*Biological Journal of the Linnean Society*, 2021, **134**, 461–473. With 4 figures.

# Brighter is darker: the Hamilton–Zuk hypothesis revisited in lizards

RODRIGO MEGÍA-PALMA[1,2,*], RAFAEL BARRIENTOS[3], MANUELA GALLARDO[4], JAVIER MARTÍNEZ[1] and SANTIAGO MERINO[5]

[1]*Universidad de Alcalá (UAH), Área de Parasitología, Departamento de Biomedicina y Biotecnología, Facultad de Farmacia, E-28805, Alcalá de Henares, Spain*
[2]*CIBIO, InBIO – Research Network in Biodiversity and Evolutionary Biology, Universidade do Porto, Campus de Vairão, Rua Padre Armando Quintas, 4485-661 Vairão, Porto, Portugal*
[3]*Road Ecology Laboratory, Departamento de Biodiversidad, Ecología y Evolución, Facultad de Ciencias Biológicas, Universidad Complutense de Madrid, José Antonio Novais 12, E-28040, Madrid, Spain*
[4]*Laboratorio de Histología, Museo Nacional de Ciencias Naturales-CSIC, c/ José Gutiérrez Abascal 2, E-28006, Madrid, Spain*
[5]*Departamento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales-CSIC, c/ José Gutiérrez Abascal 2, E-28006, Madrid, Spain*

*Received 12 April 2021; revised 9 May 2021; accepted for publication 11 May 2021*

Several studies of lizards have made an erroneous interpretation of negative relationships between spectral brightness and parasite load, and thus provided misleading support for the Hamilton–Zuk hypothesis (HZH). The HZH predicts that infected hosts will produce poorer sexual ornamentation than uninfected individuals as a result of energetic trade-offs between immune and signalling functions. To test whether there is a negative relationship between spectral brightness and pigment content in the skin of lizards, we used spectrophotometry to quantify the changes in spectral brightness of colour patches of two species after chemically manipulating the contents of orange, yellow and black pigments in skin samples. Carotenoids were identified using high-performance liquid chromatography. In addition, we compared the spectral brightness in the colour patches of live individuals with differential expression of nuptial coloration. Overall, the analyses demonstrated that the more pigmented the colour patch, the darker the spectrum. We provide a comprehensive interpretation of how variation in pigment content affects the spectral brightness of the colour patches of lizards. Furthermore, we review 18 studies of lizards presenting 24 intraspecific tests of the HZH and show that 14 (58%) of the tests do not support the hypothesis.

ADDITIONAL KEYWORDS: brightness – colour – high-performance liquid chromatography – spectrophotometry.

## INTRODUCTION

The significance of the colours displayed during mating interactions in a multitude of animal species has been explored by researchers ever since Darwin's first sexual selection studies. According to theory, animal coloration can have multiple adaptive functions, including camouflage (Marshall *et al.*, 2015) and aposematism (Tullberg *et al.*, 2005), both of which are adaptive by reducing attacks from predators. Vivid and/or contrasting coloration can also evolve as a signal of individual quality and can influence the outcome of both agonistic interactions and mate selection (Berglund *et al.*, 1996; Burley *et al.*, 2018). Thus, sexual selection can influence the evolution of animal coloration (Deutsch, 1997; Pérez i de Lanuza *et al.*, 2013; Dale *et al.*, 2015). Lizards are no exception to this rule; properties of their coloration can be correlated with immunological, morphological and behavioural traits (Sacchi *et al.*, 2007; Langkilde & Boronow, 2010). These relationships are often explained by the idea that production and maintenance of coloration are costly in terms of pigment and energy allocation to colour patches (Megía-Palma *et al.*, 2016b, 2018b).

*Corresponding author. E-mail: rodrigo.megia@gmail.com

Thus, besides defensive functions, colour patches in lizards might also provide honest visual signals of individual quality used for assessment of rivals or mate selection (Olsson, 1994; Stapley & Whiting, 2006; Megía-Palma *et al.*, 2018c).

At the population level, the expression of individual coloration displayed during social interactions in lizards can vary from complete polymorphism, where individuals express discrete coloration or morphs (Sinervo & Lively, 1996; Teasdale *et al.*, 2013), to continuous variation of a single colour hue (e.g. see intrapopulation variation of blue coloration in fig. 1 of Stoehr & McGraw, 2001).

In the past, the coloration of individuals was scored using the Munsell comparative method for colour classification (Zucker, 1988). In this method, the coloration of individuals is scored by comparison with colour charts using the naked eye (Ressel & Schall, 1989; Olsson, 1994). In the last 20 years, the advent of relatively affordable spectrophotometers, with sensitivity in the near ultraviolet range where the visual system of lizards is also sensitive, has made a quantitative assessment of the reflectance properties of lizard colour patches possible (Stapley & Whiting, 2006; Pérez i de Lanuza *et al.*, 2013). There are several methods used to analyse the spectral data obtained by spectrophotometry; of these, the segment classification method was proposed by Endler (1990) and later adapted by others (e.g. Montgomerie, 2006). In this method, three spectral components (brightness, chroma and hue) are calculated to assist in interpretation of the spectral properties of the quantified colour (e.g. Molnár *et al.*, 2013; Pérez i de Lanuza *et al.*, 2014; Megía-Palma *et al.*, 2018b).

In this study, we focus on the brightness spectral component calculated in the segment classification method, because we have identified some publications with a potential misinterpretation of this variable. According to the original terminology, 'brightness' is interpreted as the total intensity of light reaching the eye from the patch at a distance *x* (Endler, 1990; Kemp *et al.*, 2015). It is an unfortunate coincidence that the term 'brightness' is used colloquially to refer to a general pattern of coloration displayed by a species or individual (e.g. Merilä *et al.*, 1999; Montgomerie, 2006; Balenger & Zuk, 2014); hence, it describes the expansion or intensity of pigmentation shown (Hamilton & Zuk, 1982), where a 'brighter' species or individual would show more pigmentation. However, based on the original nomenclature of the segment classification method (Endler, 1990), this can also be interpreted in the opposite way, such that brighter colour patches reflect more light; hence, they have less pigmentation (see Cuervo *et al.*, 2016).

Strikingly, this ambiguous terminology led to misleading support for the predictions of the parasite-mediated sexual selection hypothesis proposed by Hamilton & Zuk (1982) (hereafter, HZH). The HZH predicts specifically that, within a population, individuals with full development of coloured ornamentation will have the lowest intensities of chronic parasitic infections (Balenger & Zuk, 2014). Thus, results strictly conforming to the HZH should render negative significant relationships between colour patch expression and infection. As a rule of thumb, conspecifics would select mates by scrutiny of the coloration of each candidate and would show a preference for the most ornamented ones. This, according to the HZH, would contribute to an increase in the frequency of genes for resistance to parasitic diseases in the genetic pool of the host population. The hypothesis has received mixed support and, based on co-evolutionary models other than the HZH (see Gandon *et al.*, 2002), it is not surprising to find examples across several taxa showing an opposite pattern, in which parasitized individuals show higher expression of coloration than uninfected ones (fish: Folstad *et al.*, 1994; birds: Zirpoli *et al.*, 2013; reptiles: Megía-Palma *et al.*, 2016a). Indeed, a posterior meta-analysis of 199 quantitative assessments of the HZH cast into doubt the generalization of its predictions (Poulin & Hamilton, 1997). Unfortunately, that study did not include any intraspecific tests on reptiles.

It is well known in both chemistry and ecology that the sum of reflectance (brightness) measured from the spectrum of a pigment decreases with the pigment concentration. Thus, the conflict in the terminology used to describe the coloration arises from the fact that most of the pigments required for the expression of vivid (i.e. highly pigmented) colour patches of lizards absorb light rather than reflect it. For example, deposition of eumelanin (a black pigment) in the basal layer of the skin of males of *Sceloporus jarrovii* that had been treated with hormones resulted in a significant reduction in the total light (i.e. spectral brightness) reflected by a blue colour patch (Quinn & Hews, 2003; Cox *et al.*, 2008). Likewise, a perceptible increment of carotenoid-based reddish belly coloration in pigment-containing cells of the upper layers of the skin was accompanied by a reduction in total brightness of the skin in *Zootoca vivipara* (Fitze *et al.*, 2009). Perhaps the most striking case was a study in *Acanthodactylus erythrurus*, in which the pterin concentration of a red patch was negatively correlated with its total (spectral) brightness (Cuervo *et al.*, 2016). Thus, individuals with redder tails were also darker. This highlights a fundamental difference from studies of bird coloration, where variation in the pigment content of feathers was associated with variations in chroma and hue, but not in spectral brightness (Saks *et al.*, 2003; but also see Laczi *et al.*, 2019). Therefore, based on evidence in reptiles, we identified some studies of lizards that

made an erroneous interpretation of the spectral variable of brightness, which led to the assumption that a negative relationship between the spectral brightness and the intensity of infection by parasites necessarily implied a confirmation of the HZH.

In the present study, we used skin samples from voucher specimens of two species of lizards with yellow, orange and blue colour patches to quantify objectively, with a spectrophotometer, the variation in spectral brightness before and after rinsing the pigments of these different colour patches with chemical treatments. Additionally, we tested whether free-ranging male lizards of the same species with differential expression of breeding coloration show significant differences in total brightness. We also revisited intraspecific tests of the HZH in lizards and discuss the trends observed to provide a conceptual framework for future experimental work. We predicted, for both rinsed skins and free-ranging males, that the poorer the pigment content of the skin, the higher its total reflectance (i.e. higher brightness). With this evidence in hand, we aim to provide a well-founded basis for interpretation of coloration in lizards.

## MATERIAL AND METHODS

### Collection of skin samples

During the lizard mating season of 2013 and 2018, skin samples were obtained from dead males of *Lacerta schreiberi* Bedriaga, 1878 ($N$ = 3) and *Psammodromus algirus* Linnaeus, 1758 ($N$ = 4) that were frozen at –20 °C until the chemical experiments. The causes of death of the seven specimens were diverse (road kills and predation). Skin samples from the blue and yellow colour patches of *L. schreiberi* and from the nuptial colour patch of *P. algirus*, which combines orange and yellow pigmentation, were removed using a scalpel.

### Extraction and identification of pigments in yellow and orange patches

After obtaining an initial spectrophotometric measurement, the skin samples with orange and yellow pigmentation were washed for ten consecutive periods of 10 min in a bath of 100% acetone to remove the carotenoid content (McGraw *et al.*, 2004; Saenko *et al.*, 2013). Washing pigments out with acetone is assumed routinely to discriminate carotenoids from pterines (Steffen & McGraw, 2007, 2009). During the acetone baths, skin strips were incubated for 5 min at room temperature with agitation, and later centrifuged at 2500 g at 40 °C for the remaining 5 min.

To characterize the carotenoids present in the samples, we used high-performance liquid chromatography (HPLC, Agilent 1200, Santa Clara, CA, USA) to analyse the dilutions obtained after the 100 min of acetone bathing. HPLC analyses were performed using a C30 bonded silica based reversed-phase column (YMC Carotenoid, YMC, Kyoto, Japan). We used the following carotenoids standards: canthaxantin, asthaxantin, and lutein (Dr. Ehrenstorfer, Manchester, NH, USA) , β-caroteno (Fluka, Munich, Germany), and β-cryptoxanthin and zeaxanthin (Extrasynthese, Genay, France).

The spectral shape of the orange pigmentation of *P. algirus* was not affected by the acetone treatment, suggesting the presence of pterins in the sample (e.g. Saenko *et al.*, 2013). To confirm this, we used separate vials to wash with 1% ammonium hydroxide ($NH_4OH$) the skin samples containing the orange pigmentation and, simultaneously, 200 heads from flies of the genus *Drosophila*, with drosopterin-based red eyes, which is a standard control (Cuervo *et al.*, 2016).

### Chemical manipulation of eumelanin and histological sections

After obtaining an initial spectrophotometric measurement, the skin samples with blue coloration from the nuptial colour patch of *L. schreiberi* were washed for ten consecutive periods of 10 min in a bath of hydrogen peroxide (33% p/v; Panreac Química S.A.U., Barcelona, Spain). The aim of this treatment was to alter the spectral properties of the eumelanin content without altering the structure of the skin. To confirm the effect of hydrogen peroxide on the eumelanin content, histological preparations were made from skin samples before and after the chemical treatment and photographed with a light microscope. To make the histological sections, skin samples from the colour patches were fixed in 4% formalin for 48 h and in Carnoy for 30 min. Next, the samples were exposed to 0.033% potassium iodide at pH 3.35 for 24 h (Gallardo *et al.*, 2015). The skin samples were then dehydrated with different alcohol concentrations: 80% ethanol for 2 h, pure ethanol for 2 h, 50% xylene solution in ethanol for 15 min, and pure xylene for 15 min. The skin samples were embedded in paraffin and cut at 7 µm thickness with a microtome (RM2045; Leica Microsystems, Wetzlar, Germany). Photomicrographs of the skin were made with a digital camera (Axio Cam MRc5; Carl Zeiss, Oberkochen, Germany).

### Quantification of variation in spectral brightness

We quantified the spectrum from all skin samples before and after each chemical wash. Before the spectral measurements, skin samples were removed from the washing solution and dried with a tissue.

We used a spectrophotometer that operated with a pulsed xenon lamp emitting light in the ultraviolet range (Jaz DPU Module; Ocean Optics, Dunedin, FL, USA). This device was connected to a glass-fibre probe that was inserted in a black holder that reduced noise from environmental light and facilitated measurements at a constant distance of 3 mm from the surface and with a constant probe angle of 90°. The perceptive visual range in lizards is 300–700 nm, where higher spectral variability was expected (Fleishman, 1997). We recorded three consecutive measurements of each colour patch that were subsequently averaged into 81 light bins of 5 nm (CLR v.1.1 software; Montgomerie, 2008). The repeatability of these spectral measurements varied from 0.74 to 0.94 (based on the study by Lessells & Boag, 1987).

The spectral brightness was calculated as $\sum R_{300–700}$, where $R$ is the average reflectance for every 5 nm light bin considered (Montgomerie, 2006). Owing to the small sample size of the skin samples, a non-parametric Friedman ANOVA for multiple dependent samples was performed to compare the measurements made at 10 min intervals for each chemical treatment, where we included the calculated spectral brightness of each skin sample as a response and the eleven 10 min intervals (0–100 min) as predictors. In addition, a non-parametric Spearman correlation was computed between the treatment duration and the average spectral brightness for every 10 min interval.

The difference in brightness of the orange patch after the single wash with $NH_4OH$ was analysed with a general nested mixed model, where we set the spectral reflectance of each of the 81 5 nm light bins as response variables and the measurement (two levels: first and second spectral measurements) as a fixed factor. We controlled for pseudoreplication by including the individual and the wavelength bins nested within individual as random terms (Millar & Anderson, 2004).

### Quantification of throat total brightness in live lizards

Lizards were captured using a noose. All individuals were sexed based on the conspicuousness of the femoral glands and the enlargement of the tail, close to the cloaca.

We captured a total of 38 adult males of *L. schreiberi*, during the 2013 breeding season (May and June), in Segovia province (Spain, 40.8864, –4.0326). Males of this species typically show an intense blue coloration, with one or two peaks in the near-ultraviolet range (Pérez i de Lanuza & Font, 2014; Megía-Palma *et al.*, 2016b). However, seven of them lacked the typical blue breeding coloration. Thus, the individuals were classified into two categories: bluish males (with vivid blue breeding coloration) and whitish (without breeding coloration). In contrast, finding males with differential expression of the yellow coloration was not possible because the yellow pigmentation is the 'background' coloration of this species and is present in every lizard.

We also analysed the orange coloration of 57 adult males of *P. algirus* that were captured during the breeding season of 2018 in Madrid province (Spain, 40.5080, –3.7673). These males were classified into two colour categories, whitish and orangish, following Díaz (1993).

We used the same spectrophotometer as above to quantify the total light reflected by the yellow and blue throat patches in *L. schreiberi* and by the orange patches in *P. algirus*. For this purpose, we followed the methodology described by Megía-Palma *et al.* (2016b). In individuals of *P. algirus* with only a small orange patch, the coloration is visible only when lizards open their mouth. Thus, we made the lizards bite a soft, black piece of rubber to measure the reflectance of the orange patch in the mouth commissure.

All of these spectral measurements were relative to a white standard with 100% reflectance in the total range of interest. The spectral brightness of the colour patches was calculated in the same way as for the skin samples ($\sum R_{300–700}$). The difference in total brightness between colour groups was tested with general linear models in STATISTICA v.10.0 (Statsoft, Tulsa, OK, USA), including total brightness as a dependent variable and colour group as a factor. Parametric assumptions for normality and homoscedasticity of the residuals of the resulting models were checked.

### Intraspecific tests of the Hamilton–Zuk hypothesis in lizards

We used Google Scholar (30 March 2021) to find intraspecific studies testing the HZH and used the following key words as search criteria: "colouration" OR "coloration" AND "colour" OR "color" OR "colour patch" OR "color patch" AND "parasite" AND "mite" AND "tick" OR "nematode" OR "disease" AND "Hamilton & Zuk" AND "lizard". We read the studies that analysed intraspecific relationships between colour expression and parasite infections thoroughly. We also consulted the reference lists of the most recent papers published on this topic. We included studies that used methods other than spectrophotometry to quantify coloration, such as photography, colorimetry or even observational methods (e.g. Schall, 1986). We considered that a parasite–colour test supported the HZH only when the relationship reported was significantly negative. We noted the species and sex of the lizards investigated and the group of parasites analysed in each test (Table 1).

**Table 1.** Summary of intraspecific tests of the Hamilton–Zuk hypothesis (HZH) in lizards ordered first by support of the HZH and second by year of publication

| Type | Method of colour quantification | HZH | Parasite analysed | Host species investigated | Sex of host | Author |
|---|---|---|---|---|---|---|
| Correlational | Photography and digital processing | Yes | Mites (families Trombiculidae and Pterygosomatidae) | *Sceloporus virgatus* | ♀ | Weiss (2006) |
| Correlational | Spectrophotometry | Yes | Ticks (*Ixodes ricinus*) | *Lacerta viridis* | ♂ | Václav *et al.* (2007) |
| Correlational | Spectrophotometry | Yes | Mites (family Trombiculidae) | *Anolis brevirostris* | ♂ | Cook *et al.* (2013) |
| Correlational | Spectrophotometry | Yes | Nematodes (unidentified) | *Lacerta schreiberi* | ♂ | Megía-Palma *et al.* (2016b) |
| Experimental | Photography and digital processing | Yes | Ticks (*I. ricinus*) | *Psammodromus algirus* | ♂ | Llanos-Garrido *et al.* (2017) |
| Experimental | Spectrophotometry | Yes | Ticks (*I. ricinus*) | *Lacerta schreiberi* | ♂ | Megía-Palma *et al.* (2018b) |
| Correlational | Colorimetry | Yes | Intestinal coccidians (*Acroeimeria sceloporis*) | *Sceloporus occidentalis* | ♂, ♀ | Megía-Palma *et al.* (2018c) |
| Correlational | Photography and digital processing | Yes | Mites (unidentified) | *Sceloporus occidentalis* | ♂ | Seddon & Hews (2020) |
| Correlational | Spectrophotometry | Yes | Ticks (*I. ricinus*) and bacteria (*Borrelia burgdorferi s.l.*) | *Lacerta schreiberi* | ♀ | Kopena *et al.* (2020) |
| Correlational | Observational | No | Haemogregarines (unidentified) | *Cnemidophorus arubensis* | ♂ | Schall (1986) |
| Correlational | Photography and Munsell colour chips | No | Haemosporidians (*Plasmodium mexicanum*) | *Sceloporus occidentalis* | ♂ | Ressel & Schall (1989) |
| Correlational | Photography and digital processing | No | Nematodes (unidentified) | *Sceloporus pyrocephalus* | ♀ | Calisi *et al.* (2008)* |
| Correlational | Spectrophotometry | No | Ticks (*I. ricinus*) | *Lacerta viridis* | ♀ | Václav *et al.* (2007) |
| Correlational | Colorimetry | No | Haematic coccidians (probably genus *Karyolysus*) | *Podarcis muralis* | ♂ | Martín *et al.* (2008)* |
| Correlational | Spectrophotometry | No | Ticks (*I. ricinus*) and haematic coccidians (*Karyolysus*) | *Lacerta viridis* | ♂ | Molnár *et al.* (2013)* |
| Correlational | Spectrophotometry | No | Haematic coccidians (*Karyolysus*) | *Gallotia galloti* | ♂ | Megía-Palma *et al.* (2016a) |
| Correlational | Spectrophotometry | No | Ticks (*I. ricinus*) and haematic coccidians (*Schellackia*) | *Lacerta schreiberi* | ♂ | Megía-Palma *et al.* (2016b)† |
| Correlational | Scanner (colour patch area) | No | Ticks (*I. ricinus*) | *Lacerta agilis* | ♂ | Lindsay *et al.* (2016) |
| Correlational | Colorimetry | No | Haemococcidians (*Lankesterella occidentalis*) | *Sceloporus occidentalis* | ♂ | Megía-Palma *et al.* (2018c) |
| Correlational | Spectrophotometry | No | Ticks (unidentified) | *Intellagama lesueurii* | ♂ | Merkling *et al.* (2018) |
| Correlational | Photography and digital processing | No | Mites (*Eutrombicula cinnabaris*) | *Sceloporus woodi* | ♂ | Orton *et al.* (2020) |

The same study is shown several times if different parasites, colour patches or sexes were studied and showed opposing relationships. The column headed 'HZH' indicates whether the test supports the parasite hypothesis.
*Articles with results that are amended based on the results of the present study.
†A study that has one result amended but also contains other relationships between parasites and colour patches correctly interpreted.

We analysed whether the outcome of the HZH tests was independent of the parasite analysed using a $\chi^2$ test that considered the parasite investigated to compare the proportion of tests that supported vs. those that contradicted the HZH.

### Data availability

The data underlying the work are available from the authors upon reasonable request.

## RESULTS

### Chemical removal of yellow and orange pigments

The acetone treatment removed the yellow pigmentation of the top skin layers of *L. schreiberi*. This was associated with an increase in the light reflected by the skin, particularly in the spectral range between 400 and 500 nm. In contrast, the orange patch of the nuptial coloration of *P. algirus* did not vanish with the acetone treatment, and the spectral shape remained constant (Fig. 1A).

The skin samples that lost pigments experienced a general increase in spectral brightness associated with the duration spent submerged in acetone (Spearman correlations: yellow patch, $\rho = 0.90$, $P = 0.0001$; orange patch, $\rho = 0.63$, $P = 0.03$). The change in spectral brightness was significant in the yellow patch of *L. schreiberi* (Friedman ANOVA: $\chi^2_{10,3} = 22.90$, $P = 0.02$), but not in the orange patch of *P. algirus* washed with acetone (Friedman ANOVA: $\chi^2_{10,3} = 15.65$, $P = 0.11$; Fig. 1B).

A single wash with ammonium hydroxide completely washed out the orangish pigmentation from both the

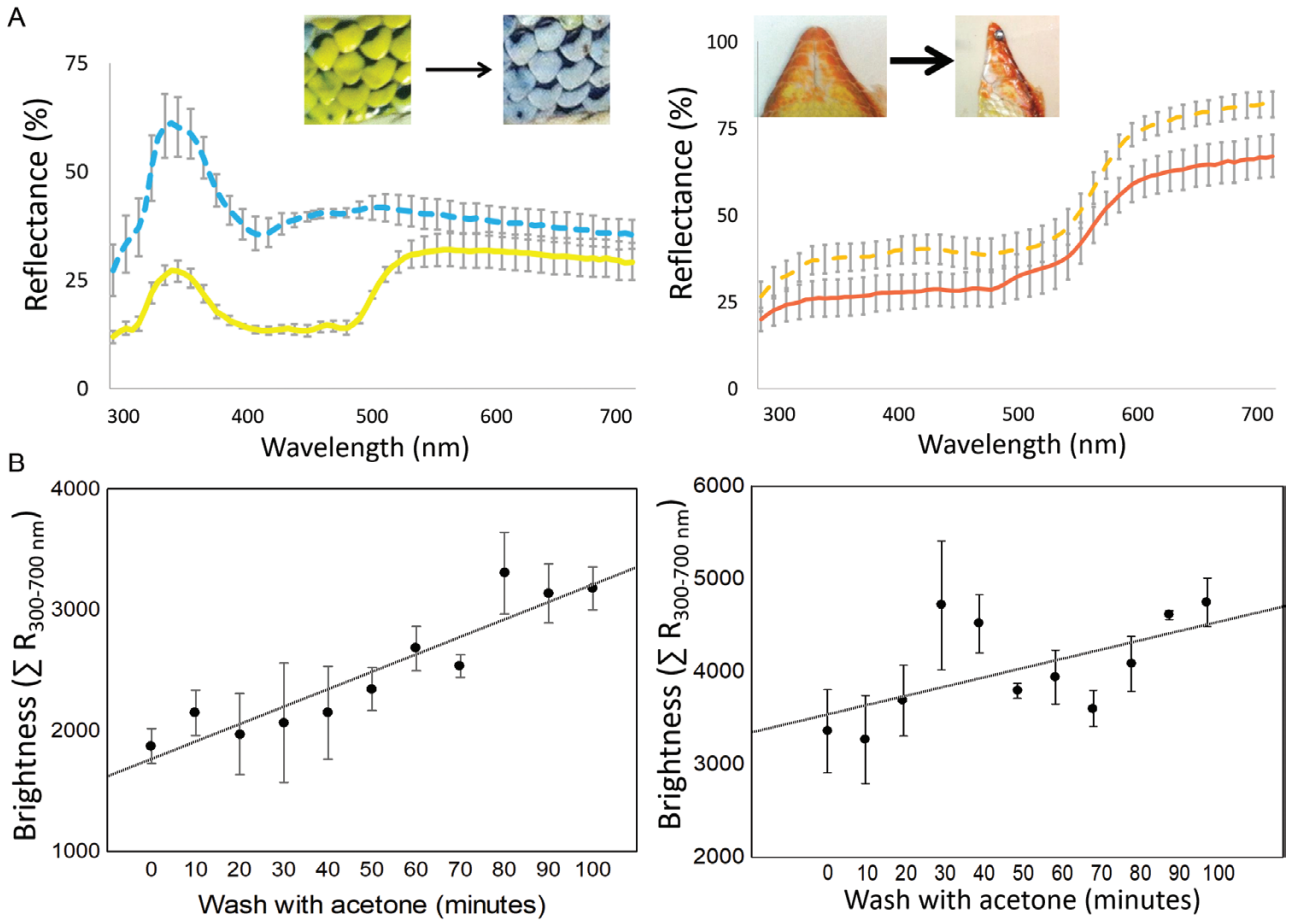


**Figure 1.** A, effect of the treatment with acetone for 100 min on the spectral brightness of the yellow patch of *Lacerta schreiberi* (left) and the orange patch of *Psammodromus algirus* (right). Continuous lines represent the mean ± SE spectra before treatment (0 min) and dotted lines the mean ± SE spectra after treatment (100 min). Pictures show the obvious change in coloration in the skin samples provoked by the treatment with acetone. B, continuous variation of spectral brightness during treatment with acetone. The plots are shown in the same order as the spectral data.

skin vouchers of *P. algirus* (Fig. 2A) and the eyes of *Drosophila*. The spectral shape of the skin changed, in particular with an increase in reflectance between 340 and 600 nm and a decrease between 600 and 700 nm (Fig. 2B). However, the overall change in the spectral brightness in the four skin samples tested was not significant ($F_{1,3}$ = 0.49, $P$ = 0.48).

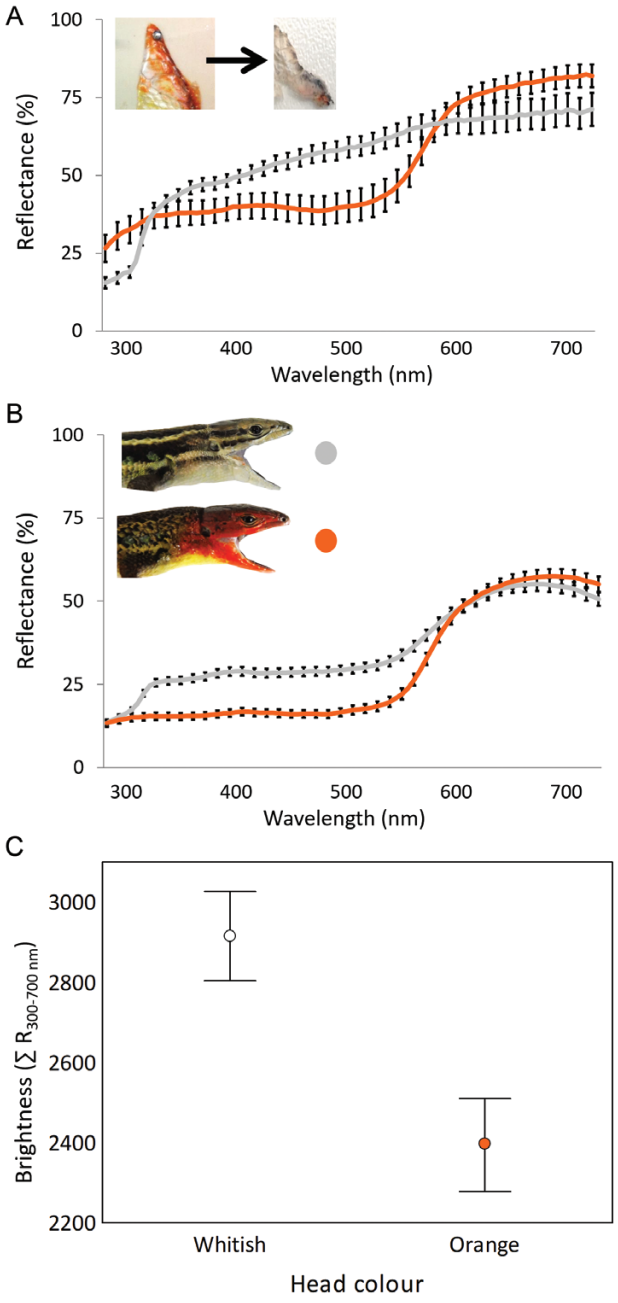


**Figure 2.** A, spectral variation after ammonium hydroxide treatment of the orange patch of *Psammodromus algirus*. The grey line represents the averaged spectra of the skin after removal of the orange pigment. B, spectral comparison of adult males without and with expression of the orange coloration. C, spectral brightness was significantly higher in the commissures without orange coloration.

## Pigment identification

The HPLC characterization of carotenoids in the skin samples of the yellow colour patch of *L. schreiberi* revealed the presence of asthaxantin (mean ± SE = 43.42 ± 4.96 µg/mL), canthaxantin (35.21 ± 9.40 µg/mL) and lutein (21.36 ± 4.44 µg/mL) (Fig. 3). The HPLC analysis revealed that at least six different carotenoids were extracted from the nuptial coloration tissues of *P. algirus* with the acetone treatment, namely asthaxantin (mean ± SE = 25.92 ± 4.63 µg/mL), canthaxantin (22.17 ± 1.61 µg/mL), zeaxanthin (16.18 ± 1.44 µg/mL), lutein (15.89 ± 2.61 µg/mL), β-carotene (11.05 ± 5.68 µg/mL) and β-cryptoxanthin (8.77 ± 4.40 µg/mL) (Fig. 3).

## Chemical manipulation of eumelanin

Histological preparations of skin samples from the nuptial blue patch showed that the melanin content of the dermis (i.e. eumelanin; Megía-Palma *et al.*, 2018a) was cleared after the chemical treatment with

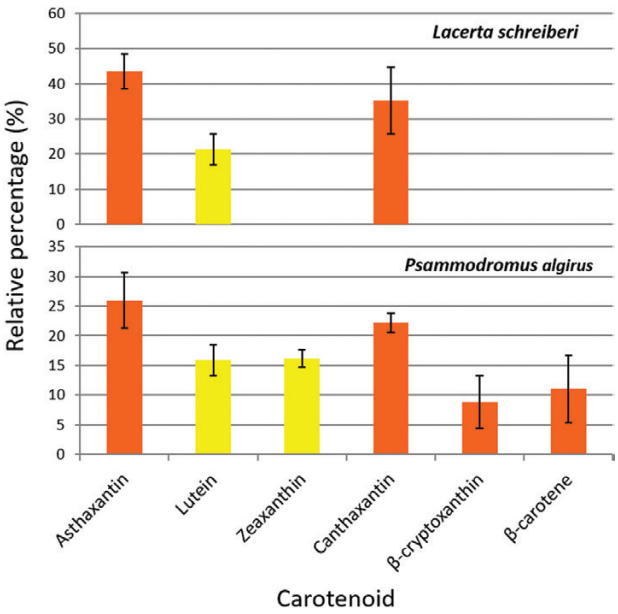


**Figure 3.** Mean ± SE percentage of carotenoids identified in the skin samples of *Lacerta schreiberi* and *Psammodromus algirus*. The relative percentage of each carotenoid was calculated as: [quantity of a specific carotenoid (in micrograms per millilitre)/total amount of carotenoids in the sample] × 100. Bars representing each carotenoid are shown in either orange or yellow according to the coloration typically presented in nature (based on the study by Khoo *et al.*, 2011).

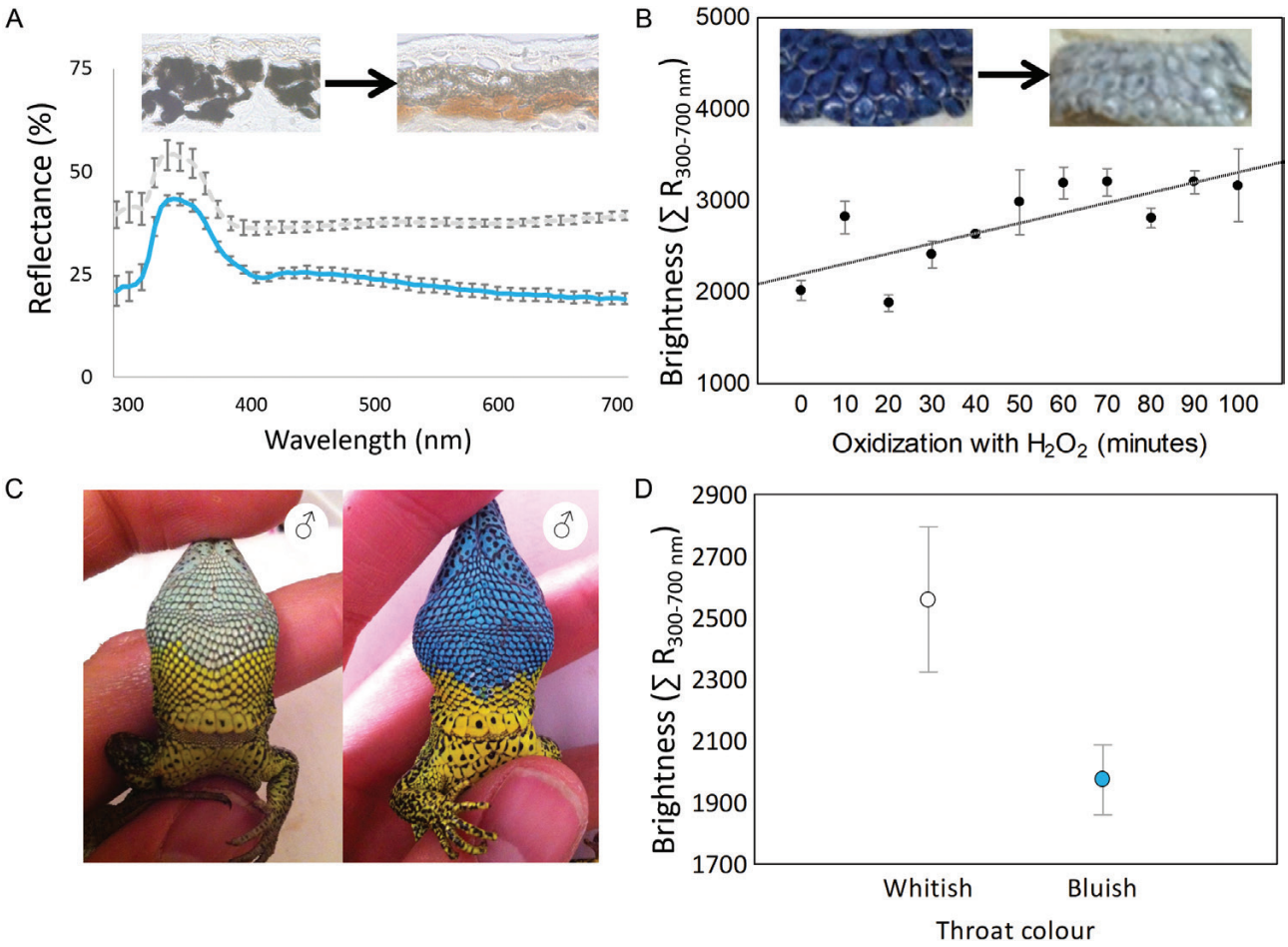


**Figure 4.** A, effect of 100 min hydrogen peroxide treatment on the spectral brightness of the blue nuptial coloration of *Lacerta schreiberi*. The continuous line represents the mean ± SE spectra before treatment (0 min) and the dotted line after treatment (100 min). Pictures show histological sections of the skin before and after the chemical treatment. B, continuous variation of spectral brightness during hydrogen peroxide treatment. Pictures show the obvious change in coloration of the skin samples provoked by hydrogen peroxide treatment. C, males of *L. schreiberi* with whitish and bluish throat patches. D, ANOVA showing significant differences of the mean ± SE spectral brightness of the throat of whitish and bluish males.

hydrogen peroxide, and the blue coloration of this colour patch turned whitish (Fig. 4A, B). There was a positive and continuous trend between the duration of hydrogen peroxide treatment and the increase in spectral brightness of the skin sample from the blue patch (Spearman correlation: blue patch in hydrogen peroxide, $\rho = 0.64$, $P < 0.0001$). The increase in spectral brightness was significant over time (Friedman ANOVA: blue patch $\chi^2_{10,3} = 20.54$, $P = 0.02$; Fig. 4B).

### Quantification of throat total brightness in live lizards

The mean ± SE brightness of *P. algirus* with whitish throats ($N = 29$) was 2915.58 ± 111.59, which was significantly brighter than the males with orange heads ($N = 28$; 2394.51 ± 115.86; $F_{1,55} = 10.5$, $P = 0.002$; Fig. 2B, C). Likewise, the mean ± SE brightness of *L. schreiberi* with whitish throats ($N = 7$) was 2559.44 ± 228.88 ($\sum R_{300–700}$), which was significantly brighter than the throats of those with intense blue throat coloration ($N = 31$; 671.33 ± 114.54; $F_{1,35} = 5.01$, $P = 0.03$; see Fig. 4C, D).

### Review of intraspecific tests of the Hamilton–Zuk hypothesis in lizards

We found 18 articles describing 24 intraspecific tests of parasite infection relative to colour expression in lizards (Table 1). Only two of them (8%) used an experimental approach (Llanos-Garrido *et al.*, 2017; Megía-Palma *et al.*, 2018b), and the other 16 were based on correlational analyses. Eight measured or estimated colour expression by spectrophotometric

methods, six by photography, two by colorimetry (with no sensitivity in the ultraviolet range), one by the naked eye, and one used a flat scanner to quantify the relative size of the colour patch studied.

Independently of the method used to quantify colour expression, 14 (58%) intraspecific correlational tests between parasite infection and colour expression in lizards provided evidence against the HZH via positive associations between infection and colour expression. Parasite infections were associated with lower expression of coloration in two experimental and eight correlational tests of the HZH. Thirteen species of lizards belonging to five families have been investigated in relationship to the HZH. The genera *Lacerta* (fam. Lacertidae) and *Sceloporus* (fam. Phrynosomatidae) received the majority of intraspecific tests of the hypothesis (75%). Also, 80% of the tests were performed on males. More than half of the tests (54%) analysed the relationship between infestation by ectoparasites (mites and ticks) and expression of sexual coloration; 33% investigated protozoans (haematic and intestinal parasites), 8% round worms (intestinal nematodes), and a single study analysed bacteria (Table 1). Interestingly, the proportion of tests that supported the HZH was independent of the parasite analysed, except for protozoans. In this group, tests providing evidence of positive relationships with colour expression were significantly higher than those supporting the HZH (Table 2).

## DISCUSSION

Our results on carotenoid- and melanin-based colour patches supported that the spectral brightness in colour patches of lizards is negatively associated with pigment content. Conforming to our predictions, the spectral brightness in the yellow and orange skin samples of the two species of lizards increased with the duration of immersion in acetone. The skin samples from the yellow patch of *L. schreiberi* changed from yellow to blue, which caused a significant increase in the total light reflected by the skin. A large proportion of this significant increase was associated with the increase in light reflected in the spectral range between 400 and 500 nm, which is the portion of the light expected to be absorbed by carotenoids (see Jacot *et al.*, 2010). The HPLC identification of the pigments rinsed out with acetone confirmed the presence of several carotenoids in the yellow skin of *L. schreiberi*. Thus, the observed spectral variation was consistent with the wash-out of carotenoids from this portion of the skin. The combination of carotenoids in *L. schreiberi* (i.e. asthaxantin, canthaxantin and lutein) was simpler than in the throat pigmentation of *P. algirus* (also containing these plus three additional carotenoids: zeaxanthin, β-carotene and β-cryptoxanthin).

The HPLC analysis detected six carotenoids in the acetone solution after treating the skin of *P. algirus*. However, this chemical treatment did not significantly alter the spectral shape of the skin of *P. algirus*, suggesting that pigments other than carotenoids produce the orange coloration in this species. The spectral shape associated with the orange coloration changed only after washing out the orange pigments in a single bath of 1% $NH_4OH$, which, in a simultaneous test, also extracted orange pigments from the eyes of *Drosophila* flies. This strongly suggests that the orange pigmentation of *P. algirus*, although also containing carotenoids, is based on pterins (Steffen & McGraw 2007, 2009). However, the observed increase in total brightness after washing out the pigments of the orange patch from the skin samples of *P. algirus* was not significant. Light reflected by the skin increased relatively between 340 and 600 nm, but decreased relatively between 600 and 700 nm (Fig. 2A). This suggests that pterins contribute to an increase in the relative reflectance in the spectral range between 600 and 700 nm, meaning that spectral chroma calculated for this spectral region ($\Sigma R_{600-700}/\Sigma R_{300-700}$) might be a good descriptor of pterin content (see also correlation coefficients in fig. 2 of the paper by Cuervo *et al.*, 2016). Nonetheless, comparison of the spectral brightness in 57 live specimens with differential expression of nuptial coloration provided key complementary information; spectral brightness was significantly lower in the individuals with a conspicuous orange patch. The reflectance in the spectral region between 600 and 700 nm relative to reflectance in the rest of the visible spectrum was high in both groups of lizards,

**Table 2.** Proportion of intraspecific tests of the Hamilton–Zuk hypothesis shown according to their support of the hypothesis and the type of parasite analysed

| Support HZH | Bacteria | Protozoans | Nematodes | Mites | Ticks |
|---|---|---|---|---|---|
| Yes | 0.04 | 0.042 | 0.042 | 0.125 | 0.167 |
| No | 0.00 | 0.292 | 0.042 | 0.042 | 0.208 |
| $\chi^2_{1,23}$ (*P*-value) | 1.02 (0.31) | 5.40 (0.02)* | 0.00 (1.00) | 1.09 (0.29) | 0.14 (0.71) |

*Significant differences for protozoans, suggesting that investigations on this group of parasites contradict the Hamilton–Zuk hypothesis (HZH).

suggesting that pterins might already be present in the skin of adult lizards with inconspicuous pigmentation, although probably at a low concentration (Fig. 2C).

The spectral brightness of the skin samples of *L. schreiberi* was significantly lower (darker) when the skin was blue than when the skin had been chemically whitened (see Fig. 4A), similar to lizards with poor expression of the blue nuptial coloration (Fig. 4C, D). Therefore, based on the results shown here, it is erroneous to assume that a negative relationship between the spectral brightness and the intensity of parasites fulfils the predictions of the HZH. Some of the tests identified here should be reinterpreted to avoid generalization of the HZH to all lizard species (Calisi *et al.*, 2008; Martín *et al.*, 2008; Molnár *et al.*, 2013; Megía-Palma *et al.*, 2016b). In these and some of the other tests identified, lizards with more parasites had darker colour patches (i.e. with higher pigment content), contradicting the HZH (see Ressel & Schall, 1989; Megía-Palma *et al.*, 2016a).

Despite the proposed amendments, the HZH has thus far received mixed support in lizards depending on the species of parasite, the colour patch or even the sex of the host evaluated within the same study (e.g. Václav *et al.*, 2007; Megía-Palma *et al.*, 2018c). Our analysis of the available intraspecific tests of the HZH in lizards suggested the existence of phylogenetic bias because only five lizard families were investigated. This indicates that the HZH remains underexplored in lizards, a taxonomic and ecologically wide group.

Interestingly, the analysis also provided evidence that positive relationships between colour patch expression and parasite infection (i.e. contrary to HZH) were significantly more frequent in studies analysing infections by protozoans. This positive relationship might be understood from a co-evolutionary perspective. Parasite virulence, measured as the cost of infection to the fitness of the host, is strongly influenced by the life-history strategies of both the host and the parasite (Gandon *et al.*, 2002; Barrett *et al.*, 2008; Paterson & Blouin-Demers, 2020). Protozoan parasites can produce chronic infections, but they can also replicate rapidly in lizards subjected to acute stress (Megía-Palma *et al.*, 2020). In this sense, a high investment in nuptial coloration and reproduction might increase replication of parasitic protozoans in lizards (Sorci *et al.*, 1996).

The seasonal production of nuptial coloration in lizards follows an increase in testosterone secretion (Cooper *et al.*, 1987; Salvador *et al.*, 1996; Cox & John-Alder, 2007). An increase in this steroid is associated with increased expression of nuptial coloration, but also metabolic rate, hence oxidative status, which is likely to have an immunosuppressant effect in lizards (Belliure *et al.*, 2004; Han *et al.*, 2020), rendering them more susceptible to some infections (Salvador *et al.*, 1996). However, selection might reward individuals that maximize their investment in nuptial coloration because this, although increasing susceptibility to some parasites, also increases their chances of reproduction (Zahavi, 1975; Díaz, 1993).

In support of these ideas, infections by protozoan parasites increased in lizards subjected experimentally to both environmental and endogenous sources of stress (Oppliger *et al.*, 1998; Megía-Palma *et al.*, 2020), although a direct effect of testosterone on the increase of blood parasitaemia in lizards remains unclear (reviewed by Roberts *et al.*, 2004; but also see Veiga *et al.*, 1998).

The putative presence of pterines, synthesized endogenously, in the nuptial coloration of *P. algirus* opens an exciting avenue of research that has scarcely been explored in lizards (Weiss *et al.*, 2012; Cuervo & Belliure, 2013; Merkling *et al.*, 2018; Andrade *et al.*, 2019). Previous studies in *P. algirus* successfully increased the orange pigmentation after testosterone supplementation (i.e. Salvador *et al.*, 1996), suggesting that pterin deposition is under hormonal control in this species, and found that mounting an immune response provokes a reduction in the intensity and extent of the orange patch (Llanos-Garrido *et al.*, 2017). This is a striking finding because it challenges previous assumptions of the relatively low energetic costs of producing pterin-based ornaments (Kemp *et al.*, 2011; Weiss *et al.*, 2011). In this sense, further exploration of the HZH might also account for costs of pigments involved in colour patches. Parasites might reduce the expression of colour patches if these are based on pigments costly to obtain, whereas colour patches based on 'cheaper' pigments might be produced by lizards despite infection by some parasites. However, this might not be a straightforward relationship because other factors, namely the availability of carotenoids in the environment (e.g. Grether *et al.*, 2005; Stuart-Fox *et al.*, 2021) or the prevalence and virulence of the associated parasites (Megía-Palma *et al.*, 2018c), might influence the relationship between colour expression and parasite infections.

Although 'brightness' was adopted to refer to human-based perception of the overall intensity of light reflected, this term is used inconsistently and sometimes confused with colour saturation (chroma) (Kemp *et al.*, 2015). We support previous authors who advocate use of 'luminance', an unequivocal term that refers to the simple quantification of the amount of light reflected by a colour stimulus (Kemp *et al.*, 2015).

In conclusion, our results support the idea that poorly pigmented patches have higher spectral brightness than fully pigmented colour patches (e.g. Cuervo *et al.*, 2016). Our findings support doubts about the generality of HZH with respect to parasite-mediated sexual selection across taxa obtained by Poulin & Hamilton (1997), highlighting that 58% of studies in lizards contradict the predictions of the HZH. However, other parasite–colour associations

might support the hypothesis even in the same lizard species, especially in cases of infection by parasites other than protozoans.

## ACKNOWLEDGEMENTS

Critical comments from two referees contributed to improvements in the manuscript. Centro de Montes y Aserradero de Valsaín and Junta de Castilla y León (SENEP/redmarca, Expte: EP/SG/625/2011; and Nª/riqosapa, Expte: EP/SG/213/2013) and Comunidad de Madrid (10/165944.9/18 and Ref.: JLZ/jwc) authorized sampling. Ministerio de Economía y Competitividad/European Agricultural Fund for Rural Development funded this research with projects CGL2012-40026-C02 (granted to S.M. and J.M.) and CGL2015-67789-C2-1-P (granted to S.M.). R.M.-P. enjoys a postdoctoral contract (CEECIND/04084/2017) provided by ICETA—Instituto de Ciências, Tecnologias e Agroambiente da Universidade do Porto and Fundação da Ciência e Tecnologia. R.B. enjoys a postdoctoral grant from Comunidad de Madrid (2018T1/AMB10374). J. A. Fargallo (Consejo Superior de Investigaciones Científicas [CSIC]) provided an *L. schreiberi* killed by a raptor. We are grateful to J. A. Hernández-Agüero (Universidad Rey Juan Carlos), who helped during the field season of 2013, E. Gabandé, who gently supplied us with Drosophila flies, and D. Luis Cuadra (CSIC), who performed the analyses of chromatography. R.M.-P., J.M. and S.M. conceived ideas and designed the methodology. R.M.-P. and R.B. collected the data. M.G. performed the histological work. R.M.-P. analysed the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

## REFERENCES

**Andrade P**, **Pinho C**, **Pérez i de Lanuza G**, **Afonso S**, **Brejcha J**, **Rubin CJ**, **Wallerman O**, **Pereira P**, **Sabatino SJ**, **Bellati A**, **Pellitteri-Rosa D**, **Bosakova Z**, **Bunikis I**, **Carretero MA**, **Feiner N**, **Marsik P**, **Paupério F**, **Salvi D**, **Soler L**, **While GM**, **Uller T**, **Font E**, **Andersson L**, **Carneiro M. 2019.** Regulatory changes in pterin and carotenoid genes underlie balanced colour polymorphisms in the wall lizard. *Proceedings of the National Academy of Sciences of the United States of America* **116:** 5633–5642.

**Balenger SL**, **Zuk M. 2014.** Testing the Hamilton–Zuk hypothesis: past, present, and future. *Integrative and Comparative Biology* **54:** 601–613.

**Barrett LG**, **Thrall PH**, **Burdon JJ**, **Linde CC. 2008.** Life history determines genetic structure and evolutionary potential of host–parasite interactions. *Trends in Ecology & Evolution* **23:** 678–685.

**Belliure J**, **Smith L**, **Sorci G. 2004.** Effect of testosterone on T cell-mediated immunity in two species of Mediterranean lacertid lizards. *Journal of Experimental Zoology Part A: Comparative Experimental Biology* **301:** 411–418.

**Berglund A**, **Bisazza A**, **Pilastro A. 1996.** Armaments and ornaments: an evolutionary explanation of traits of dual utility. *Biological Journal of the Linnean Society* **58:** 385–399.

**Burley NT**, **Hamedani E**, **Symanski C. 2018.** Mate choice decision rules: Trait synergisms and preference shifts. *Ecology and Evolution* **8:** 2380–2394.

**Calisi RM**, **Malone JH**, **Hews DK. 2008.** Female secondary coloration in the Mexican boulder spiny lizard is associated with nematode load. *Journal of Zoology* **276:** 358–367.

**Cook EG**, **Murphy TG**, **Johnson MA. 2013.** Colourful displays signal male quality in a tropical anole lizard. *Naturwissenschaften* **100:** 993–996.

**Cooper WE Jr**, **Mendonca MT**, **Vitt LJ. 1987.** Induction of orange head coloration and activation of courtship and aggression by testosterone in the male broad-headed skink (*Eumeces laticeps*). *Journal of Herpetology* **21:** 96–101.

**Cox RM**, **John-Alder HB. 2007.** Increased mite parasitism as a cost of testosterone in male striped plateau lizards *Sceloporus virgatus*. *Functional Ecology* **21:** 327–334.

**Cox RM**, **Zilberman V**, **John-Alder HB. 2008.** Testosterone stimulates the expression of a social color signal in Yarrow's spiny lizard, *Sceloporus jarrovii*. *Journal of Experimental Zoology. Part A: Ecological and Integrative Physiology* **309A:** 505–514.

**Cuervo JJ**, **Belliure J. 2013.** Exploring the function of red coloration in female spiny-footed lizards (*Acanthodactylus erythrurus*): patterns of seasonal colour change. *Amphibia-Reptilia* **34:** 525–538.

**Cuervo JJ**, **Belliure J**, **Negro JJ. 2016.** Coloration reflects skin pterin concentration in a red-tailed lizard. *Comparative Biochemistry and Physiology. Part B: Biochemistry and Molecular Biology* **193:** 17–24.

**Dale J**, **Dey CJ**, **Delhey K**, **Kempenaers B**, **Valcu M. 2015.** The effects of life history and sexual selection on male and female plumage coloration. *Nature* **527:** 367–370.

**Deutsch JC. 1997.** Colour diversification in Malawi cichlids: evidence for adaptation, reinforcement or sexual selection? *Biological Journal of the Linnean Society* **62:** 1–14.

**Díaz JA. 1993.** Breeding coloration, mating opportunities, activity, and surivival in the lacertid lizard *Psammodromus algirus*. *Canadian Journal of Zoology* **71:** 1104–1110.

**Endler JA. 1990.** On the measurement and classification of colour in studies of animal colour patterns. *Biological Journal of the Linnean Society* **41:** 315–352.

**Fitze PS**, **Cote J**, **San-José LM**, **Meylan S**, **Isaksson C**, **Andersson S**, **Rossi JM**, **Clobert J. 2009.** Carotenoid-based colors reflect the stress response in the common lizard. *PLoS One* **4:** e5111.

**Fleishman LJ**, **Bowman M**, **Saunders D**, **Miller WE**, **Rury MJ**, **Loew ER. 1997.** The visual ecology of Puerto Rican anoline lizards: habitat light and spectral sensitivity. *Journal of Comparative Physiology A. Neuroethology, sensory, neural, and behavioral physiology* **181:** 446–460.

**Folstad I**, **Hope AM**, **Karter A**, **Skorping A. 1994.** Sexually selected color in male sticklebacks: a signal of both parasite exposure and parasite resistance? *Oikos* **69:** 511–515.

**Gallardo M**, **Monsalve M**, **Valladolid M. 2015.** Dos procedimientos para el estudio de las plumas en microscopía óptica. *Boletín de la Real Sociedad Española de Historia Natural, Sección Biología* **109:** 65–69.

**Gandon S**, **Agnew P**, **Michalakis Y. 2002.** Coevolution between parasite virulence and host life-history traits. *The American Naturalist* **160:** 374–388.

**Grether GF**, **Cummings ME**, **Hudon J. 2005.** Countergradient variation in the sexual coloration of guppies (*Poecilia reticulata*): drosopterin synthesis balances carotenoid availability. *Evolution; international journal of organic evolution* **59:** 175–188.

**Hamilton WD**, **Zuk M. 1982.** Heritable true fitness and bright birds: a role for parasites? *Science* **218:** 384–387.

**Han X**, **Hao X**, **Wang Y**, **Wang X**, **Teng L**, **Liu Z**, **Zhang F**, **Zhang Q. 2020.** Experimental warming induces oxidative stress and immunosuppression in a viviparous lizard, *Eremias multiocellata*. *Journal of Thermal Biology* **90:** 102595.

**Jacot A**, **Romero-Díaz C**, **Tschirren B**, **Richner H**, **Fitze PS. 2010.** Dissecting carotenoid from structural components of carotenoid-based coloration: a field experiment with great tits (*Parus major*). *The American Naturalist* **176:** 55–62.

**Kemp DJ**, **Herberstein ME**, **Fleishman LJ**, **Endler JA**, **Bennett ATD**, **Dyer AG**, **Hart NS**, **Marshall J**, **Whitin MJ. 2015.** An integrative framework for the appraisal of coloration in nature. *The American Naturalist* **185:** 705–724.

**Kemp DJ**, **Herberstein ME**, **Grether GF. 2011.** Unrevealing the true complexity of costly color signals. *Behavioral Ecology* **23:** 233–236.

**Khoo HE**, **Prasad KN**, **Kong KW**, **Jiang Y**, **Ismail A. 2011.** Carotenoids and their isomers: color pigments in fruits and vegetables. *Molecules* **16:** 1710–1738.

**Kopena R**, **López P**, **Majlathova V**, **Martín J. 2020.** Sexually dichromatic coloration of female Iberian green lizards correlates with health state and reproductive investment. *Behavioral Ecology and Sociobiology* **74:** 1–12.

**Laczi M**, **Hegyi G**, **Kötél D**, **Csizmadia T**, **Lőw P**, **Török J. 2019.** Reflectance in relation to macro- and nanostructure in the crown feathers of the great tit (*Parus major*). *Biological Journal of the Linnean Society* **127:** 113–124.

**Langkilde T**, **Boronow KE. 2010.** Color as a signal: the relationship between coloration and morphology in male Eastern fence lizards, *Sceloporus undulatus*. *Journal of Herpetology* **44:** 261–271.

**Lessells CM**, **Boag PT. 1987.** Unrepeatable repeatabilities: a common mistake. *The Auk* **104:** 116–121.

**Lindsay WR**, **Wapstra E**, **Silverin B**, **Olsson M. 2016.** Corticosterone: a costly mediator of signal honesty in sand lizards. *Ecology and Evolution* **6:** 7451–7461.

**Llanos-Garrido A**, **Díaz JA**, **Pérez-Rodríguez A**, **Arriero E. 2017.** Variation in male ornaments in two lizard populations with contrasting parasite loads. *Journal of Zoology* **303:** 218–225.

**Marshall KL**, **Philpot KE**, **Damas-Moreira I**, **Stevens M. 2015.** Intraspecific colour variation among lizards in distinct island environments enhances local camouflage. *PLoS One* **10:** e0135241.

**Martín J**, **Amo L**, **López P. 2008.** Parasites and health affect multiple sexual signals in male common wall lizards, *Podarcis muralis*. *Naturwissenschaften* **95:** 293–300.

**McGraw KJ**, **Wakamatsu K**, **Ito S**, **Nolan PM**, **Jouventin P**, **Dobson FS**, **Austic RE**, **Safran RJ**, **Siefferman LM**, **Hill GE**, **Parker RS. 2004.** You can't judge a pigment by its color: carotenoid and melanin content of yellow and brown feathers in swallows, bluebirds, penguins, and domestic chickens. *Condor* **106:** 390–395.

**Megía-Palma R**, **Arregui L**, **Pozo I**, **Žagar A**, **Serén N**, **Carretero MA**, **Merino S. 2020.** Geographic patterns of stress in insular lizards reveal anthropogenic and climatic signatures. *Science of the Total Environment* **749:** 141655.

**Megía-Palma R**, **Jorge A**, **Reguera S. 2018a**. Raman spectroscopy reveals the presence of both eumelanin and pheomelanin in the skin of lacertids. *Journal of Herpetology* **52:** 67–73.

**Megía-Palma R**, **Martínez J**, **Merino S. 2016a.** A structural colour ornament correlates positively with parasite load and body condition in an insular lizard species. *The Science of Nature* **103:** 52.

**Megía-Palma R**, **Martínez J**, **Merino S. 2016b.** Structural- and carotenoid-based throat color patches in males of *Lacerta schreiberi* reflect different parasitic diseases. *Behavioral Ecology and Sociobiology* **70:** 2017–2025.

**Megía-Palma R**, **Martínez J**, **Merino S. 2018b.** Manipulation of parasite load induces significant changes in the structural-based throat color of male Iberian green lizards. *Current Zoology* **64:** 293–302.

**Megía-Palma R**, **Paranjpe D**, **Reguera S**, **Martínez J**, **Cooper RD**, **Blaimont P**, **Merino S**, **Sinervo B. 2018c.** Multiple color patches and parasites in *Sceloporus occidentalis*: differential relationships by sex and infection. *Current Zoology* **64:** 703–711.

**Merilä J**, **Sheldon BC**, **Lindström K. 1999.** Plumage brightness in relation to haematozoan infections in the greenfinch *Carduelis chloris*: bright males are a good bet. *Écoscience* **6:** 12–18.

**Merkling T**, **Chandrasoma D**, **Rankin KJ**, **Whiting MJ. 2018.** Seeing red: pteridine-based colour and male quality in a dragon lizard. *Biological Journal of the Linnean Society* **124:** 677–689.

**Millar RB**, **Anderson MJ. 2004.** Remedies for pseudoreplication. *Fisheries Research* **70:** 397–407.

**Molnár O**, **Bajer J**, **Mészáros B**, **Török J**, **Herczeg G. 2013.** Negative correlation between nuptial throat colour and blood parasite load in male European green lizards supports the Hamilton–Zuk hypothesis. *Naturwissenschaften* **100:** 551–558.

**Montgomerie R. 2006**. Quantifying colors. In: Hill GE, McGraw KJ, eds. *Bird coloration, vol 1. Mechanism and measurements*. Cambridge: Harvard University Press, 41–89.

**Montgomerie R. 2008.** *CLR 1.1 software. Analyzing colours*. Available at: https://bobmontgomerie.wordpress.com/resources/analyzing-colours/

**Olsson M. 1994.** Nuptial coloration in the sand lizard, *Lacerta agilis*: an intra-sexually selected cue to fighting ability. *Animal Behaviour* **48:** 607–613.

**Oppliger A**, **Clobert J**, **Lecomte J**, **Lorenzon P**, **Boudjemadi K**, **John-Alder H. 1998.** Environmental

stress increases the prevalence and intensity of blood parasite infection in the common lizard *Lacerta vivipara*. *Ecology Letters* **1:** 129–138.

**Orton RW**, **Kinsey CT**, **McBrayer LD. 2020.** Mite load predicts the quality of sexual color and locomotor performance in a sexually dichromatic lizard. *Ecology and Evolution* **10:** 3152–3163.

**Paterson JE**, **Blouin-Demers G. 2020.** High tolerance of two parasites in ornate tree lizards reduces the fitness costs of parasitism. *Journal of Zoology* **312:** 102–110.

**Pérez i de Lanuza G**, **Carazo P**, **Font E. 2014.** Colours of quality: structural (but not pigment) coloration informs about male quality in a polychromatic lizard. *Animal Behaviour* **90:** 73–81.

**Pérez i de Lanuza G**, **Font E. 2014.** Now you see me, now you don't: iridiscence increases the efficacy of lizard chromatic signals. *Naturwissenschaften* **101:** 831–837.

**Pérez i de Lanuza G**, **Font E**, **Monterde JL. 2013.** Using visual modelling to study the evolution of lizard coloration: sexual selection drives the evolution of dichromatism in lacertids. *Journal of Evolutionary Biology* **26:** 1826–1835.

**Poulin R**, **Hamilton WJ. 1997.** The Hamilton and Zuk hypothesis revisited: a meta-analytical approach. *Behaviour* **134:** 299–320.

**Quinn VS**, **Hews DK. 2003.** Positive relationship between abdominal coloration and dermal melanin density in phrynosomatid lizards. *Copeia* **2003:** 858–864.

**Ressel S**, **Schall JJ. 1989.** Parasites and showy males: malarial infection and color variation in fence lizards. *Oecologia* **78:** 158–164.

**Roberts ML**, **Buchanan KL**, **Evans MR. 2004.** Testing the immunocompetence handicap hypothesis: a review of the evidence. *Animal Behaviour* **68:** 227–239.

**Sacchi R**, **Rubolini D**, **Gentilli A**, **Pupin F**, **Razzetti E**, **Scali S**, **Galeotti P**, **Fasola M. 2007.** Morph-specific immunity in male *Podarcis muralis*. *Amphibia-Reptilia* **28:** 408–412.

**Saenko SV**, **Teyssier J**, **van der Marel D**, **Milinkovitch M. 2013.** Precise colocalization of interacting structural and pigmentary elements generates extensive color pattern variation in *Phelsuma* lizards. *BMC Biology* **11:** 105.

**Saks L**, **McGraw K**, **Hõrak P. 2003.** How feather colour reflects its carotenoid content. *Functional Ecology* **17:** 555–561.

**Salvador A**, **Veiga JP**, **Martin J**, **Lopez P**, **Abelenda M**, **Puerta M. 1996.** The cost of producing a sexual signal: testosterone increases the susceptibility of male lizards to ectoparasitic infestation. *Behavioral Ecology* **7:** 145–150.

**Schall JJ. 1986.** Prevalence and virulence of a haemogregarine parasite of the Aruban whiptail lizard, *Cnemidophorus arubensis*. *Journal of Herpetology* **20:** 318–324.

**Seddon RJ**, **Hews DK. 2020.** Melanization, α-melanocyte stimulating hormone and steroid hormones in male western fence lizards from nine populations. *General and Comparative Endocrinology* **285:** 113287.

**Sinervo B**, **Lively CM. 1996.** The rock–paper–scissors game and the evolution of alternative male strategies. *Nature* **380:** 240–243.

**Sorci G**, **Clobert J**, **Michalakis Y. 1996.** Cost of reproduction and cost of parasitism in the common lizard, *Lacerta vivipara*. *Oikos* **76:** 121–130.

**Stapley J**, **Whiting M. 2006.** Ultraviolet signals fighting ability in a lizard. *Biology Letters* **2:** 169–172.

**Steffen JE**, **McGraw KJ. 2007.** Contributions of pterin and carotenoid pigments to dewlap coloration in two anole species. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **146:** 42–46.

**Steffen JE**, **McGraw KJ. 2009.** How dewlap color reflects its carotenoid and pterin content in male and female brown anoles (*Norops sagrei*). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **154:** 334–340.

**Stoehr AM**, **McGraw KJ. 2001.** Ultraviolet reflectance of color patches in male *Sceloporus undulatus* and *Anolis carolinensis*. *Journal of Herpetology* **35:** 168–171.

**Stuart-Fox D**, **Rankin K**, **Lutz A**, **Elliott A**, **Hugall A**, **McLean C**, **Medina I. 2021.** Pteridine pigments compensate for environmental availability of carotenoids. *Authorea Preprints*.

**Teasdale LC**, **Stevens M**, **Stuart-Fox D. 2013.** Discrete colour polymorphism in the tawny dragon lizard (*Ctenophorus decresii*) and differences in signal conspicuousness among morphs. *Journal of Evolutionary Biology* **26:** 1035–1046.

**Tullberg BS**, **Merilaita S**, **Wiklund C. 2005.** Aposematism and crypsis combined as a result of distance dependence: functional versatility of the colour pattern in the swallowtail butterfly larva. *Proceedings of the Royal Society B: Biological Sciences* **272:** 1315–1321.

**Václav R**, **Prokop P**, **Fekiač V. 2007.** Expression of breeding coloration in European green lizards (*Lacerta viridis*): variation with morphology and tick infestation. *Canadian Journal of Zoology* **85:** 1199–1206.

**Veiga JP**, **Salvador A**, **Merino S**, **Puerta M. 1998.** Reproductive effort affects immune response and parasite infection in a lizard: a phenotypic manipulation using testosterone. *Oikos* **82:** 313–318.

**Weiss SL. 2006.** Female-specific color is a signal of quality in the striped plateau lizard (*Sceloporus virgatus*). *Behavioral Ecology* **17:** 726–732.

**Weiss SL**, **Foerster K**, **Hudon J. 2012.** Pteridine, not carotenoid, pigments underlie the female-specific orange ornament of striped plateau lizards (*Sceloporus virgatus*). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **161:** 117–123.

**Weiss SL**, **Kennedy EA**, **Safran RJ**, **McGraw KJ. 2011.** Pterin-based ornamental coloration predicts yolk antioxidant levels in female striped plateau lizards (*Sceloporus virgatus*). *Journal of Animal Ecology* **80:** 519–527.

**Zahavi A. 1975.** Mate selection—a selection for a handicap. *Journal of Theoretical Biology* **53:** 205–214.

**Zirpoli JA**, **Black JM**, **Gabriel PO. 2013.** Parasites and plumage in Steller's jays: an experimental field test of the parasite-mediated handicap hypothesis. *Ethology Ecology & Evolution* **25:** 103–116.

**Zucker N. 1988.** A method for rapid and precise documentation of lizard coloration. *Herpetological Reviews* **19:** 13–16.