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ORIGINAL ARTICLE

Sexually dichromatic coloration of female Iberian green lizards correlates with health state and reproductive investment

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Abstract

Females often have chromatic patterns that differ from those of males. The evolution and function of female coloration is less known than those of males. Female ornaments can result from non-adaptive intersexual genetic correlation, but they may also signal female quality, health state, or potential fecundity. We examined whether the spectral characteristics of the sexually dichromatic coloration of female Iberian green lizards (*Lacerta schreiberi*) correlate with their condition, parasite load (ticks, hemoparasites and *Borrelia* infection) and the inflammatory response, and predict reproductive investment and offspring quality. Females with more intense UV-blue throat structural coloration and with more intense carotenoid-dependent yellow chests had less hemoparasites, and females with more saturated green dorsal coloration had lower tick loads. Also, females with greener backs seemed less prone to *Borrelia burgdorferi* s. l. infection. Surprisingly, in females not infected with *Borrelia*, there was a negative relationship between tick load and carotenoid content in the chest, which was not obvious in infected females. Characteristics of the females' coloration may also predict several variables of reproductive investment, such as egg laying date, incubation time, hatchling number, hatching success, and body size and condition of their hatchlings. Because this is a polygynandric species and mating investment is costly for males, we suggest that males might use female coloration to select potential mates. However, we can expect that the physiological basis of coloration is similar in both sexes and, therefore, genetic correlation and sexual selection may act synergistically.

Significance statement

Despite widespread misconception that males are brightly colored and females are dull, females of some species also signal their quality with colorful ornaments. However, the function of female coloration is less often studied, although it may be important to fully understand sexual selection. Coloration of female Iberian green lizards is not so bright as that of males, but spectral characteristics of female colors can reflect body condition, parasite load, and immune response and, moreover, predict the number and quality of her future offspring. These correlations might help males to choose among female partners when selecting a good mate is a time and energy demanding task, increasing males' fitness. This would support direct sexual selection of female ornaments.

Keywords *Borrelia* infection · Female coloration · Lizards · Offspring quality · Parasite burden · Reproductive success

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Introduction

The evolution and function of male sexual signals are the topics of many research studies (reviewed in Andersson 1994; Andersson and Simmons 2006). However, in many species, females also often have sexual ornaments, but their evolution and function are understudied (Clutton-Brock 2009; Wright et al. 2015). In the past, female bright coloration was considered only a result of a non-adaptive process of intersexual genetic correlation due to the effects of sexual or natural selection on males' genome, which are inherited by females as well (Lande 1980). According to this, bright colors should have negative effects on female survival and reproductive success due to increased predation risk (e.g., Freeman-Gallant et al. 2014). However, female bright coloration can also evolve by direct selection. In some species, female coloration can indicate competitiveness for males (Berglund and Rosenqvist 2001). Also, females can compete between them for resources such as a breeding territory or a nesting place (Hegyi et al. 2008; Griggio et al. 2010).

Moreover, in some species, female coloration can signal female quality, informing about health state, strength of the immune system (e.g., Kelly et al. 2012), parasite burden (e.g., Potti and Merino 1996), and condition (e.g., Johnsen et al. 1996). Moreover, female colors can show potential fecundity and investment into the offspring (e.g., Potti et al. 2013). Genetic correlation and direct selection provide opposite predictions between female signals and fitness, but these are not mutually exclusive phenomena. Vestigial traits resulting from genetic correlation can be the base on which female ornaments can later evolve by sexual selection. Furthermore, there could be cooperation of these two processes; choosing a female partner with brighter or bigger signals can have beneficial fitness results for a male by producing not only daughters but also sons with brighter or bigger signals (Amundsen 2000).

Evolution of male mate choice can be influenced by the species' breeding system, parental investment, cost of pair searching, and the variance of quality among females (Bateson 1983). When females mate with more than one male and "sperm mixing" exists, the males that produce higher quantity of sperm are more successful in sperm competition, increasing their probability of fertilization (Parker et al. 1996). However, although production of a single sperm load is cheap (Dewsbury 1982), in polygynandrous or promiscuous mating systems, the increased requirement of sperm transfer can be costly (Savalli and Fox 1999). Thus, males may invest more sperm into more ornamented females (Cornwallis and Birkhead 2007). Theoretically, evolution of male mating preferences is favored and males have direct benefits if females' quality has high variance, the cost of mate searching is low, or if males' parental investment is high (Chenoweth et al. 2006). In most of species where females have ornaments, females have direct benefits from multiple pair mating (Clutton-Brock 2009). When mating rate, quality variance, or parental care are similar in both sexes, mutual choosiness is expected (Burley 1977; Johnstone et al. 1996). The higher the parental investment of a sex is, the choosier it is, and lower quality individuals discriminate less than good quality ones (Burley 1977). However, the real reason for choosiness may not be parental care per se, but rather the "time-out" of sexual competition (e.g., time invested in mate guarding) (Parker and Simmons 1996).

Honesty of female signals might function through a cost as it occurs in males (Amundsen 2000). However, there can be a trade-off between elaboration of ornaments and fecundity, which may lead males to avoid females with too elaborated ornaments (Nordeide 2002; Nordeide et al. 2006). Thereby, stabilizing selection can affect female sexual signals (Fitzpatrick et al. 1995). Nevertheless, female traits can signal directly their potential investment into the offspring. In some species, body condition of females can predict number or size of eggs (Bonduriansky 2001; Steiger 2013). Females can influence the quality of their offspring by genetic and non-genetic mechanisms. Females allocate nutrients and important biochemicals, such as antibodies, antioxidants, and hormones, into the embryos through the placenta or into the egg yolk (e.g., Thompson and Speake 2002). Rapid embryonic growth rate results in high oxidative stress inside the eggs (Blount et al. 2000; Surai et al. 2001a). Thus, the antioxidant content of eggs is very important for embryonic development and survival (Johnston et al. 2007). Conspicuous color traits can signal antioxidant body levels which can be related with antioxidant allocation inside eggs (Blount et al. 2002; McGraw et al. 2005). Female striped plateau lizards (*Sceloporus virgatus*) have pteridin-based orange throat patches and this trait is preferred by males (Weiss 2002). The size of throat patches positively relates with the body condition and negatively with parasite load, while orange chroma positively relates with body size, thus indicating phenotypic and genetic qualities (Weiss 2006). Furthermore, throat patch size positively correlates with the mass and antioxidant content of the eggs (Weiss 2006; Weiss et al. 2011) and predicts a higher condition and running speed of the offspring (Weiss et al. 2009). Similarly, ultraviolet crown coloration of female Blue tits can predict egg size and fledgling number (Szigeti et al. 2007; Henderson et al. 2013).

Here, we examined the potential functions of multiple colorful traits of female Iberian green lizards (*Lacerta schreiberi*), a diurnal lacertid lizard from the Iberian Peninsula (Marco 2015). This lizard shows strong sexual dichromatism; males have bright blue heads yellow chests and vents, and green backs with small black spots, while females have white throat coloration, yellow chest coloration, and brown or, sometimes, greenish dorsal coloration with large black spots. Interindividual variation in the males' coloration may signal morphology, health state, dominance, and pairing status (Martín and López 2009; Kopena et al. 2014a, b, 2017), but the possible signaling function of female color traits remains unexplored (but see Kopena 2017). The mating system of *L. schreiberi* is polygynandrous, and males often mate guard females (Marco and Pérez-Mellado 1999). The requirement of high sperm quantity and the loss of time-out of mating by mate guarding suggest the possible existence of some male mate choice in this species.

We measured coloration, health state, and reproductive traits of female *L. schreiberi* lizards to investigate: (i) the costs of vitellogenesis for female condition and health, (ii) whether characteristics of coloration of females could be related with their morphology and health state (inflammatory response to phytohaemagglutinin, ecto- and hemoparasite burden and bacterial infection by *Borrelia burgdorferi*), (iii) whether female quality traits could be related with reproductive success and offspring quality, and (iv) whether characteristics of coloration of mothers could predict their reproductive investment and the quality of their offspring.

Materials and methods

Study area and species

In June 2012, we captured 21 gravid female *L. schreiberi* in 'Valle de La Fuenfría' in the Guadarrama Mountains (40° 44′ N, 4° 02′ W; Madrid Province, Spain) and we immediately transferred them to "El Ventorrillo" field station (MNCN-CSIC) (Madrid province, Spain), 5 km from the capture site. Lizards were individually kept in outdoor 51 × 36 × 28 cm PVC terraria containing coconut fiber substratum and rocks for cover. Terraria were placed at a half-shady forest area, where a natural population of this lizard is also found. Every day, lizards were fed mealworm larvae (*Tenebrio molitor*) and house crickets (*Acheta domesticus*), dusted with calcium and vitamin powder, and water was provided ad libitum. We measured females' snout-to-vent length (SVL) to the nearest 1 mm (mean ± SE = 106 ± 1 mm; range = 95–116 mm), body mass just after egg laying (mean ± SE = 24.3 ± 0.6 g; range = 17.5–29.5 g), and calculated body condition as the residuals of the ln(BodyMass) regression on ln(SVL). Females were held in captivity until they laid eggs (time since capture until laying: mean ± SE = 27 ± 3 days; range = 11–49 days) and two to four days after, females were released to their capture sites after ensuring they were in good health condition. To minimize observer bias, blinded methods were use when all data were measured and/or analyzed.

Color measurements

To ensure that we could compare different individual females captured at different times, we only used measures of coloration taken at the same stadium of all females (i.e., immediately after egg laying). Thus, we avoided possible temporal changes of coloration associated to the different stadiums of gravidity of females. Nevertheless, pilot repeated measures of coloration of the same individual females through their pregnancy period did not show significant temporal variations in coloration. We measured the reflectance of females' coloration from 300 to 700 nm using an Ocean Optics USB2000 spectroradiometer with a DT-1000-MINI Deuterium–Halogen light source (Ocean Optics, Inc., Dunedin, FL, USA) following procedures described in Kopena et al. (2014a). We measured coloration of the whitish 'throat' (between the last chin shields and the collar), the yellowish 'chest' (at the middle of the second row of scales prior to the collar), and the brownish or greenish 'back' (background coloration, avoiding black spots, at the middle point above the two forelimbs). We measured three nearby points at each body location and calculated average values of each individual. All measurements took place when lizards were fully active and had been able to attain an optimal body temperature; thus, we avoided a potential influence of temperature on coloration.

We made three different PCAs, one for each body part, to mathematically summarize the complex reflectance spectra into three independent PCs (Cuthill et al. 1999; Montgomerie 2006) (Table 1). To explore the role of carotenoids in the chest "yellowish" coloration, we calculated the ratio of violet-blue (400–515 nm) and background (575–700 nm) reflectance ($R_{\text{violet-blue}}/R_{\text{background}}$) as an index of absolute carotenoid chroma reflecting the carotenoid content (Jacot et al. 2010; San-Jose et al. 2013).

Inflammatory response to phytohaemagglutinin

After egg laying, we used a pressure-sensitive spessimeter to measure the inflammatory response of the skin of female lizards in vivo by using the phytohemagglutinin (PHA) injection test (Smits et al. 1999) following procedures in Kopena et al. (2014b). We used this measure (i.e., the amount of skin swelling at the hindlimb foot pad after 24 h due to inflammation by the PHA injection) as a standardized index of immunocompetence that, independently of the type of immune cells involved (Martin et al. 2006), may reflect health state (but see Adamo 2004 for problems of interpreting measurements of immunity).

Parasite load

We counted the number of ectoparasites (*Ixodes ricinus*, Ixodidae) fixed on each female at capture time (tick prevalence = 80%, median intensity = 3.0, $CI_{95\%} = 3.2–9.1$, and range = 1–20). We also counted hemoparasites (hemogregarines) by using blood smears following Merino and Potti (1995) and Amo et al. (2004) (prevalence = 80%; median intensity = 29 infected red blood cells/2000 cells, $CI_{95\%} = 20.8–56.8$, and range = 1–142). Because values of tick and hemogregarine intensities of infection were not normally distributed, we used square-root transformed data (Kolmogorov–Smirnov test for both variables: $d = 0.11$, $P > 0.20$; Q-Q plot showed normal distribution).

To examine infection by *Borrelia burgdorferi* sensu lato, we isolated DNA from collar scale samples by alkaline

Table 1 Results and interpretation of principal component analyses (PCA) of female coloration on different body parts

		Eigenvalue	% of the variation	Relationship of the variables with PC scores		Interpretation
				Positive	*Negative*	
Throat	PC1	62.87	77.20	–	300–700 nm	Lower PC scores-- > brighter throat
	PC2	9.84	12.14	300–465 nm	470–700 nm	Higher PC scores-- > more saturated UV-blue throat
	PC3	4.21	5.19	365–605 nm	300–360 nm 610–700 nm	Higher PC scores-- > higher relative amount of short-middle wavelengths comparing to very short and long wavelengths
	Total	76.91	94.96			
Chest	PC1	49.37	60.96	–	300–700 nm	Lower PC scores-- > brighter chest
	PC2	17.76	21.92	300–490 nm	495–700 nm	Lower PC scores-- > more saturated yellow chest
	PC3	9.96	12.29	385–580 nm	300–380 nm 585–700 nm	Lower PC scores-- > higher relative amount of very short and long wavelengths comparing to short-middle wavelengths
	Total	77.09	95.17			
Back	PC1	54.28	67.01	–	300–700 nm	Lower PC scores-- > brighter back
	PC2	18.73	23.13	300–475 nm	480–700 nm	Lower PC scores-- > more saturated green or brown back
	PC3	4.02	4.96	370–615 nm	300–365 nm 620–700 nm	Higher PC scores-- > higher relative amount of short-middle wavelengths comparing to very short and long wavelengths (i.e., more UV-green back)
	Total	77.03	95.10			

hydrolysis (Guy and Stanek 1991). PCR amplification was performed in a 25 μl reaction mixture from 5 μl of 5x HOT FIREPol® Blend Master Mix Ready to Load with 10 mM MgCl2 (Solis BioDyne, Tartu, Estonia),13 μl nuclease-free water, 1–1 μl forward and reverse primer, and 5 μl DNA template. Presence of *B. burgdorferi* s. l. in samples were detected by amplifying a portion of the 5S (rrfA)-23S (rrlB) rDNA intergenic spacer (IGS) (Derdakova et al. 2003). Negative and positive controls were included in each reaction. Amplicons were separated on a 1.5% agarose gel (Sigma-Aldrich, Buchs, Switzerland) in 1x TAE Buffer (40 mM Tris, pH 7.8, 20 mM acetic acid, and 2 mM EDTA). The gel was stained by Good View II nucleic acid stain (Ecoli, Bratislava, Slovak Republic) and afterwards was visualized using a UV transilluiminator. We found lizards infected with *B. burgdorferi* s. l. (prevalence = 60%). Lizards can be reservoir hosts of this bacterium, which although has no or just mild effect on reservoir host, has immune inhibitor effect (Tracy and Baumgarth 2017) that may influence the immune response to other pathogens. For this reason, we examined the effect of the interaction of *B. burgdorferi* s. l. with tick and hemogregarine infection.

Eggs and hatchling husbandry

Near the expected laying dates (27th June to 24th July), we checked the terraria every day looking for eggs buried in the substrate. Twenty females laid a total of 281 eggs (mean ± SE = 13.5 ± 0.43 eggs/clutch; range = 10–18 eggs/clutch). When a clutch was found, the eggs were immediately transferred to closed plastic boxes (13 × 13 cm^2; one box per clutch) filled with moistened perlite (1 g perlite:1 g water). We placed boxes in an incubator at 25.5 °C (IRE-160; 94 × 60 × 60 cm; Raypa, Barcelona, Spain) following previous studies of egg incubation in this lizard species (Marco et al. 2004; Köhler 2005; see also Monasterio et al. 2013).

When incubation of eggs was close to the end (incubation time, mean ± SE = 53.4 ± 0.1 days, range = 51–60 days), the clutches were monitored every two hours between 0800 and 2200 h searching for newborns. Immediately after hatching (between 20th August and 13th September), the 181 born offspring (hatching success = 64.4%) were measured with digital calipers (to the nearest 0.1 mm) (SVL, mean ± SE = 33.5 ± 0.1 mm; range = 29.6–36.5 mm) and their 'body mass' weighed with a digital scale (to the nearest 0.01 g) (mean ± SE = 0.95 ± 0.01 g; range = 0.66–1.23 g). Body condition at hatching was estimated using the residuals of the regression of ln(body mass) on ln(SVL). We observed in some of the offspring morphological abnormalities in the feet and tail, which may indicate stress due to bad conditions during development inside the mother or during external incubation (Ji et al. 2002). The absence/presence of undeveloped or curved tail and of supernumerary toes in each of the four feet was noted to make an index of "abnormality degree" (ranging from 0 to 5, depending on the number of abnormalities observed) (mean ± SE = 0.36 ± 0.05; range:0–3). After measurement, offspring were placed in outdoor 51 × 36 × 28 cm PVC terraria in the same conditions as females for further observations and later released at the capture sites of their mothers.

Data analyses

Because female size may affect clutch size and hatchling numbers, we calculated relative clutch size and relative hatchling numbers using the residuals of the regression of clutch size or hatchling numbers on female SVL. Furthermore, since clutch size may strongly influence laying date and incubation time of eggs and offspring traits, in further analyses, we used these variables corrected in relationship to clutch size.

To estimate costs of vitellogenesis for female condition and health, we used two backward stepwise general regression models (GRM) in STATISTICA Software (StatSoft, Inc., Tulsa, OK, USA) to analyze whether body condition or the inflammatory response after egg laying (dependent variables in separated models) depended on female size, tick and hemogregarine load, and relative clutch size (independent variables) and on the presence/absence of *Borrelia* infection (fixed factor), and we included the double interactions of *Borrelia* infection with tick load and of *Borrelia* infection with hemogregarine burden.

Moreover, we analyzed the relationships of coloration with morphology and health state of females using backward stepwise GRMs with each of the PC scores describing coloration or carotenoid chroma as dependent variables in separated models and the female quality traits (SVL, condition after egg laying, tick and hemoparasite load, *Borrelia* infection, and double interactions of *Borrelia* infection with tick or with hemogregarine load) as independent variables.

Finally, we explored the relationships of mother quality traits and coloration with reproductive parameters and offspring traits. We used different backward stepwise GRMs to investigate whether reproductive parameters (hatching success, hatchling number) and offspring traits (SVL, body condition, abnormality degree, and incubation time; the average values of all the offspring of each female) as dependent variables in separated models can be predicted by the female quality traits mentioned above or by female coloration (PC scores) as independent variables.

In all the above GRM models, we also included initially the number of days that females were held in captivity before egg laying to correct for any negative effect of captivity on the reproductive output. Nevertheless, this variable was not included in the final models in any case. All models were checked for collinearity (all variance inflation factors, VIF < 3) and residuals fulfilled the normality and homoscedasticity assumptions. To control for Type I error inflation in the relationships between dependent and independent variables in the GRM models, we applied a false discovery rate (FDR) tablewise correction to the significance levels (Hochberg and Benjamini 2000; García 2003, 2004).

Results

Costs of vitellogenesis for female condition and health

Female body condition after laying was negatively and significantly related with relative clutch size (Model 1; Table 2). Thus, females that laid relatively larger clutches had a lower body condition after laying. No other variable (i.e., body size, parasite load) was included in the final model. Inflammatory response of females after egg laying was positively and significantly correlated with SVL (Model 2; Table 2), but there were no significant relationships with parasite load, body condition, or relative clutch size.

Relationships of coloration with morphology and health state of females

With respect to throat coloration, females with higher throat PC2 scores (i.e., more intensive UV and bluish throat coloration) had significantly lower hemoparasite loads (Model 3; Table 2; Fig. 1a).

Considering the chest coloration, females with lower chest PC2 scores (i.e., more saturated yellowish chest coloration) had significantly longer body sizes (Model 4; Table 2; Fig. 1b). Also, females with higher chest PC3 scores (i.e., more intensive yellowish chest coloration) had significantly lower hemoparasite load (Model 5; Table 2; Fig. 1c). Similarly, absolute carotenoid chroma of the chest was significantly and negatively related to SVL of females, supporting that bigger females had more carotenoids in the chest skin (Fig. 1d). Also, females infected by *Borrelia* had significantly more carotenoids (Fig. 1e). However, we also found a significant interaction between *Borrelia* infection and tick load (Model 6; Table 2). Thus, in females not infected by *Borrelia*, there seemed to be a stronger negative non-significant relationship between tick load and carotenoid content ($r = -0.22$, $P = 0.57$; black dots and continuous line in Fig. 1f) than in infected females ($r = -0.09$, $P = 0.80$; white dots and dashed line in Fig. 1f).

Regarding the back coloration of females, back PC2 scores were significantly related with tick load and *Borrelia* infection (Model 7; Table 2). Thus, females with higher saturation of back brown or green coloration had lower tick loads (Fig. 1g) and were more prone to be infected by *Borrelia* (Fig. 1h). Also, back PC3 was significantly related with body length and *Borrelia* infection (Model 8; Table 2). Accordingly, females with greener and more intensive back coloration were longer (Fig. 1i) and were less likely to be infected by *Borrelia* (Fig. 1j).

The rest of coloration traits (PC scores) were not significantly correlated with any variable describing morphology or health state of females (i.e., no variable was included in the final models).

Table 2 Results of backward stepwise general regression models (GRM). Significant probabilities after applying a sharpened false discovery rate (FDR) correction (corrected threshold: $\alpha \leq 0.012$) are italicized

Model. Dependent variable	Explanatory variables	β	t	P
Costs of vitellogenesis for female condition and health				
M1. Body condition				
($R^2 = 0.30$, $F_{1,18} = 9.02$, $P = 0.008$)	Relative clutch size	−0.58	−3.00	*0.008*
M2. Immune response:				
($R^2 = 0.31$, $F_{1,17} = 8.90$, $P = 0.008$)	SVL	0.59	2.98	*0.008*
Relationships of coloration with morphology and health state of females				
M3.Throat PC2				
($R^2 = 0.29$, $F_{1,18} = 8.93$, $P = 0.008$)	Hemoparasites	−0.58	2.99	*0.008*
M4. Chest PC2:				
($R^2 = 0.31$, $F_{1,18} = 9.65$, $P = 0.006$)	SVL	−0.59	−3.11	*0.006*
M5. Chest PC3				
($R^2 = 0.19$, $F_{1,18} = 5.48$, $P = 0.031$)	Hemoparasites	−0.48	−2.34	*0.008*
M6. Chest carotenoid chroma				
($R^2 = 0.65$, $F_{3,16} = 12.76$, $P = 0.0002$)	SVL	−0.93	−5.75	*<0.0001*
	Borrelia	0.87	3.27	*0.005*
	*Borrelia**tick load	−0.82	−2.83	*0.012*
M7. Back PC2				
($R^2 = 0.47$, $F_{2,17} = 7.54$, $P = 0.0045$)	Tick load	0.54	3.01	*0.008*
	Borrelia	0.51	2.84	*0.011*
M8. Back PC3:				
($R^2 = 0.55$, $F_{2,17} = 10.28$, $P = 0.0012$)	SVL	0.63	3.80	*0.001*
	Borrelia	0.47	2.83	*0.012*
Relationships of mother quality traits with reproductive success and offspring traits				
M9. Hatchling body condition				
($R^2 = 0.28$, $F_{1,16} = 7.72$, $P = 0.01$)	*Borrelia**tick load	−0.57	−2.78	*0.01*
Relationships of mother coloration with reproductive success and offspring traits				
M10. Hatchling number				
($R^2 = 0.27$, $F_{1,18} = 7.90$, $P = 0.012$)	Back PC2	−0.55	−2.81	*0.012*
M11. Hatching success				
($R^2 = 0.21$, $F_{1,18} = 6.14$, $P = 0.023$)	Back PC2	−0.50	−2.48	0.023
M12. Egg laying date				
($R^2 = 0.36$, $F_{1,18} = 11.66$, $P = 0.003$)	Back PC2	0.63	3.41	*0.003*
M13. Incubation time				
($R^2 = 0.31$, $F_{2,15} = 4.82$, $P = 0.02$)	Chest PC2	−0.50	−2.37	0.03
	Back PC3	−0.54	−2.57	0.02
M14. Average offspring SVL				
($R^2 = 0.67$, $F_{3,14} = 12.61$, $P = 0.0003$)	Throat PC3	−0.51	−3.62	*0.003*
	Back PC1	0.39	2.76	0.015
	Back PC2	−0.46	−3.25	*0.006*
M15. Hatchling body condition				
($R^2 = 0.72$, $F_{5,12} = 9.81$, $P = 0.0006$)	Throat PC1	0.53	3.88	*0.002*
	Throat PC2	0.39	3.02	*0.011*
	Throat PC3	−0.48	−3.42	*0.005*
	Chest PC1	0.56	4.15	*0.001*
	Chest PC2	−0.32	−2.47	0.03

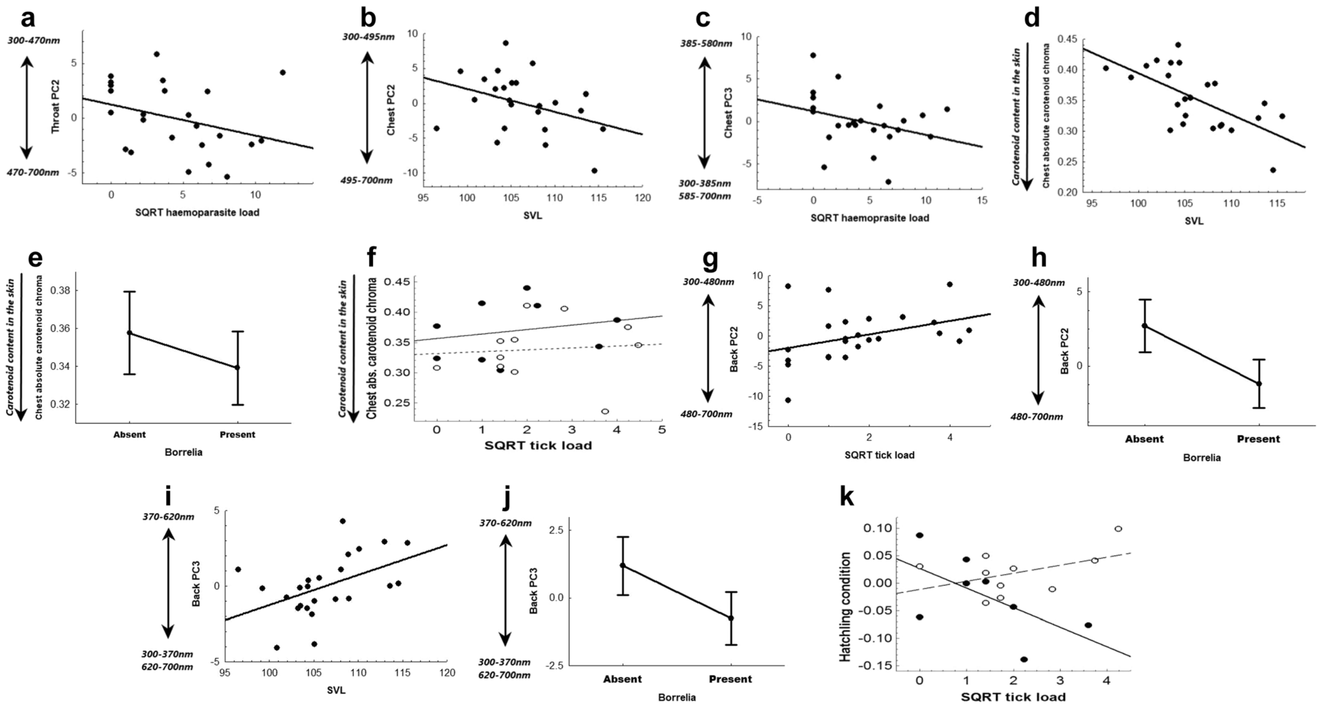

Fig. 1 Relationships of morphology and health state of females with their coloration and the condition of their offspring. Relationships between PC scores or absolute carotenoid chroma describing the throat, chest, and back coloration of female lizards *L. schreiberi* with their **a** and c hemoparasite load; **b**, **d**, and **i** body size (snout-to-vent length); **e**, **h**, and **j** *Borrelia* infection status; **f** tick load in function of *Borrelia* infections status (infected: white dots and dashed line; uninfected: black dots and continuous line); or **g** tick load. Relationship between **k** tick load and average hatchling body condition in function of *Borrelia* infection status (infected: white dots and dashed line; uninfected: black dots and continuous line). Only those relationships included in the final models are illustrated (see Table 2)

Relationships of mother quality traits with reproductive success and offspring traits

Relative clutch size, hatchling number, and hatching success were not significantly influenced by parasite load. Neither incubation time, egg laying date, offspring SVL, or offspring average abnormality degree was significantly related with any mother state variables. However, the interaction of *Borrelia* infection and tick load showed a significant interactive effect on average body condition of hatchlings (Model 9; Table 2). Thus, in females that had no *Borrelia* infection, a greater tick load non-significantly resulted in hatchlings with average lower body condition ($r = -0.59$, $P = 0.12$; black dots and continuous line in Fig. 1k), but this effect tended to be the opposite in females infected by *Borrelia* ($r = 0.45$, $P = 0.19$; white dots and dashed line in Fig. 1k).

Relationships of mother coloration with reproductive success and offspring traits

Both hatchling number (Model 10; Table 2) and hatching success (Model 11; Table 2) were significantly and negatively related with back PC2 coloration of females, which suggested that females with higher proportions of medium and long wavelengths (475–700 nm) on back coloration had more offspring, both considering the absolute numbers of juveniles born (Fig. 2a) or, although the relationship did not reach significance after a FDR correction, their numbers corrected by clutch size (i.e., higher hatching success) (Fig. 2b).

Egg laying date (adjusted for clutch size) was significantly and positively related with back PC2 coloration of females (Model 12; Table 2). Thus, females with higher proportions of medium and long wavelengths (475–700 nm) in back coloration laid their eggs earlier (Fig. 2c).

Furthermore, incubation time (adjusted for clutch size) was negatively related with the mother's chest PC2 and back PC3 coloration scores, but these relationships did not reach significance after a FDR correction (Model 13; Table 2). Thus, eggs from females with more yellowish chests and less intense UV and green back coloration non-significantly had longer incubation times (Fig. 2d, e).

Average offspring SVL (adjusted for clutch size) was significantly and negatively related with throat PC3 and back PC2 coloration scores of their mothers, and, although without reaching significance after correction, positively related with back PC1 coloration (Model 14; Table 2). Thus, females with higher proportions of very short (300–375 nm) UV wavelengths in throat coloration and duller but greater proportions of medium and long wavelengths (475–700 nm) in back coloration had on average larger hatchlings (Fig. 3a–c).

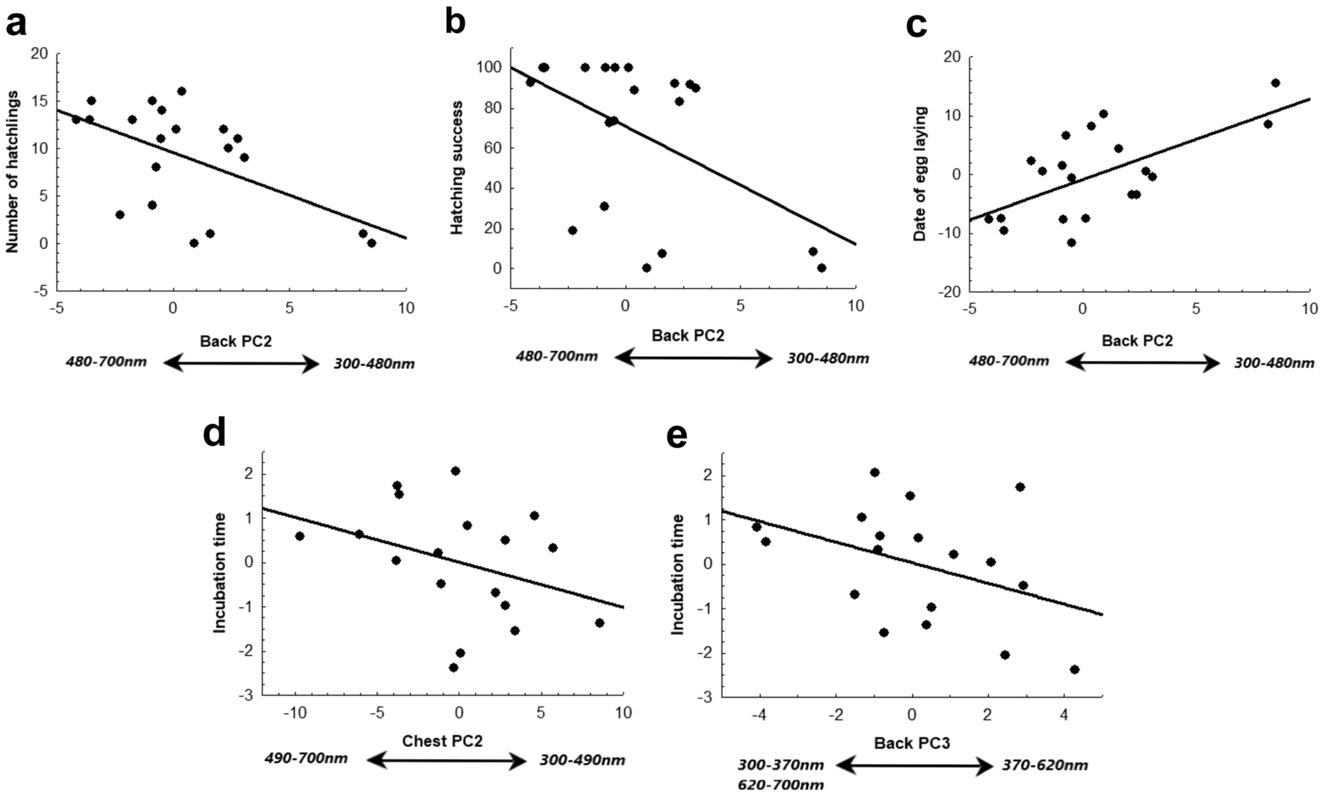

Fig. 2 Relationships of mother coloration with reproductive success. Relationships between PC scores describing the chest and back coloration of female lizards *L. schreiberi* with **a** number of hatchlings, **b** hatching success, **c** date of egg laying, or **d** and **e** incubation time. Only those relationships included in the final models are illustrated (see Table 2)

Body condition of hatchlings was significantly and positively related with throat PC1, throat PC2, and chest PC1, and significantly and negatively related with throat PC3 and, without reaching significance after correction, with chest PC2 of their mothers (Model 15; Table 2). Thus, mothers with duller but more intensive UV and bluish throats and duller but

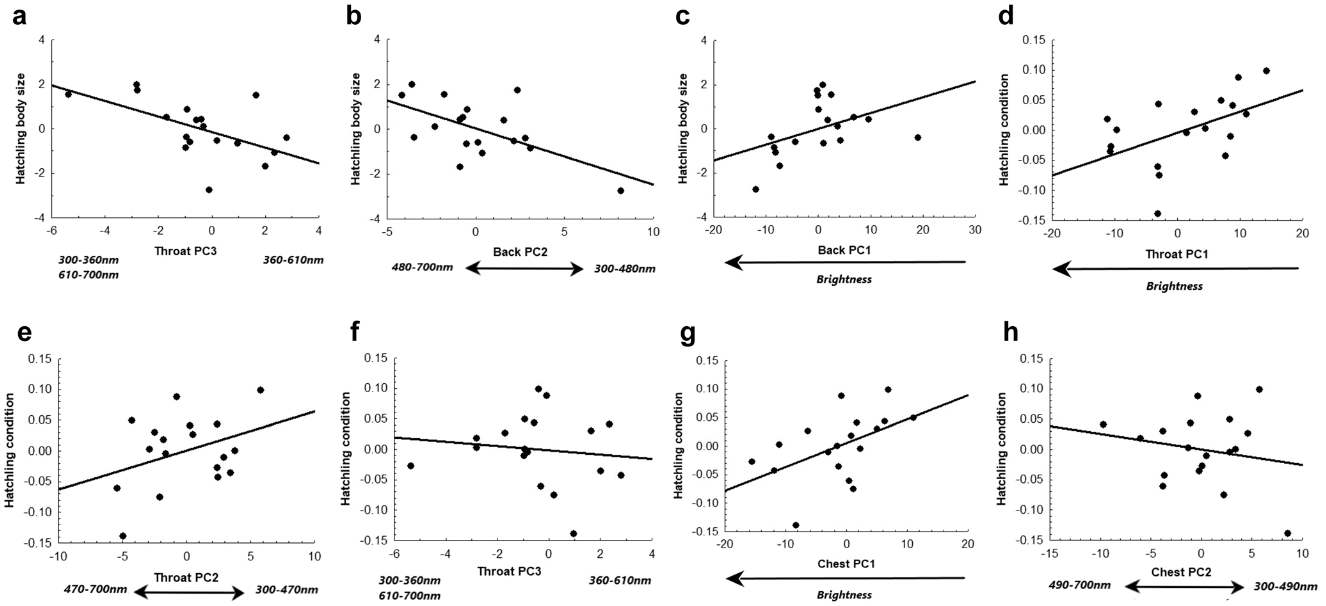

Fig. 3 Relationships of mother coloration with offspring traits. Relationships between PC scores describing the throat, chest, and back coloration of female lizards *L. schreiberi* with **a**, **b**, and **c** average hatchling body size, or with **d**, **e**, **f**, **g**, and **h** average hatchling body condition. Only those relationships included in the final models are illustrated (see Table 2)

more yellowish chests had offspring with a higher body condition (Fig. 3d–g). Average abnormality degree of hatchlings was not significantly related to any color trait of their mothers.

Discussion

Our results strongly suggest that some characteristics of the sexually dimorphic coloration of female *L. schreiberi* lizards are correlated and, therefore, might potentially signal their health state and may also reliably predict their reproductive investment and the quality of their offspring. We also found that costs of vitellogenesis (relative clutch size) may seriously affect body condition of females, although not their inflammatory response. Therefore, the current reproductive investment can increase current reproductive success, but at the cost of decreasing body condition, which may decrease the future reproductive success, such as it occurs in *Lacerta vivipara* lizards (Bleu et al. 2012). The inflammatory response was only dependent on body size, a frequent finding in lizards and birds (see e.g., Tobler et al. 2011; Vinkler et al. 2012). Nevertheless, in the largest (oldest) lizards, there may be a negative effect of senescence on the immune response (Massot et al. 2011; Plasman et al. 2019).

Our results suggest that coloration of female *L. schreiberi* might signal their health state; females with more hemoparasites had less intense UV/blue throats and less carotenoid-dependent yellow chests. Hemoparasites can elicit a physiological stress response (Tomás et al. 2005), increase lipid peroxidation in the blood (Saleh 2009), and decrease carrier molecules of lipophilic antioxidants (Das et al. 1996). Coloration may be affected by these negative effects on carotenoids and other antioxidants, which are needed for the production of melanin and the correct placement of reflective platelets in iridophores (Saenko et al. 2013; San-Jose et al., 2013), and for expressing the carotenoid-dependent coloration. Signaling parasite burden by coloration is very frequent in females of other animals. For example, brighter red female arctic chars (*Salvelinus alpinus*) (Skarstein and Folstad 1996) and female lizards *S. virgatus* with larger orange throat patches (Weiss 2006) have lower parasite burden. Female terrapins (*Mauremys leprosa*) with brighter limb stripes have a better inflammatory response (Ibáñez et al. 2013).

Bigger female lizards had more saturated yellow chests, a relationship that is similarly found in carotenoid signals of some birds (Jawor et al. 2004; Freeman-Gallant et al. 2014). Bigger (older) female lizards might increase carotenoid intake by their longer life experience, which could also contribute to develop stronger immune responses (Chew and Park 2004), although carotenoid supplementation did not increase the inflammatory response of male *L. schreiberi* (Kopena et al. 2014b).

We also found that female *L. schreiberi* lizards infected by *B. burgdorferi* s. l. had higher carotenoid content in the chest skin and that, surprisingly, this could change the negative effect of ticks on carotenoids. Both results could be explained by the immune inhibiting capability of *B. burgdorferi* s. l. At least in mammalian hosts, this bacterium can inhibit the activation pathways of the complement cascade of the innate immune system and destroy the humoral immune system (Tracy and Baumgarth 2017), which might also release antioxidants (e.g., carotenoids) from their immune role and allow them to be allocated to coloration. Also, with respect to dorsal coloration, female *L. schreiberi* lizards with more intense UV and green dorsal coloration were larger and had lower rates of *Borrelia* infection.

Clutch size of female *L. schreiberi* was determined mainly by mother size, but not by parasite burden. Similarly, parasites neither affected, hatching success, laying date, or incubation time. However, we found that *Borrelia* infection can change the negative trend between tick load and body condition of hatchlings. We suggest that this result is also the consequence of the immune inactivating capability of this bacterium. As explained above, antioxidants (e.g., carotenoids) released from their immune role might allow females to allocate them to the eggs resulting in offspring with better condition. Similarly in female striped plateau lizards (*S. virgatus*), throat patch size predicts mass and antioxidant content of the eggs and higher condition of offspring (Weiss 2006; Weiss et al. 2009, 2011).

Characteristics of the coloration of *L. schreiberi* females may also predict several variables of reproductive investment; females with more saturated dorsal coloration had relatively larger clutch sizes, shorter incubation times, more hatchlings, and higher hatching success. Melanin production for coloration can be influenced by protein or calcium consumption (reviewed in McGraw 2008), which can also increase egg development inside the mother body. Nevertheless, coloration may just be an indicative of female quality and capability of resource allocation to clutch production. Coloration is also related to reproductive parameters in other animals; in northern cardinals (*Cardinalis cardinalis*), more colorful females have earlier clutches and more fledglings (Jawor et al. 2004). Female pied flycatcher (*Ficedula hypoleuca*) with bigger white wing patches breed earlier and have more hatchlings, while the presence of a white forehead patch predicts more fledglings (Morales et al. 2007).

Furthermore, eggs from female *L. schreiberi* with more yellowish chests tended to have longer incubation times. Due to fast metabolism, production rate of free radicals is very high inside the eggs, and, thus, embryos require large amounts of antioxidants for their normal development (Blount et al. 2000; Surai et al. 2001a). Antioxidant capacity of carotenoids is higher in lower oxygen pressure, as it occurs inside the eggs (Surai et al. 2001b). Thus, allocating carotenoids into the yolk can increase egg survival and offspring quality. Thus, in female lizards *S. virgatus*, pterin-based orange throat patch size and chroma predict average egg mass and more carotenoids and antioxidants in the eggs (Weiss 2006; Weiss et al. 2011). "Carotenoid-rich" *L. schreiberi* females might invest more antioxidants into eggs, resulting in longer incubation times that would provide more time for development of the embryo and produce hatchlings with better condition. In contrast, greener backed females laid eggs with shorter incubation times. However, we did not find negative effects of a shorter incubation on offspring quality, and this result contradicts why these females had lower rate of *Borrelia* infection. It is possible that these embryos might have a faster developing rate, allowing earlier hatching and more time for feeding and reaching a better condition before the first hibernation period, increasing winter survival (Civantos and Forsman 2000; Grenot et al. 2000).

Female lizards with higher UV throat intensity and more saturated but less bright dorsal coloration had average bigger hatchlings. Similarly, females with more saturated but less bright UV-blue throats and more yellowish saturated but less bright chests had hatchlings with a better condition. Because developmental stress can influence structural UV coloration (Kemp and Rutowski 2007), maintaining a coloration with higher UV intensity may require allocating more antioxidants to reduce stress. Indeed, a supplement of carotenoids and vitamin E increased structural-based throat brightness of male *L. schreiberi* (Kopena et al. 2014a). Thus, these females might also be able to invest more antioxidants into eggs to promote offspring size and condition (Biard et al. 2005). Also, in other animals, structural coloration may indicate a higher investment in reproduction, such as in blue tits, where females with more intensive UV crown have bigger egg size (Szigeti et al. 2007) and more fledglings (Henderson et al. 2013). Furthermore, lower UV-blue-throated *L. schreiberi* females had more hemoparasites, and diseases can decrease carotenoid investment into the egg yolk (Surai et al. 2001b). Moreover, these females may have more antioxidants to be able to afford both a more intensive structural throat coloration and a chest with more carotenoids. Similarly, female lizards *S. virgatus* with bigger orange throat patches have more antioxidants in the egg yolk and produce offspring with better condition (Weiss et al. 2009, 2011). Also, carotenoid-treated females of blue tits have bigger offspring with better immune systems (Biard et al. 2005).

Although there are no data about male choice of females in this lizard species, it is likely that males could obtain very complex information about female quality and reproductive investment using color traits of females. Male preference for female coloration is a very under-researched subject, but it has been found in some fish (Amundsen and Forsgren 2001) and birds (Griggio et al. 2010). Also, male striped plateau lizards (*S. virgatus*) prefer females with bigger orange throat patches (Weiss 2002; Weiss and Dubin 2018) that reflect egg quality (Weiss et al. 2011) and offspring condition (Weiss et al. 2009). However, female coloration does not trigger male preference if it is not correlated with female quality or mother investment (Nordeide 2002; Nordeide et al. 2006).

In summary, female coloration of *L. schreiberi* predicts several components of female quality and reproductive investment. These color patterns might support direct sexual selection of female ornaments. Future studies should examine the existence of male preferences and whether female choice increases the males' fitness. Nevertheless, color physiological basis of males and females may be similar and, therefore, genetic correlation is not excluded. However, these two phenomena would increase even synergistically the fitness of individuals that prefer females with more intense colors.

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Author's contributions All authors conceived the ideas and designed methodology; RK, JM and PL collected the data; VM made analyses of *Borrelia* infection; and RK and JM analyzed the data and led writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The captures and experiments enforced all the present Spanish laws and were performed under license (permit number: 10/072913.9/12) from the Environmental Organisms of Madrid Community. Animal welfare standards and protocols of this study were supervised by the Bioethical Committee of the Spanish Research Council (CSIC).

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