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Original Article

Male rock lizards may compensate reproductive costs of an immune challenge affecting sexual signals

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Sexual signals can be evolutionarily stable if they are condition dependent or costly to the signaler. One of these costs may be the trade-off between maintaining the immune system and the elaboration of ornaments. Experimental immune challenges in captivity show a reduction in the expression of sexual signals, but it is not clear whether these detrimental effects are important in nature and, more importantly, whether they have reproductive consequences. We designed a field experiment to challenge the immune system of wild male Carpetan rock lizards, *Iberolacerta cyreni*, with a bacterial antigen (lipopolysaccharide). The immune challenge decreased relative reflectance of ultraviolet structural and melanin-dependent sexual coloration in the throat and the lateral ocelli, whereas the carotenoid-dependent dorsal green coloration was not affected. Immune activation also decreased proportions of ergosterol and cholesta-5,7-dien-3-ol in femoral secretions. These results support a trade-off between the immune system and both visual and chemical sexual ornaments. Moreover, the reproductive success of males, estimated with DNA microsatellites, depended on the expression of some color and chemical traits. However, the immune challenge did not cause overall differences in reproductive success, although it increased with body size/age in control but not in challenged males. This suggests the use of alternative reproductive strategies (e.g., forced matings) in challenged males, particularly in smaller ones. These males might consider that their survival probabilities are low and increase reproductive effort as a form of terminal investment in spite of their "low-quality" sexual signals and potential survival costs.

Key words: **coloration, DNA microsatellites, femoral gland secretions, immune challenge, lizards, LPS, paternity, reproductive success.**

INTRODUCTION

Signals used in sexual selection can be evolutionarily stable if they are honest and condition dependent or costly to the signaler, with this cost being correlated with the signaler's quality (Zahavi 1975; Pomiankowski 1988; Grafen 1990, but see Penn and Számadó 2020). Sexual signals are often reliable because there is a condition-dependent trade-off between the costs of producing and maintaining components of both sexual advertisement and the immune system (Wedekind 1992; Wedekind and Folstad 1994; Sheldon and Verhulst 1996; Lochmiller and Deerenberg 2000). Therefore, only individuals in good condition could simultaneously produce a strong immune response and extravagant sexual ornaments (Westneat and Birkhead 1998).

Within this context, many animal species have evolved a variety of colorful sexual ornaments that are used in intraspecific relationships (e.g., Cooper and Greenberg 1992). For example, in many lizards, the size and color of males' visual badges may determine success in male–male competition (Thompson and Moore 1991; Olsson 1994; Zucker 1994; Martín and Forsman 1999; Bajer et al. 2011) or mate acquisition (Díaz 1993; Baird et al. 1997; Kwiatkowski and Sullivan 2002; Anderholm et al. 2004). Many studies of birds and lizards have suggested that only healthier males may afford to produce more elaborate visual colorful displays (e.g., Blount et al. 2003; Faivre et al. 2003; Martín et al. 2008; Molnar et al. 2012), probably because of the trade-off between allocation of costly pigments, such as carotenoids, to both regulation of the immune and antioxidant function and ornamentation (Blount et al. 2003; Faivre et al. 2003; McGraw 2005; López et al. 2009a).

Similarly, many species use chemical signals in social relationships and mate choice (Mason 1992; Johansson and Jones 2007;

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Wyatt 2014). Some studies have suggested that only healthier males may afford to produce more attractive "chemical ornaments" (Penn and Potts 1998; Rantala et al. 2002, 2003; Zala et al. 2004; López et al. 2009b), probably because compounds allocated to the chemical signal are often diverted from their metabolic roles in the immune and antioxidant functions (Martín and López 2006b, 2015; Kopena et al. 2014a). Both visual and chemical signals may be important in different contexts within the same species (López and Martín 2001; López et al. 2002, 2003) and, therefore, if both types of signals are reliable, its reliability might be based on similar trade-offs. Female preference for characteristics of sexual signals that are costly to males could be a form of mate selection (Bateson 1983) also in lizards (e.g., Martín et al. 2007; Martín and López 2012; Kopena et al. 2011). In this way, the cost assumed by the male to produce high-quality sexual signals would result in increased reproductive success. However, it remains little explored whether the production of costly (honest) and attractive sexual signals is the only way to increase the reproductive success or whether alternative behavioral strategies (e.g., forced matings) might be used when it is not possible to invest in sexual signals.

The Carpetan rock lizard, *Iberolacerta cyreni*, is a small (70–80 mm adult snout-to-vent length, SVL) lacertid lizard that inhabits rocky habitats of some high mountains of the Iberian Peninsula. These lizards show a clear sexual, seasonal and ontogenetic dimorphism in coloration (i.e., adult males have green dorsal patterns, especially during the mating season, whereas young males and females are brown). Moreover, males with highly saturated green dorsal coloration have higher reproductive success (Salvador et al. 2008), and lateral blue ocelli of males are used in intrasexual relationships (López et al. 2004). This suggests that coloration could be considered as a secondary sexual signal as in other lizards (Cooper and Greenberg 1992; Stapley and Keogh 2006). Males also produce abundant femoral gland secretions during the mating season, which, as in other lizards, may reliably convey chemical information about a male's quality or genetic compatibility (Olsson et al. 2003; Martín and López 2006a,b, 2015). Females may use this information to choose prospective mates. For example, female Carpetan rock lizards prefer scent marks of males with high proportions of ergosterol and cholesta-5,7-dien-3-ol (provitamin D) in their femoral secretions (Martín and López 2006a, 2012), which may signal a better immune response (López et al. 2006). Males also use chemical signals in intrasexual agonistic relationships (Aragón et al. 2001; Martín and López 2007). However, the potential importance of investing in some specific sexual traits to increase reproductive success remains little explored.

In this paper, we explored the relationship between the ability of male *I. cyreni* to produce the sexual ornaments that seem to be preferred by females or that may confer advantages in agonistic contests with other males and their actual reproductive success. We designed a field experiment to explore 1) whether costs of immunity affected the expression of both chemical and visual ornaments of male lizards *I. cyreni*, which would allow honest signaling of male condition via multiple types of signals, and 2) whether these changes in sexual signals affected the actual reproductive success of males.

To test these questions, we experimentally challenged the immune system of male lizards with a bacterial antigen (lipopolysaccharide, LPS), without pathogenic effects, and measured its effects on the characteristics of their visual and chemical sexual signals. We predicted that, if there existed a trade-off between the immune function and the elaboration of sexual ornaments, an activation of the immune system should reduce the expression of both visual and chemical displays. Then, we captured gravid females from the study plot to obtain their clutches and the hatchlings born from them and estimated paternity of the experimental males using DNA microsatellites. We predicted that, if the characteristics of sexual, visual and chemical ornaments affected females' mate choice and/or intrasexual competition, experimentally challenged males would have lower reproductive success than control males (C-males).

Previous experiments on the cost of an immune challenge have been mainly performed in captivity to ensure that all individuals have similar environmental conditions, such as similar access to food and thermoregulation opportunities (López et al. 2009a,b; Tobler et al. 2015; Zamora-Camacho et al. 2015, 2016; Kopena et al. 2017). Captivity conditions also ensured that differences in the quality of the individuals or of their home ranges did not affect or compensate for the effects of the activation of the immune system. Therefore, it is unknown whether the detrimental effects observed in captivity are important in wild populations for the actual development of sexual signals and for reproductive success. Thus, we considered several alternative predictions for our experiment. First, in the field, challenged individuals might be able to compensate the costs of the immune response through behavioral modifications (e.g., by increasing antioxidants in the diet or by reducing activity) and, in that case, the expected detrimental effects on the development of sexual signals would not be important or might even not appear at all. Alternatively, limitations of the natural environment, for example, in terms of diet or thermoregulatory opportunities, might increase the detrimental costs of the immune challenge on sexual signals in a greater extent or affect different aspects than those observed in captivity.

MATERIALS AND METHODS

Field and experimental procedure

We made the field study in a 100 × 100 m marked plot close to "Puerto de Navacerrada" at 1850 m asl (Guadarrama Mountains, Central Spain). The habitat consisted of a homogeneous natural mix of rock gravels, low shrubs (*Juniperus communis* and *Cytisus oromediterraneus*) and grassy areas (e.g., *Festuca* sp.). We walked the plot daily when weather conditions were favorable. Between 3 and 27 May 2016, we captured by lassoing 34 resident adult male lizards (SVL: mean ± standard error [SE] = 69 ± 6 mm; range = 63–77 mm). This is a tried-and-true technique widely used for capturing lizards that does not cause any injury to animals (Dodd 2016). At this time of the year, lizards had just recently emerged from hibernation and the mating season had not yet started. For this reason, male lizards had not yet fully developed sexual coloration and were not actively producing femoral secretions. Every day, captured lizards were transported by car inside individual cloth bags to "El Ventorrillo" field station, 5 km from the study site (a 5-min short journey), for processing. Lizards were kept in captivity for just a few hours in individual terraria inside a climatic chamber, with basking spots and programmed light and temperature cycles mimicking the natural ones in the study area. Water and food (crickets) were provided ad libitum. All lizards were in healthy condition during this short captivity time.

From each individual, we measured SVL with a metallic ruler to the nearest 0.1 cm, the height, width, and length of the head with a digital caliper to the nearest 0.01 mm, and body mass with a digital scale to the nearest 0.01 g. We summarized these morphological measures using a principal component analysis (PCA), which produced a single principal component (PC) that was strongly correlated with all the variables (proportion of variation explained = 81.4%; factor loadings SVL = 0.92, head height = 0.91, head width = 0.77, head length = 0.95, and body mass = 0.94) and, thus, the body size PC scores were used as a general measure of body size.

Although there are clear evidences of a correlation between parasite load or health state and the elaboration of sexual signals (e.g., Ressel and Schall 1989; Saks et al. 2003; Martín et al. 2008; Megía-Palma et al. 2016a), costs of immunity per se can be confounded with the costs of the pathogenic effects of parasites. Thus, to look for actual immunity costs, many experimental studies have used antigens that are identified by the immune system as a pathogen and that elicit an immune response without being actually pathogenic (e.g., Bonneaud et al. 2003; Alonso-Alvarez et al. 2004; Staszewski and Boulinier 2004; Loyau et al. 2005; Uller et al. 2006). We challenged the immune system of male lizards using an injection of LPS from the cell wall of the bacteria *Escherichia coli* (serotype 0111-B4; Sigma-Aldrich, St. Louis, MO). We used LPS because it induces an inflammatory immune response by nonspecifically activating B and T lymphocytes and by producing specific anti-LPS antibodies, which have no pathogenic effects (Janeway et al. 2001). Nevertheless, LPS also induces immunopathological effects, such as fever (Deen and Hutchison 2001; Zamora-Camacho et al. 2016), alters the oxidative state (Tobler et al. 2015) and may reduce reproductive output and growth in females (Uller et al. 2006). We randomly assigned male lizards to one of the treatments; 18 experimental males ("LPS-males") were injected intraperitoneally with LPS (2.5 µg/g of body weight) diluted in 0.05 mL of phosphate buffered saline (PBS), whereas 16 control males ("C-males") were injected with the same volume (0.05 mL) of PBS alone. The LPS serotype was similar, and the concentration similar or lower than those previously used to stimulate the immune system in other lizards (Deen and Hutchison 2001; Uller et al. 2006; López et al. 2009a,b). There were not significant differences in initial body size of lizards between treatments (SVL, C-males: mean ± SE = 68 ± 1 mm; LPS-males: 70 ± 1 mm; general lineal model [GLM]: $F_{1,32} = 0.60$, $P = 0.44$; body size PC scores; $F_{1,32} = 0.22$, $P = 0.64$).

All lizards were marked permanently with a passive integrated transponder (PIT) microchip (Germano and Williams 1993; Dodd 2016). We used the smallest available PIT tags (High Performance MiniHPT8; Biomark, Inc., Boise, ID; length = 8.4 mm, diameter = 1.4 mm, weight = 0.03 g). The weight of a PIT tag represents less than 0.5% of the mean body mass of a typical adult lizard. We gently implanted PIT tags subcutaneously in the upper right side of the body. For this, we made a small puncture at around 3 cm from the snout using replacement stainless steel needle (Biomark N165 needle; length = 5.1 cm, needle diameter = 1.49 mm), cleaned and disinfected with alcohol before and after puncturing each individual, which was fitted to a specially designed syringe style implanter (Biomark MK165 syringe). We checked after each use that the needle was not blunt and, if the slightest blunting was observed, the needle was replaced with a new one. Otherwise, a new needle was used after every 10 individuals. We gently lifted the skin from the underlying muscle and then inserted the transponder subcutaneously using the implanter. During the insertion of the PIT tag, the needle was maintained parallel to the body to ensure that the tag remained under the skin and did not enter the coelomic cavity. The injection site was immediately disinfected with alcohol after the implant. Although lizards showed a small aversive response to the puncture with the needle, we did not observe any subsequent additional negative behavioral responses (e.g., stress, immobility, forced unnatural movements, or attempts to remove the tag; Warwick et al. 2013). We used a handheld portable reader (Biomark 601 Reader) to read the individual unique code of the tag from recaptured lizards. Lizards were also painted with a four color dorsal spots code for individual temporal identification at long distance.

Finally, we also took a little piece of the tip of the tail tissue (<1 cm) from every individual for paternity analyses. For this, we used disinfected scissors and made a clean and quick cut. Tissues were stored in absolute ethanol until DNA extraction. Tail-tip clipping is a standard method performed to obtain DNA in different lizard species (e.g., Noble et al. 2013; Halliwell et al 2017), including some arboreal species with prehensile tail (Herrel et al. 2012), with no apparent detrimental effect. Moreover, *I. cyreni* exhibits tail autotomy and species with this trait also show adaptations to reduce the negative effect of partial tail loss (e.g., tail regeneration). We avoided the use of local anesthesia during the processing of lizards because both tag insertion and tail-tip removal are minor interventions and because safety and efficacy of local anesthetic drugs have not been investigated in reptiles yet (Chatigny et al. 2017). Some commonly used local anesthetics (e.g., lidocaine) have been shown to have undesired side effects in lizards (e.g., a clinically relevant increase in heart rate; Ferreira et al. 2019), so caution should be taken before their use. However, we maintained lizards with relatively cold body temperatures immediately before and while being processed (8 °C for about half an hour) in an attempt to provide some pain relief as there is evidence that cold can be an anesthetic in ectothermic vertebrates (Shine et al. 2015; Lillywhite et al. 2017). We do not really know whether cooling reduced lizard nociception in our case because this has never been studied in *I. cyreni*, but at least we are confident that it was harmless to lizards as they experience this temperature at a daily basis in the wild (mean maximum and minimum temperatures at "Puerto de Navacerrada" in May 2016 were 10.7 and 3.2 °C, respectively; data obtained from https://opendata.aemet.es).

Subsequently, within the same day of capture, lizards were released in good health condition at their exact capture points to let them mate freely. Lizards behaved normally when they were released after being processed. In subsequent recaptures, all lizards had started regeneration of the tail and we did not detect injuries or health problems (e.g., infection, sores, and bleeding) from the marking and sampling procedures in any case.

We recaptured every male that we found in the plot or its surroundings at the beginning of June (between 1 and 10 June), in the middle of the mating season, some days after we carried out the experimental treatment. The time interval between applying the treatment and recapture did not significantly differ between treatments (C-males: mean ± SE = 18 ± 3 days, $N = 10$; LPS-males: 17 ± 2 days, $N = 11$; GLM, $F_{1,19} = 0.39$, $P = 0.54$). We transported lizards to the field station where they were temporally housed as previously described to weigh them, to measure their now fully developed nuptial coloration, and to take a sample of femoral gland secretions, which was now abundantly produced (see below). Lizards were thereafter released at their recapture points within the same day. Within the same period, we also captured and marked every new male occasionally found in the plot that had not been captured and marked in May. These males were probably transient individuals in the plot that were resident in adjacent areas. Nevertheless, as we had not applied the experimental treatment to these males, we considered them as unmanipulated males (["U-males"]; $N = 19$), and their morphology, coloration, and chemical signals were also measured as we did with the other males. These U-males did not differ significantly from the recaptured manipulated males in body size or mass (U-males, mean ± SE; SVL: 68 ± 1 mm; GLM: $F_{2,37} = 1.42$, $P = 0.25$; body mass: 6.9 ± 0.3 g; GLM: $F_{2,37} = 0.82$, $P = 0.45$). We also took a tissue sample for DNA analyses as these males that were not resident in the study plot could also have access to females in the plot.

Visual signals

At recapture time, at the beginning of June, we measured reflectance of the coloration (from 300 to 700 nm) of the C- and LPS-males that could be recaptured and of the new captured U-males. We measured four areas of the body: the "whitish" to "bluish" throat ("gular") and submandibular ("mental") region, the "greenish" areas of the "dorsal" part of the body, and the lateral dark "bluish" "ocelli". We used an Ocean Optics USB2000 spectroradiometer with a DT-1000-MINI Deuterium-Halogen light source (Ocean Optics, Inc., Dunedin, FL). Coloration was measured always by the same person. To exclude ambient light and standardize measuring distance and angle (45°) to the skin, a cylindrical metallic tube was mounted on the bifurcated fiber optic probe (Montgomerie 2006). At each body part, we measured coloration on three nearby points. Reflectance (R) was calculated relative to a white standard (WS-1-SS) with the OOIBase32 software (Ocean Optics, Inc.). Mean reflectance was summarized over 5-nm steps ("binned"; Grill and Rush 2000) and we calculated average values of reflectance for the three measurements of each body part of each individual before statistical analysis.

We used PCAs to summarize the reflectance spectra of the different body parts (Cuthill et al. 1999; Grill and Rush 2000). We used this method because it makes no assumptions about how reflectance variation is perceived or which aspects of the spectrum might be important (Cuthill et al. 1999). Typically, in the PCA of spectral data, the PC1 represents variation in intensity of coloration or brightness, and the subsequent PCs represent combinations of hue and chroma (Cuthill et al. 1999; Grill and Rush 2000). We made four PCAs, one for the spectra of each body part of all males. Then, we used general lineal models (GLMs) to examine the variation in each of the PC scores as the response variable and treatment (C-males vs. LPS-males vs. U-males) as a fixed factor.

Chemical signals

Immediately after recapture in the mating season of C- and LPS-males, or at the first capture in case of U-males, we gently pressed with forceps around the femoral pores to extract samples of femoral gland secretions collected directly into 1.1-mL total recovery glass vials (ref. V2275, Análisis Vínicos S.L., Tomelloso, Spain). We closed vials with Teflon-lined stoppers and stored them at −20 °C until analyses. Samples were analyzed with a gas chromatograph (Agilent 7890A, Santa Clara, CA) equipped with a Agilent HP5-MS column programmed (50 °C for 10 min, 50 to 280 °C at 5 °C/min, and 280 °C for 30 min) coupled to a mass spectrometer (Agilent 5975C with triple axis detector). We identified chemicals by comparison of mass spectra in the NIST/EPA/NIH 2002 library, and later confirmed them with authentic standards (from Sigma-Aldrich Chemical Co., St. Louis, MO).

From the chromatograms, we calculated, using the Xcalibur software, the percentage of the total ion current (TIC) to determine the relative amount of each compound (García-Roa et al. 2018). We specifically examined whether the experimental immune challenge affected the proportion of cholesterol, cholest-5,7-dien-3-ol (provitamin D_3), and ergosterol (provitamin D_2) in femoral secretions of males because previous experiments suggested that these steroids may be important as secondary sexual signals in this and other lacertid lizard species (Martín and López 2007, 2012, 2014; López and Martín 2012). We transformed areas of these steroids following the formula log[(proportion)/(1 − proportion)] to correct the problem of nonindependence between proportions (Aitchison 1986; Aebischer et al. 1993). Transformed areas were used as response variables in separated GLMs testing the effects of the treatment (C-males vs. LPS-males vs. U-males), which was included as a fixed factor. Pairwise comparisons (for both chemical and visual signals) used Tukey's honestly significant difference tests (Sokal and Rohlf 1995).

Reproductive success

At the end of June, when the mating season had finished, we collected all the gravid females that we could find at the plot ($N = 33$; SVL: mean ± SE = 72 ± 7 mm). Females were transported and maintained at "El Ventorrillo" field station until the clutch took place. Females were housed individually in outdoor terraria with a layer of coconut fiber as substrate (12 cm depth) and a clay brick for shelter. Water and food (mealworms and crickets) dusted with calcium were provided ad libitum. The diet was supplemented with a vitamin complex once a week. All females survived with good health condition and, after laying the eggs, were released at their capture sites.

Near the expected laying dates (from 11 July to 4 August), we checked the terraria every day looking for eggs buried in the substrate. When a clutch was found, the eggs were extracted from the coconut fiber and allocated individually in closed plastic containers (170 mL) filled with moistened perlite (1:1 proportion of water and perlite) and covered with a lid to keep the humidity. The eggs were kept at 27.5 °C in an incubator (FC-B2V-M/FC404 incubator, Friocell, Santa Rosa Jáuregui, Querétaro, México) until hatching. When juveniles hatched (incubation time, mean ± SE = 36 ± 2 days), we took a little piece of their tail (<3 mm) for paternity analyses as previously described. This extremely small amount of tail removed was quickly regenerated, precluding that potential negative effects of tail loss were relevant. Juveniles were kept in captivity for 1 month for other studies (they were housed in individual terraria with light and temperature cycles mimicking those in the study area and with water and crickets provided ad libitum) and subsequently were released at the capture sites of their mothers. Although we could not track survivorship of juveniles in the field because they were not marked, given that juveniles were released with good condition in their preferred microhabitats and in a date that allowed to store fat before hibernation, we were confident that they were in similar or even better condition to grow and survive than juveniles born in the field.

DNA was extracted from tail tissue samples using DNeasy® Blood and Tissue Kit (QIAGEN®, Germantown, MD). Every individual was genotyped at 7 *I. cyreni*-specific microsatellite loci (Icy1, Icy2, Icy3, Icy4, Icy5, Icy6, and Icy7; Bloor 2006). Primer pairs were combined to allow the amplification of seven loci in two multiplex reactions (reaction 1 = Icy2, Icy3, Icy4, and Icy7; reaction 2 = Icy1, Icy3, Icy5, and Icy6). Polymerase chain reactions (PCRs) were performed in a 10-μL reaction volume containing 1 μL DNA solution, 5 μL of Quiagen® Type-it Kit, 3 μL of water, and 1 μL of a mix including all primers in each multiplex at 2 μM. PCRs started with an initial denaturation step at 95 °C for 5 min, followed by 28 cycles of denaturation at 95 °C for 30 s, annealing at 54 °C for 90 s, and extension at 72 °C for 30 s. All reactions were finished with a final extension at 72 °C for 30 min. One primer from each pair was fluorescently labeled to distinguish PCR products from one another. Genotyping was carried out at the SECUGEN company facilities (Madrid, Spain). PCR products were sized using Sanger sequencing and analyzed using GeneMapper v.5 (Thermo Fisher Scientific Inc., Waltham, MA).

Parentage assignment was performed with the program CERVUS v. 3.0.7 by calculating "logarithm of odds" (LOD) scores from the natural logarithm of the overall likelihood ratio (Kalinowski et al. 2007). LOD scores were determined by means of simulation parentage analysis with 10 000 cycles ran at two levels of confidence, strict (≥0.95) and relaxed (≥80). The parentage assignment of the father given the known mother at the strict level of confidence showed a 71% success rate and, at the relaxed level, a 96% success rate. Paternity was assigned to the males with positive highest LOD scores and with significant simulation test at least in the relaxed criterion. The mean number of alleles observed by locus was 13.86 and the mean proportion of loci typed was 0.99. The heterozygosity observed (HObs) was higher than the expected one (HExp) in all the markers except in Icy1 and Icy2 (Table 1), and the Hardy–Weinberg equilibrium test was not significant in any locus except in Icy1 (Table 1).

We used two measures that provided different aspects of the reproductive success of C- and LPS-males: 1) the "total number of offspring sired" by one individual and 2) the "number of females fertilized" estimated from the number of clutches in which a male sired some offspring. Although we had paternity data of some U-males with females from the study plot, these measures were not considered reliable of their total reproductive success as these U-males were not resident and probably accessed mainly other females outside of the study plot that we did not capture.

To test whether reproductive success differed between experimental groups (C- vs. LPS-males) while controlling for body size, we made separated generalized linear models (GLZ), with a Poisson distribution and a log link function, for each of the two reproductive success measures (number of offspring sired or number of females fertilized) as the response variable using the LPS treatment as explanatory factor and male's body size PC scores as a continuous covariate. We computed two kinds of models, either including all the individual males or using only those males that sired some offspring, to look for effects only distinguishable in males that were fathers.

To examine the relationships between the expression of visual and chemical signals and reproductive success, independently of the experimental treatment, we computed two forward stepwise Poisson regression analyses (GLZ). As the response variables, we used either the number of offspring or the number of fertilized females of C- and LPS-males for which we also had measures of their sexual signals (C- males, $N = 10$; LPS-males, $N = 11$). As the predictors to be potentially included in the model, we considered initially the whole set of variables potentially involved in visual (the nine PC scores describing gular, mental, dorsal, and ocelli coloration) and chemical signaling (transformed proportions of cholesterol, cholest-5,7-dien-3-ol, and ergosterol). The predictor selection was performed adding in each step that variable that had the highest partial X^2 value in each singular model. We also used the

Table 1

Loci of *Iberolacerta cyreni* lizards (named from Bloor 2006) used in the paternity analyses. *k*: number of alleles at the locus; *N*: number of individuals typed at the locus; HObs: observed heterozygosity; HExp: expected heterozygosity; PIC: polymorphic information content; NE-1P: average nonexclusion probability for one candidate parent given the genotype of a known parent of the opposite sex; NE-2P: average nonexclusion probability for a second candidate parent given the genotype of a known parent of the opposite sex; NE-PP: average nonexclusion probability for a candidate parent pair; NE-I: average nonexclusion probability for identity of two unrelated individuals; NE-SI: average nonexclusion probability for identity of two siblings; HW: Hardy–Weinberg equilibrium test (*Significant *P*-value <0.01; NS = nonsignificant; ND = not done); *F*(null): estimated null allele frequency

Locus	*k*	*N*	HObs	HExp	PIC	NE-1P	NE-2P	NE-PP	NE-I	NE-SI	HW	*F*(null)
Icy1	12	234	0.748	0.821	0.798	0.519	0.346	0.163	0.053	0.354	*	0.0482
Icy2	22	233	0.884	0.887	0.876	0.370	0.227	0.078	0.023	0.313	NS	0.0004
Icy3	10	233	0.794	0.793	0.764	0.576	0.397	0.209	0.070	0.372	NS	−0.0021
Icy4	8	234	0.641	0.616	0.580	0.779	0.601	0.404	0.183	0.488	NS	−0.0188
Icy5	9	234	0.735	0.733	0.707	0.646	0.459	0.252	0.096	0.408	NS	0.0018
Icy6	18	233	0.931	0.920	0.913	0.285	0.166	0.045	0.013	0.294	ND	−0.0073
Icy7	18	235	0.800	0.789	0.765	0.566	0.387	0.192	0.068	0.373	NS	−0.0069

Akaike information criteria corrected for small sample size (AICc) to consider the most parsimonious model in each regression step. The likelihood-ratio test (LRT) was used as an omnibus test for intermediate and final models.

RESULTS

Body mass

LPS- and C-males did not significantly differ in initial body mass and all males lose weight in a similar way since the first capture before starting the mating season to their recapture in the middle of the mating season (mean ± SE; C-males: from 7.8 ± 0.5 g to 7.3 ± 0.4 g; LPS-males: from 7.7 ± 0.4 g to 7.2 ± 0.4 g; repeated measures GLM, time effect: $F_{1,19} = 21.98$, $P < 0.0002$; treatment: $F_{1,19} = 0.09$, $P = 0.77$; time × treatment: $F_{1,19} = 0.31$, $P = 0.58$).

Visual signals

The PCAs on average reflectance data of all spectra made separately for each body part produced in each case three PCs that together accounted in all cases for more than 92% of the variation in the original spectra. In all cases, PC1 explained more than 60% of variance and represented achromatic brightness variation in the original spectra because coefficients relating PC1 to the original reflectance data (factor loadings) were all negative and of similar magnitude (Figure 1). The comparison between LPS-, C-, and U-males did not show any significant differences in PC1 (brightness) for any body part (GLM; gular, $F_{2,37} = 0.05$, $P = 0.95$; mental, $F_{2,37} = 0.23$, $P = 0.79$; dorsal, $F_{2,37} = 0.38$, $P = 0.69$; ocelli, $F_{2,37} = 0.76$, $P = 0.47$; Figure 2).

The second PC (PC2) explained more than 20% of variance in all cases and represented, depending on the body part, variation in the relative reflectance of medium wavelengths to both very short (i.e., ultraviolet [UV]) and very long wavelengths (for gular and mental coloration) or in the relative reflectance of short to long wavelengths (for dorsal and ocelli coloration; Figure 1). There were significant differences among treatments in the PC2s of gular (GLM, $F_{2,37} = 6.44$, $P < 0.004$) and mental coloration ($F_{2,37} = 5.17$, $P = 0.01$; Figure 2), which indicated that LPS-males showed relatively lower reflectance of very short wavelengths (UV coloration) in the throat in comparison with C-males (Tukey's test, $P < 0.02$ for both body parts) and U-males ($P = 0.005$ for gular and $P < 0.02$ for mental), which did not differ from each other ($P \geq 0.95$ for both). Similarly, significant differences in the PC2 of ocelli coloration (GLM, $F_{2,37} = 5.82$, $P = 0.006$) indicated that the ocelli of LPS-males had relatively lower reflectance of short wavelengths (i.e. UV and blue) than C-males and U-males (Tukey's tests, $P = 0.01$ for both comparisons), which did not differ from each other ($P = 0.91$; Figure 2). Finally, the PC2 of dorsal coloration was not significantly affected by the immune challenge and manipulated C- and LPS-lizards did not differ from U-males (GLM, $F_{2,37} = 0.82$, $P = 0.45$; Figure 2).

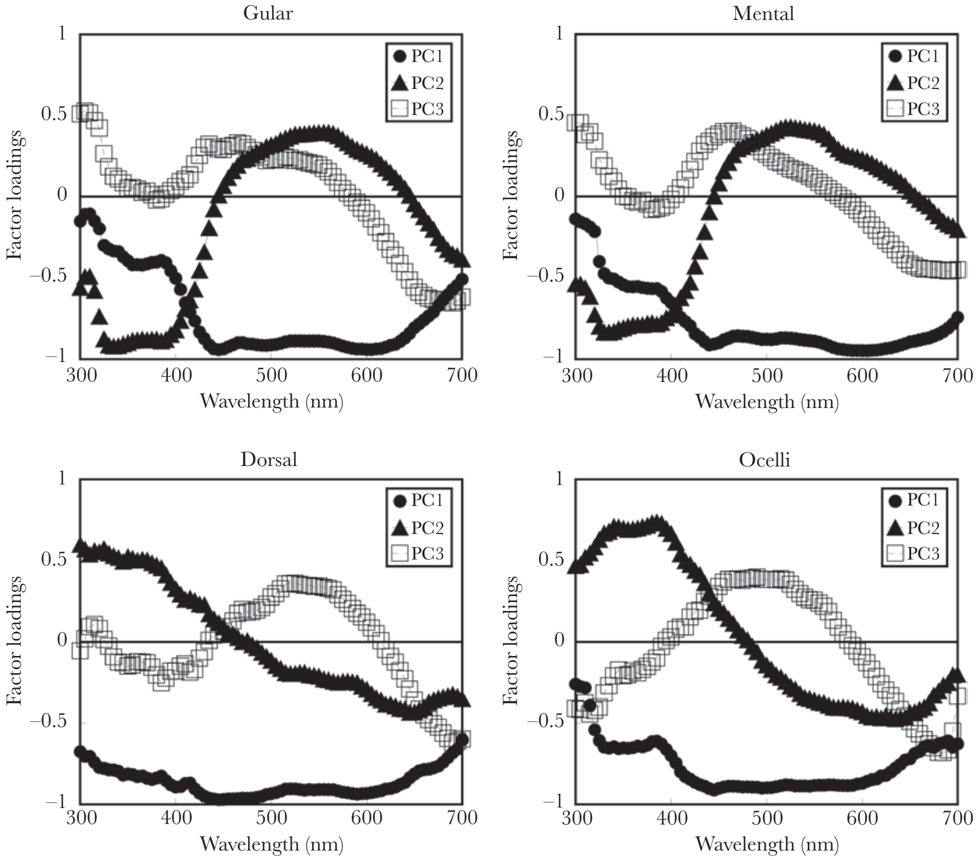

Figure 1
Loadings of the first three factors from PCAs on reflectance of coloration measured between 300 and 700 nm on four different body parts of wild male lizards *I. cyreni*.

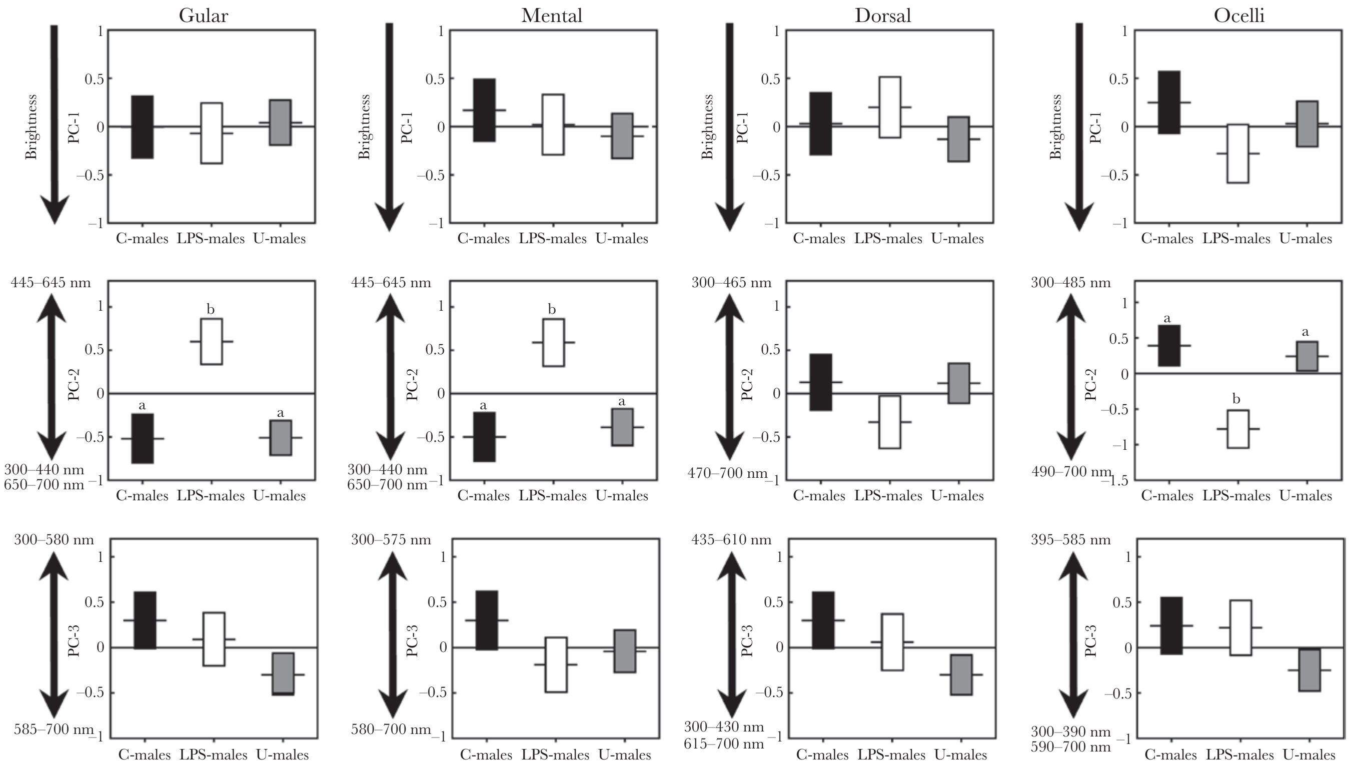

Figure 2

Mean (±SE) PC scores describing characteristics of coloration of different body parts of wild male lizards *I. cyreni* measured some days after the injection of control PBS (C-males; black boxes) or LPS (LPS-males; open boxes) and of a group of unmanipulated males (U-males; gray boxes) measured at the same time. When there were significant differences among groups, means with the same letter above the bars did not differ significantly in post hoc Tukey's tests ($P > 0.05$).

The third PC (PC3) explained more than 7% of variance in all cases and represented, depending on the body part, variation in the relative reflectance of short to long wavelengths (for gular and mental coloration) or in the relative reflectance of medium wavelengths to both very short (i.e., UV) and very long wavelengths (for dorsal and ocelli coloration; Figure 1). There were no significant differences in PC3 among treatments in any part of the body (GLM; gular, $F_{2,37} = 1.84$, $P = 0.17$; mental, $F_{2,37} = 0.65$, $P = 0.53$; dorsal, $F_{2,37} = 2.19$, $P = 0.13$; ocelli, $F_{2,37} = 1.19$, $P = 0.32$; Figure 2).

Chemical signals

There were significant differences in relative proportions of ergosterol in femoral gland secretions among treatments (GLM, $F_{2,37} = 8.26$, $P = 0.001$) (Figure 3a). LPS-males had significantly lower proportions of ergosterol than C-males (Tukey's test, $P = 0.006$) and U-males ($P = 0.0016$), which did not differ from each other ($P = 0.99$). Similarly, there were significant differences in relative proportions of cholesta-5,7-dien-3-ol in femoral gland secretions among treatments (GLM, $F_{2,37} = 16.90$, $P < 0.0001$) (Figure 3b), with LPS-males having significantly lower proportions of cholesta-5,7-dien-3-ol than C-males (Tukey's test, $P < 0.0002$) and U-males ($P < 0.0002$), which did not differ from each other ($P = 0.35$). In contrast, there were not significant differences among treatments in relative proportions of cholesterol (GLM, $F_{2,37} = 1.26$, $P = 0.30$; Figure 3c).

Effects of the immune challenge on reproductive success

The 33 females captured in the plot laid clutches with a mean (±SE) of 6.1 ± 0.3 eggs, from which 144 offspring (4.4 ± 0.4 per female) were born. From these, a total of 128 offspring sired by 38 known males were identified (LOD score > 0 and significant confidence test), and only 16 offspring could not be assigned to any known male. Multiple paternity occurred in 21 from 31 (67.7%) of the clutches in which fathers could be identified, ranging the number of different fathers per clutch from 1 to 5 (mean ± SE = 2.3 ± 0.1).

We assigned 75 offspring to 17 of the 34 C- or LPS-males in our experiment that were resident in the study plot. Thus, 7 from 16 C-males (43.7%) and 10 from 18 LPS-males (55.5%; X^2 test = 0.01, $P = 0.96$) did not apparently sire any offspring or fertilized females that we did not capture. The rest of the offspring ($N = 53$) were sired by 21 known U-males that were only occasionally seen in the borders of the plot and very likely were resident outside of the study plot and visited this plot as transient males.

Considering all the males that participated in the experiment, we did not find significant overall effect of the immune challenge in the total number of offspring sired by C-males (mean ± SE = 2.6 ± 0.9 offspring/male; $N = 16$) and LPS-males (1.8 ± 0.6 offspring/male; $N = 18$). However, we found that larger males sired significantly more offspring and the interaction was not significant (Table 2).

When considering only the individual "males that had sired any offspring," the model confirmed the lack of overall significant effect of the immune challenge (C-males: 4.7 ± 1.2 offspring/male;

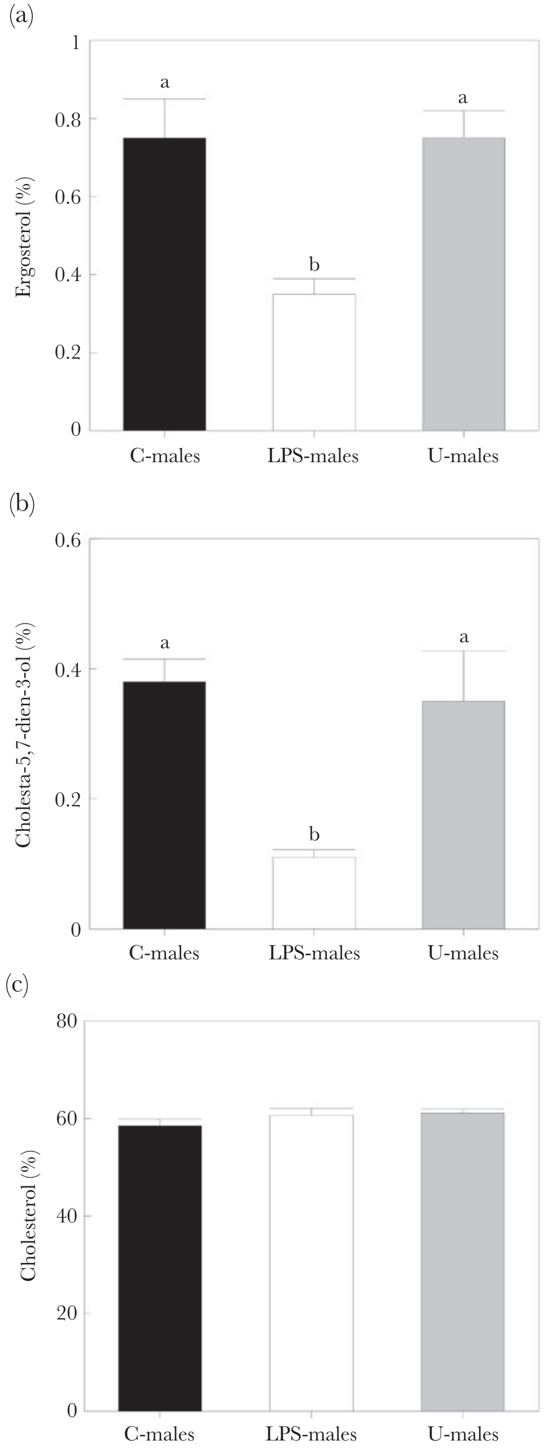

Figure 3
Mean (±SE) relative proportions (%) of (a) ergosterol, (b) cholesta-5,7-dien-3-ol, and (c) cholesterol in femoral gland secretions of wild male lizards *I. cyreni*, measured some days after the injection of control PBS (C-males; black bars) or LPS (LPS-males; open bars) and of unmanipulated males (U-males; gray bars) measured at the same time. When there were significant differences among groups, means with the same letter above the bars did not differ significantly in post hoc Tukey's tests ($P > 0.05$).

$N = 9$; LPS-males: 4.1 ± 0.9 offspring/male; $N = 8$) and the significant positive relationship between reproductive success and male size (Table 2). However, interestingly, the model also showed a significant interaction between experimental treatment and size. This interaction showed that the number of offspring produced had a different relationship with body size depending on the treatment (Figure 4a). Thus, while for C-males there was a strong steep positive significant relationship between offspring number and body size (Spearman rank correlation, $r_s = 0.79$, $P = 0.0012$) with larger males siring most of the offspring, in LPS-males, there was not a significant relationship with body size ($r_s = 0.38$, $P = 0.34$) with both larger and smaller individuals siring a similar number of offspring (Figure 4a).

The number of females fertilized, considering "all males" in the experiment, did not significantly differ between treatments (C-males: 1.4 ± 0.4 females/male; LPS-males: 1.0 ± 0.3 females/male). However, there was a significant effect of male body size, with larger males fertilizing a higher number of females, and the interaction was not significant (Table 2). Furthermore, when considering only those "males that had sired some offspring," the model confirmed the lack of differences between treatments in the number of fertilized females (C-males: 2.6 ± 0.5 females/male; LPS-males: 2.2 ± 0.4 females/male); there was not a significant overall effect of body size, but the interaction was significant (Table 2). Thus, larger C-males fertilized more females than smaller C-males ($r_s = 0.86$, $P = 0.003$), but the size of LPS-males was not significantly related ($r_s = -0.10$, $P = 0.81$) to the number of females that they fertilized (Figure 4b).

The males that fertilized more females had more offspring (GLZ, $X^2 = 19.62$, $P < 0.001$; $r_s = 0.81$, $P < 0.0001$), with a mean (±SE) of 1.8 ± 0.2 offspring/female (range = 1–4). This relationship did not significantly vary between treatments ($X^2 = 0.28$, $P = 0.59$) and the interaction was not significant ($X^2 = 0.08$, $P = 0.77$).

Relationship between sexual signals and reproductive success

The final model for offspring number, obtained at the end of the forward stepwise procedure, included as significant variables the PC2 dorsal, PC3 dorsal, PC2 ocelli, and cholest-5,7-dien-3-ol (LRT, $X^2 = 35.71$; df = 1,5; $P < 0.0001$; AICc = 96.62, AICc [null model] = 120.55; $D^2 = 43.81\%$; Table 3). During the stepwise procedure, the rest of variables were not included in the model. Therefore, males that produced a higher number of offspring were those with greener dorsal coloration (PC2 and PC3 dorsal), with ocelli with more saturated blue and UV coloration (PC2 ocelli) and with femoral secretions with higher relative proportions of pro-vitamin D_3.

The final model for the number of females fertilized included as significant variables the PC3 mental, PC3 ocelli, and cholest-5,7-dien-3-ol (LRT, $X^2 = 34.25$; df = 1,4; $P = 0.0024$; AICc = 64.62, AICc [null model] = 70.71; $D^2 = 38.64$; Table 3), showing that males that fertilized more females had submandibular mental areas with more short wavelength and UV coloration, ocelli with more UV coloration and femoral secretions with more pro-vitamin D_3, whereas all other variables were not included during the stepwise procedure.

DISCUSSION

Our experiment, made under natural conditions, showed that an experimental activation of the immune system in wild range male lizards *I. cyreni* resulted in changes in some, but not all, characteristics of both their visual and chemical sexual signals. This suggests that, in their natural environment, there may be a trade-off

Table 2

GLZ models for the effects of the immune challenge (LPS treatment, C-males vs. LPS-males) and body size PC scores of males on the number of offspring sired and number of females fertilized considering all males that participated in the experiment (N = 34) or only those that sired some offspring (N = 17). Significant effects are marked in bold

Model	Explanatory variables	df	X^2	P
All males				
Offspring number	Treatment	1	2.40	0.12
	Size	1	**28.04**	**<0.0001**
	Treatment × size	1	0.05	0.83
Females fertilized	Treatment	1	1.34	0.25
	Size	1	**9.29**	**0.002**
	Treatment × size	1	0.11	0.73
Siring males				
Offspring number	Treatment	1	0.28	0.59
	Size	1	**5.11**	**0.024**
	Treatment × size	1	**8.02**	**<0.005**
Females fertilized	Treatment	1	0.16	0.68
	Size	1	0.38	0.54
	Treatment × size	1	**4.70**	**0.03**

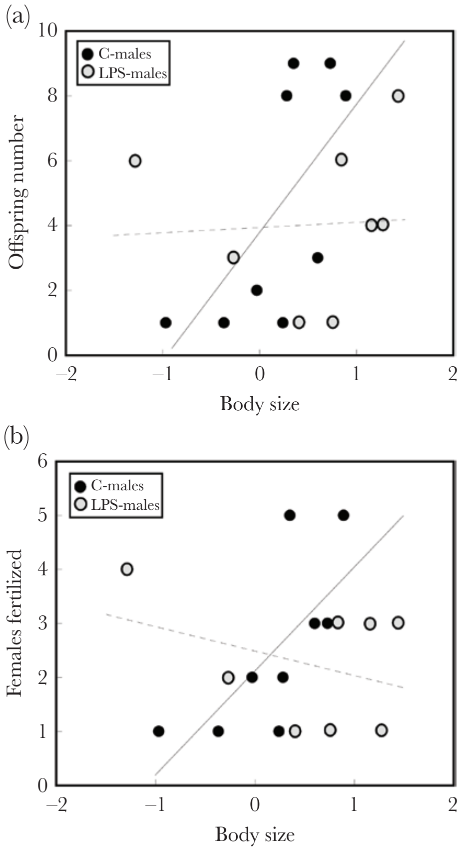

Figure 4

Relationship between (a) number of offspring sired or (b) number of females fertilized and body size PC scores in wild male lizards *I. cyreni* (C-males, black points, continuous line; LPS-males, gray points, dashed line) that sired some offspring.

between the activation of the immune system and the elaboration of visual and chemical ornaments in this lizard. We predicted that these modifications of the sexual signals should affect sexual selection and have reproductive consequences. In fact, we found that, independently of the immune challenge, males with a "higher" expression of some visual and chemical signals sired more offspring and fertilized more females. However, we did not find overall differences between treatments in reproductive success caused by the experimental immune challenge. In contrast, male's body size (age) appeared to be an important factor determining a higher reproductive success. Nevertheless, this relationship was observed in control but not in challenged lizards, suggesting the existence of alternative reproductive strategies as a response to the immune challenge in at least some males.

The effects of the immune activation on coloration are partly similar to those found in other animal species in previous experiments made under controlled captivity conditions (birds [Faivre et al. 2003; Alonso-Alvarez et al. 2004]; lizards [López et al. 2009a]; turtles [Ibáñez et al. 2014]) and also in some wild birds (Torres and Velando 2007). Similarly, an immune challenge also changed the composition of chemical signals in other lizard (López et al. 2009b) and mammal species (Kimball et al. 2014). Nevertheless, contrary to expectations, captive male lizards *Lacerta schreiberi* that suffered an immune challenge, but also received supplementary vitamin E in the diet, were able to increase saturation of UV-blue throat coloration and to maintain the characteristics of their chemical signals (Kopena et al. 2017). This further suggests that optimal captivity conditions may modify the effects of the immune challenge and that studies made under natural conditions (which could also reveal interannual variability) are needed to understand the actual relationships between the immune system and the sexual signals in the wild.

With respect to visual signals, in our field experiment, challenged LPS-males showed "whitish" throats (gular and mental areas) and lateral "bluish" ocelli with less saturated structural and melanin-dependent UV coloration than C-males and U-males, whereas the carotenoid-dependent dorsal "greenish" coloration was not significantly affected by the immune challenge. In contrast, in captive *Podarcis guadarramae* lizards, an immune challenge decreased the carotenoid dependent yellow component of the ventrolateral coloration but did not affect its UV and blue-green components (López et al. 2009a). Changes in throat UV coloration might be caused by the negative effect of the immune challenge on antioxidant levels inside the body (Mészáros et al. 2019), which are needed for the placement of reflective platelets in iridophores and the production of melanin (Quinn and Hews 2003; Grether et al. 2004; Saenko et al. 2013; Kopena et al. 2017).

Table 3

Final GLZ model resulting from a forward stepwise procedure that considered the potential effect of all variables describing visual and chemical signals (see Methods) on the number of offspring sired by the C- and LPS-males for which we also had measured their sexual signals ($N = 21$). Proportion of deviance explained = deviance retained/deviance null model. Significant effects are marked in bold

Model	Explanatory variables	df	X^2	P	Proportion of deviance explained
Offspring number	PC2 dorsal	1	**19.47**	**<0.0001**	**0.239**
	PC3 dorsal	1	**4.61**	**0.032**	**0.057**
	PC2 ocelli	1	**8.84**	**0.002**	**0.108**
	Cholest-5,7-dien-3-ol	1	**13.82**	**0.0002**	**0.170**
Females fertilized	PC3· mental	1	**5.22**	**0.022**	**0.140**
	PC2 ocelli	1	**8.35**	**0.004**	**0.225**
	Cholest-5,7-dien-3-ol	1	**11.15**	**0.0008**	**0.300**

In green lizards *Lacerta viridis*, the throat UV coloration of males seems to be the visual signal most preferred by females during mate choice (Bajer et al. 2010, 2012; Molnár et al. 2012). Also, UV-blue throat saturation of male green lizards is linked with dominance behavior (Martín and López 2009; Bajer et al. 2011). If UV coloration had similar roles in *I. cyreni*, the changes in UV coloration induced by the immune challenge might be important in both intrasexual and intersexual selection and have reproductive consequences as suggested by the relationship between reproductive success and UV coloration found in our study. Indeed, in male *I. cyreni*, the UV-blue lateral ocelli are at least important in intrasexual relationships (López et al. 2004), but the role of throat and mental UV coloration in mate choice has not been explored in this species.

In contrast to a previous captivity study where ventrolateral orange coloration of *P. guadarramae* lizards was affected by an immune challenge (López et al. 2009a), we did not find any significant effect in the carotenoid-dependent dorsal green coloration of wild *I. cyreni*, a trait that seems to confer a higher reproductive success (Salvador et al. 2008 and this study). In other captivity studies of birds and turtles, an induced immune activity diverted carotenoids from blood and decreased carotenoid-based coloration of the skin or beak (Faivre et al. 2003; Alonso-Alvarez et al. 2004; Ibáñez et al. 2014), although not of feathers (Navara and Hill 2003; Fitze et al. 2007). These contrasting results might indicate that, in our field study, the carotenoids needed by lizards to develop this visual signal were in enough supply in natural prey to meet the demands of both developing dorsal coloration and other possible physiological roles induced by the immune activation. Therefore, natural conditions might allow lizards in the wild to compensate for some detrimental effect on this signal at least under favorable environmental conditions. Alternatively, some of these carotenoids used for ornamentation might not be effective as immunostimulants as it has been suggested in other lizards (Kopena et al. 2014b) and birds (Fitze et al. 2007). Therefore, these carotenoids might not be needed for the immune response and, independently of the immune challenge, could be allocated to the visual signal, which would explain the lack of effect of the experiment.

The immune challenge also affected notoriously the quality of chemical signals of *I. cyreni* lizards. The activation of the immune system resulted in decreased proportions of cholesta-5,7-dien-3-ol and ergosterol, but not of cholesterol, in femoral secretions of males. Similarly, proportions of cholesta-5,7-dien-3-ol in femoral secretions of male lizards *P. guadarramae* decreased after an experimental immune challenge in captivity (López et al. 2009b). Moreover, in several species of lacertid lizards, there is often a natural positive relationship between proportions of these steroids in secretions and their immune responses (López and Martín 2005; López et al. 2006; Martín and López 2006a, 2015). These results may indicate that there is a trade-off in lizards between the immune system and the elaboration of chemical sexual signals that is also showed in nature. Ergosterol and cholesta-5,7-dien-3-ol are precursors of two active forms of vitamin D, which, at least in humans and other mammals, is a potent immune system modulator with a variety of effects on immune system function, such as an enhancement of innate immunity and inhibition of the development of autoimmunity (Griffin et al. 2003; Hayes et al. 2003; Fraser 2005). Therefore, activation of the immune system might require lizards to allocate larger amounts of systemic provitamin D, later transformed into vitamin D after exposure to the sun, to the immune function and, consequently, less provitamin D would be available to be allocated to femoral secretions. Female lizards can detect changes in concentration of these lipids in secretions of males (Martín and López 2006c; López and Martín 2012) and prefer scent marks of males with high proportions of them (López and Martín 2005; Martín and López 2006a, 2012). Therefore, it is likely that these compounds reliably signal male health status, affecting female mate choice decisions and determining, at least partly, the reproductive success of males as suggested by the relationships found in this study. Similarly, immune activation changes volatile composition of urine in rodents (Kimball et al. 2014; Boillat et al. 2015) and of sweat in humans (Olsson et al. 2014), eliciting in both cases avoidance or aversion from conspecifics.

At least in females of other lizard species, reproduction is suppressed or reduced after the experimental activation of the immune system with LPS injections (Uller et al. 2006) or after cutaneous wound healing (French et al. 2007a, b). Thus, an alternative explanation to our results might be that, in our experiment, LPS-male lizards simply did not develop nuptial coloration and chemical secretions as C-males did because LPS injection suppressed reproduction. However, during the mating season, both LPS- and C-males developed nuptial coloration, produced abundant femoral gland secretions, and showed reproductive behaviors (i.e., fights with other males and mating with females) and, moreover, both types of males sired some offspring. This suggests that all males were sexually active irrespective of the experimental treatment and that changes in sexual signals of LPS-males were not simply a consequence of suppressed reproduction.

Although most previous studies on this topic showed results consistent with those found here, namely a decrease in sexual signal expression as a consequence of an immune challenge (see references above), in some cases, the opposite result has been found. As already mentioned, an experimental immune challenge with LPS in captivity conditions in *L. schreiberi* did not affect UV coloration and males could even increase UV expression if they had supplementary antioxidants in the diet (Kopena et al. 2017). Interestingly, in the lizards *Gallotia gallotia* and *L. schreiberi*, the natural immune challenge caused by some parasites may rather increase UV saturation of some sexual ornaments (Megía-Palma et al 2016a,b, 2018). These contradictory results suggest that the trade-off between the immune response and the expression of sexual signals can be context dependent, varying as a function of the energetic state of the animal (French et al. 2007a; Ruiz et al. 2011) or the different types of parasitic diseases (Megía-Palma et al. 2016b), which, in the wild, will depend on seasonal and interannual environmental differences. Moreover, apparently sick challenged animals might decide to invest in sexual signals, or in reproductive behavior per se, in spite of being costly for their immune function. This would be a form of terminal investment in the current reproduction if they considered that their survival probabilities for the next breeding season are very low due to the immune challenge (Weil et al. 2006; Cote et al. 2010; Kopena et al. 2017). Therefore, in spite of potential survival costs, the observed reproductive consequences of an immune challenge in nature might not be as strong as expected considering only its apparent detrimental effects for the sexual signals.

In our experiment, the detrimental effects of the immune challenge on sexual signals of male *I. cyreni* did not result in significantly decreased overall reproductive success. One first possible explanation might be that the decrease in the expression of sexual signals produced by the immune challenge was not large enough as to have negative consequences for the effectiveness of sexual signaling. Also, it might be argued that the absence of differences in reproductive success could be due to the inexistence of actual sexual signals. However, the relationships found in this study between characteristics of sexual signals and reproductive success seem to support the results of previous studies on the role of sexual signals in intrasexual competition and females' preferences in this lizard (e.g., Martín and López 2000, 2006a,b, 2007, 2012; López and Martín 2012). Therefore, the absence of overall differences between treatments in reproductive success strongly points to the presence of alternative factors acting on mating.

The results showed that, at least in the control group, the reproductive success was directly and strongly related to body size/age, with larger, generally older, males siring the highest number of offspring. A similar relationship has been found in several species in different animal groups (e.g., Shine et al. 2000; Kovach and Powell 2003). However, interestingly, the interaction between size and treatment showed differences in this relationship depending on the experimental challenge. Thus, we found that both small and large LPS-males, independently of their size, obtained a similar reproductive success and fertilized a similar number of females, although apparently slightly lower than large C-males. These different size-reproductive success relationships probably explained the lack of overall differences between treatments when considering males of all body sizes/ages. Furthermore, the differences in the quality of visual and chemical signals and in the body size–reproductive success relationship suggest the existence of alternative condition-dependent reproductive strategies (Calsbeek and Sinervo 2008). C-males might rely more on the signaling strategy, developing high-quality signals to defeat rival males and to attract and be chosen by females. In contrast, in LPS-males, particularly in those of smaller body size, the low quality of their sexual signals and the apparent state of weakness induced by the immune challenge might suggest them a lower prospect of survival for future reproductive seasons. This may induce some small-sized/younger LPS-males to invest in mating (likely using sneaking forced matings), and not in signal production or home range defense, as a form of terminal investment (Clutton-Brock 1984) in spite of survival costs. A similar strategy occurs in other animals in response to an immune challenge (Bonneaud et al. 2004; Derting and Virk 2005; Weil et al. 2006; Sköld-Chiriac et al. 2018). In fact, a previous study of personalities showed that *I. cyreni* male lizards with a worse health state (i.e., higher infection intensity by blood parasites) took more risk than their healthier conspecifics (Horváth et al.2016). In this way, this "last resort" reproductive strategy of some LPS-males could ensure to achieve some reproductive success if exploiting more efficiently every encounter with females, independently of male–male competition and mate choice decisions of females based on the quality of the males' sexual signals. Nevertheless, large LPS-males still seemed to achieve lower reproductive success than the similarly large C-males as we predicted. Also, because of the high multipaternity on this species, the results might be also affected by processes of sperm competition independently of the experimental treatment.

We conclude that an immune challenge may affect the development of sexual signals and also have fitness consequences as suggested by the observed relationships between expression of sexual traits and reproductive success. However, some individual males are apparently able to compensate these costs, at least partly, by adopting alternative mating strategies, probably as a form of terminal investment. Future studies should examine the behavioral strategies followed by challenged animals of different ages and how different environmental and social conditions affect them.

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Authors' contributions: J.M., G.R.R., J.O., P.L, and A.S. conceived the ideas and designed the methodology. All authors worked in the field and collected the data. J.O. and G.R.R. made the genetic analyses. G.R.R. and J.M. analyzed the data and led the writing of the manuscript. All authors contributed critically to the manuscript drafts and gave final approval for submission.

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Data accessibility: Analyses reported in this article can be reproduced using the data provided by Rodríguez-Ruiz et al. 2020.

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