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RESEARCH ARTICLE

Geographic divergence and colour change in response to visual backgrounds and illumination intensity in bearded dragons

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ABSTRACT

Animals may improve camouflage by both dynamic colour change and local evolutionary adaptation of colour but we have little understanding of their relative importance in colour-changing species. We tested for differences in colour change in response to background colour and light intensity in two populations of central bearded dragon lizards (*Pogona vitticeps*) representing the extremes in body coloration and geographical range. We found that bearded dragons change colour in response to various backgrounds and that colour change is affected by illumination intensity. Within-individual colour change was similar in magnitude in the two populations but varied between backgrounds. However, at the endpoints of colour change, each population showed greater similarity to backgrounds that were representative of the local habitat compared with the other population, indicating local adaptation to visual backgrounds. Our results suggest that even in species that change colour, both phenotypic plasticity and geographic divergence of coloration may contribute to improved camouflage.

KEY WORDS: Animal coloration, Camouflage, Predator avoidance, Phenotypic plasticity, Local adaptation

INTRODUCTION

Many animals possess the capacity to change colour, an ability that has the potential to increase fitness by improving camouflage (e.g. Stuart-Fox and Moussalli, 2009; Stuart-Fox et al., 2006). The phenotypic flexibility afforded by physiological colour change makes it particularly suitable for avoiding detection from visual predators because it enables a dynamic response to perceived predatory threats. However, selection for colour change to enhance camouflage may vary spatially in relation to environmental characteristics. For example, populations of widespread species may differ in the species composition or abundance of predators with different visual capabilities, thereby affecting predation risk (Endler, 1980, 1988; Hopper, 2001). Moreover, the visual characteristics of the habitats of widespread species may also vary in their general colour, appearance and complexity, favouring geographic divergence in any mechanisms affecting visibility. For rapidly changing species, more visually complex environments should favour the evolution of an increased capacity for colour change to accommodate the greater visual diversity that an animal experiences during its lifetime (Stuart-Fox and Moussalli, 2009). Therefore, in species that use cryptic coloration as an anti-predatory tactic, environmental differences may be important drivers for the evolution of population differences in both coloration and colour change (Endler, 1978; Stuart-Fox et al., 2006). However, geographic variation in the extent and nature of colour change has seldom been quantified.

Colour change enhances camouflage by general matching of the colour and appearance of the background (Chiao et al., 2005; King et al., 1994; Osorio and Srinivasan, 1991). Consequently, background colour and pattern is a common environmental cue eliciting colour change in a variety of taxa (Chiao and Hanlon, 2001; Choi and Jang, 2014; Stevens et al., 2014a). Background colour may also interact with other environmental cues such as illumination to influence colour change and camouflage. Light environments vary greatly between different types of habitats and throughout the day, and individual conspicuousness may vary depending on the light environment (Endler, 1993; Endler and Thery, 1996; White and Kemp, 2016). Moreover, illumination affects an animal's ability to gather visual information from its environment and, therefore, to accurately match its background. For example, cuttlefish and Moorish and Mediterranean geckos are unable to match their background at low light levels, instead adopting a light coloration independent of background colour (Buresch et al., 2015; Vroonen et al., 2012; Zaidan and Wiebusch, 2007). In addition to influencing colour change through variation in visual perception, light can directly stimulate dermal chromatophores through the direct activation of photoreceptors in the skin (Oshima, 2001). It is therefore of interest to examine the effects of both background colour and illumination on colour change and resulting camouflage.

We examined the effect of background colour and illumination intensity on the capacity for colour change in two populations of the central bearded dragon lizard, *Pogona vitticeps* Ahl 1926. These two populations were chosen because they differ most in body coloration and habitat visual properties occupied by the species. Central bearded dragons are well known for their capacity to change colour, and dorsal coloration varies geographically from cream to grey to orange–red (Smith et al., 2016b). The species occupies a diversity of visual environments across its large geographical range (covering a large part of central Australia). For example, habitats from the northern end of the range are characterised by red desert sand dunes and sparse vegetation cover composed of spinifex (*Triodia* sp.) and ghost gums (*Eucalyptus papuana*), whereas the habitats of southern populations are characterised by yellow to grey substrates and semi-arid mallee woodlands composed mostly of silver emu-bush (*Eremophila scoparia*) and blue-leafed mallee (*Eucalyptus polybractea*). Using wild-caught males from populations occupying these habitats towards the northern and southern extremes of the species' range, we conducted laboratory experiments to quantify the extent of colour change in response to different cues. Specifically, we tested whether

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bearded dragon populations vary in how they change their skin colour in response to their background or to illumination intensity and whether this is consistent with local variation in natural substrate colour.

MATERIALS AND METHODS

Animals

Male bearded dragons were hand captured from the vicinities of Alice Springs (23°42′S, 133°52′E; *N*=11) and Mildura (34.18°S, 142.15°E; *N*=11) in September and October 2012. We focused on males because they are often more conspicuous while patrolling territories and to remove potential variation due to sex. Following capture, the animals were transported to housing facilities in the School of Biosciences at The University of Melbourne. Each lizard was housed in a terrarium fitted with a UV lamp and an incandescent basking lamp set to a 12 h light:12 h dark photoperiod (lights on at 07:00 h). Terraria contained a yellow sand substrate (Fig. 1; Fig. S1; see below for a description of natural and artificial background colours), a hiding place and a natural tree branch for perching (dark brown). A temperature gradient between 25 and 50°C was maintained inside the terrarium during the light phase, allowing for behavioural thermoregulation. These temperatures are within the natural range experienced by bearded dragons in the wild (Smith et al., 2016a).

Lizards were provided with water *ad libitum* and fed a diet of crickets and commercial bearded dragon food (10.04 URS Lizard Food, Ultimate Reptile Supplies, Burton, SA, Australia) mixed with green leafy vegetables, carrots and pumpkin, three times a week. Bearded dragons were obtained under Parks and Wildlife Commission Northern Territory permit number 44582 and Department of Sustainability and Environment Victoria permit number 10006453. Experimental procedures were approved by the Animal Ethics Committee of The University of Melbourne (protocol no. 1212547). Experiments were initiated approximately 3 months after capture, when lizards had fully adjusted to captive conditions.

Experimental procedures

All experiments were conducted inside a temperature-controlled room set to 35°C to ensure that ambient temperature did not influence individual colour (de Velasco and Tattersall, 2008; Smith et al., 2016b). Before each experiment, lizards were placed in a small incubator (Exo-Terra Thermoelectric Egg Incubator, Rolf C. Hagen Corp., Mansfield, MA, USA) at a temperature of 35°C for a minimum of 45 min to allow for thermal equilibrium at the bearded dragon's preferred body temperature (Cadena and Tattersall, 2009).

Background colour

To test whether the colour of bearded dragons changes in response to their background, we used digital photography to measure the colour of lizards placed for 45 min on three different sand backgrounds: orange, yellow and black (Fig. 1; Fig. S1). Although standard digital photographs only capture colour in the human-visible spectrum and diurnal lizards such as bearded dragons (and many of their predators) are likely to be able to see in the UV (Olsson et al., 2013), these lizards and their backgrounds have minimal UV reflectance; photographs capture most of the colour variation (Smith et al., 2016a).

The sand used for the orange background was obtained from the vicinity of Alice Springs and is representative of the most red/orange sands on which bearded dragons were caught (Fig. 1), while the sand used for the yellow background was similar to the yellow/cream sands predominant around Mildura (Play Sand, Richgro Garden Products, WA, Australia; Fig. 1). Black sand was purchased from a local pet store and was used as an extreme comparison to gauge the extent of colour change (Fig. 1). The experimental arenas were constructed from plywood (60×60×45 cm) and consisted of four walls coated with either the orange, yellow or black sand using spray adhesive, and a bottom tray filled with approximately 2 cm of the same sand as the walls. Three 50 W full-spectrum lamps (mercury vapour lamps; model 645001, Sylvania, Sydney, Australia) were suspended equidistantly 130 cm above the arena and yielded irradiance (400–700 nm) of 5.107 µmol

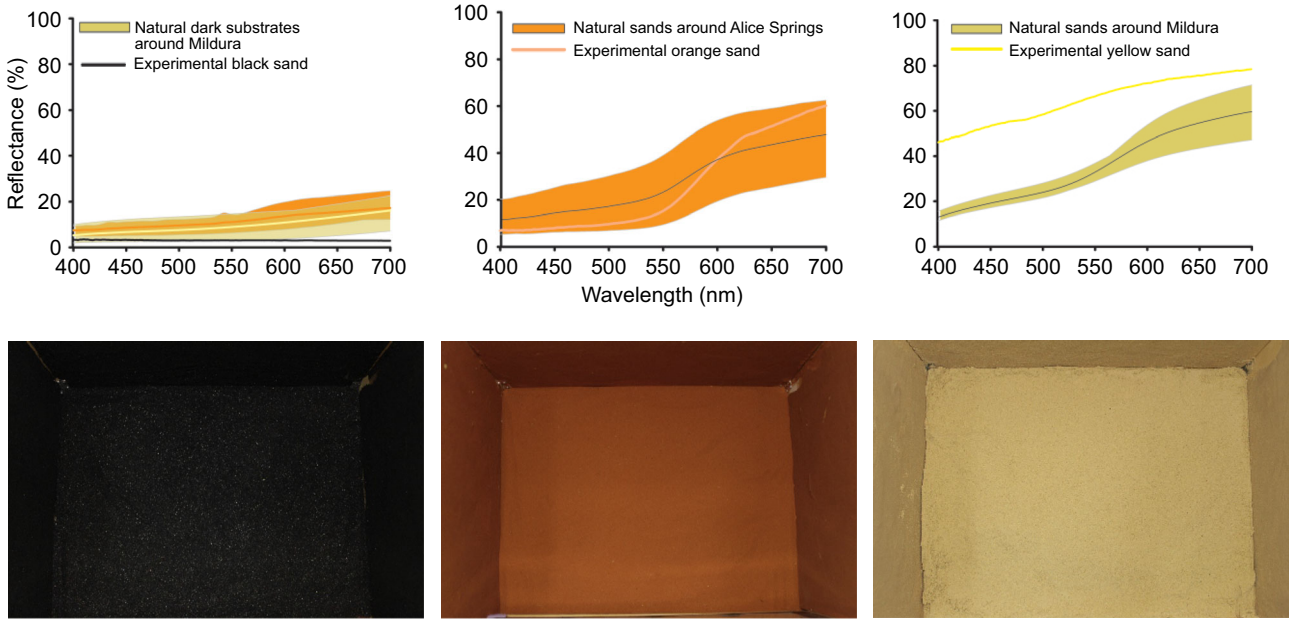

Fig. 1. Reflectance spectra and digital photographs for the black, orange and yellow (experimental and natural) backgrounds used during the experiments. Shaded areas for the natural backgrounds show the range (maximum and minimum reflectance).

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photons m^{-2} s^{-1} at lizard level. The temperature inside the arena was continuously monitored and maintained at 34.7±0.8°C (no difference between background treatments; one-way ANOVA: F_2=0.236, P=0.8), similar to typical field temperatures during the active season. To obtain digital photographs of the lizards for colour analysis, a digital camera (EOS 600D, Canon, Sydney, Australia; ISO: 400; shutter speed: 1/15; aperture: 3.5) facing the bottom of the enclosure was set directly above the arena. Each lizard was exposed to all three background treatments in a randomised order with 3.8± 5.3 days between experiments.

After the initial 45 min warming period inside the incubator, the lizard was placed inside one of the three coloured experimental arenas, and the experimenter left the room to prevent further disturbance to the animal. Handling stress affects the colour of bearded dragons (Fig. S2) and analysis of the images showed a rapid change in skin coloration during the first few minutes in the arena that may have been related to handling (Fig. S3). For this reason, and to allow enough time for bearded dragons to reach a stable coloration on the corresponding background, the first 10 min of each experiment were considered an acclimation period (Fig. S3). Lizards remained inside the arena for a further 35 min, during which digital images were remotely recorded with a camera (EOS Utility software, Canon) every 30 s. All images were recorded in RAW format at a 12 megapixel resolution.

Illumination intensity

In a separate set of experiments, we investigated the effect of three different illumination intensity treatments on lizard skin coloration. We used yellow sand for these experiments because it is available to both lizard populations even though orange sands are more common in Alice Springs.

The illumination intensity was manipulated by powering one, two or three of the mercury vapour lamps suspended above the enclosure (LL1, LL2 and LL3, irradiance of 2.084, 3.133 and 5.107 µmol photons m^{-2} s^{-1}, respectively; 400–700 nm). Temperature inside the arena was monitored and maintained at 34.9±0.5°C (no differences in temperature between illumination levels; one-way ANOVA: F_2=0.12, P=0.89).

As with the background colour experiments, the initial 10 min were considered the acclimation period, followed by an additional 35 min at the experimental lighting conditions. Photographs were taken remotely every 30 s (Camera settings: LL1: ISO 1600, shutter speed: 1/15, aperture: 5; LL2: ISO 800, shutter speed: 1/15, aperture: 4; LL3: ISO 400, shutter speed: 1/15, aperture: 3.5).

Processing and analysis of photographic data

Digital images obtained during the experimental period were subsampled every 2.5 min such that 14 images were processed and analysed for each experiment. Because colour was relatively stable during the 35 min experimental period (no sudden changes in colour, in comparison to the habituation period; Fig. S3), the 14 images provide an accurate representation of lizard colour throughout the experiment. Average red (R), green (G) and blue (B) values were obtained from the head and back of the lizard using a custom Matlab script (Mathworks Inc., Natick, MA, USA) written by J.A.E. RGB values were calibrated with respect to radiance and light intensity using custom Matlab scripts written by J.A.E. The raw RGB values were first calibrated against known reflectance values of six different grey standards (ColorChecker Passport, X-Rite, Grand Rapids, MI, USA) obtained with the same camera and under the same experimental and lighting conditions as the experiment. This procedure ensured a linear relationship between camera responses (R, G and B) and reflectance. To linearise camera responses, we applied a function in the form of $y=a\times\exp^{bx}+c\times\exp^{dx}$, where y is the linearised pixel value, and a, b, c and d are empirically derived constants specific to our camera (Garcia et al., 2013). We applied linearisation parameters specific to each illumination.

We chose to restrict our analysis to corrected RGB values to minimise additional data transformations and assumptions regarding receiver vision associated with mapping RGB values to photoreceptor stimulation (Kemp et al., 2015), particularly for tetrachromatic receivers. This is particularly important given that our data were restricted to the human-visible spectrum (400–700 nm; although UV reflectance of lizards and backgrounds is minimal; Fig. S1). Previous experiments on temperature-dependent colour change showed 1–2% change in UV reflectance across all individuals (Smith et al., 2016a), despite greater visible colour change than detected here. Separate experiments (Smith et al., 2016a) quantifying colour change during handling based on near-simultaneous photographs and spectral measurements confirmed that the distribution of colours of lizards in RGB colour space was statistically similar to the distribution of colours in avian or lizard visual colour space (Smith et al., 2016a). Those experiments were done using the same captive lizards from Mildura and Alice Springs as used in this study and validate the use of linearised and equalised RGB data from standard photographs to approximate variation perceived by avian predators or conspecifics (Smith et al., 2016a).

Achromatic contrast (AC) of the lizard to each experimental background was estimated by calculating the relative difference in luminance between the lizard and background as follows:

$$\mathrm{AC} = (I_L - I_B)/(I_L + I_B),$$

where I is the intensity or luminance, calculated as the sum of corrected R+G+B values and the subscripts L and B denote lizard and background, respectively.

The degree of chromatic contrast (CC) was estimated by calculating the Euclidean distance between the RGB values of the lizard and the background:

$$\mathrm{CC} = \sqrt{((\mathrm{R_L} - \mathrm{R_B})^2 + (\mathrm{G_L} - \mathrm{G_B})^2 + (\mathrm{B_L} - \mathrm{B_B})^2)},$$

where R, G and B values for the lizard (subscript L) and background (subscript B) were first standardised to equal total luminance (R+G +B=1).

Achromatic contrast and chromatic contrast were estimated based on the average from the three consecutive photographs in which the lizards most closely matched their backgrounds (as measured by the differences in RGB values) to assess the background similarity at the endpoint of colour change.

We also examined the extent of within-individual colour change during the 45 min the lizards were exposed to a single background; specifically, the change in achromatic and chromatic background contrast of each lizard between the beginning of the experiment (during the habituation period) at the moment when they exhibited the least similarity (achromatic or chromatic) to the background, and when they were most similar to the background.

Lastly, we examined within-individual change between the three background treatments by comparing the differences in luminance (as calculated for chromatic contrast between the lizard and background) and Euclidean difference of RGB values for an individual lizard on each pair of backgrounds (black versus orange, black versus yellow and yellow versus orange) at the time that it showed greatest similarity to its background.

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Statistical analysis

We compared the degree of background similarity, the extent of individual colour change during each trial and individual colour difference between background treatments and populations using two-way repeated measures analyses of variance (RM ANOVA) with treatment (background colour or light level), population and their interaction as factors. Significant differences revealed by RM ANOVA were further explored using Tukey *post hoc* tests. Homoscedasticity of the data was verified through visual examination of residual and *Q*–*Q* plots (Zuur et al., 2010). All statistical analyses were performed using SigmaPlot statistical and graphing software (version 12, Systat Software Inc., San Jose, CA, USA). Unless stated otherwise, all values are means±s.e.m.

RESULTS

Extent of background similarity

At the endpoints of colour change, lizards from both populations were achromatically most similar to the orange background and least similar to the yellow and black backgrounds. Chromatically, lizards from each population were equally similar to the yellow and black backgrounds and least similar to the orange background (Fig. 2B).

The extent of chromatic but not achromatic background similarity differed between populations (Fig. 2; Table 1). Lizards from Mildura, where yellow sands predominate, were chromatically more similar to the yellow background than Alice Springs lizards (Tukey, *P*=0.001; Fig. 2), while lizards from Alice Springs, where orange sands occur, were chromatically more similar to the orange and black backgrounds than lizards from Mildura (Tukey, *P*=0.048 and *P*=0.002; Fig. 2B). As these measures of similarity are for the endpoints of colour change, they indicate local adaptation (or potentially long-term plasticity) rather than population variation in short-term plasticity.

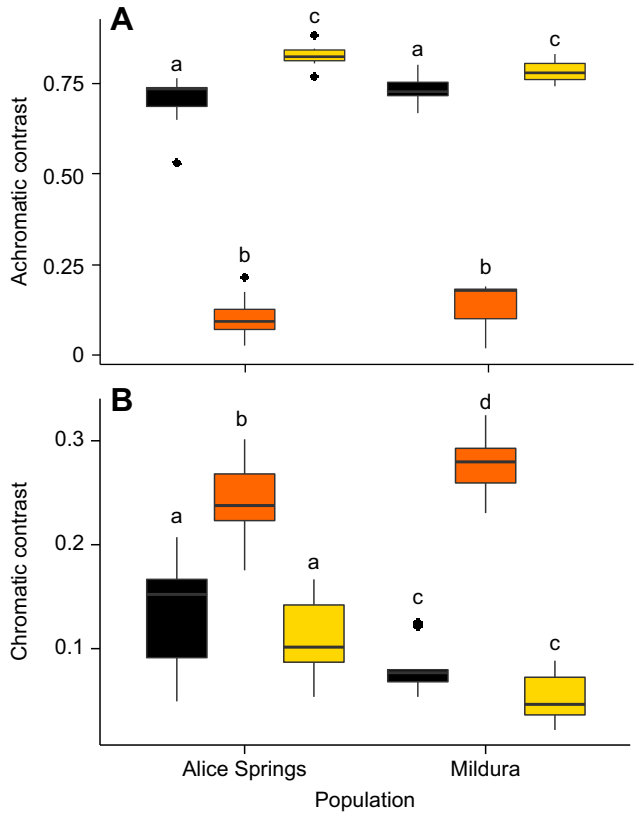

Fig. 2. Degree of background similarity for two populations of bearded dragons on the three different backgrounds. Alice Springs population, *N*=11; Mildura population, *N*=11. (A) Achromatic background similarity was estimated by calculating the absolute difference of luminance (red+green +blue, R+G+B) between the background and the lizard. (B) Chromatic background similarity was obtained by calculating the Euclidean distance between the RGB values of the lizard and the background. Different letters indicate significant differences (Tukey *post hoc* test, *P*<0.05).

Table 1. Effect of background colour and population on achromatic and chromatic similarity to the background (achromatic and chromatic contrast), and achromatic and chromatic change within experiments and between background treatments

		Statistical analyses	
Dependent variable	Factor	$F_{d.f.}$	*P*
Achromatic contrast	Background	$1072.34_{20,2}$	**<0.0001**
	Population	$0.05_{2,1}$	0.83
	Background×population	$4.15_{2,1}$	**0.02**
Chromatic contrast	Background	$134.79_{20,2}$	**<0.0001**
	Population	$9.12_{2,1}$	**0.007**
	Background×population	$8.66_{2,1}$	**0.0008**
Change in achromatic contrast within treatment	Background	$14.26_{20,2}$	**<0.0001**
	Population	$0.0002_{2,1}$	0.99
	Background×population	$1.13_{2,1}$	0.33
Change in chromatic contrast within treatment	Background	$8.41_{20,2}$	**0.001**
	Population	$2.80_{2,1}$	0.11
	Background×population	$0.17_{2,1}$	0.85
Achromatic Change between treatments	Background pairs	$216.19_{20,2}$	**<0.0001**
	Population	$1.11_{2,1}$	0.30
	Background pairs×population	$0.66_{2,1}$	0.53
Chromatic change between treatments	Background pairs	$9.41_{20,2}$	**0.0006**
	Population	$<0.0001_{2,1}$	1.00
	Background pairs×population	$1.60_{2,1}$	0.22

Significant *P*-values are in bold.

Within-individual contrast change on a single background

The extent of within-individual achromatic or chromatic contrast change on any given background did not differ between populations (Fig. 3; Table 1). Both populations exhibited greater improvements in achromatic background similarity on the black and orange backgrounds than on the yellow background (Fig. 3A; Tukey, *P*<0.05).

Chromatically, both populations exhibited a larger improvement in background similarity when exposed to the black than to either the orange or yellow backgrounds (Fig. 3B; Tukey, *P*=0.04), with no difference between the orange and yellow backgrounds.

Within-individual change in response to different backgrounds

We found no differences between populations in the extent of within-individual colour (achromatic and chromatic) changes in response to background treatments (black versus orange, black versus yellow and yellow versus orange) recorded at the time when they were most similar to the background (Table 1). For both Alice Springs and Mildura lizards, the extent of both achromatic and chromatic change between black versus orange backgrounds was smaller than that between orange versus yellow or yellow versus black backgrounds (Tukey, *P*<0.05). There was substantial variability in the degree of within-individual change on different backgrounds. While the average maximum chromatic and achromatic difference between any two treatments was 0.06±0.01

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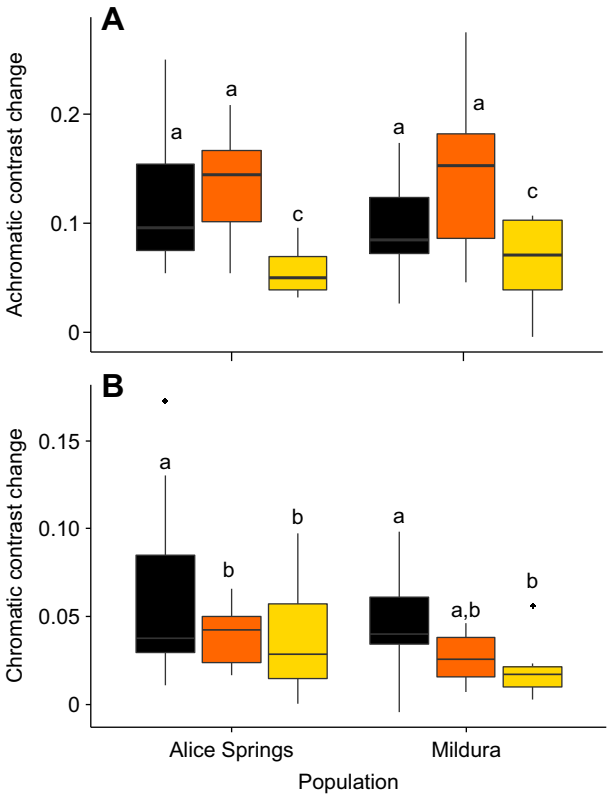

Fig. 3. Change in background similarity in the two populations of bearded dragons during exposure to the three backgrounds. Alice Springs population, *N*=11; Mildura population, *N*=11. Change was calculated as the difference in the degree of achromatic (A) or chromatic (B) similarity between the moment when the lizard was least similar and when it was most similar. Different letters indicate significant differences (Tukey *post hoc* test, $P<0.05$).

and 0.53±0.01, respectively (Fig. 4), the maximum change exhibited by a lizard was 0.08 and 0.62, respectively.

Effect of illumination intensity

Bearded dragons were darkest at the highest illumination level (LL3; Table 2). There was a significant difference in achromatic background similarity between backgrounds and populations as well as a significant interaction between the two factors (Table 3). Achromatic contrast against the background was greatest (i.e. similarity was lowest) at the highest illumination level in both populations. Alice Springs lizards had similar, moderate achromatic contrast in the medium and low illumination treatments. In Mildura lizards, achromatic contrast increased with increasing illumination intensity and was lower (i.e. higher similarity) for medium and low illuminations than in lizards from Alice Springs (Fig. 5A). We also observed a significant difference in chromatic background similarity in the Mildura population between LL2 and the two other light levels (Table 3; Fig. 5B).

DISCUSSION

Our data clearly show that bearded dragons change colour in response to the visual background and that the populations differ in endpoint background similarity, consistent with differences in the local substrate colour. At colour change equilibria, lizards from Mildura, where yellow sands predominate, were chromatically more similar to the yellow background than lizards from Alice Springs, while lizards from Alice Springs, where orange sands occur, were more similar to the orange background than lizards from Mildura.

Fig. 4. Within-individual colour change during exposure to different colour backgrounds. An individual lizard exhibiting a colour change of ~10% in chromatic values (Euclidean difference of standardised RGB values) when exposed to black versus orange and black versus yellow backgrounds and a difference of approximately 5% between yellow and orange backgrounds.

Within-individual colour change during an experiment (i.e. on a single background) was similar in the two populations but differed between backgrounds. Lizards from both populations showed more luminance change on orange and black backgrounds than on yellow, and showed most chromatic change on the black sand background, which does not occur naturally in either population and differs most from natural backgrounds. For both populations, individual colour change between backgrounds was greatest for pairs of backgrounds that differed the most achromatically (yellow versus orange or black) rather than chromatically (orange versus black), suggesting than achromatic variation in background colour may be a stronger cue for colour change. Lizards showed achromatic change in

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Table 2. Luminance of experimental backgrounds and of Alice Springs and Mildura bearded dragon populations on these backgrounds, and at different light intensities

	Background	Alice Springs	Mildura
Background			
Black	0.08±0.00	0.47±0.02^{a}	0.53±0.02^{a,c}
Orange	0.39±0.00	0.41±0.02^{a}	0.52±0.02^{c}
Yellow	1.46±0.00	0.14±0.02^{b}	0.17±0.02^{d}
Light intensity			
1	1.27±0.00	0.38±0.02^{a}	0.49±0.02^{c}
2	1.44±0.00	0.39±0.02^{a}	0.50±0.02^{c}
3	2.57±0.00	0.15±0.02^{b}	0.20±0.02^{b}

Three different backgrounds were used – black, orange or yellow sand – and three different light treatments, with 1 being the darkest and 3 the brightest. Values are means±s.e.m. Different letters indicate significant differences between values (Tukey, $P<0.05$).

response to illumination intensity, exhibiting greater background similarity at the two lower illumination intensities than at the highest illumination. This may reflect greater predation risk at low light levels (dawn or dusk) when many predators are most active. Alternatively, lizards may be less able to escape predators if low light levels are associated with lower ambient (and body) temperatures. Overall, our results indicate that background colour and illumination intensity are both important environmental cues triggering colour change and demonstrate phenotypic plasticity and local divergence in colour–background interactions.

Changes in both colour and luminance are important for camouflage (Choi and Jang, 2014; King et al., 1994; King and King, 1991; Stevens et al., 2014a,b; Vroonen et al., 2012); even small changes (e.g. <2% increase in reflectance) can ultimately improve the level of background matching of an animal to its natural habitat (Stevens et al., 2013). For the observed colour change to improve camouflage requires that the primary predators of bearded dragons (birds of prey) perceive those differences. Given that birds of prey (Olsson et al., 2013) are likely to have good colour vision (Hunt et al., 2009), it is likely that the observed increase in background similarity could improve camouflage from a predator's point of view.

Despite the capacity for colour change, there is a limit to colour lability. Lizards were able to match some backgrounds better than others, depending upon the source population. Mildura lizards were more chromatically similar to the yellow and black backgrounds than were lizards from Alice Springs, and lizards from Alice Springs were chromatically more similar to the orange background than were lizards from Mildura. Yellow sands predominate around Mildura and orange sands are absent, whereas orange sands are commonly found around Alice Springs, although yellow sands also occur in the area. Although black sand is not present in the habitats of either population, very

Table 3. Effect of illumination intensity and population on achromatic and chromatic similarity to the background (repeated measures ANOVA)

		Statistical analyses	
Dependent variable	Factor	$F_{d.f.}$	P
Achromatic contrast	Illumination	$555.83_{19,2}$	**<0.0001**
	Population	$14.08_{2,1}$	**0.001**
	Background×population	$4.27_{2,1}$	**0.02**
Chromatic contrast	Illumination	$8.32_{19,2}$	**0.001**
	Population	$3.17_{2,1}$	0.091
	Background×population	$12.59_{2,1}$	**<0.001**

Significant P-values are in bold.

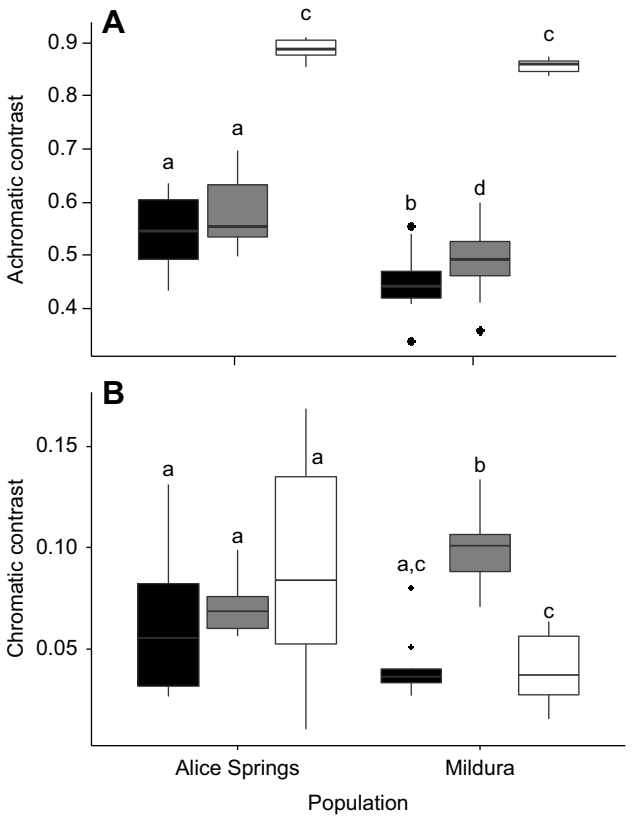

Fig. 5. Background similarity in the two populations of bearded dragons exposed to different light intensities. Alice Springs population, N=11; Mildura population, N=11. Light intensities were achieved by turning on one (LL1, black), two (LL2, grey) or three (LL3, white) mercury vapour lamps suspended above the experimental arena. (A) Achromatic background similarity (absolute difference in luminance between the lizard and the background). (B) Chromatic background similarity (Euclidean difference in RGB between the lizard and the background). Different letters indicate significant differences (Tukey *post hoc* test, $P<0.05$).

dark brown–grey branches or tree trunks are often found in both, and bearded dragons often use these as perches (V.C. and D.S.-F., personal observation). However, Mildura habitats are much more densely vegetated, such that dark brown–grey is a more common background than at Alice Springs. These results suggest possible local evolutionary adaptation of coloration to visually distinct habitats, although this needs to be confirmed with greater spatial replication. It is also possible that population differences reflect long-term chromatic adaptation (i.e. colour change) to different backgrounds; however, this seems unlikely because lizards retained population differences in colour over 2 years of captivity on the same background (V.C., unpublished data) and amateur breeding of different colour variants of bearded dragon for the pet trade suggests heritable colour variation.

Lizards from both populations showed greatest similarity to the background at the lower illuminations, which most resemble illumination intensities in the early morning or late afternoon. Such colour change in response to illumination intensity could be associated with antipredatory mechanisms. Crepuscular/nocturnal Moorish and Mediterranean geckos are also able to more closely match the luminance of their background when exposed to dimly lit conditions than when in full darkness (Vroonen et al., 2012; Zaidan and Wiebusch, 2007); this is functionally significant because camouflage is less relevant in the absence of light. Although

bearded dragon lizards are strictly diurnal and live in semi-arid well-lit habitats, they may experience higher predation risk at low light intensities as a result of greater predator activity or reduced escape speed (e.g. early in the morning before reaching active body temperature, or later at night when body temperature has already lowered).

Pogona vitticeps exhibits circadian changes in skin luminance, with darker coloration during the light phase of the daily cycle; light acts as a cue for eliciting these changes (Fan et al., 2014). Darkening of the skin early in the morning (lower illumination intensity) aids in thermoregulation by increasing radiative heat absorption, allowing bearded dragons to become active earlier in the day (Smith et al., 2016a,b). Darkening of the skin results from the dispersion of melanosomes within the melanophores, induced by melanocortins such as alpha-melanophore-stimulating hormone (α-MSH), while aggregation of melanosomes is probably associated with secretion of melatonin (a melanosome aggregator) during darkness causing skin lightening (Bagnara and Hadley, 1973). It is likely that the changes in skin luminance we observed in response to changes in illumination are the result of the proportional release of melanosome dispersant and aggregator hormones (although catecholamine neurotransmitters may also be involved; reviewed in Ligon and McCartney, 2016).

The extent of colour change observed in this study is likely to be an underestimation of the true capacity for colour change in bearded dragons. The prolonged time in captivity on a single visual background in the absence of natural predators, and the absence of any threat during experiments, may have dampened anti-predatory responses, which might be more pronounced in lizards in the wild. It has been suggested that colour change in reptiles is energetically costly (Stuart-Fox and Moussalli, 2009), as has been demonstrated for fish (Rodgers et al., 2013), and these costs should be minimised when the benefits are minimal. In our experimental arenas, it is likely that the need for camouflage was not sufficiently great to evoke the full extent of the colour change. Indeed, the response of captive bearded dragons to temperature is more pronounced and more consistent in recently captured animals (within 2 weeks of capture) than in lizards that have remained in captivity for several months (V.C. and K.R.S., unpublished data).

We have shown that bearded dragons change colour in response to the visual background and illumination intensity. This is likely to be an adaptive response to reduce the probability of visual detection by predators. Rapid physiological colour change in bearded dragons has the potential to offset trade-offs with other potential functions of colour, such as thermoregulation and signalling (Smith et al., 2016a,b). For example, an individual may adopt cryptic coloration when predation risk is high but change to a different colour for thermoregulatory or signalling purposes in the absence of predators. Bearded dragons are certainly capable of such rapid colour change, which can occur within minutes (Smith et al., 2016b) and is clearly evident when individuals are approached in the wild (V.C., K.S. and D.S.-F., personal observation). However, our results also demonstrate limits to colour plasticity and indicate geographic divergence in coloration of populations that differ in the visual characteristics of local habitats.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
V.C., J.A.E. and D.S.-F. contributed to the research conception and design; V.C. and K.R.S. performed the experiments; J.A.E. provided photo analysis methods and Matlab scripts; V.C. processed all raw data and conducted statistical analysis; V.C. and D.S.-F wrote the manuscript; all authors revised the manuscript.

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References
Bagnara, J. T. and Hadley, M. E. (1973). *Chromatophores and Color Change: the Comparative Physiology of Animal Pigmentation*. Englewood Cliffs: Prentice Hall Inc.

Buresch, K. C., Ulmer, K. M., Akkaynak, D., Allen, J. J., Mäthger, L. M., Nakamura, M. and Hanlon, R. T. (2015). Cuttlefish adjust body pattern intensity with respect to substrate intensity to aid camouflage, but do not camouflage in extremely low light. *J. Exp. Mar. Biol. Ecol.* **462**, 121-126.

Cadena, V. and Tattersall, G. J. (2009). Decreased precision contributes to the hypoxic thermoregulatory response in lizards. *J. Exp. Biol.* **212**, 137-144.

Chiao, C.-C. and Hanlon, R. T. (2001). Cuttlefish camouflage: Visual perception of size, contrast and number of white squares on artificial checkerboard substrata initiates disruptive coloration. *J. Exp. Biol.* **204**, 2119-2125.

Chiao, C.-C., Kelman, E. J. and Hanlon, R. T. (2005). Disruptive body patterning of cuttlefish (*Sepia officinalis*) requires visual information regarding edges and contrast of objects in natural substrate backgrounds. *Biol. Bull.* **208**, 7-11.

Choi, N. and Jang, Y. (2014). Background matching by means of dorsal color change in treefrog populations (*Hyla japonica*). *J. Exp. Zool. A Ecol. Genet. Physiol.* **321**, 108-118.

de Velasco, J. B. and Tattersall, G. J. (2008). The influence of hypoxia on the thermal sensitivity of skin colouration in the bearded dragon, *Pogona vitticeps*. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **178**, 867-875.

Endler, J. A. (1978). A predator's view of animal color patterns. *Evol. Biol.* **11**, 319-364.

Endler, J. A. (1980). Natural selection on color patterns in *Poecilia reticulata*. *Evolution* **34**, 76-91.

Endler, J. A. (1988). Frequency-dependent predation, crypsis and aposematic coloration. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* **319**, 505-523.

Endler, J. A. (1993). The color of light in forests and its implications. *Ecol. Monogr.* **63**, 1-27.

Endler, J. A. and Thery, M. (1996). Interacting effects of lek placement, display behavior, ambient light, and color patterns in three neotropical forest-dwelling birds. *Amer. Nat.* **148**, 421-452.

Fan, M., Stuart-Fox, D. and Cadena, V. (2014). Cyclic colour change in the Bearded Dragon *Pogona vitticeps* under different photoperiods. *PLoS ONE* **9**, e111504.

Garcia, J. E., Dyer, A. G., Greentree, A. D., Spring, G. and Wilksch, P. A. (2013). Linearisation of RGB camera responses for quantitative image analysis of visible and UV photography: a comparison of two techniques. *PLoS ONE* **8**, e79534.

Hopper, K. R. (2001). Flexible antipredator behavior in a dragonfly species that coexists with different predator types. *Oikos* **93**, 470-476.

Hunt, D. M., Carvalho, L. S., Cowing, J. A. and Davies, W. L. (2009). Evolution and spectral tuning of visual pigments in birds and mammals. *Philos. Trans. R. Soc. B Biol. Sci.* **364**, 2941-2955.

Kemp, D. J., Herberstein, M. E., Fleishman, L. J., Endler, J. A., Bennett, A. T. D., Dyer, A. G., Hart, N. S., Marshall, J. and Whiting, M. J. (2015). An integrative framework for the appraisal of coloration in nature. *Amer. Nat.* **185**, 705-724.

King, R. B. and King, B. (1991). Sexual differences in color and color change in wood frogs. *Can. J. Zool.-Rev. Can. Zool.* **69**, 1963-1968.

King, R. B., Hauff, S. and Phillips, J. B. (1994). Physiological color change in the green treefrog-Responses to background brightness and temperature. *Copeia* **2**, 422-432.

Ligon, R. A. and McCartney, K. L. (2016). Biochemical regulation of pigment motility in vertebrate chromatophores: a review of physiological color change mechanisms. *Curr. Zool.* **62**, 237-252.

Olsson, M., Stuart-Fox, D. and Ballen, C. (2013). Genetics and evolution of color patterns in reptiles. *Semin. Cell Dev. Biol.* **24**, 529-541.

Oshima, N. (2001). Direct reception of light by chromatophores of lower vertebrates. *Pigm. Cell. Res.* **14**, 312-319.

Osorio, D. and Srinivasan, M. V. (1991). Camouflage by edge enhancement in animal color patterns and its implications for visual mechanisms. *Proc. R. Soc. B Biol. Sci.* **244**, 81-85.
Rodgers, G. M., Gladman, N. W., Corless, H. F. and Morrell, L. J. (2013). Costs of color change in fish: food intake and behavioural decisions. *J. Exp. Biol.* **216**, 2760-2767.
Smith, K. R., Cadena, V., Endler, J. A., Kearney, M. R., Porter, W. P. and Stuart-Fox, D. (2016a). Color change for thermoregulation versus camouflage in free-ranging lizards. *Amer. Nat.* **188**, e668-678.
Smith, K. R., Cadena, V., Endler, J. A., Porter, W. P., Kearney, M. R. and Stuart-Fox, D. (2016b). Colour change on different body regions provides thermal and signalling advantages in bearded dragon lizards. *Proc. R. Soc. B Biol. Sci.* **283**, 9.
Stevens, M., Rong, C. P. and Todd, P. A. (2013). Colour change and camouflage in the horned ghost crab Ocypode ceratophthalmus. *Biol. J. Linnean. Soc.* **109**, 257-270.
Stevens, M., Lown, A. E. and Denton, A. M. (2014a). Rockpool gobies change colour for camouflage. *PLoS ONE* **9**, e110325.
Stevens, M., Lown, A. E. and Wood, L. (2014b). Color change and camouflage in juvenile shore crabs *Carcinus maenas*. *Front. Ecol. Evol.* **2**, 1-14.
Stuart-Fox, D. and Moussalli, A. (2009). Camouflage, communication and thermoregulation: lessons from colour changing organisms. *Philos. Trans. R. Soc. B Biol. Sci.* **364**, 463-470.
Stuart-Fox, D., Whiting, M. J. and Moussalli, A. (2006). Camouflage and colour change: antipredator responses to bird and snake predators across multiple populations in a dwarf chameleon. *Biol. J. Linnean. Soc.* **88**, 437-446.
Vroonen, J., Vervust, B., Fulgione, D., Maselli, V. and Van Damme, R. (2012). Physiological colour change in the Moorish gecko, *Tarentola mauritanica* (Squamata: Gekkonidae): effects of background, light, and temperature. *Biol. J. Linnean. Soc.* **107**, 182-191.
White, T. E. and Kemp, D. J. (2016). Color polymorphic lures target different visual channels in prey. *Evolution* **70**, 1398-1408.
Zaidan, F., III and Wiebusch, P. L. (2007). Effects of temperature and illumination on background matching in Mediterranean geckos (*Hemidactylus turcius*). *Tex. J. Sci.* **59**, 127-136.
Zuur, A. F., Ieno, E. N. and Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods. Ecol. Evol.* **1**, 3-14.