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Plastron Pigmentation Variation in a Coastal Turtle Species of Conservation Concern (*Malaclemys terrapin*)

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ABSTRACT: *Malaclemys terrapin* inhabits salt marshes of the coastal United States and has been historically divided into seven subspecies. Two of the described subspecies, *M. t. pileata* and *M. t. littoralis*, occur along the western and eastern coastlines of Louisiana, respectively. To better understand potential boundaries of these two subspecies, we documented pigmentation variation of the plastron along the coast of Louisiana where the subspecies supposedly intergrade. Photographs of the plastron were taken of captured individuals and we quantified pigment with a photoprocessing software (ImageJ). We found pigmentation differences between sexes, with females having lighter plastrons than males. The plastron pigmentation of females decreased with increasing plastron length, whereas that of males varied by site but not length. Plastron pigmentation did not correlate with longitude in either sex, indicating that there is no geographical cline in this trait. We suggest that large females might be released from the predation pressure of gape-limited predators, and therefore, can stop production of melanin as they grow. However, males and smaller females maintain cryptic, dark plastrons. Little is known about the mechanisms behind melanization in freshwater turtles, but understanding the patterns of pigmentation variation in *M. terrapin* might be important for taxonomists and conservation managers.

Key words: Coloration; Diamond-Backed Terrapins; Intraspecific variation; Sexual dichromatism

INTRASPECIFIC color variation in freshwater turtles has been attributed to sex (Thomas 2002; Ibáñez et al. 2013), body size (Thomas 2002; Ibáñez et al. 2013), immune response (Ibáñez et al. 2013), and substrate color (McCaugh 2008; Rowe et al. 2009). Freshwater turtles often have high contrast colors on their limbs, heads, and necks and in some cases, these colorations have been found to be sexually dichromatic and play a vital role in courtship (Ibáñez et al. 2014). Many species also have color variation on their shells and these colors have been less well studied despite their potential to be under similar selective pressures as the skin coloration.

Diamond-Backed Terrapins, *Malaclemys terrapin*, inhabit brackish coastal waters from Texas to Massachusetts, and an isolated population occurs on the island of Bermuda (Parham et al. 2008; Ernst and Lovich 2009). The species has historically been divided into seven subspecies on the basis of morphological characteristics like carapace and plastron coloration, body shape, presence of tubercles on the carapace, and skin coloration (see Ernst and Lovich 2009). Three of these subspecies—*M. t. macrospilota*, *pileata*, and *littoralis*—occur along the Gulf of Mexico from Florida Bay to Corpus Christi Bay. Within Louisiana, it is presumed that *M. t. pileata* occurs in the eastern portion of the state, whereas *M. t. littoralis* occurs in the western portion of the state, and the two supposedly differ in their plastron coloration (Ernst and Lovich 2009). However, recent genetic evidence has indicated that the subspecific delineations based on phenotypic traits are not congruent with genotypic data (Hart et al. 2014; Petre et al. 2015). Hart et al. (2014)

grouped terrapins into four discrete populations defined as genetic units, and the three Gulf Coast subspecies were coalesced into one management unit. This change was supported by evidence within Louisiana that there was no genetic structuring, even between the most distant populations (Drabeck et al. 2014; Petre et al. 2015). To date, however, no rigorous investigation has been done to document or explain the variation in color described between or within regions. Here, we investigate plastron pigmentation variation along the Louisiana coastline to determine if coloration is affected by longitude, body size, or sex in *M. terrapin*.

MATERIALS AND METHODS

Individual terrapins were captured in 2013 and 2014 as a part of long-term studies of the terrapin populations in coastal Louisiana (Selman et al. 2014). The specific trapping methods and site descriptions are described in Selman and Baccigalopi (2012) and Selman et al. (2014), respectively. For each individual captured at eight sites along the Louisiana coastline (Fig. 1; Table 1), we recorded plastron length (PL in cm) and mass (g). We sexed individuals on the basis of males having longer precloacal tail lengths compared with females (Butler et al. 2006). Along with sex and morphological data, we used a digital camera to photograph the plastron of each individual in sunlight (Fig. 2).

To determine the quantity of plastron pigmentation, we measured the percentage of the plastron covered by dark pigment in each photograph. After converting each photo to a RGB-stacked image, we manually adjusted the threshold until all pigmented areas were highlighted. We then outlined the plastron (excluding the limbs, head, and underside of the carapace) and measured the percentage of the plastron that was highlighted. All photo measurements and manipulations were completed in ImageJ (Schneider et al. 2012). Two of the authors (BR and WS) analyzed each photo two separate

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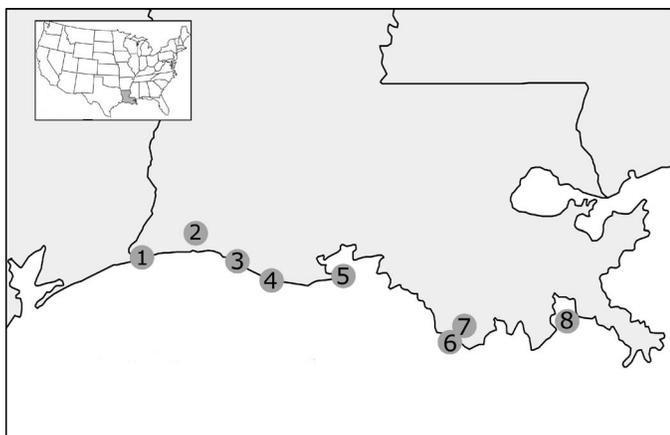


FIG. 1.—Map showing eight capture sites for *Malaclemys terrapin* along the coast of Louisiana, USA (inset). Numbers correspond to the sites listed in Table 1.

times and the average of these four measurements was used for analyses. Individuals whose results differed by greater than 15% between the observers ($n = 21$ of 189 total individuals) were removed from the analysis. These problems were typically attributable to glare on the plastron that made color determination ambiguous.

Because *M. terrapin* is sexually dimorphic, we first used a t -test to determine if the amount of plastron pigmentation differed by sex. If a significant difference existed between sexes, we separated them for the remainder of the analyses because dichromatism might mean that the sexes are under different selective pressures. We then conducted a backward stepwise model selection using the corrected Akaike's information criterion with all measured predictor variables that might have an effect on plastron coloration according to a priori hypotheses to determine the model with the best fit. Initial predictor variables included plastron length, site, and longitude. All statistical analyses were performed in R programming language v3.4.2 (R Core Team 2016).

RESULTS

A total of 189 turtles was captured and photographed across eight sites in Louisiana. Because the percentage of pigmented plastron was not normally distributed across individuals, we used a permutation test to sample from 1000 random draws of the response variable without replacement.

TABLE 1.—Percent pigmentation of the plastron of Diamond-Backed Terrapins (*Malaclemys terrapin*) as a function of sex among eight sampling sites along coastal Louisiana, USA. Site numbers correspond to those depicted in Fig. 1.

Site	Males		Females	
	n	Mean \pm 1 SD	n	Mean \pm 1 SD
1. Sabine	1	48.05 \pm 0	4	35.88 \pm 25.4
2. Rabbit/Calcasieu	7	26.36 \pm 26.52	14	15.45 \pm 20.24
3. Mermentau	23	63.66 \pm 27	18	39.96 \pm 22.45
4. Rockefeller	19	43.61 \pm 30.26	30	37.75 \pm 26.47
5. Marsh Island	0	NA ^a	4	29.32 \pm 24.41
6. Jack Stout	4	18.13 \pm 15.44	6	23.13 \pm 17.66
7. Redfish	3	26.2 \pm 32.47	4	10.63 \pm 5.1
8. Grand Terre	12	68.00 \pm 28.1	19	27.88 \pm 23.04

^a NA = not applicable.

In all cases, results from our permutation tests were not qualitatively different, so the original models were robust enough to handle violations of normality. Plastron coloration varied by sex at any size ($t = -4.43$, $df = 121.84$, $P < 0.0001$). Males were darker than females ($\bar{X}_{\text{males}} = 50.62$, $\bar{X}_{\text{females}} = 30.71$). The best-fit model for females used PL and longitude as the predictor variables, but only PL significantly predicted female pigmentation ($df = 1$, $F = 20.85$, $P < 0.0001$). Larger adult females had less pigment than smaller adult females ($t = -4.563$, $P < 0.001$; Fig. 3). Adult females were less pigmented than juvenile females ($t = -4.58$, $df = 30.25$, $P < 0.001$, $\bar{X}_{\text{adult}} = 24.86$, $\bar{X}_{\text{juv}} = 51.16$). The model with the best fit for males included only site as a predictor variable ($df = 7$, $F = 3.54$, $P = 0.003$), with the Mermentau and Grand Terre populations having the highest percent pigmentation (Table 1).

DISCUSSION

We did not find longitudinal variation in pigmentation that would be indicative of a gradient in this trait across coastal Louisiana, including *M. t. pileata* to the east and *M. t. littoralis* to the west. This conclusion is also supported by recent genetic evidence that indicated a single population in Louisiana (Petre et al. 2015). Hart et al. (2014) found four distinct genetic clusters across the range of Diamond-Backed Terrapins that were incongruent with the seven currently described subspecies. Our findings also support the absence of phenotypic differentiation between the two subspecies ranging along coastal Louisiana. Because we found that plastron pigmentation varies by sex, PL, and site, it seems possible that other phenotypic traits previously used to diagnose terrapin subspecies might be under similar control. Additionally, because considerable phenotypic variability is possible within a single site, some managers or researchers might believe that multiple terrapin subspecies are present at a single site (e.g., sites in Louisiana; W. Selman, personal observation) even though they are part of the same genetic population (Petre et al. 2015).

We detected significant differences in plastron pigmentation between males and females. Ibáñez et al. (2013) suggested that sexual dichromatism in Spanish Terrapins (*Mauremys leprosa*) does not occur on the shell because both male and female shells are subject to the same selective pressure of predation. The differences in shell coloration that we found between the sexes might be explained by exposure to different predation pressures. For example, primary predators of *M. terrapin* in Louisiana are likely alligators and large predacious fish (e.g., alligator gar, redfish) whose prey consumption is gape limited. Males and small female *M. terrapin* are darker than large female individuals, indicating that they might be under a predation pressure that is no longer constraining when females reach a certain size. Further support for this contention is evidence of adult females tending to travel into deeper waters and more frequently farther from the shoreline than adult males or juveniles (Roosenburg et al. 1999; W. Selman, personal observation).

We found that larger females are significantly lighter than smaller females (Fig. 3). Although not documented in turtles, calcium has been shown to increase melanin coloration in birds and mammals (McGraw 2008). Older



FIG. 2.—Examples of variation in plastron pigmentation of *Malaclemys terrapin* collected from the same site. The subjects shown in these images were all collected at Grand Terre (Site 8) and vary from nearly 0% to nearly 100% in the expression of melanin on the surface of the plastron. From left to right: female, 168-cm plastron length; female, 169-cm plastron length; female, 173-cm plastron length; male, 112-cm plastron length. A color version of this figure is available online.

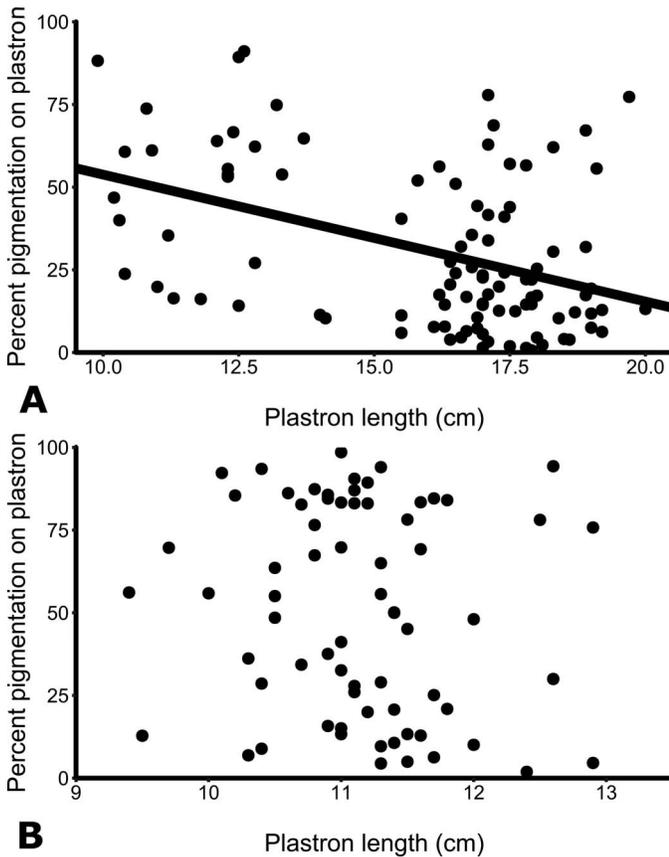


FIG. 3.—Percent pigmentation on the plastron of Diamond-Backed Terrapins (*Malaclemys terrapin*) as a function of body size (measured as straight-line plastron length) for individuals collected from sites along coastal Louisiana, USA. (A) Adult females ($df = 1$, $F = 20.85$, $P < 0.0001$, $n = 99$); (B) adult males ($n = 69$).

females might become calcium limited over time from egg production, and thus might deposit less melanin into their plastrons as they grow. However, this does not explain why a similar trend is not common in all long-lived turtles. Also, the diet of terrapins is predominated by calcium-rich gastropods and crustaceans so it does not seem likely that they would become calcium limited as they age (Tucker et al. 1995). In fact, these findings differ from documented melanization patterns in other turtles, especially the *Trachemys* group. Males of this genus exhibit ontogenetic melanization to the extent that male body stripes and shell colors are obscured as the individual ages, whereas females may show slight ontogenetic melanization of the shell (Lovich et al. 1990; Thomas 2002; Hays and McBee 2009; Bager et al. 2010). *Trachemys* males also show a change in courtship behaviors and foreclaw growth associated with melanization (Lovich et al. 1990; Thomas 2002). Stone et al. (2015) hypothesized that males might become melanistic as they approach the size of mature females for sex recognition, but this hypothesis does not hold for *M. terrapin* because small females and males show similar melanization. The mechanisms behind ontogenetic melanization are unknown, so a causal explanation for *M. terrapin* females showing a pattern that contrasts with *Trachemys* requires further research, as do the associated behavior and growth changes.

We found that male plastron pigmentation varied significantly by site (Table 1). It is unclear why we found two distant sites—Mermentau and Grand Terre—to be darker than the other sample sites. Background matching through plastic melanization of the turtle carapace has been documented in freshwater turtles (Rowe et al. 2009, 2014), but evidence is lacking to suggest that plastron coloration is phenotypically plastic enough to match down-welling light. Although we did not collect environmental variables like water clarity or substrate type, it seems possible that such variables might play a role in the degree of male plastron pigmentation given the variability observed across sites (Table 1). Alternatively, in some animals, pollution or

environmental contamination can result in melanization because melanin can bind trace elements (Chatelain et al. 2014; Goiran et al. 2017). For instance, sea snakes (*Emydocephalus annulatus*) in polluted waters exhibit increased melanization, and more melanistic snakes had higher concentrations of trace elements, and a higher frequency of ecdysis, than paler snakes (Goiran et al. 2017). To date, however, there is no evidence that this binding can occur in turtle shells and more data would be necessary to determine if pollutants are present in the sites with more melanistic males.

The histological mechanisms of pigment development in freshwater turtles are largely unknown, and this adds additional difficulty in explaining patterns of melanization. Generally, the coloration of plastron tissue is caused by epidermal melanocytes and dermal melanophores (Alibardi 2013). In *M. terrapin*, it appears that the dark pigment extends from the borders of scutes inward (Fig. 2), but the mechanism for the reduction of this coloration with size in females remains unknown. Alibardi (2013) hypothesized that as scutes expand, the dermal melanophores extend and the pigment that is visible from the epidermis “bleeds” through the more superficial corneous layers. Because we observed the opposite pattern, it is necessary to conduct histological analyses specifically of the pigmentation in *M. terrapin* to determine whether the same pigment formation mechanisms are occurring. Following this determination, other processes that might affect pigment distribution, such as growth rates or temperatures, can be further investigated and mechanistically informed.

We found that plastron pigmentation in *M. terrapin* from coastal Louisiana differed between the sexes, among body sizes in females, and among sites in males but not along a longitudinal gradient. These data indicate that there is no pigmentation gradation along the coast between the two previously described subspecies (Ernst and Lovich 2009). Our data further support previous studies that have identified genetic similarities among Louisiana populations (Hart et al. 2014; Petre et al. 2015). We suggest that the trends in pigmentation documented among our study populations might be a product of differential predation pressures among different sexes or body sizes, whereas male coloration is better predicted by location. Further exploration of the mechanisms behind pigmentation variation might provide relevant information for resource managers and biologists monitoring terrapin populations, as well as for taxonomists delimiting subspecific boundaries.

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