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Color Change for Thermoregulation versus Camouflage in Free-Ranging Lizards

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ABSTRACT: Animal coloration has multiple functions including thermoregulation, camouflage, and social signaling, and the requirements of each function may sometimes conflict. Many terrestrial ectotherms accommodate the multiple functions of color through color change. However, the relative importance of these functions and how color-changing species accommodate them when they do conflict are poorly understood because we lack data on color change in the wild. Here, we show that the color of individual radio-tracked bearded dragon lizards, *Pogona vitticeps*, correlates strongly with background color and less strongly, but significantly, with temperature. We found no evidence that individuals simultaneously optimize camouflage and thermoregulation by choosing light backgrounds when hot or dark backgrounds when cold. In laboratory experiments, lizards showed both UV-visible (300–700 nm) and near-infrared (700–2,100 nm) reflectance changes in response to different background and temperature treatments, consistent with camouflage and thermoregulatory functions, respectively, but with no interaction between the two. Overall, our results suggest that wild bearded dragons change color to improve both thermoregulation and camouflage but predominantly adjust for camouflage, suggesting that compromising camouflage may entail a greater potential immediate survival cost.

Keywords: color change, near-infrared, camouflage, thermoregulation.

Introduction

An important trade-off faced by terrestrial ectotherms is between thermoregulation and camouflage. Ectotherms must frequently expose themselves to direct sunlight to reach and maintain an active body temperature necessary for all essential functions (e.g., foraging, mating, escape from predators; Dunham et al. 1989; Seebacher and Franklin 2005), yet doing so may simultaneously increase exposure to predators or compromise camouflage (Endler 1978). Some ectotherms, however, are capable of rapid physiological color change to accommodate the competing functions of coloration—for example, by matching different backgrounds or becoming darker or lighter to increase or decrease absorption of solar radiation, respectively (Stuart-Fox and Moussalli 2009; Umbers et al. 2014). When requirements of thermoregulation and camouflage conflict, individuals may accommodate one requirement (e.g., camouflage) at the expense of the other (e.g., thermoregulation) or use different parts of their bodies for different functions (Smith et al. 2016*b*). However, if appropriate backgrounds are available, individuals may choose backgrounds that simultaneously optimize camouflage and thermoregulation (e.g., a light background when hot or a dark background when cold; Kronstadt et al. 2013). Although temperature- and background-dependent color changes in terrestrial ectotherms are anecdotally widespread and have been documented separately in some laboratory experiments (Walton and Bennett 1993; King et al. 1994; Silbiger and Munguia 2008; Umbers 2011; Langkilde and Boronow 2012; Vroonen et al. 2012; Munguia et al. 2013; Choi and Jang 2014), their joint occurrence, relative importance, and interaction in the wild remain speculative.

The thermal effects of color change depend on how the skin changes its reflectance of the spectrum of direct solar radiation, encompassing UV-visible (300–700 nm) and near-infrared (NIR; 700–2,600 nm) wavelengths (Porter 1967; Porter and Norris 1969). Reflectance in the NIR (700–2,600 nm) can have a substantial effect on rates of heating and cooling and on steady state body temperatures (Porter and Norris 1969) because more than half of the energy in direct sunlight falls within the NIR (Norris 1967; Christian et al. 1996), yet the NIR has no influence on camouflage because the visual systems of animals are insensitive to these wavelengths (Warrant and Johnsen 2013). Visible reflectance is often a poor

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predictor of NIR reflectance (Norris 1967; Gates 1980; Nussear et al. 2000), such that the shape of reflectance spectra in these two parts of the spectrum could change in different ways in response to different stimuli. Conceivably, therefore, an animal may modulate near-infrared and visible reflectance separately to accommodate requirements of thermoregulation and camouflage (Teyssier et al. 2015). For example, a hot animal on a dark background could increase reflectance of near-infrared solar radiation to prevent overheating while retaining low visible reflectance (i.e., dark coloration) for camouflage. However, the relationship between visible and near-infrared reflectance change has only recently been systematically studied (Smith et al. 2016*b*) and has never been studied for changes in response to different stimuli.

In this study, we examined the interaction between color change for thermoregulation and camouflage in radio-tracked bearded dragon lizards, *Pogona vitticeps*, in the wild. *Pogona vitticeps* is an ideal species for this study because it is well known for the ability to change color on both dorsal and ventral surfaces (Greer 1989; Houston 1998; de Velasco and Tattersall 2008; Smith et al. 2016*b*) and in response to circadian rhythms (Fan et al. 2014). The species is a relatively large (ranging in size across the species' geographic range; 15–25-cm snout-vent length; 150–600 g) semiarboreal, diurnal, omnivorous, sit-and-wait forager and occupies a broad range of semiarid habitats in central-eastern Australia. Additionally, *P. vitticeps* has been shown to behaviorally thermoregulate in a laboratory setting (Cadena and Tattersall 2009), and studies on a closely related species (*Pogona barbata*) show that these lizards actively thermoregulate in the wild (Schauble and Grigg 1998). Males defend territories from conspicuous perches during the breeding season. Thus, this species may benefit from multiple functions of color change in the wild, including thermoregulation, camouflage, and communication.

We radio tracked 12 males over 2 months during the breeding season (October–November). To test whether bearded dragons change color for camouflage and thermoregulation in the wild, we quantified the relationship between repeated measurements of individual body and background coloration from digital photos (to minimize disturbance to the animal), skin temperature from thermal images, and core body temperature from implanted temperature telemeters. To test whether bearded dragon lizards adaptively modulate NIR reflectance, we conducted a separate laboratory study on the influence of background color and temperature and their interaction on animal-visible (300–700 nm) and near-infrared (700–2,100 nm) reflectance change. We provide novel evidence of temperature- and background-dependent color change in free-ranging terrestrial animals but show that conflicts between requirements of thermoregulation and camouflage are not ameliorated by changes in near-infrared reflectance.

Material and Methods

Study Site

Twelve adult male lizards were captured by hand north of Walpeup, Australia (35°08′10″S, 142°01′30″E), during the breeding season (September–November 2013). We focused on males during the breeding season because they are likely to show the greatest color change due to sexual and territorial signaling (Castrucci et al. 1997). The environment at the field site is semiarid mallee woodland comprised mostly of silver emu bush (*Eremophila scoparia*) and blue-leafed mallee (*Eucalyptus polybractea*). Lizards have a wide variety of substrates available to them, ranging from tan to yellowish sand and gray to brown leaf litter to dark gray bark of tree trunks, stumps, and dead logs and dry yellow grass. Lizards were transported in cloth bags to the Mallee Research Station (Walpeup, Victoria), where they were temporarily maintained in captivity (University of Melbourne Animal Ethics Committee permit 1212547.2; Department of Environment and Primary Industries Victoria permit 10006829). Each lizard was weighed, measured, and housed individually in a white plastic bin (60 cm × 45 cm × 20 cm) with a bark hide, food and water dishes, and a heat lamp (during natural daylight hours) providing a naturalistic thermal gradient of 23.1°–38.2°C within the enclosure and was fed live mealworms and chopped leafy green vegetables daily.

Surgery and Telemetry

Lizards were surgically implanted with calibrated temperature-sensitive VHF transmitters (Sirtrack Ultimate Lite Implant telemeters) in the peritoneal cavity. Prior to surgery, each telemeter was calibrated in a water bath against a mercury thermometer. Each telemeter was tested at 20°, 30°, and 40°C, and the number of pulses per minute at each temperature was recorded. A specific quadratic equation ($T = ax^2 + bx + c$) was created for each telemeter, where x is the number of pulses per minute and T is the corresponding temperature (°C). In the field, the receiver and directional antenna located the lizards by picking up a pulsed radio signal transmitted by the specific frequency of each telemeter (all telemeters had separate tuning frequencies). The implanted telemeter weighed ~5 g and was always less than 5% of the body mass of the lizard (mean mass: 289.6 ± 15.7 g, range: 169.4–350.1 g; mean snout-vent length: 21.4 ± 0.53 cm, range: 17.0–23.5 cm).

Prior to surgically implanting telemeters, all surgical instruments, benches, and telemeters were sterilized (autoclaved or placed in hydrogen peroxide sterilizing solution; benches were sterilized using a 70% ethanol solution), and all instruments and telemeters were rinsed with sterile saline solution before use. The surgical technique used was similar to that used by Schauble and Grigg (1998) and Cadena and Tattersall

(2009). Briefly, lizards were administered an injectable anaesthetic of Alfaxan (10 mg/kg intravenous or 15 mg/kg intramuscular). Once the lizard had lost feet-pinching reflexes, it was intubated and ventilated using a small rodent ventilator (Inspira safety ventilator; Harvard Apparatus, Holliston, MA) at a rate of 4–6 times per minute and a tidal volume of 3–5 mL. We then made an incision just large enough for the telemeter implant parallel to the abdominal midline (~1 cm long). The skin was then blunt dissected from the muscle, and an incision approximately the same size as the one on the skin was made through the muscle wall and peritoneum. The telemeter was then inserted into the peritoneal cavity, after which the muscle and skin layers were separately sewn up using sterile 4-0 gauge Monocryl absorbable sutures (Ethicon). Each lizard was then given a subcutaneous injection of Meloxicam (0.2 mg/kg) as an analgesic. Lizards were placed on a heating pad and remained intubated until they were able to breathe on their own and a righting reflex was recovered. After surgery was complete, lizards were returned to separate clean enclosures and monitored for a recovery period of 7–12 days. All lizards resumed normal behavior (including eating) within a few hours of emergence from anaesthesia.

Lizards were released at their site of capture within 14–17 days after capture and were temporarily marked on the tail with a Sharpie oil-based white paint marker. Once released, each lizard was radio tracked using a Sirtrack Ultra Receiver and directional antenna (Sirtrack, Havelock North, New Zealand). After the initial release, one lizard was never located using radio telemetry (which is why $N = 11$ in “Results”). Field and surgical procedures were approved by the Animal Ethics Committee of the University of Melbourne (protocol 1212547).

Field Observations

Lizards were observed and measurements were recorded remotely at least once every other day during the 8-week study (October 9–November 28, 2013). For each observation, we immediately recorded the GPS waypoint of the lizard (Garmin Oregon 550, 3.2-m resolution) and core body temperature (T_b; °C) using the implanted telemeters.

To quantify the color of free-ranging lizards, we took a photo as soon as the lizard was sighted (Canon EOS 350D; 35–105-mm or telephoto 75–300-mm ultrasonic Canon zoom lens). A photo of a digital gray/white/black card (Digital Grey Kard; DGK Color Tools, Boston) with 20% gray reflectance was taken at the same position and direction of the observed lizard immediately after taking the initial photo of the lizard.

To assess the relationship between skin temperature and core body temperature, thermal images of the lizard and surrounding habitat were recorded using a thermal imaging camera (T420; FLIR Systems, Wilsonville, OR). Average dorsal skin temperature (T_s; °C) was extracted from the photos for the head, back, and tail regions by drawing regions of interest using FLIR ResearchIR software (FLIR Systems).

Analysis of Photographs

To assess lizard skin coloration, red (R), green (G), blue (B), and luminance values were extracted from gray/white/black cards, the immediate background (substrate; e.g., sand, leaf litter, grass, or perch; tree trunk or log), and the entire head, back, and tail regions of the lizard for all photos (i.e., derived mean RGB values for each region of interest), using a Matlab (MathWorks) script written by J. A. Endler. We then linearized RGB values using a biexponential function in the form of $y = a \times \exp^{b \times x} + c \times \exp^{d \times x}$, where y is the linearized pixel value and a, b, c, and d are empirically derived constants specific to a given camera (Garcia et al. 2013). Due to variable lighting conditions in natural environments (sun vs. shade) and the use of different lenses (35–105-mm or 75–300-mm lens) depending on the distance of the lizard, we derived four different linearization equations, one for each of the following four conditions: (1) full sun, normal lens; (2) full shade, normal lens; (3) full sun, telephoto lens; and (4) full shade, telephoto lens. We used photographs of a color checker standard (X-Rite, Grand Rapids, MI) obtained under each of these four conditions to derive the linearization function for R, G, B, and luminance channels based on the relationship between camera responses to the six grayscale squares of the color checker and their measured reflectance values (Stevens et al. 2007). Linearized R, G, B, and luminance values were then equalized relative to the reflectance of the gray standard in each photo (Stevens et al. 2007).

We calculated the standardized differences between the red (R) and green (G) channels as $(R - G)/(R + G + B)$ and between the green and blue (B) channels as $(G - B)/(R + G + B)$ to provide a two-dimensional representation of color space, where the distance from the origin corresponds to chroma and the angle relative to the axis corresponds to hue (Endler 1990; Grill and Rush 2000). Physical chroma was calculated as $r = (x^2 + y^2)^{1/2}$ and physical hue as $\Theta = \tan^{-1}(y/x)$, where x and y are the standardized differences between red-green (R-G) and green-blue (G-B) channels, respectively (Endler 1990), using the MATLAB function cart2pol. Luminance was calculated as the sum of the linearized and equalized R, G, and B values. As the color of lizards was relatively uniform, we calculated mean dorsal coloration (mean of head, back, and tail regions) for each color component (hue, chroma, and luminance). We used hue, chroma, and luminance values derived from corrected RGB values to minimize additional data transformations and assumptions regarding receiver vision associated with mapping RGB values to photoreceptor stimulation (Kemp et al. 2015), particularly for tetrachromatic receivers (e.g., birds, the primary predators of *Pogona vitticeps*). This is particularly important given that our data are restricted to the human-visible spectrum (400–700 nm; although UV reflectance of lizards and backgrounds is minimal; fig. A1; figs. A1–A3, B1 are available online). However, we also empirically confirmed that the colors of lizards and their natural backgrounds (derived from photos then mapped in RGB color space) have a statistically similar distribution to near-simultaneously collected spectral data mapped in avian and agamid lizard visual color space (see app. B and fig. B1 for full details; apps. A–C are available online).

Statistical Analysis

We tested whether mean dorsal lizard color (hue, chroma, or luminance) was predicted by background color (hue, chroma, or luminance) and lizard temperature (core body temperature from telemeters or skin temperature from thermal images) using generalized linear mixed models in SAS 9.3 (PROC MIXED; SAS Institute). Lizard ID was included as a random factor in all models to account for repeated measures of individual lizards. We compared models with core body temperature (T_b; °C) versus skin temperature (T_s; °C) as predictors; that is, we did not include both T_b and T_s in the same model, because the two variables were highly correlated ($R^2 = 0.865$, $P \leq .0001$; fig. A2). To test for body temperature–dependent background choice, we assessed the relationship between background color (hue, chroma, or luminance) and core body temperature, again including lizard ID as a random variable.

Laboratory Experiments

We used 10 male lizards hand-captured in October 2012 from the same region as the field experiment, brought into captivity and housed in the School of Biosciences at the University of Melbourne. Lizards were individually housed in sand-filled terraria, each fitted with UV and incandescent basking lamps set to a 12L∶12D photoperiod (lights on at 0700 hours). Terraria were also fitted with a hiding place and a natural branch for perching. A temperature gradient between 25° and 50°C was maintained inside the terrarium during the light phase, allowing for behavioral thermoregulation. Lizards were provided water ad lib. and fed a diet of crickets and commercial bearded dragon food (10.04 URS Lizard Food; Ultimate Reptile Supplies, Adelaide, Australia) mixed with green leafy vegetables, carrots, and pumpkin three times a week (they are omnivores). Bearded dragons were captured under the Department of Sustainability and Environment Victoria permit 10006453. All experimental procedures were approved by the Animal Ethics Committee of the University of Melbourne (protocol 1212547).

We placed lizards in a temperature-controlled incubator (32 cm × 36 cm × 45 cm; Exo-Terra; Rolf C. Hagen, Mansfield, MA) set at either 15° or 40°C, with either light or dark background (i.e., four treatments: hot-dark, hot-light, cold-dark, cold-light). To achieve the light or dark backgrounds, the bottom and walls of the incubator were lined with either yellow or black sand, corresponding to the approximate extremes of background reflectance in the lizards' natural habitat (fig. A3). Each lizard was tested in each of the four treatments in random order with a minimum of 24 h between experiments.

After 45 min in the incubator, we quickly (<20 s) measured the skin reflectance of the head, back, beard, and chest using a dual spectrometer system (Ocean Optics, Dunedin, FL; Smith et al. 2016*b*) comprising two spectrometers (USB2000 [300–1,000 nm] and NIRQuest [1,000–2,150 nm]) with two light sources (PX-2 pulsed xenon light for the UV-visible range; HL-2000 tungsten halogen lights for the NIR range) connected with a quadrifurcated optical fiber ending in a single probe (measurement area of 5 × 3-mm oval). The probe was held in an Ocean Optics RPH-1 probe holder at a constant angle (45°) and distance (approx. 1 cm) from the lizard skin, and each measurement was expressed relative to a Spectralon 99% white reflectance standard (Labsphere, North Sutton, NH).

From reflectance spectra, we calculated the average reflectance for UV-visible (Vis; 300–700 nm) and NIR (700–2,100 nm) wavelengths and the standardized difference between them ($(\mathrm{NIR} - \mathrm{Vis})/(\mathrm{NIR} + \mathrm{Vis})$). As the color of lizards was relatively uniform, we calculated mean values for dorsal (head and back) and ventral (beard and chest) regions and used these in subsequent analyses. We tested the effect of background color and temperature and their interaction on reflectance using a general linear mixed model (PROC MIXED; SAS Institute) with lizard ID as a random factor to account for repeated measures on individual lizards.

Results

Color Change for Thermoregulation and Camouflage in the Wild

Lizards showed a significant and consistent relationship between their dorsal skin coloration, background coloration, core body temperature (T_b; table 1), and skin temperature (T_s; table C1, available online). Lizards expressed very strong background matching (fig. 1), with a strong relationship between the background and lizard color in terms of hue, chroma, and luminance (fig. 2). Background color explained 13%, 50%, and 38% of the variation in the hue, chroma, and luminance of lizards, respectively, when T_b was included as a covariate (table 1; fig. 2). Data underlying figure 2 are deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061

Table 1: Relationship between lizard color (hue, chroma, luminance) and lizard core body temperature (T_b; °C) and color of the background (Bkgd)

Dependent variable and independent variables	F_{df}	P	Partial R^2
Lizard hue:			
T_b	$17.45_{1,68}$	$<.0001^*$	.17
Bkgd hue	$15.57_{1,68}$	$.0002^*$	.13
Lizard chroma:			
T_b	$10.62_{1,68}$	$.002^*$	.09
Bkgd chroma	$80.69_{1,68}$	$<.0001^*$	.50
Lizard luminance:			
T_b	$8.34_{1,69}$	$.005^*$	.08
Bkgd luminance	$45.87_{1,69}$	$<.0001^*$	.38

Note: Lizard ID was a random variable in each model. Partial R^2 refers to the proportion of variation accounted for by each factor relative to a model containing only the random effect. Results for the effect of skin temperature (T_s; °C) on hue, chroma, and luminance are shown in table C1. Asterisks denote values that remain significant after false discovery rate correction for multiple tests. The interaction between T_b and background color was initially included but dropped from final models, as no interaction term was significant.

/dryad.dg27h (Smith et al. 2016*a*). The color of lizards was unrelated to their mass (hue: $F_{1,71} = 0.22$, $P = .64$; chroma: $F_{1,71} = 2.64$, $P = .11$; luminance: $F_{1,71} = 0.26$, $P = .61$); therefore, mass was not included as a covariate in final models.

In addition to matching the background, lizards were markedly lighter (increasing chroma and luminance, more reflective) and yellower (decreasing hue) with increasing temperature (both T_b and T_s; figs. 1, 2). Although both T_b and background color independently explain variation in lizard color, there is no evidence for a correlation between T_b and background color (hue: $F_{1,70} = 0.26$, $P = .61$; chroma: $F_{1,70} = 1.19$, $P = .28$; luminance: $F_{1,70} = 0.25$, $P = .62$), suggesting that these lizards do not choose lighter backgrounds at higher body temperatures and darker backgrounds at lower body temperatures (i.e., they do not exhibit body temperature–dependent background choice).

Interestingly, T_s accounted for more variation in the lizards' visible hue and chroma (21% and 14%, respectively; table C1) than did T_b (17% and 9%, respectively; table 1); however, T_b accounted for slightly more variation in luminance (8%; table 1; fig. 2*C*) than skin temperature (6%; table C1). The skin surface reaches higher temperatures faster than T_b, which reaches an asymptote at 40°C as T_s continues to increase to a maximum of almost 50°C (fig. A2).

Visible and NIR Reflectance Change in the Laboratory

We tested whether color-changing animals may accommodate conflicting requirements of thermoregulation and camouflage through modifying near-infrared reflectance relative to visible reflectance. Specifically, we quantified the effects of temperature and background color and their interaction on UV-visible (300–700 nm) and near-infrared (700–2,100 nm) reflectance change. Captive lizards showed a marked dorsal increase in both visible and NIR reflectance at 40° compared to 15°C (UV: $F_{1,27} = 40.05$, $P < .0001$; NIR: $F_{1,27} = 30.98$, $P < .0001$) and increased reflectance on light backgrounds compared to dark backgrounds (UV: $F_{1,27} = 18.77$, $P = .0002$; NIR: $F_{1,27} = 8.06$, $P = .009$).

In contrast to the field data, the change in response to temperature was greater than in response to background color (fig. 3*A*; table 2). There was no interaction between temperature- and background-dependent color change (UV: $F_{1,27} = 1.54$, $P = .23$; NIR: $F_{1,27} = 1.07$, $P = .31$), with lizards changing color on both backgrounds in response to temperature (cf. fiddler crabs; Kronstadt et al. 2013). Notably, there was no consistent change in reflectance for ventral body regions (fig. 3*B*; table 2), even though this species shows the most marked color change on ventral body regions when signaling, with the chest and beard changing from cream to black (Smith et al. 2016*b*). Contrary to predictions, for dorsal body regions, the relative proportion of NIR reflectance (measured as the difference between NIR and visible reflectance standardized for total reflectance) was higher at 15° than 40°C ($F_{1,27} = 17.82$; $P = .0002$) and on dark than light backgrounds ($F_{1,27} = 10.64$; $P = .003$), with no interaction between temperature and background color ($F_{1,27} = 0.7$, $P = .41$; fig. 4).

Discussion

The importance of animal coloration for thermoregulation remains controversial despite over 100 years of research (Umbers et al. 2013; Zeuss et al. 2014). Furthermore, we have little understanding of how animals use color to accommodate competing functions of thermoregulation and camouflage, especially when these functions conflict. Animals that change color provide the opportunity to assess multiple functions of color and the interaction between them without confounding factors inherent in comparing individuals. Our results indicate that in the wild, bearded dragons change color in response to both temperature and background color, with background color having a stronger effect. Temperature-dependent color change in free-ranging lizards has not previously been demonstrated, despite the notion being widespread in physiological ecology (Seebacher and Franklin 2005; Clusella Trullas et al. 2007). However, there is a much stronger relationship between dorsal coloration and background color than either core or skin temperature, suggesting that lizards predominantly use color change for camouflage rather than for thermoregulation. Once they have reached an active body temperature, lizards may rely on behavioral thermoregulation rather than color change. We suggest that in the wild, color-changing ectotherms are likely

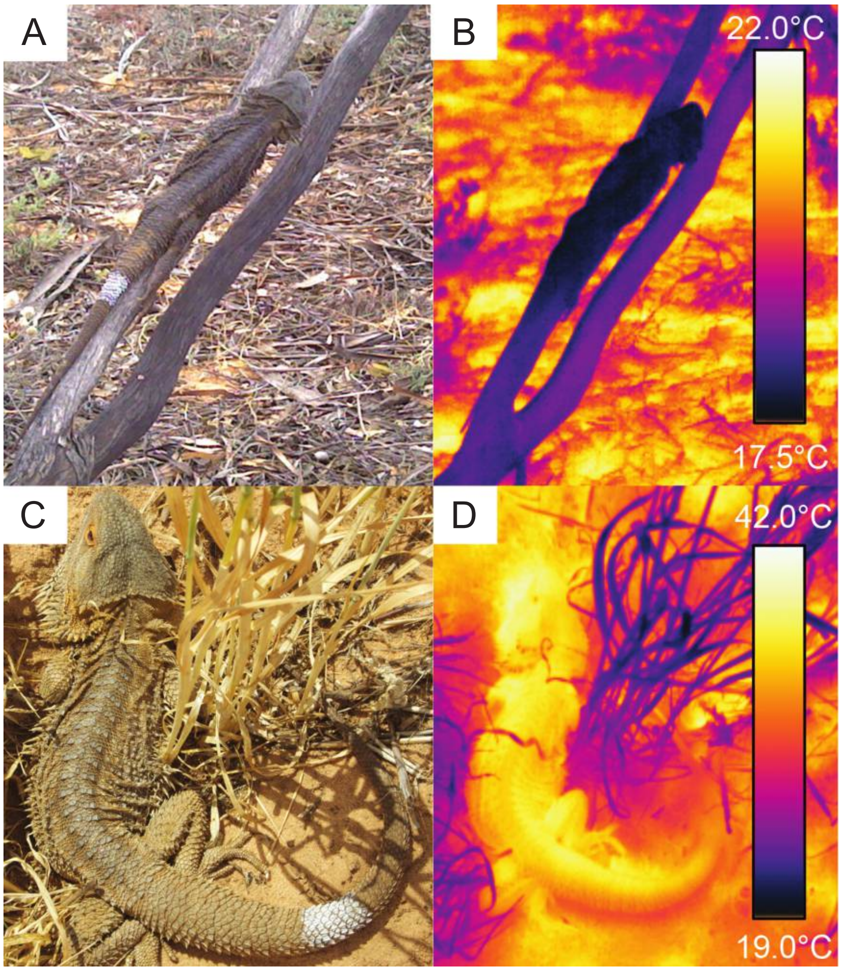

Figure 1: Photographs of the same individual at a cool core body temperature with dark coloration (top left; $T_b = 18.45$°C; *A*) and at a hot core body temperature with light coloration (bottom left; $T_b = 34.26$°C; *C*) with corresponding thermal images (*B* and *D*, respectively). Note the background matching in *A* and *C*.

to show a stronger color change response to background color than body temperature, which would suggest in turn that predation risk may present a greater immediate survival cost compared to thermoregulatory requirements. This pattern may be less marked in very extreme thermal environments.

Although bearded dragons primarily change color for camouflage in the wild, our results suggest that color change may also aid in thermoregulation. The observed color change should provide a thermoregulatory advantage because it increases absorption of solar radiation at low temperatures and decreases absorption at high temperatures (Clusella Trullas et al. 2007; Bohorquez-Alonso et al. 2011; Smith et al. 2016*b*). Furthermore, the spectral range of maximum color change (approx. 400–1,400 nm based on laboratory experiments) corresponds to the range of maximum solar irradiance such that change in this spectral range has the greatest influence on thermal balance. In general, there was a tighter relationship between dark dorsal coloration at low core body temperature than light coloration at high core body temperature, suggesting that lizards primarily use color change to increase heating rates when cold (by becoming darker; fig. 2). Dark coloration may enable the lizard to reach active body temperatures faster, essential for escaping predators and also giving more time for foraging. Biophysical models confirm that color change can provide a significant thermoregulatory advantage by substantially reducing basking time required to reach active body temperatures during the breeding season for this population (Smith et al. 2016*b*). Once an individual reaches an active body temperature, it may rely more on behavioral mechanisms (e.g., active microhabitat selection and postural adjustments) than color change for thermoregulation, with color change primarily used to facilitate camouflage.

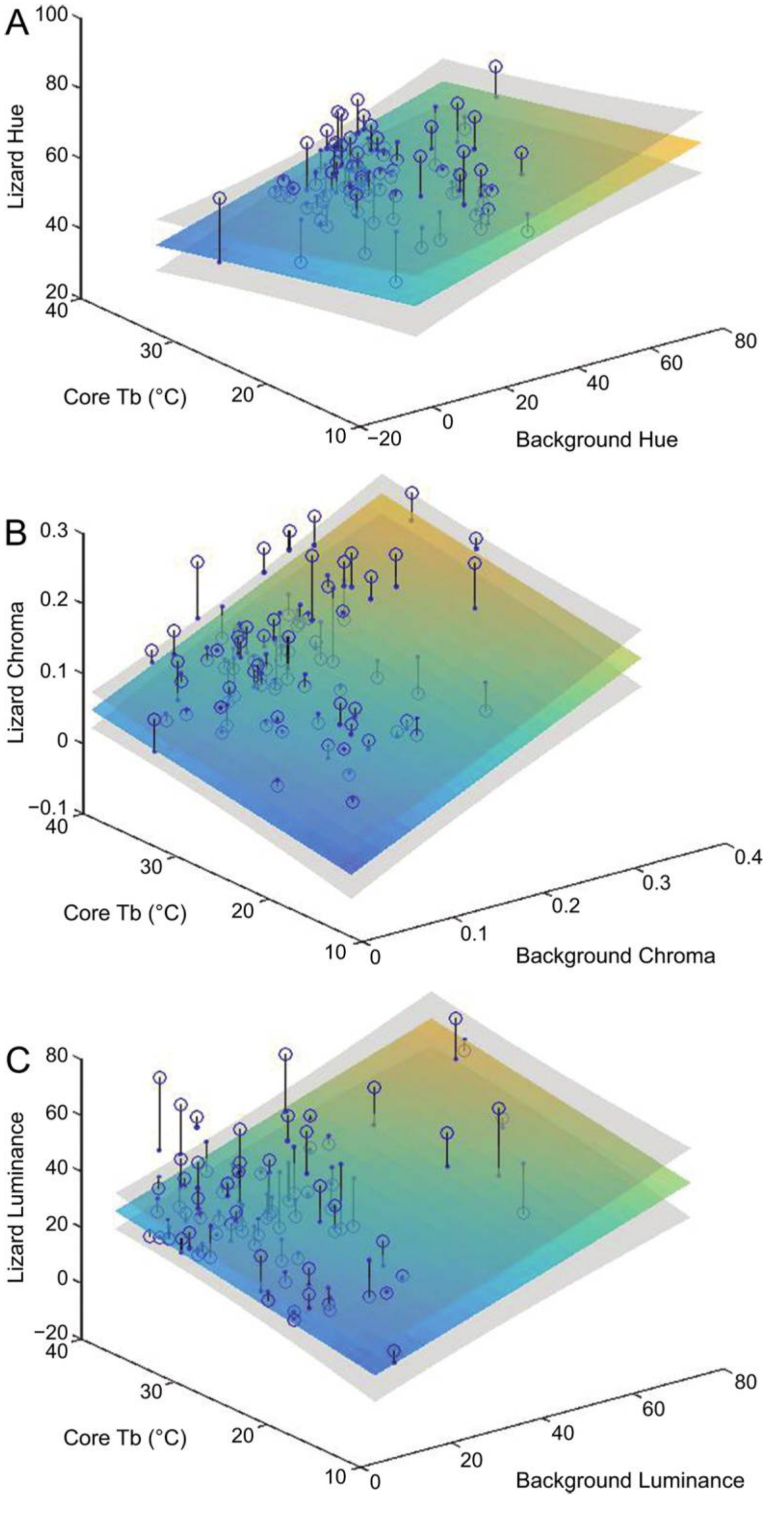

Figure 2: Relationship between the color of the lizard, the color of the background, and lizard core body temperature (T_b; °C) for hue (*A*), chroma (*B*), and luminance (*C*). The main colored plane represents the general linear mixed model prediction, the gray shaded planes represent the 95% confidence limits around the prediction, the open circles represent the data points for all lizards, the dots represent the values on the plane corresponding to the prediction, and the vertical lines represent the residuals. In each case, the relationship between the color of the lizard and the color of the background is positive, indicating color change to match the background. The relationship between lizard color and core body temperature is positive for hue and negative for chroma and luminance, indicating that lizards are darker and grayer when cold and lighter and yellower when hot. Note the smaller residuals at low body temperatures, indicating a tighter relationship between dark dorsal coloration at low core body temperature than light coloration at high core body temperature.

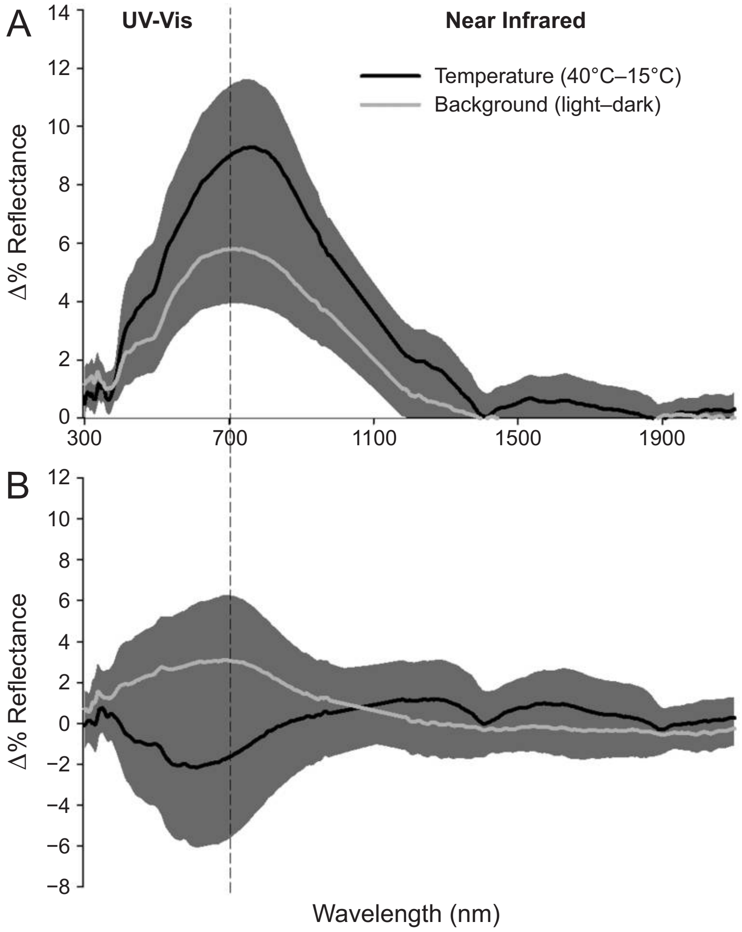

Figure 3: Dorsal (*A*) and ventral (*B*) reflectance change in response to temperature (40°–15°C) and background (light, dark) as a function of wavelength. The black line shows the mean ± SE difference in reflectance at 40° compared to 15°C (background color treatments combined), while the gray line shows the mean ± SE difference in reflectance on the light background compared to the dark background (temperature treatments combined). Values above 0 for a given wavelength indicate reflectance change in response to the treatment.

Interestingly, while skin temperature accounted for more variation in dorsal hue and chroma than core body temperature, core body temperature accounted for slightly more of the variation in luminance than skin temperature. The skin surface reaches higher temperatures faster than core body temperature; thus, bearded dragons might use skin temperature as a way to actively regulate body temperature, using it as a trigger for physiological or behavioral thermoregulation. More chromatic coloration at higher skin temperatures may be due in part to effects of temperature on mechanisms of dermal color change (Sherbrooke and Frost 1989) and because heat flux is sensed by peripheral sensors to enable rapid response (Kenton et al. 1971; Crawford and Barber 1974; Tattersall et al. 2006). However, luminance is the most important color component affecting absorption of solar radiation. Therefore, core body temperature may ultimately dictate the need to use color change for thermoregulation.

We found no evidence that bearded dragons accommodate conflicting requirements of thermoregulation and camouflage through temperature-dependent background choice. Specifically, we might expect individuals to choose a light background when hot or a dark background when cold to simultaneously optimize camouflage and thermoregulation (Kronstadt et al. 2013). Many animals select backgrounds nonrandomly to improve crypsis (Briffa and Twyman 2011; Kang et al. 2012; Lovell et al. 2013; Nafus et al. 2015). Similarly, many color-changing species exhibit behaviors that improve crypsis (Ellis et al. 1997; Garcia et al. 2003; Ryer et al. 2008; Kelley et al. 2012) because there are limits to their capacity to change color and match the background. Although we found no overall correlation between body temperature and background color, the relationship between lizard and background color was stronger (less variable) when the lizards were cold, suggesting that cold, dark lizards are more likely to be found on darker backgrounds. Crypsis is

Table 2: Effects of background color (light vs. dark) and temperature (40° vs. 15°C) and their interaction on UV-visible (Vis; 300–700 nm) and near-infrared (NIR; 700–2,100 nm) reflectance and reflectance in the NIR relative to the Vis (measured as the standardized difference between them)

Body region, dependent variable, and predictor(s)	$F_{1,27}$	P
Dorsal:		
Vis:		
Temperature	40.05	<.0001*
Background	18.77	.0002*
Temperature × background	1.54	.23
NIR:		
Temperature	30.98	<.0001*
Background	8.06	.009*
Temperature × background	1.07	.31
(Vis − NIR)/(Vis + NIR):		
Temperature	17.82	.0002*
Background	10.64	.003*
Temperature × background	.7	.41
Ventral:		
Vis:		
Temperature	.92	.35
Background	2.13	.16
Temperature × background	.03	.86
NIR:		
Temperature	3.17	.09
Background	2.90	.10
Temperature × background	1.39	.25
(Vis − NIR)/(Vis + NIR):		
Temperature	6.90	.01
Background	.89	.35
Temperature × background	2.23	.15

Note: Asterisks denote values that remain significant after false discovery rate correction for multiple tests.

likely to be particularly important when lizards are below active body temperature, because their ability to flee from predators is impaired (Norris 1967). However, backgrounds surrounding burrows where cold lizards emerge to bask may tend to be darker and less variable than other sites; therefore, the propensity of cold, dark lizards to be found on dark backgrounds may not be due to active background choice (examples of bearded dragon burrows found were holes dug in brown sand underneath leaf litter, in old rabbit burrows, at the bases of trees, and inside spinifex plants). Experimental tests would be required to confirm whether lizards show active background choice to improve crypsis when below active or preferred body temperatures.

We complemented our field study with a laboratory experiment to quantify both visible and near-infrared (NIR) reflectance change during color change. Because visible reflectance influences both camouflage and thermoregulation, whereas NIR reflectance influences only the latter, we hypothesized that lizards may be able to differentially modify the relative amount of visible and NIR reflectance to accommodate thermoregulatory and camouflage functions of color. Specifically, we would expect hot lizards to have higher relative NIR reflectance than cold lizards, particularly on dark backgrounds. For example, a hot animal on a dark background could increase the relative proportion of NIR reflectance to prevent overheating while retaining low visible reflectance (i.e., dark visible coloration) for camouflage. However, we observed no such pattern in the laboratory, and in fact the change in dorsal coloration in response to temperature was greater than the response to background color (in contrast to field observations). This may reflect the habituation of the lizards to their captive environment and the perceived absence or acclimation to a potential predatory threat (human observer) during experiments. Our laboratory data indicate that lizards modify their visible and NIR reflectance in parallel in response to temperature and background color. That is, the change in the relative proportion of NIR reflectance appears to be primarily a function of total reflectance (relative NIR decreases with increasing total reflectance). Nevertheless, the dorsal color change in response to temperature and background clearly supports a function for thermoregulation and camouflage, respectively. The stronger response to background color than temperature in the wild (in contrast to the laboratory) is consistent with the greater potential survival cost of compromising camouflage, and we suggest that this pattern is likely to hold more generally for color-changing terrestrial ectotherms.

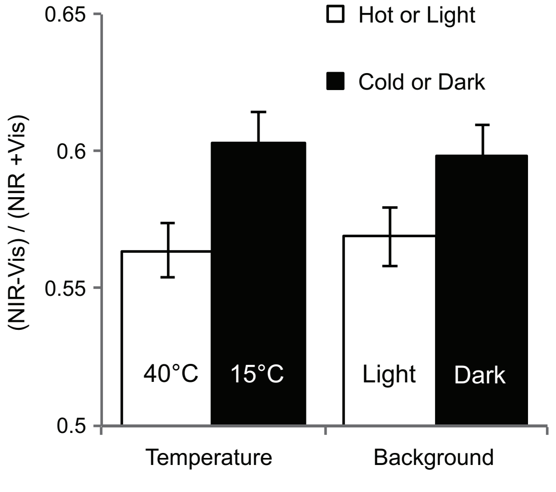

Figure 4: Relative near-infrared reflectance (NIR; measured as the standardized difference between NIR and animal-visible reflectance [Vis]) on light or dark backgrounds and at hot or cold temperatures (40° vs. 15°C) in laboratory experiments. Only main effects are shown because the interaction between temperature and background was not significant ($F_{1,27} = 0.7$, $P = .41$).

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