

Melanin-based colouration as a potential indicator of male quality in the lizard *Zootoca vivipara* (Squamata: Lacertidae)

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Abstract. In many animals, aspects of colouration are hypothesized to convey information on the body condition or quality of individuals. This idea has been tested primarily for the carotenoid-based component of body colouration. The significance of other pigments in this context has received far less attention. In the common lizard, *Zootoca vivipara*, the degree of black patterning on the ventrum and throats is sexually dimorphic and varies considerably among individuals. In this study, we examine whether this melanin-based component of body colouration may reflect individual differences in quality (SVL, condition, immune response). We find that males (but not females) with a higher degree of ventral patterning mount a stronger phytohemagglutinin-induced immune response. The amount of black patterning does not correlate with body size, body condition, aspects of dorsal colouration or parasite load. We conclude that in male *Zootoca vivipara*, melanin-based ventral colouration may signal an aspect of immune capacity to sexual rivals or potential partners.

Keywords: melanism, reptile, sexual selection, signal function.

Introduction

Sexual dichromatism, the condition in which males and females of a species show distinct colours or colour patterns, is common in many animal groups, including amphibians (Bell and Zamudio, 2012) and reptiles (Cooper and Greenberg, 1992). Several hypotheses have been proposed to explain the phenomenon. First, the differences may serve in sex recognition. Mate search is a dangerous and a time- and energy-consuming activity, and therefore misdirected sexual behaviour should be avoided as much as possible. In visually oriented species, colours may help in doing so. However, the sex recognition hypothesis cannot explain why in most species, one of the sexes (usually the male) carries bright and conspicuous colours, while the other sex (usually the female) is more dully coloured. In these cases, sexual dichromatism is generally thought to reflect sex-specific natural

selection and sexual selection. In the choosing sex, natural selection favours the dull colours that aid crypsis and thermoregulation; in the displaying sex, sexual selection may promote bright, prominent colours that increase mating success.

Sexual selection models of the origin of sexual dichromatism assume that the colours of the displaying sex reliably communicate some aspect(s) of the signaller or its environment (Searcy and Nowicki, 2005). For a signal to be reliable, producing it must imply a fitness cost that can only be carried by individuals of sufficient quality or in good condition (Zahavi, 1975). In the case of colours, a relationship between signalling intensity and individual quality may arise if pigments are in limited supply, or expensive to produce or maintain. Because they seem to fulfil these conditions, carotenoids (the pigments responsible for yellow, red and orange colouration of many vertebrates) have received considerable attention in this respect (Olson and Owen, 1998). Most animals cannot synthesise carotenoids *de novo* and must obtain them through their diet (Griffith, Parker and Olson, 2006). In carotenoid-poor environments, yellow/red/orange colouration may thus signal foraging efficiency. In addition, carotenoids play a

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part in enhancing immune response and reducing oxidative stress, and investments in colourful ornaments must thus be traded off against these functions. Several studies have suggested a role of carotenoid-based pigmentation in mate selection in reptiles (e.g. Kwiatkowski and Sullivan, 2002; Fitze et al., 2009) and amphibians (e.g. Ogilvy, Preziosi and Fidgett, 2012).

Several other classes of biological pigments affect body colours and colour patterns in amphibians and reptiles (Cooper and Greenberg, 1992), but these have received less attention in a signal-evolution context (Font, Pérez i de Lanuze and Sampredo, 2009; Weiss, Foerster and Hudon, 2012). Recent studies on *Anolis* (Steffen and McGraw, 2009) and *Sceloporus* (Weiss, Foerster and Hudon, 2012) lizards suggest a possible role for pteridine pigments. Melanins could also be relevant to indicator mechanism methods. Although melanins can in principle be assembled *de novo* from amply available aminoacid precursors, their production seems to require a careful management of the antioxidant machinery (Garratt and Brooks, 2012). In contrast to earlier beliefs, melanins may thus fulfil the role of honest indicators of health status (Stoehr, 2006). Moreover, it has been shown that in birds and mammals, melanin-based pigmentation correlates with a plethora of physiological, behavioural and immunological variables (Ducrest, Keller and Roulin, 2006), and melanin-based signals are used in social contexts (e.g. Senar and Quesada, 2006; Roulin and Altweg, 2007; McGraw, 2008; Roulin, 2009).

In this study, we examine the possible role of ventral black patterning in a lizard, *Zootoca vivipara*, as a signal of gender and individual quality. We examine in how far adult males and females of this species differ in colouration and colour patterning, and whether interindividual differences in these variables correlate with proxies of quality (body size, body mass, condition, ectoparasite load and immune response). A substantial amount of research on body colour variation of *Zootoca vivipara* has

revealed the complexity of its causation and of its meaning in social signalling (Meylan et al., 2007; Fitze et al., 2009; Lepetz et al., 2009; Cote et al., 2010; San-Jose et al., 2012, 2013). However, the bulk of these studies have centred on the carotenoid-based component of colouration. Much less is known on the role of melanin-based pigmentation.

Material and methods

Study species

Zootoca vivipara is a small, diurnal lacertid lizard (adult snout-vent length [SVL]: 40–60 mm). Ranging over much of the Eurasian continent, it has the largest geographical distribution of all reptile species. It inhabits a wide variety of humid habitats, including meadows, heathlands and peat-bogs. The individuals used in this study (37 adult males, 14 females), were captured by hand in Brecht (Belgium) during the mating season, between 30 March 2011 and 6 April 2011. Lizards were transported individually in cloth bags to the University of Antwerp, where they were placed separately in terrariums (20 × 30 × 20 cm) and were given water and food (vitamin-dusted crickets *Acheta domestica*) *ad libitum*. After experimentation, all lizards were released at the exact site of capture.

General procedures

Snout-vent length (SVL) was measured with digital calipers (Mitutoyo, Kawasaki, Japan; accuracy: 0.01 mm) and body mass was determined using an electronic balance (Scout Pro SPU202, Ohaus Corporation, New Jersey, USA; accuracy: 0.01 g). Body condition was calculated as the residual of the linear regression of SVL to body mass (both log₁₀ transformed). Body condition has been shown to correlate with energy storage, general health, male attractiveness and reproductive success in reptiles (Bonnert and Naulleau, 1994; Forsman and Lindell, 1996; Lopez, Muñoz and Martin, 2002; Hofmann and Henle, 2006; Salvador et al., 2007).

Prior to the morphometric measurements, we counted the number of ticks (*Ixodes ricinus*) present on the lizards. Ectoparasite estimates are available only for the lizards caught on the second day (35 individuals). Ticks were the only macroscopic ectoparasites observed on the lizards. We used a standard protocol (detailed in Oppliger, 2004) to estimate haemogregarine blood parasite numbers, but because none of the smears contained parasites, we did not consider this variable in further analyses.

We used the skin swelling test to assess the phytohemagglutinin (PHA) immune response. We injected 15 μl PHA solution (30 mg PHA solved in 5 ml PBS) subcutaneously in the left hind footpad. As a control, an equal amount of pure PBS solution was injected into the right hind footpad.

The thickness of the footpads was measured shortly before and 24 h after the injection, using digital callipers (Mitutoyo, Kawasaki, Japan; accuracy: 0.01 mm). On both occasions, the footpad was measured thrice and the median of these measurements was used in subsequent analyses. The immune response was calculated as the change in thickness of the PHA injected foot minus the change in the control foot. The animals showed no signs of stress or pain during these experiments. This technique is commonly used to assess the immunocompetence in lizards (e.g. Oppliger et al., 2004; Calsbeek et al., 2008; Huyghe et al., 2010). The PHA-induced swelling mirrors the individual general pro-inflammatory potential (Vinkler et al., 2010) and is related to heightened immune cell activity, but may involve both innate and acquired immunity (Martin et al., 2006). Therefore the test may not constitute an unambiguous index of T-cell mediated immunity per se (but see Tella et al., 2008 and Munoz et al., 2009), but can be considered a multi-faceted index of the cutaneous immune activity (Martin et al., 2006). We used the skin-swelling test in this study because we wanted to obtain a standardized index of immunocompetence, independent of the immune cells involved.

Colour patterning

Common lizards vary considerably in the amount of ventral black patterning (fig. 1). To obtain an individual index of degree of blackening, we photographed the ventrum and throat of each lizard three times using a digital reflex camera (Nikon D70s, Japan) fitted with a 105 mm micro-nikkor lens

(Nikon, Japan). Lizards were placed on their dorsum, so that viewing angle was always perpendicular to the ventrum or throat. All pictures were taken by the same person, in standard light conditions and using the same background and distance.

The pictures were uploaded in the GNU Image Manipulation Program, GIMP 2.6 (GNU project, <http://www.gimp.org>) and the procedure outlined by Ounpraseuth et al. (2009) was used to obtain an estimate of how much of the lizard's ventral surface and collaria were coloured black.

Because we took three photographs of each lizard, we were able to assess the repeatability of the method used to estimate the degree of black patterning. Following procedures outlined by Lessells and Boag (1987), we obtained a repeatability of 98.3% ($N = 50$). In subsequent analyses, we used the mean of the three measurements obtained for each individual to index the degree of dark patterning for that individual.

Reflectance measurements

The reflectance spectra of the lizards were measured with an Avantes spectrometer (AvaSpec-2048-USB2-UA-50 (range 250-1000 nm), Avantes, Eerbeek, The Netherlands). The probe was held perpendicular to the skin surface. The measurements were expressed relative to a white reference tile (WS2, Avantes, Eerbeek, The Netherlands). The lizards' colouration was assessed by measuring the reflectance at thirteen positions: on the head (spot 1); dorsal, between the fore limbs (spot 2); mid-mid on the dorsum (spot 3); mid-lateral (both left and right; spots 4 and 5); lateral (both left



Figure 1. Pictures of the ventrum of two male and one female *Zootoca vivipara*, demonstrating the extent of the variation in the percentage of ventral melanin-based colouration. This figure is published in colour in the online version.

and right; spots 6 and 7); dorsal, between the hind limbs (spot 8); throat (gular region, spot 9); ventral, between the fore limbs (spot 10); mid-mid on the ventrum (spot 11); ventral, between the hind limbs (spot 12) and ventral, on the tail base (spot 13). The reference tile was measured between each individual. For the dorsum and ventrum, we calculated respectively the mean reflectance of spot 1 till 8 and spot 10 till 13 for every individual between 300-700 nm. For the throat (spot 9), we also used only the reflectance between 300 and 700 nm.

Following Endler (1990) and San-José et al. (2013), we calculated the following colour variables for each body region: brightness (mean reflectance between 300-700 nm, $R_{300-700}$), UV-reflectance ($R_{300-400}$), violet-blue reflectance ($R_{400-515}$), absolute carotenoid chroma ($R_{\text{violet-blue}}/R_{\text{background}}$) and hue (the wavelength with maximal reflectance, $\lambda_{R_{\text{max}}}$).

Statistical analyses

All statistical analyses were performed in IBM SPSS Version 20 (IBM Software group, Chicago, USA). We used the Shapiro-Wilk statistic to test for normality. Variables that were not normally distributed were transformed. If transformation failed to normalize data, non-parametric tests were used. We used principal component analyses (on the correlation matrix, using the VARIMAX rotation) to condense the information on body color variation into a manageable number of composite variables. We employed Bartlett's test to assess the significance of the first component (Peres-Neto et al., 2005), and the broken stick procedure for judging the relevance of subsequent axes (Jackson, 1993). Factor loadings with absolute values above 0.7 were considered significant. We ran principal component analyses with data for the two sexes combined, and for males and females separately.

Because the degree of black patterning differed strongly between the sexes, relationships between colouration and other individual variables were assessed separately for males and females. We used a forward stepwise linear modeling approach to predict variation in immune response, using SVL, mass, degree of ventral patterning and the scores on the three PC axes as potential predictors. The information criterion (AICC) was used as a criterion for entry/removal of effects (significance set at 0.05).

Table 1. Intersexual differences in morphometrics, immune response, black patterning and colour variables of the dorsum, ventrum and throat in *Zootoca vivipara*. PC1 to PC3 are average scores on the three axes generated by a principal component analysis on the raw colour variables (brightness, hue, relative reflectances in the UV and in violet-blue regions and absolute carotenoid chroma). Significant *P*-values are shown in bold.

	Males			Females			$F_{1,47}$	<i>P</i>
	x	sd	<i>n</i>	x	sd	<i>n</i>		
SVL (mm)	48.73	2.64	35	51.26	3.98	14	6.79	0.012
Body mass (g)	3.14	0.45	35	2.71	0.64	14	7.41	0.009
Swelling response	0.067	0.05	36	0.068	0.043	13	0.001	0.98
% black patterning (ventrum)	22.57	6.5	36	0.3	0.36	13	150.34	<0.0001
% black patterning (collar)	12.40	7.25	36	0.24	0.32	13	36.01	<0.0001
PC1 (throat colours)	0.12	0.98	36	-0.29	1.01	14	1.71	0.20
PC2 (dorsal colours)	0.21	0.16	36	-0.52	0.26	14	5.91	0.02
PC3 (ventral colours)	-0.99	0.92	36	0.25	1.19	14	1.22	0.27

Results

Sexual dimorphism

In our sample, males were on average smaller than females, but weighed more (table 1). The relationship between \log_{10} -transformed body mass and SVL was similar for the two sexes (ANCOVA, homogeneity of slopes: $F_{1,45} = 2.02$, $P = 0.162$), but for any given length, males were significantly heavier ($F_{1,46} = 44.84$, $P < 0.001$).

Males exhibited much more ventral and collar black patterning than females (table 1). In our sample, none of the females had more than 2% of their ventral surface covered with black, whereas the percentage of blackening in males varied between 6 and 39%.

Principal component analysis on the colour variables measured on all lizards resulted in three significant axes, which explained 42.7, 20.1 and 14.6% of the total variation respectively. The first axis described variation in brightness, UV-reflectance, violet-blue reflectance and absolute carotenoid chroma of the throat. The second and third axes related to the same variables, but for the dorsum and the ventrum, respectively. Only the scores on the second axis differed significantly between the sexes (table 1). Compared to females, males had a brighter dorsal colouration that reflected more in the violet-blue and UV-regions and exhibited higher absolute carotenoid chroma.

In females, percentage of ventral black patterning is unrelated to SVL ($r = 0.14$, $n = 13$, $P = 0.65$), body mass ($r = 0.221$, $P = 0.47$), or body condition ($r = 0.21$, $P = 0.49$). The variation in swelling response among females could not be predicted on the basis of SVL, body mass, colour (as indexed by the PCA scores) or ventral patterning (forward stepwise linear modelling, no predictors retained in the final model). The swelling response in females also did not correlate with tick load (Spearman rank correlation, $\rho = 0.231$, $P = 0.45$).

Discussion

Our results show that the European common lizard displays clear sexual dichromatism. Males tend to have a brighter dorsal colouration which reflects more in the UV and violet blue regions and has higher absolute carotenoid chroma than in females. However, the most obvious difference occurs in the amount of black patterning on the collar and ventrum, which is much more pronounced in males than in females. Sexual dichromatism in reptiles has been attributed to both natural and sexual selection.

Natural selection may produce dichromatism when sexes differ in predator avoidance strategies (e.g. Shine and Madsen, 1994). We did not estimate the degree of background matching in our study animals, so we cannot assess whether the differences reported here make either sex more vulnerable to visual hunting predators, and by how much. The females' duller colouration may render them more cryptic when not moving (as is the case in vipers, Shine and Madsen, 1994); this would certainly benefit their strategy of staying put when pregnant (Bauwens and Thoen, 1981). It is more difficult to understand how the males' bright colours and UV reflectance could evolve in response to natural selection (predation pressure). It also seems unlikely that the differences in ventral patterning originate from differential selection on crypsis, because this part of the body will rarely be visible to (aerial) predators. It seems more

likely that the differences in ventral colouration function in a sexual context, for instance in sex recognition.

Studies on a variety of species have demonstrated that lizards at least partly rely on colours to identify the sex of conspecifics (Cooper, 1984; Cooper and Burns, 1987; Regalado, 2003; Robertson and Rosenblum, 2010). A preliminary experimental study on *Z. vivipara* (Bauwens et al., 1987) showed that males use (but do not rely exclusively on) colouration to identify females. However, the study also suggests that a combination of ventral and dorsal pigmentations is required to elicit courtship in male *Z. vivipara*: males with bellies painted to resemble females were not identified as females. Our finding that males and females differ in colouration and patterning of different body parts seems to corroborate this assertion.

Most studies of sex recognition in squamates found that males and females differ in aspects of the colouration of body parts that are readily visible to conspecifics (dorsum, flanks) or that can be displayed by adopting special postures (e.g. throats, dewlaps, ventrolateral patches; Meyers et al., 2006; Whiting et al., 2006). In contrast, Cuervo and Shine (2007) argue that the coloured patches on the abdomens of water dragons *Physignathus lesueurii* are unlikely to function as gender signals, because their position makes them difficult to discern. This argument seems to apply to the ventral black patterning in our study species. Detailed observations will have to verify if, during intraspecific encounters, common lizards somehow expose their ventrum to signal their sex (or other features). Alternatively, most signalling may occur through collar (and gular) pigmentation.

There is ample evidence that colours may reveal more about an individual lizard than just its gender identity. In a variety of species, colours function as armaments, signalling dominance and/or fighting capacity to sexual rivals (e.g. Meyers et al., 2006; Stapley and Whiting, 2006; Bajer, 2011). Lizard colours may also, and perhaps simultaneously (Meyers et al., 2006), op-

erate as ornaments, communicating aspects of a male's quality to females (Healy et al., 2008; Swierk, Ridgway and Langkilde, 2012). Is it possible that the colours or colour patterns in male common lizards act as sexual ornaments or armaments?

Although not territorial, male *Z. vivipara* will behave aggressively to other males, especially in the breeding season (Gvozdik and Van Damme, 2003). In lacertids, as in many animals, body size is a major component influencing the outcome of contests (e.g. Huyghe et al., 2005; Sacchi et al., 2009). Our results suggest that aspects of dorsal correlation (PC2) may signal body size. It would be interesting to stage fights between common lizard males that exhibit natural variation in dorsal colour patterns, or to manipulate the colours experimentally and observe the effect on the individual's dominance status. In contrast, we did not find a relationship between SVL and the degree of ventral black patterning.

An alternative (but not mutually exclusive) hypothesis is that the colours and/or black patterning constitute ornaments. Until fairly recently, female mate choice in lizards was considered rare or non-existent (Tokarz, 1995; Olsson and Madsen, 1995; Hamilton and Sullivan, 2005), but in the last couple of years, it has become increasingly clear that in many species, females prefer to associate with males that have large bodies or heads (e.g. Karsten et al., 2009; Fitze et al., 2010), display frequently (Baird et al., 2007; Kelso and Martins, 2008) or exude particular chemical signals (e.g. Lopez and Martin, 2012). In a variety of lizard species, females rely at least partly on aspects of male colouration to select mates (e.g. Lancaster et al., 2009; Bajer et al., 2010). UV-reflectance (structural colouration, Bajer et al., 2010; LeBas and Marshall, 2000) and red/orange/yellow colouration (carotenoid pigmentation, Healey et al., 2008; Lancaster et al., 2009) seem especially informative. Our results may provide an example of a melanin-based intersexual signal. Males in our sample differed considerably in three inter-

related aspects that have been shown to connect to melanin production: ventral black patterning (Morrison, Rand and Frost-Mason, 1995; Quinn and Hews, 2003), dorsal UV and blue reflection (Quinn and Hews, 2003; Font, Pérez i de Lanuze and Sampedro, 2009) and immune response (Roulin, 2004; Gasparini et al., 2009a, b; Jacquin et al., 2011). Although melanins have received less attention in studies of sexual selection than have carotenoid pigments, recent work suggests that they are expensive to synthesise (Jawor and Breitwisch, 2003) and thus may signal individual quality or condition.

If UV-reflectance and black patterning are signalling an aspect of male quality to female common lizards, then it must be a feature unrelated to body size or condition (both of which are implicated in female mate choice in this species: Fitze et al., 2008; Fitze, Cote and Clobert, 2010; Richard et al., 2009), because we found no correlation between these variables and ventral pigmentation. Age is a candidate. In many species of lizards, males acquire a characteristic (ventral) colouration when reaching maturity (e.g. Pinto, Wiederhecker and Colli, 2005; Galan, 2008), possibly as a result of increasing levels of androgenic sexual hormones (Rand, 1992; Abell, 1998). Moreover, several studies have shown that aspects of colouration may signal age in adult male lizards and that females prefer older males. Female common lizards prefer middle-aged sexual partners (Richard et al., 2005, 2009), but it is unclear which traits (other than body size) they use to judge the age of the males. Melanin content in this species varies ontogenetically (common lizards are born completely black and acquire their adult colouration in their second year of life), but we know of no study that has shown that ventral blackening is a reliable indicator of age after maturation.

An attractive possibility is that the amount of ventral blackening is signalling immune response. The general idea linking sexual colour signals and immune function is that because pigments are costly to acquire or to produce and function as immune-enhancers and anti-

oxidants, only high quality males can afford to allocate these molecules to sexual ornamentation rather than to defence systems (Lozano, 1994; von Schantz et al., 1999; Blount et al., 2003). Colourful ornaments would thus function as honest signals of health. While the bulk of empirical work on this topic has centred on carotenoid-based traits, recent findings suggest that melanin-based colouration may evolve on similar grounds (Metcalfe and Alonso-Alvarez, 2010). The following simple predictions emerge from these ideas: (1) parasites should reduce the showiness of sexual signals and (2) flashy males should demonstrate higher immune responses. Our results do not support the former prediction: we found no relationship between the amount of black colouration and ectoparasite load. This contrasts with earlier findings in other lacertid species: in *Lacerta viridis*, saturation of blue throat colour in males decreases with increasing level of tick infestation (Vaclav et al., 2007); in *Psammodromus algirus*, males with larger patches of breeding colour proved more susceptible to ectoparasite infestation (Salvador et al., 1996); and in our study species, males with redder bellies carry smaller parasite loads (Cote et al., 2010). Our data do support the prediction that showy males (here more black patterning) should have higher immune responses. Similar results have been described for *Lacerta schreiberi*, in which males with higher UV-colouration on the chest mount higher PHA immune responses (Martin and Lopez, 2009). In *Z. vivipara*, males with redder bellies also have higher cellular immune responses (Cote et al., 2010). Our results thus suggest that in the common lizard, both carotenoid and melanin pigments are implicated in signalling aspects of immune capacity (and thus health or good genes).

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