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Geographic Color Variation and Physiological Color Change in Eastern Collared Lizards (*Crotaphytus collaris*) from Southern New Mexico, USA

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ABSTRACT: Many factors can contribute to geographic color variation, including natural selection, sexual selection, and genetic drift. In squamates, phenotypic plasticity can also play an important role in color variation because color can change during maturation, across seasons, and over short time scales in response to environmental stimuli. Here, we quantify geographic color variation and assess the contribution of physiological plasticity (rapid color change) in populations of Eastern Collared Lizards (*Crotaphytus collaris*). First, we quantified the dorsal coloration of four geographically distinct populations of *C. collaris*: two putatively melanistic populations on lava flows, and two typically colored populations from the surrounding desert. Second, we quantified the degree of physiological color change occurring in response to stress and temperature, two factors known to affect reptile coloration over short time scales. We found that populations differed in overall coloration, but that only one of the two lava-flow populations was darker than its neighboring population. Both lava-flow populations exhibited less contrast with their respective substrates than the desert populations. All populations exhibited some physiological color change, but the degree of color plasticity was narrower than the differences in color across populations. Future studies on the genetics of coloration will be necessary to understand the specific mechanisms contributing to color variation in *C. collaris*, but our results indicate that natural selection for background matching likely plays a role in this system.

Key words: Background matching; Iguanidae; Lava flow; Melanism; Physiological color change; Temperature

BIOLOGISTS have long noted that coloration is important for crypsis through background matching (Darwin 1794; Cott 1940; Endler 1978). In fact, background matching is often assumed to be evidence for natural selection (Darwin 1794; Poulton 1890; Lewis 1949); however, coloration also serves other functions. For example, color patches in squamates can act as social signals, and sexual selection on these signals can produce dramatic variation in coloration within and among species (Zucker 1989; Losey 2003). Moreover, coloration is important for thermoregulation in squamates, as body color can affect the efficiency of heat absorption from—or reflection to—the environment (Gunn 1998; Clusella-Trullas et al. 2007; Langkilde and Boronow 2012).

Squamate coloration can change in response to environmental conditions over multiple time scales. When the coloration is heritable, coloration can evolve across generations at the population level. However, color can also change at the individual level over shorter time scales via phenotypic plasticity (Price 2006). For example, many squamates change coloration over ontogenetic time scales that usually coincide with sexual maturation or seasonal cues (Ferguson 1976; Carpenter 1995; Wilson et al. 2007). Over the shortest time scales, many squamates can change coloration rapidly with temperature and/or stress (termed physiological color change; Hadley and Goldman 1969; Luke 1994; Rosenblum 2005), usually through the aggregation and dispersal of melanin granules (Hadley and Goldman 1969; Stuart-Fox and Moussalli 2009).

To understand the causes and consequences of color variation in the wild more fully, it is essential to first document geographic patterns of color variation and to assess the role of plasticity. Documenting geographic color variation can lay a foundation for exploring the mechanisms

underlying intraspecific variation. Assessing physiological color change is also an important preliminary step toward an understanding of whether or not observed patterns of color variation can be explained by organismal response to short-term environmental stimuli.

Eastern Collared Lizards (*Crotaphytus collaris*) are an appropriate model taxon for understanding intraspecific and geographic color variation in squamates. *Crotaphytus collaris* populations throughout the southwestern United States vary in dorsal coloration (McCoy et al. 1997; Macedonia et al. 2004). Although previous research with this species has suggested that coloration might play a role in intraspecific signaling and/or crypsis to avoid visually oriented predators (McCoy et al. 1997; Macedonia et al. 2002; Baird 2008), little is known about the contribution of plasticity to observed color variation in *C. collaris*. To understand the factors that contribute to color variation in this species, we examined geographic variation in dorsal coloration and the potential for physiological color change in four populations of *C. collaris* in New Mexico—two desert populations and two lava-flow populations where the lizards have been noted as melanistic in coloration (Lewis 1949; Best et al. 1983). Our study had three objectives. First, we quantified geographic color variation in the four focal populations of *C. collaris*. We hypothesized that lizards on lava flows would be darker than lizards found on typical desert substrates. Second, we quantified degree of substrate matching in *C. collaris* when at active body temperature. We hypothesized that darker coloration of lava-flow lizards would minimize the contrast with their dark basalt substrates. Finally, we quantified the contribution of physiological color change to color variation in *C. collaris*. Both temperature and stress can trigger reptile color change (e.g., increased stress can cause lizards to darken in coloration [Hadley and Goldman 1969], and increased temperature can cause lizards to lighten [Luke 1994; Rosenblum 2005]). Therefore, we used both temperature

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and acute physiological stress treatments to assess color variation in *C. collaris* over short time scales. We hypothesized that physiological color change would not fully explain geographic color variation in the four focal populations of *C. collaris*.

MATERIALS AND METHODS

Lizard Capture and Photography

We collected *Crotaphytus collaris* from four populations in south-central New Mexico during June and July 2013 (Fig. 1). We collected lizards from two lava-flow populations: the Pedro Armendariz lava flow (33.528950°N, 106.883146°W; in all cases, datum = WGS84; hereinafter referred to as Lava 1; sample size $n = 15$, 6 male, 9 female) and the Carrizozo lava flow (33.707654°N, 105.932644°W; hereinafter, Lava 2; $n = 19$, 13 male, 6 female). We also collected lizards from populations adjacent to the lava flows in the Chihuahuan Desert: Elephant Butte State Park (33.137365°N, 107.177106°W; hereinafter, Desert 1; $n = 22$, 17 male, 5 female) near Lava 1, and the Oscura Mountains (33.692935°N, 106.248983°W; hereinafter, Desert 2; $n = 19$, 12 male, 7 female) near Lava 2. We captured the lizards by noose and transported them back to a field laboratory for analysis. We only used adult *C. collaris* that showed no obvious signs of ecdysis for this study. We classified lizards as adults if they exceeded the minimum body size at reproduction established for populations from Arizona, New Mexico, and west Texas (snout–vent length >64 mm for females [Ballinger and Hipp 1985] and >88 mm for males [Parker 1973]). We sexed the adult lizards based on the presence of hemipenes, enlarged femoral pores and enlarged postanal scales. We also took a sample of the same rock on which the lizard was first seen for substrate color quantification. Lizards were never found on a substrate other than a rock.

We used photographs to quantify lizard and substrate coloration. We photographed all lizards and substrates in a standardized manner adapted from Stevens et al. (2007) with the use of a Panasonic Lumix DMC-FZ28. Lizards were always photographed within 4 h of capture. To avoid diel coloration changes, we took the photographs in natural light between 1200 h and 1500 h local time under a cloudless sky. To maintain constant and diffused lighting for each photograph and a constant distance from the subject, we photographed the lizards and substrates inside an upside-down, white 18-L bucket via a hole cut into the bottom of the bucket. We photographed all lizards on a white posterboard background with white, gray, and dark gray standards (Adorama QPcard 101) present. We chose a white background to standardize across populations without approximating the natural background color of any one population, although background matching via visual stimuli is not known to occur in *C. collaris*.

Our aim was to assess physiological color change in *C. collaris* under a realistic range of temperature and stress conditions, representative of conditions that animals may experience in nature. As such, each lizard was subject to four treatments of temperature/stress: low/high, high/high, low/low, high/low. For the temperature treatments, we measured lizard coloration at temperatures above ($\sim 38^\circ\text{C}$) and below ($\sim 30^\circ\text{C}$) mean active body temperature. Both the high- and

low-temperature treatments were within the range of observed temperatures for these lizards in nature (personal observation; Brattstrom 1965). For the stress treatments, although none of the lizards were completely unstressed, we created high-stress and low-stress conditions. Specifically, we measured lizard coloration after extensive handling (high stress) and after no handling (low stress), as handling is known to induce stress responses in lizards (e.g., Langkilde and Shine 2006).

To minimize undue stress of repeatedly heating and cooling lizards, and to ensure that lizards were heated only twice during our experiments (once for each treatment), we applied treatments in a standardized order. First, within 4 h of capture from the field, we measured the lizards at low temperature and high stress. We cooled the subjects by keeping them, in their enclosures, in the shade. We stressed the cool lizards for approximately 10 min by extensive handling, including taking morphology measurements and a tail clip for future DNA analyses. We then took each subject's cloacal temperature with an Omega HL-11 thermocouple (mean ± 1 SE = $31.7 \pm 2.6^\circ\text{C}$, $n = 60$), ensuring that each lizard was near 30.0°C (body temperature $< 36.3^\circ\text{C}$; Brattstrom 1965). We then took the low-temperature/high-stress photograph of the subject. When necessary, we lowered the subjects' temperatures by placing them, in their enclosure, in a refrigerated cooler for < 5 min. It is unlikely that subjects recovered from being stressed during this interval, as recovery to baseline stress levels takes more than 2 h (Langkilde and Shine 2006). Second, we photographed the lizards under the high-temperature/high-stress treatment. We warmed the lizard in the sun to a measured body temperature near 38.0°C (body temperature $> 36.3^\circ\text{C}$; mean = $37.4 \pm 1.2^\circ\text{C}$, $n = 57$). In order to maintain higher levels of stress during the warming period, we warmed lizards in our hands or in a cage that was within < 1.5 m of a human and frequently moved. Third, we photographed the lizards under the low-temperature/low-stress treatment. We allowed the lizards to rest in a cage out of sight of a human long enough to return to a baseline unstressed condition (> 2 h; Langkilde and Shine 2006). To minimize stress, we did not handle lizards for more than 1 min. We measured cloacal temperature (mean = $28.8 \pm 3.5^\circ\text{C}$, $n = 69$) and then took the low-temperature/low-stress photograph of the subject. Fourth, we photographed the lizard under the high-temperature/low-stress treatment. We allowed the low-temperature/low-stress lizards to warm up in the upside-down bucket exposed to direct sunlight but out of human sight for approximately 5 min, until their temperature was over 36°C . To avoid prematurely removing the lizard and inducing extra handling stress, we first noted their temperatures with the use of an infrared thermometer (Nubee NUB8380). We then took the high-temperature/low-stress photograph and lastly measured the subject's cloacal temperature (mean = $38.2 \pm 1.8^\circ\text{C}$, $n = 71$).

Photograph Analyses and Color Space Creation

To extract color measurements from our photographs, we first standardized the colors of each photograph with the known values of the color standards present in each photo with the use of the function Curves (Whiteley et al. 2009) in Adobe Photoshop CS6 (Stevens et al. 2007). We confirmed linearization by plotting the measured reflectance of the

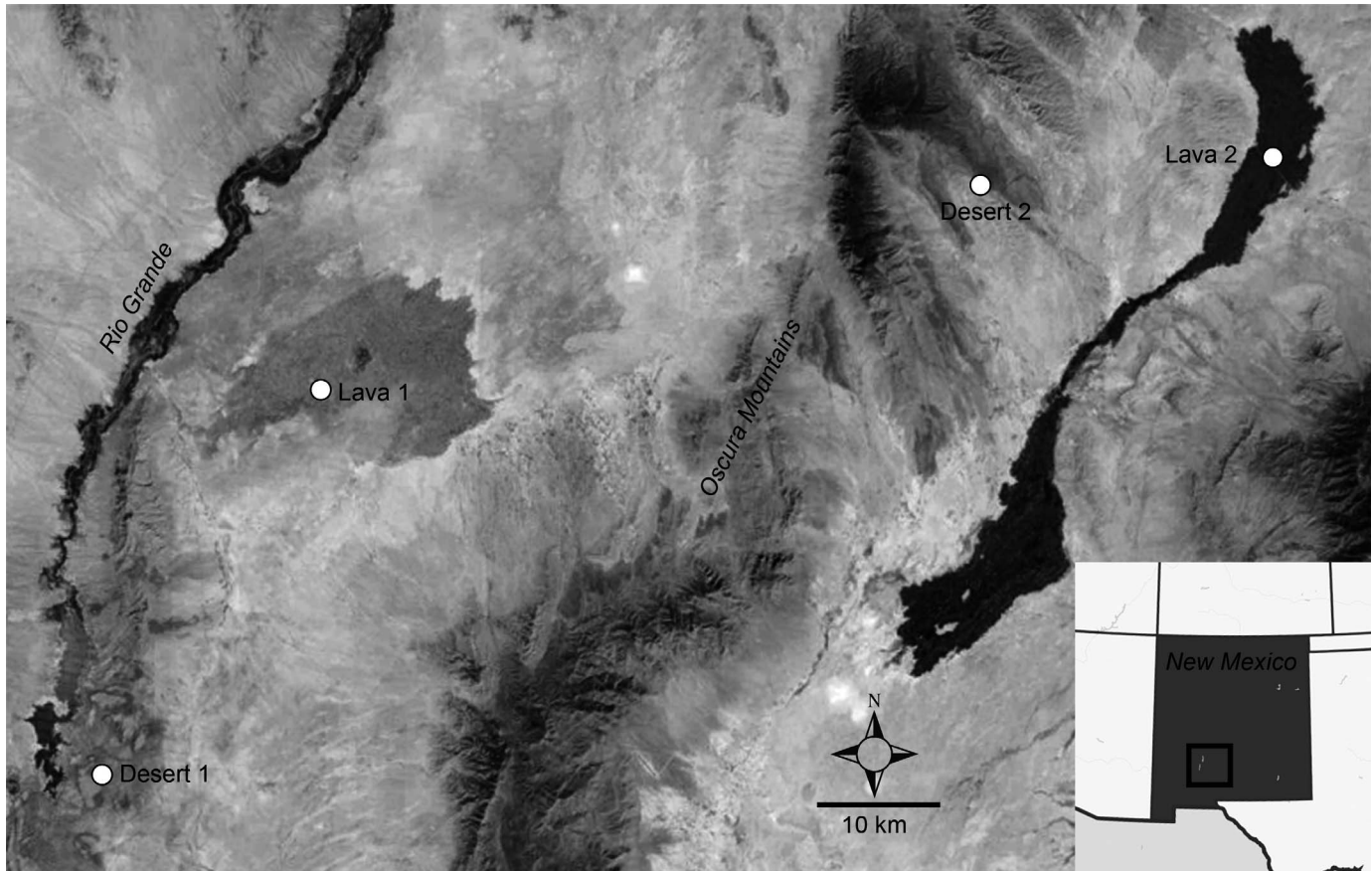


FIG. 1.—A satellite image showing locations in southern New Mexico (inset) where Eastern Collared Lizards (*Crotaphytus collaris*) were sampled. Lava 1 = the Pedro Armendariz lava flow. Lava 2 = the Carrizozo lava flow. Desert 1 = Elephant Butte State Park. Desert 2 = the Oscura Mountains. Satellite image courtesy of Google Earth.

white, gray, and dark gray standards in each photograph against the known reflectance values of the standards (adjusted $R^2 = 0.991$, 0.991 , and 0.990 for red, green, and blue channels, respectively). With the use of the plugin RGB Measure in ImageJ (v1.47, National Institutes of Health, Bethesda, MD), we then extracted the three color values for each pixel of a photograph: red, green, and blue (RGB).

We focused our analysis on dorsal coloration because this is the body region most exposed to visually oriented avian predators and thus most relevant for crypsis. We analyzed a 75×75 pixel square over the central dorsal midline of the lizards (Position Five in Macedonia et al. 2004:Fig. 1), which equated to 0.64 cm^2 of the dorsal area of each lizard. We focused on this location and sized area to: (1) average over mottling patterns on the dorsum and have a proxy for coloration as viewed directly from above (relevant for the avian predators of *C. collaris* in New Mexico; Macedonia et al. 2002); (2) avoid sexual signaling patches present on the legs and lateral areas (Macedonia et al. 2002); and (3) have a standard-sized area across all individuals. The present study focused on color, not pattern, so we averaged color values from each pixel over the 75×75 pixel square. We averaged R (red) values, G (green) values, and B (blue) values separately.

Next, we converted the RGB values into measurements meaningful to the vertebrate visual system. Vertebrates use differences in luminance to detect motion and use differ-

ences between the R, G, and B channels to detect hue and chroma (Endler and Mielke 2005; Endler 2012). We converted our RGB values into three axes: one for luminance and two for the differences between the R, G, and B channels, as in Endler (2012) and McKay (2013). The z-axis was $R + G + B$ and represented luminance, or brightness. The x-axis was $(R - G)/(R + G)$, or the difference between the red and green channels. The y-axis was $(G - B)/(G + B)$, or the difference between the green and blue channels. Thus, the dorsal color of each lizard could be described by a point in three-dimensional color space. To simplify interpretation, we converted each point in the color space to luminance (z-axis), hue, and chroma values (as in Endler and Mielke 2005). The angle between the point in the color space and the x-axis indicated hue, with a hue of 0° corresponding to red. The distance between the point and the y-axis indicated chroma. Thus, luminance was a measure of brightness (increasing with increasing brightness), hue was a measure of dominant wavelength (increasing as color becomes less red), and chroma was a measure of saturation (increasing with increasing saturation).

Statistical Analyses

We structured our analyses into three parts to quantify geographic color variation in *C. collaris* and the contribution of physiological color change to the observed color variation. First, we asked whether populations differed in coloration.

We analyzed data from lizards when they were under the high-temperature/low-stress treatment, as this is closest to the conditions that they experience throughout the day (Brattstrom 1965). To get a general understanding of whether lizard coloration varied geographically, we put the three axes of our color space into a scaled principle components analysis (PCA). All three axes of our color space contributed to PC1 (which explained 45.4% of the variance in the data set), indicating that PC1 provided a reasonable understanding of overall color. We then ran analyses of variance (ANOVA) with PC1 as the dependent variable and sex, population and their interaction as independent variables. Given that we were most interested in whether the lava-flow lizards were darker than the lizards from the surrounding desert, we also conducted ANOVAs with luminance as the dependent variable and sex, population, and their interaction as independent variables. Finally, we conducted post hoc Tukey tests to determine which populations were different in luminance from each other.

Second, we investigated the degree of contrast between the subjects and the substrates on which they were caught. Again, we analyzed lizards from the high-temperature/low-stress treatment only. To determine if the substrates from different localities varied in overall coloration, we conducted a PCA with the color space values from the substrates on which the lizards were initially encountered. All three axes of our color space contributed to PC1 (which explained 66.2% of the variance in the data set), again indicating that PC1 provided a reasonable understanding of overall substrate color. We conducted ANOVA with PC1 as the dependent variable and population as the independent variable. Again, given that we were most interested in determining whether or not lava substrates were darker than the surrounding desert substrates, we conducted an ANOVA with luminance as the dependent variable and population as the independent variable. We also conducted post hoc Tukey tests to determine which substrates differed in luminance. Next, we calculated the Euclidean distance (ED) between the lizards and their substrate following Endler and Mielke (2005). To determine if the degree to which lizards contrast from their substrate differed across populations, we used ANOVAs with the ED values as the dependent variable and population and sex as independent variables. We then conducted post hoc Tukey tests to determine which populations had different degrees of contrast. Finally, to determine whether lizards were lighter than their substrates, we ran *t*-tests for luminance between the lizards and the substrate where they were initially encountered (McKay 2013).

Third, we assessed the effect of temperature and stress on lizard coloration for each population. We conducted a PCA including color measurements of subjects under all four temperature/stress treatments. All three axes of color space contributed to PC1 (which explained 54.1% of the variance in the data set), again indicating that PC1 provided a reasonable understanding of overall substrate color. We then performed ANOVAs with PC1 as the dependent variable and sex, temperature, population, and stress to determine whether temperature and/or stress affected lizard coloration. We also grouped the data by population to determine whether the effects of temperature and/or stress were different among the four populations. We were most

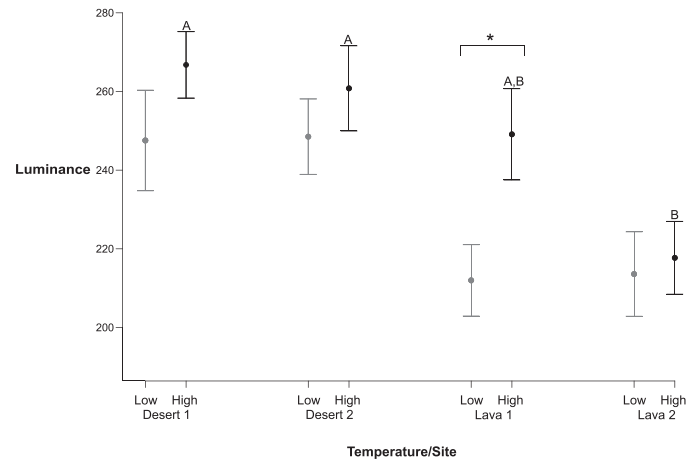


FIG. 2.—Physiological color change and geographic color variation in *Crotaphytus collaris*. Mean values (± 1 SE) are shown for luminance at high (black) and low (gray) temperature treatments. Larger luminance values indicate brighter coloration. For physiological color change, statistically significant changes in luminance between high- and low-temperature treatments are indicated with an asterisk (*, post hoc Tukey test P value < 0.001). Geographic differences among individuals representing different populations were assessed for individuals in the high temperature treatment only, wherein different letters indicate statistically distinct response values (post hoc Tukey test P value < 0.05).

interested in determining whether lizards changed in brightness with temperature or stress, so we also conducted ANOVAs as above on each population, but with luminance as the dependent variable. Because the temperature/stress analyses included more than one measurement per individual (e.g., across multiple treatments), we avoided effects of pseudoreplication by treating the individual subject as a random effect. To quantify subject color change as a function of temperature, we calculated the ED between the subject's warm coloration and cold coloration. We tested whether populations of lizards differed in their degree of physiological color change as a function of temperature using ANOVAs with ED values for each subject as the dependent variable and population, stress, and sex as the independent variables, with the subject as a random effect.

Sexual dichromatism in *C. collaris* (McCoy et al. 1997; Macedonia et al. 2004) is less pronounced in populations occurring in southern New Mexico (Macedonia et al. 2002). Nevertheless, we included sex, and its interaction with other variables, as well as values pooled across sexes, in all of the above models to determine whether (1) observed color variation could be explained by sexual dichromatism and (2) our treatments affected male and female coloration differently. All statistical analyses were conducted in R (v3.2.3; R Development Core Team 2015).

RESULTS

Crotaphytus collaris populations in our study area differed in coloration. Populations varied in overall color (PC1; $F = 6.84$, $df = 3$, $P < 0.001$) and specifically in luminance ($F = 5.06$, $df = 3$, $P = 0.003$). Tukey tests revealed that Lava 2 was darker than both Desert populations (Lava 2–Desert 1 luminance, $P = 0.003$; Lava 2–Desert 2 luminance, $P = 0.02$; Fig. 2). Sex was not a reliable predictor of color variation among populations (with

PC1: $F = 2.06$, $df = 1$, $P = 0.15$; with luminance: $F = 0.59$, $df = 1$, $P = 0.44$).

The substrates on which lizards were found also varied across localities in overall color (PC1; $F = 20.90$, $df = 3$, $P < 0.001$) and luminance ($F = 23.26$, $df = 3$, $P < 0.001$). Lava substrates were darker than Desert substrates (Lava 1–Desert 1 luminance, $P < 0.001$; Lava 1–Desert 2 luminance, $P < 0.001$; Lava 2–Desert 1 luminance, $P < 0.001$; Lava 1–Desert 2 luminance, $P < 0.001$). Substrates from the two Desert localities were similar in luminance, as were substrates from the two Lava localities (Lava 1–Lava 2, $P = 0.87$; Desert 1–Desert 2, $P = 0.26$).

The degree of background matching between lizards and their substrates differed among populations ($F = 5.03$, $df = 3$, $P = 0.003$). Post hoc Tukey tests revealed that lizards from both Lava populations exhibited less contrast with their backgrounds than those from Desert 1 (Lava 1–Desert 1, $P = 0.01$; Lava 2–Desert 1, $P = 0.03$), but not Desert 2 (Lava 1–Desert 2, $P = 0.09$; Lava 2–Desert 2, $P = 0.21$). Individuals from Desert 1 and Desert 2 were brighter than their substrates (Desert 1, $t = -6.15$, $df = 1$, $P > 0.001$; Desert 2, $t = -4.07$, $df = 1$, $P > 0.001$), whereas individuals from the Lava populations were similar to their substrates in this respect (Lava 1, $t = 0.74$, $df = 1$, $P = 0.46$; Lava 2, $t = -0.96$, $df = 1$, $P = 0.34$).

With PC1 used to evaluate pooled values for overall coloration, we found that dorsal coloration varied with temperature ($F = 15.69$, $df = 1$, $P > 0.001$), population ($F = 11.31$, $df = 3$, $P > 0.001$), and sex ($F = 8.15$, $df = 3$, $P = 0.004$), but not with stress, or any of the interaction terms. When analyzing each population separately, only Lava 1 individuals were brighter at higher temperatures ($F = 8.95$, $df = 1$, $P = 0.01$; Fig. 2). Evaluating only the luminance aspect of coloration, brightness did not vary with stress, sex or the interaction terms in any of the populations. Analyses with ED values also indicated that the degree of color change was similar across populations and sexes.

DISCUSSION

We found differences in coloration among *C. collaris* populations over relatively short geographic distances (i.e., populations separated by < 30 km). The Lava 2 population (Carrizozo) was darker than all other populations (Fig. 2), consistent with our hypothesis that lava-flow lizards would be darker to match their substrate. Lava 1 lizards exhibited similar values for brightness as those from Lava 2 and both Desert sites (Fig. 2). Lizards from both lava-flow populations showed less overall contrast with their substrates than those from the two desert populations, indicating a higher degree of background matching in the lava-flow populations. There are several possible explanations for why Lava 1 lizards are not as dark as Lava 2 lizards. Lava 2 (Carrizozo) is geologically young (5200 yr; Dunbar 1999) and characterized by dark basalt rocks with little vegetation. Lava 1 (Pedro Armendariz) is much older (760,000 yr old; Bachman and Mehnert 1978) and has more vegetation and patches of lighter sand between rocky areas. Although the lava substrate is comparable in color for the two lava flows, lizards at Lava 1 might be lighter to decrease contrast in a more heterogeneous habitat (Merilaita et al. 1999). It is also possible that demographic factors (e.g., levels of gene flow between desert

and lava populations) could differ between Lava 1 and Lava 2 and also affect coloration of individuals at each site.

The lower contrast in lizards from lava-flow populations indicates that coloration might serve an adaptive function for crypsis in these populations. Baird (2008) showed that *C. collaris* might be cryptic to both predators and prey. It is possible that lizards on nonlava substrates might be cryptic in other aspects of color not measured here (such as hue and chroma), or through use of different behaviors, despite contrasting in luminance. Studies that measure visibility of different color morphs on different backgrounds in appropriate predator visual systems could be used to assess the possible contribution of natural selection for substrate matching in this system more explicitly. Coloration also likely plays an important role in these populations with regard to thermoregulation. The dark lava-flow rocks likely absorb more heat than the adobe rocks of the surrounding desert and thus create a different thermal environment for *C. collaris*. If thermoregulation were the dominant process affecting lizard coloration on lava flows, one would expect lizards to be lighter in color in order to increase reflectance. Given that we did detect this pattern among lizards on lava flows, it is possible that there is a trade-off between thermoregulation (i.e., to avoid overheating on the darker rocks) and crypsis in the melanistic population.

Sexual dichromatism is common in other populations of *C. collaris* (McCoy et al. 1997; Macedonia et al. 2004), but previous studies have found reduced sexual dichromatism in *C. collaris* from New Mexico compared to other desert populations of *C. collaris* (e.g., Macedonia et al. 2002). We did not find evidence for sexual dichromatism in our populations despite collecting our data during the breeding season for these populations (Ballinger and Hipp 1985), the time when sexual dichromatism is expected to be greatest (McGuire 1996). Whereas Macedonia et al. (2002) detect sexual dichromatism within the species, only one of our analyses revealed an effect of sex on lizard coloration.

The dorsal coloration of individuals in all of our populations tended to lighten with increasing temperature, as has been documented in many reptile species (e.g., Hadley and Goldman 1969; Hedges et al. 1989; Rosenblum 2005). We found no evidence that our stress treatments effected lizard coloration. However, physiological color change that we attribute to temperature does not explain population-level differences in coloration. Even when lizards from the Lava 2 population were warm and at their brightest, they were still darker than subjects from both Desert populations (Fig. 2). Thus, physiological color change contributes to color variation in this system, but does not explain the geographic differences in coloration among populations.

As hypothesized by Lewis (1949), we found that *C. collaris* coloration varied across populations and that lizards from the Carrizozo lava flow are melanistic. Our results add to the growing body of knowledge about melanistic vertebrate species found at the Carrizozo lava flow (e.g., Hoekstra and Nachman 2003; Rosenblum et al. 2007; Hardwick et al. 2013). To improve our understanding of why lizards on lava flows are often melanistic, future studies in this system should focus on the heritability of color traits, trade-offs between crypsis and thermoregulation, habitat use, and

levels of gene flow across habitats to determine what factors produce the observed differences in coloration.

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