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ORIGINAL PAPER

Steroid correlates of multiple color traits in the spiny lizard, *Sceloporus pyrocephalus*

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Abstract Conspicuous coloration in females is less well studied compared to that in males. Adult female Mexican boulder spiny lizards (*Sceloporus pyrocephalus)* have conspicuously colored throat, or gular, regions, ranging from red to yellow, while adult males only weakly express such color in their gular region. Both sexes have dark blue–black gular stripes and venter stripes. Understanding proximate mechanisms underlying trait expression can aid in understanding trait function. To characterize the proximate mechanisms potentially influencing color variation among field-captured male and female *S. pyrocephalus*, we quantified three aspects of color (hue, saturation, brightness) for three body locations (gular region, gular stripes, venter stripes) and then assessed how color was related to reproductive state and concentrations of the plasma steroid hormones testosterone (T) and corticosterone (CORT) in males and T, CORT, and 17-β estradiol (E_2) in females. Testes volume was not related to variation in color or in hormones, perhaps because most males were in peak reproductive condition. Large vitellogenic follicles as opposed to oviductal eggs were associated with higher E_2 in females. Males with more dull gular stripes and females with dull venter stripes had significantly higher CORT. Females with red gular regions and pale grey gular stripes had higher T and E_2 concentrations compared to females with a more yellow gular region and darker gular stripes. Thus, gular region color in females could communicate reproductive state; dull gular stripes in males and dull venter stripes in females could communicate stress status.

Keywords *Sceloporus pyrocephalus* · Multiple color traits · Sex steroid hormones

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Introduction

Color signals expressed in one sex frequently are driven by sexual selection (Andersson 1994). In many species, sexual selection has contributed to the evolution of such conspicuous traits in males, while natural selection often favors inconspicuous traits in females (Darwin 1871; Fisher 1954; Lande 1980; Kirkpatrick 1982; Andersson 1994). However, some species have bright female as well as bright male coloration. One hypothesis for this phenomena is that female coloration is a genetically correlated response to selection on male coloration (Lande 1980). However, if different selection pressures act on males and females, the sexes may differ in color evolution (e.g., Heinsohn et al. 2005). For example, females may vary in secondary color coincident with reproductive cycles (fish: Rowland et al. 1991; Baird 1988; reptiles: Medica et al. 1973; Mitchell 1973; Ferguson 1976; Cooper et al. 1983; Cooper and

Greenberg 1992; Watkins 1997; Cuadrado 2000; Hager 2001; Weiss 2002; Calisi 2006; birds: Montgomerie and Thornhill 1989; Roulin et al. 2001; mammals: Dixson 1983; Setchell and Wickings 2004). Coloration that is associated with reproductive cycles may be advantageous to both sexes by reducing or avoiding time- and/or energy-costly courtship displays, copulation acts, altered thermoregulation and metabolism, and other costs associated with predation risk (Cooper and Greenberg 1992). Other hypotheses formulated for the expression of male-typical color traits should also be examined in females, including the badges-of-status hypothesis in which aggression is communicated via trait size (Maynard Smith and Harper 1988), the recognition hypothesis in which trait variation facilitates individual recognition (Whitfield 1986), and the immunocompetence hypothesis in which the production of traits communicates aspects of immune function to potential mates (Folstad and Karter 1992).

Multiple signals are receiving increasing attention from evolutionary biologists. Such signals may each communicate different information, different traits may amplify the same information, or some traits may be non-functional remnants of past evolution (Moller and Pomiankowski 1993; Johnstone 1995; Brooks and Couldridge 1999; Whiting et al. 2003). One species that expresses multiple color traits, and does so in both sexes, is the Mexican boulder spiny lizard, *Sceloporus pyrocephalus*. This species exhibits some color patterns (Fig. 1) that are expressed in both sexes including blue/black gular stripes and dark venter stripes. Although much variation in gular stripe and venter color is evident within the sexes, this area of color appears to vary within an individual during the breeding season more so in females than males. In addition, females also express a conspicuous coloration underlying the bars in the gular region that changes and varies from yellow to red within a breeding season, while males only weakly express such gular region color (pale yellow to white). Both males and females inflate their gular region and flatten their ventral area laterally in aggressive interactions with same-sex conspecifics (R. Calisi, personal observation), suggesting color of the gular region, gular stripes, and venter stripes are visible during at least some social interactions. Color perception in this species is unknown, but many reptiles have retinas with cones involved in true color perception (Fleishman et al. 1993; Yokoyama and Yokoyama 1996; Yokoyama 1997). Thus, these colorations could function in social signaling.

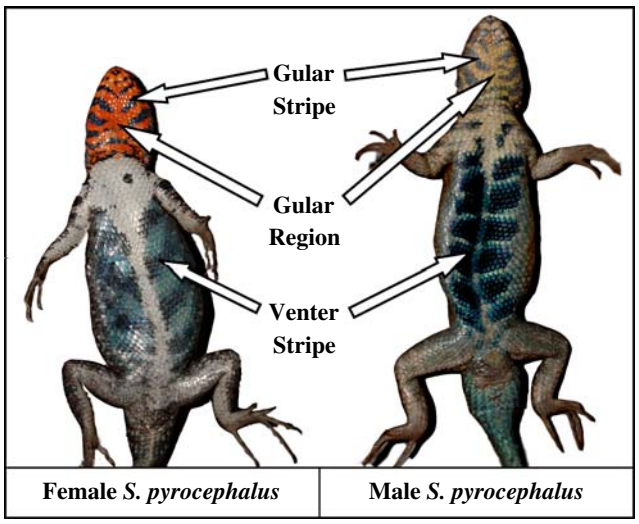

Fig. 1 Gular and venter color variation exhibited by male and female *S. pyrocephalus*

Hormones such as the sex steroids testosterone (T), 17-β estradiol (E_2), and the ''stress hormone'' corticosterone (CORT) can affect secondary color in vertebrates, including reptiles (e.g., Cooper and Greenberg 1992; Hews and Moore 1995; Salvador et al. 1996; Hews and Quinn 2003). Reproductive state, such as testes size in males and ovarian stage in females, can be related to changes in sex steroid hormones (e.g., Crews 1979a, b; Moore and Lindzey 1992; Whittier and Tokarz 1992) and can therefore also be associated with color variation in some species (Cooper and Greenberg 1992; Watkins 1997; Weiss 2002). Although numerous studies have examined hormone associations with one secondary color trait in one sex, there is a dearth of studies examining multiple color traits and hormonal and reproductive correlates in both sexes.

Using a correlational approach we examined potential hormonal mechanisms influencing the multiple secondary color traits in adults of *S. pyrocephalus.* We measured T and CORT in males and females of *S. pyrocephalus*, as well as E_2 in females because of the role of E_2 in follicle maturation (Whittier and Tokarz 1992), to assess if and how these hormones were related to variation in color traits (namely, the gular region, gular stripes, and venter stripes). We also examined relationships between hormone concentrations and testes size or ovarian state. Due to the individual variation of color change seen more so in females than males during the breeding season, we suspect that female, as opposed to male, hormone fluctuations may be more highly associated with color change and could be indicative of reproductive state. If indeed aspects of the hormonal profile correlate with variation in reproductive state, then endocrine mechanisms may mediate color variation.

Materials and methods

Fieldwork was done during the mid-breeding season (Ramirez-Bautista and Olvera-Becerril, 2004) of this oviparous species, *S. pyrocephalus*, in tropical dry forest habitat

during 17 June–6 July 2004. We sampled blood between 1600 and 1800 h, from eight different locations, averaging 620 m in elevation, within a 40 km radius of Lombardia, Michoacan, Mexico (751 m 19.17662 N, 102.66351 W). Immediately following capture, blood samples from 53 adults (25 males and 28 females) were taken from the retro-orbital sinus using heparinized microcapillary tubes. The time to capture the lizard and complete collection of blood (''capture and bleed time'') was recorded and examined for correlations to hormone concentrations. Body size, as measured by snout-to-vent (SVL) length, was also measured. Blood samples were kept on wet ice in a ice cooler until hand centrifuged (within 12 h), and plasma was removed and stored in liquid nitrogen until placed in a –20°C freezer at the Universidad Autonoma de Mexico (UNAM) for five months, when they were transferred on dry ice to Indiana State University (ISU) and then stored at –20°C prior to assay two months later.

Color measurements

Immediately after capture and bleeding, individuals were photographed. Digital images were taken of the throat and venter using an Olympus C-700 digital camera in a shaded area. Lighting was standardized by using a camera flash, and each lizard was photographed on a neutral grey-colored cardstock board that served as a color standard. The distance between camera lens and lizard was standardized and ranged from 10 to 12 cm. Digital images were saved as TIFF files.

Using Adobe Photoshop Elements (Version 3.0) software, we collected color data for several characteristics (hue, saturation, brightness) from these field images of each lizard (Fig. 1). Two measurements from the gular area included: (1) the larger reddish area in females (or the yellow–white area in males), hereafter gular region; (2) the dark, contrasting horizontal stripes on the throat, hereafter gular stripes. A third color area we measured was the dark, black or gray bars on the abdomen, hereafter venter stripes. Hue, saturation, and brightness values for the gular region, gular stripes, and venter stripes were measured by taking an average color sample from four arbitrarily chosen 5×5 pixels areas. For *hue*, measured in degrees, smaller values indicated more red (less orange–yellow) gular regions, darker gular stripes (more black/blue), and darker venter patches (more black/dark blue). *Saturation*, given in a percent value, measures the height and width, or sharpness, of the associated reflectance peak. In this species, we observed that deep red colors had tall, narrow peaks, corresponding to high saturation values. Oranges and yellows had shorter, wider peaks, corresponding to lower saturation. The opposite was true for the dark blue/black coloration of the gular stripes and venter patches: there is no peak for a black (zero reflectance) spectral curve and therefore dark black coloration is considered unsaturated, whereas lighter blue or gray express a higher degree of saturation. Brightness, given in a percent value, captures the amount of white, or total reflectance, of a color, with higher values indicating brighter colors. When needed, hue, saturation, and brightness were adjusted using the background standard to maintain the same color standard values between digital images. This process ensured any difference in ambient lighting during photographing was greatly lessened or eliminated.

Reproductive stage

Lizards were preserved in 70% ethanol on day of capture and were necropsied two months later. Female reproductive stage was dichotomized as either vitellogenic or oviductal, as all females collected had large vitellogenic follicles or eggs in the oviduct (oviductal). Late vitellogenic as well as oviductal stages are common for this time of year in this species (Ramirez-Bautista and Olvera-Becerril 2004). The volume of each follicle or egg also was calculated, using the formula for a prolate spheroid:

$$V = 4/3\pi(\text{greatest length}/2) \times (\text{greatest width}/2)^2.$$

The average volume of follicles in vitellogenic females was 191.0 mm^2 ± 43.23, $N = 9$, and the average volume of eggs in oviductal females was 251.3 mm^2 ± 81.99, $N = 16$.

All males collected were sexually mature with waxy femoral pore secretions and enlarged hemipenes, both of which are known to be activated by higher plasma levels of T (e.g., Hews and Moore 1995) associated with sexual maturity. Male reproductive stage was assessed by measuring testes volume in males to the nearest 0.1 mm using the formula previously stated for a prolate spheroid; the average of left and right testis volume was analyzed for each male.

Due to a simultaneous study involving the examination of the reproductive tract in these lizards, follicle/egg or testes sizes were inadvertently omitted from measurement in one male and three females; thus, a total of 24 males and 25 females were used for analyses associated with reproductive state.

Hormone measurement

We measured total plasma concentrations of testosterone (T), 17-beta estradiol (E_2), and corticosterone (CORT) in females, and T and CORT in males (following Wingfield and Farner 1975, as modified by Moore 1986; Hews et al. 1994). Samples were analyzed in two assays; the first assay (assay 1) was for plasma from females and the

second assay (assay 2) for plasma from males. We first equilibrated plasma samples (usually 20 µl, minimum 10 µl volume) overnight with 2,400 cpm of each tritiated steroid for calculations of steroid recoveries from individual samples. We extracted the steroids from the plasma samples with diethyl ether (2 × 2 ml), dried the ether phase in a 37°C bath with nitrogen gas, resuspended the steroids in 10% ethyl acetate pseudosaturated with ethylene glycol in isooctane, and refrigerated the samples overnight at 4°C. For chromatographic separation of steroids we added the samples to celite microcolumns (celite: propanediol: ethylene glycol [4:1:1 w:v:v] in three aliquoits over celite:water [3:1 w:v] in one aliquot). Neutral lipids, progesterone (data not included because of assay failure), T, E_2, and CORT were eluted from the columns with increasing concentrations of ethyl acetate in isooctane to separate steroids according to polarity (neutral lipids, progesterone, T, E_2, and CORT in 3.0, 3.6, 4.5, 4.5, 4.0 and 4.0 ml of 0, 1, 10, 20, 40 and 52% of ethyl acetate in isooctane, respectively). Short columns were used for assay 2 in which steroids were eluted from the columns with increasing concentrations of ethyl acetate in isooctane to separate steroids according to polarity: neutral lipids and DHT (not assayed), T, and CORT in 1.5, 2.2 and 2.8 ml of 10, 20, and 52% of ethyl acetate in isooctane, respectively. After evaporating the organic solvents, we resuspended the steroids in phosphate buffered saline (0.1%) with gelatin and sodium azide and refrigerated the samples overnight at 4°C before performing the radioimmunoassay. Duplicate aliquots of each sample were then incubated overnight at 4°C with antibodies (testosterone, WLI-T3003, from RDI Division of Fitzgerald Industries Int., Concord MA, USA; 17-B estradiol, E26-47, from Endocrine Sciences, Calabasas Hills, CA, USA; corticosterone, B3-163, from Esoterix Inc., Calabasas Hills, CA, USA) and tritiated steroids (NEN Life Sciences, now PerkinElmer Life Sciences; testosterone NET 553, 17-B estradiol NET 517, corticosterone NET 399). Unbound steroids were then removed by adding Dextran-coated charcoal and centrifuging after 10 min. We added a toluene-based scintillation fluid to the supernatant and counted radioactivity after a minimum 12-h hold. Calculations of steroid concentrations were corrected for individual sample recovery and individual plasma volume. For assay 1, average individual recoveries were 85.0% for T, 84.3% for E_2, and 53.1% for CORT. Within-assay coefficient of variation, based on six standard samples run with the plasma samples, was 2.0% for T, 6.3% for E_2, and 16.4% for CORT. For assay 2, average individual recoveries were of 84.8% for T, and 75.4% for CORT, and the intra-assay coefficient of variation of six standard columns was 2.0% for T and 9.4% for CORT.

Statistical analyses

We confirmed equality of variance and normality of $\log_{10}$-transformed data for all variables. All statistical tests were two-tailed and alpha was set at 0.05. For each color trait (gular region, gular stripe, venter stripe) we used principal components analyses (PCA) in SYSTAT 8.0 to extract factor scores from the three attributes we obtained from the digital photos (hue, saturation, brightness). The factor loading scores were then used in multiple linear regression analyses (general linear model procedure, GLM) to examine the relationships between the color trait value, plasma hormone concentrations, and reproductive state (testes volume in males; vitellogenic versus oviductal stage in females). We assessed effects of possible covariates, including the time it took to capture and bleed a lizard (capture and bleed time), body size (SVL), collection date, collection location, collection time (of day), and, for females, clutch size. These variables would be included as covariates if significant.

We also individually analyzed hue, saturation or brightness color attribute for each color trait (gular region, gular stripe, and venter stripe) and how each was associated with plasma hormone concentrations and with reproductive stage (testes volume or vitellogenic versus oviductal). We consider these individual GLM analyses to be exploratory, because of the number of tests conducted, and set alpha at 0.05.

Results

The time to capture and bleed ranged from two to nine minutes in both males (mean ± SD = 4.57 ± 2.08) and females (4.75 ± 1.71). Hormone concentrations were not affected by capture and bleed time in either males ($F_{2,25} = 0.305$, $P = 0.740$) or females ($F_{3,24} = 0.799$, $P = 0.507$), nor were they affected by SVL (males: $F_{2,25} = 1.558$, $P = 0.230$; females: $F_{3,24} = 0.129$, $P = 0.942$), collection date (males: $F_{2,25} = 2.322$, $P = 0.119$; females: $F_{3,24} = 0.2155$, $P = 0.120$), collection location (males: $F_{2,25} = 0.115$, $P = 0.892$; females: $F_{3,24} = 0.422$, $P = 0.739$), time of day of collection (males: $F_{2,25} = 0.548$, $P = 0.588$; females: $F_{3,24} = 1.499$, $P = 0.258$), or clutch size in females ($F_{3,21} = 0.471$, $P = 0.706$).

Males

The average plasma concentration of T in males was 36.121 ng/ml (range: 0.04–77.83). Plasma CORT averaged 4.084 ng/ml (range: 0.300–25.14). Plasma concentrations of T and CORT in males were not significantly related to each other ($F_{1,26} = 0.107$, $P = 0.747$). Body size (SVL)

was not related to color variables (hue, saturation, and brightness) of the gular region ($F_{3,20} = 1.383$, $P = 0.277$), the gular stripe ($F_{3,20} = 0.326$, $P = 0.807$) or venter stripe ($F_{3,20} = 0.608$, $P = 0.617$).

The PC1 for gular region color in males (analyzing hue, saturation, and brightness) explained 56.5% of the total variance (Eigen value = 1.70), with head hue demonstrating a large positive loading (0.917) and head saturation demonstrating a large negative loading (-0.924); the loading value for head brightness was 0.036. The PC2 for gular region color explained 34.7% of the total variance (Eigen value = 1.043), with gular brightness demonstrating a large negative loading (-0.992). The PC1 for gular stripe color (analyzing hue, saturation, and brightness) explained 51.9% of the total variance (Eigen value = 1.56), with stripe saturation demonstrating a large negative loading (-0.885) and stripe brightness demonstrating a large positive loading (0.785); the loading value for stripe hue was 0.399. The PC2 for gular stripe color explained 33.5% of the total variance (Eigen value = 1.006), with stripe hue demonstrating a large negative loading (-0.891). The PC1 for venter stripe color (analyzing hue, saturation, and brightness) explained 43.2% of the total variance (Eigen value of 1.30), with venter hue demonstrating a large negative loading (-0.817) and venter saturation demonstrating a large positive factor loading (0.782); the loading value for venter saturation was 0.134. The PC2 for venter color explained 34.4% of the total variance (Eigen value of 1.03), with venter saturation demonstrating a large negative loading (-0.957). All Eigen values for the third principle components (PC3) were less than 1.0 for each body location; therefore, PC3 was not subjected to further analysis for any data set.

Reproductive condition–color relationships

Testes volume did not explain the significant variation in PC1 or PC2 factor loadings for either gular region, gular stripes, or venter stripes (gular region, $F_{2,18} = 2.857$, $P = 0.084$; gular stripe, $F_{2,18} = 0.063$, $P = 0.939$; venter stripe, $F_{2,18} = 0.625$, $P = 0.546$; Table 1). In the exploratory GLM analyses, variation in testis volume did not explain significant variation in any of the individual color attributes for the gular region (hue, $F_{1,19} = 2.121$, $P = 0.162$; saturation, $F_{1,19} = 0.861$, $P = 0.365$; brightness, $F_{1,19} = 3.488$, $P = 0.077$), for the gular stripe (hue, $F_{1,19} < 0.001$, $P = 0.985$; saturation, $F_{1,19} = 0.335$, $P = 0.569$; brightness, $F_{1,19} = 0.738$, $P = 0.401$) or for the dark venter stripe (hue, $F_{1,19} = 0.022$, $P = 0.884$; saturation, $F_{1,19} = 0.694$, $P = 0.415$; brightness, $F_{1,19} = 0.809$, $P = 0.380$; Table 1).

Hormone–color relationships

Models examining the association of T or CORT with the PC1 and PC2 factor loading scores for gular region, for gular stripe, and for venter stripe color were not significant (Table 2). Similarly, in the exploratory GLM analyses, variation in hormone concentrations and color was not related in this sample of males (Table 1); plasma hormone concentrations did not significantly explain variation in hue, saturation, or brightness of the gular region, the gular stripes, or the venter stripes. However, results suggest that gular stripe brightness was negatively associated with increasing CORT (Table 1; Fig. 2; model, $P = 0.076$; within model results for CORT, $P = 0.038$).

In sum, male variation in only one color trait may be associated with variation in a hormone. Specifically, brightness of the gular stripes tended to be negatively associated with higher plasma CORT. The reproductive variable of testis volume did not contribute to explaining male color variation for any trait.

Females

Average plasma concentrations of T, E_2, and CORT were 5.216 ng/ml (range: 0.01–26.15), 0.234 ng/ml (range: 0.010–1.277) and 9.599 ng/ml (range: 3.380–32.291), respectively. Plasma concentrations of T and E_2 were positively related to each other and negatively related to CORT ($F_{2,25} = 5.474$, $P = 0.011$). Body size (SVL) was not related to color variables (hue, saturation, and brightness) of the gular region ($F_{3,23} = 2.353$, $P = 0.138$), the gular stripe ($F_{3,23} = 1.325$, $P = 0.291$), or venter stripe ($F_{3,23} = 1.764$, $P = 0.182$).

Reproductive state was significantly predicted by plasma E_2, which was higher in females with vitellogenic follicles compared to females with oviductal eggs (Fig. 3; F_1,23 = 10.772, $P = 0.003$). The relationship between reproductive state and plasma T concentrations was similar, although only marginally significant (Fig. 3; $F_{1,23} = 4.235$, $P = 0.051$). Reproductive state and CORT were not significantly associated ($F_{1,23} = 0.045$, $P = 0.956$). Variation in the other reproductive measure for females (follicle/egg size) was positively related to E_2 ($F_{1,23} = 7.151$, $P = 0.018$) but did not predict plasma concentrations of T ($F_{1,23} = 2.172$, $P = 0.154$) or of CORT ($F_{1,23} = 0.084$, $P = 0.774$).

In females, the PC1 for gular region color (hue, saturation, and brightness) explained 61.8% of the total variance (Eigen value = 1.86), with head hue, saturation, and brightness demonstrating factor loading scores of 0.928, –0.567, and 0.819, respectively. The Eigen value was less than 1.0 for the gular region and was therefore not subjected to further analysis. For the gular stripe color analysis

Table 1 Exploratory analyses of individual color attributes (hue, saturation, brightness) and hormones in males

	DF	R^2	*F*	Sig.
Gular Region Hue				
Model	2,22	0.068	0.807	0.459
	Coef.	**Std. Coef.**	**t**	**Sig.**
T	0.040	0.245	1.187	0.248
CORT	–0.021	–0.073	–0.355	0.726
Gular Region Saturation				
Model	2,22	0.035	0.401	0.675
	Coef.	**Std. Coef.**	**t**	**Sig.**
T	–0.041	–0.143	–0.681	0.503
CORT	0.055	0.110	0.524	0.606
Gular Region Brightness				
Model	2,22	0.111	1.369	0.275
	Coef.	**Std. Coef.**	**t**	**Sig.**
T	0.005	0.143	0.709	0.486
CORT	–0.017	–0.289	–1.432	0.166
Gular Stripe Hue				
Model	2,22	0.040	0.464	0.635
	Coef.	**Std. Coef.**	**t**	**Sig.**
T	0.022	0.133	0.634	0.533
CORT	0.048	0.162	0.774	0.447
Gular Stripe Saturation				
Model	2,22	0.079	0.950	0.402
	Coef.	**Std. Coef.**	**t**	**Sig.**
T	–0.019	–0.097	–0.472	0.641
CORT	0.088	0.257	1.252	0.224
Gular Stripe Brightness				
Model	2,22	0.209	2.906	0.076^{+}
	Coef.	**Std. Coef.**	**t**	**Sig.**
T	0.008	0.152	0.798	0.433
CORT	–0.037	–0.419	–2.202	0.038*
Venter Stripe Hue				
Model	2,22	0.010	0.109	0.897
	Coef.	**Std. Coef.**	**t**	**Sig.**
T	–0.005	–0.057	–0.270	0.790
CORT	0.012	0.076	0.359	0.723
Venter Stripe Saturation				
Model	2,22	0.040	0.455	0.640
	Coef.	**Std. Coef.**	**t**	**Sig.**
T	–0.008	–0.052	–0.248	0.806
CORT	0.050	0.188	0.898	0.379
Venter Stripe Brightness				
Model	2,22	0.056	0.651	0.531
	Coef.	**Std. Coef.**	**t**	**Sig.**
T	–0.032	–0.106	–0.511	0.615
CORT	–0.116	–0.220	–1.059	0.301

Analyses using general linear models examined each color attribute of each color trait (gular region, gular stripe, venter stripe) in relation to plasma levels of T (testosterone) and CORT (corticosterone)

of hue, saturation, and brightness, PC1 explained 51.1% of the total variance (Eigen value = 1.53), with stripe saturation demonstrating a large negative loading (–0.878) and brightness demonstrating a large positive loading (0.872); the factor loading score for stripe saturation was 0.872. The PC2 for gular stripe color explained 34.2% of the total

Table 2 Color, as summarized in principle components analyses, and relationships with plasma hormones, for males and females

PC1					PC2				
	DF	R^2	*F*	Sig.		*DF*	R^2	*F*	Sig.
Males									
Gular Region Color					**Gular Region Color**				
Model	2,22	0.060	0.707	0.504	Model	2,22	0.098	1.196	0.321
	Coef.	**Std. Coef.**	**t**	**Sig.**		**Coef.**	**Std. Coef.**	**t**	**Sig.**
T	0.263	0.213	1.029	0.314	T	–0.146	–0.118	–0.583	0.566
CORT	–0.228	–0.106	–0.510	0.615	CORT	0.604	0.280	1.380	0.181
Gular Stripe Color					**Gular Stripe Color**				
Model	2,22	0.135	1.722	0.202	Model	2,22	0.111	1.375	0.247
	Coef.	**Std. Coef.**	**t**	**Sig.**		**Coef.**	**Std. Coef.**	**t**	**Sig.**
T	0.204	0.166	0.832	0.414	T	–0.060	–0.049	–0.242	0.811
CORT	–0.679	–0.315	–1.585	0.127	CORT	–0.719	–0.334	–1.655	0.112
Venter Stripe Color					**Venter Stripe Color**				
Model	2,22	0.026	0.297	0.746	Model	2,22	0.053	0.621	0.547
	Coef.	**Std. Coef.**	**t**	**Sig.**		**Coef.**	**Std. Coef.**	**t**	**Sig.**
T	–0.041	–0.033	–0.157	0.876	T	0.010	0.008	0.040	0.968
CORT	–0.348	–0.161	–0.765	0.453	CORT	–0.496	–0.230	–1.107	0.280
Females									
Gular Region Color					**Gular Region Color**				
Model	3,24	0.127	1.164	0.344	Eigen value < 1.0				
	Coef.	**Std. Coef.**	**t**	**Sig.**					
T	–0.451	–0.299	–1.307	0.204					
CORT	–0.808	–0.198	–1.025	0.316					
E_2	0.137	0.234	1.030	0.313					
Gular Stripe Color					**Gular Stripe Color**				
Model	3,24	0.172	1.665	0.201	Model	3,24	0.236	2.478	0.086
	Coef.	**Std. Coef.**	**t**	**Sig.**		**Coef.**	**Std. Coef.**	**t**	**Sig.**
T	–0.640	–0.424	–1.904	0.069$^+$	T	0.794	0.526	2.462	0.021*
CORT	–0.150	–0.037	–0.195	0.847	CORT	0.204	0.050	0.277	0.784
E_2	0.246	0.419	1.894	0.070$^+$	E_2	–0.262	–0.446	–2.100	0.046*
Venter Stripe Color					**Venter Stripe Color**				
Model	3,24	0.335	4.024	0.019*	Model	3,24	0.181	1.764	0.181
	Coef.	**Std. Coef.**	**t**	**Sig.**		**Coef.**	**Std. Coef.**	**t**	**Sig.**
T	–0.263	–0.174	–0.873	0.391	T	–0.043	–0.029	–0.130	0.898
CORT	0.826	0.203	1.200	0.242	CORT	–1.700	–0.417	–2.225	0.036*
E_2	–0.252	–0.430	–2.169	0.040*	E_2	0.030	0.051	0.231	0.819

For each sex, first and second principle components for the color (hue, saturation, and brightness) of each body location (gular region, gular stripe, and venter stripe) were extracted and used to assess relationships of factor loading scores with T (testosterone) and CORT (corticosterone) for males, and T, CORT and E_2 (17-β estradiol) for females by using general linear models

variance (Eigen value = 1.025), with gular stripe hue demonstrating a large negative loading score (–0.990). The PC1 for the venter stripe color analysis of hue, saturation, and brightness explained 57.0% of the total color variance (Eigen value = 1.71), with venter stripe hue, saturation, and brightness loading scores of 0.465, 0.965, and –0.750, respectively. The PC2 for venter stripe color explained 38.3% of the total variance (Eigen value = 1.149), with venter stripe hue, saturation, and brightness loading scores of 0.868, 0.068, and 0.626, respectively. All Eigen values for the third principle components (PC3) were less than 1.0 for each body location; therefore, PC3 was not subjected to further analysis for any data set.

Reproductive condition–color relationships

In females, reproductive state did not explain variation in color. The PC1 and PC2 factor loadings for each of the

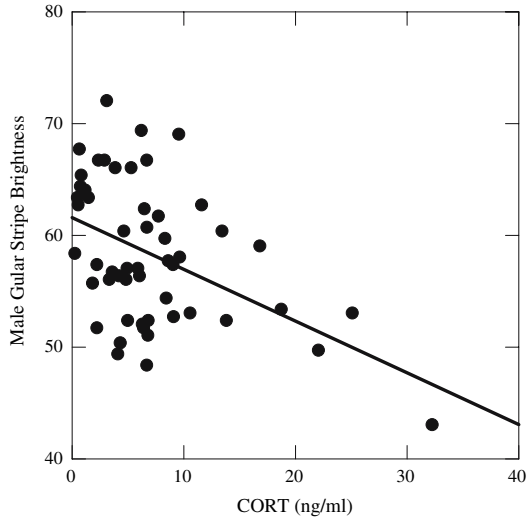

Fig. 2 Male gular stripe brightness and corticosterone (CORT). The overall model assessing how variation in male gular stripe brightness was explained by plasma hormone concentrations was close to significant ($P = 0.07$), and CORT variation explained significant variation in male color ($P = 0.037$), suggesting males with high CORT have venter stripes that are less bright

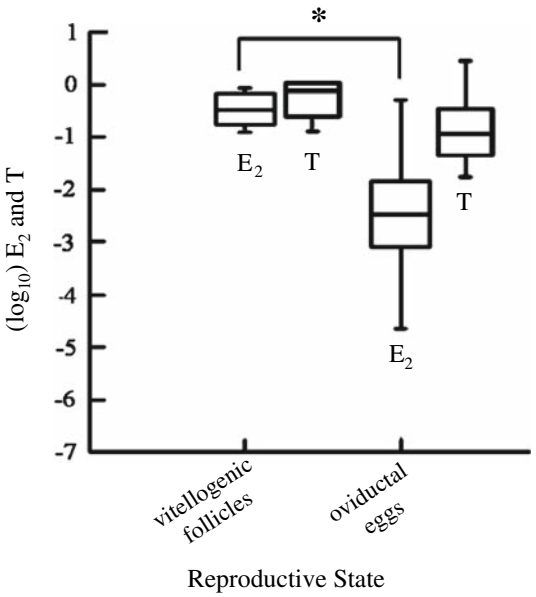

Fig. 3 Mean (± SE) plasma concentrations of 17-β estradiol (E_2) and testosterone (T) in females with large vitellogenic follicles versus oviductal eggs. Only the between-state difference in E_2 was statistically significant (*$P = 0.023$); although, the difference in T was nearly significant ($P = 0.051$)

three-color traits were not significantly associated with reproductive state (gular region, PC1: $P = 0.807$; gular stripe, PC1: $P = 0.620$, PC2: $P = 0.889$; venter stripe, PC1: $P = 0.183$, PC2: $P = 0.706$). Similarly, the PC1and PC2 factor loadings for each of the three-color traits were not significantly associated with follicle/egg size variation (gular region, PC1: $P = 0.807$; gular stripe, PC1: $P = 0.296$, PC2: $P = 0.725$; venter stripe, PC1: $P = 0.243$, PC2: $P = 0.736$).

In the exploratory GLM analyses, reproductive state in females did not explain variation in any of the individual color attributes of the gular region (hue, $F_{1,23} = 0.357$, $P = 0.556$; saturation, $F_{1,23} < 0.001$, $P = 0.992$; brightness, $F_{1,23} = 0.010$, $P = 0.922$), of the gular stripe (hue, $F_{1,23} = 0.002$, $P = 0.965$; saturation, $F_{1,23} = 0.207$, $P = 0.653$; brightness, $F_{1,23} = 0.182$, $P = 0.673$) or of the venter stripe (hue, $F_{1,23} = 0.453$, $P = 0.508$; saturation, $F_{1,23} = 1.468$, $P = 0.238$; brightness, $F_{1,23} = 1.574$, $P = 0.222$).

Hormone–color relationships

In the principle components analysis there were significant relationships between female color traits and only one hormone (Table 2): variation in factor loadings for venter stripe PC1 was significantly related to E_2 (model, $P = 0.019$; within model results for E_2, $P = 0.040$). Neither the factor loadings for gular region PC1 nor the loadings for gular stripe PC1 and PC2 or venter stripe PC2 were associated with variation in hormone titers.

In the exploratory GLM analyses, hormone concentrations in females were significantly related to, or exhibited trends, with variation in some aspects of gular color (Table 3). For gular stripe, hue tended to be more grey and less black with higher T and E_2 levels (model: $F_{3,24} = 2.478$, $P = 0.086$; within model results for T, $P = 0.027$; within model results for E_2, $P = 0.056$), and saturation tended to decrease (be more pale) with higher T and E_2 levels (model: $F_{3,24} = 2.848$, $P = 0.059$; within model results for T, $P = 0.017$; within model results for E_2, $P = 0.019$). In contrast, for the gular region, saturation tended to increase (get more red) with increasing T and E_2 (model: $F_{3,24} = 2.792$, $P = 0.062$; within model results for T, $P = 0.014$, within model results for E_2, $P = 0.030$; Fig. 4). Neither gular region brightness nor gular region saturation varied significantly with any hormones (Table 3).

Some variation in venter stripe color attributes was also explained by variation in hormones (Table 3). For venter stripes, higher plasma E_2 was associated with increased saturation (model $P = 0.047$; within model results, $P = 0.078$) and increased brightness (model $P = 0.013$, within model results $P = 0.062$). By contrast, decreased brightness of the venter stripe was significantly associated with increasing CORT (model, $P = 0.013$; within model results for CORT, $P = 0.027$; Fig. 5).

In sum, specific aspects of different color traits in females were associated with different hormones. Higher E_2 and T were significantly related to more red gular regions and pale grey gular stripes, and higher CORT was signif-

Table 3 Exploratory analyses of individual color attributes (hue, saturation, brightness) and hormones in females

	DF	R^2	*F*	*Sig.*
Gular Region Hue				
Model	3,24	0.050	0.418	0.742
	Coef.	**Std. Coef.**	**t**	**Sig.**
T	0.069	0.151	0.632	0.533
CORT	–0.200	–0.161	–0.799	0.432
E_2	0.017	0.094	0.398	0.694
Gular Region Saturation				
Model	3,24	0.259	2.792	0.062$^+$
	Coef.	**Std. Coef.**	**t**	**Sig.**
T	0.038	0.561	2.662	**0.014***
CORT	–0.001	–0.004	–0.024	0.981
E_2	0.013	0.484	2.312	**0.030***
Gular Region Brightness				
Model	3,24	0.093	0.816	0.497
	Coef.	**Std. Coef.**	**t**	**Sig.**
T	–0.006	–0.117	–0.503	0.619
CORT	–0.039	–0.269	–1.364	0.185
E_2	0.002	0.088	0.380	0.707
Gular Stripe Hue				
Model	3,24	0.237	2.478	0.086$^+$
	Coef.	**Std. Coef.**	**t**	**Sig.**
T	0.314	0.505	2.363	**0.027***
CORT	–0.183	–0.109	–0.604	0.552
E_2	0.103	0.426	2.008	0.056$^+$
Gular Stripe Saturation				
Model	3,24	0.263	2.848	0.059$^+$
	Coef.	**Std. Coef.**	**t**	**Sig.**
T	0.044	0.542	2.576	**0.017***
CORT	–0.034	–0.156	–0.879	0.388
E_2	0.016	0.524	2.508	**0.019***
Gular Stripe Brightness				
Model	3,24	0.093	0.818	0.497
	Coef.	**Std. Coef.**	**t**	**Sig.**
T	–0.013	–0.180	–0.772	0.447
CORT	–0.044	–0.218	–1.104	0.281
E_2	0.006	0.192	0.830	0.415
Venter Stripe Hue				
Model	3,24	0.149	1.398	0.268
	Coef.	**Std. Coef.**	**t**	**Sig.**
T	–0.027	–0.107	–0.474	0.640
CORT	–0.192	–0.286	–1.497	0.147
E_2	–0.017	–0.178	–0.792	0.436
Venter Stripe Saturation				
Model	3,24	0.277	3.070	**0.047***
	Coef.	**Std. Coef.**	**t**	**Sig.**
T	0.093	0.169	0.811	0.425
CORT	–0.290	–0.194	–1.102	0.281
E_2	0.082	0.381	1.841	0.078$^+$
Venter Stripe Brightness				
Model	3,24	0.358	4.456	0.013*
	Coef.	**Std. Coef.**	**t**	**Sig.**
T	0.023	0.114	0.581	0.567
CORT	–0.208	–0.390	–2.351	0.027*
E_2	0.029	0.381	1.956	0.062$^+$

Analyses using general linear models examined each color attribute of each color trait (gular region, gular stripe, venter stripe) in relation to plasma levels of T (testosterone), CORT (corticosterone) and E_2 (17-β estradiol)

icantly associated with dull venter stripes. Both E_2, and to a lesser extent T, were also associated with variation in reproductive state.

Discussion

Males

Color and hormones

None of the PC1 factor loadings extracted from hue, saturation, and brightness variables, for gular region, for gular stripe, or for venter stripe significantly explained variation in steroid hormone plasma concentrations. In the exploratory analyses of individual color attributes, there also was no suggestion that color traits likely to have a strong melanin component (gular stripe, venter stripe) varied with androgen level. Other studies have found that androgens contribute to expression of melanin-based color traits, but typically they have focused on animals that exhibit a greater range of plasma T than we captured in this study (e.g., Kimball and Erpino 1971; Cox et al. 2005), or focused on earlier organizational actions of androgens (e.g., juveniles versus adults; summarized in Cooper and Greenberg 1992; Hews and Quinn 2003; Cox et al. 2005). That adult levels of plasma T did not vary with other aspects of coloration (e.g., blues or reds) is also consistent with other studies of adult coloration in Phrynosomatid lizards. For example, most work suggests that androgens have little activational effects on abdominal blue patches of males in several *Sceloporus* species (see Hews and Quinn 2003; Cox et al 2005), or on red labial coloring in red-lipped prairie lizards (*Sceloporus undulatus erythrocheilus*, Rand 1992). Orange (vs. brown) head coloration in the wall lizard, *Psammodromus algirus*, was not affected by elevating exogenous testosterone (Salvador et al. 1997). However, relatively few endocrine studies of lizard coloration have carefully quantified of variation in color attri-

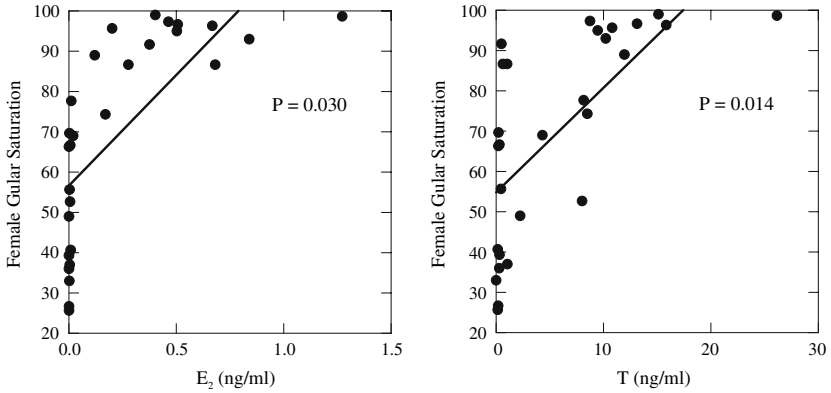

Fig. 4 Saturation of gular region in females in accordance with concentrations of 17-β estradiol (E_2) and testosterone (T). The statistical model examining the relationship between gular saturation with E_2, T, and corticosterone was not significant ($P = 0.062$); however, within model results indicated that high concentrations of E_2 and T are associated with high gular saturation. High saturation corresponds to red–orange, whereas lower saturation indicates yellow–orange. Therefore, females with high E_2 and T were generally red–orange, while females with low E_2 and T were generally yellow–orange

butes of individual color traits such as in this study, and thus more such work is warranted.

Although variation in plasma T did not explain color variation, plasma CORT tended to be associated with dull (low brightness) gular stripes in the exploratory analyses of individual color attributes for each trait. In green anoles lizards (*Anolis carolinensis*) higher stress is associated with body darkening from green to brown (Summers and Greenberg 1994) but this color change, due to melanosome movements within dermal melanophores, may be mediated primarily by catecholaminergic signals that, in part, are stimulated by increased CORT (Greenberg 2002). Chronically high CORT can negatively affect immune function (Dhabhar and McEwen 1997; French et al. 2006). If the brightness of venter stripes in males correlates with some aspect of health, female mate choice could be based on this color trait. If high CORT correlates with infection by communicable parasites, such female choice could confer immediate fitness benefits, good genes for parasite resistance to offspring, or both (c.f. Lopez and Martin 2005). Female mate choice based on male traits, however, seems to be uncommon or absent in territorial polygynous lizard species (Olsson and Madsen 1995; Tokarz 1995).

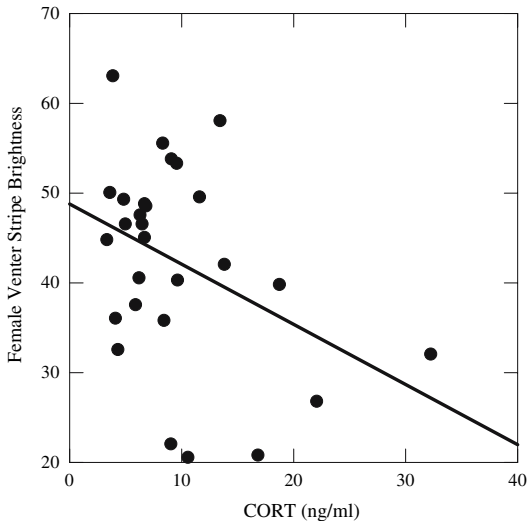

Fig. 5 Female venter stripe brightness and corticosterone (*CORT*). Variation in venter stripe brightness was significantly explained by variation in plasma hormone concentrations (model $P = 0.013$); CORT was the only significant component within the model ($P < 0.027$). Females with high plasma CORT had less bright venter stripes

Reproductive stage and hormones

Male testis volume was not significantly related to concentrations of T or CORT. Although testis size in this species increases over the breeding season (Ramirez-Bautista and Olvera-Becerril 2004), testis volume may have been maximized in the populations in this small geographic area during the time of collection, therefore limiting volume variation. Similarly, variation in testis size among reproductively competent males can have little ability to predict steroidogeneic capacity (Moore and Lindzey 1992).

Color and reproductive stage

A relationship was not evident between color and reproductive stage in males. Male color may arise due to organizational effects of steroid hormones acting in a critical period during development, rather than be temporarily activated after sexual maturation (Arnold and Breedlove 1985; c.f. Hews and Quinn 2003 for lizard coloration). Our data suggest the there is little color variation among males

that vary in testis size, although we did not sample males across a very wide range of reproductive states.

Females

Color and hormones

Our results suggest that the PC1 for venter stripe color may be correlated to concentrations of E_2. Results from exploratory analyses show mostly trends suggesting high E_2 and low CORT are associated with bright venter stripes. High T and E_2 also may be related to red (as opposed to yellow) gular regions and pale grey (as opposed to black) gular stripes.

Reproductive stage and hormones

Concentrations of E_2 were high in females carrying vitellogenic follicles, while E_2 was low in females carrying oviductal eggs. Although T and CORT were not significantly related to reproductive stage, the three hormones were significantly interrelated: concentrations of T and E_2 were positively related to each other and negatively related to CORT. One explanation for these associations could be steroid hormone mediation of follicle maturation, in addition to any direct effects of hypothalamic and/or pituitary hormones (involving the hormones regulation of either the gonadal or interrenal axes). Concentrations of E_2 typically show positive correlations with ovarian follicle size in reptiles (e.g., Crews 1980; Whittier and Tokarz 1992). An increase in T could facilitate an increase and/or maintain high concentrations of E_2 if converted via aromatase enzymes (Whittier and Tokarz 1992; Baum 2002). Increased CORT may inhibit production of sex steroid hormones such as T and E_2 via negative interactions between the hypothalamic-pituitary-interenal axis and the hypothalamic-pituitary-gonadal axes (Greenberg and Wingfield 1987; Tokarz 1987; Rivier and Rivest 1991; DeNardo and Licht 1993). However, contrary to our results, some studies show a positive interaction between the HPI and HPG axes during extended times of reproductive activity (Wilson and Wingfield 1994; Knapp and Moore 1997; Moore et al. 2000; Leary et al. 2004) and/or when associated with transitions between reproductive tactic expression, as seen in some anurans (Leary et al. 2004, 2006). This suggests that testosterone may mediate expression of certain energetically costly traits that require CORT-mediated mobilization of energy stores (Wingfield et al. 1995; Moore and Jessop 2003). Concentration amount of CORT may be of importance, as an increasing number of studies have reported that moderate increases in adrenocorticoids may positively affect reproduction, while extremely high concentrations may inhibit reproduction (see review: Moore and Jessop 2003). It is also important to note that concentrations of adrenocorticoids, like CORT, may elicit a more or less effective response on the reproductive axis in reptiles, as well as amphibians and birds, depending on whether the animal is in its breeding season or not (Romero 2002).

Reproductive stage and color

Color was not directly related to follicle or egg size. However, plasma E_2 was significantly higher in vitellogenic females than in oviductal females (as was T, though not significantly so), and E_2 and T tended to be higher in females with a red gular region, pale gray gular stripes, and bright venter stripes. These relationships may be consistent with the following scenario, which posits a time lag involved with color change. A female experiences increased T and E_2, perhaps directly functioning in follicle maturation, which causes her gular region to change from yellow to red. When T and E_2 are highest, the gular region is most red. Once the follicles enter the oviduct to become fertilized, T and E_2 drop, no longer needed to mature the follicle. While T and E_2 may drop quickly, color change occurs relatively slowly, and a female with fertilized, oviductal eggs may still express a red gular region due to this time lag but have lower T and E_2. Testing these hypotheses will require longitudinal studies of changes in color and hormone concentrations in females across the ovarian cycle and manipulative studies to assess the contribution of changing plasma hormone concentrations to female color.

These associations of female color and reproductive state suggest a role for the color in signaling reproductive condition. Little is known about the mating system of *S. pyrocephalus*. Fertilization most likely takes place as the vitellogenic follicle leaves the ovary and enters the oviduct (Whittier and Tokarz 1992). A female *S. pyrocephalus* could signal receptivity, as she is intense red in her gular region, pale/gray in her gular stripe, and bright in her venter stripe when E_2 and T are relatively high, which occurs during these late vitellogenic stages. A yellow gular region, dark gular stripes, and dull venter stripes may thus signal non-receptivity. In accordance with the courtship stimulation hypothesis (Cooper and Greenberg 1992), signaling non-receptivity to a male may reduce many costs potentially associated with courtship and copulation. Assessment of female color by males may also, in some scenarios, be favored by reduced courtship costs to males. Females often appear to resist male courtship and copulation attempts ($n > 50$ copulation attempts observed), by lunging and biting at the male. This is consistent with results from other studies of orange–red breeding colorations in lizards (Cooper 1986; Watkins 1997), which mostly all support the courtship rejection hypothesis when the

breeding coloration is most intense. Future work should experimentally explore this hypothesis in *S. pyrocephalus*. The aggressive behaviors could also ensure that only stronger males (which could be indicative of good genes for phenotypic vigor) can subdue a female and father her offspring, favoring good quality sons (''the sexy son hypothesis,'' Weatherhead and Robertson 1979).

We found that attributes of the color traits in female *S. pyrocephalus* correlated with endocrine profiles that characterize late vitellogenic versus oviductal females. While this supports reproductive signaling hypotheses, other hypotheses deserve consideration. For example, in striped plateau lizards, *Sceloporus virgatus*, orange female reproductive color also correlates with body size and egg mass, as well as body condition and ectoparasite load (Weiss 2006). We did not find an association of color with body size, nor with average egg/follicle size. Color and parasite relationships should also be examined *S. pyrocephalus*. The expression of androgen-mediated traits can reduce immune function (Folstad and Karter 1992). If aspects of female coloration are mediated by androgens, consistent with the positive association we observed, then coloration may also provide information about parasite loads to conspecifics. This information could be used by males in mate choice, which has been suggested for a few lizard species (Tokarz 1992; Olsson 1993; Whiting and Bateman 1999), although not in the territorial Phrynosomatids.

Information about health or parasite loads also may be used by potential opponents in female-female aggressive interactions. Female *S. pyrocephalus* exhibit aggressive intra and inter-sexual behavioral interactions during the breeding season (Calisi 2006). Like males, females extend their gular region and flatten their ventral area laterally when confronted with conspecific females. Also like males, females lunge and bite at the dorsal areas of same-sex opponents, usually ending with one lizard leaving/being driven off the particular boulder of the occurrence. They also will lunge and bite at courting males (R. Calisi, personal observation). High plasma T and E_2 have been associated with increased female aggression in lizards (Woodley and Moore 1999a, b; Rubenstein and Wikelski 2005), and further behavioral endocrine studies may help to elucidate possible relationships between secondary sexual characteristics, hormonal state and behavior.

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