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Variation in male ornaments in two lizard populations with contrasting parasite loads

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Abstract

In the context of the immunocompetence handicap hypothesis, we explored how differences in parasite load affect the way in which sexual ornaments codify information about individual quality. We studied variation in sexual signals in two Iberian populations of the lizard *Psammodromus algirus*, a species in which sexually active males display a red head coloration. In one population, males were free of tick nymphs, whereas in the other one all males were tick-infested (mean of 12.7 tick nymphs/individual).At the onset of the breeding season, the red-coloured surface was larger in the non-parasitized population than in the parasitized one, whereas the opposite was true for colour saturation. We experimentally simulated a bacterial infection (by intraperitoneal injection of lipopolysaccharide) to examine the effects of immune activation on the expression of this sexual ornament. In the non-parasitized population, our treatment caused a reduction in the red-coloured surface of experimental males, whereas in the parasitized population it caused a decrease in colour saturation. In the parasitized population, males that displayed sexual coloration were larger, and had fewer parasites, than uncoloured ones, and inflammatory response to lipopolysaccharide injection in the palm of the hind paw was negatively correlated with colour saturation, but not with colour extension. Thus, we suggest parasites not only constrained the expression of sexual ornaments, but they also changed the signal properties that conveyed useful information about the quality of their bearers.

Introduction

The handicap hypothesis was proposed by Zahavi (1975) to explain the evolution and maintenance of costly sexual ornaments as honest signals of genetic quality. Since then, many researchers have expanded good-gene models to show how exaggerated sexual signals advertise overall heritable fitness (Hill, 1991; Iwasa, Pomiankowski & Nee, 1991; Maynard-Smith, 1991). The basic idea is that the development of sexual ornaments may be involved in trade-offs with different aspects of organismal performance such as predation risk (Giery & Layman, 2015), locomotor efficiency (Møller & Lope, 1994) or immune system activation (Hamilton & Zuk, 1982), in such way that only high quality individuals would be able to express the ornaments without compromising their fitness. Concerning the hypothesis that male ornaments are reliable signals of genetic resistance to parasites and diseases, testosterone was subsequently proposed as a link between sexually selected traits and associated immune costs: only immunocompetent males would be able to increase their testosterone levels, thereby deviating resources from immunity towards the production of an attractive signal, while controlling their pathogen load (Folstad & Karter, 1992).

Previous research has supported the immunocompetence handicap hypothesis in a wide variety of organisms and signals: tail length in barn swallows (Møller, 1988), song rate of starlings (Casagrande *et al.*, 2015), chemical signals of burying beetles (Chemnitz *et al.*, 2015) and rock lizards (Martín & López, 2014), or sexual coloration of fish (Milinski & Bakker, 1990), lizards (Salvador *et al.*, 1996), or birds (Zuk, Thornhill & David-Ligon, 1990; Blount *et al.*, 2003). Most of these studies are based either on correlational approaches showing negative associations between immune system and ornamentation, or on experiments that manipulate either the intensity of sexual ornaments or the activation of the immune system (Møller, 1988; Zuk *et al.*, 1990; Salvador *et al.*, 1996; Casagrande *et al.*, 2015). However, few studies have taken advantage of ecological differences among populations of the same species to investigate the selective pressures that affect the evolution of sexual ornaments (Endler & Houde, 1995; Giery & Layman, 2015).

Here, we examine inter-population differences in sexual signals potentially mediated by parasite load. We test the hypothesis that differences between a non-parasitized and a heavily parasitized population can change the signal features that convey useful information about immune quality. In our model organism, the lacertid lizard *Psammodromus algirus*, males display a red coloration in the head during the breeding season whose degree of enlargement has been related to body size (Díaz, 1993), testosterone levels (Díaz, Alonso-Gómez & Delgado, 1994; Salvador *et al.*, 1996) and reproductive success (Díaz, 1993; Martín & Forsman, 1999). We searched for potentially correlated differences in parasite load and sexual signals between two populations separated by a 600-m elevational gradient. At low-elevation, males were free of *Ixodes sp.* tick nymphs, and they showed a more conspicuous sexual coloration. At high-elevation, parasite load was high, and sexual coloration was less conspicuous. The reason why only high-elevation lizards were parasitized, despite ticks being also present at low-elevation, remains unknown, but it might be related to differences in the availability of alternative intermediate hosts (Casher *et al.*, 2002). Both immunocompetence handicap and the importance of parasitism by tick nymphs of the genus *Ixodes* were previously shown in *P. algirus* by Salvador *et al.* (1996), in an experiment in which testosterone-implanted males increased the conspicuousness of the red coloration of the head, but suffered a higher parasite load (associated with higher mortality) than control males.

In this study, we elicited an immune response in male lizards from both populations by intraperitoneal injection of a bacterial lipopolysaccharide solution (hereafter LPS), which simulates a bacterial infection. We aimed to unravel what particular traits of the sexual ornament could be employed as an honest signal of immune quality in each population. We discuss our results in the light of the hypothesis that, if signaling by means of the extension of colored area is limited by parasites, then other aspects of the ornament should be selected to signal immune quality.

Materials and Methods

Study species and study areas

Psammodromus algirus (Linnaeus, 1758) is a medium-sized (snout-vent length 60–90 mm; mass 6–16 g) lacertid lizard that occupies shrub and woodland Mediterranean habitats. Males display a reddish coloration on the head during the breeding season (April–June; Veiga & Salvador, 2001), which ranges from relatively inconspicuous and restricted to the posterior supralabial or infralabial scales in subordinate lizards, to a brilliant patch on the sides of head, mental scutes and throat in dominant, large individuals (Díaz, 1993).

Our first study area was located at ‘El Pardo’ (Spain: 40°31′N, 03°47′W; 650 m elevation). This site is a holm oak (*Quercus ilex* L.) forest in which offshoots of *Q. ilex* dominate the shrub layer together with open areas covered by annual herbs. The second site was located at Navacerrada (Spain: 40°44′ N, 4°00′ W; 1300 m elevation), at a linear distance of 32 km from the first one. This area is a deciduous Pyrenean oak (*Quercus pyrenaica* Willd.) forest with a high cover of shrub patches. Lizards from both populations show no apparent genetic differentiation (Díaz *et al.*, 2017), but they differ in phenotypic traits such as escape-tactics, sexual dimorphism, and life history (Iraeta *et al.*, 2010, 2011; Iraeta, Salvador & Díaz, 2013).

Collection and husbandry of animals

Sample size consists of 39 males from high elevation, captured in 2014 and 2015, and 24 males from low elevation captured in 2014. Lizards were transferred to the lab, measured (snout-vent length [SVL], head length, head width, and average length of hindlimbs), weighed (body mass), and individually caged in terraria (40 × 60 cm and 30 cm high) in a room with natural photoperiod. Heat was supplied by a spotlight bulb, which created a thermal gradient allowing lizards to thermoregulate within their preferred temperature range. Food (crickets *Acheta domestica*, sprinkled with a commercial diet supplement) and water were supplied *ad libitum*.

Tick infestation

We used a database containing information on 157 adults of both sexes captured in 2005 to analyse interpopulation, seasonal and sexual variation in the prevalence and intensity of infestation by nymph ticks (identified as *Ixodes* spp. by Salvador *et al.*, 1996). This database, which includes two sampling periods, one in the early breeding season (March 30–April 26) and the other one in the mid-late breeding season (May 11–June 7), has already been used in previous studies (Iraeta *et al.*, 2006, 2011, 2013). However, tick data have not been published before. Prevalence was defined as the percentage of individuals with at least one tick on their body surface, and parasite intensity was defined as the number of ticks per infested individual.

Immune system activation and measurement of inflammatory response

In 2014, we performed an experiment to examine the effects of immune system activation on the expression of sexual coloration at the peak (early May) and end (late June)of the breeding season. Two subgroups (experimental and control) were generated at random within each of the two populations. Lizards in the experimental subgroups ($N = 8$ and 11 for high- and low-elevation respectively) were intraperitoneally injected a dose of 2.5 mg of LPS of *Escherichia coli* serotype O111: B4, diluted in 0.05 ml saline solution per gram of body mass. Control individuals ($N = 6$ and 8 for high- and low-elevation respectively) were injected with the same volume of saline solution. This procedure is similar to those previously employed with other lizards (Uller, Isaksson & Olsson, 2006; López, Gabirot & Martín, 2009). Experimental and control males did not differ significantly in coloured surface, colour saturation, structural size, or physical condition, even after controlling for the effects of population of origin or date of capture (all P’s > 0.12). To quantify the effects of immune system activation on sexual coloration, we examined two digital images (see ‘Sexual coloration’ below) taken immediately before and 2 weeks after injection of the antigen.

In 2015, lizards were photographed upon arrival to the lab to measure the colored surface and colour saturation, as explained in the following section. We subsequently estimated inflammatory response by subcutaneously injecting all males with 0.1 mg of LPS diluted in 0.01 ml of serum in the palm of the left hind paw (see Zamora-Camacho *et al.*, 2014 for a similar procedure). We used a caliper to measure the thickness of the palm just before injection of LPS and four hours later, that is, at the moment of the expected peak of immune system activation. In 19 of 20 males, the thickness of the inoculated palm increased 4 h after the subcutaneous administration of LPS (repeated measurements ANOVA: $F_{1,19} = 52.72$, $P < 0.001$). Previous studies have shown that the thickness of the inflammation is associated with the strength of the immune response (Parmentier, De Vries Reilingh & Nieuwland, 1998; Zamora-Camacho *et al.*, 2014).

Sexual coloration

Coloration data were obtained from digital images of the ventral view of the head, throat and neck of each male, with all individuals immobilized in the same position. Pictures were taken in a dark room with a table supplemented by two side light sources at the same distance from the lizard and a holder to fix the camera at a standardized position. Although this method does not allow detecting ultraviolet components that could be present in the sexual signal, we chose to obtain coloration data from digital images instead of performing live measures with a spectrophotometer because of the lack of repeatability of spectrophotometry in non-static surfaces and the difficulties in characterizing irregularly distributed colour patches (Stevens *et al.* 2007).

We used Adobe Photoshop CS6 for image processing (see Fig. S1). We standardized the analysed area using the 'magnetic lasso' tool to delimit the surface comprised within the lines defined by the snout and the posterior edges of the infralabial scutes. The extent of red coloured surface was measured with the 'magic wand' tool (at 30% tolerance) after randomly clicking at the middle of the red area. We subsequently used the 'similar' option of the magic wand tool (at 30% tolerance) to select all areas with similar RGB values, and we measured coloured surface as the percentage of coloured pixels in the analysed area. We calculated colour saturation of the coloured surface, using the ratio R/(R+G+B), where R, G and B are the red, green and blue channels of the graphics card; red saturation is 100% if such ratio is equal to 1. Colour measurements were taken blindly with respect to population of origin and treatment.

Statistical analyses

We checked the assumptions of parametric tests and, when necessary, we log- or arcsine-transformed the variables. We analysed the 2014 data, using ANOVAs with the difference between colour measurements before and 2 weeks after treatment as the dependent variable, and treatment, population and date as the categorical predictors. We used contrast analysis (i.e. planned comparisons) to test the significance of a predicted specific effect within our larger statistical design, namely that males from both populations should differ in their responses to LPS injection while controlling for the effects of date (in other words, that the effects of the interaction between treatment and population on colour variation should be significant).

We estimated the structural size of each male by computing its score on a Principal Component (hereafter PCsize) that combined all size measurements (SVL, head length, head width, and average hind limb length; retained variance = 0.785). Physical condition was estimated using the residuals of the regression of log-body mass on log-SVL.

The effect of parasite load on the sexual colouration of high elevation males was analysed, using a model selection approach based on the Akaike information criterion. Because 12 of 20 males had no sexual coloration at all, we used a logistic regression (generalized linear model with logit as the link function) with presence or absence of sexual coloration as the binomial response variable, and structural size, body condition, and parasite load as continuous predictors.

Finally, we employed a partial least squares regression (PLS) to examine variation in the inflammatory response. Extension of coloured surface, colour saturation, together with parasite load and morphological traits (size and condition) were used as predictors. All statistical analyses were executed with the Statistica (StatSoft) software package.

Results

Tick infestation levels and associated ecoimmunological scenarios

Tick nymphs were detected only on high-elevation lizards (data from the 2005 sample: overall prevalence of 78.3% and 0.0% at high- and low-elevation, with 95% confidence intervals of 67.6–86.3% and 0–6.1%, respectively; $\chi^2 = 98.9$, d.f. = 1, $P < 0.001$). Parasite load was on average 7.9 nymphs per infested individual. Prevalence increased during the breeding season, from 62.8% in March–April to 95.0% in May–June; $\chi^2 = 12.7$, d.f. = 1; $P < 0.001$). Parasite load was higher for males than for females, particularly at the end of the breeding season, when there was a dramatic increase in the mean number of nymphs carried by males (Fig. 1). In 2015, on a sample of 20 males captured in late June, tick prevalence was of 100%, but load (8.2 mites/individual) was lower than in May–June 2005. By the end of the breeding season, and pooling together the 2005 and 2015 samples, overall parasite intensity was 12.7 tick nymphs per high-elevation male (vs. no tick nymphs at low elevation). Occasional visits between 2005 and 2015 confirmed that whereas low-elevation lizards never carry tick nymphs, most lizards are parasitized at high elevation.

Inter-population differences

In May, the red coloured area was greater in males of the non-parasitized population than in males of the parasitized

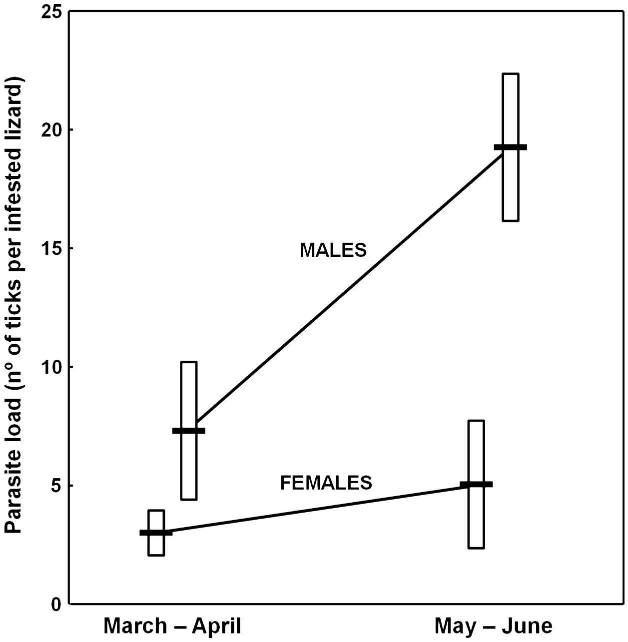

Figure 1 Parasite load (mean number of ticks per infected lizard ±95% confidence interval) for male and female lizards of the parasitized, high-elevation population at the beginning (March–April) and end (May–June) of the breeding season.

one ($F_{1,18} = 5.63$, $P = 0.029$; non-parasitized, mean ± SD = 35.1 ± 20.8%; parasitized: 13.8 ± 17.8%), whereas the opposite was true for coloir saturation ($F_{1,18} = 6.58$, $P = 0.019$; non-parasitized: 54.5 ± 3.7%; parasitized: 60.0 ± 5.8%). Colour saturation of males of the non-parasitized population increased along the breeding season, rising from 54.5 ± 3.7% in early May to 61.4 ± 4.8% in late June (two-way ANOVA with date and population as factors; date: $F_{1,39} = 9.15$, $P = 0.004$; population: $F_{1,39} = 3.82$, $P = 0.058$; interaction: $F_{1,39} = 4.17$, $P = 0.048$). The surface of the coloured area did not vary significantly between May and June in either population ($F_{1,39} = 0.34$, $P = 0.561$).

Experimental males of the non-parasitized population responded to the intraperitoneal injection of LPS by reducing the red-coloured surface on their heads, whereas experimental males of the parasitized population did not show such response (Fig. 2, planned comparison for the interaction between the effects of treatment and population: $F_{1,25} = 5.77$, $P = 0.024$). On the other hand, experimental males of the parasitized population responded to LPS with a significant reduction in colour saturation, whereas experimental males of the non-parasitized population did not show such response (Fig. 2, planned comparison: $F_{1,25} = 5.57$, $P = 0.026$).

Parasite-mediated trade-offs in high-elevation males

Our model selection approach with the 2015 data produced a logistic regression (Table 1) in which the ability of males to express sexual coloration was predicted by structural size (log-likelihood = −12.57, $\chi^2 = 25.1$, d.f. = 1, $P < 0.001$) and parasite load (log-likelihood = −4.84, $\chi^2 = 9.7$, d.f. = 1, $P = 0.002$). Consistently, males that expressed sexual coloration were larger than those that did not show head coloration (mean SVL ± SD = 73.4 ± 2.6 mm and 68.2 ± 3.1 mm, respectively; $F_{1,18} = 15.11$, $P = 0.001$). For a given structural size, coloured males had less parasites (mean number of ticks adjusted for body size ± SD: 1.6 ± 2.5 ticks per individual) than non-coloured ones (12.1 ± 7.7 ticks per individual; ANCOVA: $F_{1,17} = 5.85$, $P = 0.027$), suggesting that parasite load negatively affected the ability of lizards to display sexual coloration.

The PLS regression analyzing coloured surface and colour saturation as predictors of inflammation produced a single factor that explained 40.4% of the variance in swelling ($F_{1,18} = 12.22$, $P = 0.003$; Fig. 3). Predictor weights showed that physical condition and colour saturation, but not structural size, parasite load or coloured surface, explained a significant amount of variation in the inflammatory response induced by the injection of LPS (Fig. 3). Thus, inflammatory response was higher for males with a better body condition (simple correlation: $r = 0.535$, d.f. = 18, $P = 0.015$). Controlling for body condition, the inflammatory response was negatively correlated with colour saturation (partial correlation: $r = -0.565$, d.f. = 17, $P = 0.012$), but not with coloured surface (partial correlation: $r = -0.128$, d.f. = 17, $P = 0.601$).

Discussion

Our 2005 data showed that parasite load was higher for males than for females, as it has been reported in other lizards (Brace, Sheikali & Martin, 2015; Reedy *et al.*, 2016). Males of *P. algirus* have larger home ranges than females, and larger and more coloured males display greater activity to improve their reproductive success (Díaz, 1993). As a consequence, their probability of becoming infested with tick nymphs should also be higher, what would lead to an increased parasite load by the end of the breeding season. Higher parasite loads in males than in females could be explained by the effects of testosterone, which increases activity and compromises immunocompetence (Folstad & Karter, 1992; Salvador *et al.*, 1996; Belliure, Smith & Sorci, 2004). Circulating testosterone levels remain high until the end of the breeding season, as spermatogenesis proceeds and sexual ornaments enlarge (Díaz *et al.*, 1994). All these processes reach a peak between April and mid-June, when the area of sexual coloration is positively correlated with circulating testosterone levels in lizards from the non-parasitized population (Díaz *et al.*, 1994). Also, tick nymph load reaches a maximum in males of the parasitized population at the end of the breeding season, especially if their testosterone levels are experimentally increased (Salvador *et al.*, 1996). Thus, the concomitant phenology of tick nymph infestation and colour enlargement supports the trade-off between sexual ornamentation and parasite load that has been described in these lizards (Salvador *et al.*, 1996) and other taxa (Malo *et al.*, 2009; Pollock, Vredevoe & Taylor, 2012).

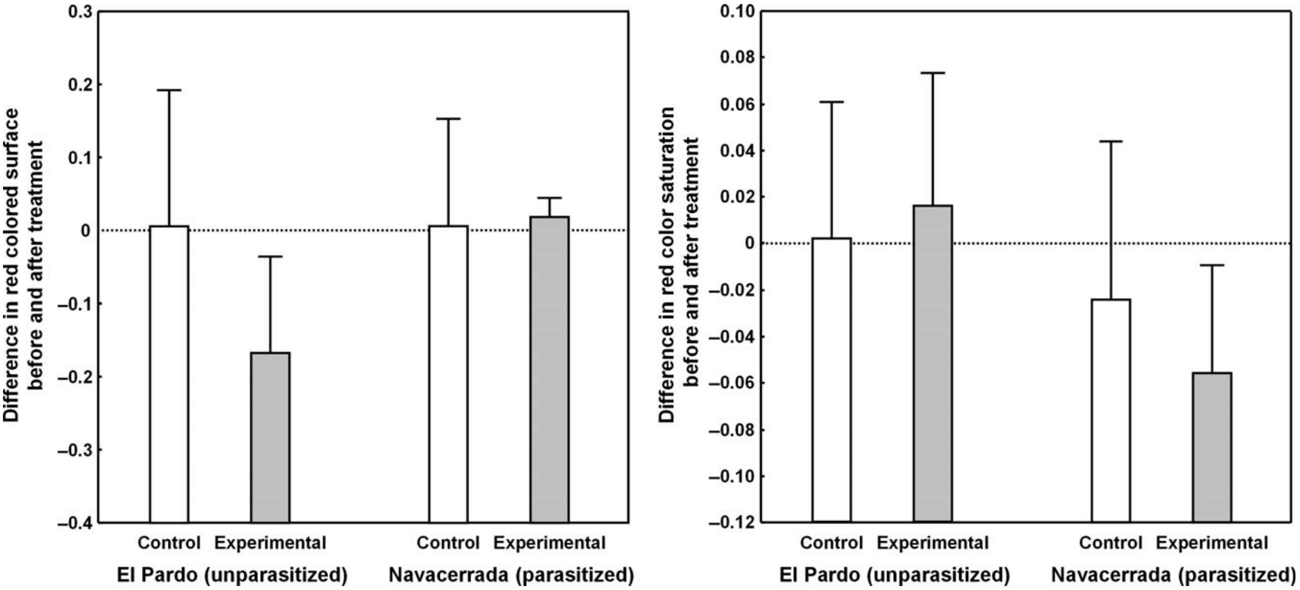

Figure 2 Differences between measurements of red-coloured surface (left) and red saturation (right) taken before (measurement 1) and 2 weeks after (measurement 2) treatment for males from the parasitized (El Pardo) and non-parasitized (Navacerrada) populations: mean values of the difference measurement 2 – measurement 1 (±95% confidence interval). Control males (white bars) were injected with saline solution whereas experimental males (gray bars) were injected with LPS.

Table 1 Model selection approach to predict the presence or absence of sexual colouration in males of the parasitized population (binomially distributed response variable) as a function of structural size, physical condition, and parasite load

Variables in model	AIC	Δ AIC	Log-likelihood χ^2
Structural size - parasite load	6.27	–	24.65
Physical condition - structural size - parasite load	8.00	1.73	26.92
Structural size	13.83	7.56	17.09
Physical condition - structural size	15.67	9.40	17.25
Physical condition	30.37	24.10	0.55
Parasite load	30.50	24.23	0.42
Physical condition - parasite load	31.14	24.87	1.79

AIC, Akaike information criterion.

Within this context of ecoimmunological differences, our experimental approach suggests that the colour trait that is traded-off against immune system activation varies between non-parasitized and parasitized populations. Our results support this interpretation in two ways. Firstly, and foremost, in the non-parasitized population experimental males responded to LPS injection by reducing the extent of the red-coloured surface, whereas in the parasitized population they reduced colour saturation. Secondly, these results were consistent with inter-population differences at the beginning of the breeding season, when the effects of sexual signals on mating success should be maximal. Thus, the features that were traded-off against the immunological activation caused by LPS could be those employed as sexual signals in each population (Hamilton & Zuk, 1982; Jacot *et al.*, 2005).

How do parasites influence these different responses? The sexual ornament was more conspicuous in low-elevation males, with no parasites, than in parasitized, high-elevation ones, which suggests that parasites limit the amount of resources available for expressing a colour signal of a certain size (Kekäläinen, Pirhonen & Taskinen, 2014). This would explain why lizards of the non-parasitized population employ coloured surface as an honest signal of male quality. The signal is effective because, in the absence of tick nymphs, it has enough inter-individual variation to remain informative, a necessary condition for the evolution of costly ornaments (Delhey & Peters, 2008). And it is honest because it is traded-off with immune response, as demonstrated by its consistent decrease in experimental males, which indicates that males may have trouble maintaining a large coloured area and simultaneously mounting an efficient immune response. The males that succeeded to produce a red-coloured sexual signal were larger (i.e. they were older and/or had grown faster) than those that did not display it; and, for a given body size, coloured males had less parasites than uncoloured ones. This is what would be expected if parasites led males to allocate to the immune system part of the resources they would otherwise invest in sexual coloration. This could also explain why colour saturation, rather than colored surface, could be used to signal male quality (as suggested by the decrease in saturation found in LPS-injected males but not in control ones).

Our reasoning is based on the assumption that, in the face of a parasitic infection, it is more demanding to grow a larger colored surface than to increase the saturation of a relatively

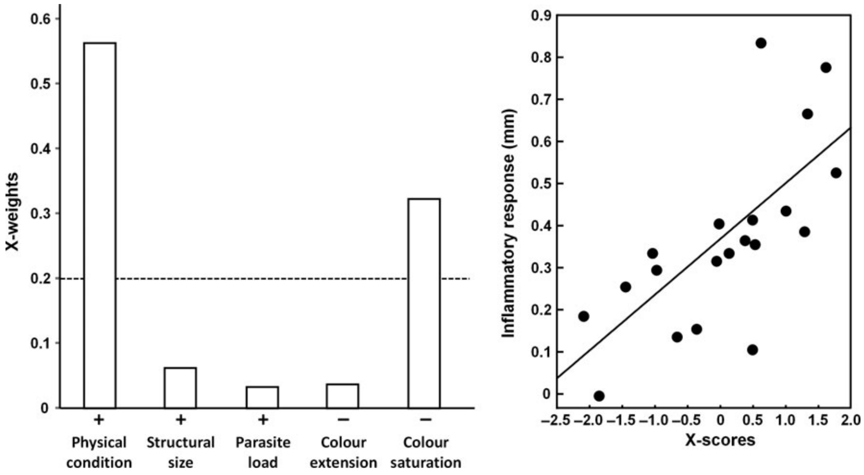

Figure 3 Results of a partial least squares analysis (PLS) used to test for the relationship between the phenotypic variables considered (physical condition, structural size, parasite load, extension of coloured surface, and colour saturation) and the inflammatory response of male lizards to the subcutaneous injection of LPS. Left: relative contribution of the phenotypic variables to the multivariate factor (*X*) predicting inflammatory response; variables with squared weights >0.20 (dashed line) are significant. Right: scatterplot depicting the regression of inflammatory response on *X*-scores.

smaller one. This assumption is consistent with the difference in the average extension of coloured surface between males of both populations (equivalent to a 2.5-fold increase), that was larger than the same difference in saturation (equivalent to a 1.1-fold increase). Also, the effect of the LPS treatment was higher for the extent of coloured area in the non-parasitized population, which experienced a 1.9-fold decrease, than for colour saturation in the parasitized one, which experienced a 1.1-fold decrease. Thus, we suggest that males express their sexual coloration at the beginning of the breeding season as much as they can, and subsequently increase the saturation of the coloured surface. When released from parasite load, they use coloured surface as a signal, and they eventually increase colour saturation with the remaining resources, perhaps in the face of new matings (second clutches are common at low-elevation; Díaz *et al*., 2007). However, when lizards are challenged by parasites and fail to grow a large coloured surface, as in the tick-infested population, they use saturation as the main signaling trait from the beginning of the breeding season. Thus, after controlling for the effects of body condition, inflammatory response was negatively correlated with colour saturation, suggesting that parasitized males had to trade-off their already compromised resources between a more attractive sexual ornament and a more effective immune response.

Finally, it should be stressed that, because our study compares only two sites that differ in altitude, inferences drawn from our results are, in a strict sense, restricted to the two sites used. However, our two-sites comparison may be representative of altitudinal effects (tick-parasitized lizards are widespread at montane oak forests, and very scarce at lowland holm oak forests; authors, personal observation), and our results may suggest general patterns that could be tested with future work. Also, other ecological differences between sites, such as habitat visibility (Endler & Houde, 1995), may have contributed to shape sexual ornaments. For example, mean distance between shrubs is larger, and cover of open areas is higher, at lower elevation (Iraeta *et al*., 2010), and this may have favoured colour surface, which can be perceived from a longer distance, as a sexual signal. At high-elevation, however, differences in red saturation would be effective only from a short distance.

In summary, we suggest that parasites may constrain the expression of sexual ornaments to the extent of regulating what trait characteristics are most informative about individual quality. Thus, parasites like tick nymphs, independently of their effects on individual survival (but see Salvador *et al*., 1996), may have a significant impact on individual fitness by influencing the expression of key sexual ornaments. Although our work suggests that the colour features measured are reliable signals of male quality, experimental approaches would be needed to clarify the link between parasites and sexual selection (female preferences and competition among males). Furthermore, we encourage future studies on other organisms in which ecoimmunological differences might be affecting signaling systems. Because parasite-host coevolution is widespread in life, this approach could shed more light on the role of parasites as modulators of the phenotypic effects of sexual selection, including qualitative changes in the traits involved in signaling systems.

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Conflict of interest

The authors declare that they have no conflict of interest.

Compliance with ethical standards

All applicable international, national and institutional guidelines for the care and use of animals were followed. All procedures performed in this study were in accordance with the ethical standards of our institution (Universidad Complutense de Madrid).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Key steps in Photoshop CS6 image processing. Above: standardized area for the analysis of ventral coloration of the head, obtained using the *magnetic lasso* tool to select the surface comprised within the triangle defined by the snout and the posterior edges of the infralabial scutes. Below: extent of red colored surface, measured using the magic wand tool. We used histograms to count the numbers of pixels in the appropriate layers, which allowed us to determine the overall amount of skin surface involved in the production of the sexual ornament, and to obtain the average value of the R, G, and B channels of the graphics card to calculate color saturation as R / (R+G+B).