Cox & Calsbeek 2010). Castration suppresses and T stimulates growth in adult male brown anoles (Cox, Stenquist & Calsbeek 2009), but the effects of T on females are unknown, as are the effects of T at earlier ages when sexual dimorphism first develops. Brown anoles are also sexually dimorphic in head shape, skeletal morphology and the size of the dewlap, an extensible structure consisting of brightly coloured skin and underlying cartilage that is used as a social and sexual signal by anoles (Harrison & Poe 2012; Sanger *et al.* 2013). The extent to which these traits are influenced by T is generally unknown for either sex, although castration slightly reduces dewlap size in adult males (Cox *et al.* 2009). We chose to focus on these traits, along with resting metabolic rate (RMR) and energy storage, because they encompass a variety of tissues and reflect both morphological and physiological sexual dimorphism, thus permitting a holistic appraisal of the pleiotropic effects of T on a variety of target tissues.

We first characterized the natural development of sexual dimorphism in a colony of brown anoles raised from hatching to adulthood in a laboratory common garden. Next, we experimentally elevated plasma T levels in males and females at approximately the age when sexual dimorphism first began to develop (5–8 months post-hatching). We used this experiment to test (i) whether multiple, sexually dimorphic traits are pleiotropically regulated by T; and (ii) whether males and females respond similarly to elevated T levels. We predicted that T would stimulate growth and mass gain and that these effects would be associated with increased RMR, increased bone growth and decreased fat storage and liver mass. We also predicted that T would stimulate enlargement of the dewlap and induce the development of the dark orange dewlap coloration characteristic of adult males from this population. To determine whether sexual dimorphism has evolved in part through the decoupling of these traits from sensitivity to T in females, we directly compared the phenotypic responses of juvenile males and females to the same level of exogenous T. We predicted that, if males and females differ in their capacity to respond to T, this would be evident as a significant sex × treatment interaction for a given trait. Using this experimental framework, we show that T exerts pleiotropic effects on a variety of physiological and morphological traits and that female anoles have retained sensitivity to T for all of these sexually dimorphic traits.

Materials and methods

STUDY ANIMALS

Animals in this study were the progeny of adults that were collected from the island of Great Exuma (23°29′N, 75°45′W) in The Bahamas in January 2012 and bred in the laboratory from April–August 2012. All animals were fed crickets (*Acheta domesticus* 'pinheads' fed daily to hatchlings, *Gryllus assimilis* fed three times per week to juveniles and adults) supplemented with Fluker's Calcium with Vitamin D_3® and Fluker's Reptile Vitamin with Beta Carotene® (Fluker's, Port Allen, LA, USA). We provided drinking water twice daily by spraying the sides of each cage with deionized water. We housed animals individually in plastic cages (29 × 19 × 18 cm, Lee's Kritter Keeper, Lee's Aquarium and Pet Products, San Marcos, CA, USA) maintained at 29 °C and 65% relative humidity. Each cage included a perch (PVC pipe), a hammock (fibreglass screen), carpet substrate and a potted plant. Each cage was situated beneath two ReptiSun 10·0 UVB bulbs (ZooMed, San Luis Obispo, CA, USA) and maintained on a 12L : 12D photoperiod.

DEVELOPMENT OF SEXUAL DIMORPHISM

As context for our experiment, we characterized the natural development of sexual dimorphism in body size, dewlap size and dewlap colour by measuring independent subsets of 10 males and 10 females at each of five age points: 3, 6, 9, 12 and 20 months of age (100 total individuals). We measured snout-vent length (SVL) to the nearest 1 mm with a ruler and body mass to the nearest 0·01 g with an electronic balance (ScoutPro SP402, Ohaus Corporation, Pine Brook, NJ, USA). We measured dewlap size by grasping the hyoid cartilage with forceps, manually extending the dewlap over graph paper, and tracing it with a pencil. The tracing was digitally scanned to measure dewlap area using the 'freehand line tool' to measure area in IMAGEJ (Schneider, Rasbabd & Eliceiri 2012). We assessed colour of the dewlap by comparing extended dewlaps to Munsell Soil Color Charts (Munsell Color X-Rite, Grand Rapids, MI, USA). A single investigator (CLC) performed this step. We then recorded saturation ('Chroma') and brightness ('Value') based upon the assigned colour chip for each individual. We used separate *t*-test for each age to test whether each trait differed between the sexes (Table S1, Supporting Information). We performed all statistical analyses in JMP v. 9.0.1 (SAS Institute, Cary, NC, USA).

TESTOSTERONE MANIPULATIONS

We manipulated testosterone (T) in lizards that ranged from 5 to 8 months of age (median 6·5 months post-hatching), at which point sexual dimorphism was just starting to develop for most traits (Fig. 1). We assigned size-matched individuals of each sex to either a control treatment (blank implant) or a T treatment (T implant; $n = 12$ per sex, per treatment). We made implants by cutting Silastic tubing (Dow Corning, Midland, MI, USA; 1·47 mm i.d., 1·96 mm o.d.) into 4-mm segments and then sealing one end of each implant with silicone adhesive. We then filled each piece of tubing with either 1 μL dimethyl sulphoxide (DMSO, control treatment) or 100 μg testosterone (T-1500, Sigma-Aldrich Inc., St. Louis, MO, USA) dissolved in 1 μL DMSO. We sealed the open end of each implant with silicone adhesive and allowed it to cure for 2 days. At this point, the DMSO had diffused out of the Silastic tubing, leaving either empty implants or implants filled with 100 μg crystalline T. Prior to surgery, lizards were anaesthetized with a 2-μL intraperitoneal injection of bupivacaine (anaesthetic and analgesic, 2·5 mg mL^{-1}), held at −20 °C for 5 min, and then immobilized on a slightly thawed chemical ice pack. We inserted the implant into the coelomic cavity via a 3-mm ventral incision that was then closed with cyanoacrylate surgical adhesive (VetClose®, Butler Schein Animal Health, Dublin, OH, USA).

GROWTH

We measured SVL and mass of each animal immediately prior to surgical treatment and again at 2 months following surgery. We calculated growth rate as the difference between initial size and final size, divided by the time (days) elapsed between measurements. We tested for effects of sex, T treatment and their interaction on growth rate using linear models with pre-treatment size (SVL or body mass) as a covariate, to account for the negative relationship between size and growth that characterizes lizards (Cox & John-Alder 2005; Cox, Skelly & John-Alder 2005). Because we detected a significant interaction between initial SVL and T treatment, we retained this interaction in our final statistical model. No other two- or three-way interactions with initial SVL or mass were detected or retained in our final models.

DEWLAP SIZE AND COLOUR

At 2 months post-treatment, we measured the size, saturation and brightness of the dewlap using the methods described above. We tested for effects of sex, T treatment and their interaction on $\log_{10}$ dewlap area using linear models with $\log_{10}$ SVL as a covariate. We tested for effects of sex, T treatment and their interaction on dewlap saturation and brightness by treating these as ordinal traits using logistic regression.

SKELETAL MORPHOLOGY

We obtained a radiograph of each lizard immediately prior to surgery and 2 months following surgery. We first cooled lizards for 10 min at 4 °C in small, ventilated plastic bags and then arranged 9–12 lizards in standardized postures (Fig. S1) on an 8·5 × 10 inch Kodak Biomax XAR film (Eastman Kodak, Rochester, NY, USA) that was exposed for 12 sec at 20 kV in an MX-20 X-Ray Specimen Radiography System (Faxitron Bioptics, Tucson, AZ, USA). We developed the film in a Konica Minolta Medical Film Processor SRX-101A (Konica Minolta, Tokyo, Japan) and scanned it for digital measurement. We used IMAGEJ (Schneider,

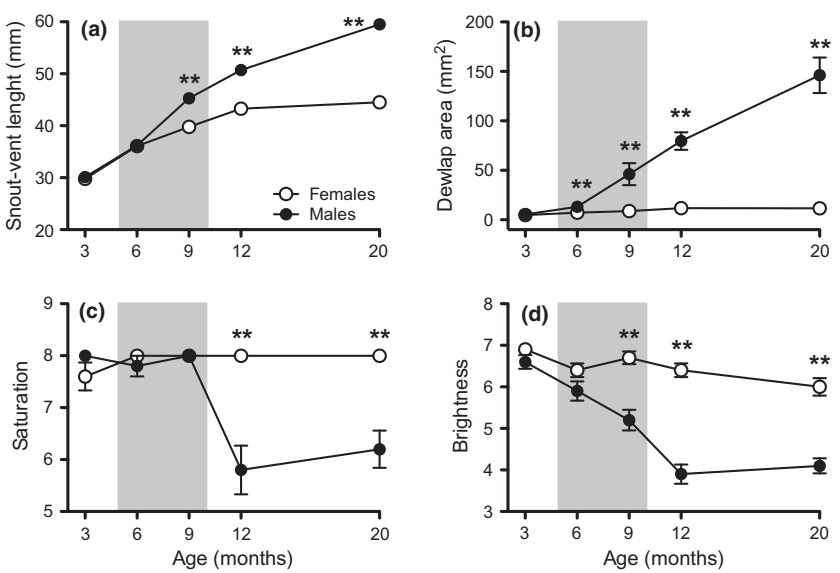

Fig. 1. Development of sexual dimorphism in (a) snout-vent length, (b) dewlap area, (c) dewlap saturation and (d) dewlap brightness for unmanipulated males and females from the source colony for the T-manipulation experiment. Shaded boxes indicate the timing of the 2-month T-manipulation experiment, ranging from the youngest age at which the experiment was initiated (5 months) to the oldest age at which the experiment concluded (10 months). Statistical differences between the sexes at each age are indicated with asterisks (**$P < 0·001$).

Rasbabd & Eliceiri 2012) to measure the linear dimensions of nine skeletal traits: head width, jaw length, pectoral width, pelvic width, humerus length, radius length, ulna length, femur length and tibia length (Fig. S1). We calculated growth as the difference between pre- and post-treatment measurements of each trait. We tested for effects of sex, T treatment and their interaction on growth in each trait using ANCOVA with the pre-treatment value of each trait as a covariate. In several instances, we detected significant interactions between initial trait values and sex or treatment, in which case we retained these interactions in our final statistical models.

RESTING METABOLIC RATE

We measured RMR at 1·5 months following surgery. RMR approximates the energetic cost of tissue maintenance and metabolism at a given temperature, without the energy expenditure associated with digestion or activity, and was measured for fasted animals at rest between 09.00 and 18.00 h. We performed stop-flow respirometry (Lighton 2008) with a Field Metabolic System (FMS, Sable Systems, Las Vegas, NV, USA), which measures water vapour pressure, O_2 concentration and CO_2 concentration. We calibrated the water vapour sensor using zero-humidity gas (nitrogen) and ambient air that was saturated with water vapour (Lighton 2008). We calibrated the O_2 sensor using the fixed-span mode with ambient air flowed over a Drierite column (Lighton 2008). We calibrated the CO_2 sensor by zeroing it with nitrogen and then spanning with custom span gas (0·05% CO_2, 99·5% nitrogen, product number NI CD5000C-Q from GTS-WELCO, Morrisville, PA, USA).

Lizards were fasted for 2 days, weighed and placed in one of eight 300-mL Plexiglass respirometry chambers (G115, Qubit Systems) at 09.00 h, shortly after the onset of the 12L : 12D photoperiod at 08.00 h. Chambers were held within a precisely regulated temperature cabinet set at 30 ± 0·2 °C (PTC-1 cabinet and PELT-5 temperature controller, Sable Systems) and plumbed through a multiplexer calibrated for stop-flow operation (RM-8, Sable Systems). After an initial acclimatization period of 1 h, chambers were closed for 50 min, during which time lizards depleted O_2 and produced CO_2. Air was then pushed through the chamber at 1000 mL min^{-1} using a Mass Flow System sensor and pump (MFS, Sable Systems). This air was then pulled through the Field Metabolic System at 200 mL min^{-1}, at which point water vapour pressure, CO_2 concentration and O_2 concentration were measured. Each chamber was then closed for another 50-min interval, and repeatedly sampled this way every hour for 7 h, so that each animal yielded seven sequential estimates of O_2 consumption between 10.00 and 18.00 h. We automated data collection using EXPEDATA software (v. 1.6.4Sable Systems) and calculated O_2 consumption (VO_2) after correcting for water vapour pressure, CO_2 concentration and the amount of time that the chamber was closed (Lighton 2008). We then selected the minimum VO_2 out of these seven values as the best estimate of RMR for each animal. We tested for effects of sex, treatment and their interaction on $\log_{10}$ VO_2 using ANCOVA with $\log_{10}$ body mass as a covariate.

FAT STORAGE AND ORGAN MASSES

At 2 months post-treatment, we euthanized animals via decapitation and collected a blood sample from each individual into a heparinized capillary tube for testosterone assay. We then dissected each individual and measured the wet mass of the abdominal fat bodies and the liver, because these organs are sites of stored energy that can be mobilized by T to facilitate anabolic processes. We tested for effects of sex, T treatment and their interaction on the relative mass of each organ using linear models with body mass as a covariate. We also included two- and three-way interactions of sex and treatment with body mass, when significant.

TESTOSTERONE ASSAY

We centrifuged blood samples for 10 min at 10 ***g*** and 4 °C and then stored separated plasma at −20 °C until extraction. We suspended 10 μL plasma per animal in 16 mL ultrapure water and then extracted it through a SepPak C18 solid phase extraction column (3 cc, 500 mg; Waters, Inc., Milford, MA, USA) attached to a 24-port vacuum manifold. We primed columns with two washes with 2 mL HPLC-grade MeOH, followed by two washes with 2 mL distilled water. We eluted total hormone from the column with two 2-mL volumes of HPLC-grade MeOH, which was then evaporated in a water bath at 37 °C under a stream of high-purity nitrogen delivered via an evaporating manifold (Evap-O-Rac, Cole Parmer, USA). We resuspended the resulting hormone residues in 2 mL assay buffer from enzyme immunoassay (EIA) kits (catalogue number 582701, Cayman Chemical, Ann Arbor, MI, USA), which we used to assay T in duplicate on two 96-well plates, including inter- and intra-assay standards. Intra-assay coefficients of variation were 0·4–5·7%, and the inter-assay coefficient of variation was 4·9%. We validated EIA kits by assessing parallelism of a serial dilution of *A. sagrei* pooled plasma with the standard curve ($t_8 = 1{\cdot}382$; $P = 0{\cdot}2403$) using a comparison of slopes test (Zar 1996). We tested for effects of sex, T treatment and their interaction on plasma T levels using ANOVA.

Results

DEVELOPMENT OF SEXUAL DIMORPHISM

At 3 months of age, males and females were statistically indistinguishable in body size, dewlap size and dewlap colour (Fig. 1; Table S1). At 6 months of age, males and females were still statistically indistinguishable in body size and dewlap colour, although males had slightly larger dewlaps than females (Fig. 1b). By 9 months of age, males were larger in SVL and mass and had larger and less bright dewlaps than females (Fig. 1). By 12 months of age, sex differences in saturation of the dewlap were also detected (Fig. 1d). Sexual dimorphism in body size and dewlap size continued to increase through 20 months of age, but sexual dimorphism in dewlap colour remained fairly constant after 12 months of age (Fig. 1).

VALIDATION OF TESTOSTERONE MANIPULATION

At 2 months post-treatment, plasma T levels were significantly elevated in T-implanted males (mean ± SEM = 2·98 ± 1·49 ng mL^{-1}) and females (1·84 ± 1·13 ng mL^{-1}) compared with lizards with a blank implant (treatment: $F_{1,42} = 44{\cdot}57$; $P < 0{\cdot}001$). Plasma T levels were higher in males (0·576 ± 0·41 ng mL^{-1}) than in females (0·366 ± 0·42 ng mL^{-1}) with a blank implant (sex: $F_{1,42} = 5{\cdot}43$; $P = 0{\cdot}025$, sex × treatment: $F_{1,42} = 2{\cdot}58$; $P = 0{\cdot}12$). Plasma T levels ranged from 1 to 6 ng mL^{-1} in T-implanted animals, well within natural variation for adult *A. sagrei* males, which have mean values ranging from 3 to 13 ng mL^{-1} (Cox *et al.* 2009; Husak & Lovern

2014) and can peak at upwards of 30 ng mL^{-1} in the breeding season (Tokarz *et al.* 1998).

TESTOSTERONE AND GROWTH

Males and females differed in growth in SVL (sex: $F_{1,39} = 10{\cdot}63$; $P = 0{\cdot}002$; SVL covariate: $F_{1,39} = 10{\cdot}25$; $P = 0{\cdot}003$), and T stimulated growth to a similar degree in both sexes (treatment: $F_{1,39} = 32{\cdot}05$; $P < 0{\cdot}001$; sex × treatment: $F_{1,39} = 2{\cdot}32$; $P = 0{\cdot}136$). Whereas control males grew more than four times faster than control females, T largely abolished this natural sex difference by stimulating growth in both sexes (Fig. 2a). Testosterone also stimulated growth in body mass (treatment: $F_{1,41} = 10{\cdot}00$; $P = 0{\cdot}003$), an effect that was more pronounced in females than in males (sex × treatment: $F_{1,41} = 12{\cdot}78$; $P < 0{\cdot}001$). Males exceeded females in the growth of most skeletal traits, and T stimulated the growth of various long bones (e.g. humerus, ulna, tibia) to a similar degree in either sex (Table 1). Testosterone did not impact head width or jaw length, despite the fact that these traits are sexually dimorphic (Table 1).

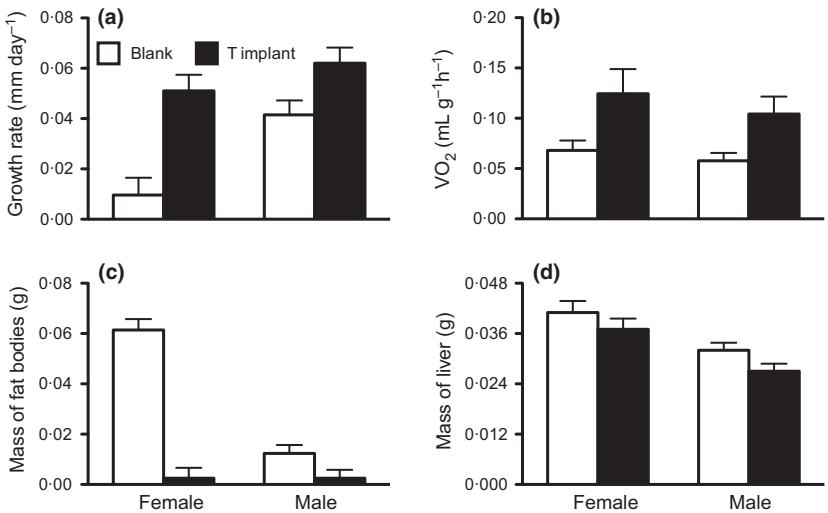

Fig. 2. Testosterone (a) stimulated growth in snout-vent length (SVL), (b) increased resting metabolic rate (VO_2), (c) decreased the wet mass of visceral fat bodies and (d) decreased the wet mass of the liver for juvenile males and females. Data in (a) are least squares means (±1 SE) from a general linear model with sex and testosterone treatment as interacting main effects and initial SVL as a covariate, including interactions of SVL with sex and testosterone treatment. For clarity, data in (b) are mass-specific means (±1 SE), although statistical analysis was conducted with log-transformed whole-animal VO_2 with interacting sex and treatment main effects and a log-transformed mass covariate. Data in (c) are least squares means (±1 SE) from a general linear model with sex and testosterone treatment as interacting main effects and mass as a covariate, including a sex × mass interaction. Data in (d) are least squares means (±1 SE) from a general linear model with sex and testosterone treatment as interacting main effects and including a body mass and all possible 2- and 3-way interactions.

Table 1. Effects of sex and testosterone treatment on the growth of nine skeletal traits (illustrated in Fig. S1). Initial values for each trait were included as covariates

		Sex		Treatment		Sex × Treatment		Initial trait size	
Skeletal trait	d.f. (error)	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Jaw length*,†	39	**14·27**	**< 0·001**	1·04	0·315	0·27	0·607	**36·72**	**< 0·001**
Head width†,‡	41	**21·74**	**< 0·001**	2·78	0·104	1·57	0·218	**58·77**	**< 0·001**
Pectoral width	28	**11·75**	**0·002**	1·68	0·206	0·07	0·795	**21·94**	**< 0·001**
Pelvic width	41	**6·29**	**0·016**	**7·89**	**0·008**	**10·87**	**0·002**	**51·70**	**< 0·001**
Humerus length	40	**17·87**	**< 0·001**	**11·13**	**0·002**	0·78	0·384	**51·20**	**< 0·001**
Radius length	34	2·53	0·121	3·09	0·088	1·47	0·233	**18·38**	**< 0·001**
Ulna length	34	**8·54**	**0·006**	**8·08**	**0·008**	0·89	0·354	**36·22**	**< 0·001**
Femur length‡	41	**46·61**	**< 0·001**	3·25	0·078	2·57	0·117	**21·32**	**< 0·001**
Tibia length	41	**35·49**	**< 0·001**	**12·12**	**0·001**	1·91	0·174	**20·47**	**< 0·001**

Bold font indicates significance at $P < 0{\cdot}05$ without adjustment for multiple comparisons. Differences in error degrees of freedom occur because, for some individuals, accurate pre- and post-treatment measurements could not be obtained for all traits.
*Significant interaction of Sex × Size included.
†Significant interaction of Treatment × Size included.
‡Significant interaction of Sex × Treatment × Size included (with two-way interactions).

TESTOSTERONE AND ENERGETICS

Males and females did not differ in RMR (sex: $F_{1,41} = 0{\cdot}38$; $P = 0{\cdot}54$), but T increased RMR to a similar extent in both sexes (treatment: $F_{1,41} = 11{\cdot}13$; $P = 0{\cdot}002$; sex × treatment: $F_{1,41} = 0{\cdot}31$; $P = 0{\cdot}58$; Fig. 2b). Females had substantially larger fat bodies than males (sex: $F_{1,40} = 50{\cdot}42$ $P < 0{\cdot}001$), and T dramatically reduced the mass of the fat bodies in both sexes (treatment $F_{1,40} = 66{\cdot}29$; $P < 0{\cdot}001$; body mass covariate: $F_{1,40} = 22{\cdot}03$; $P < 0{\cdot}001$; Fig. 2c). Due to the extreme sex difference in the mass of fat bodies in control animals, T reduced fat stores to a greater degree in females than in males (sex × treatment: $F_{1,40} = 33{\cdot}82$; $P < 0{\cdot}001$). For a given body mass, females also had relatively larger livers than males (sex: $F_{1,38} = 16{\cdot}47$; $P < 0{\cdot}001$; body mass covariate: $F_{1,38} = 25{\cdot}98$; $P < 0{\cdot}001$). Testosterone reduced liver mass similarly in both sexes (treatment: $F_{1,38} = 4{\cdot}64$; $P = 0{\cdot}038$; sex × treatment: $F_{1,38} = 0{\cdot}01$; $P = 0{\cdot}941$; Fig. 2d).

TESTOSTERONE AND DEVELOPMENT OF THE DEWLAP

Testosterone roughly doubled the size of the dewlap in each sex (treatment: $F_{1,41} = 68{\cdot}16$; $P < 0{\cdot}001$; sex × treatment: $F_{1,41} = 0{\cdot}17$; $P = 0{\cdot}68$; Fig. 3a), although dewlaps of males were always larger than those of females for any given body size (sex: $F_{1,41} = 11{\cdot}13$; $P = 0{\cdot}002$; SVL covariate: $F_{1,41} = 17{\cdot}49$; $P < 0{\cdot}001$; Fig. 3a). The colour of the dewlap was also impacted by T to a similar extent in each sex, decreasing in both saturation (treatment: $\chi^2 = 37{\cdot}39$; $P < 0{\cdot}001$; sex × treatment: $\chi^2 = 3{\cdot}71$; $P = 0{\cdot}054$) and brightness (treatment: $\chi^2 = 13{\cdot}27$; $P < 0{\cdot}001$; sex × treatment: $\chi^2 = 1{\cdot}58$; $P = 0{\cdot}21$; Fig. 3b). Males and females did not differ in dewlap saturation (sex: $\chi^2 = 37{\cdot}39$; $P < 0{\cdot}001$) or dewlap brightness (sex: $\chi^2 = 3{\cdot}71$; $P = 0{\cdot}054$), in part because sexual dimorphism in dewlap coloration is minor before 9 months of age (Fig. 1).

Discussion

Sex steroids facilitate the development and expression of sexual dimorphism in numerous traits across diverse vertebrate taxa (e.g. Owens & Short 1995; Badyaev 2002; Cox, Stenquist & Calsbeek 2009). This necessarily involves the coupling of a sexually dimorphic trait to a steroid that is secreted in sex-specific fashion, but it is generally unknown whether the evolution of sexual dimorphism also typically proceeds by functionally decoupling the same trait and steroid in the opposite sex. In brown anoles, we found that T influences the development of a suite of sexually dimorphic traits, including growth, body size, skeletal development, fat storage, and the size and colour of the dewlap (Fig. 4). Despite pronounced sexual dimorphism, female brown anoles have retained sensitivity to T with respect to each of these traits, exhibiting phenotypic responses of similar or greater magnitude than those observed in males (Fig. 4). This implies that the evolution of androgen-mediated sexual dimorphism in brown anoles has been achieved primarily by coupling trait expression to T in males without decoupling these traits from T in females. Thus, sex differences in T production and secretion are likely of greater significance than sex differences in tissue-specific responsiveness to T, at least for these traits. Additionally, these results illustrate how sex steroids can act as pleiotropic regulators of sexual dimorphism in diverse traits, ranging from body size and metabolic rate to dewlap size and colour.

Testosterone has historically been viewed as a growth-promoting hormone, based largely upon studies of model species in agriculture, aquaculture and medical research, most of which exhibit male-biased sexual size dimorphism (Wehrenberg & Giustina 1992; Gatford *et al.* 1998; Holloway & Leatherland 1998; Cox & John-Alder 2005). In squamate reptiles (lizards and snakes), T can either stimulate or inhibit growth, with T stimulating male growth in species with male-biased sexual size dimorphism and inhibiting male growth in species with female-biased sexual size dimorphism (Lerner & Mason 2001; Cox & John-Alder 2005; Cox, Skelly & John-Alder 2005; Cox, Stenquist & Calsbeek 2009). Consistent with this framework and with previous work on adult *A. sagrei* males (Cox, Stenquist & Calsbeek 2009), we found that T stimulated growth in length and mass in a species with extreme male-biased

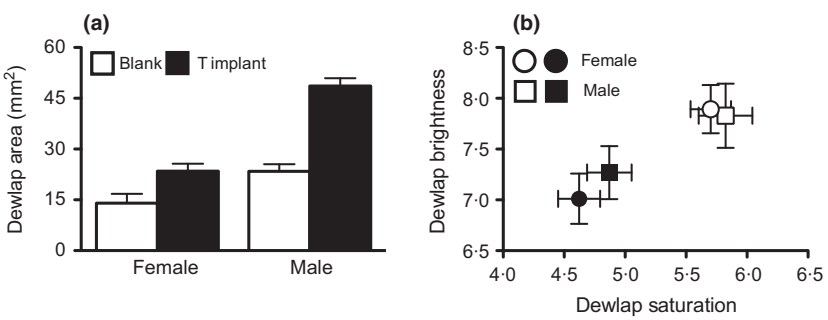

Fig. 3. Testosterone (a) increased dewlap area and (b) decreased the saturation and brightness of the dewlap in juvenile males and females. Data in (a) are least squares means (±1 SE) from an ANCOVA model with sex and testosterone treatment as main effects with interaction and snout-vent length (SVL) as a covariate. Data in (b) are means (±1 SE). Open symbols in (b) correspond to the control groups and filled symbols correspond to the T-implanted groups.

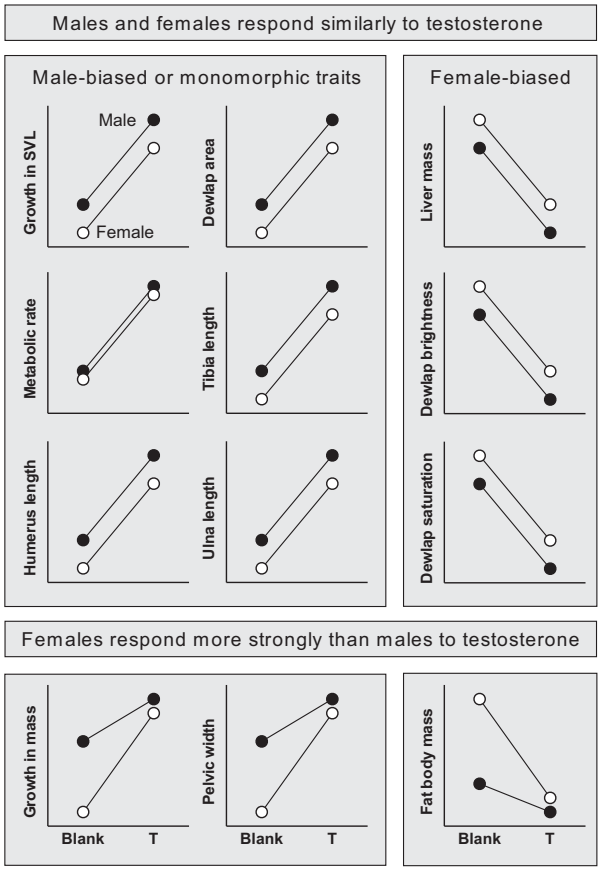

Fig. 4. Graphical summary of the relative direction and magnitude of responses to T by juvenile males (black symbols) and females (white symbols). Females exhibited responses to T that were similar to those of males (top panels, no significant sex-by-treatment interaction) or stronger than those of males (bottom panels, $P < 0{\cdot}05$ for sex-by-treatment interaction). Testosterone stimulated the expression of male-biased traits (traits for which males had larger values, left panels) and one monomorphic trait (metabolic rate, no overall sex effect) and decreased the expression of female-biased traits (traits for which females had larger values, right panels). All slopes and trait values are abstracted for simplicity and illustrative clarity.

sexual size dimorphism. Our results extend those of previous studies in that we demonstrate stimulatory effects of T on growth at the precise ontogenetic stages when sexual size dimorphism naturally begins to develop. We also show that females possess the physiological and genetic potential to grow at rates comparable to males when exposed to elevated plasma T levels at this ontogenetic stage.

Our results also clarify the physiological and energetic mechanisms underlying both natural sex differences in growth and T-mediated increases in growth. First, we found that whole-animal patterns of growth in SVL and mass were reflected in growth of individual skeletal elements, particularly long bones in the limbs (Table 1). This confirms that T stimulates skeletal growth in addition to mass gain and overall body elongation. However, we did not detect an effect of T on the development of sexually dimorphic skull shape (Table 1). This is consistent with recent evidence suggesting that oestrogens may play a larger regulatory role than androgens in sexual dimorphism of skull shape in other *Anolis* species and that their effects may occur at developmental stages earlier than those investigated in our experiments (Sanger *et al.* 2014). Secondly, we found that stimulation of growth by T was accompanied by an increase in RMR. Comparable stimulatory effects of T on standard or RMR have been observed in other lizards and some birds (Gupta & Thapliyal 1985; Buchanan *et al.* 2001; Tobler, Nilsson & Nilsson 2007), although other studies report no effect of T (Marler *et al.* 1995; Buttemer & Astheimer 2000) or even suppression of metabolic rate following T administration (Wikelski *et al.* 1999). Finally, we found that stimulation of growth by T was accompanied by a decrease in the mass of fat bodies and liver, two tissues associated with energy storage. This implies that T stimulates growth by shifting energy from fat storage and towards growth, as observed in many species. Moreover, intact males and females differed naturally in fat storage, suggesting that sexual size dimorphism is related in part to a difference in energy allocation to storage vs. growth that is mediated by T and evident prior to sexual maturation.

Our results provide an interesting comparison to the large body of research exploring the effects of sex steroids on behaviour and development of the nervous system. Both androgens and oestrogens are generally important for neural development and behaviour, and for the establishment of sex differences therein (Schlinger 1997; Holloway & Clayton 2001; Trainor & Marler 2002; Wade 2005). Whereas exogenous androgens can shift females towards male-like patterns of behaviour and development, much of this potential for responsiveness is canalized into sex-specific trajectories early in ontogeny due to the organizational effects of sex steroids (Breedlove & Arnold 1983; Konishi & Akutagawa 1988; Winkler & Wade 1998; Beck & Wade 2009; Remage-Healey *et al.* 2010). Our results suggest that the potential for sex-specific development in *A. sagrei* is not rigidly canalized early in ontogeny, given that juvenile females retain responsiveness to testosterone well into the natural developmental period of sexual divergence for a variety of morphological and physiological traits.

The dewlap is an important social and sexual signal in anoles and can be used to deter predators, to signal aggression to conspecifics, to facilitate species recognition, and to court and attract mates (Jenssen 1977; Williams & Rand 1977; Leal & Rodriguez-Robles 1997; Nicholson, Harmon & Losos 2007). Despite the importance of the dewlap in social and sexual signalling and the prevalence of anoles as ecological model systems (Losos 2009), the effects of T on dewlap morphology have only been characterized for adult males of two species (*Anolis carolinensis*: Lovern *et al.* 2004; Husak *et al.* 2007; *A. sagrei*: Cox *et al.* 2009). We found that T enlarged the dewlap and induced male-typical dewlap coloration in juvenile males and females, although females never developed dewlaps that were comparable in

size to those of males. In analogous fashion, T stimulates the development of large and colourful throat and belly patches that serve as social and sexual signals in other lizards (Hews & Moore 1996; Salvador *et al*. 1996; Cox *et al*. 2005; Cox, Zilberman & John-Alder 2008). As in brown anoles, treatment of these species with exogenous T is often sufficient to induce male-typical ventral coloration in juveniles of either sex (Cox *et al*. 2005; Cox, Zilberman & John-Alder 2008). Beyond squamate reptiles, testosterone stimulates the development of male sexual signals in many vertebrates (Fernald 1976; Richards 1982; Lank, Coupe & Wynne-Edwards 1999; Gonzalez *et al*. 2001; Mougeot *et al*. 2004), though in some cases, elaborate male coloration or plumage develops as the default phenotype unless suppressed by oestrogens (Owens & Short 1995).

Our results clearly indicate that females have retained responsiveness to T for a variety of sexually dimorphic traits, though we cannot exclude the possibility that these responses are mediated by oestrogen-receptor signalling following conversion of T to estradiol (the same caveat applies to effects of exogenous T on males). Nonetheless, the overall similarity in responsiveness of males and females argues that common pathways are likely involved in most cases, whether these involve androgen- or oestrogen-receptor signalling by T or its downstream metabolites, which include both androgens and oestrogens. We chose to manipulate T because it is the primary circulating androgen for anoles and other squamates (Crews *et al*. 1978; Hews & Klime 1978), though future work could explicitly test the involvement of androgen receptors using 5α-dihydrotestosterone (DHT), which cannot be converted to estradiol. Likewise, the complementary role of oestrogens in mediating sexual dimorphism remains an important, though understudied, topic for future research (Starostová *et al*. 2013; Sanger *et al*. 2014).

In the light of a growing focus on the role of androgens in modulating diverse traits in females (Schwabl 1996; Whittingham & Schwabl 2002; Jawor 2007), recent work has explored the response of females to T as a potential constraint on the evolution of T-mediated traits in males (Clotfelter *et al*. 2004; Zysling *et al*. 2006; O'Neal *et al*. 2008). Given that female anoles retain responsiveness to T for most traits, including those that are subject to sexually antagonistic selection (e.g. body size, Cox & Calsbeek 2010), evolutionary changes in circulating T levels could have antagonistic fitness effects for males and females. Though relatively little is known about the extent to which T production and secretion are genetically correlated between males and females of any species, circulating androgen levels themselves are heritable (Lubritz, Johnson & Robinson 1991), and recent studies of bank voles indicate that males with high T levels have sisters with low fitness (Mills *et al*. 2009; Mokkonen *et al*. 2011, 2012). Our results suggest an underlying mechanism that could generate similar genetic variation in fitness and are therefore broadly consistent with the emerging view that sexual conflict may often persist even for traits that are regulated by mechanisms, such as sex steroids, that facilitate a high degree of sex-specific expression (Cox 2014).

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Data accessibility

Data on growth, metabolism and organ size for this study are deposited in the Dryad Digital Repository (doi: 10.5061/dryad.5r7p8) (Cox *et al*. 2014).

References

Badyaev, A.V. (2002) Growing apart: an ontogenetic perspective on the evolution of sexual size dimorphism. *Trends in Ecology and Evolution*, **17**, 369–378.

Beck, L.A. & Wade, J. (2009) Morphology and estrogen receptor alpha mRNA expression in the developing green anole forebrain. *Journal of Experimental Zoology*, **311A**, 162–171.

Breedlove, S.M. & Arnold, A.P. (1983) Hormonal control of a developing neuromuscular system. II. Sensitive periods for the androgen induced masculinization of the rat spinal nucleus of the bulbocavernosus. *Journal of Neuroscience*, **3**, 424–432.

Buchanan, K.L., Evans, M.R., Goldsmith, A.R., Bryant, D.M. & Rowe, L.V. (2001) Testosterone influences basal metabolic rate in male house sparrows: a new cost of dominance signalling? *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **268**, 1337–1344.

Buttemer, W.A. & Astheimer, L.B. (2000) Testosterone does not affect basal metabolic rate or blood parasite load in captive male White-plumed Honeyeaters *Lichenostomus penicillatus*. *Journal of Avian Biology*, **31**, 479–488.

Chenoweth, S.F., Rundle, H.D. & Blows, M.W. (2008) Genetic constraints and the evolution of display trait sexual dimorphism by natural and sexual selection. *The American Naturalist*, **171**, 22–34.

Clotfelter, E.D., O'Neal, D.M., Gaudioso, J.M., Casto, J.M., Parker-Renga, I.M., Snajdr, E.A. *et al*. (2004) Consequences of elevating plasma testosterone in females of a socially monogamous songbird: evidence of constraints on male evolution? *Hormones and Behavior*, **46**, 171–178.

Cox, R. (2014) Integrating costs of reproduction between the sexes. *Integrative Organismal Biology* (eds L.B. Martin, C.K. Ghalambor & H.A. Woods), pp. 153–168. John Wiley and Sons, Hoboken, NJ.

Cox, R.M. & Calsbeek, R. (2009) Sexually antagonistic selection, sexual dimorphism, and the resolution of intralocus sexual conflict. *The American Naturalist*, **173**, 176–187.

Cox, R.M. & Calsbeek, R. (2010) Sex-specific selection and intraspecific variation in sexual size dimorphism. *Evolution*, **64**, 798–809.

Cox, R.M. & John-Alder, H.B. (2005) Testosterone has opposite effects on male growth in lizards (*Sceloporus* spp.) with opposite patterns of sexual size dimorphism. *Journal of Experimental Biology*, **208**, 4679–4687.

Cox, R.M., Skelly, S.L. & John-Alder, H.B. (2003) A comparative test of adaptive hypotheses for sexual size dimorphism in lizards. *Evolution*, **57**, 1653–1669.

Cox, R.M., Skelly, S.L. & John-Alder, H.B. (2005) Testosterone inhibits growth in juvenile male eastern fence lizards (*Sceloporus undulatus*): implications for energy allocation and sexual size dimorphism. *Physiological and Biochemical Zoology*, **78**, 531–545.

Cox, R.M., Stenquist, D.S. & Calsbeek, R. (2009) Testosterone, growth and the evolution of sexual size dimorphism. *Journal of Evolutionary Biology*, **22**, 1585–1598.

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*. *Journal of Experimental Zoology*, **309**, 505–514.

Cox, R.M., Skelly, S.L., Leo, A. & John-Alder, H.B. (2005) Testosterone regulates sexually dimorphic coloration in the eastern fence lizard, *Sceloporus undulatus*. *Copeia*, **2005**, 597–608.

Cox, R.M., Stenquist, D.S., Henningsen, J.P. & Calsbeek, R. (2009) Manipulating testosterone to assess links between behavior, morphology, and performance in the brown anole *Anolis sagrei*. *Physiological and Biochemical Zoology*, **82**, 686–698.

Cox, C.L., Hanninen, A.F., Reedy, A.M. & Cox, R.M. (2014) Data from: female anoles retain responsiveness to testosterone despite the evolution of androgen-mediated sexual dimorphism. *Dryad Digital Repository*, doi:10.5061/dryad.5r7p8.

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

De Ridder, E., Pinxten, R., Mees, V. & Eens, M. (2002) Short- and long-term effects of male-like concentrations of testosterone on female european starlings (*Sturnus vulgaris*). *The Auk*, **119**, 487–497.

DeBold, J.F. & Miczek, K.A. (1981) Sexual dimorphism in the hormonal control of aggressive behavior of rats. *Pharmacology Biochemistry and Behavior*, **14**, 89–93.

Dulka, J.G. & Maler, L. (1994) Testosterone modulates female chirping behavior in the weakly electric fish, *Apteronotus leptorhynchus*. *Journal of Comparative Physiology A*, **174**, 331–343.

Fairbairn, D.J. (1997) Allometry for sexual size dimorphism: pattern and process in the coevolution of body size in males and females. *Annual Review of Ecology and Systematics*, **28**, 659–687.

Fernald, R.D. (1976) The effect of testosterone on the behavior and coloration of adult male cichlid fish (*Haplochromis burtoni*, Gunther). *Hormone Research*, **7**, 172–178.

Gatford, K.L., Egan, A.R., Clarke, I.J. & Owens, P.C. (1998) Sexual dimorphism of the somatotrophic axis. *Journal of Endocrinology*, **157**, 373–389.

Gonzalez, G., Sorci, G., Smith, L.C. & de Lope, F. (2001) Testosterone and sexual signalling in male house sparrows (*Passer domesticus*). *Behavioral Ecology and Sociobiology*, **50**, 557–562.

Gupta, B.B.P. & Thapliyal, J.P. (1985) Role of thyroid and testicular hormones in oxidative metabolism of the Indian garden lizard, *Calotes versicolor*. *General and Comparative Endocrinology*, **58**, 20–27.

Harrison, A. & Poe, S. (2012) Evolution of an ornament, the dewlap, in females of the lizard genus *Anolis*. *Biological Journal of the Linnean Society*, **106**, 191–201.

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

Hews, E.A. & Klime, D.E. (1978) Testicular steroid synthesis by the green lizard *Lacerta viridis*. *General and Comparative Endocrinology*, **35**, 432–435.

Hews, D.K. & Moore, M.C. (1996) A critical period for the organization of alternative male phenotypes of the tree lizards by exogenous testosterone? *Physiology and Behavior*, **60**, 425–429.

Hews, D.K. & Quinn, V.S. (2003) Endocrinology of species differences in sexually dimorphic signals and aggression: using the organization and activation model in a phylogenetic framework. *Lizard Social Behavior* (eds S.F. Fox, T.A. Baird & J.C. McCoy), pp. 253–277. Johns Hopkins University Press, Baltimore, Maryland.

Holloway, C.C. & Clayton, D.F. (2001) Estrogen synthesis in the male brain triggers the development of the avian song control pathway *in vitro*. *Nature Neuroscience*, **4**, 170–175.

Holloway, A.C. & Leatherland, J.F. (1998) Neuroendocrine regulation of growth hormone secretion in teleost fishes with emphasis on the involvement of gonadal sex steroids. *Reviews in Fish Biology and Fisheries*, **8**, 409–429.

Husak, J.F. & Lovern, M.B. (2014) Variation in steroid hormone levels among Caribbean *Anolis* lizards: endocrine system convergence? *Hormones and Behavior*, **65**, 408–415.

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

Jawor, J.M. (2007) Testosterone response to GnRH in a female songbird varies with stage of reproduction: implications for adult behaviour and maternal effects. *Functional Ecology*, **21**, 767–775.

Jenssen, T.A. (1977) Evolution of anoline lizard display behavior. *American Zoologist*, **17**, 203–215.

Kelley, D.B. & Pfaff, D.W. (1976) Hormone effects on male sex behavior in adult South African clawed frogs, *Xenopus laevis*. *Hormones and Behavior*, **7**, 159–182.

Ketterson, E.D., Nolan, V.J. & Sandell, M. (2005) Testosterone in females: mediator of adaptive traits, constraint on sexual dimorphism, or both? *The American Naturalist*, **166**, S85–S98.

Konishi, M. & Akutagawa, E. (1988) A critical period for estrogen action on neurons of the song control system in the zebra finch. *Proceeding of the National Academy of Sciences of the United States of America*, **85**, 7006–7007.

Lande, R. (1980) Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution*, **34**, 292–307.

Lank, D.B., Coupe, M. & Wynne-Edwards, K.E. (1999) Testosterone-induced male traits in female ruffs (*Philomachus pugnax*): autosomal inheritance and gender differentiation. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **266**, 2323–2330.

Leal, M. & Rodriguez-Robles, J.A. (1997) Antipredator responses of the Puerto Rican giant anole, *Anolis cuvieri* (Squamata:Polychrotidae). *Biotropica*, **29**, 372–375.

Lerner, D.T. & Mason, R.T. (2001) The influence of sex steroids on the sexual size dimorphism in the red-spotted garter snake, *Thamnophis sirtalis concinnus*. *General and Comparative Endocrinology*, **124**, 218–225.

Lighton, J.R.B. (2008) *Measuring Metabolic Rates*. Oxford University Press, Oxford.

Losos, J.B. (2009) *Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles*. University of California Press, Berkeley, California.

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

Lubritz, D., Johnson, B. & Robinson, O.W. (1991) Genetic parameters for testosterone production in boars. *Journal of Animal Science*, **69**, 3220–3224.

Mank, J.E. (2009) Sex chromosomes and the evolution of sexual dimorphism: lessons from the genome. *The American Naturalist*, **173**, 141–150.

Marler, C.A., Walsberg, G., White, M.L. & Moore, M.C. (1995) Increased energy expenditure due to increased territorial defense in male lizards after phenotypic manipulation. *Behavioral Ecology and Sociobiology*, **37**, 225–231.

McGlothlin, J.W. & Ketterson, E.D. (2008) Hormone-mediated suites as adaptations and evolutionary constraints. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **363**, 1611–1620.

Mills, S.C., Grapputo, A., Jokinen, I., Koskela, E., Mappes, T., Oksanen, T. A. *et al.* (2009) Testosterone-mediated effects on fitness-related phenotypic traits and fitness. *The American Naturalist*, **173**, 475–487.

Mokkonen, M., Kokko, H., Koskela, E., Lehtonen, J., Mappes, T., Martiskainen, H. *et al.* (2011) Negative frequency-dependent selection of sexually antagonistic alleles in *Myodes glareolus*. *Science*, **334**, 972–974.

Mokkonen, M., Koskela, E., Mappes, T. & Mills, S.C. (2012) Sexual antagonism for testosterone maintains multiple mating behaviour. *Journal of Animal Ecology*, **81**, 277–283.

Mougeot, F., Irvine, J.R., Seivwright, L., Redpath, S.M. & Piertney, S. (2004) Testosterone, immunocompetence, and honest sexual signalling in male red grouse. *Behavioral Ecology*, **15**, 930–937.

Murphy, C.A. & Stacey, N.E. (2002) Methyl-testosterone induces male-typical ventilatory behavior in response to putative steroidal pheromones in female round gobies (*Neogobius melanostomus*). *Hormones and Behavior*, **42**, 109–115.

van Nas, A., Thakurta, D.G., Wang, S., Yehya, N., Hovarth, S., Zhang, B. *et al.* (2009) Elucidating the role of gonadal hormones in sexually dimorphic gene coexpression networks. *Endocrinology*, **150**, 1235–1249.

Nicholson, K.E., Harmon, L.J. & Losos, J.B. (2007) Evolution of *Anolis* lizard dewlap diversity. *PLoS ONE*, **2**, e274.

O'Neal, D.M., Reichard, D.G., Pavilis, K. & Ketterson, E.D. (2008) Experimentally-elevated testosterone, female parental care, and reproductive success in a songbird, the Dark-eyed Junco (*Junco hyemalis*). *Hormones and Behavior*, **54**, 571–578.

Owens, I.P.F. & Short, R.V. (1995) Hormonal basis of sexual dimorphism in birds: implications for new theories of sexual selection. *Trends in Ecology and Evolution*, **10**, 44–47.

Peterson, M.P., Rosvall, K.A., Choi, J.-H., Ziegenfus, C., Tang, H., Colbourne, J.K. *et al.* (2013) Testosterone affects neural gene expression

differently in male and female juncos: a role for hormones in mediating sexual dimorphism and conflict. *PLoS ONE*, **8**, e61784.
Peterson, M.P., Rosvall, K.A., Taylor, C.A., Lopez, J.A., Choi, J.-H., Ziegenfus, C. *et al*. (2014) Potential for sexual conflict assessed via testosterone-mediated transcriptional changes in liver and muscle of a songbird. *The Journal of Experimental Biology*, **217**, 507–517.
Remage-Healey, L., Coleman, M.J., Oyama, R.K. & Schlinger, B.A. (2010) Brain estrogens rapidly strengthen auditory encoding and guide song preference in a songbird. *Proceedings of the National Academy of Sciences*, **107**, 3852–3857.
Richards, C.M. (1982) The alteration of chromatophore expression by sex hormones in the Kenyan reed frog, *Hyperolius viridiflavus*. *General and Comparative Endocrinology*, **46**, 59–67.
Salvador, A., Veiga, J.P., Martin, J. & Lopez, P. (1996) Testosterone supplementation in subordinate, small male lizards: consequences for aggressiveness, color development, and parasite load. *Behavioral Ecology*, **8**, 135–139.
Sanger, T.J., Sherrat, E., McGlothlin, J.W., Brodie, E.D. III, Losos, J.B. & Abzhanov, A. (2013) Convergent evolution of sexual dimorphism in skull shape using distinct developmental strategies. *Evolution*, **67**, 2180–2193.
Sanger, T.J., Seav, S.M., Tokita, M., Langerhans, R.B., Ross, L.M., Losos, J.B. *et al*. (2014) The oestrogen pathway underlies the evolution of exaggerated male cranial shapes in *Anolis* lizards. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **281**, 2014039, online early.
Schlinger, B.A. (1997) Sex steroids and their actions on the birdsong system. *Journal of Neurobiology*, **33**, 619–631.
Schneider, C.A., Rasbabd, W.S. & Eliceiri, K.W. (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, **9**, 671–675.
Schwabl, H. (1996) Environment modifies the testosterone levels of a female bird and its eggs. *The Journal of Experimental Zoology*, **276**, 157–163.
Starostová, Z., Kubička, L., Golinski, A. & Kratochvíl, L. (2013) Neither male gonadal androgens nor female reproductive costs drive development of sexual size dimorphism in lizards. *The Journal of Experimental Biology*, **216**, 1872–1880.
Staub, N.L. & De Beer, M. (1997) The role of androgens in female vertebrates. *General and Comparative Endocrinology*, **108**, 1–24.
Tobler, M., Nilsson, J.-Å. & Nilsson, J.F. (2007) Costly steroids: egg testosterone modulates nestling metabolic rate in the zebra finch. *Biology Letters*, **3**, 408–410.
Tokarz, R.R., McMann, S., Seitz, L. & John-Alder, H.B. (1998) Plasma corticosterone and testosterone levels during the annual reproductive cycle of male brown anoles (*Anolis sagrei*). *Physiological Zoology*, **71**, 139–146.
Trainor, B.C. & Marler, C.A. (2002) Testosterone promotes paternal behaviour in a monogamous mammal via conversion to oestrogen. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **269**, 823–829.
Wade, J. (2005) Current research on the behavioral neuroendocrinology of reptiles. *Hormones and Behavior*, **48**, 451–460.
Wehrenberg, W.B. & Giustina, A. (1992) Basic counterpoint: mechanisms and pathways of gonadal steroid modulation of growth hormone secretion. *Endocrine Reviews*, **13**, 299–308.
Whittingham, L.A. & Schwabl, H. (2002) Maternal testosterone in tree swallow eggs varies with female aggression. *Animal Behaviour*, **63**, 63–67.
Wiens, J.J. (1999) Phylogenetic evidence for multiple losses of a sexually selected character in phrynosomatid lizards. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **266**, 1529–1536.
Wikelski, M., Lynn, S., Breuner, J., Wingfield, J. & Kenagy, G. (1999) Energy metabolism, testosterone and corticosterone in white-crowned sparrows. *Journal of Comparative Physiology A*, **185**, 463–470.
Williams, E.E. & Rand, A.S. (1977) Species recognition, dewlap function and faunal size. *American Zoologist*, **17**, 261–270.
Winkler, S.M. & Wade, J. (1998) Aromatase activity and regulation of sexual behaviors in the green anole lizard. *Physiology and Behavior*, **64**, 723–731.
Zar, J.H. (1996) *Biostatistical Analysis*, 3rd edn. Prentice Hall, Upper Saddle River, New Jersey.
Zysling, D.A., Greives, T.J., Breuner, C.W., Casto, J.M., Demas, G.E. & Ketterson, E.D. (2006) Behavioral and physiological responses to experimentally elevated testosterone in female dark-eyed juncos (*Junco hyemalis carolinensis*). *Hormones and Behavior*, **50**, 200–207.

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Supporting Information

Additional Supporting information may be found in the online version of this article:

Fig. S1. Radiograph of a juvenile brown anole, illustrating the landmarks used to measure nine skeletal traits.
Table S1. Statistical summary of the ontogeny of sexual dimorphism in snout-vent length, body mass, dewlap size, dewlap brightness and dewlap saturation.