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Testosterone Regulates Sexually Dimorphic Coloration in the Eastern Fence Lizard, *Sceloporus undulatus*

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Adult male Eastern Fence Lizards (*Sceloporus undulatus*) possess blue and black ventral patches that function in sex recognition and intrasexual social signaling, but this ventral coloration is absent or greatly reduced in females and juvenile males. Adult males also exhibit a relatively uniform, reddish brown dorsal coloration, while females and juvenile males are cryptically colored, with two rows of dark brown or black chevrons set against a background of gray and brown. In the present report, we show that sexual divergence in ventral coloration is temporally correlated with sexual divergence in plasma testosterone in free-living juvenile males and females, supporting the hypothesis that sexual dichromatism is regulated at least in part by testosterone. We experimentally tested this hypothesis by (1) removing the primary source of circulating testosterone in juvenile males via surgical castration, (2) restoring testosterone in castrated males with tonic-release implants, and (3) implanting intact juvenile females with exogenous testosterone. As predicted by our hypothesis, the development of blue and black ventral coloration in *S. undulatus* was (1) inhibited by castration in juvenile males, (2) restored by exogenous testosterone following castration in juvenile males, and (3) promoted by exogenous testosterone in juvenile females. The expression of male-specific dorsal coloration was also (1) inhibited by castration and (2) restored by exogenous testosterone following castration in juvenile males. Our results are consistent with established literature supporting the critical role of androgens in the mediation of sexually dimorphic coloration among phrynosomatid lizards.

SEXUALLY dimorphic coloration, or sexual dichromatism, occurs when males and females of a given species or population differ in coloration or color pattern. This phenomenon is widespread among lizards and is generally assumed to facilitate sex recognition and intraspecific social signaling (reviewed in Cooper and Greenberg, 1992). Recent studies of coloration in phrynosomatid lizards have ranged in focus from proximate cellular regulatory mechanisms (e.g., Morrison et al., 1995; Quinn and Hews, 2003) to behavioral use and social significance (e.g., Cooper and Burns, 1987; Smith and John-Alder, 1999; Quinn and Hews, 2000) and, ultimately, the evolution of sexual dichromatism across populations and species (e.g., Wiens, 1999; Wiens et al., 1999; Hews and Quinn, 2003). With few exceptions, these studies share a common organizing theme: the central role of sex steroids in the integration of proximate and ultimate causation of sexual dichromatism. Numerous lines of evidence implicate sex steroids as physiological mediators of sexually dimorphic coloration in lizards (reviewed in Cooper and Greenberg, 1992) and recent attention has focused on the central role of the endocrine system in coordinating sex-specific coloration and the expression of correlated morphological, behavioral, physiological, and life-history traits (e.g., Hews and Moore, 1995; Sinervo et al., 2000; Hews and Quinn, 2003). In the present report, we contribute to this expanding literature with experimental evidence for the testosterone-dependent expression of sexually dimorphic dorsal and ventral coloration in the Eastern Fence Lizard, *Sceloporus undulatus*.

Adult male *Sceloporus undulatus* exhibit laterally paired blue throat and abdominal patches, which are bordered medially by black pigment (Fig. 1, Control Male). Typically, these patches are visible only when broadcast via postural adjustments (i.e., elevation of the head and body, lateral compression of the body) during stereotyped behavioral displays. Blue and black ventral patches are absent or greatly reduced in female *S. undulatus* and function in sex recognition and intrasexual male agonistic signaling (Cooper and Burns, 1987). In addition to sexual dimorphism in ventral coloration, adult *S. undulatus* exhibit striking sexual dimorphism in dorsal coloration. Adult females and juveniles of both sexes are cryptically colored, with two parallel rows of dark brown or black chevrons set against a background of gray and brown (Fig. 1, Control Female). In contrast, adult males exhibit a more uniform, reddish brown dorsal coloration that is achieved by a lightening of the chevrons and dorsolateral area from dark

Fig. 1. Digital scans of the ventral (top panels) and dorsal (bottom panels) surfaces of individual *Sceloporus undulatus*, illustrating the typical coloration of each treatment group at 420 d post-treatment. Castrated Males exhibited dorsal and ventral coloration similar to Control Females, while Testosterone Males resembled Control Males. Lettered ellipses indicate the areas that we sampled for our digital analyses of hue, saturation, and brightness (see Fig. 5); (A) blue throat patches, (B) blue abdominal patches, (C) dorsal chevrons, (D) dorsolateral area.

brown and black to yellowish red (Fig. 1, Control Male).

Juvenile males in our New Jersey population develop male-specific dorsal and ventral coloration between May and September of their first full summer (age 9–13 mo), such that it is well developed upon reproductive maturity the following spring (age 20–21 mo). This morphological color change occurs roughly coincident with maturational increases in territorial aggression and movement patterns (S. Skelly, unpubl. data), which are influenced by the sex steroid testosterone in many phrynosomatids (DeNardo and Sinervo, 1994; Marler and Moore, 1989, 1991; Quinn and Hews, 2003; Klukowski et al., 2004). Further, several studies of morphologically distinct populations of *Sceloporus undulatus* (*sensu lato,* Leache and Reeder, 2002) have implicated testosterone as an important regulator of male-specific ventral coloration (Rand, 1992; Quinn and Hews, 2003). Thus, we hypothesized that the development of male-specific coloration is regulated by testosterone in *S. undulatus.* Accordingly, we predicted that (1) the development of male-specific coloration would be accompanied by an increase in plasma testosterone in free-living males, and (2) manipulations of circulating testosterone would influence the development and expression of male-specific coloration.

In the present report, we show that sexual divergence in ventral coloration is temporally correlated with divergence in plasma testosterone among free-living juvenile males and females. We also experimentally demonstrate that the development of blue and black ventral coloration in *Sceloporus undulatus* is (1) inhibited by castration in juvenile males, (2) restored by exogenous testosterone following castration in juvenile males, and (3) promoted by exogenous testosterone in juvenile females. Further, we show that the expression of male-specific dorsal coloration by juvenile males is (1) inhibited by castration and (2) restored by exogenous testosterone following castration.

Materials and Methods

We present the results of several studies conducted from 2000–2004 in either a natural ''field'' environment or in ''laboratory'' captivity. We did not have any *a priori* expectations that treatment effects on coloration would differ between field and laboratory, but we subsequently distinguish between the two because of underlying differences in experimental design. We collected lizards from several locations in the vicinity of the Rutgers University Pinelands Research Station in New Lisbon, Burlington County, New Jersey (41°N, 74°35′W). Animals were captured by hand or hand-held noose and were given a unique toe clip for permanent identification. Sex was determined by the presence (male) or absence (female) of enlarged post-anal scales.

Ontogeny of plasma testosterone levels.—To describe natural developmental changes in plasma testosterone, we collected blood samples from free-living juvenile males and females (see Testosterone assay, below). We sampled the natural population at 4 ontogenetic stages during a single year: ages ca. 2 mo (October), 9.5 mo (May), 11.5 mo (July), and 14 mo (October). To compare developmental changes in plasma testosterone and coloration, we also recorded the ventral coloration of each animal using a categorical scale (see Quantification of coloration, below). Hereafter, we refer to these animals as ''free-living'' to distinguish them from the ''experimental'' animals in our manipulation studies.

Field experiment.—We collected juvenile male *Sceloporus undulatus* (late May, age 9 mo) and assigned them to one of three size-matched treatment groups: (1) Castrated Males, (2) Testosterone Males, and (3) Control Males (see Surgical treatments, below). We also included a Control Female group. Animals were temporarily housed in laboratory cages (see Laboratory experiment, below) until surgical treatments were completed. We released the experimental animals into a large (ca. 6000 m^2) enclosed area of natural habitat at the Rutgers University Pinelands Research Station (Cox et al., in press). We periodically recaptured experimental animals from June through August and recorded blue and black ventral coloration using a categorical scale (see Quantification of coloration, below). In July of the following summer, we recaptured all surviving animals (age 23 mo, 420 d post-treatment) and housed them briefly in lab cages before digitally scanning the dorsal and ventral surface of each animal (see Quantification of coloration, below).

Laboratory experiment.—We collected hatchling male and female *Sceloporus undulatus* (September, age 1 mo) and raised them individually in 10-gallon glass aquaria at Rutgers University. To ensure social isolation, we visually separated all cages with opaque barriers. Animals were maintained on a 12L:12D photoperiod with continuous access to a 25 W incandescent lamp for basking during photophase. We offered each

animal several small crickets (*Acheta domestica*) daily and made water available *ad libitum*. In February (age 6 mo), we assigned each male to one of three size-matched treatment groups: (1) Castrated Males, (2) Testosterone Males, and (3) Control Males. Females were separated into two size-matched groups: (1) Testosterone Females and (2) Control Females (see Surgical treatments, below). As in our field experiment, we periodically recorded blue and black ventral coloration using our categorical scale. We did not monitor dorsal coloration in this experiment.

Testosterone implants.—We constructed tonic-release testosterone implants from 5 mm lengths of Silastic® tubing (Dow Corning, 0.058'' i.d., 0.077'' o.d.). After sealing one end of each tubule with silicone adhesive gel (NuSil Technology, MED-1037), we used a Hamilton® syringe to inject 3 μL of a solution of testosterone (Sigma, T-1500) dissolved in dimethyl sulfoxide (DMSO, 100 μg T μL^{-1}) into the open end of each implant. We then sealed each tubule with silicone adhesive and waited several days for the DMSO to evaporate and diffuse through the tubing, 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 evaporation and diffusion.

Surgical treatments.—We anaesthetized animals with an intramuscular injection of ketamine (Ketaset®, Fort Dodge Laboratories, 130 mg kg^{-1}). We then exposed the gonads via bilateral or medial ventral incisions and bilaterally castrated (orchiectomized) both Castrated Males and Testosterone Males by ligating each spermatic cord with surgical silk, ablating each testis, and cauterizing each ligated spermatic cord after removal of the testes. For Control Males, Control Females, and Testosterone Females, we performed ''sham'' surgeries in which we made identical incisions to expose and manipulate the testes and ovaries while leaving the gonads completely intact. We then inserted either a testosterone implant (Testosterone groups) or a placebo implant (Castrated and Control groups) into the coelomic cavity and closed the incision(s) with surgical silk sutures (lab study) or Nexaband® (Veterinary Products Laboratories) surgical glue (field study). We verified our treatments by assaying plasma testosterone levels at both 47 and 420 d post-treatment in our field experiment, and at 65 d post-treatment in our lab experiment (see Testosterone assay, below). We also verified the success of our castration surgeries via necropsy and visual inspection of the reproductive tract.

Testosterone assay.—We used heparinized microhematocrit capillary tubes (Fisher Scientific) to collect 40–80 μL of blood from the postorbital sinus of each sampled animal. Owing to the small size of free-living juveniles at 2 mo posthatching (1–2 g body mass), we collected blood samples via decapitation at this age. All blood samples were collected from animals within 2 min of capture. We held the samples on ice until they could be centrifuged (within 8 h of collection) and stored the separated plasma at −20 C until subsequent assays. We performed radioimmunoassays (RIAs) for plasma testosterone following the methods of Smith and John-Alder (1999). Samples were extracted twice in diethyl ether, dried under a stream of ultra-filtered air, and reconstituted in phosphate-buffered saline with gelatin (PBSG). Reconstituted samples were assayed with tritiated testosterone as a radiolabel (PerkinElmer Life Sciences Inc.) and testosterone antiserum (1:18,000 initial dilution) developed in rabbits by A. L. Johnson (The University of Notre Dame, Indiana). We did not use chromatography to separate testosterone from other androgens prior to RIA, so our ''plasma testosterone'' values should be interpreted with the caveat that they reflect any additional binding of the testosterone antibody to other androgens (e.g., 5α-dihydrotestosterone, 50% cross-reactivity). Samples from separate years were analyzed in separate assays (typical inter-assay and intra-assay variation of 6 and 7%, respectively; Smith and John-Alder, 1999). Limits of detection ranged from 5–14 pg testosterone per assay tube. Individuals whose plasma testosterone ($n = 3$) fell below the limit of detection were assigned a plasma testosterone level of zero and included in subsequent analyses.

Quantification of coloration.—At several time points in each experiment, we recorded the ventral coloration of each animal as a categorical value between 0 (no coloration) and 5 (typical adult male coloration). We gave each animal a separate score for blue versus black coloration, but we did not distinguish between throat and abdominal coloration with this method. Hereafter, we use the terms ''throat'' and ''abdominal'' to refer exclusively to those body regions, while we use the term ''ventral'' in reference to both of the above body regions.

At the conclusion of our field experiment (420 d post-treatment), we used a digital scanner (Epson® Perfection® 1240U) to obtain an

image of the dorsal and ventral surface of each animal. From these images, we used Adobe® PhotoShop® software (version 7.0.1) to estimate the hue, saturation, and brightness of each animal's (A) blue throat patches, (B) blue abdominal patches, (C) dorsal chevrons, and (D) dorsolateral area (Fig. 1). We first circumscribed the area of interest using the ''elliptical marquee'' tool (see Fig. 1) 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 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) and used the mean of each animal's right and left halves in all subsequent analyses.

We also used our digital images to measure the size of blue and black throat and abdominal patches. We counted an individual scale as ''blue'' if it was covered by >50% blue pigment, and ''black'' if it was covered by >50% black pigment. We calculated patch size for each animal as the number of scales of a given color, and summed across both body halves for each patch size measure.

Statistical analyses.—All statistical analyses were conducted using SAS (version 8.2, SAS Institute Inc.). For most comparisons among male treatment groups, we used one-way ANOVA with treatment (Castrated, Control, Testosterone) 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 treatment groups. For most comparisons between female treatment groups, we used one-way ANOVA with treatment (Control, Testosterone) as the main effect. For analyses involving our categorical measures of ventral coloration, we employed non-parametric Kruskal-Wallis tests. We did not include Females in any of our analyses involving Castrated Males and Testosterone Males, since the absence of complementary surgical treatments among females would have precluded valid inferences with regard to the source of any observed effects.

Results

Ontogeny of plasma testosterone levels.—Plasma testosterone was low and did not differ between free-living juvenile males and females at 2 months post-hatching (Fig. 2A). However, by 11.5 months of age (July), plasma testosterone in free-living males reached a seasonal peak and was significantly higher than in females. This sexual divergence in plasma testosterone was paralleled by a similar divergence in ventral coloration. Neither sex exhibited blue or black ventral coloration at 2 months post-hatching, but this coloration was significantly more pronounced in juvenile males by 11.5 months of age (Fig. 2B–C). Females developed faint blue ventral coloration (Fig. 2B) but never expressed black ventral coloration (Fig. 2C).

Experimental plasma testosterone levels.—In both experiments, we observed a significant elevation in plasma testosterone among Testosterone Males relative to both Castrated Males and Control Males (Fig. 3). Plasma testosterone was also elevated in Testosterone Females relative to Control Females (Fig. 3). Our experimentally induced plasma testosterone levels were comparable to values measured in free-living juvenile males of similar age (Fig. 2, July). However, in both the lab and field experiments, we were unable to detect differences in plasma testosterone between Control Males and either Castrated Males or Control Females (Fig. 3), despite sex differences in plasma testosterone for free-living juveniles of similar age (Fig. 2, May–July). Plasma testosterone levels of Testosterone Males were still elevated at 420 d post-treatment (mean = 14.2 ng ml^{-1}), which suggests that our implants were still functional at this time. Further, testosterone implants salvaged upon necropsy contained visible crystals, which we subsequently dissolved in ethanol and identified as testosterone via RIA. Salvaged placebo implants contained neither visible crystal nor detectable testosterone. Upon dissection at 420 d post-treatment, we did not find any discernable gonadal tissue in either Castrated Males or Testosterone Males (n = 7), which suggests that our surgical castrations were successful.

Qualitative color results.—Figure 1 illustrates the inhibitory effects of castration on the development and expression of male-specific ventral and dorsal coloration in male *Sceloporus undulatus.* This figure also demonstrates that these inhibitory effects are prevented by chronic treatment of castrated males with exogenous testosterone. At 420 d post-treatment, Testosterone Males and Control Males exhibited ventral and dorsal coloration typical of adult male *S. undulatus.* However, ventral and dorsal coloration were so profoundly altered by castration that we initially mistook most, if not all, Castrated Males for females prior to inspection of

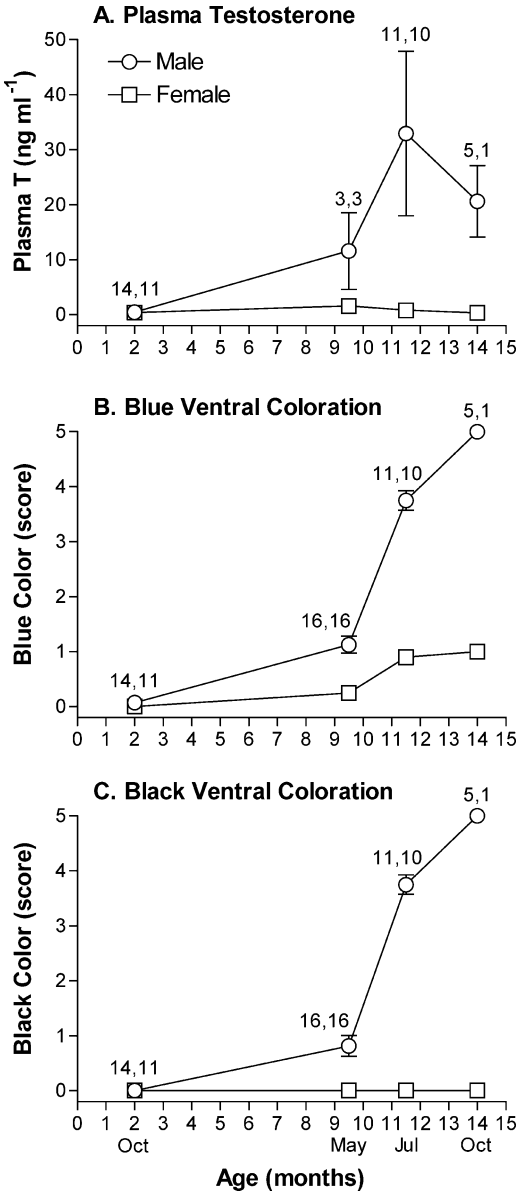

Fig. 2. Mean ± 1 SE (A) plasma testosterone and categorical scores for (B) blue and (C) black ventral coloration vs. age for free-living juvenile male and female *Sceloporus undulatus*. Numbers above symbols indicate sample sizes for males and females, respectively. At 2 months of age, males and females did not differ in plasma testosterone ($F_{1,23} = 0.28$; $P = 0.298$), blue coloration ($\chi^2 = 0.79$; $P = 0.375$), or black coloration ($\chi^2 = 0.00$; $P = 1.00$). By 11 months of age, males had significantly more plasma testosterone ($F_{1,19} = 4.67$; $P = 0.022$), blue coloration ($\chi^2 = 18.15$; $P < 0.001$), and black coloration ($\chi^2 = 19.24$; $P < 0.001$) than females. Blue coloration increased with age in both males ($\chi^2 = 35.26$; $P < 0.001$) and females ($\chi^2 = 19.35$; $P < 0.001$), but black coloration increased with age only in males ($\chi^2 = 33.36$; $P < 0.001$).

the post-anal scales (R. Cox and H. John-Alder, pers. obs.).

Categorical color scores.—Our categorical measures of blue and black ventral coloration did not differ among male groups prior to treatment ($P > 0.15$ for all comparisons). However, by the conclusion of each experiment, our blue ventral coloration scores were consistently lower for Castrated Males than for either Testosterone Males or Control Males (Fig. 3). Black ventral coloration was rarely observed in Castrated Males and was generally more pronounced in Testosterone Males than Control Males by the conclusion of our experiments (Fig. 3). Further, the initial development of both blue and black ventral coloration was accelerated in Testosterone Males relative to Control Males, despite their similar scores by the conclusion of our experiments (data not shown). We observed very little blue or black ventral coloration in Control Females, but treatment with exogenous testosterone resulted in the atypical development of both blue and black ventral coloration among Testosterone Females (Fig. 3D). At 65 d post-treatment, our categorical measures of blue and black ventral coloration were comparable between Testosterone Males and Testosterone Females (Fig. 3D), despite pre-treatment sexual differences in both blue ($\chi^2 = 10.50$; $P = 0.001$) and black ($\chi^2 = 8.46$; $P = 0.004$) ventral coloration.

Ventral patch size.—At 420 d post-treatment, we did not detect any difference in blue throat patch size among our male treatment groups (Fig. 4A). However, Castrated Males had significantly smaller blue abdominal patches than Control Males and Testosterone Males (Fig. 4B). We also observed significant differences in the size of black throat (Fig. 4A) and abdominal (Fig. 4B) patches among all male treatment groups. Black ventral coloration was almost entirely absent in Castrated Males, while Testosterone Males possessed enlarged black throat and abdominal patches relative to the intermediate patch sizes exhibited by Control Males.

Ventral hue, saturation, and brightness.—At 420 d post-treatment, Castrated Males exhibited significantly brighter (i.e., relatively more white than black) blue throat patches than Testosterone Males, although neither group differed significantly from Control Males (Fig. 5A). We did not detect any significant treatment effect on the saturation of blue throat patches, although Castrated Males tended to exhibit less saturation than either Control Males or Testosterone

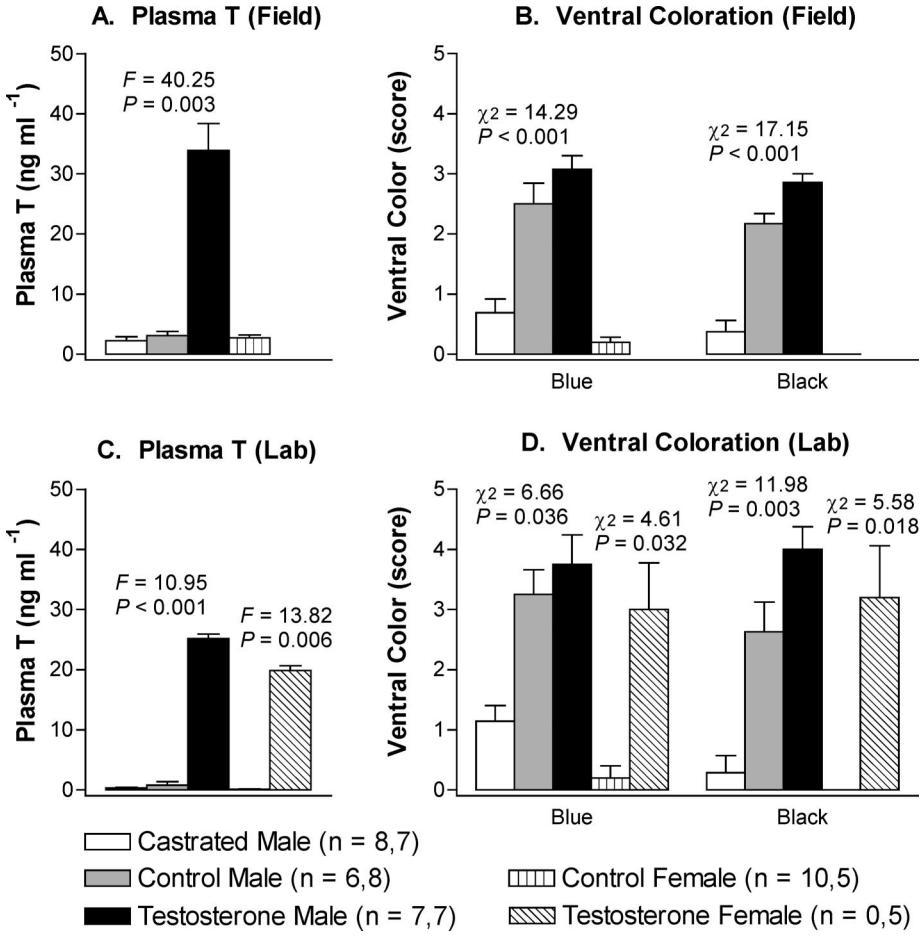

Fig. 3. Mean ± 1 SE plasma testosterone (left panels) and categorical scores for blue and black ventral coloration (right panels) in *Sceloporus undulatus* treatment groups in our field (A–B; 47 d post-treatment) and lab (C–D; 65 d post-treatment) experiments. Sample sizes are reported for the field and lab experiments, respectively. Statistics are reported for ANOVA (plasma testosterone) and non-parametric Kruskal-Wallis analyses (coloration scores) among male treatment groups (Castrated, Control, Testosterone) or between female treatment groups (Control, Testosterone). Note that Testosterone Females were not included in the field experiment.

Males (Fig. 5A). Castrated Males exhibited significantly brighter (i.e., relatively more white than black) and less saturated (i.e., relatively more gray than blue) blue abdominal patches than either Control Males or Testosterone Males, which did not differ in either regard (Fig. 5B). Hue of blue throat and abdominal patches did not differ among male treatment groups.

Dorsal hue, saturation, and brightness.—At 420 d post-treatment, Castrated Male chevrons exhibited a lower hue (i.e., ''very dark brown'' vs. ''yellowish red''; Munsell Soil Color Charts, 1994, GretagMacbeth, New Windsor, NY) and were less bright than those of Control Males and Testosterone Males (Fig. 5C). Control Males exhibited significantly brighter dorsolateral coloration than either Castrated Males or Testosterone Males (Fig. 5D), whereas Testosterone Males exhibited a significantly lower dorsolateral hue than either Castrated Males or Control Males (i.e., ''reddish brown'' versus ''yellowish red''; Munsell colors; Fig. 5D). Saturation of dorsal chevrons and dorsolateral area did not differ among male treatment groups.

Discussion

In free-living juvenile *Sceloporus undulatus*, the development of sexual dichromatism is paralleled by a similar sexual divergence in plasma

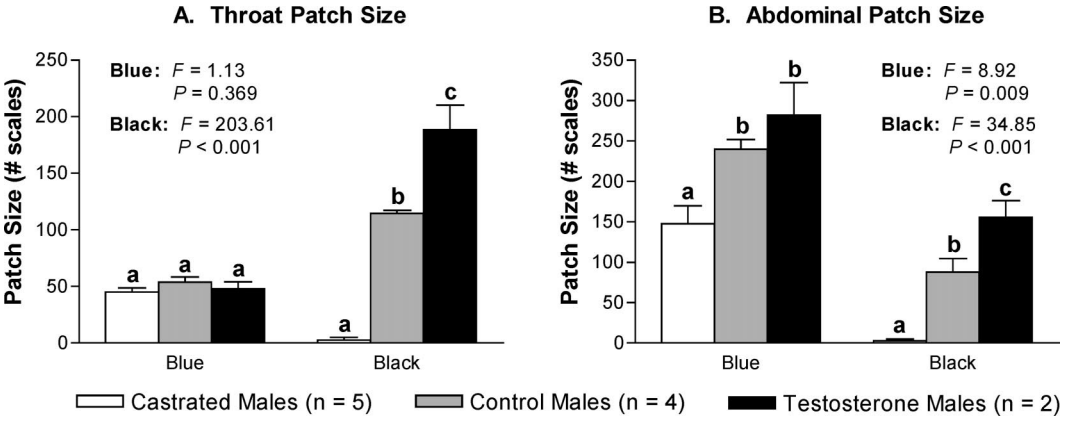

Fig. 4. Patch size (mean ± 1 SE) of blue and black (A) throat and (B) abdominal patches among male *Sceloporus undulatus* treatment groups at 420 d post-treatment. Statistics are reported for ANOVA of patch size with treatment (Castrated, Control, Testosterone) as the main effect. Lowercase letters denote *post hoc* statistical separation of treatment groups (Ryan-Einot-Gabriel-Welsch test).

testosterone, which suggests a functional relationship between testosterone and coloration. Our experiments verify this functional relationship: (1) castration inhibits the development of male-specific dorsal and ventral coloration in juvenile males, (2) exogenous testosterone restores the expression of male-specific dorsal and ventral coloration in juvenile males, and (3) exogenous testosterone promotes the atypical development of male-specific ventral coloration in juvenile females. The ecological relevance of our induced plasma testosterone levels is supported by their close agreement with mean plasma testosterone measured in field-active juvenile males during the period when male-typical coloration develops. However, plasma testosterone was atypically low in intact Control Males, compared to field-active juvenile males of similar age. We can only speculate as to the cause of this unexpected result, but it is conceivable that our experimental conditions (e.g., confinement in a field enclosure or lab cage) may have introduced a ''caging'' artifact by suppressing plasma testosterone. Despite the similar plasma testosterone levels of Control Males, Castrated Males, and Control Females, the latter two groups never developed male-typical coloration. One possibility is that these groups differed in ''physiological activity'' of testosterone (e.g., free versus bound hormone, receptor saturation, conversion to other steroids) despite uniformly low plasma testosterone levels. A related possibility is that these treatment groups differed with regard to other androgens (e.g., 5α-dihydrotestosterone) that have been shown to influence male-specific coloration in lizards (Hews and Moore, 1995). While male-specific coloration may be fully expressed even at low plasma testosterone levels, its development is clearly suppressed by castration, and our results unambiguously show that exogenous testosterone promotes the development of such coloration in both females and castrated males. Thus, we conclude that androgens are critical for the development and expression of sexually dimorphic dorsal and ventral coloration in *S. undulatus*.

Our conclusion is consistent with evidence supporting the critical role of androgens in the mediation of blue and black ventral coloration in other phrynosomatids (reviewed in Cooper and Greenberg, 1992; Quinn and Hews, 2003). Castration inhibits expression of blue ventral coloration in males of sexually dichromatic species (e.g., Kimball and Erpino, 1971; Hews et al., 1994), while exogenous androgens typically induce development of blue ventral coloration in both males and females (e.g., Rand, 1992; Hews and Moore, 1995; Quinn and Hews, 2003). In sexually monochromatic *Sceloporus jarrovii*, both males and females exhibit blue throat and abdominal patches, presumably due to the evolutionary gain of blue ventral coloration in females from an ancestral condition of sexual dichromatism (Wiens, 1999). As in sexually dichromatic species, the expression of blue throat coloration in juvenile male *S. jarrovii* is inhibited by castration, but promoted by exogenous testosterone (R. Cox, V. Zilberman, and H. John-Alder, unpubl. data). However, treatment of juvenile male or female *Sceloporus virgatus* with exogenous testosterone does not produce blue abdominal patches (Abell, 1998), which have been evolutionarily lost in males of this

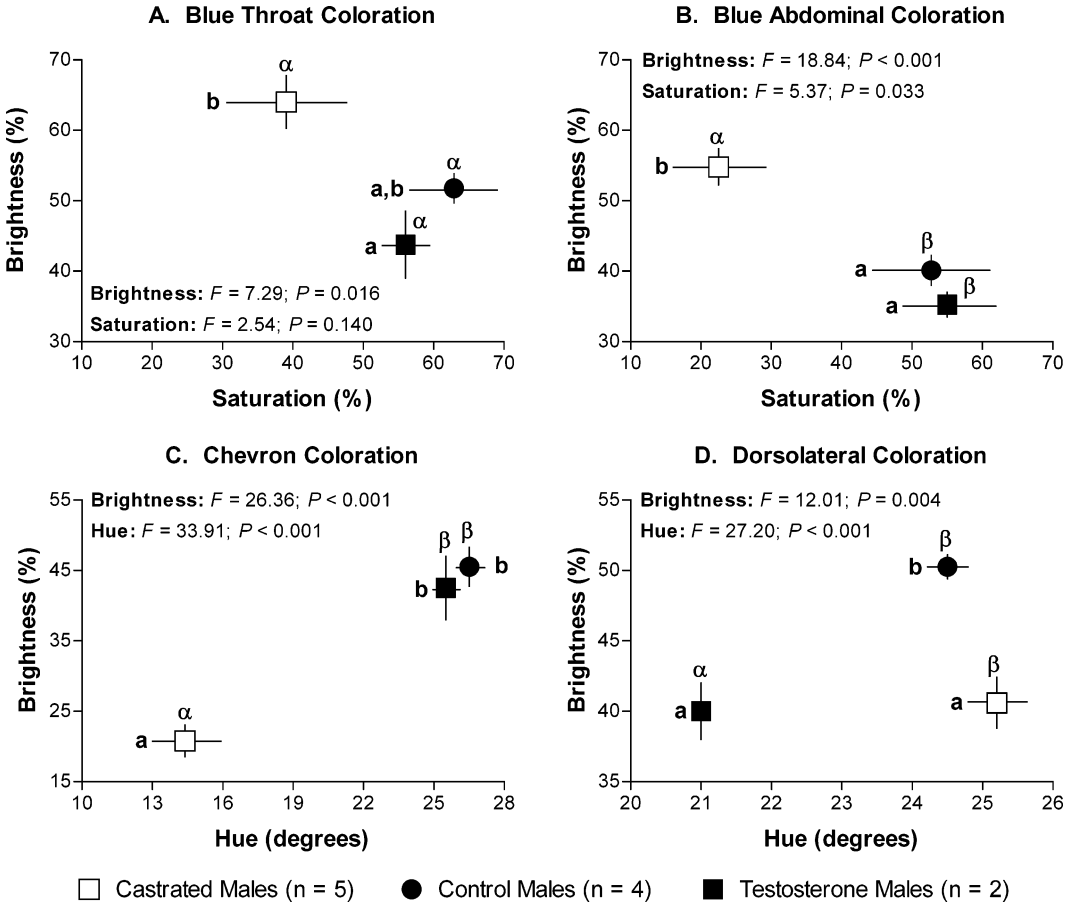

Fig. 5. (A–B) Brightness and saturation (mean ± 1 SE) of blue (A) throat, and (B) abdominal patches among male *Sceloporus undulatus* treatment groups at 420 d post-treatment. Hue of blue throat and abdominal patches did not differ among treatment groups. (C–D) Brightness and hue (mean ± 1 SE) of (C) dorsal chevrons, and (D) dorsolateral area. Saturation of dorsal chevrons and dorsolateral area did not differ among treatment groups. Statistics are reported for ANOVA of hue, saturation, and brightness with treatment (Castrated, Control, Testosterone) as the main effect. Greek letters denote *post hoc* statistical separation (Ryan-Einot-Gabriel-Welsch test) of treatment groups along the horizontal axis (saturation or hue), and lowercase letters denote *post hoc* statistical separation along the vertical axis (brightness). Note that Castrated Males (open symbols) differ significantly from Control Males and Testosterone Males (filled symbols) for most traits.

sexually monochromatic species (Wiens, 1999). Thus, in species that exhibit blue ventral coloration in one or both sexes, the expression of this coloration in both males and females may be critically dependent upon androgens. However, the cellular elements necessary for this response to androgens may be absent in species where ventral blue patches have been evolutionarily lost (see Quinn and Hews, 2003; Hews and Quinn, 2003).

In some phrynosomatids, ontogeny influences the sensitivity of blue ventral coloration to circulating androgens. For example, hatchling female *Urosaurus ornatus* develop male-specific blue abdominal coloration in response to exogenous 5α-dihydrotestosterone, but adult females do not respond to the same treatment (Hews and Moore, 1995). In other phrynosomatids, the effects of castration and/or exogenous androgens on blue ventral coloration are often less pronounced in adult animals than in juveniles (reviewed in Hews and Quinn, 2003). Further, adult male ventral patch expression typically exhibits little seasonal variation, despite dramatic differences in circulating androgen levels between breeding and non-breeding males (reviewed in Cooper and Greenberg, 1992). We were able to induce the development of male-specific ventral coloration in lab-reared female *Sceloporus undulatus* by administering ex-

ogenous testosterone as late as 6 months of age, at which point intact Control Males were already developing ventral coloration. Thus, the ontogenetic window for androgen organization of ventral blue coloration apparently extends at least until the onset of physiological maturation. We do not know whether older adult female *S. undulatus* would be insensitive to exogenous androgens, as in *Urosaurus ornatus* (Hews and Moore, 1995). However, Rand (1992) observed a stimulatory effect of exogenous testosterone on blue ventral coloration in adult female *S. u. erythrocheilus* (*sensu lato,* Leache and Reeder, 2002), and similarly concluded that this trait is organized at sexual maturation.

In contrast to the existing literature on ventral coloration, our study is the first to provide direct experimental evidence that testosterone influences the expression of male-specific dorsal coloration in *Sceloporus undulatus*. Unlike ventral coloration, dorsal coloration is capable of short-term physiological change and typically varies with season (breeding versus non-breeding) and social status (dominant versus subordinate) in males of this and other related species (Carpenter, 1962; Castrucci et al., 1997; Smith and John-Alder, 1999). Other seasonally variable lizard sexual dichromatisms are similarly influenced by androgens (reviewed in Cooper and Greenberg, 1992). Adult females of many lizard species develop bright orange or yellow coloration when gravid, and exogenous testosterone (as well as progesterone and possibly estradiol) promotes the expression of this coloration (Cooper and Ferguson, 1972; Cooper and Clarke, 1982; Cooper and Crews, 1987). Adult males of some *Eumeces* and *Sceloporus* species develop bright orange heads during the breeding season; the expression of this coloration is inhibited by castration of adult males and promoted by exogenous testosterone administered to females or castrated males (Edgren, 1959; Cooper et al., 1987; Rand, 1992). Seasonal fluctuations in circulating testosterone correlate with the production of orange pteridine pigments in *S. u. erythrocheilus*, suggesting seasonal hormonal activation of xanthophores and pteridine biosynthesis (Morrison et al., 1995). Similar mechanisms may influence yellowish red dorsal chevron coloration in male *S. undulatus*.

Our study reinforces the prevailing consensus that sex steroids are critical to the regulation of sexual dichromatism in lizards. However, we still lack a thorough understanding of the underlying physiological mechanisms whereby sex steroids coordinate the expression of complex sexual dimorphisms in coloration. For example, what combination of physiological mechanisms enable androgens to promote the permanent development of dark ventral coloration while simultaneously regulating the transient expression of light dorsal coloration? The expression of blue ventral coloration requires both (1) the reflection of short (blue) wavelengths of light by superficial iridophores and (2) the absorption of other wavelengths by dispersed melanin in underlying melanophores; black ventral coloration requires only the latter (Cooper and Greenberg, 1992; Morrison et al., 1995; Hews and Quinn, 2003). In *Sceloporus*, androgens may permanently organize iridophore structure at maturation to reflect blue light (Morrison et al., 1995; Quinn and Hews, 2003), and circulating androgens may be required to maintain melanophore expansion and the vivid expression of both black and blue coloration (Kimball and Erpino, 1971). Our experimental results agree with the hypothesis that ventral patch expression involves both the organization of iridophore structure and the activation of melanophore expansion. Juvenile males that are castrated after some ventral coloration has developed maintain faint blue throat and abdominal patches, but lose all ventral black coloration (Fig. 1, Castrated Male). Further, when adult male *S. undulatus* with fully developed ventral patches are castrated, ventral blue coloration becomes less vivid (John-Alder et al., 1996). Basal testosterone levels in non-breeding adult males (or experimental Control Males, see above) are presumably sufficient to maintain ventral patch expression, which varies little between seasons (Rand, 1992).

Our observed effects of testosterone on dorsal coloration may be largely indirect, involving general activation of the sympathetic system to enable physiological color response to seasonal and social stimuli. During the breeding season, resident male *Sceloporus undulatus* become lighter in response to experimentally induced territorial intrusions by subordinate males (Smith and John-Alder, 1999). This response is not observed in females or in males outside of the breeding season, suggesting that seasonal and sex differences in circulating testosterone may influence the sensitivity of the color response to social stimuli (see also Castrucci et al., 1997 in *Urosaurus ornatus*). Socially induced stress can produce either melanin aggregation (lightening) or melanin dispersion (darkening), depending upon whether alpha- or beta-adrenergic receptors are predominantly stimulated in target melanophores (reviewed in Cooper and Greenberg, 1992). In *S. undulatus*, dorsal lightening can be induced *in vitro* by alpha-adrenergic stimulation and *in vivo* by epinephrine

and the specific alpha-agonist fluoronorepinephrine, while dorsal darkening can be induced by beta-adrenergic stimulation and by stress-related adrenocorticotrophic hormone and melanophore-stimulating hormone (H. John-Alder, R. Tsao, M. Gleba, and L. Smith, unpubl. data). The complexity of this system may enable a single stimulus to effect opposite color responses in tissue-specific fashion. For example, tissue-specific (dorsal vs. ventral) differences in adrenergic receptor type could explain our observation that testosterone promotes both melanin aggregation (dorsal lightening) and melanin dispersion (ventral blue and black patch expression) in *S. undulatus*. In contrast, castration may remove an important component of sympathetic arousal, thereby inhibiting color responses involving either receptor type.

Much work remains to be done to integrate proximate cellular and physiological regulatory mechanisms within the larger ecological and evolutionary context of sexual dichromatism. However, most studies to date have demonstrated that sex steroids play an important regulatory role in the expression of sex-specific coloration in reptiles (Cooper and Greenberg, 1992). Our own experimental results convincingly show that testosterone is critical to the development and expression of male-specific dorsal and ventral coloration in *Sceloporus undulatus*. In addition to regulating proximate cellular color responses in sex-specific fashion, sex steroids coordinate the expression of sex differences in other correlated traits (e.g., social behavior, body size, immune response). Thus, focus on the endocrine regulation of coloration should enable future investigators to integrate their findings within a larger ecological context encompassing behavior, morphology, physiology, and life history.

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