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Feeling the heat: Extreme temperatures compromise constitutive innate humoral immunity and skin color in a desert dwelling lizard

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ABSTRACT

Environmental temperature, particularly in habitats with extreme temperature fluctuations, may shape selection pressures on life history traits. Especially in ectotherms, temperature affects performance, physiology, and in some species, skin color. Skin color can be a sexual ornament signaling the bearer's ability to resist infections, when only high-quality individuals are able to invest both in high immune defense and elaborate ornament expression. However, how the information content of these sexual traits may vary with environmental conditions has been less studied. Dickerson's collared lizard (*Crotaphytus dickersonae*) males are blue and have a black and white collar. This conspicuous coloration signals performance and immune response, and is related to body temperature. Here, by maintaining males at higher, lower, and mean environmental temperatures we evaluated whether temperature variation influences color and constitutive innate humoral immunity (agglutination and lysis titers, estimated through hemolysis-hemagglutination assays), and whether extreme temperatures impose trade-offs between color and humoral immunity. We found that at low and high temperature treatments males had lower agglutination and lysis titers, and at low temperature, blue chroma from the dorsum declined and males became greener. Interestingly, at low and control temperature treatments, agglutination titer and blue coloration were positively correlated, whereas high temperatures revealed a trade-off between increasing agglutination titers and displaying bluer skin color. Our results suggest that in the Dickerson collared lizard even short-term variation of environmental temperature affects performance of constitutive innate humoral immunity and the brilliant blue skin color. Particularly, high temperatures may compromise some components of male's immunity and sexual signaling.

1. Introduction

In ectotherms, environmental temperature is the main determinant of body temperature and has been a driver and a constraint in their evolutionary history. Body temperature affects physical and physiological performance, influencing a variety of traits such as locomotor performance, growth, metabolic rate, and fecundity (reviewed in Angilletta, 2009). Enzyme activity increases with temperature, leading to increased performance, yet enzymes may denature at extreme high temperatures (Tattersall et al., 2012; Abram et al., 2017). Therefore, generally there is an optimal body temperature at which a given performance trait is maximized, and at lower and higher temperatures performance level drops (Huey, 1982; Angilletta, 2009). Accordingly, in ectotherm vertebrates, the immune response typically declines at extreme temperatures (e.g. Mondal and Rai, 2001; Merchant and Britton, 2006; Rollins-Smith and Woodhams, 2011; Palacios et al., 2013). Additionally, the abundance of enzymes and immune cells declines at low temperatures, further decreasing the immune response (Maniero and Carey, 1997; Raffel et al., 2006). Hence, environmental temperature variation imposes fitness challenges that ectotherms have to cope with, either by increasing thermoregulation effort or risk facing a reduced performance level.

Interestingly, temperature can also influence the color reflected by the skin. Ectotherms are often darker in cold climates, which allows for shorter time to heat up and longer activity period (Clusella-Trullas et al., 2007). However, at high temperatures, dark colors can lead to overheating and therefore some animals have developed the ability to lighten their colors (Sherbrooke, 1997; Smith et al., 2016). Structural coloration (blue or ultraviolet) may also change with temperature (Morrison et al., 1996; Hettyey et al., 2009; Bajer et al., 2012). For

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example, males of the Eastern fence lizard (*Sceloporus undulatus*) change the color of their status badge in throat and ventrum from green to blue when body temperature increases (Langkilde and Boronow, 2012). These physiological color changes may be rapid and are reversible (Duellman and Trueb, 1994; Langkilde and Boronow, 2012).

If color and performance traits fluctuate with temperature, color may be a dynamic signal of current performance level (Hutton et al., 2015; Robinson and Gifford, 2018). However, the production and maintenance of conspicuous colors and the activation of immune defenses by the host are expected to be costly (Hasselquist and Nilsson, 2012; Tibbetts, 2014) and an allocation trade-off may occur (Lochmiller and Deerenberg, 2000; Hasselquist and Nilsson, 2012; but see Flatt and Heyland, 2012). Color traits often function as sexual signals, and they can be reliable indicators of immunocompetence, because the cost of immune function constrains low quality individuals from producing elaborate sexual traits (Hamilton and Zuk, 1982; Folstad and Karter, 1992; Anderson, 1994). Accordingly, many species display colorful sexual traits that honestly convey the strength of immune response related traits of the bearer (e.g. Griffith et al., 2006; Velando et al., 2006; Shawkey et al., 2007; Gasparini et al., 2009; Griggio et al., 2010; Pérez-Rodríguez et al., 2013).

Extreme conditions can generate additional costs and expose allocation trade-offs (Lazzaro and Little, 2009). For example, extreme high temperatures will require increased thermoregulation effort (Vickers et al., 2011), activate the heat shock response (Sørensen et al., 2003), and increases metabolic rate (Gillooly et al., 2001), leading to stronger competition for resources between fitness traits. Accordingly, temperature stress revealed a trade-off between survival and reproduction in *Drosophila* (Marshall and Sinclair, 2009). Also, at higher temperatures and when food resources were scarce the butterfly *Bicyclus anynana* showed lower phenoloxidase activity and haemocyte abundance, both traits related with the immune response (Karl et al., 2011). Natural temperatures animals experience can show a considerable amount of variation, especially in extreme environments, like deserts where temperatures can steeply rise, and these changes could have a great impact on the performance of organisms. Changes in temperatures could cause variation in dynamic skin colors (e.g. structural or melanin-based) and immune performance, or expose trade-offs between these fitness traits, within an individual during short periods.

In the Dickerson's collared lizard *Crotaphytus dickersonae,* males display a bright blue coloration over their entire body, whereas females are dull brown with yellow legs and tail (McGuire, 1996; Macedonia et al., 2009). Both sexes have a collar consisting of two black bands divided by a white one. The conspicuous male coloration is most likely involved in sexual selection. In a correlational study, we found that males with a bluer dorsum and darker black collars had greater scores on traits related to resource-holding power (Plasman et al., 2015). Also, males with darker black collar had fewer parasites and responded with more inflammation to an antigen (phytohemagglutanin; Plasman et al., 2015). Interestingly, males with high body temperatures had stronger inflammation response to phytohemagglutanin and were bluer, although the black in their collar was lighter (Plasman et al., 2015). Therefore, it is possible that coloration of males fluctuates with temperature, perhaps in synchronization with the immune response.

To investigate the influence of temperature on sexual color, constitutive innate humoral immunity (agglutination and lysis titers), and a potential trade-off between the expressions of these traits, we manipulated environmental temperature within the natural range to which Dickerson collared lizards are exposed. We predicted that the expression of both traits would be lower at extreme temperatures compared to control. Especially high temperatures should be detrimental, as these animals already live at temperatures close to their critical upper temperature limit (46.5 °C in *Crotaphytidae*; Clusella-Trullas et al., 2011).

2. Materials and methods

The study was conducted from May to June 2012 in Bahía de Kino in the state of Sonora, Mexico. During the reproductive period of *C. dickersonae*, 18 adult males were captured between Bahía de Kino and Punta Chueca, and brought in individual bags to a research station in Bahía de Kino, located within the geographic range of this species. Therefore, during captivity, lizards experienced lighting (approximately 14 h of sunlight) and humidity (14 ± 7%) as in their natural habitat.

On the day of capture the male's snout-vent length (± 1 mm; hereafter SVL) and body mass (± 0.1 g; PESOLA scale) were measured. Body condition index (BCI) was calculated as the residuals of the general linear model between log-transformed SVL and log-transformed body mass. Because parasite load can affect traits of the immune function (Goldberg and Holshuh, 1992; Huyghe et al., 2010; Plasman et al., 2015), we collected and counted all ectoparasites found on the body of each male and on the bags in which the males were transported on the day of capture. Males were kept in individual outdoor cages (60 × 60 × 60 cm) made of a wooden frame covered with mesh. Canvas between adjacent cages and at the back of each cage avoided visual contact between males. Every morning, individuals were fed with one or two live crickets and cages were misted, water was provided *ad libitum*.

2.1. Temperature treatments

To assess the effect of environmental temperature on the males' color traits and constitutive innate humoral immunity, each male endured three environmental temperature treatments: low, control and high temperatures. In the low temperature treatment, cages were placed in the shade with a cold white light (a neon light bulb) placed in each cage to reduce light differences with cages from other treatments. In the control treatment, cages were directed towards the west. In the high temperature treatment, cages were oriented towards the south, to attain direct sun light for a longer period of time compared to the other two treatments. Males were maintained in each treatment for 48 h and the order in which they entered treatments was determined at random. All cages were fitted with a refuge to reduce stress and to offer an escape from lethal temperatures. Per treatment, two dataloggers (Hobo Temperature Datalogger H02-001-02) that measured environmental temperature every 30 min were placed in separate cages. Within a treatment, temperatures measured at the same time in two different cages were similar (Pearson correlation: $r = 0.97, 0.98, 0.97$ for high, control and low temperature treatment respectively, n = 196, 96 and 384 respectively, $P < 0.01$ in all cases), and repeatability between days was high (Pearson correlation for low temperature treatment: $r > 0.53$, measurements of 9 days (48 measures per day), $P < 0.01$, control: $r > 0.60$, comparing 5 days, $P < 0.01$, high temperature: $r > 0.86$, comparing 9 days, $P < 0.01$).

The temperatures experienced by the lizards in each treatment fell within natural temperature ranges of the coastal area of the state of Sonora, Mexico, the natural habitat of our subject species (Table 1; http://smn.cna.gob.mx). Maximum temperatures were significantly different between all temperature treatments, but average temperature between the control and high temperature treatments did not differ (Table 1). Night-time temperatures tended to be lower in the high temperature treatment compared to the low temperature treatment (Table 1).

After 48 h in a temperature treatment, between 13:00 and 14:00 h, a blood sample was taken from each male for later estimation of agglutination and lysis titers, and cloacal temperature (± 0.1 °C; Miller and Weber, Inc) and color (details below) were measured. Blood samples (100 µl) were taken from the caudal vein in the tail and were immediately centrifuged during 10 min at 6000 rpm to separate plasma from the cellular fraction. Plasma was stored in liquid nitrogen and later in a deep-freezer (−80 °C) until immune analyses were performed.

Table 1
Environmental temperatures of the temperature treatments. The average night-time, average day-time (8:00–18:00) and maximum temperature (°C; average ± standard deviation) are given for the low, control, and high temperature treatments. Statistics of ANOVA-test are shown.

	Low	Control	High	df	*F*	*P*
Night-time	25.5 ± 1.82^{a}	$25.4 \pm 0.79^{a,b}$	23.3 ± 2.41^{b}	2,22	3.67	0.04
Day-time	26.3 ± 1.62^{a}	37.2 ± 3.31^{b}	39.0 ± 1.04^{b}	2,22	108.70	< 0.01
Maximum	29.0 ± 2.10^{a}	51.6 ± 7.92^{b}	61.0 ± 2.79^{c}	2,22	134.60	< 0.01

Different letters indicate significant differences between the treatments as specified by post-hoc tests (Tukey).

Handling of a lizard took approximately 5 min, and males were then placed in the next treatment.

2.2. *Color measurements*

To evaluate the effect of environmental temperature on male color traits, color measurements of the blue dorsum and the black collar were taken with a portable spectrophotometer that determines reflectance from 360 to 740 nm at 10 nm intervals (Minolta CM-2600d, Osaka Japan). Measurements were standardized to a white standard. For each male, we took three measurements of the blue color (one on each side of the male, and one in the dorsum) in areas big enough to permit a lecture without including any white spots. From the mean reflectance curve of these measurements we calculated the blue chroma as the sum of reflectance from 400 to 480 divided by the sum of total reflectance (Plasman et al., 2015). Males with higher blue chroma are deeper cobalt blue than those with lower blue chroma. From the collar, one measurement from each of the two black bands was taken at a wide place in the band that allowed a reading of only black skin. The lightness of the black collar bands was calculated as the sum of total reflectance from 360 to 740 nm from the mean reflectance curve of both black bands. High lightness indicates a relative light black collar and low lightness a darker black collar.

2.3. *Constitutive innate humoral immunity*

Constitutive innate humoral immunity was estimated by a hemolysis/hemoagglutination assay as described by Matson et al. (2005). This assay is based on two components of the constitutive innate humoral immunity: natural antibodies (NAbs) and complement pathway. Briefly, NAbs opsonise invading micro-organisms: they mark the microorganisms for destruction by phagocytes, and trigger lysis by activating the complement pathway. At high NAb concentration in the plasma lysis occurs, but when concentration declines, NAbs only mark the microorganisms and agglutination can be observed, whereas at very low concentrations microorganisms are not affected (Matson et al., 2005).

For the hemalysis-hemagglutination assay we followed Matson et al. (2005) protocol. In a 96-well plate (Corning #3795) plasma samples from *C. dickersonae* were serially diluted (1:2) with PBS from column 1 to 11; the 12th column functioned as a negative control. To each well we added 25 μl of rabbit whole blood (conserved in Alsever; Dibico 1602 FC), washed and taken to a concentration of 1% with PBS. Plates were incubated (1602H Hova-Bater incubator) for 90 min at 31 °C for samples from the low temperature treatment, at 37 °C for samples from the control treatment, and at 39 °C for samples from the high temperature treatment. Incubation temperatures were determined as the average body temperature of the males in each temperature treatment. After incubation plates were placed at an angle of 45° for 20 min and then scanned (HP deskjet F4180). From the pictures, the column of the highest dilution at which agglutination still took place was determined (hereafter, agglutination titer). Plates were then rested 70 min at room temperature and scanned a second time to determine maximum lysis (hereafter, lysis titer). When the cessation of agglutination or lysis was unclear, half-scores between the two dilutions were given. To determine repeatability, each plate included a pool sample and for eight samples duplicates were repeated in different plates. Repeatability between plates was high ($r = 0.84$ for agglutination and $r = 0.90$ for lysis, $n = 12$, $P < 0.01$ for both tests). Agglutination and lysis titers were evaluated independently by two observers with high reliability intra (mean = 96% for agglutination and 99% for lysis) and inter observer (90% for agglutination and 98% for lysis). The two observers were unaware of the treatment that each sample belonged to. For statistical analyses the average agglutination and lysis values of the two observers were used.

2.4. *Statistical analyses*

First, to determine the influence of environmental temperature treatments on male skin color and constitutive innate humoral immunity we performed repeated measure models for each of the following traits: blue chroma, lightness of the black collar and agglutination and lysis titers. Significant effects of temperature treatment were further investigated using Tukey for post-hoc pairwise comparisons. Second, to evaluate the potential relationship between male coloration and agglutination and lysis titers, and whether these relationships vary with environmental temperature and individual traits, we used general linear mixed models (GLMM). Response variable was male color. Initial models included as independent variables: temperature treatment, body temperature, SVL, body condition index, number of ectoparasites, and either agglutination or lysis titers, and the second and third level interactions between immune components, treatment and number of ectoparasites. Parasite load was included in the interactions because it has been found to affect inflammation response to PHA in this lizard (Plasman et al., 2015), and may affect levels of NAbs due to a possibly priming effect. Individual identity was included as random factor. The different measures of individual traits were not correlated ($P > 0.22$). Final models were obtained by stepwise backwards elimination, comparing the Akaike Information Criterion corrected for low sample size (AICc) of the models following Zuur et al. (2009). Analyses were performed in R version 3.2.0 (R Core Team, 2017), GLMM were performed in the package nlme (Pinheiro et al., 2018), Tukey post hoc tests were performed with multcomp package (Hothorn et al., 2008). Mean ± standard deviations are given throughout the results.

3. Results

3.1. *Environmental temperature and male body temperature*

Environmental temperature at which males were exposed affected their body temperature ($F_{2,34} = 36.92$, $P < 0.01$), particularly for males in the low temperature treatment. Male body temperature was significantly lower in the low temperature treatment (29.5 ± 4.5 °C), compared to body temperature of males in the control (36.6 ± 1.6 °C; Tukey: $z = -7.16$, $P < 0.01$), and high temperature treatments (37.0 ± 1.6 °C; Tukey: $z = 7.43$, $P < 0.01$, $n = 18$). Body temperature of males from the control and high temperature treatments did not differ (Tukey: $z = 0.68$, $P = 0.77$); however, for the few males that had not entered refuge at the time of measurements, body temperature was higher in the high temperature treatment (38.6 ± 0.6 °C) than in the

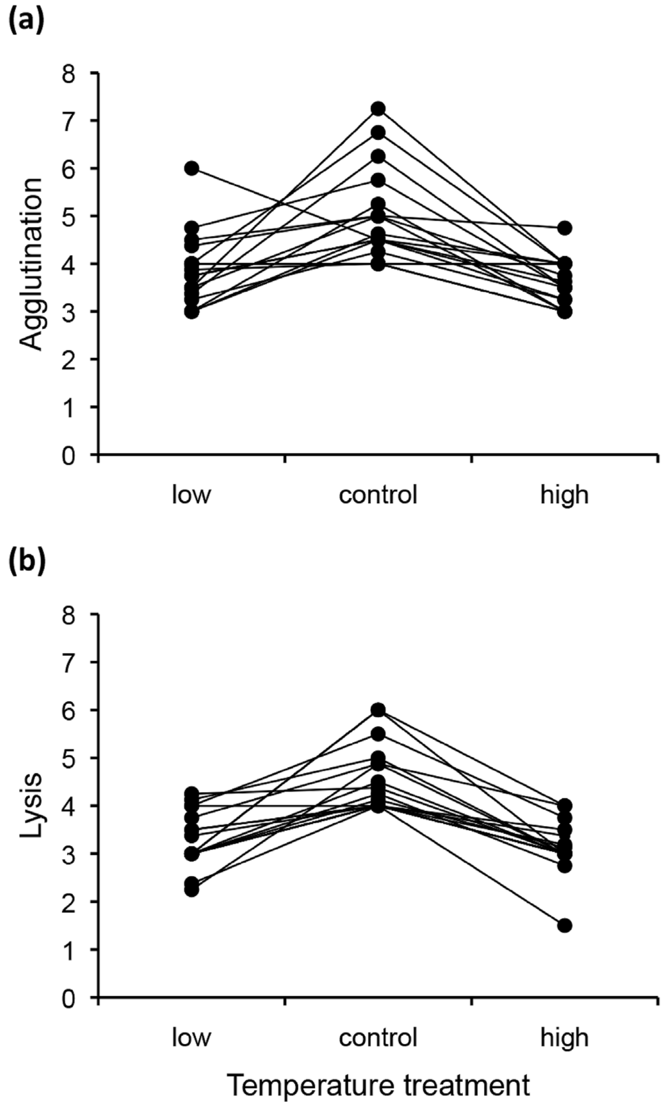

Fig. 1. Constitutive innate humoral immunity at different temperature treatments (low, control and high) of *Crotaphytus dickersonae* males. Constitutive innate humoral immunity was evaluated as (a) agglutination and (b) lysis titers. The titer responds to the highest dilution at which agglutination or lysis still took place, with 1 being a low dilution (1:1), indicating a weak immune performance, and 8 a high dilution (1:128) referring to a strong immune performance. Each line indicates an individual male (n = 18).

control treatment (36.6 ± 1.6 °C, n = 5).

3.2. Effects of environmental temperature on male constitutive innate humoral immunity

Male constitutive innate humoral immunity varied with temperature (agglutination: $F_{2,34} = 16.21$, $P < 0.01$; lysis: $F_{2,34} = 34.01$, $P < 0.01$; Fig. 1). Compared to the control treatment (agglutination = 4.95 ± 0.97, lysis = 4.53 ± 0.70), both agglutination and lysis titers were lower at low temperature (agglutination = 3.87 ± 0.74, Tukey: $z = -4.11$, $P < 0.01$; lysis = 3.28 ± 0.57, Tukey: $z = -6.76$, $P < 0.01$) and high temperature treatments (agglutination = 3.66 ± 0.51, Tukey: $z = -5.84$, $P < 0.01$; lysis = 3.13 ± 0.53, Tukey: $z = -8.24$, $P < 0.01$; Fig. 1). At low and high environmental temperature, agglutination and lysis titers did not differ (agglutination: Tukey: $z = -1.44$, $P = 0.31$; lysis: Tukey: $z = -1.07$, $P = 0.53$). Agglutination and lysis titers were highly correlated (Pearson correlation: $r = 0.86$, $P < 0.01$).

3.3. Effects of environmental temperature on male color traits

Environmental temperature influenced blue dorsal color of males, with males in the low temperature treatment becoming greener (Fig. 2). Blue chroma was significantly lower at low temperatures ($F_{2,34} = 16.29$, $P < 0.01$; Fig. 3; blue chroma = 0.25 ± 0.02, Tukey: compared to control: $z = -5.34$, $P < 0.01$; compared to high temperature treatment: $z = 4.38$, $P < 0.01$), but no differences between blue chroma of males in the control (0.28 ± 0.02) and high temperature treatments were found (0.27 ± 0.03; Tukey: $z = -0.82$, $P = 0.69$). Interestingly, males that were bluer in the control treatment showed a greater decrease in blue chroma in the low temperature treatment (Pearson correlation: $r = -0.61$, $P = 0.01$, $n = 18$); a similar, but not significant, trend was found in the high temperature treatment ($r = -0.43$, $P = 0.07$, $n = 18$). The lightness of the black stripes did not differ between treatments ($F_{2,32} = 1.01$, $P = 0.37$). As only blue chroma was affected by the environmental temperature treatment, only this color variable was further analyzed.

3.4. Trade-off between constitutive innate humoral immunity and color mediated by environmental temperature

Agglutination titer had a temperature-dependent relation with blue chroma (interaction agglutination × treatment; Table 2). At low temperatures and in the control treatment males with higher agglutination titer were also bluer. In contrast, at high temperatures there was a negative relation between agglutination titer and blue chroma (Fig. 4). Body temperature and the number of ectoparasites had a positive relation with blue chroma (Table 2). Male blue chroma was unrelated to BCI ($F_{1,16} = 0.17$, $P = 0.44$) and SVL ($F_{1,15} = 0.04$, $P = 0.83$). There was no relation between lysis and blue chroma when males were maintained at different temperatures (lysis: $F_{1,32} = 1.56$, $P = 0.22$; interaction lysis × treatment: $F_{2,30} = 1.54$, $P = 0.23$).

4. Discussion

Results from our experiment show that in the Dickerson's collared lizard the blue skin color and constitutive humoral immunity of males varies with environmental temperature. At low and high temperature treatments, males had a weaker agglutination and lysis response, suggesting that even short-term changes in environmental temperature can impact constitutive innate humoral immunity. At low temperature, blue chroma from the dorsum skin also declined. Furthermore, at high temperatures, there was a negative relationship between agglutination titers and blue chroma of males, while at lower temperatures the opposite trend was found. Hence, within natural variation, extreme temperatures weakened the level of agglutination, and high temperatures compromised the level of agglutination mediated by NAbs versus displaying a brilliant blue color.

In ectotherms, performance capacity depends on body temperature (Angilletta, 2009). In our study, compared to the control treatment, males had lower agglutination and lysis titers after 48 h of exposure to low and high temperatures. In low temperatures, low kinetic energy may cause a weaker immune response, whereas at high temperatures, enzymes may (partially) denaturalize, lowering reaction efficiency (Tattersall et al., 2012; Abram et al., 2017). Furthermore, the decline at high and low temperatures of lysis titers may implicate other physiological key process for the organism, as proteins of the complement pathway have been recently found to be involved in the regulation of key metabolic pathways of cells (Kolev and Kemper, 2017). Interestingly, in our experiment, between-individual variation in agglutination and lysis titers was greater in the control treatment compared to the low and high treatments, suggesting that extreme environmental temperatures may constrain the responses of constitutive innate humoral immunity, which might influence the outcomes of host-parasite interactions (Lazzaro and Little, 2009; Thomas and Blanford, 2003).

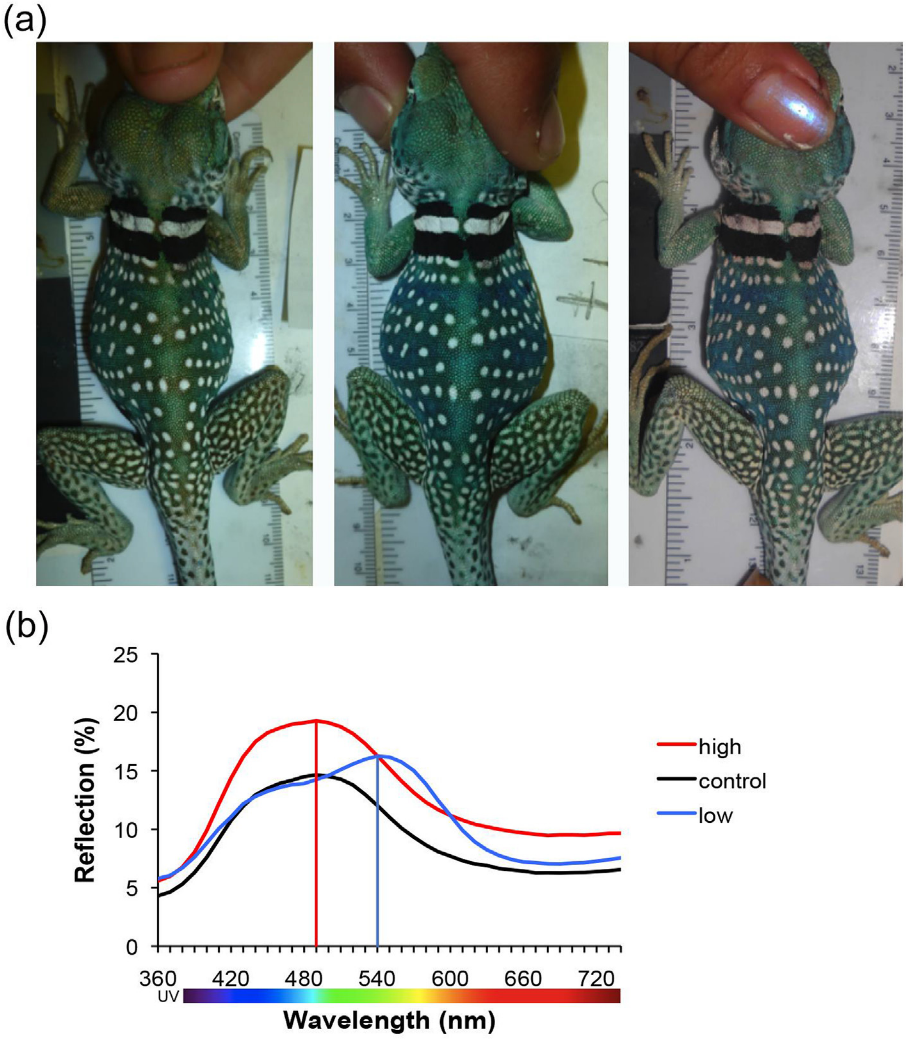

Fig. 2. (a) An example of a male *Crotaphytus dickersonae* lizard in the three temperature treatments. The same male is depicted in from left to right: low, control and high temperature treatment. (b) Mean reflectance curves of this male in low (blue; peak value 540 nm in the green range), control (black; peak value 490 nm in the blue range) and high (red; pick value 490 nm in the blue range) temperature treatments. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

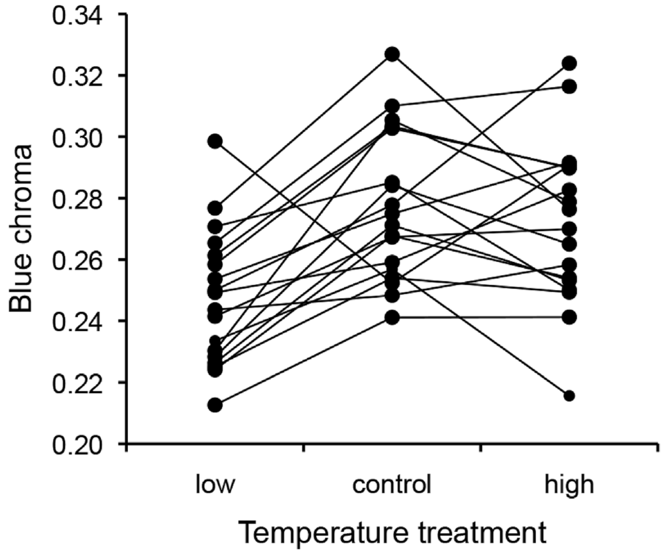

Fig. 3. Within-individual effect of temperature treatment (low, control and high) on male blue chroma. Each line indicates an individual male (n = 18).

Table 2

The model with the best fit that explains male blue chroma. The analysis was performed with a General Linear Mixed Model with normal error distribution. P-values are shown for all main effects and interactions. Only the best model is shown (AICc = −256.62), other models had ΔAICc > 2. Initial model also included SVL and body condition index.

Variables included in final model	estimate ± SE	*P*
Treatment		< 0.001
low	−0.0622 ± 0.0358	
high	0.0421 ± 0.0427	
Agglutination	0.0013 ± 0.0060	0.046
Ectoparasites	0.0002 ± 0.00005	0.007
Body temperature	0.0017 ± 0.0008	0.017
Agglutination x Treatment		0.018
low	0.0118 ± 0.0073	
high	−0.0128 ± 0.0102	

Additionally, under natural conditions, costs of mounting an immune response at extreme temperatures may involve changes in behavioral thermoregulation that may influence predation risk, foraging time and mating opportunities (Dunham et al., 1989; Lochmiller and Deerenberg, 2000).

Short-term variation in environmental temperature also affected the

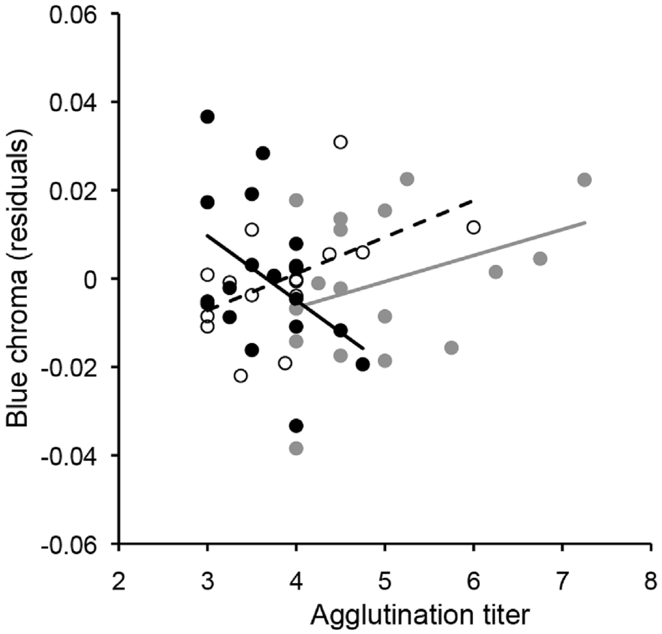

Fig. 4. The relation between agglutination and blue chroma at each temperature treatment. Low, control and high temperature treatments are indicated by open, grey and black circles respectively. The titer of agglutination responds to the highest dilution at which agglutination still took place, with 2 being a low dilution (1:2), indicating a weak immune performance, and 8 a high dilution (1:128) indicating a strong immune performance. Blue chroma is shown as the residuals of the final model excluding the interaction Agglutination x Treatment (see Table 2).

blue coloration of the skin displayed by males, which highlights the dynamic nature of this color trait. Although most studies on color change refer to changes in melanin-dependent color traits (dark colors; e.g. Clusella-Trullas et al., 2007), we did not find evidence that temperature influenced the black stripes of the collar. Generally, darkening of the skin is caused by melanin dispersal in melanophores over other layers of cromatophores (Bagnara et al., 1968). Probably the lack of other color layers in the collar reduces the possibility to darken. In structural colors, such as the blue coloration shown by Dickerson's collared lizards, temperature may affect the space between platelets in the iridophores, which determines the wavelength that is reflected (Morrison et al., 1996). At lower temperatures, the space between platelets in the iridophores increases such that higher wavelengths are reflected, and animals look greener (Morrison et al., 1996). Dickerson's collared lizard males did indeed appear to be greener at lower temperatures (Fig. 2), suggesting a similar mechanism underlying color variation with low temperature. Alternatively, aggregation of melanin, below the structural color, may reduce light reflected and intensify the blue color (Shawkey et al., 2007). The lack of significant differences in blue chroma of males between the control and high temperature treatments may be due to behavioral thermoregulation of some males in the high temperature treatment that were in the refuge when captured for color measurement; this is a natural behavior to high and possibly lethal temperatures, and many lizards seek refuge during the hottest hours of the day. In addition, structural constraints (e.g. the temperature at which the animal achieves the minimal distance between platelets) may limit further blue chroma variation at higher temperatures.

In the Dickerson's collared lizard blue skin color probably plays an important role in sexual communication (Plasman et al., 2015). Our current findings that both sexual color and levels or response of the natural antibodies change with temperature, suggests that blue color may signal current performance level more honestly (Lazzaro and Little, 2009). Color change may also facilitate thermoregulation (Smith et al., 2016), and blue has been suggested as protection to overheating (Sternberg, 1996). Whether the color change influences thermoregulation of Dickerson's collared lizards needs to be investigated in future studies. Additionally, the greener color at lower temperatures may make these lizards less conspicuous, allowing them to lessen the negative effects of the low performance level by using alternative, more cryptic, behavior strategies (reviewed in Duarte et al., 2017).

Extreme temperatures can be stressful to ectotherms. At low temperatures performance is reduced, but risk of reaching critical temperatures is probably small compared to exposure to high temperatures (Clusella-Trullas et al., 2011). The high environmental temperatures of the desert, however, may reach above lethal temperatures and may strongly augment thermoregulation effort (Cole, 1943; Huey, 1982; Vickers et al., 2011; Tattersall et al., 2012). Additionally, at higher temperatures, the metabolic rate of ectotherms increases and thus augments energy costs of body maintenance (Gillooly et al., 2001). Animals may then fail to optimize temperature-related traits and are liable to compromises. Accordingly, Dickerson's collared lizard males exposed to high temperatures had a strong negative relation between agglutination titer and sexual color, revealing a temperature-dependent trade-off between these traits. In contrast, when males were kept at low and control temperature treatments the relationship between immune response and blue chroma was positive, suggesting that blue chroma may be an honest signal of innate immune performance.

Contrary to agglutination, lysis titer did not show a relation with blue chroma. Agglutination is the result of the action of NAbs, whereas lysis is due to the interactions of NAbs with the complement system (Matson et al., 2005). Perhaps an allocation trade-off is more likely to occur with NAbs than with the complement system, either because production of NAbs may require more energy or it responds faster to external factors. Further, different components of the immune response may have different costs and even compete with each other (Krams et al., 2012), and changes in the environment may alter these costs differently (Zamora-Camacho et al., 2016), which may lead to different trade-offs. Interestingly, in a previous study we found no relation of blue chroma with the inflammation response to an injection with PHA, whereas the inflammation did correlate with the blackness of the collar (Plasman et al., 2015). In the present study, we found the opposite when considering natural antibodies titers. It has been suggested that different colors traits may relate with different components of the immune system and form part of a multiple signaling system (Bro-Jørgensen, 2010). Accordingly, in the Dickerson collared lizard, the black in the collar may be related to the strength of the cellular immune response (Plasman et al., 2015), while the blue skin color displayed in the dorsum may be a signal of innate humoral immunity performance.

In conclusion, Dickerson's collared lizard males blue color expression and constitutive innate humoral immunity vary with temperature, and high temperatures compromises investment between these two traits. We suggest that the blue dorsal color of males may be a dynamic signal of innate immune performance. The extreme high temperatures experienced by these lizards in the Sonoran Desert probably imposes high costs, forcing males to compromise between a good immune response to increase chance of survival or a striking sexual color to increase chances of reproduction. The rise in environmental temperature due to climate change (Coumou and Rahmstof, 2012; IPCC, 2012) will likely increase the challenge of thermoregulation (Clusella-Trullas et al., 2011), and may immunocompromise this diurnal desert dwelling lizard, increasing its vulnerability to infectious diseases. The results highlight how even short-term variation of a pervasive environmental factor such as temperature, influences some components of male's innate humoral immunity and sexual signaling, with higher temperatures revealing a trade-off between these fitness-related traits.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2019.05.012.

Ethics

All applicable institutional and/or guidelines for the care and use of animals were followed. Permission to carry out the research was granted by Secretaría del Medio Ambiente y Recursos Naturales (permit number SGPA/DGVS/03365/12).

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Author contributions

MP and RT conceived and designed the experiments; MP performed the experiments and analyzed the data; MP and RT contributed equally to the writing of the manuscript.

Conflicts of interest

Authors declare they have no conflict of interest.

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