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Authors: Christopher D. Robinson , and Matthew E. Gifford

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Intraseasonal Changes of Patch Color in Prairie Lizards (*Sceloporus consobrinus*)

CHRISTOPHER D. ROBINSON¹ AND MATTHEW E. GIFFORD

Department of Biology, University of Central Arkansas, Conway, AR 72035, USA

ABSTRACT: Many sexually selected traits are static throughout the breeding season. Some vary within the breeding season, however, typically in response to physiological changes. Most work attempting to understand the mechanisms underlying phenotypic changes has occurred in the laboratory, with comparatively less research examining how changes to physiology affect signal production in natural populations. Here, we report intraseasonal plasticity in abdominal and throat patch color of Prairie Lizards (*Sceloporus consobrinus*), and examine how changes in body size, body condition, and testosterone underlie intraseasonal variation. We found that changes in testosterone best explain why throat patches of Prairie Lizards become less blue throughout the breeding season. If females reproduce with males based on patch color, then individual changes in patch phenotypes could have important consequences if signaling morphology is asynchronous among males.

Key words: Allometry; Condition; Phrynosomatidae; Plasticity; Spectrometry; Testosterone

SEXUALLY selected traits can manifest themselves as large or colorful ornaments. Often, these traits show little variation over time. Some sexually selected traits are quite dynamic, however, both inter- (Moore and Marler 1986; Middleton 1993) and intraseasonally (Ferguson 1976; Weiss 2002; Jessop et al. 2009). Understanding intraseasonal variation, and the resulting selection consequences (McGraw and Hill 2004), in sexually selected phenotypes can inform evolutionary biologists why variation is maintained among individuals even if some phenotypes are preferred by mates over others. Several mechanisms have been experimentally tested in isolation to understand how physiology can affect signaling morphology, including hormone variability (Rand 1990; Jessop et al. 2009) and changes in condition (Hamilton and Zuk 1982; Weiss 2006). However, interactions among physiological traits can confound inferences from these isolated experiments when downstream effects are not controlled. For example, the production of free radicals can be a consequence of both testosterone, via increased metabolic rate, and age, as antioxidant capacity decreases (reviewed in Metcalfe and Alonso-Alvarez 2010). By not estimating the effect of both predictors on free-radical production, important variation can go unexplained. Studies using repeated-measures designs in natural populations could help clarify which of many mechanisms best explain phenotypic changes within a season.

Prairie Lizards (*Sceloporus consobrinus*) are ideal animals to use in studies investigating the role of physiological changes in signaling morphology. Generally, individuals stay within a defined home range (Haenel et al. 2003), which allows for reliable recaptures and, therefore, repeated measurements of individuals. Additionally, testosterone (John-Alder et al. 2009) and condition (Goldberg 1974) decrease throughout the breeding season, so intraseasonal changes in color might be related to the degree of change in these traits. Finally, this species exhibits sexually dimorphic coloration on their abdomens and throats (males have blue, females are generally white; Cooper and Burns 1987), suggesting patch morphology (color and size) might be under sexual selection. Indeed, female *S. undulatus*, a

closely related species, use throat patch area, in conjunction with other traits and in the absence of territorial cues, to assess males (Swierk et al. 2012). Additionally, both abdominal and throat patch color reflects sprinting ability in *S. consobrinus* (Robinson and Gifford 2018), which, in another lizard species, is associated with reproductive success (Husak et al. 2006).

Many studies have examined the importance of color in *Sceloporus*, considering both proximate and ultimate mechanisms. The ancestral state of *Sceloporus* color is likely sexual dichromatism, with multiple evolutionary losses and gains of color among species (Wiens 1999). Despite several evolutionary losses of color, behavioral responses have been conserved. As examples, males from species that do not have colorful patches still respond submissively to signalers with color, and the presence of a color patch elicits aggressive responses in sexually dichromatic species (Cooper and Burns 1987; Quinn and Hews 2000, 2010). Developmentally, testosterone induces male-like coloration (Kimball and Erpino 1971; Rand 1990, 1992; Quinn and Hews 2003; Cox et al. 2005). Specifically, testosterone increases the quantity of melanophores that lie deep to structural iridophores (Kimball and Erpino 1971; Morrison 1995) and that are necessary to absorb relatively long wavelength light (i.e., greens and reds), so only blue is reflected. Furthermore, melanophores are more abundant in association with blue abdominal and throat patches than other areas of the skin (Morrison 1995). Interestingly, the iridophore layer is comprised of “nonideal” iridophores that can alter peak reflectance by changing the distance between stacked iridophores (Fujii 1993). Spacing between iridophores, which influences the light-scattering ability of this layer, is temperature dependent in some phrynosomatids (Morrison et al. 1996), such that at higher temperatures iridophores are closer together and therefore reflect shorter wavelengths (appear more blue; Morrison et al. 1996; Langkilde and Boronow 2012; Stephenson et al. 2016; Robinson and Gifford 2018). The research concerning the ontogeny and plasticity of color in male *Sceloporus* allows for the formulation of testable hypotheses about intraseasonal changes within individuals.

¹ CORRESPONDENCE: e-mail, cdrobinsontx@gmail.com

Although these patches are permanent (Rand 1992; Cox et al. 2005), color expression might vary within a season if the expression of color is tied to physiological traits that also exhibit seasonal variation. Chin color of male *Sceloporus undulatus erythrocheilus* (Rand 1990, 1992) and throat patch color of female *S. virgatus* (Weiss 2002) and *S. graciosus* (Ruiz et al. 2011) vary within the breeding season, in association with testicular recrudescence/senescence and state of gravidity, respectively. To date, however, no studies have documented how seasonality might affect the color of male abdominal and throat patches in natural populations (but see Hews and Quinn 2003 for evidence against seasonal activation of color by hormones in experimental populations).

Here, we test whether patch coloration in Prairie Lizards exhibits intraseasonal variation. We present three hypotheses that, although not mutually exclusive, explore potential mechanisms underlying variation in patch color. First, we hypothesize that as males grow, patches will become less blue (reflect longer wavelengths). If iridophores are costly to produce, maintaining a sufficient density of iridophores to reflect blue (Morrison et al. 1996) as patches grow would become increasingly difficult, especially if patches grow under positive allometry (Ossip-Draho et al. 2018). Next, we hypothesize that males with higher testosterone will reflect shorter wavelengths, and that as testosterone decreases throughout the breeding season (John-Alder et al. 2009), patches will become less blue. We made this prediction because testosterone can influence melanin deposition (Kimball and Erpino 1971; Cooper and Greenberg 1992; Castrucci et al. 1997), so changes in testosterone could alter the melanophore layer that lies deep to the iridophores, changing patch reflectance. Finally, we hypothesize that males in better condition will be bluer and, as condition decreases throughout the breeding season, patches will reflect lower quality.

MATERIALS AND METHODS

Animal Subjects

In the 2017 breeding season (~March–June), we captured 47 male *S. consobrinus* by noose or hand at the Sylvan Hills Prayer Walk in Sherwood, Pulaski County, AR, USA (34.857814N, -92.223018W; datum = WGS84). In this population, copulation can occur as early as 19 March, with male territorial behaviors starting earlier (personal observations). In 2016–2018, we documented the first clutches being oviposited on 6–9 May. Males are observed actively courting females until early June, after which time males become relatively inactive. Females can continue to lay eggs through mid-July, with hatchling emergence occurring from early July through mid-late September.

We measured snout–vent length (SVL) with the use of a clear ruler (± 0.5 mm), mass with a Pesola spring scale (± 0.1 g), patch morphology, and testosterone for each recaptured male. We calculated body condition as the residuals from a linear regression of \log_{10} -transformed mass on \log_{10} -transformed SVL. Mass, SVL, and patch morphology were quantified for 21 males once, 15 males twice, 5 males 3 times, and 6 males 4 times. For testosterone measurements, sample sizes are slightly different because samples were lost or concentrations were too low to detect (in June): 24 males

were measured once, 11 males were measured twice, 6 males were measured 3 times, and 4 males were measured 4 times.

Patch Morphology

Because patch color in Prairie Lizards is thermally sensitive, we incubated lizards in a VL-36 incubator (Percival Scientific, Perry, IA, USA) at 34°C (mean [± 1 SD] field body temperature in 2016 = 34.2 \pm 2.3°C; Robinson and Gifford 2018) for 1 h prior to measurement. We quantified color with the use of an Ocean Optics Flame miniature spectrometer with a X-2 Pulse xenon light source (Ocean Optics, Inc., Dunedin, FL, USA). The probe was held perpendicular to the surface of the subject's body, and a probe holder was used to maintain a fixed distance of 5 mm from the reflective surface. We took three measurements from the left abdominal patch (anterior, central, and posterior) and one from each throat patch. We averaged values within patch type (i.e., abdominal and throat) to obtain representative spectra for each subject. We used the pavo package (Maia et al. 2013) in R (v3.4.1; R Core Team 2017) to average spectra over 5-nm bins (following Stuart-Fox et al. 2006), smoothed them using locally weighted smoothing, and extracted brightness, saturation, and hue (Montgomerie 2006; Maia et al. 2013). Brightness represents the total reflectance over the spectral range (Delhey et al. 2003; Siefferman and Hill 2005; Andersson and Prager 2006), with higher brightness appearing closer to white (total reflectance). Saturation is calculated as the difference between the wavelengths of maximum and minimum reflectance divided by the brightness (Smiseth et al. 2001; Andersson et al. 2002; Andersson and Prager 2006) and represents color intensity. Hue is defined as the wavelength at which the peak reflectance occurs (Andersson 1999; Keyser and Hill 2000; Andersson and Prager 2006). Lizards that were shedding were excluded from color quantification.

To quantify patch area (mm²), we took ventral photographs with a Casio Exilim EF-X1 camera after spectral measurements. We imported these images into ImageJ (Schneider et al. 2012) and used the freehand tool to draw around each patch. Because some individuals did not have distinct left and right throat patches (the patches were large and had grown together), we added the two patch measurements together for individuals with distinct patches to obtain one measurement. Although each lizard had distinct abdominal patches, we added left and right abdominal patch measurements together to yield a total blue area for the abdominal region.

Testosterone Quantification

We collected approximately 100 μ L of blood from the right postorbital sinus within 2 min of capture to minimize the potential interactions between testosterone and corticosterone (Moore et al. 1991). We stored samples on ice and obtained plasma from samples via centrifugation at 8000 rpm. Plasma was stored at -80°C until quantification. Samples were triple-extracted with the use of dichloromethane, dried under compressed N₂, and reconstituted in ELISA buffer. Testosterone was quantified with the use of a testosterone ELISA Kit (No. 582701, Cayman Chemical) according to manufacturer specifications. This kit has a detectability range from 3.9–500 pg/mL, and a cross-reactivity with 5 α -dihydrotestosterone and 5 β -dihydrotestos-

TABLE 1.—Mean values (± 1 SE) for phenotypic traits measured in male Prairie Lizards (*Sceloporus consobrinus*) throughout the 2017 breeding season (March–June) in Pulaski County, AR. Bolded terms represent significant phenotypic change across months ($P < 0.05$).

	Month			
	March	April	May	June
Body condition	0.03 \pm 0.02	-0.02 \pm 0.01	0.01 \pm 0.01	-0.02 \pm 0.02
Testosterone (ng/mL)	186.76 \pm 18.73	134.44 \pm 15.01	82.54 \pm 12.34	36.44 \pm 10.99
Abdominal hue (nm)	484.92 \pm 3.71	504.11 \pm 2.92	509.47 \pm 3.14	511.67 \pm 4.61
Throat hue (nm)	470.13 \pm 3.41	486.03 \pm 3.09	489.00 \pm 2.55	494.09 \pm 3.54

terone of 27.4 and 18.9%, respectively. Although this kit has not been validated for use in *Sceloporus*, it has been validated in other genera of lizards (e.g., *Anolis*, Cox et al. 2015; *Heloderma*, Carruth 2015). Klukowski (2011) has validated a similar kit (Assay Designs, Inc. Correlate-EIA™ Testosterone kit 900-065, Ann Arbor, MI, USA) for *S. undulatus*. Both kits use mouse monoclonal antibodies and, therefore, should perform similarly.

We ran our standard curve in duplicate. We diluted samples by a factor of 250, added 50 μ L of the plasma dilution to wells in triplicate, and combined those samples with 50 μ L testosterone AchE and 50 μ L testosterone ELISA antiserum. Then, we placed the 96-well plate on an orbital shaker for 2 hr at room temperature. We then washed the plates and added 200 μ L of Ellman's Reagent prior to incubating the plates on an orbital shaker in the dark for 1 hr at room temperature. We used a photospectrometer to quantify the absorbance for each well at 415 nm. Intra-assay variation was 15.9%.

Statistical Analyses

All analyses were performed in R. To examine effects of date, SVL, testosterone, and condition on patch color, we used linear mixed-effects models available for the package lmerTest (Kuznetsova et al. 2016). Models included one color variable as the dependent variable and individual ID as a random effect. Additionally, we regressed each of these variables against date to detect intraseasonal changes. When date significantly predicted a variable, we included date as a covariate in subsequent analyses.

To remove the effects of multicollinearity between date and other variables, we used sequential regression (Graham 2003). We used the lmodel2 package (Legendre 2014) to calculate ordinary least squares (OLS) slopes for allometric relationships (Kilmer and Rodríguez 2017). Although OLS slopes can be calculated as the slope from a linear regression including \log_{10} transformed variables, the lmodel2 package reports 95% CIs, which are useful when assessing relationships that might be close to isometric. We also used multiple regression to parse out the variables that exerted the strongest influence on color. In multiple-regression models, we scaled variables by subtracting the mean value from each datum and dividing by the standard deviation, resulting in a mean value of 0 and a standard deviation of 1. For temporally variable color metrics having multiple significant predictors (as determined by date being a significant covariate in earlier analyses), we started with a model that included all significant predictors. We then compared each model to a model with one fewer term with the use of AIC (Burnham and Anderson 2004). A model was considered better than another if it had an AIC value at least 2 points lower than the model with which it was being compared. For

our best model, we calculated the partial regression coefficient for each predictor variable to assess the relative importance of each variable to the model.

RESULTS

As the breeding season progressed, mean values for testosterone ($F_{1,78.98} = 22.08$, $P < 0.001$) and body condition ($F_{1,57.01} = 5.84$, $P = 0.01$) decreased, whereas abdominal hue ($F_{1,53.10} = 43.70$, $P < 0.001$), throat hue ($F_{1,52.01} = 46.09$, $P < 0.001$), and throat brightness ($F_{1,61.16} = 7.83$, $P < 0.001$) all increased (linear mixed-effects model with subject ID as random effect; Table 1, Fig. 1). Furthermore, larger males had throat patches that appeared greener ($F_{1,52.34} = 6.90$, $P = 0.01$) and were brighter ($F_{1,50.35} = 7.49$, $P = 0.01$). Abdominal patch color was not related to body size (Table 2). Testosterone was not related to SVL ($F_{1,79.99} = 0.33$, $P = 0.57$), but was negatively related to throat hue (males with higher testosterone had bluer patches; $F_{1,48.18} = 4.28$, $P = 0.04$). No color metric varied with body condition. In a multiple regression including testosterone and SVL to predict throat hue, both variables influenced throat hue (Table 3), with testosterone having the strongest effect (partial correlations; testosterone = -0.54; SVL = 0.27). The area of both patch types exhibited positive allometry with body size (OLS slope [95% CI]: abdominal patch = 3.78 [3.40–4.17]; throat patch = 3.04 [2.07–4.00]).

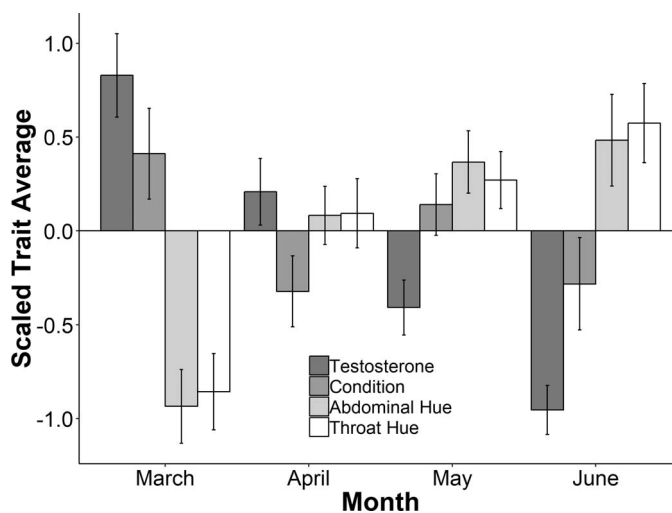


FIG. 1.—Scaled values for phenotypic traits measured in male Prairie Lizards (*Sceloporus consobrinus*) throughout the 2017 breeding season (March–June) in Pulaski County, AR. Variables were scaled to mean of 0 and standard deviation of 1. The increase of hue over the breeding season corresponds with patches becoming greener (reflecting longer wavelengths). Error bars represent ± 1 SE.

TABLE 2.—Summary results from univariate mixed-effects models examining phenotypic traits in male Prairie Lizards (*Sceloporus consobrinus*) collected throughout the 2017 breeding season (March–June) in Pulaski County, AR. All models include individual ID as a random effect. Bolded rows represent significant effects ($P < 0.05$). Terms followed by an asterisk (*) represent the independent variable for a given model (SVL = snout–vent length).

	F value	P value
Body condition*		
Abdominal hue	1.21	0.28
Abdominal brightness	0.58	0.45
Abdominal saturation	1.19	0.28
Abdominal patch area	<0.001	0.95
Throat hue	0.90	0.35
Throat brightness	0.94	0.34
Throat saturation	<0.001	0.96
Throat patch area	2.55	0.12
SVL*		
Abdominal hue	0.43	0.51
Abdominal brightness	<0.001	0.99
Abdominal saturation	2.79	0.10
Abdominal patch area	200.52	<0.001
Throat hue	6.90	0.01
Throat brightness	7.49	0.01
Throat saturation	1.19	0.28
Throat patch area	19.59	<0.001
Testosterone	0.54	0.46
Testosterone*		
Abdominal hue	0.41	0.53
Abdominal brightness	0.05	0.83
Abdominal saturation	3.75	0.06
Abdominal patch area	0.89	0.35
Throat hue	4.28	0.04
Throat brightness	0.06	0.81
Throat saturation	0.34	0.56
Throat patch area	3.35	0.08
Body condition	2.28	0.14

DISCUSSION

We have provided evidence that patch color in *S. consobrinus* is temporally variable in response to testosterone as measured at 34°C, an ecologically important temperature for *Sceloporus* (Robinson and Gifford 2018). As testosterone decreased, patches reflected longer wavelengths (appeared less blue). Testosterone and condition varied within individuals, but we eliminated condition as a mechanism underlying intraseasonal change because it did not co-vary with color. Body size, although related to throat color, did not change significantly throughout the breeding season and, therefore, is unlikely to explain intraseasonal variation. Indeed, multiple regression analyses indicated that hue was related to body size, but to a lesser extent than plasma testosterone level.

Testosterone could explain temporal variation in color for many reasons. First, it is required for patch development (Kimball and Erpino 1971; Rand 1990, 1992; Quinn and Hews 2003; Cox et al. 2005), and subjects with high testosterone had the bluest throat patches, suggesting that testosterone might influence iridophores, melanophores, or the interaction between them. Although patches are permanent (Rand 1992; Cox et al. 2005), the underlying structures are dynamic (Taylor and Bagnara 1972). In Prairie Lizards, testosterone might interact with α -melanocyte-stimulating hormone (α -MSH) to influence melanin dispersion (Bagnara and Hadley 1973; Castrucci et al. 1997; Calisi and Hews 2007), which, in turn, affects iridophore

TABLE 3.—Comparison of models predicting throat hue in male Prairie Lizards (*Sceloporus consobrinus*) measured throughout the 2017 breeding season (March–June) in Pulaski County, AR. The model with the lowest AIC is bolded (Test = testosterone, SVL = snout–vent length).

Model	Model predictors	AIC	Δ AIC
1	Test + SVL	633.72	0.00
2	Test	646.01	12.29
3	SVL	664.15	30.43

reflectance (Taylor and Bagnara 1972; Morrison 1995; Macedonia et al. 2000). Decreasing levels of testosterone could result in lower levels of α -MSH and, therefore, fewer melanophores deep to the iridophore layer. This would result in patches appearing greener because the longer wavelengths typically absorbed by the melanophores can instead be reflected (Taylor and Hadley 1970). Alternatively, testosterone can cause oxidative stress. Individuals with high testosterone levels use more energy (Marler et al. 1995), which leads to an increased free radical load. Testosterone might also reduce resistance to oxidative stress (Alonso-Alvarez et al. 2007). Therefore, individuals with high testosterone could produce more free radicals while having a depressed antioxidant capacity, potentially compounding the effects of oxidative stress. Iridophores can be damaged by free radicals (Cahn et al. 2015), so individuals with high testosterone levels might benefit from displaying attractive signals early in the season, but incur a reproductive cost later if they have fewer iridophores as a function of oxidative stress (i.e., appearing greener).

Body size, which is generally correlated with age, could also affect the reflectance of individual patches. Older individuals have higher amounts of free radicals than younger ones (Olsson et al. 2008). Additionally, antioxidant capacity decreases with age (Alonso-Alvarez et al. 2006), so larger (likely older) individuals have a decreased response to oxidative stress. This might manifest itself in longer wavelength (less blue) reflectance of patches if oxidative stress damages iridophores (Cahn et al. 2015). Alternatively, if iridophores are costly to produce (San-Jose et al. 2013; Cahn et al. 2015), larger individuals might reflect longer wavelengths (appear less blue) because of the inability to sustain iridophore density as patches grow disproportionately fast (under positive allometry; Ossip-Drahos et al. 2018).

One potential mechanism left unaddressed in our study is how parasitism might affect individual color change (Hamilton and Zuk 1982). Parasite load has been correlated with many variables examined in this study, including testosterone levels (Klukowski and Nelson 2001; Cox and John-Alder 2007), body condition (Sorci and Clobert 1995; Weiss 2006), and color (Hamilton and Zuk 1982; Ressel and Schall 1989; Calisi et al. 2008; Cook et al. 2013). Consequently, individual rates of infestation could explain some variance in intraseasonal color change. We do not, however, expect this to be the case. In our population of Prairie Lizards, trombiculid mites are by far the most abundant ectoparasite, followed by ticks (Ixodidae; personal observations). The reproductive phenology of trombiculids at our study site is such that their abundance does not peak until the start of the summer (Sasa 1961; personal observations), and a similar pattern holds for populations in Tennessee (Klukowski 2004). Because this infestation does not occur until after increases in patch hue

have occurred (becoming less blue; Fig. 1), it is unlikely that trombiculid mite load could have a strong effect. However, we are unable to rule out the influence of any internal parasite activity (Ressel and Schall 1989; Calisi et al. 2008).

Understanding plasticity and its underlying mechanisms provides insights into the evolution of sexual traits. Similarly, it helps explain why intrapopulational variation can be maintained despite female preferences for certain phenotypes. Single measurements of individuals within the breeding season might miss important intra-individual variation that results in asynchronous mating windows for different males. Future studies should expand the use of repeated measurements to elucidate how phenotypic selection changes over time, and how behavior (e.g., thermoregulation) can mediate individual responses to intraseasonal variation.

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