Female secondary coloration in the Mexican boulder spiny lizard is associated with nematode load

R. M. Calisi[1], J. H. Malone[1] & D. K. Hews[2]

1 Department of Biology, The University of Texas at Arlington, Arlington, TX, USA
2 Department of Biology, Indiana State University, Terre Haute, IN, USA

Abstract

Male secondary sexual traits purportedly facilitate female choice of a potential mate's quality. One example of male quality is the ability to resist detrimental infections by parasites; sexual traits that reflect parasite infection can allow females to select for parasite resistance in future offspring. High parasite numbers can constrain the exaggeration of male traits, but few studies have examined the effects of parasites on female secondary traits. Female Mexican boulder spiny lizards *Sceloporus pyrocephalus*, undergo a change in secondary sexual coloration over their breeding season and express red gular regions and gray gular stripes in association with late stages of follicle maturation during the reproductive cycle, as well as blue ventral stripes. We examined if color change associated with the female reproductive cycle varied in hue, saturation and brightness in relation to high nematode loads. Our results suggest that high nematode loads of particular areas of the body are correlated with dull, as opposed to bright, secondary sexual coloration in females of *S. pyrocephalus*. One reason females could be most vulnerable to nematodes during late reproductive stages is because of corresponding high concentrations of circulating testosterone known to occur in this species at this time. Testosterone has demonstrated immunosuppressive effects in many species; however, results have varied and conclusive evidence for its role in immune function remains controversial. Using a subset of individuals from this study, we found that high concentrations of plasma testosterone were significantly related to high nematode loads. These findings prompt further studies examining the physiological attributes of parasite infestation, the honest signaling capabilities of nematode loads and their potential role in sexual selection for coloration in females.

Introduction

Theoretical and empirical studies suggest that sexual selection has driven the evolution of conspicuous traits in males, while natural selection has driven the evolution of inconspicuous traits in females (Darwin, 1871; Fisher, 1954; Lande, 1980). However, females of some species (in addition to those exhibiting sex-role reversal) also exhibit bright coloration (reviews in Amundsen & Pärn, 2006; Omland & Hofmann, 2006). Such female coloration may result as a genetically correlated response to selection on male coloration (Lande, 1980), although current evidence in passerine birds suggests that selection producing colorful female plumage has acted on males and females independently (Amundsen & Pärn, 2006). Bright coloration in females often may indicate reproductive stage, as seen in fish (Baird, 1988; Rowland, Baube & Horan, 1991), reptiles (reviewed in Cooper & Greenberg, 1992; and see Watkins, 1997; Cuadrado, 2000; Hager, 2001; Weiss, 2002; Calisi, 2006), birds (Montgomerie & Thornhill, 1989; Roulin *et al.*, 2001a)

and mammals (Dixson, 1983; Setchell & Wickings, 2004). Such reproductive coloration may benefit females as signaling nonreceptivity to a male may reduce courtship and copulation-associated costs such as energy expenditure, predation risk and altered thermoregulation and metabolism (Cooper & Greenberg, 1992), thus selecting for the trait, and perhaps for male responses to female color variation. While female secondary coloration often indicates female reproductive receptivity, it is less clear how often such secondary sexual coloration correlates with female health, or 'quality,' and how commonly such information is used in same-sex and between-sex signaling contexts.

In males, secondary sexual traits may communicate resistance for parasitic infections. Females choosing mates based on this signal may gain direct or indirect fitness benefits (Hamilton & Zuk, 1982; Kirkpatrick & Ryan, 1991), and males may assess color of other males because it imparts key information about condition and dominance (Senar, 2006). One prediction of parasite-mediated sexual selection hypothesis is that relatively high parasite loads should constrain trait

exaggeration (Zuk *et al*., 1990). Although studies have documented the predicted trait–parasite relationships (reviews in Andersson, 1994; Owens, 2006), the prevalence and the strength of parasite-mediated sexual selection are both widely debated because comparative studies are contradictory and experimental data have been difficult to generate (McLennan & Brooks, 1991; John, 1995; Getty, 2002; Owens, 2006).

Until recently, comparatively fewer studies examined female coloration in the context of sexual selection (Getty, 2002; Amundsen & Pärn, 2006; Senar, 2006). As in males, color signals in females may function both in intersexual selection (e.g. Roulin *et al*., 2001*a*) and in intrasexual selection (e.g. Trail, 1990; Dijkstra, Seehausen & Groothuis, 2008; Rosvall, 2008). Comparatively little work has detailed the role of parasites in female color signals. To better understand the functions of female coloration, we need more studies on conspicuous female coloration that examine factors affecting expression of these traits, and to detail whether and how these traits are used in inter- and intrasexual interactions.

We thus explored nematode–coloration relationships in females of a species in which females also have showy secondary sexual coloration. We studied parasites and coloration in female Mexican boulder spiny lizards *Sceloporus pyrocephalus*, which express different color patterns that covary with the reproductive cycle (Calisi & Hews, 2007). *Sceloporus pyrocephalus* is an oviparous species (Ramírez-Bautista & Olvera-Becerril, 2004) in which both sexes express bright ventral coloration, that is, within the genus, more often only expressed in males (Wiens, 2000; Hews & Quinn, 2003). Both sexes exhibit blue-black stripes on the throat (gular) and venter, but females also express a conspicuous red coloration within the gular region (varying from red to yellow during their breeding season, Fig. 1), while males only weakly express such color in the gular region. In females, a combination of red coloration in the gular region and pale gray gular stripes correlates with late

vitellogenic follicles, while yellow gular coloration with black gular stripes is related to early vitellogenic stages (Calisi & Hews, 2007). Males and females both exhibit intra- and intersex aggression by inflating their gular region and flattening their ventral area laterally when confronted with a conspecific (R. M. Calisi & J. H. Malone, pers. obs.), suggesting that color of the gular region and the gular and venter stripes are all visible during encounters with opposing individuals. Color perception in this species is unknown, but many reptiles have retinas that express cones involved in color perception (Fleishman, Loew & Leal, 1993; Yokoyama & Yokoyama, 1996; Yokoyama, 1997), and thus variation in male and female secondary coloration may be a trait on which social selection acts.

Testosterone can influence secondary characteristics such as color (e.g. Hews & Quinn, 2003) but may also reduce immunocompetence (Folstad & Karter, 1992) and perhaps leave an individual more subject to parasitic infection. The plasma concentrations of testosterone, as well as 17β-estradiol, in females of *S. pyrocephalus* are highest during mature follicle stages of reproduction, and individuals exhibit red gular regions (as opposed to yellow) and gray (as opposed to black) gular stripes during this time (Calisi & Hews, 2007). Thus, we also reconfirm a relationship found by Calisi & Hews (2007), who found that high testosterone was significantly correlated with red gular regions and gray gular stripes. Finally, we assessed whether plasma testosterone concentration and nematode load are positively correlated. We predict that (1) female reproductive cycle color change will vary in hue, saturation and/or brightness in association with nematode load and (2) high nematode loads will be associated with high-circulating plasma concentrations of testosterone.

Materials and methods

Fieldwork was performed in tropical dry forest in Michoacan, Mexico, during the breeding season months of June–July (Ramírez-Bautista & Olvera-Becerril, 2004) in 2004 and 2005. Fifty-two mature females (13 in 2004, 39 in 2005) of *S. pyrocephalus* were captured throughout the study by noosing or hand between 14:00 and 18:00 h from eight different locations within a 40 km radius of the town of Lombardia, Michoacán, México (elevation 751 m; 19.17662°N, 102.66351°W). The snout-to-vent length (SVL) was measured with a ruler to the nearest 1.0 mm.

Blood sampling

We sampled blood from the retro-orbital sinus using heparinized microcapillary tubes. Blood samples were collected from a subset of 11 females immediately following capture in association as a part of another study examining color and hormone physiology in 2004 (see Calisi & Hews, 2007). All females were taken from the same location on the same day during the time between 16:00 and 17:30 h. The time that elapsed from capture to the completion of blood collection (mean = 5 min, range = 3–8 min) was examined
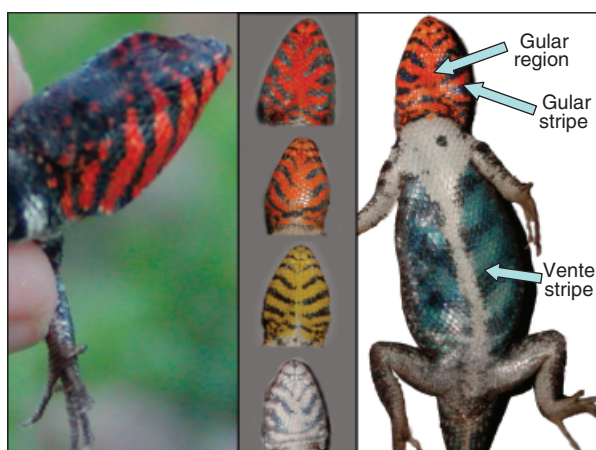

Figure 1 *Left*: a profile view of a female *S celoporus pyrocephalus*. *Middle*: female coloration of the gular region ranges from red to yellow during the breeding season. *Right*: the ventral side of a female *S. pyrocephalus*. Three regions of secondary sexual coloration were measured: the gular, gular stripe and venter stripe regions.

for possible confounding correlations to testosterone concentrations. Blood samples were kept in an ice cooler until hand centrifuged (within 12 h). Plasma was stored in liquid nitrogen until placed in a −20 °C freezer at the Universidad Autónoma de México, and transferred to Indiana State University on dry ice for analysis.

Color measurements

Digital images were taken of the gular and ventral areas immediately following capture of each lizard using an Olympus C-700 digital camera (Olympus Imaging America Inc., Center Valley, PA, USA) in a shaded area. Lighting was standardized by using the camera flash, and each lizard was photographed on a neutral gray-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. We used this method to quantify color because it allowed for immediate documentation upon field capture (some lizards can change in color when being handled for long periods of time; Norris, 1967) and greater objectivity as compared with human-assigned values using color chips (Hamilton *et al.*, 2005). This technique was also used in a previous study to quantify color in this species (Calisi & Hews, 2007).

Color data were collected on several traits of each lizard. Two measurements were taken from the gular region: one of the larger reddish area (gular region) and the other of the dark, contrasting lines that horizontally stripe the gular (gular stripes). A separate color value was taken of the dark venter stripes (Fig. 2). We assessed the color values using Adobe Photoshop Elements, Version 3.0 software (cf. Calisi & Hews,

Figure 2 A box plot of female gular hue in relation to reproductive stage. Females are most red during late-vitellogenic stages, which could possibly signal fertilization receptivity to males. A Tukey HSD multiple comparison *post hoc* test revealed significant differences between gular hue in mid-vitellogenic versus late-vitellogenic stages (**$P=0.002$) and late-vitellogenic versus oviductal stages (*$P=0.037$).

2007). Hue, saturation and brightness values for the gular area, gular stripes and venter stripes were measured by taking an average color sample from four randomly chosen 5×5 pixel areas. For hue, smaller degrees indicated redder (less orange-yellow) gular regions (average color variable value ± average SD among the four pixel areas = $17.44 \pm 2.62°$), black gular stripes (less gray = $208.81 \pm 5.84°$) and black/dark blue venter stripes (less gray/light blue = $155.79 \pm 16.85°$). Saturation measures the height and width, or sharpness, percentage of the associated reflectance peak. In this species, we found that deep red colors had tall, narrow peaks, indicating high saturation ($82.19 \pm 2.21\%$). Oranges and yellows had shorter, wider peaks, and were therefore considered less saturated. The opposite was true for the dark blue/black found in the gular stripes ($23.33 \pm 4.58\%$) and venter patches ($30.42 \pm 7.48\%$). There is no peak for a black, zero-reflectance, spectral curve. Therefore, dark black coloration was considered unsaturated, whereas lighter blue and gray shades express a higher percentage of saturation. A brightness measurement captures the amount of white, or total reflectance, of a color, with higher values indicating brighter colors (gular region: $67.78 \pm 2.43\%$, gular stripe: $34.54 \pm 3.63\%$, venter stripe: $38.74 \pm 5.39\%$). Individuals with low brightness values were termed 'dull.'

Parasite load

Lizards were euthanized by an intraperitoneal injection of Nembutal® (sodium pentobarbital), and the mouth, peritoneal cavity and all internal organs were examined for internal helminth parasites (*sensu* Mata-López, García-Prieto & León-Règagnon, 2002) at the Universidad Nacional Autonóma de México, México City by parasitologists E. Matínez-Salazar, M. A. Arízmendi-Espinósa and R. Mata-López in the laboratory of V. León-Règagnon. Roundworms (Phylum: Nematoda) were generally the only macroscopic parasite found, although one individual contained a large number (100+) of cestode larvae and no nematodes; this individual, considered an outlier, was not used in analyses. The locations of nematodes within the body, the number of individuals that had nematodes (nematode prevalence) and the number of nematodes per individual (nematode abundance) were documented.

Reproductive state

Females were necropsied to determine their stage of reproduction. The volume of each egg/follicle was estimated using the formula for a prolate spheroid:

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

Egg volume and position within the body cavity after fertilization is unknown in lizards of *S. pyrocephalus*; however, the point of fertilization may occur when follicles enter the oviduct (now termed 'eggs'). Females were categorized both visually and by using their average follicle/egg volume into having pre- ($<10\,\text{mm}^3$), mid- ($10–60\,\text{mm}^3$), late- ($>60\,\text{mm}^3$) vitellogenic follicles, or oviductal eggs.
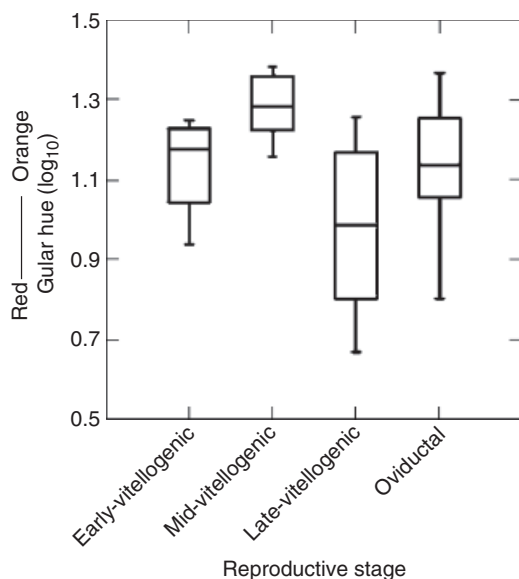
Hormone analysis

We measured total plasma concentrations of testosterone and corticosterone by radioimmunoassay (RIA) after chromatographic separation on microcolumns of diatomaceous earth (following Wingfield & Farner, 1975; as modified by Moore, 1986; Hews, Knapp & Moore, 1994). Briefly (details in Calisi & Hews, 2007), the Wingfield–Farner method involved first separating steroid hormones from each other and from neutral interfering lipids using ether extraction and phase partition column chromatography. The purified steroid hormone fractions were dried, resuspended in assay buffer and assayed in duplicate using RIA. Individual samples were corrected for plasma volumes and for individual recoveries, as assessed by adding a small amount of radiolabeled steroid hormone to each sample before the ether extraction. The standard curve was run in triplicate. Average individual recoveries for testosterone and corticosterone were 84.8 and 53.1%, respectively with 2.0 and 16.4% intra-assay coefficients of variation, respectively.

Statistical analyses

SYSTAT 8.0 (SPSS Inc., Chicago, IL, USA, 1998) software was used for all analyses. We checked for normality in the data, with log_{10} transformations of follicle and egg volume, color measurements, SVL, parasite load and testosterone concentrations successfully fulfilling normality and equal variance assumptions. We used general linear regression analyses (GLM) to model relationships among color, reproductive state, testosterone and parasite load. Color variables (hue, saturation and brightness for the gular region, gular stripe and venter stripe) and parasite loads (of the cloaca, intestine, stomach and a total load of these three areas) were individually checked for possible confounding relationships by the independent variables of the year of collection, date of collection, location of collection (in reference to one of eight possible locations in which lizards were collected), time of day of collection and SVL. Each color variable was then examined in a model with independent variables including parasite loads of the cloaca, intestine, stomach and/or the total loads. Many times, the overall model, but not individual independent variables, was statistically significant. In this case, we reduced the model to locate the independent variable that was explained by the dependent color variable. Linear regression was also used to examine the relationship between testosterone concentrations and total parasite loads. Because of the exploratory nature of these analyses, α was set at 0.05, although most results involving multiple comparisons were significant at $P < 0.01$.

Results

Reproductive state and color

Gular hue was related to reproductive stage in females ($F_{3,48} = 4.572$, $P = 0.007$). Early vitellogenic follicles were associated with orange-red gular hues, mid-vitellogenic follicles with orange hues, late-vitellogenic follicles with red hues and oviductal eggs with a range from red-orange hues (Fig. 2). This result has been previously observed in this species (Calisi & Hews, 2007). Gular saturation and brightness, as well as hue, saturation and brightness for the gular stripe and venter stripe regions were not related to reproductive state ($P > 0.30$ in all cases).

Nematode load and color

Mean total nematode abundance and standard deviation were 7 ± 18 (81% prevalence), and mean totals per body area examined were 4 ± 15 for the cloaca (52% prevalence), 2 ± 4 for the intestine (31% prevalence) and 4 ± 15 for the stomach (21% prevalence). Neither color variables nor parasite loads were related to the date of collection, location of collection, time of day of collection or SVL ($P > 0.20$ in all cases). Certain color variable and nematode load relationships were apparent from the results of reduced models (for full and reduced model results, see Table 1).

Gular region

The models consisting of gular hue ($F_{4,47} = 7.807$, $P < 0.001$), gular saturation ($F_{4,47} = 4.139$, $P = 0.006$) and gular brightness ($F_{4,47} = 3.144$, $P = 0.023$), and their relationships with cloacal, intestinal, stomach and total nematode loads were significant overall. Reducing these models indicated a negative relationship between gular hue and both total and cloaca nematode load ($F_{2,49} = 9.400$, $P < 0.001$), a positive relationship between gular saturation and cloaca nematode load ($F_{1,50} = 16.643$, $P < 0.001$), and a negative relationship between gular brightness and stomach nematode load ($F_{1,50} = 10.443$, $P = 0.002$, Fig. 3). In general, high cloaca nematode loads were related to highly saturated red, though less bright, gular regions.

Gular stripe

The model consisting of gular stripe saturation and its relationship with cloacal, intestinal, stomach and total nematode loads and gular stripe brightness was significant overall ($F_{4,47} = 2.783$, $P = 0.037$). The model consisting of gular stripe brightness showed a weak relationship, although not significant, with parasite loads ($F_{4,47} = 2.336$, $P = 0.069$). Reducing these models indicated a positive relationship between gular stripe saturation and cloaca nematode load ($F_{1,50} = 11.729$, $P = 0.001$), and a negative relationship between gular stripe brightness and stomach nematode load ($F_{1,50} = 6.519$, $P = 0.014$). Gular stripe hue was not related to nematode loads ($F_{4,47} = 0.752$, $P = 0.562$). In general, pale gray gular stripes were associated with high cloaca and stomach nematode loads.

Venter stripe

The models consisting of venter stripe hue ($F_{4,47} = 0.295$, $P = 0.025$), venter stripe saturation ($F_{4,47} = 0.600$, $P = 0.664$) and venter stripe brightness ($F_{4,47} = 0.267$, $P = 0.892$), and

Table 1 Results of full and reduced models depicting relationships between color variables and nematode loads

	d.f.	R^2	F	Sig.
Gular hue				
Model:	4,47	0.319	7.807	<0.001*
	Coef.	Std. coef.	t	Sig.
Total	−0.621	−1.307	−1.990	0.052+
Cloaca	−0.717	−1.273	−2.313	0.025*
Intestine	0.564	0.809	1.820	0.075
Stomach	0.262	0.485	0.937	0.353
Gular hue: reduced model				
Model:	2,49	0.277	9.400	<0.001*
	Coef.	Std. coef.	t	Sig.
Total	−0.229	−0.483	−3.526	0.001*
Cloaca	−0.298	−0.530	−3.867	<0.001*
Gular saturation				
Model:	4,47	0.260	4.139	0.006*
	Coef.	Std. coef.	t	Sig.
Total	0.147	0.412	0.566	0.574
Cloaca	0.081	0.191	0.312	0.756
Intestine	0.098	0.258	0.381	0.705
Stomach	0.129	0.232	0.557	0.580
Gular saturation: reduced model				
Cloaca	1,50	0.250	16.643	<0.001*
Gular brightness				
Model:	4,47	0.211	3.144	0.023*
	Coef.	Std. coef.	t	Sig.
Total	−0.063	−1.121	−1.490	0.143
Cloaca	0.060	0.906	1.436	0.158
Intestine	0.063	0.759	1.490	0.143
Stomach	−0.029	−0.448	−0.755	0.454
Gular brightness: reduced model				
Stomach	1,50	0.173	10.433	0.002*
Gular stripe hue				
Model:	4,47	0.060	0.752	0.562
	Coef.	Std. coef.	t	Sig.
Total	−0.395	−0.498	−0.606	0.547
Cloaca	0.310	0.329	0.478	0.635
Intestine	0.328	0.281	0.505	0.616
Stomach	0.524	0.582	0.899	0.373
Gular stripe saturation				
Model:	4,47	0.191	2.783	0.037*
	Coef.	Std. coef.	t	Sig.
Total	0.038	0.139	0.183	0.856
Cloaca	0.100	0.311	0.488	0.628
Intestine	0.043	0.107	0.208	0.836
Stomach	0.042	0.136	0.227	0.821
Gular saturation: reduced model				
Cloaca	1,50	0.190	11.729	0.001*
Gular stripe brightness				
Model:	4,47	0.166	2.336	0.069+
	Coef.	Std. coef.	t	Sig.
Total	−0.053	−0.633	−0.818	0.417
Cloaca	0.043	0.432	0.666	0.509
Intestine	0.024	0.197	0.377	0.708
Stomach	0.010	0.104	0.171	0.865
Gular stripe brightness: reduced model				
Cloaca	1,50	0.115	6.519	0.014*
Venter stripe hue				
Model:	4,47	0.025	0.295	0.879

Table 1. Continued.

	d.f.	R^2	F	Sig.
	Coef.	Std. coef.	t	Sig.
Total	−0.100	−0.210	−0.251	0.803
Cloaca	0.116	0.207	0.294	0.770
Intestine	0.132	0.189	0.334	0.740
Stomach	0.016	0.030	0.046	0.964
Venter stripe saturation				
Model:	4,47	0.049	0.600	0.664
	Coef.	Std. coef.	t	Sig.
Total	0.070	0.106	0.128	0.899
Cloaca	−0.229	−0.292	−0.421	0.676
Intestine	−0.030	−0.030	−0.054	0.957
Stomach	−0.079	−0.105	−0.161	0.873
Venter stripe brightness				
Model:	4,47	0.023	0.267	0.892
	Coef.	Std. coef.	t	Sig.
Total	0.018	0.076	0.090	0.928
Cloaca	0.008	0.029	0.042	0.967
Intestine	−0.050	−0.140	−0.248	0.805
Stomach	−0.020	−0.074	−0.113	0.911

When full models were significant, the models were reduced to determine the specific body area(s) of nematode load related to the color variable in question.
*$P<0.05$.
+Trend ($P<0.10$).
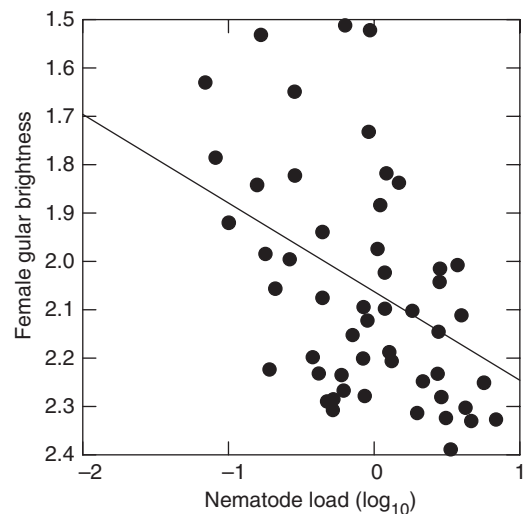Coef., Coefficient; Std. coef., Standard coefficient; Sig., Significance.

Figure 3 Female gular brightness is negatively correlated with nematode load ($F_{1,50}=10.433$, $P=0.002$).

their relationships with cloacal, intestinal, stomach and total nematode loads were not significant.

Hormones and nematode load

Total nematode load was positively related to plasma testosterone concentrations ($F_{1,9} = 6.333$, $P = 0.033$, Fig. 4).
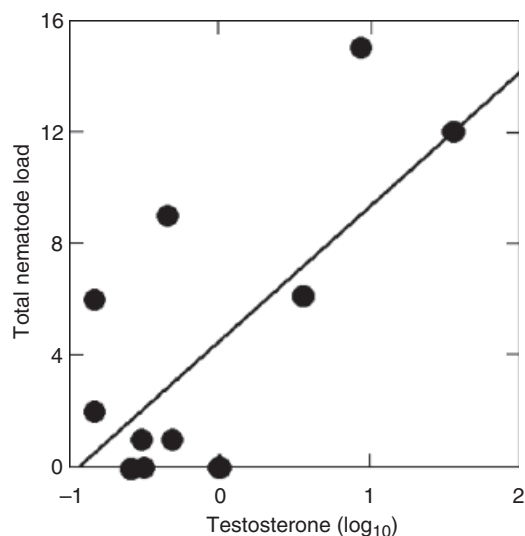
Figure 4 Female plasma testosterone concentrations are positively correlated with total nematode load ($F_{4,47} = 6.333$, $P = 0.033$).

The time it took to capture and obtain a blood sample did not affect testosterone concentrations ($F_{1,9} = 0.004$, $P = 0.950$). However, there was no significant relationship between nematode load and plasma corticosterone ($F_{1,9} = 0.034$, $P = 0.855$). Capture and bleed times also did not explain variation in plasma corticosterone in this small sample of females ($F_{1,9} = 0.629$, $P = 0.437$).

Discussion

Our results indicate that some secondary color traits in *S. pyrocephalus* females correlate with parasite load and with reproductive state. Females with dull red gular regions and dull, pale gray gular stripes had high nematode loads and were in late vitellogenic stages of reproduction. Late vitellogenic stages, a red gular region and pale gray gular stripes have been associated with high plasma testosterone concentrations in this species (Calisi & Hews, 2007). Finally, our results reveal that high testosterone is correlated with high nematode load.

In *S. pyrocephalus*, females exhibit aggressive inter- and intrasexual interactions during the breeding season (R. M. Calisi & J. H. Malone, pers. obs.; Calisi & Hews, 2007), and thus color had the potential to be a social signal involved in aggression. Males court females and then grasp the skin on the female's neck before copulation (often dragging her some distance into a rock crevasse). Females are mostly, and many times successfully, resistant to male courting and copulation attempts, lunging and biting at the male (R. M. Calisi, pers. obs.). Such female behaviors could increase the likelihood that a strong male (which could indicate genetic quality) succeed in mating and father her offspring, resulting in phenotypically vigorous (good quality) sons ('the sexy son hypothesis,' Weatherhead & Robertson, 1979). Alternatively, females may exhibit mate choice based on other male criteria. Regardless, because such intersexual interactions

during mating appear to be energetically costly to both sexes, selection may have acted to reduce these costs via honest signaling, as when female coloration communicates reproductive status (Cooper & Greenberg, 1992). In this study, we found that female *S. pyrocephalus* with high nematode load were likely to have red but dull gular regions as well as pale, dull gray gular stripes. Thus, red coloration could signal female fertilization receptivity, and the brightness of the overall gular area (both the gular region and the gular stripes) could signal female nematode load or even 'quality' (in a handicap model: high load but high expression of secondary sexual trait). Secondary color traits, and male responses to the traits, may have evolved because of (1) the costs incurred by males when courting nonreceptive females (Cooper & Greenberg, 1992) and (2) to select for parasite resistance in offspring and/or avoid contracting parasites, or both.

In a previous study, duller venter stripes in females were correlated with higher levels of the glucocorticoid, corticosterone (Calisi & Hews, 2007). We did not find a relationship between venter stripe dullness and a trait that might be expected to be associated with 'physiological stress,' nematode load. However, these negative results are difficult to interpret, given the array of reproductive states of this small sample of females, and the known effects of reproductive state and body condition on plasma corticosterone in female lizards (e.g. Woodley & Moore, 2002; Cartledge & Jones, 2007).

Aggressive female–female interactions have also been observed in this species (R. M. Calisi & J. H. Malone, pers. obs.). As in male–male interactions, females inflate their gular region and flatten their ventral area laterally when confronted with a conspecific female, and females will lunge and bite at female opponents. This often ends with one lizard leaving or being driven from the immediate site (R. M. Calisi, pers. obs.). If there is competition for resources (e.g. feeding, basking or oviposition sites; mates) among female *S. pyrocephalus*s, females could benefit by conserving energy and reducing the risk of predation by signaling fighting ability, rather than engaging in fighting *per se*. As in male–female interactions, the association of red, dull gular areas could convey information to opponents about reproductive state, nematode load or body condition. Delimitating the relative roles of inter- and intrasexual selection in female color evolution will be challenging, especially given the multicomponent nature of female color patterns in this species.

We also reconfirmed the finding (Calisi & Hews, 2007) that female gular region coloration in *S. pyrocephalus* varies with reproductive stage. Female gular regions appear most red during late-vitellogenic stages, when testosterone and 17β-estradiol are both high (Calisi & Hews, 2007). Females redden in their gular region as their follicles mature, perhaps expressing their deepest red coloration at the optimal time for fertilization, which is unknown for this species. In other lizard species, mature follicles about to leave/leaving the ovary are capable of being fertilized (Whittier & Tokarz, 1992). Females carrying oviductal eggs are less red (more

orange) and have significantly lower testosterone and 17β-estradiol compared with females carrying late-vitellogenic follicles (Calisi & Hews, 2007). Egg maturation may no longer require elevated levels of these circulating hormones, as eggs are close to oviposition. Finally, females with early vitellogenic follicles were redder than those with mid-vitellogenic follicles, possibly due to effects from previously elevated steroid levels in this multiply-clutching species, consistent with a time-lag in color alterations (Cooper & Greenberg, 1992; Weiss, 2002). Thus, color is a mechanism by which males, or other females, could discriminate among females of different reproductive states (cf. Cooper & Greenberg, 1992).

Signals that are regulated, at least in part, by endocrine status could ultimately yield fitness benefits via honest reproductive signaling, but costs may accompany these benefits. We show that testosterone, while related to female color and the reproductive cycle in *S. pyrocephalus* (Calisi & Hews, 2007), was also positively related to nematode load (this study). High concentrations of testosterone are associated with high estradiol concentrations and follicle maturation in *S. pyrocephalus* (Calisi & Hews, 2007). Generally, reptile estradiol concentrations are positively correlated with ovarian follicle size (Crews, 1980; Whittier & Tokarz, 1992). An increase in testosterone could be because it is a precursor to estradiol and can be converted to estradiol via aromatase enzymes (Whittier & Tokarz, 1992; Baum, 2002). Thus, high testosterone could maintain high levels of estradiol necessary for follicle maturation. Furthermore, high concentrations of testosterone could benefit a female by increasing her reproductive effort and altering her color, allowing honest signaling of fertilization receptivity. However, there may be tradeoffs in energy allocation, such as reduced immunocompetence and increased susceptibility to parasitic infection (Grossman, 1985; Folstad & Karter, 1992; Peters, 2000; Roberts, Buchanan & Evans, 2004), as seen with malarial parasites in female birds (*Parus major*; Oppliger, Celerier & Clobert, 1996a; Oppliger, Christe & Richner, 1997) and haematozoan parasites in female lizards (*Lacerta vivipara*, Sorci, Clobert & Michalakis, 1996). Blood parasites of *L. vivipara* are linked to other physiological costs such as low levels of hemoglobin, decreased resting oxygen consumption, reduced running speed (Oppliger, Christe & Richer, 1996b; Sorci *et al.*, 1996), as well as to reduced tail regeneration (Oppliger & Clobert, 1997). Finally, we found no association between corticosterone and nematode load. As indicated earlier, these negative results for corticosterone are difficult to interpret, given the array of reproductive states of this small sample of females. Behavioral competition in lizards is sometimes associated with elevated corticosterone (e.g. Comendant *et al.*, 2003), but not always (e.g. Langkilde, Lance & Shine, 2005). Thus, detailed study of corticosterone, social behavior, female coloration and parasites in *S. pyrocephalus* is clearly warranted, as it is for many colorful lizard species (Moore & Jessop, 2003).

Increases in testosterone can correlate with increased mobility in lizards (Olsson *et al.*, 2000; Sinervo *et al.*, 2000),

as can elevated corticosterone in some reptiles (e.g. Cease, Lutterschmidt & Mason, 2007). Female *S. pyrocephalus* with red gular regions (high testosterone and large late-vitellogenic follicles) daily move greater distances during the breeding season than more orange-yellow females (low testosterone and small pre-vitellogenic follicles; Calisi, 2006). Thus, red females may travel more and/or have larger home ranges, and such differences in female movement may allow them to encounter more resources, such as mates, food and/or egg deposition sites. Increased movement may also increase exposure to parasites such as nematodes, thus contributing to the association between nematode loads and testosterone.

Parasite-mediated signaling in females

Resistance to parasites is presumably a major component of fitness, and conspicuous female coloration could signal immune ability in social contexts. Studies testing this signaling role for color, in either sex, assess how color relates to parasite loads or to immune challenge responses. Similar to the findings for *S. pyrocephalus* females, negative relationships have been found between female color and parasite infections in some bird species (e.g. Horak *et al.*, 2001; Piersma *et al.*, 2001; Roulin *et al.*, 2001b). All of these studies, including this, are correlational and thus cannot exclude the hypothesis that variation in parasite exposure, and not just female quality, contributes to the observed patterns. Indeed, movement data on female *S. pyrocephalus* suggest the parasite exposure hypothesis is viable. Manipulative studies, including experimentally infecting animals, are stronger tests. Some have yielded predicted associations (e.g. Harper, 1999; Hill, 2002) but others have not (e.g. Pärn *et al.*, 2005). Clearly, more such rigorous tests are needed.

Few studies have assessed the role of parasite-mediated female coloration in social signaling. Several studies suggest a role of female coloration in male mate choice. In barn owls, males may choose female mates based on their spottedness (Roulin *et al.*, 2001a). Furthermore, indirect selection is possible in this species, as nestling antibody responses are related to the amount of maternal spotting (Roulin *et al.*, 2000), although maternal yolk effects have not been excluded. A role for female coloration in intrasexual competition has been reported for several vertebrates, especially for birds (reviewed in Amundsen & Pärn, 2006) but also in lizards (e.g. Comendant *et al.*, 2003) and fish (Dijkstra *et al.*, 2008). Experimental tests show that condition affects female coloration (Hill, 2002; Siefferman & Hill, 2005), but more work is needed assessing the role of parasites in such female coloration and potential use in signaling.

In lizards, there is very little work combining examination of female color, parasites and social signaling. Female color in striped plateau lizards, *Sceloporus virgatus*, varies with body condition, ectoparasite load, body size and egg mass (Weiss, 2006), suggesting a role for color in signaling female condition or quality, and males in this species assess female coloration (Weiss, 2002). In female European green lizards, *Lacerta viridis* yellow chest color saturation increased with

residual tick numbers, after correcting for the effects of morphology on skin coloration and tick load (Vaclav, Prokop & Fekiac, 2007), but the signaling role is also unknown, as for *S. pyrocephalus*. A number of studies on side-blotched lizards have identified physiological (e.g. Comendant *et al.*, 2003) and fitness differences of female color morphs (e.g. Sinervo, Bleay & Adamopoulou, 2001), but direct behavioral tests of signaling function of female color in intra- and intersexual contexts in this species are needed. Such behavioral studies assessing the roles of female coloration in conspecific signaling are also warranted in *S. pyrocephalus*, given we have demonstrated an association among parasites, color and hormones. In addition, manipulative studies are needed to directly gauge nematode influence over secondary color characteristics and physiology, including the potential role of glucocorticoids, which could also contribute to the relationships among aggression, body condition and coloration in females.

Acknowledgments

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