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Multiple color patches and parasites in *Sceloporus occidentalis*: differential relationships by sex and infection

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Abstract

Parasites generally have a negative influence on the color expression of their hosts. Sexual selection theory predicts resistant high-quality individuals should show intense coloration, whereas susceptible low-quality individuals would show poor coloration. However, intensely colored males of different species of Old and New World lizards were more often infected by hemoparasites. These results suggest that high-quality males, with intense coloration, would suffer higher susceptibility to hemoparasites. This hypothesis remains poorly understood and contradicts general theories on sexual selection. We surveyed a population of *Sceloporus occidentalis* for parasites and found infections by the parasite genera *Lankesterella* and *Acroeimeria*. In this population, both males and females express ventral blue and yellow color patches. *Lankesterella* was almost exclusively infecting males. The body size of the males significantly predicted the coloration of both blue and yellow patches. Larger males showed darker (lower lightness) blue ventral patches and more saturated yellow patches that were also orange-skewed. Moreover, these males were more often infected by *Lankesterella* than smaller males. The intestinal parasite *Acroeimeria* infected both males and females. The infection by intestinal parasites of the genus *Acroeimeria* was the best predictor for the chroma in the blue patch of the males and for hue in the yellow patch of the females. Those males infected by *Acroeimeria* expressed blue patches with significantly lower chroma than the uninfected males. However, the hue of the yellow patch was not significantly different between infected and uninfected females. These results suggest a different effect of *Lankesterella* and *Acroeimeria* on the lizards. On the one hand, the intense coloration of male lizards infected by *Lankesterella* suggested high-quality male lizards may tolerate it. On the other hand, the low chroma of the blue coloration of the infected males suggested that this coloration could honestly express the infection by *Acroeimeria*.

Key words: animal communication, coloration, Hamilton and Zuk, parasites, sexual selection.

In 1982, Hamilton and Zuk proposed a co-evolutionary mechanism to explain how sexual selection favors resistance against parasitic diseases in nature: The parasite hypothesis (Hamilton and Zuk 1982). This Hamilton–Zuk (H–Z) hypothesis predicts a negative relationship between the expression of the host's secondary sexual characters and the degree of its parasitic infections (Balenger and Zuk 2014). Thus, sexual selection favors resistance against parasites because individuals would mate with highly ornamented individuals (Hamilton and Zuk 1982). Evidence supporting the parasites hypothesis (Hamilton and Zuk 1982) exists for insects (Zuk 1988; Worden et al. 2000), fish (Ward 1988; Houde and Torio 1992), and birds (Merilä et al. 1999; Hörak et al. 2001; del Cerro et al. 2010). In all these host–parasite systems, the infected individuals usually show poor breeding coloration (Thompson et al. 1997; Martínez-Padilla et al. 2007, but see also Skarstein and Folstad 1996) and suffer reduced survival (Merino et al. 2000; Martínez-de la Puente et al. 2010). However, studies of both Old and New World lizards have produced mixed results. On the one hand, studies investigating the relationships between the expression of the breeding coloration and the infection by ticks or nematodes in lizards suggest that these parasites negatively affect the expression of breeding coloration in male lizards (Václav et al. 2007; Megía-Palma et al. 2016a, 2017a; Llanos-Garrido et al. 2017). On the other hand, studies considering chronic infections produced by hematic parasites contradict previous results and, therefore, the parasite hypothesis. In these studies, male lizard hosts with more hematic parasites usually have more intense colorations (Ressel and Schall 1989; Megía-Palma et al. 2016a, 2016b). Similarly as it occurs in birds, coloration in New and Old World lizards reflect individual quality, and males with more intense colors may be of better quality because they are usually bigger, keep larger home ranges, and have better mating success than paler individuals (Díaz 1993; Salvador and Veiga 2001; Langkilde and Boronow 2010). Thus, the importance of the parasite hypothesis to sexually dimorphic coloration remains poorly understood in lizards.

Visual communication is important in lizards and plays a key role in male intrasexual selection (Bajer et al. 2010; Ábalos et al. 2016). Dominant males have intense colors (Martín and López 2009), and females might also prefer these intensely colored males (Salvador and Veiga 2001). However, costs and benefits of coloration may differ between sexes (Svensson et al. 2009; Swierk and Langkilde 2013). In New World lizards of the family Phrynosomatidae, males use the coloration of conspecifics for sex discrimination (Cooper and Burns 1987). Indeed, in some populations where males and some females show similar colorations, those females that have male-like coloration suffer reduced fitness because males invest more time courting females with female-like phenotypes (Swierk and Langkilde 2013). That said, selection may also act on female coloration if it varies with reproductive status and may serve to either attract or dissuade potential mates (Cooper and Crews 1988).

Parasites can affect males and females differently owing to sexual differences in behavior or hormone levels (Duneau and Ebert 2006). Thus, given these or other sexual differences in life-history traits, we might also expect sexual differences in the level or the sign of the relationship between the degree of infection and the color expression, even in cases where both sexes show similar ornaments (e.g., Megía-Palma et al. 2016 b). Thus, studying species where both sexes show color patches offers a good opportunity to investigate sexual differences in the expression of shared traits in relation to parasitic infections. Here, we analyzed the spectral reflectance of blue and yellow coloration of lizards in the genus *Sceloporus* in relation to the infection by 2 closely related hematic and intestinal parasites in a population where both males and females express color patches. The aim of the study was to test whether the different color patches in males and females reflect information on these parasitic infections and also whether the observed relationships have a negative sign as expected by the H–Z hypothesis. Considering previous results of the parasite hypothesis from studies of lizards, we predicted that males with more intense coloration will be infected by hemoparasites. However, we might expect a negative relationship between the male's color expression and the infection by intestinal parasites. In addition, we expect different relationships of parasitic infections, with color expression depending on the sex of the host.

Material and Methods

The host model

Sceloporus occidentalis (Squamata: Phrynosomatidae) is usually described as sexually dimorphic. Adult males express blue ventral patches, whereas females typically show gray bellies (e.g., Stebbins and McGinnis 2012). However, in some populations of this lizard species, both sexes present ventral blue patches (Bell and Price 1996). In a closely related species, *S. jarrovi*, patches of males had reduced lightness, greater saturation and a higher angular hue (i.e., were more bluish) than ventral patches of females (Cox et al. 2008). In addition to the blue coloration, *S. occidentalis* has yellow patches on their forelimbs that are fundamentally based on pterins in lizards of the family Phrynosomatidae (Morrison et al. 1995; Weiss et al. 2012; Haisten et al. 2015). The function of pterine-based coloration as a potential intraspecific signal has been documented in female lizards of the genus *Sceloporus* (Weiss 2002; Weiss et al. 2012). However, there is little evidence for its function in male lizards of this genus where, on the contrary, blue coloration seems to have received a major attention (e.g., Cooper and Burns 1987; Martins 1993; Smith and John-Alder 1999). The presence of multiple color patches in males and females in some populations of *S. occidentalis* makes this species a good model to test whether variation in social coloration in lizards of both sexes could reflect chronic parasitic infections as expected by the H–Z hypothesis. Several intestinal and hematic parasite genera were described as infecting *S. occidentalis* (Bonorris and Ball 1955; Clark 1970; Ressel and Schall 1989). Recently, we molecularly characterized the blood and the intestinal parasites found in the population of *S. occidentalis* that we will study here and we classified them, respectively, in the genera *Lankesterella* (Eimeriorina: Lankesterellidae) and *Acroeimeria* (Eimeriorina: Eimeriidae) (Megía-Palma et al. 2015, 2017b). The intestinal parasites, genus *Acroeimeria*, that infect *S. occidentalis* undergo direct horizontal transmission among hosts without the intervention of a vector. In contrast, hemoparasites of the genus *Lankesterella* are transmitted by hematophagous arthropods. All of these endoparasites undergo reproduction in internal organs of the host and break down the host's tissues with every reproductive cycle, causing negative effects (Telford 2008).

Sampling methods

We collected 68 *S. occidentalis bocourtii* (45 males and 23 females) in May 2014, corresponding to the breeding season of this species. Lizards were captured in a linear transect of 400 m (from 36.985270, −122.061440 to 36.985287, −122.056934) on the campus of the University of California, Santa Cruz, CA (UCSC).

Each lizard was individually identified using a xylene-free marker with a blue number that was written in the gray area of the belly out of the color patches as examples in the Appendix. The lizards were transported in a cooler to the lab in the UCSC facilities to perform all the color measurement under standardized light conditions (see below). Snout-to-vent length (SVL) for each lizard was measured with a ruler to the nearest millimeter and the individuals were weighed to the nearest centigram with a digital balance (Scout Pro SP202, Ohaus Corp., NJ, USA). A body condition index (BCI) was calculated as the residuals of the regression of log mass on log SVL (Dunlap and Mathies 1993). The sex of the individuals was determined by the presence of enlarged post-anal scales (Cox et al. 2005; Langkilde and Boronow 2012). No lizard died during the study, and all were released at their site of capture following data collection.

Screening of blood smears for parasites

Blood samples were collected from the base of the tail of each lizard using sterilized needles (Megía-Palma et al. 2013, 2014). In the case of male lizards, blood was collected by carefully avoiding the area of the hemipenis (by bleeding the tail at least 2 cm from the cloaca). The drop of blood obtained by this method was collected with a heparinized microcapillary tube (BRAND, 75×1.1 mm, Na-heparinized). These blood samples were used to make thin-layer blood smears. The dried blood smears were fixed with methanol and stained for 40 min with Giemsa 1:10 at pH 7.2. The same person (RMP) screened 15,000 red blood cells of each individual lizard at $\times 1,000$ magnification (Megía-Palma et al. 2017b) for diagnosing the presence of parasites in the blood of the lizards.

Screening of fecal samples

Fecal samples were collected directly into 1.5-mL micro-centrifuge tubes by briefly massaging the belly of the lizards (e.g., Megía-Palma et al. 2016a). These fecal samples were stored in 1 mL potassium dichromate (Duszynski and Wilber 1997). We applied Sheather's sugar flotation technique (Megía-Palma et al. 2015) to concentrate intestinal parasites. Each sample was screened at $\times 600$ magnification.

Measuring reflectance of color patches

We measured the reflectance from the blue patch on the right side of the belly; and the yellow patch located on the anterior part of the right forelimb (Appendix). We avoided the blue markings that we used to identify the individuals during the color measurements. All spectral measurements of the colorful patches were obtained by spectrophotometry from 400 to 700 nm. Unfortunately, we could not measure ultraviolet (UV) reflectance due to a problem with the UV lamp in the spectrophotometer and, thus, it remained off during the measurements (the UV lamp is independent of the deuterium–tungsten light used for the visible range). Thus, our analyses are centered exclusively in the reflection of the visible range of the spectrum. It is known that blue ventral coloration of Sceloporus lizards peaks in the blue–green spectral area and the reflectance is residual in the near-UV range (see Figures 1a and 1b in Stoehr and McGraw 2001). Pterins—and not carotenoids—are known to be the main pigment that produce the yellow or orange-based coloration of phrynosomatids (Morrison et al. 1995; Weiss et al. 2012; Haisten et al. 2015) and, apparently, no significant biological information is contained in the near-UV region of color patches based on this pigment (Haisten et al. 2015; Cuervo et al. 2016). The spectrophotometer, an USB2000 Ocean Optics, was connected to a fiber-optic probe
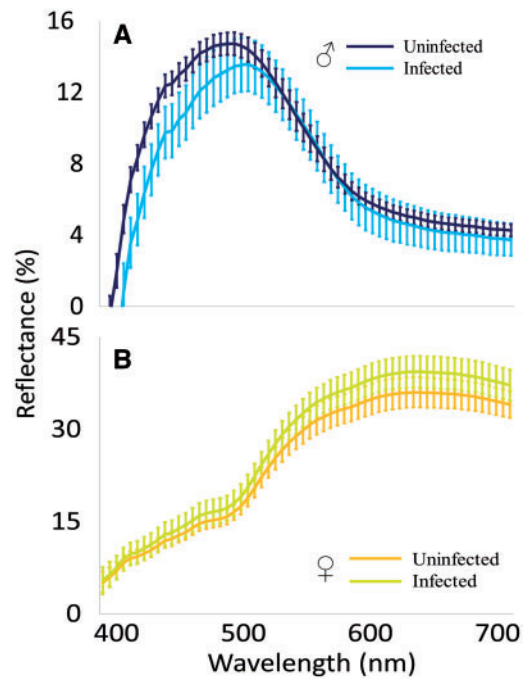
Figure 1. (A) Comparison of the mean ± SE spectra of the blue patches of males taking in consideration infection by parasites of the genus *Acroeimeria*. (B) Comparison of mean ± SE spectra of the yellow patches of females considering infection by the same parasite.

(Ocean Optics Inc., Dunedin, FL, USA). The light source used was a deuterium–tungsten light (MINI DT1000A-112; Analytical Instruments System, Inc., Ringoes, NJ, USA). We used an integration time of 10 ms, a boxcar width of 5, and 10 averaged readings per spectrum. In a darkened room, we measured the reflectance from the color patches with a probe at 45° of inclination and a constant distance of 3 mm from the skin surface. We recorded 3 spectra per patch. Ventral blue coloration shifts from blue to green depending on the body temperature in the closely related species, *S. undulatus* (Langkilde and Boronow 2012). To account for this, all individuals remained at room temperature ($\sim 24^\circ$C) for ~ 20 min before we measured coloration. All measurements were relative to a 99% WS-1 white reflectance standard.

Statistical analyses

The mean reflectance spectra of each color patch and lizard were summarized in 1-nm bin-size spectra using the CLR v1.1 software for analyzing reflectance spectra (Montgomerie 2009). Thereafter, the data of the 3 spectral measurements were averaged in Microsoft Excel (2010) per patch and lizard. Spectral data of color reflectance can be broken down into 3 variables for its analysis: that is, lightness, chroma, and hue (Montgomerie 2006). The total lightness for each spectrum was calculated as $\Sigma Q_{400\text{-}700}$, with Q being the percentage of reflectance for a given wavelength (λ). Lightness is the total amount of light reflected by a surface and it is interpreted as how dark or light a surface is (Montgomerie 2006). Hue can be interpreted as a categorical variable of color describing a surface (blue, green, yellow, red, etc). The hue variable for the blue and the yellow patches was defined as the value of λ for the Q_{max} (i.e., λ_{max}; Montgomerie 2006). Thus, lower values of hue in the blue patch define more bluish coloration, whereas higher values of hue in the yellow patch define orange-like patches. For chroma calculation of

each color patch, we selected the defined range of blue and yellow in the visible range (Endler 1990; Grill and Rush 2000), which assigns wavelength ranges of 75 nm to each color (i.e., blue, green, yellow, and red) and we calculated $\Sigma Q_{segment}/\Sigma Q_{400-700}$. Thus, we obtained a relative reflectance, or saturation, of the segment of interest for each color patch (i.e., 400–475 nm in the blue patch and 550–625 nm in the yellow patch).

We analyzed the relationships among the chromatic variables, the morphological variables, and the presence of parasites using Akaike's information criterion (AIC) to select the model with the best fit (= with the lowest AIC, Burnham and Anderson 2004). Furthermore, we performed model averaging using MuMIn (Barton 2013) and calculated the relative importance of each predictor summing the weights of all the models where the term appears. For that, we considered sufficiently informative all the models with Δ AIC ≤ 2 (Burnham and Anderson 2004). In addition, we calculated the significance of the coefficients for each predictor in the final models. For those predictors that were significant (<0.05), we calculated the maximum likelihood estimate and its standard error. Each chromatic variable was independently tested in 2 sets of tests. First, we ran the analyses for both males and females together. The sex and the presence of *Acroeimeria* were fixed as factors. The SVL, BCI, and interaction sex*Acroeimeria were the independent predictors. Second, we tested the relationships among the chromatic variables, the morphological variables, and the presence of parasites in males and females separately. As we found *Lankesterella* in only 2 females, we tested for the effects of infection of the hemoparasite on males only. We tested differences in SVL and BCI between individuals of both sexes with absence/presence of *Acroeimeria* with one-way ANOVA.

Results

Parasites detected

We found parasites of the genus *Acroeimeria* in the feces and parasites of the genus *Lankesterella* in the blood of the lizards (Megía-Palma et al. 2015, 2017). No infection by malarial parasites was detected. We found a similar prevalence of *Acroeimeria* (33.8%) in the sample ($\chi^2_{1, 68} = 0.71$). There were no differences between sexes in the prevalence of *Acroeimeria*: 31% (14/45) of the males and 39% (9/23) of the females were infected ($\chi^2_{1, 68} = 0.3$, $P = 0.6$). We found 30.8% (21/68) prevalence of *Lankesterella* in the sample. However, only 2 females were infected and, therefore, the prevalence was significantly higher in males (42.2%) than in females (8%; $\chi^2_{1, 68} = 7.7$, $P = 0.005$).

Parasites, body size, and body condition

There were no differences in SVL among males or females with respect to the presence/absence of *Acroeimeria* (differences of SVL in males: $F_{1, 43} = 0.23$, $P = 0.63$; difference of SVL in females: $F_{1, 21} = 0.35$, $P = 0.55$. In contrast, the males infected by *Lankesterella* were significantly larger (mean SVL $\pm$ SE = 61.7 ± 1.0 mm) than the uninfected ones (mean SVL $\pm$ SE = 57.7 ± 1.4 mm; $F_{1, 43} = 4.78$, $P = 0.03$). Significant differences were present neither in BCI between infected and uninfected males with respect to *Acroeimeria*, nor in males ($F_{1, 42} = 0.09$, $P = 0.75$) or in females ($F_{1, 20} = 0.18$, $P = 0.66$). Neither were the differences in BCI significant with respect to *Lankesterella* ($F_{1, 42} = 1.55$, $P = 0.21$).

Sexual dimorphism and dichromatism

Males and females did not significantly differ in SVL (males mean SVL $\pm$ SE = 59.4 ± 0.9 mm, range = 41.0–69.0; females mean SVL $\pm$ SE = 56.4 ± 1.0 mm, range = 48.0–68.0; $F_{1, 66} = 3.78$, $P = 0.05$). Our model-selection procedure produced a set of models (Table 1) that included the sex of the individuals as the best predictor for the chromatic variables in the blue patch (best models for the blue patch: lightness $\sim$ sex + SVL, AICc = 927.04, weight = 0.49; chroma $\sim$ *Acroeimeria* + sex + SVL, AICc = −186.55, weight = 0.28; hue $\sim$ *Acroeimeria* + sex + *Acroeimeria**sex, AICc = 179.62, weight = 0.22). The coefficients in our model-averaging procedure suggested that the sex of the individuals was a significant predictor for the lightness (relative importance = 1.0, $P < 0.001$) and the hue of the blue patch (relative importance = 1.0, $P < 0.001$), whereas it was nearly significant in relation to the blue chroma (relative importance = 0.68, $P = 0.05$). The blue patches of the males were significantly darker (the mean $\pm$ SE lightness for the blue patch was 494.6 ± 27.9 for the males and $1,154.6 \pm 61.6$ for the females; $F_{1, 66} = 126.09$, $P < 0.0001$) and significantly more bluish (the mean $\pm$ SE hue for the blue patch was 494.7 ± 3.0 for the males and 535.8 ± 9.3 for the females; $F_{1, 66} = 27.41$, $P < 0.0001$) compared with those of females (Table 1). Thus, the blue patch was sexually dichromatic. In addition, the SVL was a significant predictor for lightness in the blue patch of males (relative importance = 1.0, $P < 0.001$; Pearson's correlation: $P < 0.0001$, $R^2 = 0.39$). Larger males had darker blue patches than smaller individuals (Table 2). Among the females, none of the predictors explored here significantly explained the chromatic variables of the blue coloration.

Although the sex of the individuals was included in the best models for all the chromatic variables in the yellow patch (best models for the yellow patch: lightness $\sim$ *Acroeimeria* + BCI + sex + *Acroeimeria**sex, AICc = 982.59, weight = 0.31; chroma $\sim$ sex + SVL, AICc = −289.62, weight = 0.27; hue $\sim$ *Acroeimeria* + BCI + sex + *Acroeimeria**sex, BCIc = −151.46, weight = 0.55), our model-averaging procedure suggested that neither sex nor the infection by *Acroeimeria* were important predictors for the chromatic variables of the yellow patch when males and females are analyzed together because their coefficients were not significant in the resulting averaged models (Table 1). Nevertheless, the SVL of the lizards significantly predicted the chroma (relative importance = 1.0, $P < 0.001$) and the hue (relative importance = 1.0, $P < 0.001$) of the yellow patch in both sexes with the same sign (Table 2). Larger individuals had higher yellow chroma and higher values of hue (i.e., orange) than smaller individuals (Pearson's correlation for chroma: $P < 0.0001$, $R^2 = 0.27$; and hue: $P = 0.002$, $R^2 = 0.12$; Figure 2). Furthermore, SVL was a significant predictor of the yellow lightness in males (relative importance = 0.78, $P < 0.01$; Pearson's correlation: $P = 0.04$, $R^2 = 0.09$). Larger males had darker yellow patches (Figure 2). In addition, BCI was a significant predictor of the hue in the yellow patch of males (relative importance = 0.96, $P < 0.01$; Pearson's correlation: $P = 0.02$, $R^2 = 0.11$). Males with better body condition had higher values of hue.

Parasite infection and coloration

Our model-selection procedure suggested that the infection by the intestinal parasites of the genus *Acroeimeria* was a good predictor for coloration when males and females were analyzed separated. The individual models for the chromatic variables of both sexes (Table 2) suggested that infection by *Acroeimeria* was a significant predictor of the chroma in the blue patches of the males (best models for the blue patch of the males: blue chroma $\sim$ *Acroeimeria*, AICc = −116.58, weight = 0.42, coefficient for *Acroeimeria* = 0.96) and the hue in the

Table 1. Akaike analyses of the chromatic variables for males and females together and summarized by model-averaging procedure

		Blue: L	SE	Blue: C	SE	Blue: H	SE	Yellow: L	SE	Yellow: C	SE	Yellow: H	SE
n (Δ AIC ≤ 2)		3		2		4		2		5		2	
N_{TOTAL_V}		4		3		4		5		5		5	
Importance	Sex	***1.00		*0.68		***1.00		0.93		*1.00		0.57	
	SVL	***1.00		0.25		0.48		**0.82		***1.00		***1.00	
	BCI	0.19		0.22		0.18		**0.79		0.40		***1.00	
	Acroeimeria	0.32		**0.90		0.43		0.56		0.51		0.56	
	Sex*Acroeimeria	0.09		**0.50		*0.29		**0.44		*0.38		***0.46	
Estimates	Sex	**619.65**	58.52			**43.27**	10.27						
	SVL	**−15.01**	4.33					**−14.33**	6.53	**<0.01**	<0.001	**1.25**	0.36
	BCI							**−1363.65**	634.72			**112.47**	35.89
	Acroeimeria			**−0.04**	0.02								
	Sex*Acroeimeria			**0.06**	0.03			**371.94**	168.45			**−25.72**	9.47

Notes: n (Δ AIC ≤ 2) = number of probable models with Δ AIC ≤ 2. N_{TOTAL_V} = total number of variables included in the models with Δ AIC ≤ 2. Importance = relative weight of the variables in the resulting averaged model. In the row "importance," values shown in bold indicate the coefficients of the maximum likelihood estimates that are significant with level of significance: *P = 0.05, **P < 0.01, ***P < 0.001. In the row "estimates," we show the slope of the coefficients and their standard error. L, C, and H are lightness, chroma, and hue, respectively.

Table 2. Akaike analyses of the chromatic variables for males and females separated and summarized by model-averaging procedure

Males		Blue: L	SE	Blue: C	SE	Blue: H	SE	Yellow: L	SE	Yellow: C	SE	Yellow: H	SE
n (Δ AIC ≤ 2)		2		2		6		7		4		4	
N_{TOTAL_V}		2		2		3		4		4		3	
Importance	SVL	***1.00		0.21		0.22		**0.78		***1.00		**0.68	
	BCI	0.19		0.21		0.64		0.38		*0.66		**0.96	
	Acroeimeria	0.19		***0.96		0.58		*0.67		*0.66		0.53	
	Lankesterella	*0.57		0.25		0.30		0.43		0.29		0.25	
Estimates	SVL	**−17.25**	3.72					**−15.13**	7.23	**<0.01**	<0.001	**0.92**	0.45
	BCI											**130.01**	49.51
	Acroeimeria			**−0.05**	0.02								
	Lankesterella												

Females		Blue: L	SE	Blue: C	SE	Blue: H	SE	Yellow: L	SE	Yellow: C	SE	Yellow: H	SE
n (Δ AIC ≤ 2)		3		3		4		3		1		2	
N_{TOTAL_V}		2		2		3		2		1		3	
Importance	SVL	0.16		0.50		0.39		0.15		**0.81		***1.00	
	BCI	0.30		0.24		0.20		*0.63		0.20		0.19	
	Acroeimeria	0.24		0.17		0.24		0.30		0.16		**1.00	
Estimates	SVL									**<0.01**	0.001	**2.99**	0.58
	BCI												
	Acroeimeria											**−16.15**	6.02

Notes: n (Δ AIC ≤ 2) = number of probable models with Δ AIC ≤ 2. N_{TOTAL_V} = total number of variables included in the models with Δ AIC ≤ 2. Importance = relative weight of the variables in the resulting averaged model. In the row "importance," values shown in bold indicate the coefficients of the maximum likelihood estimates that are significant with level of significance: *P = 0.05, **P < 0.01, ***P < 0.001. In the row "estimates," we show the slope of the coefficients and their standard error. L, C, and H are lightness, chroma, and hue, respectively.

yellow patches of the females (yellow hue $\sim$ *Acroeimeria* + SVL, AICc = −40.57, weight = 0.47, coefficient for *Acroeimeria* = 1.00). The males infected with parasites of the genus *Acroeimeria* had blue patches with significantly less chroma (mean ± SE = 23.2 ± 0.01) than the uninfected males (mean ± SE = 28.7 ± 0.01; $F_{1, 43}$ = 7.31, P = 0.009; Figure 1A); meanwhile, the females that were infected by *Acroeimeria* expressed yellow patches that had lower values of hue (yellow-like patches: mean ± SE = 629.4 ± 7.5) than the uninfected females (orange-like patches: mean ± SE = 641.4 ± 6.6), but this difference was not significant ($F_{1, 21}$ = 1.77, P = 0.19; Figure 1B).

Discussion

We found partial evidence supporting the parasite hypothesis (Hamilton and Zuk 1982) in this population of *S. occidentalis*.

Males and females infected by intestinal parasites of the genus *Acroeimeria* expressed lower coloration as predicted by the H–Z hypothesis, although the differences observed in the females were not significant. The infected males expressed blue patches with a mean 5.5% less chroma than the blue patch of the uninfected males. Hormonal differences between infected and uninfected males might explain the chromatic differences observed in the blue patch. In this sense, reduction of testosterone level by castration of male *S. jarrovi* induced a significant reduction in chroma in the blue coloration (Cox et al. 2008). Similarly to the castrated males in the study of Cox et al. (2008), male *S. occidentalis* that were infected by *Acroeimeria* expressed blue patches with low chroma. Castrated male *S. jarrovi* reduced their territorial and sexual behavior (Moore 1987), and malarial parasites have similar effects in *S. occidentalis* (Dunlap and Schall 1995) because they hinder their ability to
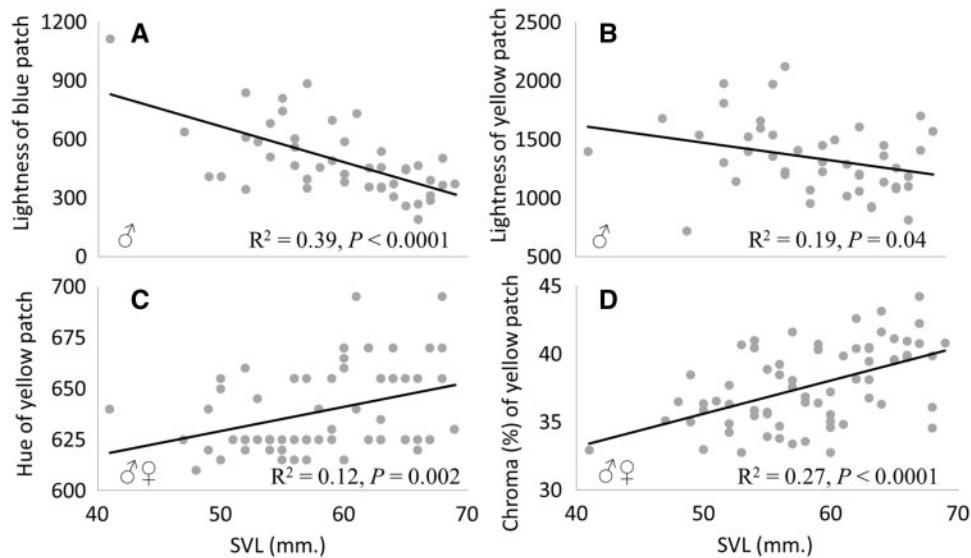
Figure 2. Relationship between the SVL and the chromatic variables of the blue (A) and the yellow (B, C, and D) patches. In each of the analyses, the sex analyzed is shown in the bottom-left corner.

compete for females or to actively patrol their home ranges (Schall and Dearing 1987; Schall and Houle 1992). Eimeriid parasites of strict intestinal life cycle, such as the genus *Acroeimeria*, are largely known to cause multiple diseases—and even death—in livestock and wild fauna (Coudert et al. 1993; Blake and Tomley 2014). Similarly to males infected by malarial parasites, the presence of low chroma in the blue patches of the males infected by *Acroeimeria* suggested that the infection might have a negative effect on the fitness of the male lizards in this population.

Besides the relationships found between the blue coloration of the males with respect to the infection by *Acroeimeria*, we found low statistical evidence that supports any relationship of the infection by *Lankesterella* with the expression of the color patches measured. Interestingly, hemoparasites of the genus *Lankesterella* infected almost exclusively males that, additionally, were larger than the uninfected ones. Larger males expressed darker blue patches (i.e., less lightness) than uninfected males and, moreover, as previously commented, had darker yellow patches, with high chroma and high hue. Langkilde and Boronow (2010) found that larger males of the closely related species, *S. undulatus*, had darker blue patches than smaller males, suggesting that the coloration might shift ontogenetically and, thus, larger males of *S. occidentalis* with intense coloration might be older. There are few reported cases of *Lankesterella* parasites in reptiles (Telford 2008), although its presence may be more common than previously thought (e.g., Maia et al. 2016; Megía-Palma et al. 2017b). To our knowledge, only 1 study describes a negative effect of *Lankesterella* parasites causing pneumonia in avian hosts (Speer et al. 1997). In *S. occidentalis*, we found low intensities of infection (i.e., mean parasite load/15,000 cells $\pm$ SE $= 4.6 \pm 1.1$, max $= 40$) in comparison to other studies of Old World lizards (e.g., Megía-Palma et al. 2014 found a mean load of *Lankesterella* in 10,000 cells $= 27.8$, with max $= 115$ in *Acanthodactylus erythrurus*). This might mean that, in this population it is important to maintain infections by *Lankesterella* under low thresholds because only high-quality individuals can tolerate it or, alternatively, it might suggest a low virulence of this hemoparasite (*sensu* Svensson and Råberg 2010). In addition, the sexual differences in infection by *Lankesterella* and the sexual dichromatism

in the blue patch could be related to sexual differences in testosterone secretion because this hormone influences dimorphism in immunocompetence in lizards (e.g., Mondal and Rai 1999) and, further, is responsible for sexual dichromatism in *Sceloporus* (Cox et al. 2005, 2008; Calisi and Hews 2007). Testosterone increases the expression of the blue coloration in males of *Sceloporus* that may be important in *S. occidentalis* because this coloration is displayed toward competitors at short distance, reduces aggressive escalation during fights, and is used by conspecific for sex discrimination (Cooper and Burns 1987; Sheldahl and Martins 2000). However, the production of testosterone reduces both macrophage production and the inflammatory response in male lizards (Mondal and Rai 1999; Belliure et al. 2004). In addition, it may augment the metabolic rate (e.g., Feuerbacher and Prinzinger 1981), increasing the oxidative stress and the cellular damage in the individuals (von Schantz et al. 1999). At the same time, oxidative stress can impair immune functions (Alonso-Álvarez et al. 2007), and renders males more susceptible to parasitic infections (Mondal and Rai 1999).

In addition to the relationships achieved between the parasitic infections and the coloration and morphology of the male lizards, we found a strong effect of SVL on the chromatic variables of the yellow coloration, with the same sign in both males and females. Large males expressed orange-like (high values of hue) yellow patches (also explained by BCI), which had more chroma and were darker than those of smaller males. Similarly, large females expressed orange-like yellow patches with more chroma than smaller females. Thus, it seems to be a non-dichromatic patch that might have similar signaling function irrespective of the sex of the bearer. The Western Fence Lizard is considered a territorial lizard species because both sexes show high site fidelity and defend their home ranges (Sheldahl and Martins 2000). One of its most frequent behaviors is a series of short push-ups that they display to potential mates or rivals from the distance (e.g., Schall and Houle 1992; Sheldhal and Martins 2000). During this display, individuals may increase the visibility of the yellow patches of the forelimbs that might inform to conspecifics on their body size. The body size of the individuals correlates with the female's fecundity in the genus

Sceloporus (Jiménez-Arcos et al. 2017) and with fighting ability in lizards in general (Carpenter 1995; Molina-Borja et al. 1998).

Based on the current knowledge of the high visual sensitivity of the visual system in closely related lizards of the family Iguanidae (i.e., Loew et al. 2002), we may speculate on the perceptual capacity of *S. occidentalis* to discriminate the chromatic characteristics of conspecifics. Unfortunately, our spectral data did not include the UV range, although probably not being relevant in the color patches of *Sceloporus* (Stoehr and McGraw 2001), it precludes the application of tetrachromatic visual models in this study and, therefore, the consecution of stronger conclusions. We can only speculate whether these differences could be discriminated by conspecifics. It seems reasonable that the visual system of lizards co-evolves with the subtle variation of the coloration of conspecifics (Stoehr and McGraw 2001), especially if those could transmit *Acroeimeria*. A proper discrimination between infected and uninfected potential mates may be important for these lizards to decrease the probability of infection whereas increasing the resistance to this parasite in the population.

In conclusion, we found partial support for the parasite hypothesis (Hamilton and Zuk 1982) in *S. occidentalis* because lizards infected with parasites of the genus *Acroeimeria* expressed duller colorations than the uninfected individuals. Although parasites might mediate the sexual selection of this species (e.g., Schall and Dearing 1987; Schall and Houle 1992), the *Lankesterella*–lizard host system studied here did not support the H–Z hypothesis because infected males were bigger and more intensely colored than the uninfected ones.

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Appendix
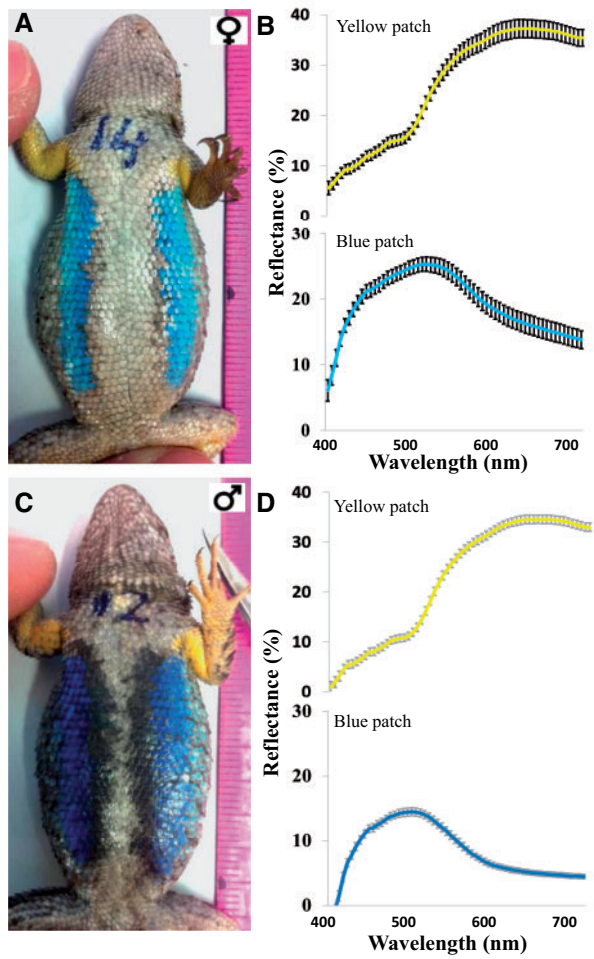
Figure A1. Ventral coloration and mean ± SE spectral profiles of both yellow and blue patches of female (A, B) and male (C, D) *Sceloporus occidentalis bocourtii*.