

Article

Manipulation of parasite load induces significant changes in the structural-based throat color of male iberian green lizards

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Abstract

The honesty of structural-based ornaments is controversial. Sexual selection theory predicts that the honesty of a sexual signal relies on its cost of production or maintenance. Therefore, environmental factors with negative impact on individuals could generate high costs and affect the expression of these sexual signals. In this sense, parasites are a main cost for their hosts. To probe the effect of parasites on the structural-based coloration of a lacertid species *Lacerta schreiberi*, we have experimentally removed ticks from a group of male Iberian green lizards using an acaricide treatment (i.e., the broad-use insecticide fipronil). All individuals were radio-tracked and recaptured after 15 days to study changes in coloration in both the ultraviolet (UV)-blue (structural-based) and UV-yellow (structural and pigment-based) ornamentations after manipulation, as well as changes in endo- and ectoparasitic load and body condition. Additionally, after the experiment, we measured the skin inflammatory response to a mitogen. The fipronil treatment was effective in reducing ticks and it was associated with a significant reduction of hemoparasite load. Throughout the season, individuals treated with fipronil tended to maintain the brightness of the UV-blue throat coloration while control lizards tended to increase it. However, individuals treated with fipronil that were not infected with hemoparasites significantly reduced the brightness of the UV-blue throat coloration. Individuals with a higher initial tick load exhibited a lower UV saturation increment (UV-blue) and a higher brightness increment (UV-yellow) during the experiment. Overall these results experimentally support the idea that parasites adversely influence the expression of the structural-based coloration of male Iberian green lizards. This adds evidence to the hypothesis that sexual ornaments in lizards function as honest signals.

Key words: animal communication, ectoparasites, Hamilton & Zuk, structural-coloration.

Sexual selection theory predicts that secondary sexual traits function as signals to conspecifics. Development of these traits should be costly, indicating the quality of the bearers through their good genes as indicated by the handicap principle (Zahavi 1975). This principle is based on the assumption that females mainly select males with genes that enhance viability. However, directional selection for male traits should eventually eliminate heritable variation in those traits.

Hamilton and Zuk (1982) proposed one specific good gene model that could explain the maintenance of heritable variation, the parasite hypothesis. In this case, male sexual traits are associated with resistance to parasites and, as parasites and hosts coevolve, the best genotype may oscillate between generations. This model is mainly useful in populations under parasitic pressure since other factors such as predation and environmental thermal constraints could also

be important selective forces to shape sexual traits (Marshall and Stevens 2014; Pérez I de Lanuza and Font 2014; Reguera et al. 2014).

Color ornaments based on pigments are mainly constituted by carotenoids and pterines that produce red, orange, and yellow colorations (but see McGraw et al. 2004). Carotenoids are not synthesized in the organism and can only be obtained through the diet (Goodwin 1984). However, they may have a key role as scavengers of reactive oxygen species (ROS) that result from metabolism (Burton 1989) or improving the immune function. Thus, there may be a trade-off between maintaining colorful carotenoid-based ornamentation and antioxidant or immune responses (von Schantz et al. 1999; Martín et al. 2008; López et al. 2009; Mougeot et al. 2009; Cote et al. 2010; Kopena et al. 2014). In this sense, the costs of production or maintenance (i.e., honesty) of carotenoid-based ornaments have been studied in different organisms (in lizards, Fitze et al. 2009; in birds, Mougeot et al. 2009; in lizards, Cote et al. 2010; in birds, Simons et al. 2012). In addition to carotenoid-based ornaments, some yellow or orange-based coloration of lizards (especially American species) are instead based on pterins (Weiss et al. 2012; Haisten et al. 2015). Thus, yellow ornaments based on pterines might be costly to produce because they are synthesized consuming purines via salvage pathways (Morrison et al. 1995; Weiss et al. 2012). In contrast with pigment-based ornaments, there is a lack of consensus on whether environmental factors (e.g., parasites, temperature, food intake) causally influence the expression of structurally based ornamentation (i.e., blue or UV-blue), although several correlational studies showed significant relationships between the inter-individual variation in structural-based ornamentation and both biotic and abiotic factors (Shawkey et al. 2007; Bajer et al. 2012; Langkilde and Boronow 2012; Molnár et al. 2013; Megía-Palma et al. 2016a). For example, structural coloration was positively related to infection by hemoparasites in two species of lizards, suggesting that UV-blue coloration in lizards might reflect the individual ability to cope with stress (Megía-Palma et al. 2016a, 2016b). Also, Bajer et al. (2012) suggested that structural coloration of green lizards honestly signals the individual's ability to procure an optimal thermal niche. This is based on evidence demonstrating that lizards saw higher expression of the structural color ornament when exposed to temperature within the optimal range of the species compared to lizards exposed to suboptimal temperatures (Bajer et al. 2012; Langkilde and Boronow 2012).

The structural-based coloration in lizards (i.e., ultraviolet-based or UV-blue colorations) may serve as social signals of quality reflecting dominance and reproductive status, mating success, fighting ability, bite force, body condition, parasite load, and thermal niche (Huyghe et al. 2005; Whiting et al. 2006; Martín and López 2009; Bajer et al. 2010, 2011, 2012; Molnár et al. 2013; Pérez I de Lanuza et al. 2014; Martín et al. 2016; Megía-Palma et al. 2016a). These kinds of ornaments may result from the combined effect of iridophore arrangement and melanin deposition in the dermal layers of the skin of lizards (Kuriyama et al. 2006; Pérez I de Lanuza 2012). Thus, the disposition and thickness of iridophores produces blue/UV-blue colorations (well-arranged iridophores) or whitish to reddish colorations (highly disordered iridophores) (e.g., Saenko et al. 2013). In combination with iridophores, melanophores may absorb those wavelengths that are not scattered by iridophores and then, modify the spectral shape and the entire reflectance (i.e., brightness) (Grether et al. 2004; Olsson et al. 2013). Melanophores play a key role in the contribution to the blue and UV-blue colorations (Cox et al. 2005, 2008). In contrast, pigment-based colorations present a

superficial layer of xanthophores/erythrophores that can contain a combination of pigments (pterines and/or carotenoids) (Kopena et al. 2013; Olsson et al. 2013; Haisten et al. 2015) and that in combination with the remaining skin layers results in yellow, orange, or some red ornaments (Grether et al. 2004; San-Jose et al. 2013). Experimental studies demonstrated the influence of hormones on the structural component (San-Jose et al. 2013) and the pigmentary component underlying the coloration of lizards (Cox et al. 2008; Cote et al. 2010). Corticosterone increased the redness of the ventral coloration in the common lizard in response to acute stress (Fitze et al. 2009; Cote et al. 2010). This color variation is produced by altering the crystal spacing within iridophores in the dermis rather than by carotenoid mobilization (San-Jose et al. 2013). Testosterone increases melanization in lower layers of the dermis (e.g., Quinn and Hews 2003) increasing the reflectance by short wavelengths in structural-based colorations (i.e., UV-blue/blue patches; Grether et al. 2004).

Throat coloration of the Iberian green lizard *Lacerta schreiberi* (Bedriaga 1878) is composed of two different color ornaments that may reflect both individual health and body condition status (Martín and López 2009; Stuart-Fox et al. 2009; Megía-Palma et al. 2016b). The UV-blue nuptial coloration may be a sexually selected trait in this species (Martín and López 2009). Males of *L. schreiberi* with darker (i.e., less brightness) and more UV saturated throats were more often found guarding females in the field (Martín and López 2009). In contrast, dominant males showed brighter UV-blue throat coloration with higher UV saturation than subordinate individuals (Martín and López 2009). However, UV-yellow coloration (i.e., carotenoid-based, Kopena et al. 2013; Megía-Palma, unpublished data) may signal immune status and reinforce information about individual condition (Martín and López 2009; Megía-Palma et al. 2016b). A recent study demonstrated that selection promotes the evolution of contrasted color patterns (i.e., UV-based and yellow- or red-based color patterns) (Pérez I de Lanuza and Font 2016). Thus, contrasted color patches (e.g., UV-yellow vs. UV-blue patches) that conform complex ornamentation of lizards should be studied together to have a broader perspective of the environmental factors that model lizard coloration.

The Iberian green lizard is found in humid habitats where ticks (Acari: Ixodidae) are common ectoparasites (Stuart-Fox et al. 2009; Megía-Palma et al. 2016b). The first phenology peak of nymphal ixodid ticks in Central Spain (Estrada-Peña et al. 2004) coincides with the reproductive peak of *L. schreiberi* (Salvador 1987). During the breeding, season adult male Iberian green lizards experience an increase in steroid hormone levels that enhances the expression of their characteristic dark UV-blue throat coloration similarly to other European green lizard species (e.g., Václav et al. 2007). In turn, steroid hormones may impair the immune system of male lizards (Folstad and Karter 1992; Alonso-Álvarez et al. 2007) making them more susceptible to ectoparasites (Olsson et al. 2000; Roberts et al. 2004; Olsson et al. 2005; Cox and John-Alder 2007; Václav et al. 2007; Megía-Palma et al. 2016b). Mounting an immune response to fight against parasites is costly because a primary response of the immune system against a challenge is to produce ROS (Halliwell and Gutteridge 2007). In this scenario, the role of carotenoids or pterines as ROS scavengers is crucial to restore homeostasis and enhance immune response (Burton 1989; Weiss et al. 2011; Kopena et al. 2014). Therefore, the benefits of fighting parasites may exceed the negative effect of these pathogens (Lochmiller and Deerenberg 2000). Moreover, in natural populations, hosts are affected by multiple parasites. Investing energy to fight against them all compete

with allocation of energy into other parts of the organism (López et al. 2009; Kopena et al. 2014; Lindsay et al. 2016).

In this study, we have performed a manipulative experiment on the tick load in males of Iberian green lizards *L. schreiberi* to test the effect of parasitism on both the structural- and the pigment-based component of the nuptial ornamentation. In addition, we quantified the hemoparasitic load and the local inflammatory response of the individuals to a mitogen. Our aim was to test the possible trade-offs between fighting parasites and producing social coloration. We predict that lizards with a reduced load of parasites will afford a better maintenance of the nuptial ornaments throughout the season.

Materials and Methods

Sampling

We collected 20 adult males of the Iberian green lizard *L. schreiberi*, in a deciduous forest in Segovia, Spain (40.88814, -4.02827), from early May to late August 2012. We captured the lizards using a noose pole. Each lizard was measured to the nearest millimeter with a ruler and weighed to the nearest decigram with a digital balance. The individual age was estimated as the number of arrested lines (LAGs) in one phalanx obtained from each lizard using common techniques in skeletochronology described in Megía-Palma et al. (2016b). Growth rate among individuals of the same age may influence the relation between length and mass in lizards (Halliday and Verrell 1988). To remove the effect of the age over body condition index (BCI) we included the number of LAGs found in the phalanxes of the individuals as a cofactor in the regression of the snout-vent length (SVL) on the mass (Schall and Pearson 2000). We used the residuals of this analysis as the new variable of BCI (Megía-Palma et al. 2016b). In addition, we counted the number of attached ticks that are mainly in the neck of the lizards (see experimental procedure below further) and we recovered blood samples from the lizards to study the presence of blood protozoa. We repeated all measurements at recapture. All lizards were experimentally manipulated in the field to avoid the stress of transportation. The procedures employed in this study were authorized by permit EP/SG/625/2011 provided by Junta de Castilla y León.

Endoparasite load quantification

We made thin-layer blood smears to survey for hemoparasites. Smears were immediately air dried and then fixed with methanol and stained for 40 min with Giemsa diluted 1:10 in buffer, pH 7.2 (Megía-Palma et al. 2013). We screened the smears to count the number of hemoparasites per 15,000 red cells. Every lizard was supplied with radio transmitters (BD-2 transmitters, 1.25 g; Holohil Systems Ltd., Ontario, Canada). Using these radio transmitters, male lizards were radio tracked allowing us to recapture all of them 14 days after the first capture. At recapture, lizards were blood sampled and we again counted the hemoparasitic load under the microscope. We obtained 2 variables of infection by hemoparasites. First, we had absence/presence of hemoparasites. Second, we had the hemoparasitic load per 15,000 cells at capture and at recapture.

Measuring skin coloration

With the aid of a spectrophotometer in the field, (Jaz DPU® Module, Ocean Optics Inc., Dunedin, Florida) we measured the throat nuptial coloration of male lizards. We measured color reflectance 3 consecutive times in a central area of each of the UV-blue (structural-based) and the UV-yellow (pigment-based) throat areas.

The spectrophotometer used one Pulsed Xenon Light Source (Jaz-PX) connected to an optical fiber. The probe was mounted within a holder that ensured that readings were taken from areas 1 mm in diameter at a constant distance of 3 mm from the skin surface at a 90° angle (Endler 1990; Martín and López 2009; Bajer et al. 2010). All the measurements were quantified relative to a 99% WS-1 white reflectance standard (all the components from Ocean Optics Inc., Dunedin, FL, USA).

We analyzed the spectral data from 320 to 700 nm in both the UV-blue and the UV-yellow throat areas from the lizards, adapting the segment classification method for spectral analysis (Endler 1990; Grill and Rush 2000; Megía-Palma et al. 2016a, 2016b). Previous studies on sexual selection of green lizards experimentally showed that the UV throat reflectance from the UV-blue nuptial coloration is an important trait that may be under selection (Martín and López 2009; Bajer et al. 2010, 2011). Thus, the UV region (320–400 nm) of the nuptial UV-blue coloration was used to calculate the UV saturation ($\Sigma Q_{320-400} / \Sigma Q_{320-700}$), where Q is the value of reflectance for each considered wavelength. In addition, the spectrum from the UV-yellow throat coloration showed a first peak in the UV region and a second peak in the yellow region. Thus, we considered the region from 320 to 400 nm to calculate UV saturation, and the region from 450 to 700 nm to calculate carotenoid chroma (Montgomery 2006). The total brightness was calculated as $\Sigma Q_{320-700}$. To calculate the variable of hue, we adapted Endler's (1990) calculation which integrates information of the full spectrum including the UV range (Pérez I de Lanuza et al. 2014; Megía-Palma et al. 2016a, 2016b). We calculated segment differences as follows: for medium to short wavelengths, $\Sigma Q_{500-600} - \Sigma Q_{320-400}$; for long to medium wavelengths, $\Sigma Q_{600-700} - \Sigma Q_{400-500}$. In this way, we can derive objective estimates of hue. The UV-blue and the UV-yellow patches of male *L. schreiberi* produce a contrasted color pattern (Figure 1) (*sensu* Pérez I de Lanuza and Font 2016). According to Pérez I de Lanuza and Font (2016), the maintenance of highly contrasted patterns may be positively selected within Lacertidae, and we hypothesized that maintaining the chromatic contrast in the throat during the season may be costly. Thus, to study if color contrast of the throat varied during the experiment, we calculated at each capture and for each lizard the difference in reflectance between the UV-blue and the UV-yellow patches of the throat as a measure of total throat contrast (Endler 1990). For this purpose, we calculated the Euclidean distance between the colorimetric variables (i.e., brightness, chroma, and hue) of the spectra of the UV-blue and the UV-yellow patches (see Endler 1990). Values above zero mean that UV-blue color was conspicuous over the UV-yellow coloration. In contrast, values of contrast below zero mean that UV-yellow was conspicuous over the UV-blue coloration because we subtracted the chromatic variables of the UV-yellow coloration to the chromatic variables of the UV-blue coloration. We log-transformed the resulting variable of contrast to comply with assumptions of normality and homocedasticity.

Tick removal treatment

Male lizards were alternatively assigned to experimental or control groups as they were captured (i.e., even captures to control and odd ones to experimental groups). In the experimental group, ticks were gently removed using tweezers and a widely used insecticide containing 0.25 g/100 mL of fipronil (Frontline Spray, MERIAL, Toulouse, France) was immediately applied with a paintbrush onto the bandage used to hold the radio transmitter. This insecticide belongs to the phenylpyrazole chemical family (Caboni et al. 2003)

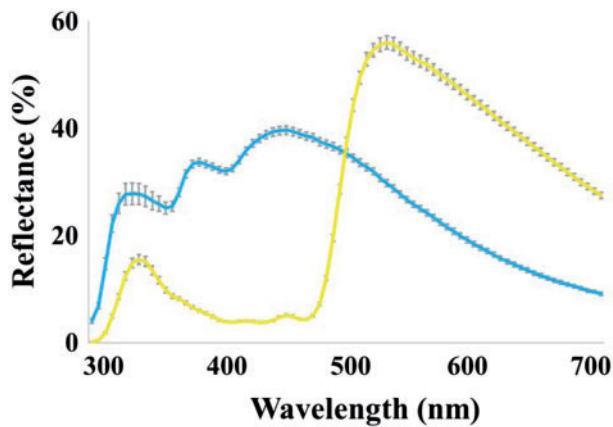


Figure 1. Mean \pm standard error of the reflectance in the throat color patches. The UV-yellow and the UV-blue patches constitute a highly contrasted ornament.

and is widely used to kill fleas and ticks. The purpose of this treatment was to prevent tick reinfestation during the experiment since ticks can easily infest cleared lizards after manipulation (Olsson et al. 2000). In the control group, ticks were not removed from the captured lizards and the bandage used to hold the radio transmitter was impregnated with distilled water.

Inflammatory skin reaction

At day 15 after the first capture, every lizard was recaptured. Blood, tick load, mass, and coloration were sampled again. We performed 3 repeated measures of the toe thickness in the rear limb with a digital spessimeter with 0.01 millimeter precision and with constant pressure (Mitutoyo 547-360, Neuss, Germany). Each lizard was injected with 0.02 mL of a phytohemagglutinin solution (PHA-P; lyophilized powder, Sigma-Aldrich Quimica SL, Madrid, Spain) in phosphate-buffered saline (PBS; 2 mg/mL) in the toe that we measured. This injection provokes a slight swelling at the point of injection that disappears after 48 h in this species (Kopena et al. 2014). To validate the protocol, we also injected the same volume of PBS (without phytohemagglutinin, as a control condition) in the same toe of the opposite rear limb, expecting an increased inflammatory reaction in the toe injected with PHA. Lizards were then released at the same spot of capture. PHA is a plant-derived mitogen that stimulates the recruitment of leukocytes involved in both adaptive and innate responses at the site of injection, producing a measurable tissue swelling (Martin et al. 2006; Forsman et al. 2010; Kopena et al. 2014). This is commonly used in evolutionary ecology to estimate T-cell-mediated immunity (Belliere et al. 2004; López et al. 2009; Martín and López 2009; Kopena et al. 2014), although it also reflects other components of the immune system such as major histocompatibility complex molecules (Moreno et al. 1999; Morales et al. 2006). Within the next 24 h (i.e., day 16), lizards were recaptured again and 3 new repeated measurements of the thickness of the toe at the point of injection were taken. The total inflammatory reaction was estimated as the difference between the average initial and average final measurements. All injections and measurements were made by the same person (R.M.-P.). As expected swelling in the experimental toe was significantly higher than in the control toe $F_{1, 40} = 8.3, P = 0.006$.

Statistical analyses

To test the effect of the treatment on Δ BCI (i.e., BCI₂ – BCI₁) and the inflammatory response to the injection of PHA, we performed

general regression (GRM) backward stepwise models in Statistica 10.0 (Statsoft Inc., Tulsa, OK, USA). In previous studies, hemococcidians covaried with tick load and the thermal behavior of lizards (Molnár et al. 2013; Paranjpe et al. 2014). Thus, we included the absence/presence of *Schellackia* hemoparasites as a cofactor in the analyses. In addition, we included the interaction of the treatment (i.e., tick removal/control) with the remaining variables in all the analyses. The threshold of significance for the GRM models was $P < 0.05$. The residuals of these models were checked for normality and homoscedasticity. As a proxy to test the difference in immunological trade-off that ticks may inflict on the lizards, we compared the load of hemoparasites between captures in the control and the fipronil-treated groups with a nonparametric Wilcoxon matched pairs test.

Finally, we performed individual backward stepwise GRM for each of the colorimetric variables. The dependent variables in these models were the increment (final capture – initial capture) of the brightness, saturation/chroma, and hue for both the UV-blue and the UV-yellow color patches (i.e., change in color). All the colorimetric variables of the UV-yellow patch and the UV saturation of the UV-blue patch were log-transformed to improve the distribution of the residuals in the resulting models. In all of these backward stepwise GRM analyses, we included the presence/absence of hemoparasites (i.e., *Schellackia*), date of capture, Δ BCI, the initial tick load, and the inflammatory response to the PHA injection. In addition, we included in all the analyses the interactions treatment**Schellackia*, treatment* Δ BCI, treatment*PHA, and treatment*date. The backward stepwise method allowed us to make a selection of the final models by removing the variables with no effect from the analysis (Cordell and Clayton 2002). Then, we performed repeated measures ANOVAs to test the within-individual effect of the treatment on tick load, BCI, and the colorimetric variables. In each analysis, we included the measurements at capture and recapture as dependent variables and as independent variables only the variables selected by the backward stepwise procedure. We show mean \pm standard error of parasitemia and number of ticks.

Results

Effect of the treatment on tick load

The initial prevalence of tick infestation was 95.0% (19/20 lizards). We randomly assigned the lizards to either the control or the fipronil-treated group ($n = 10$ lizards per group). There were no significant differences between experimental groups in initial tick load (ANOVA initial tick load: $F_{1, 18} = 0.28, P = 0.86$). Lizards in the control group had 21.1 ± 5.4 (minimum=0; maximum=46) ticks and the fipronil-treated group had 19.9 ± 4.5 (min=2; max=44) ticks at the beginning of the experiment. The effect of treatment on the ticks was significant (ANOVA treatment: Δ ticks: $F_{1, 18} = 26.69, P < 0.0001$). As expected, lizards from the experimental groups had no ticks at recapture. In the control group, the mean \pm SE of the tick load at recapture was 5.8 ± 1.69 (min=0; max=17). In addition, we performed a within-effect analysis (repeated measures) including the initial and the final tick load as dependent variables with the treatment as factor. This analysis was significant ($F_{1, 18} = 26.69, P < 0.0001$). Reduction of tick load was significant in the fipronil-treated group.

Effect of the treatment on hemoparasite load

We found hemococcidians of genus *Schellackia* (Apicomplexa: Eimeriorina) (see Megía-Palma et al. 2013). The prevalence of

Schellackia parasites was 60% (12/20). The initial mean parasitemia \pm standard error between experimental groups did not differ (control group: 3.3 ± 1.66 , range 0–14, fipronil-treated group: 5.10 ± 2.86 (0–29); Mann–Whitney test for independent samples: $U = 43.5$, $P = 0.63$). At recapture the parasitemia had significantly decreased in the fipronil-treated group (Wilcoxon test: $Z = 2.02$, $P = 0.04$; fipronil-treated group: 3.0 ± 2.04 (0–21); Figure 2), but there were no significant differences in the control group (Wilcoxon test: $Z = 1.09$, $P = 0.27$; control group: 4.20 ± 2.13 (0–22)).

Effect of the treatment on inflammatory reaction (PHA) and body condition

The injection of PHA had no effect on the swelling of toes. However, lizards that showed higher local inflammatory response to the injection of PHA after the experiment (at day 16), also had reduced body condition during the experiment (GRM PHA: $F_{1, 18} = 7.07$, $P = 0.01$; $\beta = -0.53$, Figure 3). The final model for Δ BCI included the inflammatory response to the PHA injection ($F_{1, 18} = 7.07$, $P = 0.01$; $\beta = -0.53$). Lizards with higher inflammatory response had lower Δ BCI. In addition, we performed a within-effect analysis (repeated measures) including the initial and the final BCI as dependent variables. We included the initial tick load as an independent variable to corroborate the results achieved by the analysis of change in BCI. This analysis was significant ($F_{1, 18} = 7.07$, $P = 0.01$).

Effect of the treatment on coloration

There were no significant differences between experimental groups in coloration at the beginning of the experiment (UV-yellow patch: brightness: $F_{1, 18} = 0.03$, $P = 0.5$; UV saturation: $F_{1, 18} = 2.85$, $P = 0.1$; carotenoid chroma: $F_{1, 18} = 0.06$, $P = 0.8$; hue: $F_{1, 18} = 0.16$, $P = 0.6$. UV-blue patch: brightness: $F_{1, 18} = 0.05$, $P = 0.8$, UV saturation: $F_{1, 18} = 0.21$, $P = 0.6$, hue: $F_{1, 18} = 0.34$, $P = 0.5$). The experiment affected the structural-based throat coloration in male Iberian green lizards. The interactions treatment*date of capture ($F_{1, 17} = 6.17$, $P = 0.02$) and treatment**Schellackia* prevalence ($F_{1, 17} = 5.56$, $P = 0.03$) explained the change in brightness of the

UV-blue coloration (Figures 4 and 5). The fipronil-treated lizards without *Schellackia* parasites had significantly lower brightness increase (and even reduced it) in the UV-blue coloration of the throat than both control lizards without *Schellackia* parasites in the blood (Fisher LSD post hoc analysis: $P = 0.02$) and fipronil-treated lizards infected with *Schellackia* parasites (Fisher LSD post hoc analysis: $P = 0.04$). In addition, although the experiment did not affect the increment of UV saturation in the UV-blue area, the initial number of ticks were significantly and negatively related to the increment of UV saturation in the UV-blue area ($F_{1, 18} = 8.70$, $P = 0.008$; $\beta = -0.57$; Figure 6). Similarly, there was a significant and positive relationship between the variation in brightness of the UV-yellow throat coloration and the number of ticks at the beginning of the experiment ($F_{1, 13} = 8.36$, $P = 0.01$; $\beta = 0.52$; Figure 7). None of the variables measured significantly explained the quantified changes in hue of the UV-blue throat coloration or change in UV saturation and hue of the UV-yellow throat coloration. Finally, the treatment did not significantly affect the variation in contrast between the throat color patches. However, the change in color contrast of the throat between the UV-blue and the UV-yellow patches was negatively and significantly explained by the initial tick load ($F_{1, 18} = 14.37$, $P = 0.001$). Thus, lizards with higher tick loads at the beginning of the treatment showed more conspicuous UV-yellow patches (i.e., negative values of change in contrast) than lizards with fewer ticks at the beginning of the experiment that had higher increase of conspicuousness in the UV-blue patch (Figure 8).

In addition, we performed within-effect analyses for each of the variables of color and color contrast using the independent variables and factors selected by the stepwise elimination procedure implemented in the analyses of change (measure 2 – measure 1). The results pointed to the same conclusions. There was a significant interaction between treatment and *Schellackia* that influenced brightness of the UV-blue patch ($F_{1, 17} = 5.56$, $P = 0.03$). There was a significant interaction between treatment and date of capture that influenced brightness of the UV-blue patch ($F_{1, 17} = 6.17$, $P = 0.02$). A higher tick load at the beginning of the experiment negatively influenced UV saturation of the UV-blue patch ($F_{1, 16} = 9.57$, $P = 0.006$), it had a positive influence on the brightness UV-yellow patch ($F_{1, 18} = 3.87$, $P = 0.06$) and reduced the contrast between the UV-blue and the UV-yellow patches ($F_{1, 18} = 14.37$, $P = 0.001$).

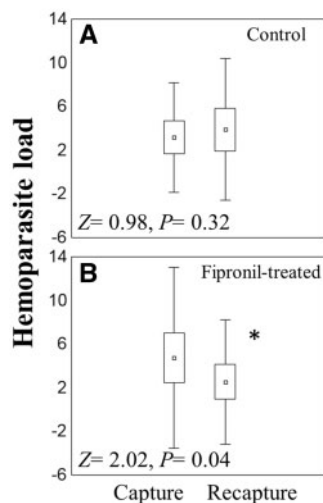


Figure 2. Differences on hemoparasite load between captures for each experimental group. (A) Non-significant differences in hemoparasite load between capture and recapture in the control group. (B) Significant differences in hemoparasite load between capture and recapture in the tick-removal (i.e., fipronil-treated) group.

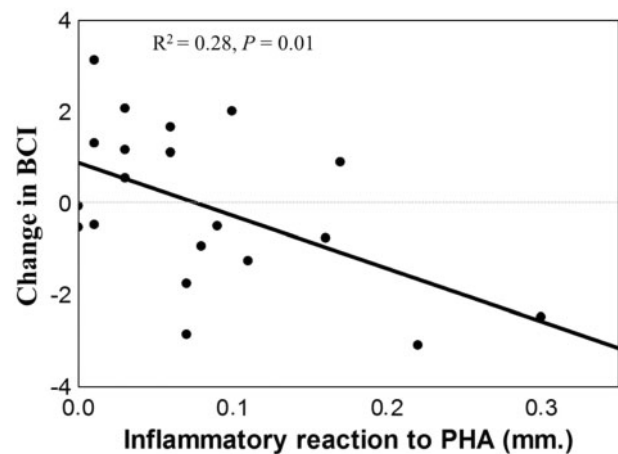


Figure 3. Relationship between the change in body condition and the reaction to the injection of phytohaemagglutinin. Individuals below the gray dotted line had a reduction in body condition during the experiment.

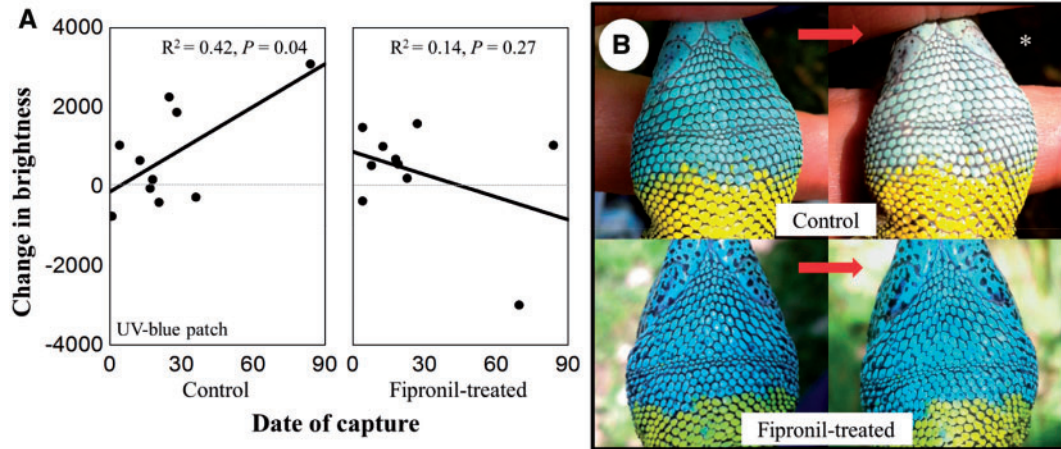


Figure 4. (A) Variation of the brightness of the UV-blue throat area along the season for control and fipronil-treated groups. Individuals below the gray dotted line had a reduction in brightness of the UV-blue throat coloration during the experiment. (B) Striking examples of male throat coloration variation across the season. The same male individual was photographed in the field before and after the experiment.

Note: The dull throat coloration in this extreme example of the control group.

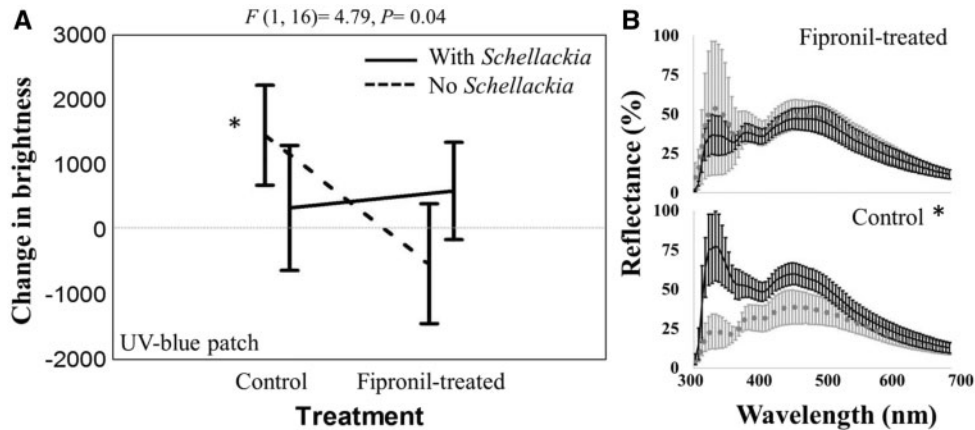


Figure 5. (A) GRM plot showing the effect of the treatment on the brightness change in the UV-blue throat coloration between captures comparing lizards infected and uninfected with *Schellackia* parasites. Individuals below the gray dotted line had a reduction in brightness of the UV-blue throat coloration during the experiment. (B) Variation of spectral profiles from the UV-blue throat area of uninfected lizards (i.e., no *Schellackia*) from each experimental group. Gray dotted line represents the initial spectra and black solid line represents the UV-blue reflectance at recapture.

Note: Control lizards without endoparasites significantly increased the brightness in the UV-blue patch.

Discussion

This study experimentally demonstrated that lizards treated with fipronil insecticide significantly reduced their tick load in relation to the control group. The experimental reduction of tick load had a significant effect on hemoparasite load, and throat coloration of male Iberian green lizards. Our experimental results suggested that the host immune response to tick infestation might trade-off with fighting against hemoparasites because only fipronil-treated lizards (i.e., with less ticks) afforded a significant reduction in parasitemia. In contrast, Molnár et al. (2013) found a negative relation between the number of ticks on the lizards and the hemoparasite load. They suggested that ticks may induce a systemic immune response in the host (Wikel et al. 1997) that favors the fight against hemoparasites (Molnár et al. 2013). If tick infestation induces a systemic immune response in male Iberian green lizards we would expect a significant effect of the treatment on the local inflammatory response measured here. However, we did not find this. Instead, we found individuals

that responded with higher local inflammation to the PHA had reduced their body condition during the experiment. Similarly, Kopena et al. (2014) tested the inflammatory skin reaction to the same mitogen in male Iberian green lizards that had been fed with a diet rich in antioxidants (carotenoids + vitamin E). Lizards that were fed with vitamin E had a significantly higher inflammatory response but, as in our study, they also showed a higher loss of body condition than control lizards (Kopena et al. 2014). These results suggest a compromise in the allocation of energy between inflammatory response and fat storage.

Our manipulative study affected the expression of the structural-based throat coloration. The treatment*date of capture interaction explained variation in brightness of the UV-blue coloration. The fipronil-treated lizards (i.e., with less ticks) afforded a significant reduction in hemoparasite load and tended to maintain or even decrease the brightness of their UV-blue ornament. In contrast, control lizards could not afford reduction of their hemoparasite load, and

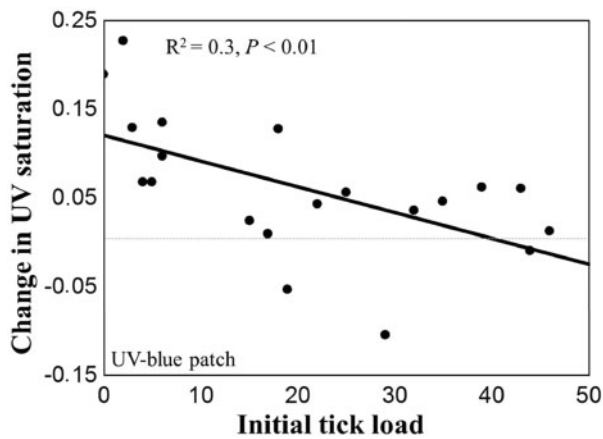


Figure 6. Relationships between variation in UV saturation in the UV-blue throat area and the initial tick load. Individuals below the gray dotted line had a reduction in UV saturation of the UV-blue throat coloration during the experiment.

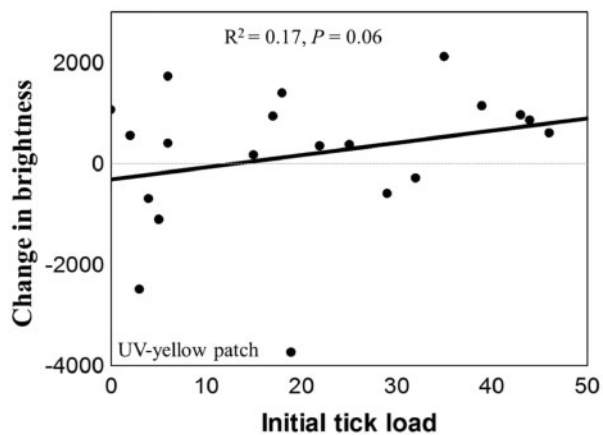


Figure 7. Relationship between variation in the brightness of the UV-yellow throat area and the initial tick load. Individuals below the gray dotted line had a reduction in brightness of the UV-yellow (i.e., carotenoid-based) coloration during the experiment.

they tended to increase the brightness of the UV-blue throat coloration. Thus, lizards with adaptations to avoid or resist tick infestation may be able to maintain darker throat coloration during longer periods along the season. Our results also suggested that lizards that avoid tick infestation may afford a significant reduction of blood parasitemia.

In addition, the treatment*prevalence interaction of *Schellackia* parasites also explained part of the variation in UV-blue brightness. *Schellackia* is a genus of hemococcidian parasites that undergoes endogenous development in the walls of the intestine of the host, where after several cycles of reproduction that destroy the epithelium it enters peripheral blood stream (Telford 2008). Our results suggest a cost of infection on the maintenance of UV-blue coloration of the host. Indeed, only lizards without *Schellackia* hemoparasites showed significant differences in brightness increment when comparing control and fipronil-treated lizards. Individuals of the fipronil-treated group that were not infected with *Schellackia* afforded maintenance, and even reduction, of brightness of the UV-blue throat coloration. Fading of the throat coloration during the

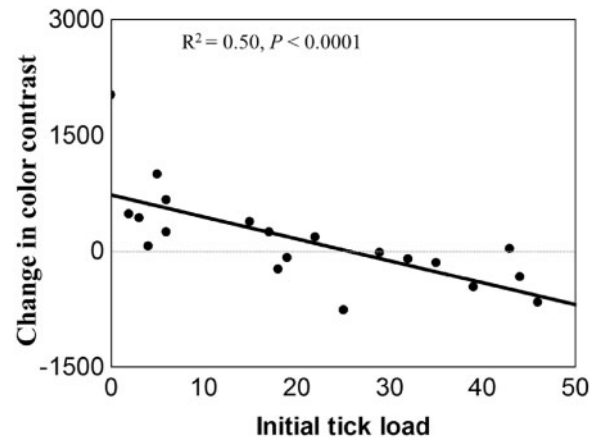


Figure 8. Relationship between the variation in the chromatic contrast in the throat and the initial load of ticks. Individuals below the gray dotted line ended the experiment with high increase of conspicuousness of the UV-yellow coloration.

season may reflect a trade-off between maintaining the nuptial coloration and fighting parasites.

This experiment demonstrated that parasites influence the expression of a structural-based ornament in lacertids. This may be a direct effect of a single parasitic or a combined effect of two parasitic diseases. Hamilton and Zuk (1982) proposed that parasites are important drivers of sexual selection in nature because mate selection based on the expression of secondary sexual traits may be affected by parasites. Therefore, this sexual ornamentation would inform conspecifics of the individual's genetic ability to avoid parasitic diseases (Hamilton and Zuk 1982). Several experimental studies demonstrated that females exert direct selection on mates based on their sexual ornaments (e.g., López and Martín 2005) that included UV-based signals (Bajer et al. 2010) in lacertids while male intra-sexual selection may drive evolution of coloration (i.e., Pérez I de Lanuza and Font 2013). For example, a previous study showed that male Iberian green lizards with darker (i.e., less brightness) UV-blue throats were more often found paired with females in the field (Martín and López 2009). Based on our results, males with UV-blue throats with less brightness might be signaling their individual quality to resist negative effects of parasites. Thus, maintaining dark UV-blue throat coloration may be advantageous for male Iberian green lizards to get access to females. The mechanism that produces the dark UV-blue coloration in green lizards during the breeding season is unknown. It is possible that the increased melanization of the dermis plays a major role in the UV-blue coloration of lacertids (e.g., Fulgione et al. 2015). Previous correlational studies in lizards reported positive relationships between the expression of structural (and melanic)-based ornaments and parasite infection (Ressel and Schall 1989; Megía-Palma et al. 2016a, 2016b). Thus, future studies should evaluate the existing trade-offs between melanin synthesis and the cost of infections.

Attending to the spectral variation of the UV-blue throat area in the individuals without *Schellackia* infection (i.e., control vs. fipronil-treated group), it seems that most of the variation in total brightness of the spectra was given by UV wavelengths (i.e., 320–400 nm). However, UV saturation was not significantly affected by the experiment. Nevertheless, producing and maintaining high UV saturation in nuptial UV-blue ornaments may be important in green lizards (Václav et al. 2007; Martín and López, 2009; Bajer

et al. 2010, 2011, 2012; Megía-Palma et al. 2016b). For example, UV saturation of the throat coloration reflected parasite load, body condition, dominance status, and pairing success in lizards including *L. schreiberi* (Whiting et al. 2006; Martín and López 2009; Bajer et al. 2010; Pérez I de Lanuza et al. 2014; Martin et al. 2016; Megía-Palma et al. 2016a).

The number of ticks at the beginning of the experiment negatively correlated with the increment in UV saturation, but it was significantly and positively related to the increment of brightness of the UV-yellow throat coloration. Previous studies in this species found no evidence relating dominance status or mating success to the UV-yellow coloration in *L. schreiberi* (Martín and López 2009; Stuart-Fox et al. 2009). However, sexual selection may favor the presence of complex and contrasted color ornaments in lacertids (Pérez I de Lanuza and Font 2016). Thus, male lizards that afforded maintenance of highly contrasted throat ornamentation (i.e., high UV saturation in the UV-blue area and low brightness in the UV-yellow area) despite supporting a high tick load may reflect their individual quality to tolerate the handicap of parasites while producing the throat signal. This idea was supported by the highly significant and negative relationship found between the initial tick load and the spectral contrast in the throat. Lizards with lower tick loads at the beginning of the experiment showed higher increment in chromatic contrast in the throat (i.e., higher conspicuousness of the UV-blue coloration over the UV-yellow coloration) than lizards with higher initial load of ticks.

In conclusion, we demonstrated the co-variation of the structural-based nuptial ornament of the Iberian green lizard with parasite load. Proper maintenance of nuptial coloration may compete with an immune response against pathogens because fipronil-treated lizards afforded both a significant reduction in hemoparasite load and tended to avoid the increase of brightness of the UV-blue throat coloration in comparison to control lizards, which may be important in this lizard species to access females. Moreover, male lizards that remained infested with ticks during the experiment tended to increase the brightness in the UV-blue throat coloration, whereas fipronil-treated lizards tended to maintain a more stable UV-blue coloration. Initial tick load in the lizards influenced variation in UV saturation of the throat and maintenance of contrasted UV-blue/UV-yellow throat complex ornamentation, respectively. Therefore, the capacity to fight parasites may be fundamental for proper expression of throat coloration in male Iberian green lizards, and hence throat coloration may reflect the quality of the individuals.

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