1   Effect of stress on female-specific ornamentation

2

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13   selection

14 Signal honesty is theorized to be maintained by condition-dependent trait expression. However,

15 the mechanisms mediating the condition-dependence of sexually selected traits are often

16 unknown. New work suggests that elevated glucocorticoid levels during physiological stress may

17 play a role in maintaining signal honesty. Here, we experimentally examine the effect of both

18 chronic and acute stress on the expression of the condition-dependent ornamentation of female

19 striped plateau lizards, *Sceloporus virgatus*. Females were stressed either chronically via

20 corticosterone implants or relatively acutely via autotomy, were sham manipulated or were left

21 unmanipulated. Both stressors resulted in elevations in corticosterone within physiologically

22 relevant levels, though the implants resulted in significantly higher levels than did autotomy.

23 Corticosterone-implanted females were less likely to produce a clutch of eggs, but those

24 individuals that did reproduce had similar reproductive output to females from other treatment

25 groups. Compared to females in other groups, the corticosterone-implanted females tended to

26 develop smaller ornaments that had less UV and orange-to-red wavelength reflectance relative to

27 medium wavelength reflectance. The sex steroid hormones testosterone and estradiol were

28 correlated to corticosterone levels, but did not appear to underlie the effect on ornament

29 expression; of the steroids measured, only corticosterone levels were negatively related to

30 ornament size and coloration. Thus, the condition-dependent ornamentation of female lizards is

31 sensitive to chronic elevations in stress hormones, supporting their importance in the

32 maintenance of signal honesty.

33

34 Honesty in sexually selected signals is often maintained by mechanisms that ensure the trait is

35 expressed in proportion to the true condition of the signaler. Theoretically, only those individuals

36 in good condition should be able to withstand the costs of signal production and/or maintenance

37 (Zahavi, 1975). Mechanistically, energetic and/or physiological trade-offs between the signal and

38 essential bodily processes may be responsible for regulating signal honesty. For instance, energy

39 allocated to ornament development is energy not available for other aspects of reproduction or

40 for growth and survival. Physiological trade-offs, such as those associated with carotenoid-based

41 and testosterone-regulated signals, often hinge on interactions with the immune system.

42 Carotenoids deposited in ornaments may compromise immune function directly as fewer

43 carotenoids are available for antioxidant function or indirectly via carotenoids' interactions with

44 more potent colorless antioxidants (Biard et al., 2009; Hartley and Kennedy, 2004; Lozano,

45 1994). The elevated testosterone levels necessary for signal development have also been

46 proposed to suppress immune function (Folstad and Karter, 1992; Braude et al., 1999;

47 Hasselquist et al., 1999).

48   Recent evidence suggests that stress hormones may also mediate the trade-offs

49 maintaining signal honesty (e.g., Husak and Moore 2008). Stress is a physiological response to

50 an environmental stressor that triggers the hypothalamic-pituitary-adrenal axis to secrete

51 glucocorticoid hormones such as corticosterone, which is the primary glucocorticoid in reptiles,

52 birds, and many mammals. Corticosterone then affects energy resource redistribution and

53 immune responses, as well as other essential physiological processes, in a context-specific

54 manner (French et al., 2007). While often considered in the context of survival in the face of

55 acute stressors, chronic exposure to elevated glucocorticoids can negatively impact immune

56 function. Indeed, the effect of elevated glucocorticoids varies greatly depending not only on the

57 duration, but also on the type, intensity and predictability of the experienced stressor (Wingfield

58 et al., 1998). Regardless, because glucocorticoids mediate both energy allocation and

59 physiological trade-offs, stress hormones are likely candidates for ensuring honesty in sexually

60 selected traits (Buchanan, 2000). For instance, glucocorticoids appear to negatively affect the

61 expression of sexually selected vocalizations (Buchanan et al. 2003; Spencer et al. 2003; Leary,

62 Garcia, and Knapp 2006) and ornaments (Calisi and Hews, 2007; Roulin et al., 2008).

63   We experimentally tested whether condition-dependent traits are susceptible to stress

64 using the striped plateau lizard, *Sceloporus virgatus* – a species that expresses female-specific

65 ornamentation during the reproductive season. Females develop orange throat patches during the

66 ovarian cycle, with peak ornament size and color occurring around the time of ovulation (Weiss,

67 2002). Though all reproductive females express some orange color, the extent of ornamentation

68 at peak expression varies among individuals and that variation may function as an honest signal

69 of female phenotype as well as the quality of her eggs and her future offspring (Weiss, 2006;

70 Weiss et al., 2009, 2011). If corticosterone mediates the expression of this honest signal, then we

71 predict that females experimentally exposed to stressors will produce smaller and less colorful

72 ornaments relative to control females. Because the form of the stressor can greatly impact the

73 physiological response, we used both the relatively brief, ecologically relevant stressor of tail

74 autotomy and the chronic elevation of stress hormone via corticosterone implants. Any effect of

75 these manipulations on female phenotype may be due to direct effects of the stressor or indirect

76 effects via interrelationships between corticosterone, sex steroid hormones, and body condition

77 (Husak and Moore 2008), thus we explore each of these variables in our analyses. We also

78 examined the effect of our manipulations on reproductive output and possible reproductive

79 suppression, as it may be adaptive for females to limit energy allocation to reproduction in the

80 face of stress (Wingfield and Sapolsky, 2003).

81

82 **Materials and methods**

83 *Lizard and Egg Care*

84 Sixty female and 15 male lizards were collected by noose in late April and early May from areas

85 surrounding the American Museum of Natural History's Southwestern Research Station

86 (SWRS), Portal, AZ, USA (elevation ~1600 m); all individuals were found within 4.8 km of

87 SWRS. Lizards were housed on SWRS property, randomly assigned to three outdoor enclosures

88 (61 X 30.5 X 40.6 cm; n = 20 females and 5 males per enclosure) outfitted with multiple perches

89 and hides (i.e., bricks, logs, and rocks). Males were simultaneously placed in enclosures 6 d prior

90 to the addition of females. Females were held in standard 10 gallon glass terraria tanks (up to 5

91 females/tank) for 1-7 d following capture until treatments were administered (see below) and

92 then were simultaneously added to the enclosures in the evening when males were inactive.

93 Approximately 6-8 g of crickets were added to enclosures three times per week and water was

94 provided *ad libitum*. Males and non-reproductive females were released to their site of capture in

95 late June. Gravid females were released to their site of capture following oviposition which was

96 induced in early July, once free-ranging females were observed to have initiated egg-laying

97 behavior, via 0.1 ml oxytocin injection. Without such induction, captive *S. virgatus* females may

98 become egg-bound; thus, it is common for work conducted on this species' eggs and hatchlings

99 to involve chemical induction of egg-laying (e.g., Abell, 1997; Qualls and Andrews; Smith et al.,

100 1995; Weiss et al., 2009). Some females (n = 4) did not lay full clutches after three oxytocin

101 injections (delivered once/d for 3 d); remaining eggs were collected by dissection. In addition,

102 during the course of the experiment, two females and one male went missing from the

103 enclosures, and seven females died (in a single event due to overheating while in temporary

104 containers). At the end of the experiment there were 12 or 13 females in each treatment group.

105   Eggs (n = 316 from 36 reproductive females) were examined for embryonic disks to

106 determine fertilization. Clutch size (total number of eggs produced) and clutch mass (mass of all

107 eggs whether fertilized or unfertilized) were recorded for each mother. All eggs were then buried

108 in moistened vermiculite (0.8 ml water per g vermiculite) in plastic containers (14 X 10 X 6.5

109 cm; one container per clutch) covered with parafilm and were haphazardly placed in an incubator

110 at 28° C. Daily checks for hatchlings began at 30 d of incubation and hatching occurred over a 2

111 wk period in late August. Upon emergence, each hatchling was sexed based on post-anal scales

112 and weighed. For each mother, we recorded hatchling number (total number of offspring

113 emerged from eggs), average hatchling body mass, and offspring sex ratio. Hatchlings were

114 released to the site of their mother's capture within 11 d (mean ± SE = 4.3 ± 0.2 d) of emergence.

115

116 *Female manipulations and measurements*

117 Females were randomly assigned to four treatment groups: no manipulation (CONTROL1),

118 blank implant (CONTROL2), autotomized, and corticosterone implant (C-IMP). The hormone

119 implants were made of Silastic tubing (Dow Corning, Midland, Michigan, USA; i.d. 1.47 mm,

120 o.d. 1.96 mm) filled with crystalline corticosterone (CORT; 10 mm steroid packing length, 15

121 mm total implant length), were sealed with silicone glue plugs, and were pierced three times with

122 a 22s gauge needle to increase the rate of diffusion. Blank implants were filled entirely with glue

123 (15 mm total length). Animals under deep hypothermic anesthesia were given small incisions,

124 implants were inserted into the peritoneal cavity, and the incision was closed with adhesive

125 (Weiss and Moore, 2004). For tail removal, pressure was placed on the tail 15 mm from the

126 lizard's vent and increased until the lizard released the tail below the point of contact. Treatments

127 were administered on 05 May when females are early in ovarian development (Rose, 1981) and

128 follicles are generally undetectable by palpation.

129   At the time of collection and once a week following treatment, we measured female snout

130 to vent length (SVL), total tail length, body mass, and ornament characteristics (see below). We

131 calculated body condition as the standardized residuals from a regression of body mass on SVL[3]

132 (e.g., Weiss, 2006). We use data from the initial measurement, Measurement 2 (at the time of

133 blood sampling) and Measurement 6 (the final measurement) in analyses. Though all females

134 were of reproductive size, not all produced clutches. Those females that produced a clutch were

135 classified as reproductive, and those that did not were classified as non-reproductive. Non-

136 reproductive females included both those that failed to initiate a clutch and those that initiated

137 vitellogenesis (as determined by palpation) but later resorbed their follicles.

138   We measured female ornamentation weekly and focused our analyses on peak ornament

139 expression (Weiss et al., 2009, 2011). To do so, the right throat patch of each female was

140 photographed weekly along with a small ruler using an Olympus C-5050 ZOOM 5-megapixel

141 digital camera set to macro mode with a Super Bright zoom F1.8 lens under standardized indoor

142 lighting, and each photo series was separately assessed by three people (EEM, SLW, and one

143 independent reviewer); peak ornament expression was determined to occur on the date that had

144 the most agreement between viewers (59% complete agreement). We then quantified peak

145 ornament size and peak ornament color. Peak ornament size was measured by selecting orange

146 pixels from the chosen photograph using the "color range" command of Adobe Photoshop 4.0

147 (Weiss, 2006) and determining the area of the selected pixels (in mm^2) in National Institutes of

148 Health Image 1.60.

149   Ornament color was measured using an Ocean Optics (Dunedin, FL, USA) USB 2000

150 spectrometer and OOI Base software (Integration time = 500 ms, Average = 5, Boxcar = 5).

151 Females were restrained ventral side up and the probe was placed 1.9 cm above the right throat

152 patch. The patch was illuminated by a PX-2 xenon light source and its reflectance was compared

153 to that of a Spectralon white standard. Spectra from the date of peak ornament expression were

154 processed by calculating median reflectance for every 20 nm bin from 300-700 nm, and

155 averaging the medians across three spectra collected for a female at the selected time point. For

156 females that did not develop any ornamentation (and thus do not have a date of peak expression),

157 we averaged the 20 nm bin medians across spectra from all six weeks of measurement.

158   From these processed spectra, we determined mean reflectance across all wavelengths for

159   each female, and we separately analyzed spectral shape via principal component analysis (PCA).

160   To quantify spectral shape independently of mean reflectance, we subtracted each female's

161   mean reflectance from her spectral data across all wavelengths before submitting the dataset to

162   PCA (Cuthill et al., 1999). Only principal components with eigenvalues greater than 1.0 are

163   considered further. The first principal component (PC1) summarized 59% of the variance in the

164   spectra and represents variation in the relative amount of long (> 480 nm) to short (< 480 nm)

165   wavelength reflectance. PC2 accounted for 35% of the variance and represents relative amounts

166   of both long (> 580 nm) and short (< 380 nm) wavelengths to medium wavelengths. Figure 1

167   provides the component loadings across wavelengths for each principal component.

168

169   *Hormone analysis*

170   To measure circulating hormone levels, we collected blood samples from each female's post-

171   orbital sinus 2.5-3 wk after the experimental treatments were applied; females were still pre- or

172   early vitellogenic at this time. The time of handling for blood collection averaged 121.2 ± 5.4 s

173   (range = 38-210 s). Blood samples were centrifuged to separate the plasma, and the plasma was

174   stored at -70°C until assayed. Between 18 and 85 µl of plasma was collected from each

175   individual. Plasma samples were assayed for corticosterone, progesterone, testosterone, and

176   estradiol by radioimmunoassay following established methods (Moore, 1986).

177   Briefly, samples were equilibrated overnight with 2,000 cpm of radioactive hormone for

178   determination of individual recoveries. Steroids were extracted from plasma using 30% ethyl

179   acetate in isooctane and separated by stepwise elution using celite chromatography. Collected

180   fractions were dried down, resuspended in PBS, and assayed using rabbit anti-progesterone and

181   rabbit anti-testosterone antibodies from Fitzgerald Industries International (Concord,

182   Massachusetts, USA), a sheep anti-estradiol antibody from Biogenesis Ltd. (Poole, UK), and a

183   rabbit anti-corticosterone antibody from MP Biomedicals (Solon, Ohio, USA). Based on an

184   average plasma volume of 47 µl, the minimum detectable values were 257 pg/ml for

185   progesterone, 56 pg/ml for testosterone, 64 pg/ml for estradiol, and 678 pg/ml for corticosterone.

186   Intra-assay coefficients of variation were 32.9% for progesterone, 5.5% for testosterone, 28.0% for

187   estradiol, and 20.6% for corticosterone.

188

189 *Statistical analysis*

190 The effect of treatment group on independent variables was analyzed by general linear models

191 when assumptions were met by untransformed or log-transformed variables and by non-

192 parametric Kruskal-Wallis tests when necessary. To determine our final statistical models

193 concerning the effect of our manipulations on ornamentation (size, mean reflectance, PC1, and

194 PC2), we first examined bivariate correlations among maternal phenotype variables (SVL, tail

195 length, body mass, and body condition) to assess which to include as model covariates while

196 avoiding issues of multicollinearity; we also used chi-square tests to examine the relationship

197 between the mother's treatment group and her reproductive status. Based on these analyses (see

198 below), our final general linear models examining ornament expression included treatment group

199 as well as the covariate 'body mass at the time of blood sampling' and reproductive state. To

200 examine the relative impact of corticosterone and the sex steroid hormones on measures of

201 ornamentation, we used linear regression. All analyses were conducted in SPSS Version 19.

202

203 **Results**

204 Females that went missing or died did not differ from females that remained in the study in

205 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

206 $= 0.32$) or body condition ($F_{2,57} = 0.58$, $p = 0.56$). However, the two missing females tended to

207 be small ($SVL_{missing} = 58.5 \pm 1.5$ mm; $SVL_{dead} = 61.4 \pm 0.6$ mm; $SVL_{remaining} = 62.1 \pm 0.4$ mm)

208 and escaped from the same enclosure, suggesting a small hole may have been present. Only

209 remaining females are considered in all further analyses.

210

211 *Hormone levels*

212 Measured hormone levels were not significantly related to handling time (r range = -0.14-0.07, p

213 range = 0.34-0.96, n = 51 for all). As expected, females differed in circulating CORT levels ($F_{3,47}$

214 $= 50.19$, $p < 0.001$; Table 1). CORT levels of C-IMP females were significantly higher than

215 those of all other groups ($p < 0.001$ for all) and autotomized females had significantly higher

216 levels than both control groups ($p < 0.04$ for both); CORT levels of the two control groups did

217 not significantly differ from each other ($p = 0.84$). Importantly, the elevated CORT levels of C-

218 IMP females (28.46 ± 3.02 ng/ml) appear to be physiologically relevant; Abell (1998) found

219 CORT to be as high as 37.74 ng/ml in a small sample of free-ranging female *S. virgatus*. CORT

8

220   levels were significantly negatively correlated to levels of testosterone ($r = -0.41$, $p = 0.003$) and

221   estradiol ($r = -0.35$, $p = 0.01$), but not progesterone ($r = -0.17$, $p = 0.22$); thus, treatment groups

222   also significantly differed in levels of testosterone ($X^2_3 = 18.03$, $p < 0.001$) and estradiol ($X^2_3 =$

223   13.63, $p = 0.003$), but not progesterone ($X^2_3 = 2.54$, $p = 0.47$; Table 1). Levels of all three sex

224   steroids were positively correlated to each other (r range: 0.77-0.94, $p < 0.001$ for all).

225

226   *Maternal phenotype*

227   Females in the four treatment groups did not significantly differ in initial SVL ($F_{3,47} = 0.27$, $p =$

228   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

229   ($F_{3,47} = 0.54$, $p = 0.66$). They also did not significantly differ in body mass or body condition at

230   the time of blood sampling or at the final measurement (all $p > 0.40$). Body mass at blood

231   sampling was the only variable both significantly positively correlated to all body shape

232   variables ($r \geq 0.34$, $p \leq 0.015$, $n = 51$ for all) and statistically uncorrelated to any of the ornament

233   variables (r range: -0.18-0.13, $p \geq 0.20$, $n = 51$) or to CORT ($r = 0.21$, $p = 0.15$, $n = 51$); thus it

234   was selected as the sole covariate to explore body shape relationships in our models investigating

235   the effect of treatment on ornamentation.

236

237   *Reproductive status and output*

238   Final reproductive state was also included in our final statistical models as ornament expression

239   is known to vary with reproductive state (Weiss, 2002) and C-IMP females were significantly

240   less likely than other females to produce a clutch of eggs ($X^2_3 = 9.04$, $p = 0.03$; Table 2). Of the

241   females that failed to produce a clutch, 53% were C-IMP females ($n = 8$), 20% were autotomized

242   ($n = 3$) and 13% were in each of the two control groups ($n = 2$/control group). Some of these 15

243   females appeared to initiate the reproductive cycle and then resorb their eggs ($n = 8$), whereas

244   five C-IMP and two autotomized females never developed detectable follicles. Among C-IMP

245   females, those that reproduced tended to have lower CORT levels ($t_{11} = -1.97$, $p = 0.07$) and had

246   higher body mass at the time of blood sampling ($t_{11} = 3.00$, $p = 0.04$) than those that did not

247   reproduce (Table 2). (We did not test for reproductive vs. non-reproductive differences within

248   other treatment groups due to low power, but the only other apparent trend was for CORT levels

249   of autotomized females to be lower in reproductive than non-reproductive subjects; see Table 2.)

250   Among those females that did successfully produce a clutch, treatment group had no significant

251   effect on any measure of reproductive output including clutch size ($F_{3,32} = 1.30$, p = 0.29), clutch

252   mass ($F_{3,31} = 0.51$, p = 0.68), hatchling number ($F_{3,32} = 0.47$, p = 0.71), average hatchling mass

253   ($F_{3,22} = 0.73$, p = 0.55), and offspring sex ratio ($F_{3,19} = 0.65$, p = 0.60).

254

255   *Effect of stress on ornament expression*

256   Peak ornament size was significantly smaller in non-reproductive females than in reproductive

257   females ($F_{1,42} = 7.11$, p = 0.01) and was marginally affected by treatment ($F_{3,42} = 2.75$, p =

258   0.055), with C-IMP females tending to have smaller ornament patches than did females in all

259   other groups (Fig. 2A); there was no reproduction X treatment interaction ($F_{3,42} = 1.26$, p = 0.30).

260   Mean reflectance at peak ornament expression (Fig. 2B) and PC1 (Fig. 2C) both were unaffected

261   by reproductive state (reflectance: $F_{1,42} = 2.77$, p = 0.10; PC1: $F_{1,42} = 0.003$, p = 0.96), treatment

262   (reflectance: $F_{3,42} = 0.98$, p = 0.41; PC1: $F_{3,42} = 0.76$, p = 0.52), and the interaction of these two

263   factors (reflectance: $F_{3,42} = 0.78$, p = 0.51; PC1: $F_{3,42} = 0.14$, p = 0.94). However, PC2 was

264   strongly affected by both factors (reproduction: $F_{1,42} = 9.13$, p = 0.004; treatment: $F_{3,42} = 6.15$, p

265   = 0.001; Fig. 2D), although not by a reproduction X treatment interaction ($F_{3,42} = 0.19$, p = 0.90).

266   Lower PC2 scores, indicating patches with less UV and orange-to-red wavelength reflectance

267   relative to medium wavelength reflectance, were found in non-reproductive females than in

268   reproductive females. PC2 scores were also lower in C-IMP females relative to unmanipulated

269   CONTROL1 females (p < 0.001) and autotomized females (p = 0.007), and were lower in blank-

270   implant CONTROL2 females than in CONTROL1 females (p = 0.02). Removing reproductive

271   state from the above statistical models, due to concerns regarding low sample size among the

272   non-reproductive group and thus low statistical power, strengthened the above patterns

273   concerning the effect of treatment on ornament expression (size: p = 0.002; reflectance: p = 0.08;

274   PC1: p = 0.33; PC2: p < 0.001).

275   Because treatment groups differed not only in CORT levels but also in sex steroid

276   hormone levels, we used regression to parse out which hormone played the strongest role in

277   mediating the effect on peak ornament size and PC2 scores. For both variables, CORT was the

278   only steroid with a significant coefficient (Table 3); elevated CORT levels were associated with

279   smaller patches ($\beta = -0.403$, p = 0.005) and lower PC2 scores ($\beta = -0.438$, p = 0.001; Fig. 3).

280

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 corticosterone levels and are predictive of both female and offspring phenotype (Weiss, 2006; Weiss et al., 2009, 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 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 (2007) found that the color of male *Sceloporus pyrocephalus* correlated to circulating CORT levels and not to androgen levels, whereas some aspects of female coloration correlated to CORT while others correlated to 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 8-9 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 1.5-2 fold CORT increase experienced by autotomized females was not sufficient to significantly impact these aspects of ornament development. We

11

312 assumed that autotomy would represent an ecologically relevant stressor as tail break frequencies

313 are around 20% in free-ranging populations (Smith, 1996), and that the stressor would be

314 relatively acute, though because we sampled at only one time point, the time course and pattern

315 of the CORT response is unknown. While autotomy has been well studied in lizards for its

316 effects on locomotion, behavior and survival (Bateman and Fleming, 2009), remarkably little

317 work has examined the response of the hypothalamic-pituitary-adrenal axis. In one study that did

318 examine this effect, autotomized female skinks, *Eulamprus heatwolei*, had elevated CORT 1 h

319 post-autotomy but levels had returned to baseline by 2 d and 14 d post-event (Langkilde and

320 Shine, 2006). Thus, the current results are novel as they document a prolonged, though relatively

321 mild, stress response following tail loss in lizards. This physiological response may nevertheless

322 mediate known compensatory behavioral changes (Cooper 2007) and growth rate reductions

323 (Smith, 1996) among autotomized *S. virgatus*.

324   Overall, the sensitivity of the ornament to chronic stress suggests ornament development

325 is associated with a physiological cost. This cost may be realized via immunosuppression, as the

326 link between chronic elevations of CORT and immune suppression has strong empirical support

327 (in contrast to the less consistent experimental support for the link between androgens and

328 immunosuppression proposed by the immunocompetence handicap hypothesis; Buchanan, 2000;

329 Folstad and Karter, 1992; Roberts et al., 2004); however the effect of chronic stress on female *S.*

330 *virgatus* immune response remains to be tested. Regardless of the underlying mechanism, the

331 mediation of ornament expression by stress hormones suggests that individuals who base

332 reproductive decisions on the assessment of these secondary sexual characteristics may benefit

333 by increasing mating opportunities with high quality, healthy individuals (Husak and Moore,

334 2008).

335   The size and color of female ornaments were also related to reproductive state, with

336 smaller and less colorful patches being associated with the suppression of reproduction that

337 occurred among some females, independent of treatment group. Though sample size of non-

338 reproductive females is small in this study, the pattern detected was expected as the ornament is

339 known to develop and fade in concert with the ovarian cycle (Weiss 2002), thereby serving as a

340 signal of reproductive state as well as phenotypic quality. Also expected was the increased rate

341 of reproductive suppression among C-IMP females, as it seems selectively advantageous for

342 limited energy resources to be shunted to survival rather than reproduction in the face of an

343 anabolic challenge. However, some female reptiles have been found to maintain reproduction in

344 such instances (reviewed by Moore and Jessop 2003) and indeed, 38% of the C-IMP females

345 successfully reproduced despite high circulating CORT levels. Thus, the effect of CORT on

346 reproductive suppression varies among individual *S. virgatus* females, possibly in a context-

347 dependent manner. In Wingfield and Sapolsky's (2003) review of the maintenance of

348 reproduction in the face of stress, they highlight that selection for stress resistance may be

349 particularly strong among seasonal breeders with only one breeding opportunity per year; indeed,

350 this is the case for the study population of *S. virgatus*. Conversely, it is interesting to note that

351 reproductive suppression also occurred among four (15%) control females with CORT levels that

352 were 7.5 times lower than that experienced by reproductive C-IMP females, and suppression

353 rates up to 25% have been found among unmanipulated *S. virgatus* females housed similarly to

354 those in the current study (Weiss et al., 2002). Reproductive suppression in response to captivity

355 may occur via mechanisms independent from CORT production.

356   Of the females that did reproduce, treatment group did not have a significant effect on

357 reproductive output, including clutch size, clutch mass and average hatchling body mass. We

358 also found no significant effect on offspring sex ratio, which might be expected under models of

359 sex allocation theory (Charnov, 1982; Correa et al., 2005; Love et al., 2005). It is possible that

360 these results are influenced by our relatively small sample size, and that our treatments may have

361 affected aspects of offspring phenotype that were unmeasured here such as morphology, growth,

362 immune response, dispersal and risk-aversion. However, specific predictions are difficult to

363 make as the direction of corticosterone-based maternal effects has been found to vary with

364 offspring sex and among species (De Fraipont et al., 2000; Hayward and Wingfield, 2004; Love

365 et al., 2005; Meylan and Clobert, 2005; Sinervo and DeNardo, 1996; Uller et al., 2009; Vercken

366 et al., 2007; Warner et al., 2009). To determine the fitness consequences of elevated maternal

367 corticosterone, it would be necessary to track the survival of females and their resultant offspring

368 (Love and Williams, 2008; Meylan and Clobert, 2005).

369

373 assessing peak ornament expression; and Ellen Ketterson and Danielle Whittaker for

374 radioimmunoassay assistance.

375

379

380 **References**

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506 **Figure legends**

507 Figure 1. Component loadings across wavelengths from the principal component analysis of

508 peak ornament color in female *Sceloporus virgatus*. PC1 (solid line) represents variation in the

509 relative amount of long to short wavelength reflectance, and PC2 (dashed line) represents

510 relative amounts of both long and short wavelengths to medium wavelengths.

511

512 Figure 2. The effect of treatment on mean + SE (A) peak ornament size, (B) mean reflectance,

513 (C) PC1 scores and (D) PC2 scores of reproductive and non-reproductive female lizards. Sample

514 sizes are at the base of bars in A.

515

516 Figure 3. Relationship between corticosterone levels of female lizards and (A) peak ornament

517 size and (B) PC2 scores.

518

Table 1. Mean (± SE) levels of steroid hormones (ng/ml) 2.5-3 wk post-treatment.

	Corticosterone	Progesterone	Testosterone	Estradiol
CONTROL1 (n = 13)	3.19 ± 0.43^a	0.93 ± 0.38	1.63 ± 0.60^a	0.98 ± 0.35^a
CONTROL2 (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 $p < 0.05$.

Table 2. Mean (± SE) CORT levels (ng/ml) and body mass (g) of females that did and did not reproduce.

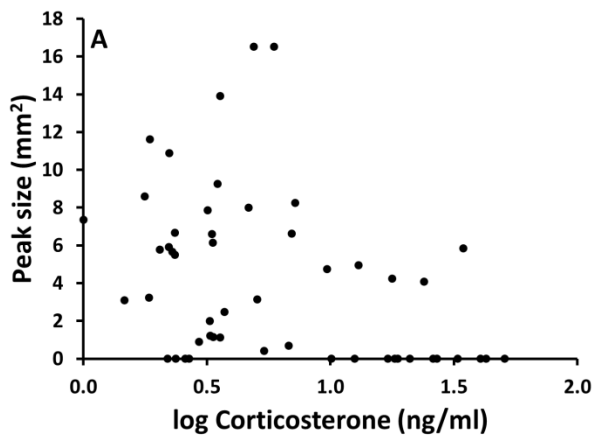|	CONTROL1			CONTROL2			Autotomized			C-IMP		
	CORT	Body mass	n	CORT	Body mass	n	CORT	Body mass	n	CORT	Body mass	n
Reproductive	3.24 ± 0.51	7.37 ± 0.34	11	3.79 ± 0.81	7.44 ± 0.37	11	4.67 ± 0.82	7.10 ± 0.41	9	21.96 ± 3.15	8.96 ± 0.65	5
Non-reproductive	2.91 ± 0.38	5.73 ± 0.29	2	2.76 ± 0.13	7.63 ± 0.37	2	9.25 ± 3.47	6.98 ± 1.42	3	32.52 ± 3.98	7.06 ± 0.31	8

Table 3. Regression models examining the effect of circulating steroid hormone levels on ornament expression. p values are listed within parentheses.

	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)

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