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Testosterone Stimulates the Expression of a Social Color Signal in Yarrow's Spiny Lizard, *Sceloporus jarrovii*

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ABSTRACT The sex steroid testosterone has been shown to regulate the development of male-specific coloration in many organisms that exhibit sexual dichromatism, but the role of testosterone is less certain for species in which both sexes express bright coloration. Lizards in the genus *Sceloporus* possess bright blue patches on their throats and abdomens. These patches, which are used in social signaling, are often regulated by testosterone and are consequently expressed only in males of most species. However, Yarrow's Spiny Lizard (*Sceloporus jarrovii*, Cope 1875) exhibits a derived condition in which both sexes express bright blue ventral patches despite dramatic sexual differences in circulating testosterone levels throughout postnatal ontogeny. In this study, we used surgical castration and hormone replacement in juvenile males to test the hypothesis that testosterone stimulates the expression of blue ventral coloration in *S. jarrovii*. In two separate experiments conducted in captivity and the natural field environment, we found that surgical castration decreased the hue and saturation while increasing the brightness of blue throat and abdominal patches. Castration also decreased the amount of black pigment bordering the blue throat patch. Treatment of castrated males with exogenous testosterone restored all aspects of ventral coloration to values similar to those of intact control males. Early organizational effects of testosterone during prenatal development may lead to the expression of blue coloration in both sexes, but the results of our present experiments indicate that subsequent effects of testosterone during sexual maturation further enhance the coloration of males. *J. Exp. Zool. 309A:505–514, 2008.*

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Many animals exhibit pronounced sexual dichromatism, a difference in coloration or color pattern between conspecific males and females. Evolutionary biologists have long studied the adaptive significance of sexual dichromatism related to sex differences in foraging ecology and predator avoidance, intrasexual signaling, mate choice, and species recognition (Andersson, '94). As predicted from this evolutionary perspective, sexually antagonistic selection pressures actively favor sexual differences in coloration or color pattern of many species (Price and Burley, '94; Forsman, '95; Forsman and Shine, '95; Forsman and Appelqvist, '99). However, the evolution of sexual dichromatism in response to sexually antagonistic selection depends upon the extent to

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which males and females can achieve sex-specific phenotypic expression of coloration in the face of underlying constraints imposed by their shared genome (Badyaev, 2002; Bedhomme and Chippindale, 2007). This requires some form of genetic (e.g., sex-linked inheritance) or epigenetic mechanism (e.g., sex-limited developmental modifiers) to differentially mediate color expression in each sex (Kimball and Ligon, '99).

Among vertebrates, sex steroids (androgens, estrogens, and progestins) are effective regulators of sexual dichromatism because they are produced and secreted in sex-specific fashion primarily by the gonads. Sex steroids have been shown to influence the development of sex-specific coloration in a variety of vertebrates, including birds (Kimball and Ligon, '99), fishes (Cardwell and Liley, '91), reptiles (Cooper and Greenberg, '92), and amphibians (Hayes and Menendez, '99). When sex-specific coloration is restricted to females, it is often induced by estrogens or progestins (Cooper and Ferguson, '72; Cooper and Clarke, '82; Cooper and Crews, '87), whereas male-specific coloration is often regulated by testicular androgens (Rand, '92; Hews and Moore, '95; Cox et al., 2005b). Moreover, the attendant fitness costs of elevated androgen levels, such as increased parasitism (Cox and John-Alder, 2007), compromised immune function (Oppliger et al., 2004), and reduced resistance to oxidative stress (Alsonso-Alvarez et al., 2007), provide mechanisms that explicitly link sex-specific coloration to androgens as an honest indicator of quality (Folstad and Karter, '92). For these theoretical and empirical reasons, it is widely accepted that sex steroids often underlie the development and expression of sexual dichromatism in vertebrates (Cooper and Greenberg, '92; Kimball and Ligon, '99).

An interesting contrast arises in species where both sexes express bright coloration. Is such sexual monochromatism the result of an evolutionary decoupling of sex steroids from the proximate cellular mechanisms that regulate coloration or the result of shared developmental effects of sex steroids in both sexes? Lizards in the genus *Sceloporus* provide an informative comparative system for such questions because they have a well-characterized phylogeny that indicates multiple evolutionary transitions between two forms of sexual monochromatism (bright blue ventral colors either present or absent in both sexes) and sexual dichromatism (bright blue ventral colors present in males only) (Wiens, '99; Hews and Quinn, 2003). In sexually dichromatic species, the development of blue ventral coloration in males is regulated by testosterone and related androgens, and females can often be induced to express male-specific coloration when treated with exogenous androgens (Kimball and Erpino, '71; Hews and Moore, '95; Quinn and Hews, 2003; Hews and Quinn, 2003; Cox et al., 2005b). By contrast, exogenous testosterone fails to induce the expression of ventral coloration in monochromatic species that have secondarily lost blue patches (Abell, '98; Hews and Quinn, 2003, R. M. Cox, unpublished data). Is the expression of coloration similarly decoupled from testosterone in species where both sexes exhibit blue ventral patches?

In this study, we address this question in *Sceloporus jarrovii*, a species in which both males and females express bright blue coloration in the throat region and in laterally paired abdominal patches (Wiens, '99; Quinn and Hews, 2003). The relationship between testosterone and coloration is uncertain in this species, given that both sexes express bright coloration despite marked sexual differences in circulating plasma testosterone levels throughout postnatal ontogeny (Cox and John-Alder, 2005). Moreover, blue throat coloration is present from birth in both sexes (R. M. Cox, pers. obs.), well in advance of marked increases in plasma testosterone levels that accompany maturation of males (Cox and John-Alder, 2005). These observations suggest that the development of coloration in this species may involve prenatal organizational effects of testosterone that similarly influence both sexes, rather than maturational increases in plasma testosterone that occur later in life and are known to influence the development of sexual dichromatism in other *Sceloporus* species (Cox et al., 2005b).

In this study, we assess the potential role of testosterone in stimulating blue throat coloration by castrating juvenile *S. jarrovii* males just prior to sexual maturation to remove the primary source of endogenous testosterone. We then compare castrated males with both intact control males and castrated males treated with exogenous testosterone to evaluate the effect of circulating androgens on blue ventral coloration in this species. If the evolution of sexual monomorphism in this species reflects either (1) decoupling of coloration from circulating testosterone levels or (2) prevalence of prenatal organizational effects of testosterone rather than later effects during sexual maturation, then we predict that coloration of males should be insensitive to castration and testosterone replacement.

MATERIALS AND METHODS

Animals and experimental design

We collected *S. jarrovii* yearlings (age ca. 3 months) from two locations in the Chiricahua Mountains, Cochise Co., AZ (USA). Animals used in our laboratory experiment were collected near Buena Vista Peak (31°54′ N, 109°16′ W, 2,500 m elevation) and those in our field experiment were studied in the North Fork of Cave Creek Canyon (31°53′ N, 109°13′ W, 1,700 m elevation). For our laboratory experiment, we immediately transported animals to our captive facility at Rutgers University, where they were housed individually in plastic cages ($36 \times 42 \times 46\,cm^3$) containing sand bedding and two bricks that were stacked to form a shelter and basking site. Water was always available in a shallow dish lined with aquarium gravel and each animal was offered three crickets per day for food (essentially ad libitum feeding, see Cox et al. 2006). Crickets were occasionally dusted with Fluker's 2:1 calcium:phosphorus dietary supplement and were found to contain 78% crude protein, 11% crude fat, and 11% ash by dry mass (Dairy One, Inc. Ithica, NY; see Cox et al. 2006). We provided heat by suspending an incandescent spotlight (Philips 65 W BR-40SP, Royal Philips Electronics, Netherlands) above each basking site. Cages were arranged under a bank of fluorescent bulbs (General Electric Chroma 50, General Electric Company, Fairfield, CT) for ultraviolet radiation. Fluorescent lights were set on timers to provide a 12-hr daily photoperiod and spotlights were set to provide a 10-hr basking period. Adjacent cages were separated with opaque barriers to prevent social interactions. For our field study, lizards were brought to the American Museum of Natural History's Southwestern Research Station for surgeries (see below) and then released at their exact site of capture.

Upon capture, we measured snout–vent length (SVL, to the nearest mm using a ruler) and body mass (to the nearest 0.1 g using a Pesola® spring scale, Pesola AG, Baar, Switzerland) of each animal. We used these measurements to assign each male to one of three, size-matched treatment groups: (1) castrated males receiving a testosterone implant (CAST+T), (2) castrated males receiving a placebo implant (CAST), and (3) intact, control males receiving a sham surgery and placebo implant (CON). For our laboratory study, we also included a reference group of females (FEM) that received a sham surgery and placebo implant, thus facilitating direct comparison with CON males.

Surgical treatments

Prior to surgery, we anaesthetized animals with an intramuscular injection of ketamine (Vetus Animal Health, MFA Inc., Columbia, MO; $130\,mg\,kg^{-1}$). We then exposed the gonads via a single ventral incision and bilaterally castrated (orchiectomized) CAST and CAST+T males by ligating their spermatic cords with surgical silk, ablating each testis, and cauterizing each ligated spermatic cord after removal of the testes. For control males (CON) and females (FEM), we performed ''sham'' surgeries in which we made identical incisions to expose and manipulate the gonads while leaving them completely intact. We then inserted either a testosterone implant (CAST +T, see below) or a placebo implant (CAST, CON, FEM) into the coelomic cavity and closed the incision with Nexaband® surgical glue (Veterinary Products Laboratories, Phoenix, AZ). On the day after surgery, we returned animals to their cages (laboratory study) or to their exact location of capture (field study). Animals appeared healthy and vigorous following surgery, and postoperative survival was high in both the laboratory (43 of 47, 93%) and the field (65 of 67, 97%).

Testosterone implants

We constructed tonic-release testosterone implants from 5 mm lengths of Silastic® tubing (Dow Corning, Midland, MI; 1.47 mm i.d., 1.96 mm o.d.). After sealing one end of each tubule with silicone adhesive gel (Dow Corning), we used a Hamilton® syringe (Hamilton Company, Reno, NV) to inject 3 μL of a solution of testosterone (Sigma, Sigma-Aldrich Inc., St. Louis, MO; T-1500) dissolved in dimethyl sulfoxide (DMSO, 100 μg T/μL) into the open end of each implant. We then sealed each tubule with silicone adhesive and waited several days for the DMSO to diffuse through the tubing and evaporate, leaving 300 μg of crystalline testosterone within the lumen (ca. 1.5 mm length) of each implant. We constructed placebo implants in identical fashion, but injected them with pure DMSO, which left an empty tubule after diffusion and evaporation.

We have previously shown that castration reduces plasma testosterone of males to basal levels typical of females, whereas our exogenous implants restore plasma testosterone of castrated males to levels typical of intact, breeding males

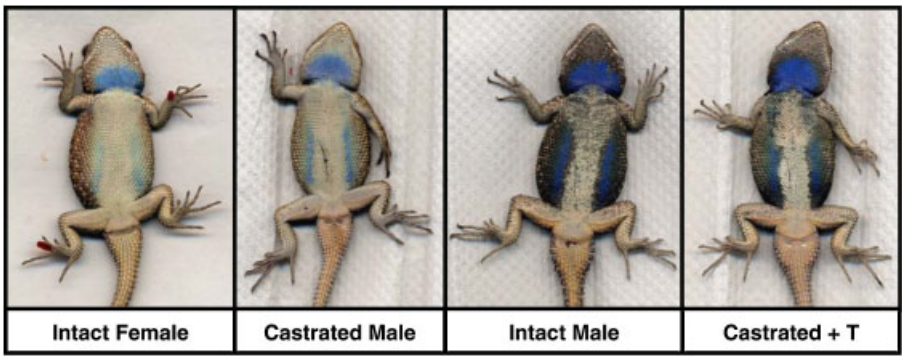

Fig. 1. Digital scans illustrating typical ventral coloration of intact females, castrated males, intact males, and castrated males treated with exogenous testosterone (T). Images of males are from free-living animals recaptured ca. 51 d posttreatment during the seasonal peak in circulating testosterone levels of intact males. Castration feminized males by reducing the intensity of blue coloration and preventing the expression of black coloration, whereas testosterone replacement restored the coloration of castrated males to values similar to intact males.

(Cox and John-Alder, 2005, 2007; Cox et al., 2005a,b, ; John-Alder and Cox, 2007; John-Alder et al., 2007). In analogous studies of wild lizards, we have found that these same implants continue to release hormone for at least 420 days and potentially much longer postimplantation (Cox et al., 2005a). Moreover, we have previously documented differences in plasma testosterone levels between the same group of field-active CAST (0.4 ± 0.2 ng/mL), CON (46.4 ± 5.9 ng/mL), and CAST+T (32.1 ± 3.9 ng/mL) males that we analyze for color in this study (Cox and John-Alder, 2005). Thus, treatment differences in hormone levels have been previously verified for our field experiment.

Although we did not measure plasma testosterone levels of our laboratory animals at the time when we measured coloration in this study, we have previously shown that captive CON males have plasma testosterone levels that are intermediate (mean $\pm$ 1SE = 19.7 ± 6.9 ng/mL) between the basal levels of CAST males (0.8 ± 0.2 ng/mL) and the elevated levels of CAST+T males (39.0 ± 7.6 ng/mL) (Cox et al., 2006). Moreover, we have previously shown that captive males of the same age have substantially higher plasma testosterone levels than captive females (Cox et al., 2008), and females from this population do not reach sexual maturity within the time frame of our captive study (Cox, 2006; Cox et al., 2008). Thus, in both laboratory and field animals, our treatments produce a substantial difference in mean circulating testosterone levels between CAST and CAST+T males. CAST males exhibit low plasma testosterone levels similar to females, whereas hormone implants restore plasma testosterone of CAST+T males to levels similar to those of intact CON males (Cox and John-Alder, 2005; Cox et al., 2006).

Quantification of coloration

At the conclusion of our laboratory (mean 49 d posttreatment) and field experiments (mean 51 d), we used a digital scanner (Epson®, Epson America Inc., Long Beach, CA; Perfection® 1240U) to obtain an image of the ventral surface of each animal (Fig. 1). We gently removed large patches of shed skin from several animals prior to scanning, but we did not otherwise censor our data as a function of shedding state. From these digital images, we used Adobe® PhotoShop® software (version 7.0.1) to estimate the hue, saturation, and brightness of each animal's blue throat and abdominal patches (Cox et al., 2005b). We first circumscribed a representative area in the center of the patch using the ''elliptical marquee'' tool and then used the ''histogram'' tool to derive the mean red, green, and blue values for all pixels within the selected area. We used the ''color picker'' tool to convert these values to the corresponding measures of hue (actual color reflected; measured on a standard 360° color wheel), saturation (purity of the color; 0% = gray, 100% = fully saturated), and brightness (lightness of the color; 0% = black, 100% = white).

To assess repeatability, we performed this procedure six separate times per image for a subset ($n = 12$, four per treatment) of our digital scans. We then performed analysis of variance (ANOVA) and measured repeatability as the

ratio of variance (sum of squares) within individuals to total variance (within individuals+across individuals). Repeatability was extremely high (>0.95) for all measures: throat hue = 0.988; throat saturation = 0.998; throat brightness = 0.996; abdominal hue = 0.959; abdominal saturation = 0.989; abdominal brightness = 0.988. Although these measures do not assess potential variation within individuals over time, they do validate our measurements of coloration from any single image as highly repeatable relative to typical variation across individuals and treatment groups.

We also used these digital images to measure the size of the blue and black throat patches. Abdominal patches were too discontinuous and irregular to permit similar analyses. In defining patch size, we included all individual scales with >50% blue or black pigment as part of the "patch." Because the shapes of the blue and black patches were irregular, we circumscribed the area of interest using the "magnetic lasso" tool in Adobe® PhotoShop®. Next, we obtained the number of pixels in the circumscribed area using the "histogram" tool. We converted this measure to an absolute metric (cm^2) on the basis of the resolution of the scanned image (13,924 pixels/cm^2). This method provides an absolute measure of patch size, so we analyzed relative measures (i.e., size of the patch relative to size of the animal) by including body size (SVL) as a covariate.

Statistical analyses

All statistical analyses were conducted using SAS (version 8.2, SAS Institute Inc.). For comparisons of posttreatment values among groups, we used one-way ANOVA with treatment as the main effect. When we observed significant treatment effects, we used the Ryan-Einot-Gabriel-Welsch test (REGWQ, SAS Institute 1989) to determine post hoc separation of groups. For analyses of patch area, we used ANCOVA with treatment as the main effect and SVL as a covariate. For our laboratory experiment, we analyzed changes from pretreatment to posttreatment values using repeated-measures ANOVA with treatment as a between-subjects effect and time as a within-subjects effect. We did not measure pretreatment coloration in our field experiment.

RESULTS

Sexual differences in coloration

Intact males (CON) and females (FEM) differed in most aspects of blue ventral coloration that we examined (Figs. 1–2). At the beginning of the captive period, males exceeded females in hue ($t = 6.50$; $P < 0.001$; df = 21) and saturation ($t = 7.46$; $P < 0.001$) of blue throat patches (Fig. 2). Males and females did not differ in brightness of blue throat patches ($t = 0.02$; $P = 0.98$). Males also exceeded females in hue ($t = 3.30$; $P = 0.003$) and saturation ($t = 2.45$; $P = 0.023$) of blue abdominal patches, whereas females exceeded males in brightness (i.e., females had relatively whiter patches; $t = 4.98$; $P < 0.001$;

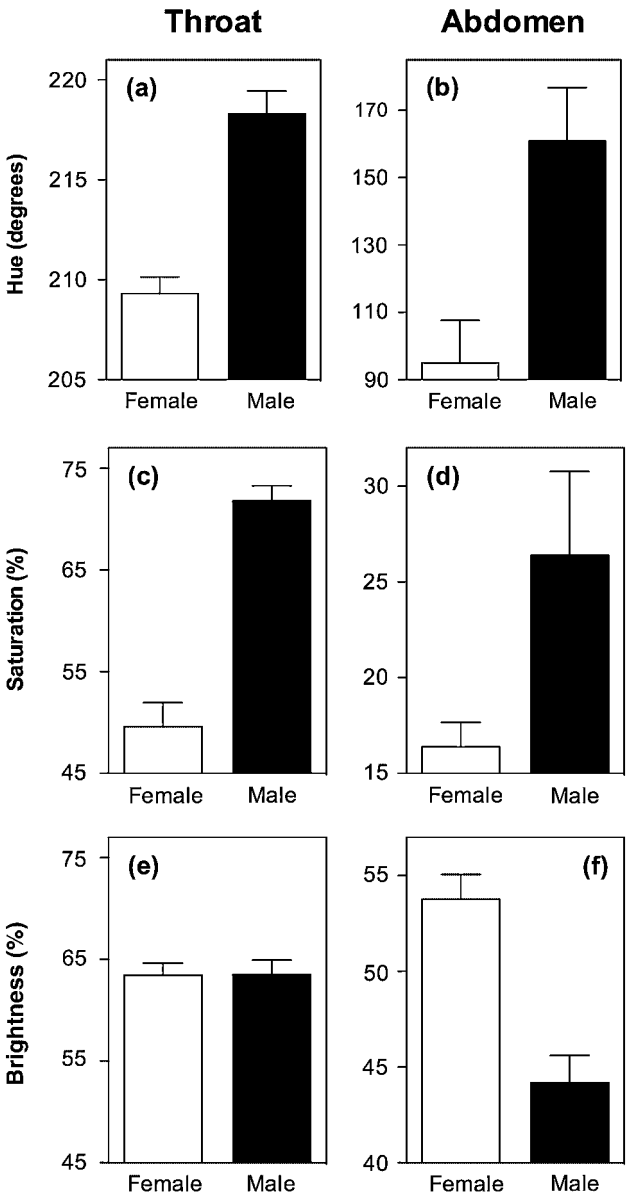

Fig. 2. Mean (+1SE) values for hue, saturation, and brightness of blue patches on the throats and abdomens of intact, captive males (CON, $n = 10$) and females (FEM, $n = 13$). Sexes differed for all measures of coloration except brightness of the throat patch.

Fig. 2). Males also had larger blue throat patches than females (ANCOVA sex; $F_{2,20} = 13.59$; $P = 0.002$) after accounting for the scaling of patch area with body size (ANCOVA body size; $F_{2,20} = 14.29$; $P = 0.001$). In addition to these strong sex effects, we detected a significant within-subjects effect of time on saturation of blue throat ($F_{1,21} = 78.58$; $P < 0.001$) and abdominal color ($F_{1,21} = 4.47$; $P = 0.047$). This indicates that blue coloration ''faded'' in both sexes by decreasing in saturation during captivity.

Treatment effects on coloration

Experimental castration and testosterone replacement influenced all aspects of blue ventral coloration that we examined (Fig. 1). In our field study, CON and CAST+T exceeded CAST in hue ($F_{2,29} = 9.15$; $P < 0.001$; Fig. 3a) and saturation ($F_{2,29} = 13.19$; $P < 0.001$; Fig. 3c), of blue throat patches, but had lower brightness ($F_{2,29} = 13.18$; $P < 0.001$; Fig. 3e). Similarly, CON and CAST+T exceeded CAST in hue ($F_{2,29} = 12.33$; $P < 0.001$; Fig. 3b) and saturation ($F_{2,29} = 12.19$; $P < 0.001$; Fig. 3d) of blue abdominal patches, but had lower brightness ($F_{2,29} = 34.03$; $P < 0.001$; Fig. 3f). We did not include a sham female treatment group in our field experiment, so we cannot assess the extent to which castration feminized male coloration via direct statistical comparison.

In our laboratory study, repeated-measures ANOVA revealed an interaction between treatment and time (presurgery vs. postsurgery) on hue ($F_{2,26} = 4.47$; $P = 0.022$) and saturation ($F_{2,26} = 7.59$; $P = 0.003$) of blue throat coloration (Fig. 4). We did not detect a similar time-by-treatment interaction for brightness of blue throat coloration ($F_{2,26} = 1.32$; $P = 0.28$). We also found weak treatment-by-time interactions with respect to hue ($F_{2,26} = 3.12$; $P = 0.061$), saturation ($F_{2,26} = 3.47$; $P = 0.046$), and brightness ($F_{2,26} = 6.53$; $P = 0.005$) of blue abdominal coloration. These analyses also revealed significant within-subjects effects, such that saturation of blue coloration decreased during captivity for throat ($F_{1,27} = 49.16$; $P < 0.001$) and abdominal patches ($F_{1,27} = 10.29$; $P = 0.004$). When we assessed posttreatment differences in ventral coloration of each male treatment group in comparison with females, we found that CAST males were statistically indistinguishable from FEM with respect to saturation and brightness of blue throat and abdominal patches (Fig. 4c–f). Although castration significantly reduced the hue of blue throat coloration relative to CON and TEST males, it did not reduce hue to the same level observed in FEM (Fig. 4a). Hue of abdominal patched did not differ among male treatment groups and was significantly greater than that observed in FEM (Fig. 4b). Thus, castration generally feminized male coloration in the sense that it shifted the male phenotype toward that of females, although some aspects of coloration remained similar to the male phenotype or intermediate between females and CON and CAST+T males (Fig. 4).

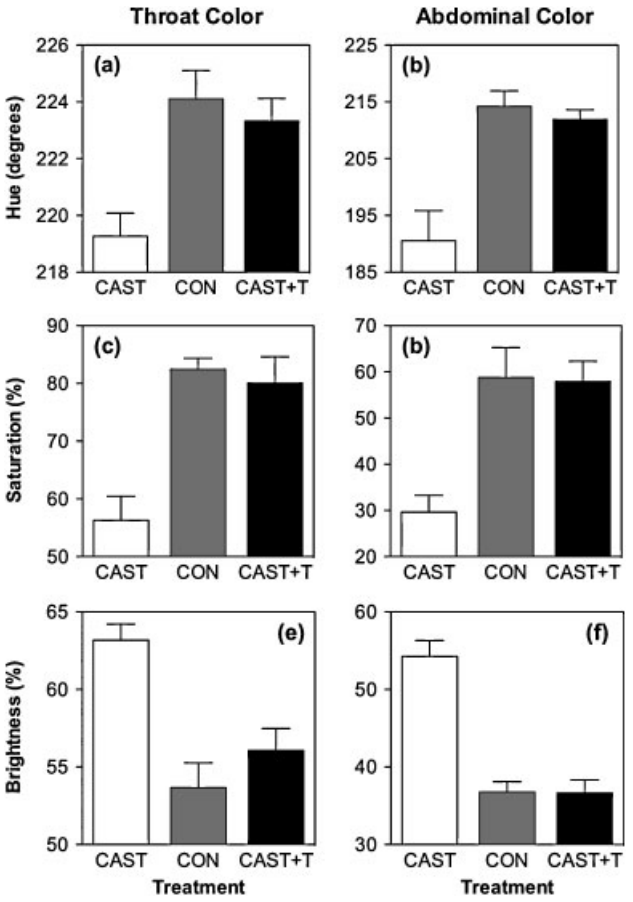

Fig. 3. Mean (+1SE) values for hue, saturation, and brightness of blue patches on the throats and abdomens of CAST ($n = 11$), CON ($n = 9$), and CAST+T males ($n = 12$). Data are shown for free-living males upon recapture at an average of 51 d posttreatment, at which point CAST differed significantly from CON and TEST for all measures of coloration.

Treatment effects on patch size

In our field experiment, CON and CAST+T males had slightly larger blue throat patches than CAST males ($F_{2,29} = 3.46$; $P = 0.045$; Fig. 5). However, this may simply reflect the fact that

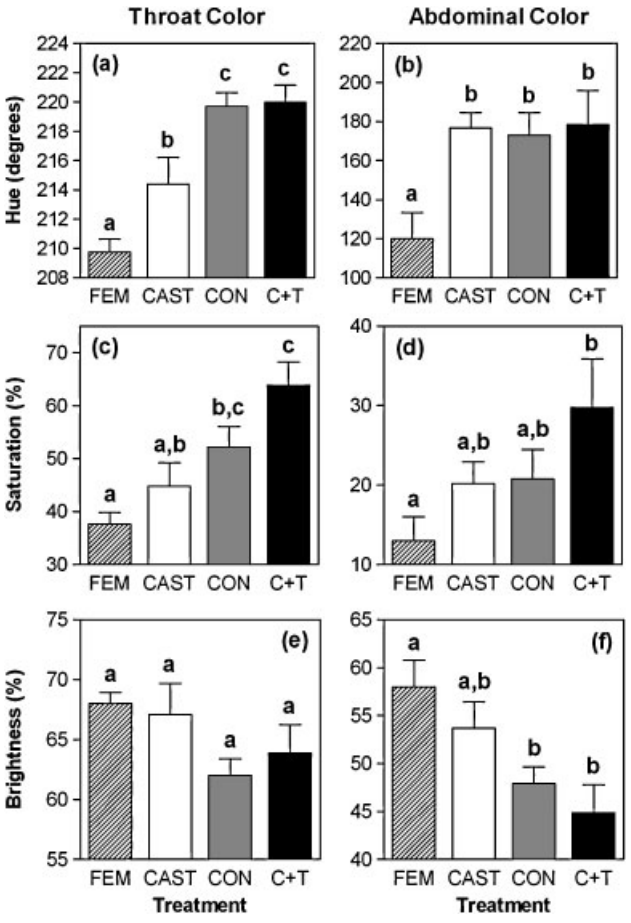

Fig. 4. Mean (+1SE) values for hue, saturation, and brightness of blue patches on the throats and abdomens of FEM ($n = 13$), CAST ($n = 10$), CON ($n = 10$), and CAST+T ($n = 9$). Data are shown for captive animals at 49 d posttreatment. Lowercase letters denote statistical separation based on ANOVA with Ryan-Einot-Gabriel-Welsch post hoc tests. Posttreatment differences are not as pronounced as in free-living males (Fig. 3) because coloration ''faded'' during captivity, but repeated-measures ANOVA revealed significant time-by-treatment interactions (i.e., effects of testosterone on coloration) despite this within-subjects effect. See text for details.

castration inhibited growth of CAST males (Cox and John-Alder, 2005), as we did not find any difference in the size of blue throat patches after controlling for differences in body size (ANCOVA treatment; $F_{3,28} = 0.17$; $P = 0.85$) and patch size increased with body size (ANCOVA SVL; $F_{3,28} = 17.11$; $P < 0.001$). Moreover, there was no effect of treatment on the size of the blue throat patch in our laboratory experiment ($F_{2,26} = 0.94$; $P = 0.40$), where treatment differences in body size were absent owing to similar growth rates of each group in captivity (Cox et al., 2006).

In our field study, experimental castration and testosterone replacement dramatically influenced the expression of black pigmentation bordering the blue throat patch (Fig. 1). Both CON and CAST+T had substantially larger black throat patches than CAST males ($F_{2,29} = 17.15$; $P < 0.001$; Fig. 5). This difference remained significant after correcting for differences in body size (ANCOVA; $F_{3,28} = 5.90$; $P = 0.007$). Similar differences in black pigment were evident around the margins of the blue abdominal patches (Fig. 1), although the irregularity of this coloration precluded quantification and formal statistical analysis. We were also unable to analyze posttreatment differences in black pigment of our laboratory animals, as long-term captivity suppressed the expression of black pigmentation.

DISCUSSION

Although both sexes of *S. jarrovii* express blue ventral coloration (Wiens, '99; Wiens et al., '99; Quinn and Hews, 2003), we found a significant difference between the vibrant, saturated patches typical of males and the whiter, less saturated patches characteristic of females (Figs. 1–2). Thus, though males and females are qualitatively monomorphic with respect to the presence of blue ventral coloration, we conclude that they are quantitatively dimorphic in its expression. Moreover, our experimental data suggest that this quantitative dimorphism is regulated in part by sexual differences in circulating testosterone. Blue ventral coloration intensifies at roughly the time when yearling males first experience elevated plasma testosterone levels, and our experiments conducted during this same ontogenetic period revealed significant effects of testosterone on coloration. Surgical castration decreased the hue and saturation while increasing the brightness of blue throat and abdominal patches (Figs. 1, 3). Castration also decreased the amount of black pigment bordering the blue throat patch (Figs. 1, 5). Thus, castration feminized males by reducing the intensity of ventral blue coloration and producing phenotypes similar to females. Treatment of castrated males with exogenous testosterone restored all aspects of ventral coloration to values similar to those of intact control males (Figs. 3–5).

In several respects, our results for *S. jarrovii* are similar to those of previous studies involving sexually dichromatic *Sceloporus* species. In both monochromatic *S. jarrovii* (this study) and dichromatic *S. undulatus* (John-Alder et al., '96; Cox et al., 2005b), castration dramatically reduces the

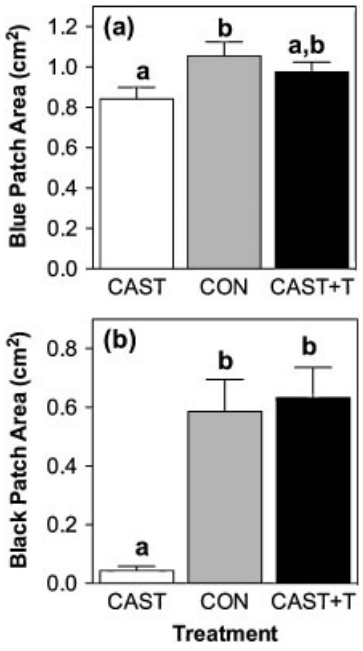

Fig. 5. Mean (+1SE) size of blue and black throat patches for CAST ($n = 11$), CON ($n = 9$), and CAST+T males ($n = 12$). Data are shown for free-living males upon recapture at an average of 51 d posttreatment. Lowercase letters denote statistical separation of treatment groups on the basis of post hoc Ryan-Einot-Gabriel-Welsch tests (REGWQ).

intensity of blue ventral coloration but does not abolish the expression of the patches themselves, provided castration occurs after the ontogenetic stage at which patches first develop. Exogenous testosterone also stimulates the expression of blue coloration in males of each species, particularly when administered at ontogenetic stages corresponding to natural maturational increases in circulating testosterone and intensity of coloration (Cox et al., 2005b). Finally, testosterone has a modest effect on the size of blue patches and a dramatic effect on the expression of black coloration bordering and underlying the blue patches in both species (Cox et al., 2005b). Collectively, these similarities indicate that the evolution of sexual monochromatism in *S. jarrovii* is not simply the result of a decoupling of coloration from its ancestral endocrine triggers. This stands in contrast to the evolution of sexual monochromatism in which both sexes lack blue coloration. For example, the absence of blue patches in males of *S. virgatus* is owing in part to the insensitivity of coloration to circulating testosterone during postnatal development (Abell, '98; Hews and Quinn, 2003, R. M. Cox, unpublished data).

Our experiments do not directly address the proximate cellular and physiological mechanisms by which testosterone mediates coloration, but our results are consistent with the prevailing model for expression of blue ventral coloration in *Sceloporus* and related lizards. Expression of vibrant blue coloration requires the reflection of blue light by superficial iridophores as well as the absorption of other wavelengths by dispersed melanin in underlying melanophores (Cooper and Greenberg, '92; Morrison et al., '95; Hews and Quinn, 2003). If these melanophores are contracted, then blue light reflecting from iridophores appears faint because other wavelengths are also reflected from underlying cells. Interspecific and intersexual differences in blue ventral coloration are associated with differences in the density of underlying dermal melanin (Quinn and Hews, 2003), and circulating androgens may be required to maintain melanophore expansion and the expression of both blue and black ventral coloration (Kimball and Erpino, '71; Cox et al., 2005b). Our results support this mechanism by demonstrating that the expression of black ventral coloration is strongly dependent upon circulating testosterone.

In several species of sexually dichromatic *Sceloporus* and closely related *Urosaurus* lizards, the atypical expression of blue ventral coloration and its underlying cellular components can be induced by treating juvenile females with testosterone or other androgens (Kimball and Erpino, '71; Hews and Moore, '95; Quinn and Hews, 2003; Hews and Quinn, 2003; Cox et al., 2005b). Given that males of *S. jarrovii* responded to testosterone in similar fashion to males of sexually dichromatic species, it seems likely that coloration of *S. jarrovii* females would also respond to exogenous testosterone administered at the appropriate ontogenetic stage. For example, social behaviors that are regulated by androgens in *S. jarrovii* males are also induced by exogenous testosterone in females (Woodley and Moore, '99a,b). However, we are unaware of any attempts to manipulate testosterone and assess coloration in females of this species. Moreover, it is unknown whether effects of testosterone on coloration involve androgen or estrogen receptor activation in target tissues, because aromatization of testosterone to estradiol may be responsible for behavioral effects of testosterone in females of *S. jarrovii* (Woodley and Moore, '99a,b). Whether natural development of blue coloration in females is under androgenic or estrogenic control, we predict that exogenous testosterone should stimulate blue coloration in females of this species.

Our experimental results clearly demonstrate that the intensity of blue ventral coloration is influenced by testosterone in *S. jarrovii*, but they do not address the question of how and why females develop blue coloration in this species. Given that blue coloration is present from birth, it seems likely that prenatal organizational effects of androgens may contribute to the development of coloration in both sexes. The viviparous reproductive mode of *S. jarrovii* may help to explain why any such effects accrue to both male and female embryos, because these embryos share a common maternal environment throughout gestation (Hews and Quinn, 2003). Female embryos would therefore be subject to androgens of maternal and/or fraternal origin throughout embryonic development, and studies of placental mammals have shown that female embryos can be masculinized in utero by androgens from adjacent male embryos (vom Saal, '89). By contrast, male and female embryos are individually sequestered within eggs of oviparous species, thus isolating them from the hormonal environment of their mothers and siblings throughout the majority of their embryonic development. Intriguingly, females with blue ventral coloration are also observed in several other *Sceloporus* species with viviparous reproduction, although this phenomenon also occurs in strictly oviparous lineages (Hews and Quinn, 2003). Future research into the development of bright ventral coloration in *Sceloporus* females should focus explicitly on the prenatal organizational effects of sex steroids and the extent to which viviparous species experience a sex-specific prenatal endocrine environment (e.g., Painter et al., 2002; Painter and Moore, 2005). Given that *S. jarrovii* females also exhibit relatively high levels of behavioral aggression (Woodley and Moore, '99a,b; Hews and Quinn, 2003), the early organizational effects of androgens may provide an elegant developmental mechanism linking social behavior, coloration, and reproductive mode.

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LITERATURE CITED

Abell AJ. 1998. The effect of exogenous testosterone on growth and secondary sexual character development in juveniles of *Sceloporus virgatus*. Herpetologica 54:533–543.

Alsonso-Alvarez C, Bertrand S, Faivre B, Chastel O, Sorci G. 2007. Testosterone and oxidative stress: the oxidation handicap hypothesis. Proc R Soc B 274:819–825.

Andersson M. 1994. Sexual selection. Princeton, NJ: Princeton University Press.

Badyaev AV. 2002. Growing apart: an ontogenetic perspective on the evolution of sexual size dimorphism. Trends Ecol Evol 17:369–378.

Bedhomme S, Chippindale AK. 2007. Irreconcilable differences: when sexual dimorphism fails to resolve sexual conflict. In: Fairbairn DJ, Blanckenhorn WU, Szekely T, editors. Sex, size and gender roles: evolutionary studies of sexual size dimorphism. Oxford, UK: Oxford University Press. p 185–194.

Cardwell JR, Liley NR. 1991. Hormonal control of sex and color change in the stoplight parrotfish, *Sparisoma viride*. Gen Comp Endocrinol 81:7–20.

Cooper Jr WE, Clarke RF. 1982. Steroidal induction of female reproductive coloration in the keeled earless lizard, *Holbrookia propinqua*. Herpetologica 38:425–429.

Cooper Jr WE, Crews D. 1987. Hormonal induction of secondary sexual coloration and rejection behaviour in female keeled earless lizards (*Holbrookia propinqua*). Anim Behav 35:1177–1187.

Cooper Jr WE, Ferguson GW. 1972. Relative effectiveness of progesterone and testosterone as inductors of orange spotting in female collared lizards. Herpetologica 28: 64–65.

Cooper WE, Greenberg N. 1992. Reptilian coloration and behavior. In: Gans C, Crews D, editors. Hormones, brain, and behavior. Chicago, IL: The University of Chicago Press. p 298–422.

Cox RM. 2006. A test of the reproductive cost hypothesis for sexual size dimorphism in Yarrow's Spiny Lizard, *Sceloporus jarrovii*. J Anim Ecol 75:1361–1369.

Cox RM, John-Alder HB. 2005. Testosterone has opposite effects on male growth in lizards (*Sceloporus* spp.) with opposite patterns of sexual size dimorphism. J Exp Biol 208:4679–4687.

Cox RM, John-Alder HB. 2007. Increased mite parasitism as a cost of testosterone in male striped plateau lizards, *Sceloporus virgatus*. Funct Ecol 21:327–334.

Cox RM, Skelly SL, John-Alder HB. 2005a. Testosterone inhibits growth in juvenile male eastern fence lizards (*Sceloporus undulatus*): implications for energy allocation and sexual size dimorphism. Physiol Biochem Zool 78: 531–545.

Cox RM, Skelly SL, Leo A, John-Alder HB. 2005b. Testosterone regulates sexually dimorphic coloration in the

eastern fence lizard, *Sceloporus undulatus*. Copeia 2005: 597–608.

Cox RM, Zilberman V, John-Alder HB. 2006. Environmental sensitivity of sexual size dimorphism: laboratory common garden removes effects of sex and castration on lizard growth. Funct Ecol 20:880–888.

Cox RM, Barrett MM, John-Alder HB. 2008. Effects of food restriction on growth, energy allocation, and sexual size dimorphism in Yarrow's Spiny Lizard, *Sceloporus jarrovii*. Can J Zool 86:268–276.

Folstad I, Karter AJ. 1992. Parasites, bright males and the immunocompetence handicap hypothesis. Am Nat 139: 603–622.

Forsman A. 1995. Opposing fitness consequences of colour pattern in male and female snakes. J Evol Biol 8:53–70.

Forsman A, Appelqvist S. 1999. Experimental manipulation reveals differential effects of colour pattern on survival in male and female pygmy grasshoppers. J Evol Biol 12:391–401.

Forsman A, Shine R. 1995. The adaptive significance of colour pattern polymorphism in the Australian scincid lizard *Lampropholis delicata*. Biol J Linnean Soc 55:273–291.

Hayes TB, Menendez KP. 1999. The effect of sex steroids on primary and secondary sex differentiation in the sexually dichromatic reedfrog (*Hyperolius argus:* Hyperolidae) from the Arabuko Sokoke Forest of Kenya. Gen Comp Endocrinol 115:188–199.

Hews DK, Moore MC. 1995. Influence of androgens on differentiation of secondary sex characters in tree lizards, *Urosaurus ornatus*. Gen Comp Endocrinol 97:86–102.

Hews DK, Quinn VS. 2003. Endocrinology of species differences in sexually dimorphic signals and aggression: using the organization and activation model in a phylogenetic framework. In: Fox SF, Baird TA, McCoy JC, editors. Lizard social behavior. Baltimore, MD: Johns Hopkins University Press. p 253–277.

John-Alder HB, Cox RM. 2007. Development of sexual size dimorphism in lizards: testosterone as a bipotential growth regulator. In: Fairbairn DJ, Blanckenhorn WU, Szekely T, editors. Sex, size and gender roles: evolutionary studies of sexual size dimorphism. London: Oxford University Press. p 195–204.

John-Alder HB, McMann S, Katz LS, Gross A, Barton DS. 1996. Social modulation of exercise endurance in a lizard (*Sceloporus undulatus*). Physiol Zool 69:547–567.

John-Alder HB, Cox RM, Taylor EN. 2007. Proximate developmental mediators of sexual size dimorphism: case studies from squamate reptiles. Integr Comp Biol 47: 258–271.

Kimball FA, Erpino MJ. 1971. Hormonal control of pigmentary sexual dimorphism in *Sceloporus occidentalis*. Gen Comp Endocrinol 16:375–384.

Kimball RT, Ligon JD. 1999. Evolution of avian plumage dichromatism from a proximate perspective. Am Nat 154: 182–193.

Morrison RL, Rand MS, Frost-Mason SK. 1995. Cellular basis of color differences in three morphs of the lizard *Sceloporus undulatus erythrocheilus*. Copeia 1995:397–408.

Oppliger A, Giorgi MS, Conelli A, Nembrini M, John-Alder HB. 2004. Effect of testosterone on immunocompetence, parasite load, and metabolism in the common wall lizard (*Podarcis muralis*). Can J Zool 82:1713–1719.

Painter DL, Moore MC. 2005. Steroid hormone metabolism by the chorioallantoic placental of the mountain spiny lizard, *Sceloporus jarrovi*, as a possible mechanism for buffering maternal–fetal hormone exchange. Physiol Biochem Zool 78:364–372.

Painter DL, Jennings DH, Moore MC. 2002. Placental buffering of maternal steroid hormone effects on fetal and yolk hormone levels: a comparative study of a viviparous lizard, *Sceloporus jarrovi*, and an oviparous lizard, *Sceloporus graciosus*. Gen Comp Endocrinol 127:105–116.

Price DK, Burley NT. 1994. Constraints on the evolution of attractive traits: selection in male and female zebra finches. Am Nat 144:908–934.

Quinn VS, Hews DK. 2003. Positive relationship between abdominal coloration and dermal melanin density in Phrynosomatid lizards. Copeia 2003:858–864.

Rand MS. 1992. Hormonal control of polymorphic and sexually dimorphic coloration in the lizard *Sceloporus undulatus erythrocheilus*. Gen Comp Endocrinol 88: 461–468.

vom Saal FS. 1989. Sexual differentiation in litter-bearing mammals: influence of sex of adjacent fetuses in utero. J Anim Sci 67:1824–1840.

Wiens JJ. 1999. Phylogenetic evidence for multiple losses of a sexually selected character in phrynosomatid lizards. Proc R Soc Lond B Biol Sci 266:1529–1536.

Wiens JJ, Reeder TW, Montes de Oca AN. 1999. Molecular phylogenetics and evolution of sexual dichromatism among populations of the Yarrow's Spiny Lizards (*Sceloporus jarrovii*). Evolution 53:1884–1897.

Woodley SK, Moore MC. 1999a. Female territorial aggression and steroid hormones in mountain spiny lizards. Anim Behav 57:1083–1089.

Woodley SK, Moore MC. 1999b. Ovarian hormones influence territorial aggression in free-living female mountain spiny lizards. Horm Behav 35:205–214.