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The Role of Phenotypic Plasticity in Color Variation of Tularosa Basin Lizards

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An experimental approach was taken to evaluate the role of phenotypic plasticity in reptile coloration for three lizard species which exhibit dramatic variation in dorsal body darkness associated with different substrate environments. In southern New Mexico, blanched color morphs of *Aspidoscelis inornata*, *Holbrookia maculata*, and *Sceloporus undulatus* inhabit the gypsum dunes of White Sands, and a melanic color morph of *S. undulatus* is found on the Carrizozo lava flow. Temperature was manipulated to determine the extent of physiological (rapid) plasticity in coloration for all three species. Color change in response to short-term stimuli did not explain the variation among lizards from different habitats. Although lizards of all color morphs were slightly darker when colder, more melanic lizards displayed a diminished capacity for rapid color change. Common garden experiments were conducted to evaluate the potential for ontogenetic plasticity in coloration for *H. maculata* and *S. undulatus*. Offspring from mothers of different substrate environments were distinguishable by color despite identical developmental conditions. Hatchlings and adults exhibited similar coloration for *H. maculata*, but a developmental trajectory in coloration was observed in *S. undulatus*. Overall, environmental variation alone did not explain differences in dorsal coloration among lizards from distinctive habitats. Results from these experiments support the hypotheses that color morphology in Tularosa Basin lizards has a strong heritable component and that observed variation is likely adaptive.

ADAPTIVE evolution requires traits with heritable variation. Although many ecologically relevant characters do have a simple genetic basis (Watt, 1983; Swanson et al., 1991; Nachman et al., 2003), environmental variation can affect phenotypes in complex ways (Via, 1994; Schlichting and Pigliucci, 1998). Studies of natural selection in the wild often assume that traits of interest are genetically based despite the potential importance of phenotypic plasticity. This presumption frequently remains untested because techniques used to evaluate the role of plasticity often rely on reciprocal transplants and large-scale breeding experiments that can be difficult to implement in natural systems.

Cases of reptile color variation have long been studied as examples of adaptive evolution (Cott, 1940; Norris and Lowe, 1964). Dorsal body darkness often varies with substrate color or temperature environment and is generally presumed to be an adaptation for crypsis or thermoregulation (Kettlewell, 1973). Coloration affects visibility to avian predators, so natural selection for substrate matching is predicted to be strong for diurnal reptiles (Norris, 1965). Coloration also impacts thermoregulation because darker reptiles are able to warm faster and maintain higher body temperatures (e.g., Bittner et al., 2002). Geographic variation in reptile coloration is frequently a consequence of changes in melanin, the pigment responsible for dorsal body darkness (Majerus, 1998).

Testing the assumption that melanin-based characters are heritable is important because patterns of pigment distribution are not necessarily fixed within individuals (Waring, 1963). Environmental conditions are known to affect melanin density at several temporal scales. Physiological (rapid) color change can occur in response to short-term stimuli (Nery and Castrucci, 1997) as existing melanin becomes dispersed (darkening) or aggregated (lightening). Ontogenetic color change can occur in response to longer-term environmental stimuli as melanin production increases (darkening) or decreases (lightening). Physiological color change has been documented in reptiles and is often associated with thermoregulatory or stress responses (Waring, 1963). Ontogenetic color change is less well-studied in reptiles, but is known to be important for melanin-based characters in other taxa. For example, melanin production can be affected in amphibians by temperature and predation regime (McCollum and Leimberger, 1997; Garcia et al., 2003) and in invertebrates by temperature, photoperiod, and population density (Kingsolver and Huey, 1998; Hazel, 2002; Solensky and Larkin, 2003)

Three lizard species, *Aspidoscelis inornata* (Little Striped Whiptail, formerly *Cnemidophorus inornatus*, Reeder et al., 2002), *Holbrookia maculata* (Common Lesser Earless Lizard), and *Sceloporus undulatus* (Eastern Fence Lizard), exhibit dramatic color variation in the Tularosa Basin of southern New Mexico. Dorsal body darkness correlates with substrate color of two geologically recent formations. Blanched forms of all three species inhabit the light gypsum dune fields of White Sands (Smith, 1943; Lowe and Norris, 1956; Dixon, 1967). Melanic populations of *S. undulatus* are found on the dark basalt rocks of the nearby Carrizozo lava flow (Lewis, 1949). In the surrounding habitat matrix and throughout the rest of their ranges, all three species have ''wildtype'' morphologies which are brown in color and well-matched to the adobe soils of the region.

Previous studies have suggested that dorsal coloration in adult Tularosa Basin lizards is static but have not rigorously tested the contribution of environment to phenotype. Early researchers kept adult *A. inornata*, *H. maculata*, and *S. undulatus* on different substrates in the lab and found dorsal darkness to be fixed (Smith, 1943; Bundy, 1955; Lowe and Norris, 1956). Lack of color change in adults does not, however, rule out environmental influence on color morphology early in life. Studies also reported that White Sands lizards became slightly darker with cold temperatures (Bundy, 1955; Lowe and Norris, 1956), but these studies did not take an experimental approach to understanding capacity for physiological color change.

Here I evaluate the role of phenotypic plasticity in dorsal body darkness for populations of *A. inornata*, *H. maculata*, and *S. undulatus*. I experimentally examine the influence of environmental variation on color morphology at the population level: within individuals and between generations. I first ask whether physiological color change can obscure differences among color morphs by manipulating lizard temperature. I then document the degree of ontogenetic plasticity in coloration among offspring of different color morphs using common garden rearing experiments.

Materials and Methods

Sampling.—*Aspidoscelis inornata*, *H. maculata*, and *S. undulatus* were collected in southern New Mexico from May 15 through June 29, 2003. Blanched lizards of all three species were collected in ''white sand'' habitat on the gypsum dunes of White Sands National Monument and White Sands Missile Range, Otero County. Wildtype lizards were collected in ''dark soil'' habitat in the yucca scrubland and blue grama grasslands of Jornada Long-Term Ecological Research Station and White Sands Missile Range, Otero, Doña Ana, and Socorro Counties. Melanic *S. undulatus* were collected in ''lava'' habitat on the basalt rocks of the Carrizozo lava flow, Lincoln County. *Aspidoscelis inornata* and *H. maculata* do not occur in the rocky lava habitat. Lizards were caught by hand or noose and transported to research facilities in Alamogordo, New Mexico. Dorsal coloration was quantified for all samples, and a subset of samples was used in physiological or ontogenetic plasticity experiments detailed below. After use in experiments, lizards were returned to capture points or accessioned as specimens to the Museum of Vertebrate Zoology, University of California at Berkeley.

Quantifying color variation.—Dorsal color readings were taken with an Ocean Optics USB 2000 spectrophotometer using a dual deuterium/tungsten halogen light source and a probe oriented at 45 degrees to the skin surface. For each individual, three spectral recordings along the dorsal midline were averaged: between the front limbs, at the center of the body, and between the hind limbs. Each spectral reading consisted of percent reflectance recordings in reference to a white standard at 0.3 nanometer (nm) intervals. Three nanometer bins were created by averaging every 10 points along the spectrum, reducing the number of variables from ~2000 to ~200 for analysis. Readings from 300–700 nm, the spectral range visible to squamates and their avian predators (Bennett and Cuthill, 1994; Cuthill et al., 1999), were excised for analysis.

Spectrophotometric measurements contain information about three aspects of coloration: brightness describes light transmission intensity, chroma describes color purity, and hue describes the wavelength of maximum slope (Endler, 1990). Principal Components Analysis (PCA) can be used to determine which aspect of coloration best explains observed phenotypic variation. Empirical findings show that Principal Components Axis 1 (PC1) corresponds to brightness while PC2 and PC3 generally contain information about chroma and hue (Grill and Rush, 2000). In all analyses of Tularosa Basin lizards, PC1 explained over 85% of observed variation. Because color morphs in this study differed primarily with respect to brightness rather than hue or chroma, a direct measure of brightness could be used. A single brightness

value for each individual was calculated as area under the spectral curve (AUC). Principal Components Axis 1 (PC1) and AUC values were analyzed with analysis of variance (ANOVA) designs specified for each experiment below. For every analysis, homogeneity of variances was tested (Cochran, 1941). The only comparison which failed to meet the necessary analysis assumptions was between white sand and dark soil *H. maculata* hatchlings. Results with and without transformations to correct for heteroscedastic variances had identical significance levels; therefore, uncorrected values are presented. If an ANOVA was significant, *post hoc* Tukey HSD tests were used to determine which groups occupied significantly different color space. Results of PCA and AUC analyses were nearly identical, so AUC is presented to avoid redundancy. All statistical analyses were executed in Statistica (StatSoft Inc.).

Physiological plasticity.—Rapid dorsal darkening, which occurs as existing melanin becomes dispersed in the dermis, can be induced by cold temperatures, stress, electrical shock, and hormonal stimulation (Norris, 1965; Filadelfi and Castrucci, 1994). Because a similar mechanism seems to underlie physiological color change in response to different stimuli, only temperature stimulation was employed in the experiments presented here. Physiological plasticity experiments were conducted in Alamogordo, New Mexico on July 27, 2003. For each of the three Tularosa Basin species, four females of each color morph were used in temperature trials. Only adults were used to remove possible ontogenetic variation in color morphology, and only females were used to remove possible effects of sexual dichromatism. Sample sizes necessary to detect differences among color morphs, given the phenotypic variance observed in natural populations, were determined by power analysis. Individuals used in the study were well within the range of typical variation observed in source populations.

Two biologically meaningful temperature treatments were chosen to represent the cold and hot activity extremes experienced by these desert animals in their natural habitats. Previous experiments at White Sands National Monument documented body temperatures of active lizards between 32.3 C and 39.0 C; below 30 C, lizards were inactive or basking, and above 40 C lizards sought shelter (Dixon, 1967; Hager, 2000). The ''cold'' treatment (20 C), therefore, was below preferred body temperature and corresponded to early morning substrate temperatures recorded at study sites (pers. obs.). The ''hot'' treatment (40 C) was above preferred body temperature but below known critical thermal maxima for focal species (Sena, 1978; Crowley, 1985). Lizard cages were heated with heat lamps and cooled in a refrigerated chamber. A thermal acclimatization period of 30 min per treatment was sufficient for animals to reach target temperatures. Treatment order was randomized for each individual, and a resting period was provided between treatments.

To ensure that treatment methods were effective and unbiased, lizard temperature was recorded at the end of each treatment with a laser thermometer (Raytek ST Pro) at a distance of 1 cm. Although laser thermometers measure surface temperature, lizard surface and body temperatures have a predictable relationship even when measurements are made at a distance of 1 m (Alberts and Grant, 1997). When laser thermometers are used at close range, as in this study, temperature readings are nearly identical to those of cloacal thermometers (pers. obs.). After the cold treatment, lizard temperature averaged 20.77 C (19.69–21.84 C, 95% CI), and after the hot treatment, lizard temperature averaged 38.89 C (38.45–39.33 C, 95% CI). Animals reached target temperatures, and mean temperatures between dark and light animals were indistinguishable. Although dark lizards may warm more quickly than light lizards, the acclimatization period was sufficient for all color morphs to obtain target temperatures.

Spectrophotometric recordings were taken immediately after removing each individual from the temperature treatment. Brightness scores were analyzed with repeated measures ANOVA to determine whether rapid color change could explain observed variation among different color morphs. Brightness values were used as the dependent variable, lizard habitat (i.e., white sand, dark soil, lava) was used as the categorical predictor variable, and temperature treatment was used as the within-subject factor. Significant between group variation would indicate that differences among habitat groups were greater than differences within habitat groups even when individuals were exposed to different temperatures. Significant within group variation would indicate the importance of temperature effects and/or temperature by habitat interactions. *Post hoc* Tukey tests were utilized to determine treatment effect size and to test the hypothesis that lizards were darker when colder. Average percent change in brightness for each color morph was calculated in reference to the mean brightness of animals when cold.

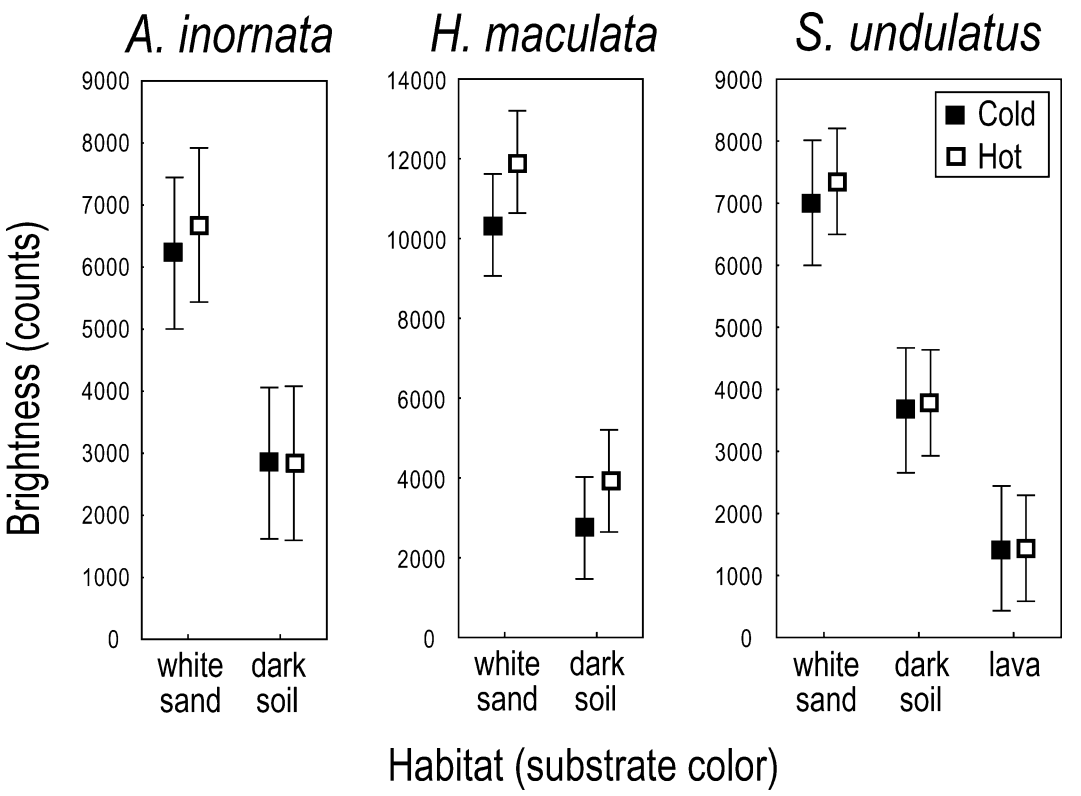

Fig. 1. The effect of physiological plasticity on dorsal color variation. Mean and 95% confidence intervals are presented for dorsal brightness (calculated as area under the spectral curve) for different color morphs at two temperature treatments.

Ontogenetic plasticity.—A common garden design was used to test the contribution of environmental factors to lizard color morphology. Regardless of coloration, gravid females, incubating eggs, and developing hatchlings were maintained in identical conditions. Homogenizing rearing conditions eliminated variation, not only in substrate color, but also in other potential factors such as diet or temperature. Single-generation common garden experiments provide a strong test of environment effects throughout ontogeny but are unable to remove possible maternal effects. To rigorously test the role of maternal effects, split-brood and multigeneration experiments are required, but, as for many natural systems, are not feasible with Tularosa Basin lizards. The potential contribution of maternal effects is discussed in more detail below.

Gravid females were caught in the wild and maintained in indoor cages on a moist substrate of peat-moss and sand. Captive lizards were provided with food, water, and basking lights to mimic natural light cycles. Cages were checked daily for egg deposition. Once laid, eggs were transferred to a Vermiculite mixture that was changed every 10 days. Eggs were housed individually and incubated at 28 C. All eggs were transported to the University of California at Berkeley on July 3, 2003. Hatchlings were then reared in University of California animal care facilities. Once hatchlings emerged, they were maintained on a substrate of intermediate brightness. Spectrophotometric readings were taken within 10 days of lizard hatching. Although Tularosa Basin lizards can be abundant in their natural habitats, sample sizes were limited by the logistical constraints of collecting and housing gravid females from three species during a short breeding season. Additionally, not all gravid females collected produced viable eggs. For *H. maculata*, eggs were successfully reared from five white sand females (12 hatchlings) and four dark soil females (13 hatchlings). For *S. undulatus*, eggs were successfully reared from two white sand (5 hatchlings), three dark soil (16 hatchlings), and four lava (21 hatchlings) females. Eggs from *A. inornata* were not successfully reared.

Three one-way ANOVAs were conducted with

TABLE 1. PHYSIOLOGICAL PLASTICITY: REPEATED MEASURES ANOVA ON DORSAL BRIGHTNESS WITH INDIVIDUALS GROUPED BY HABITAT (DARK SOIL, WHITE SAND, AND LAVA) AND TEMPERATURE AS A TREATMENT EFFECT. Degrees of freedom (df), mean squares (MS), F statistics (*F*), and significance levels (*P*) are provided; * indicates statistically significant results.

Source of variation	df	MS	*F*	*P*
A. inornata				
Between groups				
Habitat	1	52781483	26.42	0.0021*
Error	6	1997715	—	
Within groups				
Temperature	1	205377	6.57	0.0427*
Temperature × Habitat	1	210018	6.72	0.0410*
Error	6	31259	—	
H. maculata				
Between groups				
Habitat	1	240384979	140.57	<0.0001*
Error	6	1710022	—	
Within groups				
Temperature	1	7589084	15.85	0.0073*
Temperature × Habitat	1	160825	0.34	0.5833
Error	6	478766	—	
S. undulatus				
Between groups				
Habitat	2	66918686	49.80	<0.0001*
Error	9	1343649	—	
Within groups				
Temperature	1	146801	8.04	0.0196*
Temperature × Habitat	2	60942	3.34	0.0824
Error	9	18262	—	

brightness scores as the dependent variable. First, all hatchlings within species were compared to determine whether they exhibited divergent color morphologies despite common garden rearing conditions. Hatchlings were grouped with maternal color morphology as the categorical predictor variable. Second, hatchlings of each color morph were compared to adults of their parental populations to determine whether there were ontogenetic trajectories in color morphology. Adult samples contained equal numbers of males and females, and sample sizes were adjusted to have a balanced design for ANOVA (12 white sand, 13 dark soil adult *H. maculata*; 7 white sand, 15 dark soil, 17 lava adult *S. undulatus*). Lizards were grouped with age class as the categorical predictor variable. Third, to better understand whether observed patterns in white sand *S. undulatus* could result from experimental treatments, 5 captive juveniles, 7 wild-caught juveniles, and 7 adults were compared. Captive juveniles were raised in the lab for 1–2 months from common garden experiment hatchlings. Wild-caught juveniles were approximately the same age as captive juveniles, but were captured in their natural habitat. In all analyses, a significant result would indicate that dorsal color variation was greater between specified groups than within groups. Significance levels were adjusted with a Bonferroni correction when multiple comparisons were performed.

RESULTS

Physiological plasticity.—In all three Tularosa Basin lizard species, animals from different habitats were significantly differentiated in dorsal coloration ($P<0.005$ for all species) regardless of temperature treatment (Fig. 1, Table 1). White sand and dark soil lizards were distinguishable even when white sand lizards were at their darkest (when cold) and dark soil lizards were at their lightest (when hot).

Although physiological color change within morphs was minimal compared to differences

TABLE 2. PHYSIOLOGICAL PLASTICITY: MEAN AND STANDARD ERROR OF THE MEAN FOR DORSAL BRIGHTNESS AND CHANGE IN DORSAL BRIGHTNESS WITH TEMPERATURE FOR LIZARDS FROM DIFFERENT HABITATS. Brightness was calculated as area under the spectral curve (AUC); color change with temperature (ΔAUC) was calculated in reference to individuals when cold.

Habitat	Treatment	Sample size	Mean AUC	SE (mean) AUC	Mean ΔAUC	SE (mean) ΔAUC
A. inornata						
Dark soil	Cold	4	2841.01	336.95	−2.55	124.71
Dark soil	Hot	4	2838.47	395.70		
White sand	Cold	4	6244.42	620.62	455.73	125.32
White sand	Hot	4	6700.15	599.34		
H. maculata						
Dark soil	Cold	4	2769.56	267.20	1176.90	582.18
Dark soil	Hot	4	3946.46	328.35		
White sand	Cold	4	10321.22	688.63	1577.93	373.95
White sand	Hot	4	11899.15	664.06		
S. undulatus						
Dark soil	Cold	4	3660.00	378.36	121.90	132.50
Dark soil	Hot	4	3781.85	265.20		
White sand	Cold	4	7009.46	656.82	345.66	85.66
White sand	Hot	4	7355.12	576.19		
Lava	Cold	4	1437.20	138.97	1.70	49.99
Lava	Hot	4	1438.90	158.81		

among morphs, a significant treatment effect was observed for all species ($P<0.05$ for all species), demonstrating that individuals were darker when held at colder temperatures (Table 2).

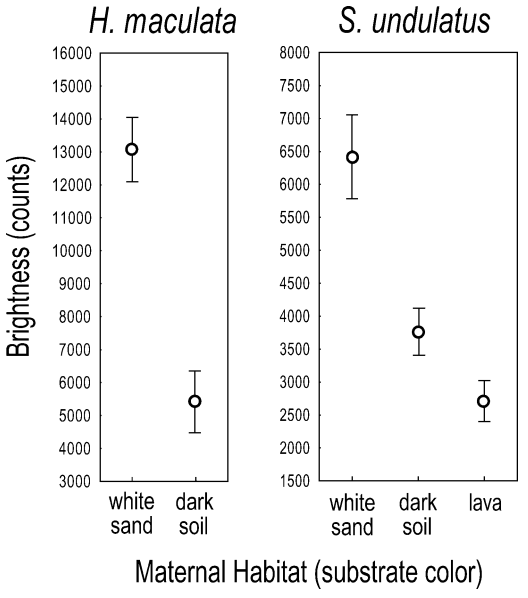

Fig. 2. Results from common garden rearing experiments. Mean and 95% confidence intervals for offspring dorsal color within the first week of hatching.

Additionally, there was a greater change in brightness with temperature for lighter color morphs than for darker color morphs in all species (Table 2). White sand *A. inornata* brightened 7% when hot, compared to only 0.1% for dark soil individuals. For *S. undulatus*, change in dorsal brightness was greatest for white sand individuals (5%), intermediate for dark soil individuals (3%), and minimal for lava individuals (0.1%). Individuals of *H. maculata* had the most color lability of the three focal species. At higher temperatures, dark soil individuals showed a greater percent color change (42% vs. 15% for white sand) but the brightening of white sand individuals was greater in raw intensity level. *Post hoc* tests revealed that the only statistically significant changes in brightness with temperature were for blanched morphs ($P<0.05$ for white sand morphs of *A. inornata* and *S. undulatus*).

Ontogenetic plasticity.—Mothers from different habitats produced offspring which were distinguishable by color morphology despite common garden rearing conditions (Fig. 2, Table 3). In *H. maculata*, white sand and dark soil hatchlings occupied completely non-overlapping regions of color space, with white sand hatchlings 141% brighter than dark soil hatchlings (Table 4). In *S. undulatus*, offspring of all three color morphs were statistically distinguish-

TABLE 3. ONTOGENETIC PLASTICITY: ANOVA ON DORSAL BRIGHTNESS. For each species, comparisons among hatchlings reared in common garden conditions and comparisons between hatchlings and adults from their source populations are presented. An additional comparison between captive-reared juveniles, wild-caught juveniles, and adults is shown for white sand *S. undulatus*. Degrees of freedom (df), mean squares (MS), F statistics (*F*), and significance levels (*P*) are provided; * indicates statistically significant results after Bonferroni correction.

Source of variation	df	MS	*F*	*P*
H. maculata				
Dark soil to white sand hatchlings				
Habitat	1	365728200	136.63	<0.0001*
Error	23	2676694		
Dark soil hatchlings to dark soil adults				
Habitat	1	1810527	2.66	0.1163
Error	24	681847		
White sand hatchlings to white sand adults				
Habitat	1	357222	0.07	0.7944
Error	22	5135946		
S. undulatus				
Dark soil, white sand, and lava hatchlings				
Habitat	2	28105635	56.58	<0.0001*
Error	39	496787		
Dark soil hatchlings to dark soil adults				
Habitat	1	7734762	9.53	0.0043*
Error	30	812008		
White sand hatchlings to white sand adults				
Habitat	1	34147233	14.44	0.0035*
Error	10	2364677		
Lava hatchlings to lava adults				
Habitat	1	20868616	65.96	<0.0001*
Error	36	316371		
White sand juveniles (wild and captive) to adults				
Habitat	2	2992264	1.43	0.2684
Error	16	2093405		

able by color; hatchlings from white sand habitat were 70% brighter than those from dark soil, and hatchlings from lava habitat were 28% darker than those from dark soil (Table 4).

Ontogenetic trajectories in color were observed in *S. undulatus* but not *H. maculata*. For *H. maculata*, hatchlings were statistically indistinguishable in color from adults of source populations (Fig. 3, Table 3). Conversely, for *S. undulatus*, hatchlings from all habitats were significantly different from adults in dorsal brightness (Fig. 3, Table 3). White sand and dark soil hatchlings were darker than respective adults (35% and 21%, respectively), while lava hatchlings were brighter than lava adults (121%; Table 4). A comparison with a third age class, juveniles, was therefore performed for blanched *S. undulatus* to further explore the observed ontogenetic trajectory in coloration. Wild and captive juveniles were statistically indistinguishable in color from each other and from wild-caught adults (Table 3). Additionally, mean brightness of juveniles was, as expected, intermediate between hatchlings and adults (Fig. 4, Table 4). Newly emerged white sand hatchlings were 35% darker than adults and subsequently brightened as they aged such that juveniles were only 13% darker than adults.

DISCUSSION

Results suggest that environmental variation alone cannot explain the dramatic color variation among lizards in different Tularosa Basin substrate habitats. Color morphs that occupy white sand, dark soil, and lava environments

TABLE 4. ONTOGENETIC PLASTICITY: MEAN AND STANDARD ERROR OF THE MEAN FOR DORSAL BRIGHTNESS OF LIZARDS FROM DIFFERENT HABITATS AND ONTOGENETIC STAGES (HATCHLINGS REARED IN COMMON GARDEN EXPERIMENTS AND ADULTS FROM THEIR SOURCE POPULATIONS). For white sand *S. undulatus*, two sets of juveniles (lab-reared and wild-caught) are also shown.

Habitat	Stage	Sample size	Mean AUC	SE AUC
H. maculata				
Dark soil	Hatchling	13	5415.59	187.05
White sand	Hatchling	12	13071.32	651.95
Dark soil	Adult	13	5943.36	264.41
White sand	Adult	12	12827.32	656.46
S. undulatus				
Dark soil	Hatchling	16	3763.61	227.75
White sand	Hatchling	5	6410.77	353.16
Lava	Hatchling	21	2717.26	102.72
Dark soil	Adult	16	4746.89	222.78
White sand	Adult	7	9832.41	709.67
Lava	Adult	17	1226.86	159.94
White sand	Juvenile (wild)	7	8742.36	434.21
White sand	Juvenile (captive)	5	8583.41	470.30

were distinguishable despite experimental manipulation at both physiological and ontogenetic time-scales. The potential contribution of environment to phenotype in *A. inornata*, *H. maculata*, and *S. undulatus* is discussed below.

Physiological plasticity.—Rapid color change in response to environmental stimuli did not account for differences among color morphs in the three focal species. Although physiological change within color morphs was minimal compared to differences among color morphs, data

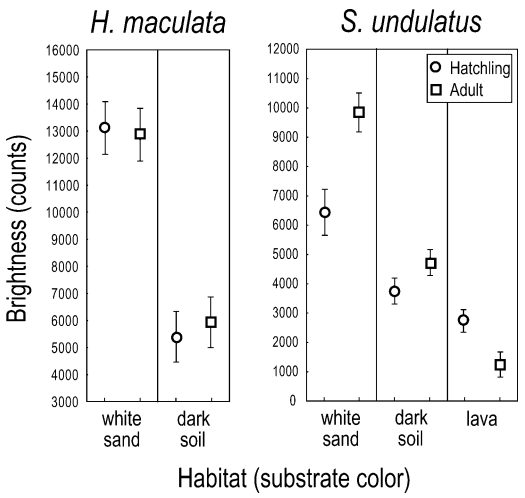

Fig. 3. Hatchling and adult color comparisons. Mean and 95% confidence intervals for dorsal color of hatchlings and adults from their parental population.

presented here support the observation that lizards tend to be darker when colder (Lowe and Norris, 1956; Sherbrooke et al., 1994). Physiological hypotheses have been advanced to suggest that this trend represents a thermoregulatory adaptation (de Jong et al., 1996). Studies in diverse taxa have demonstrated that melanic animals heat faster and can reach higher body temperatures than non-melanic conspecifics (e.g., Pearson, 1977; Forsman, 1995). Rates of warming are important if desert lizards are more vulnerable to predation early in the day before they are sufficiently active to escape. It may then be advantageous for lizards to be darker in colder temperatures to facilitate heat gain and allow optimal body temperatures to be reached more quickly (Norris, 1965; Watt, 1968). Conversely, lightening responses at higher temperatures may reduce heat loads during hot conditions (Benson, 1933).

Furthermore, results indicate that lighter color morphs may have more color lability than darker morphs. Norris (1965) also found that the darkening response triggered by experimental stimulation varied in intensity for different color morphs of the Common Side-blotched Lizard, *Uta stansburiana*. He detected negligible color change in melanic lava flow lizards but extensive plasticity in lighter lizards. The trend that darker lizards have reduced capacity for color change could indicate a trade-off between increased melanin production for substrate matching and decreased ability to aggregate and disperse melanin granules in response to short-term stimuli. Similar trade-offs between lo-

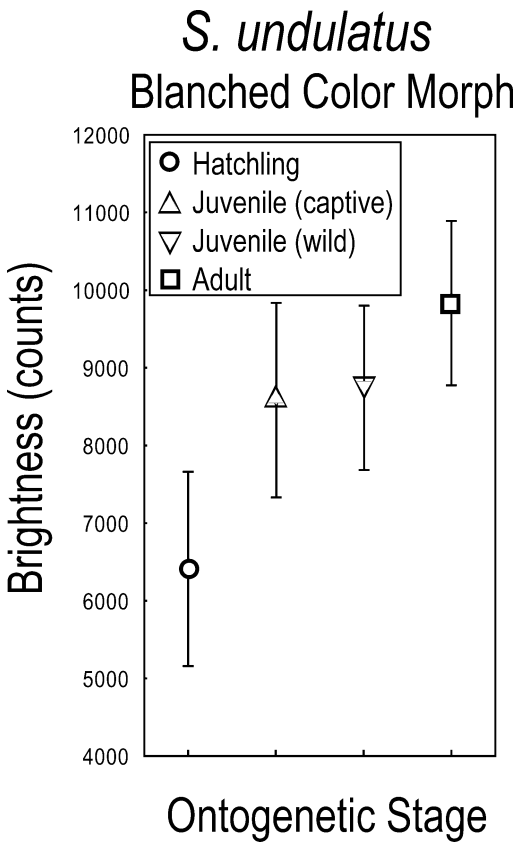

Fig. 4. Ontogenetic trajectory in brightness for white sand populations of *S. undulatus*. Mean and 95% confidence intervals for dorsal color of hatchlings raised in the lab, juveniles raised in the lab, juveniles caught in the wild, and adults caught in the wild.

cal adaptation and phenotypic plasticity have been documented in many taxa (Schlichting and Pigliucci, 1998).

Ontogenetic plasticity.—Common garden experiments with *H. maculata* and *S. undulatus* suggest that color differences among lizards in diverse substrate environments cannot be explained by environmental variation alone. Parents from different habitats produced offspring that were easily distinguishable by color morphology in both *H. maculata* and *S. undulatus*. Because eggs laid by females of all color morphs were maintained under identical conditions, these differences are expected to be due to genetic rather than environmental effects.

In *S. undulatus*, significant differences between hatchling and adult coloration likely indicate an ontogenetic trajectory in color morphology. An alternative explanation for differences in color between hatchlings and adults is that common garden conditions shifted hatchlings to less extreme mean phenotypes. One test to distinguish between treatment effects and a true ontogenetic trajectory is to measure color variation between captively-reared and wild-caught individuals at the same developmental stage. Direct comparisons between hatchlings or adults were impossible; newly emerged hatchlings are not readily caught in the wild, and several years of maturation are necessary for a comparison between captively-reared and wild-caught adults. However, a direct comparison between captively-reared and wild-caught animals was feasible at the juvenile stage and indicates that patterns observed do not represent a treatment effect, but rather an ontogenetic trajectory in brightness for *S. undulatus*. Developmental trajectories in color morphology have been documented in other species (e.g., Garcia et al., 2003). Further work is necessary to determine whether patterns observed in *S. undulatus* result from developmental constraints or variable selection pressures through ontogeny.

Although results suggest that phenotypic plasticity cannot explain dorsal color variation in Tularosa Basin lizards, the present study is unable to exclude the possible contribution of maternal effects. Maternal environment is known to affect a number of offspring characteristics in lizards such as size (Swain and Jones, 2000), growth rate (Wapstra, 2000), dispersal ability (Massot et al., 2002), locomoter performance (Sinervo and Huey, 1990; Sorci and Clobert, 1997), predator response (Shine and Downes, 1999), and immune function (Uller and Olsson, 2003). Maternal effects on coloration, however, are under-studied in reptiles. In avian taxa, maternal provisioning of eggs with pigments has been documented (Blount et al., 2000). However, all recorded cases of maternal effects on pigmentation involve carotenoids, pigments which are acquired dietarily and produce primarily yellow and red coloration (e.g., Blount et al., 2002). Dorsal brightness of Tularosa Basin lizards is affected by melanin, not carotenoids. Unlike carotenoids, melanin pigments are not acquired from the environment; cells destined to be melanophores are dedicated early in development from neural crest tissue (Bagnara and Hadley, 1973). No mechanism is known by which maternal provisioning can affect melanin density, although further study is necessary to test this explicitly.

Although common garden experiments were not conducted with *A. inornata*, a recent candidate gene study provides strong evidence of the

heritability of color variation in this species as well (Rosenblum et al., 2004). One amino acid substitution at the melanocortin-1 receptor (*Mc1r*), a gene known to be important for melanin production in mammals and birds, is highly associated with blanched coloration in white sand populations of *A. inornata*. Patterns of linkage disequilibrium, distributions of allele frequencies, and levels of population structure inferred from an unlinked marker are also consistent with recent selection at the *Mc1r* locus. Therefore, there is evidence in all focal species for an underlying genetic basis to observed color variation. Because environmental variation cannot explain patterns of dorsal coloration in Tularosa Basin lizards, it is appropriate to consider the role of natural selection for local substrate matching in shaping observed phenotypic variation.

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