

Multiple colour traits signal performance and immune response in the Dickerson's collared lizard *Crotaphytus dickersonae*

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Abstract Conspicuous male coloration is often an honest signal of individual quality. In Dickerson's collared lizard (*Crotaphytus dickersonae*), males are striking blue, have a black and white collar and have white spots on their bodies. In this study, we investigated whether this extravagant colour pattern is an indicator of male performance in variables related to resource-holding power (bite force, running speed and endurance) and immune condition (cellular response and ectoparasite load). Also, we assessed direct and indirect effects of testosterone levels and body temperature on colour traits. We found that males with bluer skin, and darker and bigger black collars performed better in variables related to resource-holding power. Also, the black colour of the collar was related to immune condition. Blue chroma was positively related to temperature, yet males with bigger and darker black collars had lower body temperatures. Testosterone had minor effects on immune response and colour traits. Our results suggest that multiple colour traits in the Dickerson's collared lizard may honestly indicate physical and physiological performance.

Keywords Honest signal · Coloration · Temperature · Testosterone · Immune condition · Resource-holding power

Introduction

According to sexual selection theory, in many species, conspicuous coloured males have evolved due to sexual selection (Anderson 1994). More colourful males are expected to have increased fitness, either because colour traits are favoured by mate choice or colour functions as a signal of status in intra-sexual competition. Communication via colour traits will be evolutionarily stable when the colour reflects performance, condition or genetic quality of the animal, and colour can thus be considered an honest signal (Guilford and Stamp Dawkins 1991). In theory, honest signalling is maintained when the potential costs of cheating are high (Számádó 2011) or when high- and low-quality individuals differ in some components of their physiological state or condition that influence their health, performance or ability to acquire resources, resulting in constraints or higher costs for lower quality individuals to produce and maintain a high intensity signal (Zahavi 1975, 1977; Hamilton and Zuk 1982; Maynard Smith 1991; Johnstone 1995). For example, conspicuous coloration can be an honest signal when only individuals in good condition can pay the costs of increased visibility to predators (Endler 1992; Martín and López 2001) or prey (Baird 2008). Additionally, development and maintenance of pigment-dependent colour traits might be costly when the pigments involved are difficult to obtain or are required for other essential functions such as the immune function (Lozano 1994). However, there is no need of costs of production for colour traits to be honest indicators of condition. Colour traits might be linked to condition when regulatory agents that stimulate

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colour expression (e.g. testosterone) simultaneously depress or share pathways with other fundamental physiological processes (Hill 2011).

Hormones have been targeted as a key mechanism controlling reproduction through their sex-specific influence on the expression of morphological and behavioural traits, including the display of secondary sexual characters such as the conspicuous colours of many animals (Kimball 2006). For instance, testosterone stimulates muscle development (Herbst and Bhasin 2004; Huyghe et al. 2009), and therefore whole-organism performance on traits that influence survival and intra-sexual competition and dominance, hence contributing to overall fitness (Ellis 1995; Husak and Fox 2008; Irschick et al. 2008). Accordingly, in lizards, testosterone levels have been found to influence running speed, endurance, and bite force, which are performance traits that influence male-male competition and dominance (Klukowski and Nelson 1998; Klukowski et al. 1998; Olsson et al. 2000; Robson and Miles 2000; Sinervo et al. 2000; Lappin and Husak 2005; Peterson and Husak 2006; Husak et al. 2007; Lailvaux and Irschick 2007; John-Alder et al. 2009). Furthermore, testosterone is often required for the development of male colour traits favoured by sexual selection (Hews et al. 1994; Salvador et al. 1997; Sinervo et al. 2000; Cox et al. 2008; Olsson et al. 2012). However, elevated levels of testosterone can also impose costs, by suppressing immunocompetence and increasing parasitism (Hamilton and Zuk 1982; Folstad and Karter 1992; Roberts et al. 2004), resulting in a decrease of individual fitness (Cox and John-Alder 2007). Particularly, it has been suggested that if testosterone is responsible for the expression of colour traits, and simultaneously has immunosuppressive effects, only individuals in good condition or with resistant genes could afford to display testosterone-dependent colourful traits (Folstad and Karter 1992). Thus, testosterone-dependent colour traits are expected to be honest signals of male competitive ability and individual condition.

In lizards, recent studies report a temperature dependence of structural coloration. Higher body temperatures are related to more reflection in the UV and blue range (Hettyey et al. 2009; Bajer et al. 2012; Langkilde and Boronow 2012). In the Eastern Fence lizards (*Sceloporus undulatus*), the male's throat and ventral colour patches changed from green to blue when the lizards were moved from low to high temperatures (Langkilde and Boronow 2012). Body temperature is very important for ectothermic animals, as it influences their growth, performance, and immune response (Bennett 1980; Huey 1982; Angilletta et al. 2002; Zimmerman et al. 2010). Typically, at optimal temperature, organisms have higher physiological and physical performance, compared to performance at lower or higher temperatures. Furthermore, animals with greater thermoregulation capacity may be active within a larger range of environmental temperatures, will have less microhabitat restriction and increased activity time, resulting

in more time to forage, territory defence and mate searching. Hence, particularly in animals that live in habitats with extreme temperatures, colour traits that depend on body temperature might be honest indicators of physiological and physical performance (Bajer et al. 2012; Langkilde and Boronow 2012).

Animal coloration consists often of multiple colour traits that in the context of sexual selection might convey information on the bearer's quality. According to the redundancy hypothesis, different colour traits reflect the same information of individual quality, allowing for a better and faster assessment of potential mates or competitors, reducing the time spent on assessment and the probability of errors, and facilitating the detection of cheaters (Møller and Pomiankowski 1993; Candolin 2003). Alternatively, the multiple messages hypothesis posits that different colours or different components of the same colour trait (e.g. hue, lightness, size or number of patches) are produced through different pathways and can therefore indicate different aspects of individual quality (Møller and Pomiankowski 1993; Candolin 2003; Grether et al. 2004). For example, red, orange and yellow colour patches are typically produced by carotenoid pigments, which are obtained from the diet, and might function as antioxidants and immunostimulants; then, carotenoid-dependent colour traits have been considered as indicators of both nutritional and immune status (Lozano 1994). Brown and black colours are produced by melanins and are frequently related to dominance status (e.g. Jawor and Breitwisch 2003). Melanins are endogenously produced and genes involved in melanogenesis affect other physiological aspects of the individual such as the immune response (Ducrest et al. 2008). Additionally, in ectotherms, melanin-dependent colours have been associated to thermoregulation, shortening the time necessary to heat up and hence increasing the time the animal can perform optimally (Clusella Trullas et al. 2007). On the other hand, structural colours, such as blue and UV, result from the selective reflectance of light by a diversity of structures in the skin (Umbers 2013). For example, in vertebrates, the arrangement of iridophores, a type of chromatophore that contains crystalline structures, may give rise to blue (Morrison et al. 1996; Umbers 2013). Structural colours will thus require precise development; as a result of this, it has been suggested that they might indicate good genes (Shawkey et al. 2003; Umbers 2013). In addition, in ectotherms, structural colours may require an adequate body temperature (Hettyey et al. 2009; Bajer et al. 2012; Langkilde and Boronow 2012). Consequently, by displaying different colour traits, individuals may signal different aspects of their condition, performance or social dominance, or may reinforce through multiple signals the same information about its quality.

The Dickerson's collared lizard *Crotaphytus dickersonae* inhabits the Sonoran desert in Mexico, an open habitat with

sparse vegetation cover and air temperatures that can reach over 50 °C. These lizards have a strong sexual dimorphism in size and colour. Males are on average 14 % larger and 63 % heavier than females (Plasman et al. 2007). Females are brownish with yellow legs and tail, while the body of males is deep cobalt blue (McGuire 1996; Macedonia et al. 2009). Both sexes have white spots on the dorsum and a collar of two black bands with a white stripe in the middle. The possible function of male coloration in this species is presently unknown. However, in the closely related species, *Crotaphytus collaris*, females have been found to prefer brighter males (Baird et al. 1997). Furthermore, in *C. collaris* displays such as full show or gular extension, in which the male exhibit its coloration (e.g. Macedonia et al. 2004), can be seen in interactions with both male and female conspecifics and have been related to the intensity of aggression and courtship frequency (Baird et al. 1997; Husak 2004; Baird 2013). Similar display behaviours have also been seen in *Crotaphytus dickersonae* (MP personal observation). Hence, the conspicuous male coloration of the Dickerson's collared lizard might be under sexual selection.

In this study, we evaluated whether male colour traits (the blue colour of the body, the white spots on the dorsum and the collar) of the Dickerson's collared lizard are indicators of performance (bite force, running speed and endurance), immune response and parasite load. Testosterone has been associated to colour displays and performance, and, in lizards, body temperature may modulate colour, performance and physiology. Therefore, we tested for direct and indirect relationships between circulating testosterone levels, body temperature, and colour and performance traits. Finally, to evaluate whether testosterone may function as an immune suppressor, we examined the relationships between testosterone and immune response and parasites load.

Methods

The study was conducted in Bahía de Kino, Sonora, Mexico. Forty eight males were captured between Bahía de Kino and Punta Chueca from April to June 2011, during the breeding season of *C. dickersonae* (McGuire 1996). Within 4 min after capture, males were blood sampled by taking 100 µl of blood with a 1-ml syringe from the caudal vein in the tail. Samples were stored on ice and within 4 h after collection were centrifuged (10,000 rpm during 10 min) to separate the plasmatic fraction from the cellular one. Plasma was stored in liquid nitrogen during fieldwork and then in a deep freezer (−70 °C) until hormone assays were performed. After blood samples were taken, water was given directly in the mouth of each animal to avoid dehydration. Animals were brought in individual cloth bags to a research station in Bahía de Kino where they were kept for the stretch of the tests. Bahía de Kino

is within their distribution range and thus allows for natural day-night rhythm and temperatures. Once in the field station and before any other manipulations that might make the parasites to leave the host, all ectoparasites present on the males and in the bags used for transportation were collected. Ectoparasites were counted per host and stored in ethanol (70 %) for later determination to family level.

In captivity, males were individually housed in outdoor cages (60×60×60 cm) made of wooden frames covered with mesh. Canvas between adjacent cages and in the back of the cages avoided visual contact among males and reduced stress produced by people passing by. Every morning, lizards were provided with food (one or two live crickets depending on their size) and water ad libitum, and cages were misted.

Colour, body temperature and morphological measurements

On the day of capture, three measurements of the background blue on the dorsum and one measurement from each of the two black bands of the collar were taken with a portable spectrophotometer that determines reflectance from 360 to 740 nm at 10-nm intervals (Minolta CM-2600d; Minolta Co. Ltd, Osaka Japan). Although some lizard species can change colour due to the stress of handling (e.g. Greenberg 2002), in the Dickerson's collared lizard no colour changes were observed between capture and colour measurements. Immediately after colour measurements, cloacal temperature, an indicator of body temperature, was measured with a cloacal thermometer (± 0.1 °C; Miller and Weber, Inc). Colour and body temperature measurements were completed within 2 to 3 min, and all measurements were taken between 15:00 and 17:00 hours. In ectotherms, body size influences the rate of heat loss, with bigger animals cooling at slower rates than smaller animals (Claussen and Art 1981). To avoid biases from differential heat loss caused by lizard size, before colour and body temperature measurements, lizards were sunbathed for 60 min to allow them to return to their preferred body temperature during the active time of the day. Body temperatures of males after sunbathing did not differ from body temperatures of male lizards measured immediately after capture in the same field site for a different study in a previous year (mean \pm SD, present study 37.33 ± 0.175 °C, $n=39$; males from 2005, 37.33 ± 0.178 °C, $n=43$ from Plasman et al. 2007; $t=-0.14$, $P=0.99$). Hence, it is unlikely that our estimates of body temperature might be biased by the size of the individual or the time elapsed between colour and body temperature measurements (roughly 1–2 min).

After colour and temperature measurements, three pictures were taken from the lateral and dorsal sides to estimate the area of the collar and the number and area of the white spots by counting the number of pixels per colour using the histogram option of Adobe Photoshop (version 8.0). Lizards were placed on a flat white surface with a ruler as a scale reference.

Pictures were taken from 40 cm distance with a Nikon Coolpix camera. For each male, the snout-vent length (hereafter SVL; ± 1 mm) and the body mass (PESOLA scale ± 0.1 g) were measured.

From the reflectance colour curves of each lizard, we calculated the blue chroma as the sum of reflectance from 400 to 480/sum of total reflectance of the mean reflectance curve of the dorsum (this index includes reflectance from violets and blue wavelengths; however, for simplicity, we will refer to it as blue chroma; Endler 1990). A lizard with higher blue chroma displays a deeper cobalt blue than those with lower blue chroma, which exhibit a more turquoise colour. Also, the lightness of the black collar bands was measured (sum of total reflectances of the mean reflectance curve of the collar); high lightness indicates a relative light black collar and low lightness a darker black collar.

Male competitive abilities: bite force, running speed and endurance

As a proxy of bite force, we estimated the size of the adductor mandibulae complex (AMC) by making the lizard open its mouth to the maximum and taking a photograph from the front. The photograph was taken after the photographs for colour measurements and included a ruler to allow for scale. The AMC was estimated from the photographs as the distance from the middle of the coronoid bone to the extreme outside of the cheek (Lappin et al. 2006) using ImageJ (version 1.44o). AMC have been found to correlate with bite force in *C. collaris* (Lappin et al. 2006).

To estimate running speed, each male was chased down a racetrack (Husak 2006). The racetrack was made of wood ($2.44 \times 0.40 \times 0.40$ m) and covered with sandpaper to improve traction. At every 25 cm, a stripe was drawn, which allowed for the estimation of running speed on parts of the racetrack. A dark bag at the end of the track stimulated the animal to target a refuge. The lizard was tested in three continuous runs. The tests were videotaped with a Sony Digital 8 Handicam. From the videos, running speed was estimated, and the faster meter in any of the three runs was taken as maximal running speed.

Endurance was estimated by forcing the lizard to walk on a treadmill (Robson and Miles 2000; Sinervo et al. 2000; Mills et al. 2008). The treadmill consisted of a continuous belt that was moved by hand at 2 km/h. A box made of wood of $50 \times 40 \times 40$ cm prevented the lizards to escape. A Plexiglas window in the frontal side of the box gave the impression of an open area and thus a direction in which to walk. The lizard was stimulated to walk by tapping it gently on the flanks and was considered exhausted when it did not respond to three continuous taps (± 1 seg; Robson and Miles 2000; Sinervo et al. 2000; Mills et al. 2008). All tests were performed during the hours the lizards were seen active in their cages (from 9:00 to 13:00 and 15:00–17:30 h). Lizards completed the running

speed and endurance tests on different consecutive days (second and third day after capture). The order in which a lizard completed these tests was at random, and the order of the tests did not influence performance ($P > 0.18$).

Cell-mediated immune response

After locomotor tests had been completed, to evaluate in vivo local cellular immune response, males were injected subcutaneously in the palm of the right front foot 37 μ l of a solution of 5 mg of Phytohemagglutinin-P lectin of the red kidney bean *Phaseolus vulgaris* (PHA; Sigma L8754, St Louis, USA) in 1 ml of phosphate buffered saline solution (PBS) (Belluire et al. 2004; Berger et al. 2005; Mills et al. 2008). The point of injection was marked with an indelible marker and swelling at the point of injection was estimated as the difference between thickness of the manus before and 12, 18 and 24 h after injection, as measured with a digital micrometer (± 0.001 mm; Mitutoyo). More swelling is related with a greater infiltration and proliferation of macrophages and lymphocyte; hence, local inflammatory response to the PHA mitogen (hereafter PHA response) has been used as an index of the strength of the cell-mediated immune response (e.g. Belluire et al. 2004; Berger et al. 2005). As inflammation was maximal at 12 h, these measurements were used for the analyses. Because after locomotor tests males were included in a mate selection test (M. Plasman and R. Torres in preparation), cell-mediated immune response was measured on average 7 days after capture.

Testosterone determination

Steroid extractions from plasma were performed by a double ether extraction. Briefly, the amount of plasma obtained was measured with a pipette (± 1 μ l), and 1 ml of anhydrous ether [$((C_2H_5)_2O)$; JT Baker, Center Valley, USA, code 9244-02] was added. Samples were then set for 30 min in a vortex and centrifuged for 5 min at 2000 rpm. Liquid and solid parts were separated. The ether was evaporated leaving the hormone behind. To the solid part, 1 ml of ether was added and the protocol repeated. To obtain a solution with the original dilution, the hormone extract was dissolved in an equal amount of EIA buffer (Cayman Chem., Ann Arbor, USA, code 400060), as has been the original plasma sample. The solution was shaken to dissolve and to recover all hormones from the tube walls. Levels of testosterone were then determined with a testosterone ELISA kit (Cayman Chem., Ann Arbor, USA, code 582701). The kit uses mouse antibodies and has a lower detection limit of 6 pg/ml and an IC_{50} of 32 pg/ml. Samples were initially diluted three times; however, to obtain values within the detection range of the ELISA, 86 % of the samples had to be diluted another ten times resulting in a final dilution of 30 times. Samples were analysed in duplicate with respect to a standard curve, and the average of duplicates was used for the

analyses. Intra-assay coefficient of variation was 6.2 % as measured from duplicates within plates, while inter-assay coefficient of variation was 5.9 % as calculated from a pooled sample included throughout the plates. Testosterone level was not correlated to the time elapsed from capture to blood sampling ($P=0.55$) or to capture date ($P=0.26$); therefore, all samples were included in the analyses.

Statistical analyses

Because physiological variables and variables that estimate male competitive abilities are probably correlated with each other, we used a path analytical approach to evaluate simultaneously the potential covariation between independent variables and their direct and indirect effects on male colour traits. Path analysis provides a method in a multiple regression framework for structuring hypothetical causal relationships between multiple traits (Wright 1921). For the path analysis, we first defined alternative models that represent different biological hypothesis of whether male colour may indicate performance and immune condition (immunocompetence and parasite load), and the potential effects of plasma testosterone level (hereafter T-level) and body temperature on colour displayed. For example, to evaluate the role of T-level as a mediator of male colour, we analysed direct effects of T-level on the colour, and possible indirect effects through the relationship of T-level with PHA response and parasite load, as testosterone is hypothesised to be an immune suppressor (e.g. Folstad and Karter 1992). In a path analysis, the strength of a direct effect is given as the direct path coefficient. This indicates the strength of the independent variable over the dependent variable, corrected for the effects of other variables included in the model. The effect of a variable on the response variable through its influence on other variables is called indirect path coefficient and is calculated by multiplying the path coefficients on the route between variables. The sum of direct and indirect path coefficients is the total path coefficient (Bentler 1989).

The different colour patches were analysed in separate path analyses. Initial models included SVL, body temperature, T-level, AMC, endurance, running speed, PHA response and parasite load. Body mass and SVL were strongly correlated ($R=0.88$, $P<0.01$, $n=48$); hence, only SVL was included in the models. T-level and endurance were log-transformed before analyses. To evaluate the potential immune suppressor effect of T-level, we assessed a model that tested the effects of T-level on PHA response and parasite load, allowing simultaneously for the effects of body temperature and SVL. Subsequently, we fixed the relationships with T-level to zero and compared models with a Wald test (Bentler 1989).

From the initial hypothetical path models, the analysis evaluates the degree of fit between the expected covariance

structures and the observed data. The goodness-of-fit of the model was evaluated with a χ^2 . A significant χ^2 ($P<0.05$) indicates that the data differs from the expected covariances and the model is rejected. Due to missing data during field work, our sample size for the analyses varied between models from 32 to 48 males. Hence, because sample size was small, we also used the comparative fit index (CFI) and the standardised root mean square residual (SRMR) to reduce type I and II errors (Hu and Bentler 1999). CFI compares the model against a baseline model in which all variables are considered independent. CFI depends on the correlations between variables and gives a higher value for higher correlations, and a CFI value of 0.96 or higher indicates that the model is in accordance to the data (Bentler 1989). SRMR calculates standardised residuals; therefore, a smaller value is better and models with SRMR smaller than 0.09 are considered a good fit to the data (Hu and Bentler 1999). Further, we used Akaike's Information Criterion (AIC) to select the best model. AIC compares between models and optimises the trade-off between complexity and variance. AIC values indicate the amount of information lost, assuming that the data was generated in the process suggested by the model, and a lower value is better (Anderson 2008). Path analyses were performed in R-program (version 2.15.2) package Lavaan (Rossee 2012).

Results

Blue chroma

Path analysis suggests that in the Dickerson collared lizard, blue chroma is related to indicators of performance, but not to parasite load or PHA response (Fig. 1). Males with bluer body

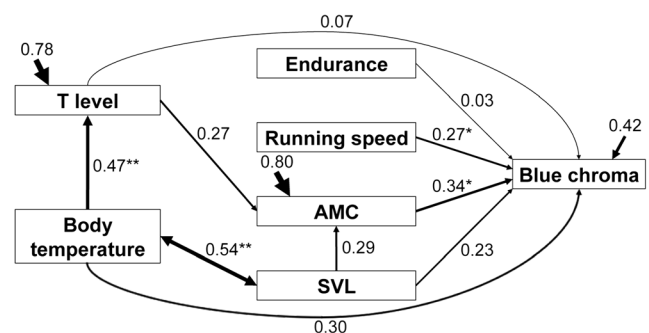


Fig. 1 Path model illustrating effects on blue chroma of performance measures (endurance, running speed and AMC), SVL, T-level and body temperature (model's $\chi^2=13.22$, $df=11$, $P=0.28$, $AIC=291.46$). Thickness of the arrows indicates strength of the relation. Double-headed arrows indicate a covariance. Single-headed arrows indicate an effect of the variable at the base of the arrow over the variable at the head of the arrow. Single-headed arrows without a box at the base of the arrow indicate effects of unmeasured factors. Standardised estimate coefficients are given (* $P<0.05$, ** $P<0.01$)

dorsum run faster and had greater AMC. Also, larger males had higher body temperatures, and both male size and body temperature were positively related to blue chroma (Fig. 1). T-level direct (path coefficient=0.07) and indirect effects (through its effect on AMC, indirect path coefficient=0.09) on blue coloration were low (Fig. 1). Hence, we did not find evidence that T-level influences the blue coloration of males.

Collar

The lightness and size of the black stripes of male collar were related to male performance and immune response. Males with darker (i.e. lower lightness) and larger black collars had higher running speed and bigger AMCs (Fig. 2). Also, males with darker collar had greater endurance, produced a higher PHA response and had lower parasite loads (Fig. 2b). PHA response had a smaller positive effect on the size of the collar, and there was only an indirect effect of parasite load (indirect path coefficient through PHA response=0.11; Fig. 2a). Interestingly, larger males had larger black collars, but SVL had only an indirect effect on the lightness of the collar (indirect path coefficient through PHA response=0.34; Fig. 2b). Body temperature was positively related to T-level and had a positive effect on collar lightness and a negative effect on collar size: males with darker and larger black collars had lower body temperature (Fig. 2). We did not find evidence that T-level influenced the size or colour of the black collar.

The white area of the collar was only directly (direct path coefficient=0.36) related to AMC and indirectly to SVL (through its affect on AMC, indirect path coefficient=0.15). The white stripe was not related to other performance measurements, immune response, T-level or body temperature.

White spots

Males with more white spots were larger (number of spots, path coefficient=0.54; area of spots, path coefficient=0.40) and had bigger AMC (area of spots, path coefficient=0.44); yet, males with more white spots had lower endurance (path coefficient=-0.41) and lower body temperatures (path coefficient=-0.28). The number and total area of white spots on the male dorsum were not related to PHA response, parasite load, T-level or running speed.

Overall, path analyses suggest that different colour patches in Dickerson's collared lizard may convey moderately different information (Fig. 3). The black collar was related with indexes of performance, and these relationships were stronger than the relationships of performance with the blue dorsal colour. Moreover, lightness of the collar was related to immune status. White spots were only related to AMC (area) and endurance (number). Body temperature influenced the colour, especially the area of the black collar, and also affected

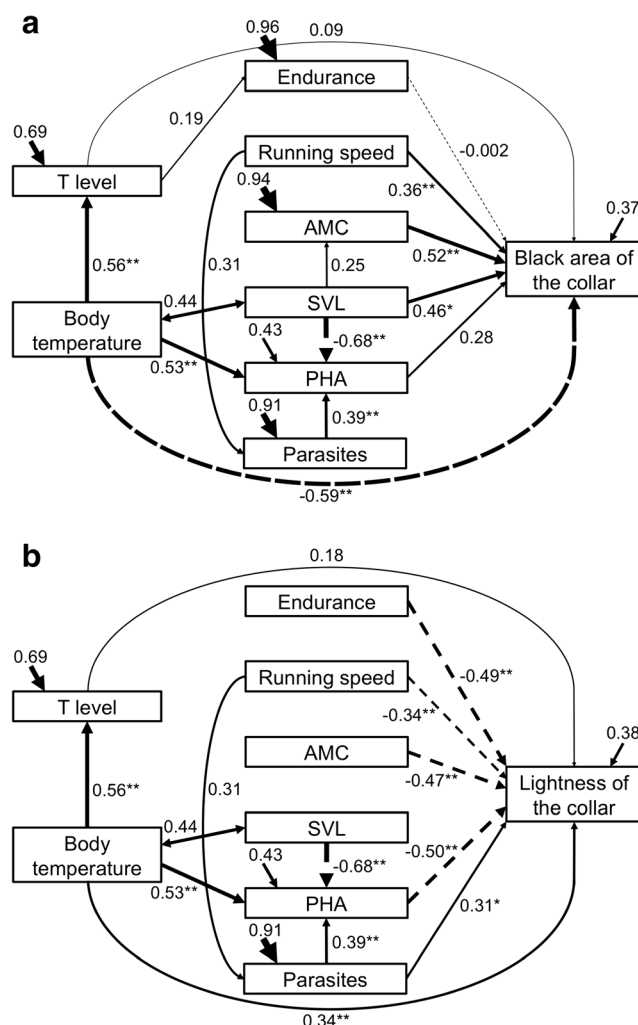
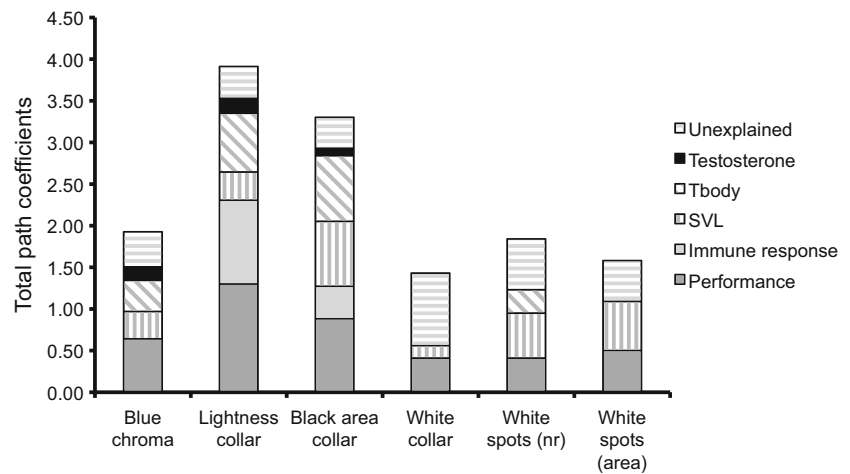


Fig. 2 Black collar indicates performance and immune response. Path model illustrating effects of performance measurements (endurance, running speed and AMC), immune response measurements (PHA-response and parasite load), SVL, T-level and body temperature on **a** size of the black collar ($\chi^2=15.43$, $df=18$, $P=0.63$, $AIC=852.26$) and **b** collar lightness ($\chi^2=19.60$, $df=20$, $P=0.48$, $AIC=676.04$). Thickness of the arrows indicates strength of the relation. Double-headed arrows indicate a covariance. Single-headed arrows indicate an effect of the variable at the base of the arrow over the variable at the head of the arrow. Single-headed arrows without a box at the base of the arrow indicate effects of unmeasured factors. Solid arrows indicate positive relations, whereas dashed arrows indicate negative relationships. Standardised estimate coefficients are given (* $P<0.05$, ** $P<0.01$)

PHA response. We found no evidence that T-level may be involved in colour display.

Finally, we evaluated whether colour traits were correlated with each other. After controlling for size, colour traits were not correlated with each other (all $P>0.05$), except for the correlations between the area of the black and white stripes of the collar ($R=0.44$, $P=0.003$), the number and area of the white spots ($R=0.47$, $P=0.001$), and a marginal correlation between the lightness of the collar and the number of white spots ($R=0.30$, $P=0.049$).

Fig. 3 Summary of path coefficients of the variables included in the models for the different colour traits. The total path coefficients for each variable were calculated as the sum of the absolute values of direct and indirect path coefficients. For visual simplicity, in the figure performance includes the added path coefficients of AMC, running speed and endurance, while immune response includes the added path coefficients of PHA response and parasite load



Parasite load and immune response

All ectoparasites found on males were mites of the family *Trombiculidae*. On average, males carried 175 mites, but there was a large variation among individuals (range 3–786). Although all ectoparasites were removed on the day of capture, roughly 7 days before the PHA test, animals with higher ectoparasite load had higher PHA response (Fig. 4a). Interestingly, bigger animals had lower PHA response (Fig. 4a, b). Effects of T-level on PHA response and parasite load were small (Fig. 4a). Furthermore, the model performed significantly better when testosterone was excluded (model including T-level AIC=567.37, model excluding T-level AIC=549.04; Wald test, $\chi^2=24.07$, $P<0.01$), suggesting that T-level had no significant effect on parasite load and PHA response.

Discussion

We found that in the Dickerson's collared lizard, multiple male colour traits indicate performance and immune condition. Male size, an important trait in determining dominance among lizard species (Tokarz 1985; Carpenter 1995; Sacchi et al. 2009), was positively correlated to the blue, the size of the black collar stripes and the number of white spots; the darkness of the collar was independent from size. However, independent of size effects, the results suggest that colour traits indicate male competitive ability and resource-holding power, while the blackness of the collar also indicates immunocompetence.

Dickerson's collared lizard males with deeper cobalt blue dorsums, larger and darker black collars and a greater number of white spots had larger AMC and performed better at the running speed test, and both of these performance traits are probably related to male competitive ability (Robson and Miles 2000; Perry et al. 2004; Huyghe et al. 2005; Husak

and Fox 2006; Husak et al. 2006a, b). Among territorial male lizards, agonistic interactions with potential rivals include rapid pursuits to chase away males from territories and, when escalation occurs, fights with fierce biting that can produce serious wounds (Lappin and Husak 2005). The size of the AMC, the jaw-closing musculature, is directly related to maximum bite force (Lappin et al. 2006), and bite force predicts dominance and reproductive success (e.g. Herrel et al. 1999; Huyghe et al. 2005; Lappin and Husak 2005; Husak et al. 2006a). Hence, if running speed and bite force are key weapons in male-male competition, indicating their performance through colour displays may be advantageous to avoid the costs associated to fighting. In a closely related species, *C. collaris*, mouth-gaping displays by adult males provide an index of bite force during close-range encounters (<1 m; Lappin et al. 2006). Dickerson's collared males typically guard their territory from a prominent rock from where complex displays that may advertise their colour traits are exchanged with potential rivals. Our data suggests that multiple conspicuous body colour traits might relay information on male fighting ability from longer distances.

In our study, males with darker collars mounted a greater PHA response and had smaller parasite load, while males with larger black collars mounted a greater PHA response; no relationship between the blue of the dorsum and the number of white spots with PHA response or parasite load was found. Black colours normally depend on melanin (Jawor and Breitwisch 2003; Kuriyama et al. 2006), and melanin-based colour patches are often implicated in social communication and linked to the immune function (Macintosh 2001; Ducrest et al. 2008). The ability to mount a cell-mediated immune response, a generalised short-term response that promotes healing of wounds and enhances resistance to infections, may have important fitness consequences during the breeding season when male-male interactions increase the probability of injury (Zuk and Johnsen 1998). In the Dickerson's collared lizard, colour and size of black stripes of the collar of males

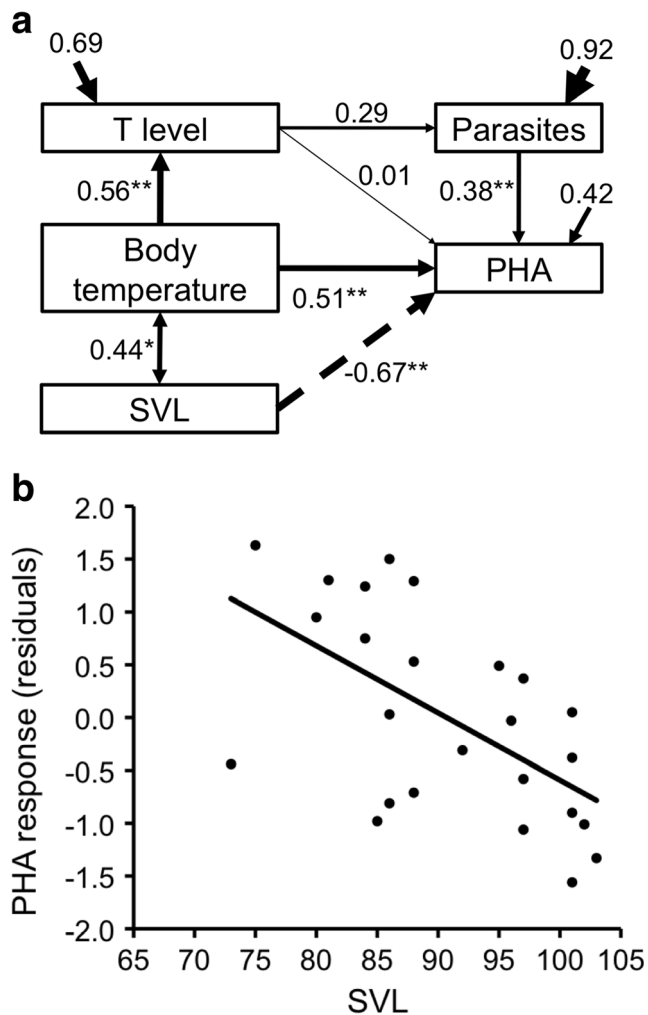


Fig. 4 **a** Path model illustrating effects of T-level and body temperature on parasite load and PHA response ($\chi^2=1.26$, $df=3$, $P=0.74$, $AIC=567.38$). Thickness of the arrows indicates strength of the relation. Double-headed arrows indicate a covariance. Single-headed arrows indicate an effect of the variable at the base of the arrow over the variable at the head of the arrow. Single-headed arrows without a box at the base of the arrow indicate effects of unmeasured factors. Solid arrows indicate positive relations, whereas dashed arrows indicate negative relationships. Standardised estimate coefficients are given (* $P<0.05$, ** $P<0.01$). **b** The relationship between PHA response and size of the animal. On the y-axis, the standardised residuals of PHA response controlling for parasite load and body temperature are shown

are related to the strength of a cell-mediated immune response and parasite load, suggesting that the collar is a signal of the immune status of the male.

Interestingly, PHA response was stronger in male lizards that carried greater parasite loads. Similarly, a positive relationship between parasite load and an inflammatory immune response has been reported in two other lizard species: *Sceloporus jarrovi* (Goldberg and Holshuh 1992) and, only late in the reproductive season, in *Podarcis melisellensis* (Huyghe et al. 2010). A positive correlation between parasite load and immune response might result from a general priming effect, i.e. when previous immune challenges lead to an

immune response that increases sensitivity of the immune system to subsequent challenges (Huyghe et al. 2010; Schmid-Hempel 2011). Hence, it is possible that due to a general priming effect, males with greater parasite load, and presumably whose immune system was recently challenged, elicited a stronger immune response, while individuals with lower parasite load elicited a weaker swelling response because their immune systems have not been equally primed (Huyghe et al. 2010). Interestingly, PHA response was strongly and negatively related to male size suggesting reduced immunocompetence with age (lizards grow during their entire life, hence size is likely related to age; Sexton et al. 1992). Future studies should investigate whether priming is of a general or specific type and whether immunosenescence occurs in Dickerson's collared lizards.

We found no evidence that plasma levels of testosterone influenced the expression of colour traits, enhanced performance or was correlated with PHA response or parasite load of Dickerson's collared lizards. Furthermore, our data did not support the immunocompetence handicap hypothesis as stated by Folstad and Karter (1992). In our study, all males had fully developed adult coloration and T-level determination was based on a single sample taken at capture. Mean level of plasma testosterone of males was within the range of values reported for other adult male lizards of similar size (e.g. Cox and John-Alder 2005; Baird and Hews 2007; John-Alder et al. 2009). Hence, it is unlikely that our estimations of T-levels were biased. In some lizard species, testosterone only influence colour during a critical period prior to or during colour development (Kimball and Erpino 1971; Hews et al. 1994; Hews and Moore 1996). It is possible that in the Dickerson's collared lizard, current male colour traits can therefore be unrelated to measured T-levels, but could instead reflect testosterone level during development or during a longer period of time. In addition, natural T-levels might fluctuate with stress, immune status at capture, or age (Folstad and Karter 1992; Moore et al. 1991). In our study, body temperature and T-level were strongly related, and body temperature and immune response were linked to size suggesting that individual variation in age and condition might be important to understand the potential trade-offs between the display of testosterone-dependent colour traits and physiological performance (Alonso-Alvarez et al. 2009; Ruiz et al. 2010). At present, we have no evidence that testosterone plays a role modulating colour traits or that compromises the strength of the inflammatory immune response.

Male body temperature influenced physiological variables and colour traits. Males with higher body temperature had a stronger PHA response and higher T-levels, and displayed bluer dorsum and smaller and lighter black collars. Body temperature was positively related to male size. In lizards, larger animals cool down at lower rates than smaller animals (Claussen and Art 1981). In our study, males were allowed

to sunbath before temperature measurements, so it is unlikely that size-related differences in cooling rate have biased the estimation of body temperature. However, larger males probably have an advantage at maintaining their optimal body temperature than smaller males. In ectotherms, body temperature is a key trait for fitness due to its effects on growth, metabolism, immune response and locomotor performance (reviewed in Angilletta et al. 2002; Zimmerman et al. 2010). Furthermore, in lizards, temperature influences sexual colour traits (Bajer et al. 2012; Langkilde and Boronow 2012). Optimal body temperature in small ectotherms is mainly achieved by behavioural thermoregulation, which may be costly in terms of time invested and increased predation risk (Huey 1982; Dunham et al. 1989; Adolph and Porter 1993). Particularly, in species with very conspicuous colours that contrast with the natural background, as is the case of the Dickerson's collared lizard, visibility to predators during sunbathing will be high (Macedonia et al. 2009). In the Dickerson's collared lizard, predation pressure appears to be low and may not have a strong selection effect (Plasman et al. 2007). However, the extreme hot environment of the Sonoran Desert may impose important constraints and challenges for thermoregulation. Hence, individuals that are able to maintain body temperature within a range that optimises physiological performance, the display of colour traits and active time should enjoy greater fitness than those that are not. Therefore, in the Dickerson's collared lizard, colours dependent on temperature may signal current physiological and physical performance.

The exact mechanisms of how temperature affects integumentary colour in lizards are unknown, but changes in structural colours, such as blue, can result from altered spacing of the iridophores platelets in the cells of the light-reflecting layer (Morrison et al. 1996). Additionally, if melanin synthesis is temperature dependent, this may influence the intensity of melanin-dependent colours, such as the black of the collar, and structural colours due to its role as a purifier layer in structural colours (Quinn and Hews 2003; Grether et al. 2004). Future studies need to evaluate in more detail the influence of temperature on individual colour variation.

In conclusion, multiple colour traits of Dickerson's collared males indicate resource-holding power and immune condition; particularly, the black stripes of the collar might function as a status badge. Interestingly, most colour traits considered in this study were not related with each other, probably because of different physiological process involved in colour production. However, multiple traits that convey the same information may be selected for the advantages of reducing the time of assessment, the likelihood of erroneous assessments and cheating (Møller and Pomiankowski 1993; Candolin 2003). Future studies need to evaluate whether male multiple colour traits are used to assess the fighting prowess of potential rivals, to assess potential mates during mate choice

or both. Furthermore, colours dependent on body temperature may advertise physical and physiological performance in ectotherms and therefore indicate status and condition. Coloration in male Dickerson's collared lizard can therefore be considered an honest signal that can be used to assess male performance and condition.

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Ethical standards The study complies with the current regulations of Mexico regarding animal welfare.

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

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