Color Variation among Habitat Types in the Spiny Softshell Turtles (Trionychidae: Apalone) of Cuatrociénegas, Coahuila, Mexico

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ABSTRACT.—Ground coloration is highly variable in many reptile species. In turtles, ground color may correspond well to the background coloration of the environment and can change over time to match new surroundings in the laboratory. Variable carapace and plastron coloration across three habitat types were investigated in the Black Softshell Turtle, Apalone spinifera atra, by measuring individual components of the RGB (Red, Green, Blue) color system. In general, A. s. atra carapaces were darker in turtles from lagoons than in turtles from playa lakes. Red and green values were significantly different among all pairs of habitat types, but blue values differed only between the playa lakes and lagoons. Mean color components (RG only) for each population were significantly correlated with corresponding values for the bottom substrate, indicating a positive association of carapace and habitat substrate color components. In contrast, plastron ground color RGB channels showed no significant differences between habitat types and no significant correlations with substrate RGB. These results suggest that dorsal background matching in A. s. atra may be responsible for some of the variation in this key taxonomic trait.

The color of an organism is an important component of many aspects of an organism’s biology and is often used as a taxonomic character (Endler, 1990; Brodie and Janzen, 1995; Darst and Cummings, 2006). Yet, an animal’s coloration may be plastic and can depend on many different biotic and abiotic factors (Endler, 1990; Bennett et al., 1994). For example, overall habitat irradiance is a common stimulus for physiological color change in reptiles, presumably as a means to become more cryptic (Norris and Lowe, 1964; Rosenblum, 2005; Rowe et al., 2006a). This physiological response occurs rapidly and uses hormonal signals to expand or contract melanophores in the dermal layer of skin (Bartley, 1971). Interestingly, laboratory tests indicate that the shells and skin of turtles, too, can change color to more closely match dark or light backgrounds, although this change occurs over weeks or months (Woolley, 1957; Bartley, 1971; Rowe et al., 2006b).

Cuatrociénegas in Coahuila, Mexico, offers a unique ecosystem in which to observe natural variation in shell color of turtles across three very different aquatic habitat types (Winokur, 1968). The region’s endemic and endangered Softshell Turtle, Apalone spinifera atra (atra meaning black or dark; Webb and Legler, 1960; Lovich et al., 1990, Fritz and Havaš, 2006), is taxonomically defined by its dark pigmentation, and the validity of A. s. atra as a full species has been challenged (Smith and Smith, 1979). Hatchlings of A. s. atra cannot be differentiated from a lighter conspecific, Apalone spinifera emoryi (Winokur, 1968), but adults show marked differences in coloration across habitats (this study), which could be a result of genetically based ontogenetic pigmentation variation among habitats or phenotypic plasticity in response to substrate color variation.

Apalone spinifera atra was originally described as an isolated species (Webb and Legler, 1960), but canal building in the late 1800s was thought to have opened the basin hydrologically, resulting in opportunities for hybridization between A. s. atra and A. s. emoryi (Smith and Smith, 1979; Ernst and Barbour, 1992). A recent genetic evaluation found no differentiation between morphologically identified A. s. atra individuals and morphologically identified A. s. emoryi individuals within and outside the basin (McGaugh and Janzen, in press). However, substantial coloration differences exist among habitats and this phenotypic variation was left unexplained by the genetic study. Examination of the color variation of the softshell turtles across the basin, in relation to background coloration of habitats (Woolley, 1957; Bartley, 1971; Rowe et al., 2006b), may provide an important morphological perspective on A. s. atra’s refuted species delimitation (McGaugh and Janzen, in press). For simplicity, and because haplotypes in the genetic study showed no morphological (i.e., light and dark turtles shared mitochondrial and nuclear DNA haplotypes) or geographic grouping, the name A. s. atra is used to refer to all Apalone in this study, even though A. s. emoryi morhps are present in
the basin (Smith and Smith, 1979; McGaugh and Janzen, in press).

In this study, I evaluated the hypothesis that background matching could be responsible for the observed color variation across habitats in *A. s. atra*. To evaluate this hypothesis, coloration of the animals’ carapace and plastron and locality substrate was measured. Background matching was expected to be probable if carapace, but not plastron coloration, was correlated to locality substrate coloration. Color was measured with digital photographs using the RGB system (red, green, blue; Stevens et al., 2007), the components of which correspond to broad bands of longwave (red), mediumwave (green), and shortwave (blue) light (Stevens et al., 2007). Possible influences of sex, size, and their interactions were also evaluated.

**Materials and Methods**

**Study Site and Field Methodology.—** Three main habitat types, playa lakes (barrial lakes), lagoons, and a river, were investigated within El Área de Protección de Flora y Fauna Cuatrociénegas, Coahuila, Mexico. This area of high endemism contains diverse habitats, including hundreds of small water bodies nestled within the Chihuahuan desert (Minckley, 1969; Meyer, 1973). Playa lakes are large, shallow lakes (<1 m deep) with sparse vegetation, large daily temperature fluctuations, and relatively high mineral content (Minckley, 1969). Lagoons are typically deeper lakes (<1–10 m) with abundant vegetation, including waterlilies (*Nymphaea*), muskrass (*Chara*), pondweeds (*Potamogeton*), and cattails (*Typha*), and relatively constant temperatures (Minckley, 1969). Rivers are flowing channels with steep banks that are up to 2.5 ms deep and have vegetation such as *Nymphaea*, *Chara*, *Potamogeton*, *Typha*, bladderworts (*Utricularia*), and sedge (*Eleocharis*) where the current is slow (Minckley, 1969). Most aquatic habitats in Cuatrociénegas have clear water with a visible bottom.

Six localities were examined for *A. s. atra*, and localities with the same habitat types (e.g., lagoons) were combined in statistical analysis except for the Pearson’s correlation analysis (described below). All habitats used in this study are stable bodies of water of unknown geologic age. No aboveground aquatic connections are known between the localities used in this study. Turtle dispersal between sites may not be frequent because sites are separated by distances (5.53–31.58 km) greater than other species of softshells typically travel territorially (Galois et al., 2002). Sampling for shell coloration included all five drainages of the basin (Evans, 2005) and spanned 30 days of trapping (4 June to 5 July 2004).

Turtles were captured in lobster or hoop traps baited with sardines. Each *A. s. atra* was tattooed with a unique pattern on the plastron. Plastron length was measured with dial calipers to the nearest millimeter, and sex was determined by tail length (Webb and Legler, 1960), with males identified by a much longer tail than females relative to their body size. All *A. s. atra* had plastron lengths over 73 mm. Hatchling and juvenile turtles were not included in the analysis. Sixty total individuals were sampled (Lagoon1: N = 16, Lagoon2: N = 6, Lagoon3: N = 13, River: N = 10, Playa lake1: N = 10, Playa lake2: N = 5).

**Digital Photography and Analysis.—** Digital photographs were taken with a Canon Power Shot G5 that was positioned directly above the turtle. Color component measurements from digital systems have been shown to be positively correlated with values from spectrometry, and digital photography has many advantages over spectrometry for data acquisition in the field (Rowe et al., 2006a; Stevens et al., 2007). A level on the tripod ensured that the camera lens was parallel to the ground. The lens-to-animal distance measure was not taken. Photos were taken out of direct sunlight and with the flash on and white balance off. Each photo was taken with the highest resolution possible for the camera (2,592 × 1,944 pixels) and converted to JPEG at the highest quality compression level available on the camera.

Digital quantification of color was performed using Jasc Paint Shop Pro9 (Corel, Eden Prairie, MN) by viewing the untransformed values of R, G, and B in the diagnostic histogram. The HSL (Hue, Saturation, Lightness) system was not used in the analysis because this system may be inaccurate (Stevens et al., 2007). In Jasc Paint Shop Pro9, the histogram displays a distribution graph of the separate channels of color in an image and allows the user to analyze the distribution. A swath of at least 100 square pixels was selected from the right middle portion of the carapace and the plastron of *A. s. atra*. All photos were analyzed at a resolution of 70.866 pixels per cm. Therefore, each *A. s. atra* sample was ≥14 mm². Some *A. s. atra* had a blotched pattern on their carapaces, and these were included in the analysis. Otherwise, their pigmentation was generally uniform. Care was taken during analysis only to sample areas with a clear view of the carapace (i.e., flash glares, algae growth, mineral deposits, or bite marks on the animal were excluded from the swath). Intraindividual color variability was not measured because of these various obstructions.
A grey color standard (paint swatch EE2054C from Lowe’s Home Improvement Warehouse) was placed in each photo. Digital quantification of the paint swatch was achieved by the same method described for the turtles. Associations between turtle RGB-values and paint swatch RGB-values were strong \( r > 0.33 \) and \( P < 0.002 \) for RB-values of carapace and RGB-values of plastron, but correlation coefficient of paint swatch G-values and carapace G-values was not significant \( [r > 0.23, P < 0.08] \); therefore, substantial light variation across photographs occurred. Thus, standardizing the RGB values from the turtles by the paint swatch was necessary, and RGB-values from the paint swatch were used as a covariate in the statistical analyses. Some of the inconsistencies of variable ambient field conditions and camera biases can be accounted for by providing a common color swatch in each picture (J. A. Endler, pers. comm.; M. Stevens, pers. comm.; but for detailed review, see Stevens et al., 2007). However, this technique does not remove inconsistencies associated with nonuniform brightness across the photograph (J. A. Endler, pers. comm.; Stevens et al., 2007). To reduce the effect of these inconsistencies on the overall analysis, the animal was consistently placed so that a landscape photo was taken with the posterior of the animal on the left side of the photograph and the snout on the right side. Finally, because only one grey color standard was used, no linearization (also called gamma correction) could be achieved; consequently, dark objects may be estimated as lighter than they are, and light objects may be estimated as darker than they actually are (see Stevens et al., 2007:fig. 6). Fortunately, any such biases render the comparisons in this study conservative.

Past studies that have evaluated the relationship between carapace color and habitat type have qualitatively described the habitats as “dark” or “light” bottomed (e.g., Rowe et al., 2006a). In this study, lagoons are dark bottomed, playa lakes are light bottomed, and the river was intermediate. To provide a more quantitative measure of this habitat descriptor, one wet substrate sample of approximately 200 ml in volume was taken from the bottom of each site and was photographed and measured for RGB-values in the same manner as the turtles. Substrate color at each site appeared relatively uniform although this assumption was not explicitly tested. Vegetation was not included. However, lagoons were the only aquatic habitats with substantial submerged vegetation, and bare substrate makes up large portions of the lagoon bottom (Webb and Legler, 1960).

**Statistical Analysis.**—To test for habitat structuring in color components, data were transformed for normality (verified through Shapiro-Wilks tests). All carapace components were log transformed, and all paint swatch components from the carapace photographs were raised to the three-quarters power to achieve normality. Plastron R and paint swatch R from the plastron photographs were raised to the three-quarters power to achieve normality, and other plastron components and paint swatch components from plastron photographs were normal (all verified through Shapiro-Wilks tests). Each response variable (R, G, or B from plastron or carapace) was analyzed with analysis of covariance (ANCOVA), using the respective paint swatch component as the covariate and plastron length, sex, habitat type, and interaction terms between all combinations of these factors as fixed factors. All factors and interaction terms that were not significant were removed from the model, and the ANCOVA was rerun until only significant factors remained. The only significant interaction that was detected was between habitat type, sex, and plastron length for blue carapace component \( (F_{2,48} = 6.17, P < 0.0038) \). To incorporate this interaction term in the model for the blue carapace color channel, all nonsignificant factors and lower-order interaction terms were left in this model. Sex was not a significant factor for plastron or carapace RGB-values \( (P > 0.09) \) for all ANCOVAs and, thus, was removed from all models, except the blue carapace component where it was previously explained to be important for a significant interaction term. Plastron length was not a significant factor for carapace color components \( (P > 0.22, F_{1, 48} < 0.151) \) for all ANCOVAs but did show a significant, or nearly significant, positive association with plastron color components \( (R: F_{1, 54} = 2.82, P < 0.10; G: F_{1, 54} = 13.86, P < 0.0005; B: F_{1, 54} = 10.36, P < 0.00022) \). Therefore, for carapace color components, the model consisted only of the color standard as a covariate and habitat type as a factor. However, for the blue color component as mentioned above and for plastron color components, the model consisted only of the color standard as a covariate and plastron length and habitat type as factors. This statistical analysis resulted in some of the models being inconsistent with others. Several additional analyses were done to ensure that results were not an artifact of the model. The blue color component and plastron components were analyzed using the most basic model for the other two carapace components (e.g., color standard as covariate and habitat type only), and overall results did not change. The alternative strategy, including all nonsignificant factors in the analysis of carapace red
and green components, resulting in important differences across pairwise habitat types being missed because the model was unnecessarily overparameterized with nonsignificant terms.

Least-squares (LS) means t-tests, a method used to assess the significance of differences among the best linear-unbiased estimates of the habitat means for the model design, were used to determine which habitat types were significantly different for certain color components. Among-site substrate samples were compared using LS means t-tests. Finally, Pearson’s product-moment correlations were used to assess the relationship between LS mean for carapace and substrate sample RGB-values and plastron and substrate sample R/G-values for each locality. All statistics were performed in R 2.4.0 (R Development Core Team, 2006) and JMP 6.0.2 (SAS Institute Inc., Cary, NC, 2006).

RESULTS

Carapace and Plastron Color Variation.—For *A. s. atra*, there were significant differences among habitat types for all carapace color components (R: $F_{2,59} = 22.35, P < 0.001$; G: $F_{2,59} = 32.60, P < 0.001$; B: $F_{2,59} = 7.37, P < 0.002$). Red and green color channels increased significantly from lagoons to the river to the playa lakes (lagoons to river: R: $t_{52} = 2.91, P < 0.003$; G: $t_{52} = 2.37, P < 0.011$; lagoons to playa lakes: R: $t_{57} = 6.57, P < 0.001$; G: $t_{57} = 8.07, P < 0.001$). The B color channel was significantly greater, or nearly so, in playa lakes than in the lagoons and the river (lagoons to river: $t_{48} = 0.694, P < 0.49$; lagoons to playa lakes: $t_{48} = 3.84, P < 0.001$; playa lakes to river: $t_{48} = 1.63, P = 0.054$). Consistently higher RGB values suggest that turtles from playa lakes are overall lighter than those from lagoons. No significant habitat structuring of plastron color components was observed ($F_{2,54} < 1.90, P > 0.16$ in all cases). Overall, results of the RGB analyses suggest that carapace color components differ significantly among most habitat types, whereas plastron color components do not.

Bottom Substrate Color Variation and Relationship between Substrate and Shell Colors.—Substrate samples showed the same trend as the turtle RGB-values and increased from lagoons to the river to playa lakes. Playa lakes R and G substrate values were significantly higher than in lagoons (playa lakes vs. lagoons: R: $t_{3} = 4.24, P < 0.012$; G: $t_{3} = 3.67, P < 0.017$, playa lakes vs. river: R: $t_{3} = 2.82, P < 0.066$; G: $t_{3} = 2.59, P < 0.096$). No significant differences existed in the blue color channel ($F_{2,5} = 4.785, P < 0.12$). Correlations of turtle carapace RG-values and substrate RG-values were strongly positive and significant (R: $r = 0.81, P < 0.026$; G: $r = 0.74, P < 0.045$). Associations for turtle carapace B and substrate B-values were positive but not significant (B: $r = 0.39, P < 0.22$). All associations of plastron RGB components and substrate RGB components were negative, but no significant correlations were detected (R: $r = -0.35, P < 0.50$; G: $r = -0.51, P < 0.15$; B: $r = -0.69, P < 0.065$).

DISCUSSION

Two conclusions can be drawn from the statistical analyses performed in this study: (1) carapace and substrate sample R and G color components differ among habitat type comparisons and are significantly, positively correlated to each other; and (2) plastron ground color components do not differ significantly across habitat types and do not significantly correlate with substrate sample color components.

The color components varied across habitats in different ways. In particular, the short wavelength reflectance (B) of the turtles showed a weaker trend of habitat structuring than the long (R) and medium (G) wavelength light (Table 1). Short wavelengths of light are often absorbed by dissolved organic matter in the water and are not available for illumination of underwater objects (Markager and Vincent, 2000). Alternatively, blue is often conspicuous in water <2 m deep (Maan et al., 2006); hence, may be constrained to maintain crypsis. Therefore, ambient lighting conditions underwater could eliminate this color channel’s relative importance to potential cryptic coloration or constrain the plasticity of this color channel to match the surroundings. Although more work remains to be done on the photic environment experienced by these turtles, either of these explanations may be consistent with an adaptive explanation for carapace color variation.

My results support a hypothesis of dorsal background matching for *A. s. atra*. Although not all comparisons were significant, the RGB color components of the sampled localities generally revealed an increase in carapace ground color darkness from playa lakes to the river to lagoons (Figs. 1, 2; Table 1), and the correlations of R and G with substrate values were positive and significant. In contrast, plastron ground color did not show any significant habitat type structuring in RGB-values or significant correlation with substrate RGB-values. If these observations of general carapace darkening were the result of a passive staining process from abiotic forces instead of background matching, plastron and carapace color should have changed in the same direction (Rowe et al., 2006a). Further, background
matching has been noted for a wide variety of reptiles (e.g., reviewed by Norris and Lowe, 1964; Cooper and Greenberg, 1992; Rowe et al., 2006b), and the pattern observed here (i.e., dorsal, but not ventral, matching) is consistent with expectations from a cryptic coloration mechanism. It is unknown, and would be interesting to investigate, whether the observed phenotypic variability is a result of short-term phenotypic plasticity such as that seen in laboratory settings (Bartley, 1971; Ernst et al., 1994) or is a result of a fixed local adaption (Rosenblum et al., 2004).

The habitat structuring of carapace RGB-values and plastron pigmentation in A. s. atra is important in a taxonomic context. Apalone spinifera atra is predominantly defined by dark dorsal coloration (Webb and Legler, 1960; Smith and Smith 1979; Lovich et al., 1990). Winokur (1968) reported that darker, A. s. atra–like animals were mainly in lagoons and that lighter, A. s. emoryi–like specimens resided in rivers (no playa lakes were examined in his analysis). He then hypothesized that ecological preferences (i.e., lagoons for the darker A. s. atra, rivers for the lighter A. s. emoryi) potentially were involved (Winokur, 1968). Here, I suggest that background color matching, whether transient and plastic or fixed and locally adaptive, explains much of the phenotypic variation among Apalone in the Cuatrociénegas basin. This hypothesis is supported by the correlation of carapace but not plastron, color components to substrate samples and experimental evidence from an earlier study that showed that dorsal background matching occurs in the laboratory in Apalone (Bartley, 1971; Ernst et al., 1994). The DNA evidence of the companion study (McGaugh and Janzen, in press), supports the decision of Fritz and Havas (2006) to demote A. s. atra from species to subspecies rank but leaves unexplained the morphological diversity seen in this species across the basin. The preliminary morphological data presented here suggests that background matching may explain the phenotypic diversity seen across habitats in the basin. However, the mechanism by which this substantial morphological variation is achieved remains enigmatic.

TABLE 1. Statistics on RGB-values of carapaces for Apalone spinifera atra in Cuatro Ciénegas, Mexico. Carapace coloration across three habitat types was analyzed by ANCOVA and LS means t-tests. Significant comparisons are denoted by an asterisk. Raw data were log transformed (carapace) and taken to the three-fourths power (color standard for carapace photographs) for all A. s. atra. Data from soil samples and their color standards were not transformed. Abbreviations are R = red (long wavelength), G = green (medium wavelength), and B = blue (short wavelength).

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<th>Habitat</th>
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<td></td>
<td>LS mean</td>
<td>LS mean</td>
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<tr>
<td>Lagoon</td>
<td>R 69.24</td>
<td>1.936</td>
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<td></td>
<td>G 68.34</td>
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<td>B 64.68</td>
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<td>Playa Lake</td>
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<td></td>
<td>G 172.56</td>
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<td>River</td>
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FIG. 1. Boxplots of carapace RGB-values for Apalone spinifera atra in different habitats in Cuatrociénegas, Coahuila, Mexico. The box indicates quartiles, and the median is indicated by the heavy line within the box. The lines illustrate points falling within 1.5 times the box size. Outliers, which were more extreme than the lines, are not shown.
such as a reciprocal transplant experiment, is needed to determine whether the pigmentation variation is plastic in the field, as is seen in the lab (Bartley, 1971; Ernst et al., 1994), or could potentially be a result of fixed genetic differences between populations.

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