

RESEARCH ARTICLE

Effect of stress on female-specific ornamentation

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SUMMARY

Signal honesty is theorized to be maintained by condition-dependent trait expression. However, the mechanisms mediating the condition dependence of sexually selected traits are often unknown. New work suggests that elevated glucocorticoid levels during physiological stress may play a role in maintaining signal honesty. Here, we experimentally examine the effect of both chronic and acute stress on the expression of the condition-dependent ornamentation of female striped plateau lizards, *Sceloporus virgatus*. Females were stressed either chronically *via* corticosterone implants or relatively acutely *via* autotomy, were sham manipulated or were left unmanipulated. Both stressors resulted in elevations in corticosterone within physiologically relevant levels, though the implants resulted in significantly higher levels than did autotomy. Corticosterone-implanted females were less likely to produce a clutch of eggs, but those individuals that did reproduce had reproductive output similar to that of females from other treatment groups. Compared with females in other groups, the corticosterone-implanted females tended to develop smaller ornaments that had less UV and orange-to-red wavelength reflectance relative to medium wavelength reflectance. The sex steroid hormones testosterone and estradiol were correlated to corticosterone levels, but did not appear to underlie the effect on ornament expression; of the steroids measured, only corticosterone levels were negatively related to ornament size and coloration. Thus, the condition-dependent ornamentation of female lizards is sensitive to chronic elevations in stress hormones, supporting their importance in the maintenance of signal honesty.

Key words: autotomy, corticosterone, reproductive suppression, *Sceloporus*, sex steroids, sexual selection.

Received 2 October 2012; Accepted 18 March 2013

INTRODUCTION

Honesty in sexually selected signals is often maintained by mechanisms that ensure the trait is expressed in proportion to the true condition of the signaler. Theoretically, only those individuals in good condition should be able to withstand the costs of signal production and/or maintenance (Zahavi, 1975). Mechanistically, energetic and/or physiological trade-offs between the signal and essential bodily processes may be responsible for regulating signal honesty. For instance, energy allocated to ornament development is energy not available for other aspects of reproduction or for growth and survival. Physiological trade-offs, such as those associated with carotenoid-based and testosterone-regulated signals, often hinge on interactions with the immune system. Carotenoids deposited in ornaments may compromise immune function directly as fewer carotenoids are available for antioxidant function or indirectly *via* carotenoids' interactions with more potent colorless antioxidants (Biard et al., 2009; Hartley and Kennedy, 2004; Lozano, 1994). The elevated testosterone levels necessary for signal development have also been proposed to suppress immune function (Folstad and Karter, 1992; Braude et al., 1999; Hasselquist et al., 1999).

Recent evidence suggests that stress hormones may also mediate the trade-offs maintaining signal honesty (e.g. Husak and Moore, 2008). Stress is a physiological response to an environmental stressor that triggers the hypothalamic-pituitary-adrenal axis to secrete glucocorticoid hormones such as corticosterone, which is the primary glucocorticoid in reptiles, birds and many mammals.

Corticosterone then affects energy resource redistribution and immune responses, as well as other essential physiological processes, in a context-specific manner (French et al., 2007). While often considered in the context of survival in the face of acute stressors, chronic exposure to elevated glucocorticoids can negatively impact immune function. Indeed, the effect of elevated glucocorticoids varies greatly depending not only on the duration, but also on the type, intensity and predictability of the experienced stressor (Wingfield et al., 1998). Regardless, because glucocorticoids mediate both energy allocation and physiological trade-offs, stress hormones are likely candidates for ensuring honesty in sexually selected traits (Buchanan, 2000). For instance, glucocorticoids appear to negatively affect the expression of sexually selected vocalizations (Buchanan et al., 2003; Spencer et al., 2003; Leary et al., 2006) and ornaments (Calisi and Hews, 2007; Roulin et al., 2008).

We experimentally tested whether condition-dependent traits are susceptible to stress using the striped plateau lizard, *Sceloporus virgatus* Smith 1938 – a species that expresses female-specific ornamentation during the reproductive season. Females develop orange throat patches during the ovarian cycle, with peak ornament size and color occurring around the time of ovulation (Weiss, 2002). Though all reproductive females express some orange color, the extent of ornamentation at peak expression varies among individuals and that variation may function as an honest signal of female phenotype as well as the quality of her eggs and her future offspring

(Weiss, 2006; Weiss et al., 2009; Weiss et al., 2011). If corticosterone mediates the expression of this honest signal, then we predict that females experimentally exposed to stressors will produce smaller and less colorful ornaments relative to control females. Because the form of the stressor can greatly impact the physiological response, we used both the relatively brief, ecologically relevant stressor of tail autotomy and the chronic elevation of stress hormone *via* corticosterone implants. Any effect of these manipulations on female phenotype may be due to direct effects of the stressor or indirect effects *via* interrelationships between corticosterone, sex steroid hormones and body condition (Husak and Moore, 2008), thus we explore each of these variables in our analyses. We also examined the effect of our manipulations on reproductive output and possible reproductive suppression, as it may be adaptive for females to limit energy allocation to reproduction in the face of stress (Wingfield and Sapolsky, 2003).

MATERIALS AND METHODS

Lizard and egg care

Sixty female and 15 male lizards were collected by noose in late April and early May from areas surrounding the American Museum of Natural History's Southwestern Research Station (SWRS), Portal, AZ, USA (elevation ~1600 m); all individuals were found within 4.8 km of SWRS. Lizards were housed on SWRS property, randomly assigned to three outdoor enclosures (61×30.5×40.6 cm; $N=20$ females and 5 males per enclosure) outfitted with multiple perches and hides (i.e. bricks, logs, and rocks). Males were simultaneously placed in enclosures 6 days prior to the addition of females. Females were held in standard 10 gallon glass terraria tanks (up to five females per tank) for 1–7 days following capture until treatments were administered (see below) and then were simultaneously added to the enclosures in the evening when males were inactive. Approximately 6–8 g of crickets were added to enclosures three times per week and water was provided *ad libitum*. Males and non-reproductive females were released to their site of capture in late June. Gravid females were released to their site of capture following oviposition, which was induced in early July, once free-ranging females were observed to have initiated egg-laying behavior, *via* 0.1 ml oxytocin injection. Without such induction, captive *S. virgatus* females may become egg-bound; thus, it is common for work conducted on this species' eggs and hatchlings to involve chemical induction of egg-laying (e.g. Abell, 1997; Qualls and Andrews, 1999; Smith et al., 1995; Weiss et al., 2009). Some females ($N=4$) did not lay full clutches after three oxytocin injections (delivered once per day for 3 days); remaining eggs were collected by dissection. In addition, during the course of the experiment, two females and one male went missing from the enclosures, and seven females died (in a single event due to overheating while in temporary containers). At the end of the experiment there were 12 or 13 females in each treatment group.

Eggs ($N=316$ from 36 reproductive females) were examined for embryonic disks to determine fertilization. Clutch size (total number of eggs produced) and clutch mass (mass of all eggs whether fertilized or unfertilized) were recorded for each mother. All eggs were then buried in moistened vermiculite (0.8 ml water per gram vermiculite) in plastic containers (14×10×6.5 cm; one container per clutch) covered with parafilm and were haphazardly placed in an incubator at 28°C. Daily checks for hatchlings began at 30 days of incubation and hatching occurred over a 2 week period in late August. Upon emergence, each hatchling was sexed based on post-anal scales and weighed. For each mother, we recorded hatchling number (total number of offspring emerged from eggs), average

hatchling body mass and offspring sex ratio. Hatchlings were released to the site of their mother's capture within 11 days (mean \pm s.e.m.=4.3 \pm 0.2 days) of emergence.

Female manipulations and measurements

Females were randomly assigned to four treatment groups: no manipulation (Control 1), blank implant (Control 2), autotomized, and corticosterone implant (C-IMP). The hormone implants were made of Silastic tubing (Dow Corning, Midland, MI, USA; i.d. 1.47 mm, o.d. 1.96 mm) filled with crystalline corticosterone (CORT; 10 mm steroid packing length, 15 mm total implant length), were sealed with silicone glue plugs, and were pierced three times with a 22s gauge needle to increase the rate of diffusion. Blank implants were filled entirely with glue (15 mm total length). Animals under deep hypothermic anesthesia were given small incisions, implants were inserted into the peritoneal cavity, and the incision was closed with adhesive (Weiss and Moore, 2004). For tail removal, pressure was placed on the tail 15 mm from the lizard's vent and increased until the lizard released the tail below the point of contact. Treatments were administered on 5 May, when females are early in ovarian development (Rose, 1981) and follicles are generally undetectable by palpation.

At the time of collection and once a week following treatment, we measured female snout to vent length (SVL), total tail length, body mass and ornament characteristics (see below). We calculated body condition as the standardized residuals from a regression of body mass on SVL³ (e.g. Weiss, 2006). We use data from the initial measurement, measurement 2 (at the time of blood sampling) and measurement 6 (the final measurement) in analyses. Though all females were of reproductive size, not all produced clutches. Those females that produced a clutch were classified as reproductive, and those that did not were classified as non-reproductive. Non-reproductive females included both those that failed to initiate a clutch and those that initiated vitellogenesis (as determined by palpation) but later resorbed their follicles.

We measured female ornamentation weekly and focused our analyses on peak ornament expression (Weiss et al., 2009; Weiss et al., 2011). To do so, the right throat patch of each female was photographed weekly along with a small ruler using an Olympus C-5050 ZOOM 5-megapixel digital camera set to macro mode with a Super Bright zoom F1.8 lens (Center Valley, PA, USA) under standardized indoor lighting, and each photo series was separately assessed by three people (E.E.M., S.L.W. and one independent viewer); peak ornament expression was determined to occur on the date that had the most agreement between viewers (59% complete agreement). We then quantified peak ornament size and peak ornament color. Peak ornament size was measured by selecting orange pixels from the chosen photograph using the 'color range' command of Adobe Photoshop 4.0 (Weiss, 2006) and determining the area of the selected pixels (in mm²) in NIH Image 1.60 (National Institutes of Health, Bethesda, MD, USA).

Ornament color was measured using an Ocean Optics (Dunedin, FL, USA) USB 2000 spectrometer and OOI Base software (integration time=500 ms, average=5, boxcar=5). Females were restrained ventral side up and the probe was placed 1.9 cm above the right throat patch. The patch was illuminated by a PX-2 xenon light source (Ocean Optics) and its reflectance was compared with that of a Spectralon white standard. Spectra from the date of peak ornament expression were processed by calculating median reflectance for every 20 nm bin from 300 to 700 nm, and averaging the medians across three spectra collected for a female at the selected time point. For females that did not develop any ornamentation (and

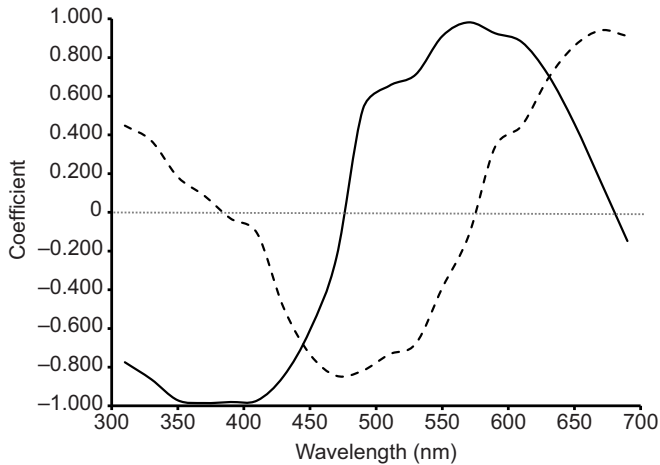


Fig. 1. Component loadings across wavelengths from the principal component analysis of peak ornament color in female *Sceloporus virgatus*. PC1 (solid line) represents variation in the relative amount of long to short wavelength reflectance, and PC2 (dashed line) represents relative amounts of both long and short wavelengths to medium wavelengths.

thus do not have a date of peak expression), we averaged the 20 nm bin medians across spectra from all 6 weeks of measurement.

From these processed spectra, we determined mean reflectance across all wavelengths for each female, and we separately analyzed spectral shape using principal component analysis (PCA). To quantify spectral shape independently of mean reflectance, we subtracted each female's mean reflectance from her spectral data across all wavelengths before subjecting the data set to PCA (Cuthill et al., 1999). Only principal components with eigenvalues greater than 1.0 are considered further. The first principal component (PC1) summarized 59% of the variance in the spectra and represents variation in the relative amount of long (>480 nm) to short (<480 nm) wavelength reflectance. PC2 accounted for 35% of the variance and represents relative amounts of both long (>580 nm) and short (<380 nm) wavelengths to medium wavelengths. Fig. 1 provides the component loadings across wavelengths for each principal component.

Hormone analysis

To measure circulating hormone levels, we collected blood samples from each female's post-orbital sinus 2.5–3 weeks after the experimental treatments were applied; females were still pre- or early vitellogenic at this time. The time of handling for blood collection averaged 121.2 ± 5.4 s (range=38–210 s). Blood samples were centrifuged to separate the plasma, and the plasma was stored at -70°C until assayed. Between 18 and 85 μl of plasma was collected from each individual. Plasma samples were assayed for corticosterone, progesterone, testosterone and estradiol by radioimmunoassay following established methods (Moore, 1986).

Briefly, samples were equilibrated overnight with 2000 c.p.m. of radioactive hormone for determination of individual recoveries. Steroids were extracted from plasma using 30% ethyl acetate in isooctane and separated by stepwise elution using celite chromatography. Collected fractions were dried down, resuspended in phosphate buffered saline, and assayed using rabbit anti-progesterone and rabbit anti-testosterone antibodies from Fitzgerald Industries International (Concord, MA, USA), a sheep anti-estradiol antibody from Biogenesis (Poole, UK) and a rabbit anti-corticosterone antibody from MP Biomedicals (Solon, OH, USA). Based on an average plasma volume of 47 μl , the minimum detectable values were 257 pg ml^{-1} for progesterone, 56 pg ml^{-1} for testosterone, 64 pg ml^{-1} for estradiol and 678 pg ml^{-1} for corticosterone. Intra-assay coefficients of variation were 32.9% for progesterone, 5.5% for testosterone, 28.0% for estradiol and 20.6% for corticosterone.

Statistical analysis

The effect of treatment group on independent variables was analyzed by general linear models when assumptions were met by untransformed or log-transformed variables and by non-parametric Kruskal–Wallis tests when necessary. To determine our final statistical models concerning the effect of our manipulations on ornamentation (size, mean reflectance, PC1 and PC2), we first examined bivariate correlations among maternal phenotype variables (SVL, tail length, body mass and body condition) to assess which to include as model covariates while avoiding issues of multicollinearity; we also used chi-square tests to examine the relationship between the mother's treatment group and her reproductive status. Based on these analyses (see below), our final general linear models examining ornament expression included treatment group as well as the covariate 'body mass at the time of blood sampling' and reproductive state. To examine the relative impact of corticosterone and the sex steroid hormones on measures of ornamentation, we used linear regression. All analyses were conducted in SPSS version 19 (IBM, Armonk, NY, USA).

RESULTS

Females that went missing or died did not differ from females that remained in the study in initial SVL ($F_{2,57}=1.96$, $P=0.15$), tail length ($F_{2,57}=1.66$, $P=0.20$), body mass ($F_{2,57}=1.17$, $P=0.32$) or body condition ($F_{2,57}=0.58$, $P=0.56$). However, the two missing females tended to be small (SVL_{missing}= 58.5 ± 1.5 mm; SVL_{dead}= 61.4 ± 0.6 mm; SVL_{remaining}= 62.1 ± 0.4 mm) and escaped from the same enclosure, suggesting that a small hole may have been present. Only remaining females are considered in all further analyses.

Hormone levels

Measured hormone levels were not significantly related to handling time ($r=-0.14$ – 0.07 , $P=0.34$ – 0.96 , $N=51$ for all). As expected, females differed in circulating CORT levels ($F_{3,47}=50.19$, $P<0.001$; Table 1). CORT levels of C-IMP females were significantly higher

Table 1. Mean (\pm s.e.m.) levels of steroid hormones (ng ml^{-1}) 2.5–3 weeks post-treatment

	Corticosterone	Progesterone	Testosterone	Estradiol
Control 1 (N=13)	3.19 ± 0.43^a	0.93 ± 0.38	1.63 ± 0.60^a	0.98 ± 0.35^a
Control 2 (N=13)	3.63 ± 0.69^a	0.86 ± 0.36	1.34 ± 0.85^a	0.69 ± 0.35^a
Autotomized (N=12)	5.82 ± 1.13^b	1.58 ± 0.59	2.79 ± 1.38^a	1.30 ± 0.49^a
C-IMP (N=13)	28.46 ± 3.02^c	0.35 ± 0.08	0.09 ± 0.02^b	0.09 ± 0.02^b

Different letters indicate significant differences among groups, per hormone, at $P<0.05$.

than those of all other groups ($P < 0.001$ for all) and autotomized females had significantly higher levels than both control groups ($P < 0.04$ for both); CORT levels of the two control groups did not significantly differ from each other ($P = 0.84$). Importantly, the elevated CORT levels of C-IMP females ($28.46 \pm 3.02 \text{ ng ml}^{-1}$) appear to be physiologically relevant; Abell (Abell, 1998) found CORT to be as high as 37.74 ng ml^{-1} in a small sample of free-ranging female *S. virgatus*. CORT levels were significantly negatively correlated with levels of testosterone ($r = -0.41$, $P = 0.003$) and estradiol ($r = -0.35$, $P = 0.01$), but not progesterone ($r = -0.17$, $P = 0.22$); thus, treatment groups also significantly differed in levels of testosterone ($\chi^2 = 18.03$, $P < 0.001$) and estradiol ($\chi^2 = 13.63$, $P = 0.003$), but not progesterone ($\chi^2 = 2.54$, $P = 0.47$; Table 1). Levels of all three sex steroids were positively correlated with each other ($r = 0.77-0.94$, $P < 0.001$ for all).

Maternal phenotype

Females in the four treatment groups did not significantly differ in initial SVL ($F_{3,47} = 0.27$, $P = 0.84$), tail length ($F_{3,47} = 1.29$, $P = 0.29$), body mass ($F_{3,47} = 0.33$, $P = 0.81$) or body condition ($F_{3,47} = 0.54$, $P = 0.66$). They also did not significantly differ in body mass or body condition at the time of blood sampling or at the final measurement (all $P > 0.40$). Body mass at blood sampling was the only variable both significantly positively correlated with all body shape variables ($r \geq 0.34$, $P \leq 0.015$, $N = 51$ for all) and statistically uncorrelated with any of the ornament variables ($r = -0.18-0.13$, $P \geq 0.20$, $N = 51$) or to CORT ($r = 0.21$, $P = 0.15$, $N = 51$); thus it was selected as the sole covariate to explore body shape relationships in our models investigating the effect of treatment on ornamentation.

Reproductive status and output

Reproductive state was also included in our final statistical models as ornament expression is known to vary with reproductive state (Weiss, 2002) and C-IMP females were significantly less likely than other females to produce a clutch of eggs ($\chi^2 = 9.04$, $P = 0.03$; Table 2). Of the females that failed to produce a clutch, ~53% were C-IMP females ($N = 8$), ~20% were autotomized ($N = 3$) and ~13% were in each of the two control groups ($N = 2$ per control group). Eight of these 15 females appeared to initiate the reproductive cycle and then resorb their eggs, whereas five C-IMP and two autotomized females never developed detectable follicles. Among C-IMP females, those that reproduced tended to have lower CORT levels ($t_{11} = -1.97$, $P = 0.07$) and had higher body mass at the time of blood sampling ($t_{11} = 3.00$, $P = 0.04$) than those that did not reproduce (Table 2). [We did not test for reproductive *versus* non-reproductive differences within other treatment groups due to low power, but the only other apparent trend was for CORT levels of autotomized females to be lower in reproductive than non-reproductive subjects (see Table 2).] Among those females that did successfully produce a clutch, treatment group had no significant effect on any measure of reproductive output, including clutch size ($F_{3,32} = 1.30$, $P = 0.29$), clutch mass ($F_{3,31} = 0.51$, $P = 0.68$), hatchling number ($F_{3,32} = 0.47$, $P = 0.71$), average hatchling mass ($F_{3,22} = 0.73$, $P = 0.55$) and offspring sex ratio ($F_{3,19} = 0.65$, $P = 0.60$).

Effect of stress on ornament expression

Peak ornament size was significantly smaller in non-reproductive females than in reproductive females ($F_{1,42} = 7.11$, $P = 0.01$) and was marginally affected by treatment ($F_{3,42} = 2.75$, $P = 0.055$), with C-IMP females tending to have smaller ornament patches than females in all other groups (Fig. 2A); there was no reproduction \times treatment interaction ($F_{3,42} = 1.26$, $P = 0.30$). Mean reflectance at peak ornament expression (Fig. 2B) and PC1 (Fig. 2C) were both unaffected by reproductive state (reflectance: $F_{1,42} = 2.77$, $P = 0.10$; PC1: $F_{1,42} = 0.003$, $P = 0.96$), treatment (reflectance: $F_{3,42} = 0.98$, $P = 0.41$; PC1: $F_{3,42} = 0.76$, $P = 0.52$) and the interaction of these two factors (reflectance: $F_{3,42} = 0.78$, $P = 0.51$; PC1: $F_{3,42} = 0.14$, $P = 0.94$). However, PC2 was strongly affected by both factors (reproduction: $F_{1,42} = 9.13$, $P = 0.004$; treatment: $F_{3,42} = 6.15$, $P = 0.001$; Fig. 2D), although not by a reproduction \times treatment interaction ($F_{3,42} = 0.19$, $P = 0.90$). Lower PC2 scores, indicating patches with less UV and orange-to-red wavelength reflectance relative to medium wavelength reflectance, were found in non-reproductive females than in reproductive females. PC2 scores were also lower in C-IMP females relative to unmanipulated Control 1 females ($P < 0.001$) and autotomized females ($P = 0.007$), and were lower in blank-implant Control 2 females than in Control 1 females ($P = 0.02$). Removing reproductive state from the above statistical models, due to concerns regarding low sample size among the non-reproductive group and thus low statistical power, strengthened the above patterns concerning the effect of treatment on ornament expression (size: $P = 0.002$; reflectance: $P = 0.08$; PC1: $P = 0.33$; PC2: $P < 0.001$).

Because treatment groups differed not only in CORT levels but also in sex steroid hormone levels, we used regression to parse out which hormone played the strongest role in mediating the effect on peak ornament size and PC2 scores. For both variables, CORT was the only steroid with a significant coefficient (Table 3); elevated CORT levels were associated with smaller patches ($\beta = -0.403$, $P = 0.005$) and lower PC2 scores ($\beta = -0.438$, $P = 0.001$; Fig. 3).

DISCUSSION

The negative correlations between CORT and both ornament size and color (PC2) in female striped plateau lizards support the idea that these orange throat patches are stress-mediated sexually selected traits, and suggest that sexual selection pressures may act directly on the hormonal stress response (Husak and Moore, 2008). The affected ornament features are sensitive to circulating CORT levels and are predictive of both female and offspring phenotype (Weiss, 2006; Weiss et al., 2009; Weiss et al., 2011). The ornament's susceptibility to the physiological effects of stress is independent of the interrelationships between CORT and body mass, as well as those between CORT and the sex steroid hormones.

Sex steroid hormones have been the focus of most work examining the hormonal regulation of lizard coloration, with evidence suggesting androgen regulation of male color (Cox et al., 2005; Rand, 1992) and (primarily) progesterone regulation of female-specific reproductive color (reviewed by Cooper and Greenberg, 1992; Jessop et al., 2009). However, these studies did not examine glucocorticoids. Given the unpredictable relationship

Table 2. Mean (\pm s.e.m.) CORT levels (ng ml^{-1}) and body mass (g) of females that did and did not reproduce

	Control 1			Control 2			Autotomized			C-IMP		
	CORT	Body mass	N	CORT	Body mass	N	CORT	Body mass	N	CORT	Body mass	N
Reproductive	3.24 \pm 0.51	7.37 \pm 0.34	11	3.79 \pm 0.81	7.44 \pm 0.37	11	4.67 \pm 0.82	7.10 \pm 0.41	9	21.96 \pm 3.15	8.96 \pm 0.65	5
Non-reproductive	2.91 \pm 0.38	5.73 \pm 0.29	2	2.76 \pm 0.13	7.63 \pm 0.37	2	9.25 \pm 3.47	6.98 \pm 1.42	3	32.52 \pm 3.98	7.06 \pm 0.31	8

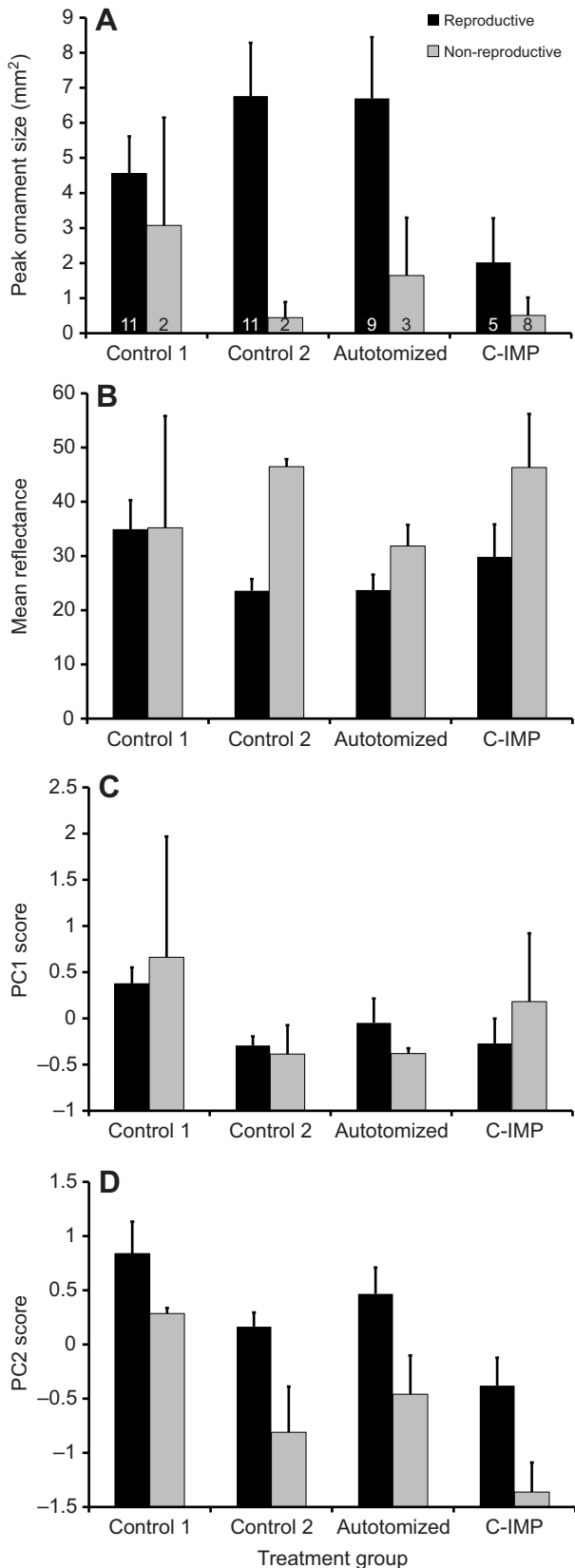


Fig. 2. The effect of treatment on (A) peak ornament size, (B) mean reflectance, (C) PC1 scores and (D) PC2 scores of reproductive and non-reproductive female lizards. Control 1, no manipulation; Control 2, blank implant; C-IMP, corticosterone implant. Data are means + s.e.m. Sample sizes are at the base of bars in A.

between gonadal and adrenal hormones (i.e. various species show positive, negative or no correlation between them), more studies are needed that examine both classes of steroids simultaneously so that we can disentangle their relative roles (Husak and Moore, 2008). In one of the only studies to do so, Calisi and Hews (Calisi and Hews, 2007) found that the color of male *Sceloporus pyrocephalus* was correlated with circulating CORT levels and not with androgen levels, whereas some aspects of female coloration were correlated with CORT while others were correlated with testosterone and/or estradiol (progesterone was not measured). Similar to our findings, elevated CORT resulted in duller display color among *S. pyrocephalus*. Such patterns suggest that the ornamentation of these sceloporine lizards can be used by conspecifics to reliably assess current condition.

The type, duration and intensity of the stressor that induces elevated CORT may influence the way in which ornament expression is affected (Roulin et al., 2008). The ornament of *S. virgatus* females was more sensitive to the chronic stressor of CORT implants and less sensitive to stress associated with autotomy. Whereas the 8- to 9-fold increase in CORT experienced by C-IMP females [which falls within CORT levels found in free-ranging females (Abell, 1998)] tended to reduce patch size and lowered the expression of UV and orange-red wavelengths relative to medium wavelengths, the 1.5- to 2-fold CORT increase experienced by autotomized females was not sufficient to significantly impact these aspects of ornament development. We assumed that autotomy would represent an ecologically relevant stressor as tail break frequencies are approximately 20% in free-ranging populations (Smith, 1996), and that the stressor would be relatively acute, though because we sampled at only one time point, the time course and pattern of the CORT response is unknown. While autotomy has been well studied in lizards for its effects on locomotion, behavior and survival (Bateman and Fleming, 2009), remarkably little work has examined the response of the hypothalamic-pituitary-adrenal axis. In one study that did examine this effect, autotomized female skinks, *Eulamprus heatwolei*, had elevated CORT 1 h post-autotomy, but levels had returned to baseline by 2 days and 14 days post-event (Langkilde and Shine, 2006). Thus, the current results are novel as they document a prolonged, though relatively mild, stress response following tail loss in lizards. This physiological response may nevertheless mediate known compensatory behavioral changes (Cooper, 2007) and growth rate reductions (Smith, 1996) among autotomized *S. virgatus*.

Overall, the sensitivity of the ornament to chronic stress suggests that ornament development is associated with a physiological cost. This cost may be realized *via* immunosuppression, as the link between chronic elevations of CORT and immune suppression has strong empirical support [in contrast to the less consistent experimental support for the link between androgens and immunosuppression proposed by the immunocompetence handicap hypothesis (Buchanan, 2000; Folstad and Karter, 1992; Roberts et al., 2004)]; however, the effect of chronic stress on female *S. virgatus* immune response remains to be tested. Regardless of the underlying mechanism, the mediation of ornament expression by stress hormones suggests that individuals who base reproductive decisions on the assessment of these secondary sexual characteristics may benefit by increasing mating opportunities with high-quality, healthy individuals (Husak and Moore, 2008).

The size and color of female ornaments were also related to reproductive state, with smaller and less colorful patches associated with the suppression of reproduction that occurred among some females, independent of treatment group. Though sample size of

Table 3. Regression models examining the effect of circulating steroid hormone levels on ornament expression

	Overall model	Adjusted R^2	Corticosterone	Progesterone	Testosterone	Estradiol
Peak size	$F_{4,46}=4.64$ (0.003)	0.23	$\beta=-0.403$ (0.005)	$\beta=-0.065$ (0.77)	$\beta=0.055$ (0.89)	$\beta=0.261$ (0.50)
PC2	$F_{4,46}=7.48$ (<0.001)	0.34	$\beta=-0.438$ (0.001)	$\beta=-0.264$ (0.19)	$\beta=0.006$ (0.99)	$\beta=0.506$ (0.16)

P -values are listed within parentheses.

non-reproductive females was small in this study, the pattern detected was expected as the ornament is known to develop and fade in concert with the ovarian cycle (Weiss, 2002), thereby serving as a signal of reproductive state as well as phenotypic quality. Also expected was the increased rate of reproductive suppression among C-IMP females, as it seems selectively advantageous for limited energy resources to be shunted to survival rather than reproduction in the face of an anabolic challenge. However, some female reptiles have been found to maintain reproduction in such instances (reviewed by Moore and Jessop, 2003) and indeed, 38% of the C-IMP females successfully reproduced despite high circulating CORT levels. Thus, the effect of CORT on reproductive suppression varies among individual *S. virgatus* females, possibly in a context-dependent manner. In Wingfield and Sapolsky's (Wingfield and Sapolsky, 2003) review of the maintenance of reproduction in the face of stress, they highlight that selection for stress resistance may be particularly strong among seasonal breeders with only one breeding opportunity per year; indeed, this is the case for the study population of *S. virgatus*. Conversely, it is interesting to note that reproductive suppression also occurred among four (15%) control females with CORT levels that were 7.5 times lower than that

experienced by reproductive C-IMP females, and suppression rates up to 25% have been found among unmanipulated *S. virgatus* females housed similarly to those in the present study (Weiss et al., 2002). Reproductive suppression in response to captivity may occur *via* mechanisms independent from CORT production.

Of the females that did reproduce, treatment group did not have a significant effect on measures of reproductive output, including clutch size, clutch mass and average hatchling body mass. We also found no significant effect on offspring sex ratio, which might be expected under models of sex allocation theory (Charnov, 1982; Correa et al., 2005; Love et al., 2005). It is possible that these results are influenced by our relatively small sample size, and that our treatments may have affected aspects of offspring phenotype that were unmeasured here, such as morphology, growth, immune response, dispersal and risk aversion. However, specific predictions are difficult to make as the direction of corticosterone-based maternal effects has been found to vary with offspring sex and among species (De Fraipont et al., 2000; Hayward and Wingfield, 2004; Love et al., 2005; Meylan and Clobert, 2005; Sinervo and DeNardo, 1996; Uller et al., 2009; Vercken et al., 2007; Warner et al., 2009). To determine the fitness consequences of elevated maternal corticosterone, it would be necessary to track the survival of females and their resultant offspring (Love and Williams, 2008; Meylan and Clobert, 2005).

ACKNOWLEDGEMENTS

We thank Carla Abrams for assistance with lizard collection; Melissa VanKleek and Julia Bass for assistance with egg induction, incubation and hatchling care; Elizabeth Hoffman for help assessing peak ornament expression; and Ellen Ketterson and Danielle Whittaker for radioimmunoassay assistance.

AUTHOR CONTRIBUTIONS

S.L.W. was involved in the conception of the study and experimental design, conducted the experimental manipulations, assisted data collection, interpreted the findings, and drafted and revised the article. E.E.M. was involved in the experimental design, data collection, analysis, interpretation and editing of the article. D.S.W. was involved in collecting data on measures of female reproductive output and editing of the article. D.K. conducted the radioimmunoassay, and assisted in data analysis, interpretation and editing of the article.

COMPETING INTERESTS

No competing interests declared.

FUNDING

Funding was provided to S.L.W. by a Murdock Charitable Trust Life Sciences Grant, and to S.L.W. and E.E.M. by the University of Puget Sound.

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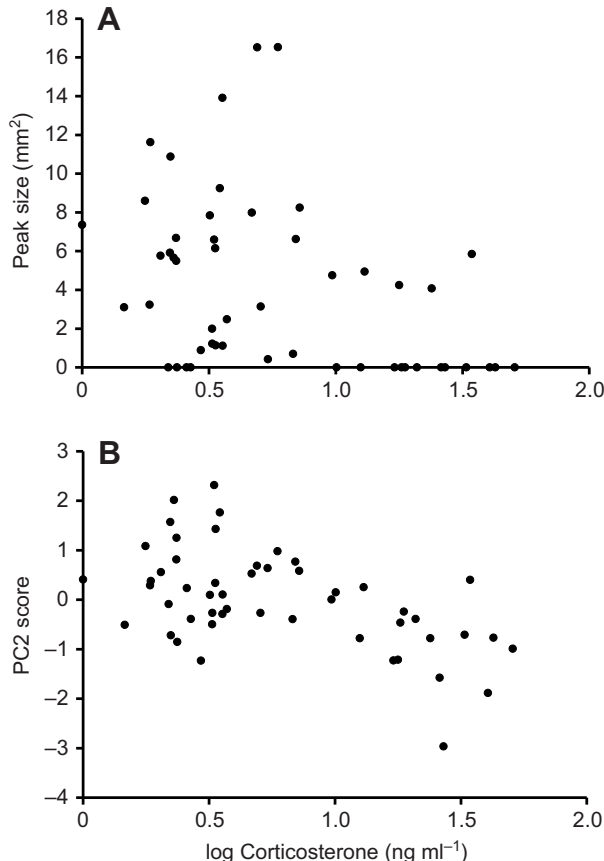


Fig. 3. Relationship between corticosterone levels of female lizards and (A) peak ornament size and (B) PC2 scores.

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