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Short Communication

Plastral Colour Fading of *Pseudemys concinna* Leconte 1830 (Testudines: *Emydidae*)

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ABSTRACT

Geometric techniques can be easily applied to many zoological problems, from recognizing subpopulations to studying sexual dimorphism. *Pseudemys concinna* (*P. concinna*) is a large terrapin species known as the Eastern River Cooter, native to North America. The hypothesis was to test if plastral pattern tends to fade with age. The present study applies geometric techniques to assess size in a sample of 20 fresh corpses of *P. concinna* (18 females and 2 males). Plastron color (red, blue, and green channels) was used to objectivize this fading pattern. A negative regression between size and color was registered, so the colour plastral intensity of *P. concinna* LeConte 1830 tends, effectively, to fade with age. Elucidating the cellular and chemical mediators and mechanisms of these slow color changes will likely require laboratory study.

1. Introduction

Some turtle and tortoise species present a high degree of individual variation and a reported susceptibility to deformation due to environmental effects[1,2]. Carotenoid-based ornaments provide a good example of ornamental traits[3].

The genus *Pseudemys* includes several species of cooters and red-bellied turtle[4]. *Pseudemys concinna (P. concinna)* LeConte 1830 is a large riverine turtle species known as Eastern River Cooter, native to North America [4,5]. The plastron is yellow and marked anteriorly with a large, darker pattern. The plastral pattern tends to fade with age. The present study aimed to describe the changes in plastron color with age in *P. concinna* using geometric morphometrics and analysis of color in channels as a potential method.

2. Materials and Methods

2.1. Sample collection

A total of 20 fresh corpses (18 females and 2 males) of specimens from *P. concinna* (range of plastron length: 102.2-241.7 mm, Figure 1) was obtained from CRARC, *Catalonia Reptiles and Amphibians Rescue Center,* in Catalonia, Spain, collection. As inclusion criteria, none of the plastrons presented malformations or lesions that could influence the development of the studied region. Each specimen was subsequently photographed once with a mounted digital camera on the ventral face (plastron).

Figure 1. A captive *Pseudemys concinna*. Photo: Albert Martínez-Silvestre

2.2. Imaging

Each turtle was leveled in accordance with a horizontal

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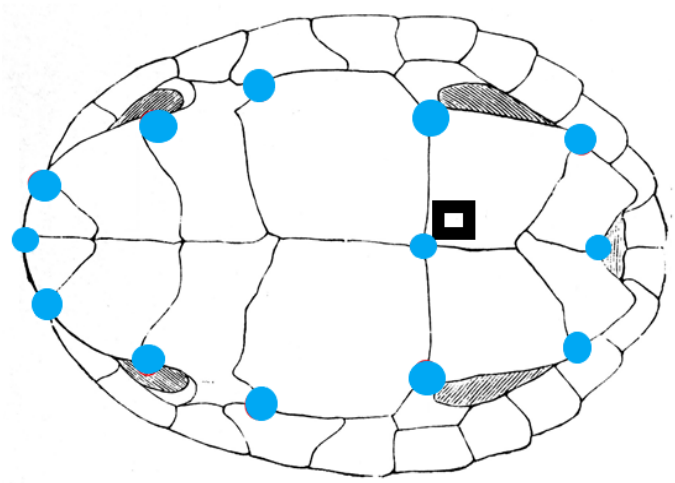

Figure 2. Landmark configuration used in this study. The set was composed of 13 discrete landmarks on 2D pictures of Pseudemys concinna plastron (3 mid-sagittal and 5 lateral pairs). The left of the image corresponds to the gular scute. Colour was analyzed on the squared area, close to the midsagittal line of the left femoral scute

plan. Image capture was performed with a Nikon® D70 digital camera (Japan) (image resolution of 2,240 x 1,488 pixels) equipped with a Nikon AF Nikkor® 28-200 mm telephoto lens. The camera was placed so that the focal axis of the camera was parallel to the horizontal plane and centered on the plastral (ventral) aspect. A scale was put over each specimen. A set of 15 landmarks (3 mid-sagittal and 6 per side) were located on each plastron (Figure 2).

2.3. Digitation and shape analysis

The images were transported to TPSUtil[6] software to convert the file for ulterior study. The digitation process was followed utilizing TPSDig2[6] software. A set of 15 discrete anatomical landmark points (3 on mid-sagittal line and 6 paired on the contour), located on intersections of different scute, was taken from the images. All measurements were carried out twice to allow measurement errors to be estimated[7]. To apply the usual GM methods, it is necessary to project shape space, which is curved, non-Euclidean, onto a linear, Euclidean space[8]. Thus, the linear tangent space was used as a location of the consensus configuration on the curved shape space[8]. A Generalized Procrustes analysis (GPA) approach eliminates the scale, and the translational, and rotational differences of the coordinate data of the landmarks among subjects[9]. The coordinate data of each specimen are usually scaled by its centroid size (CS, the square root of the sum of squared distance between each landmark and the plastron centroid,[10]. The CS and GPA-scaled coordinates are the expressions of size and shape, respectively[9]. Centroid size was used as a proxy for size.

2.4. Colour analysis

The RGB values for red, green, and blue colour channels of pixels represent the tissue properties of the plastron. Its analysis was done in two independent replica sessions. A two-way Non parametric multivariate analysis of variance (NPMANOVA) using Gower distances (9,999 permutations) were assessed differences between replicas and sexes.

2.5. Correlation with colour

A multivariate analysis of three colour channels against CS as independent variable was done (all values log-transformed) and a one-way ANCOVA (Analysis of COVAriance) compared the change for each colour according to size.

2.6. Statistical analysis

MorphoJ software (version 1.07a,11) and PAST software (version 2.17c)[12] were used for all statistical analyses. A $p < 0.05$ was regarded as statistically significant. For color analysis, GIMP 2.6 software (version 11) was used.

3. Results

NPMANOVA reflected no statistical differences between replicas ($p = 0.927$) and sexes ($p = 0.068$, Table 1). Red presented the highest values, followed by green. Size values appeared correlated to colors (multiple $R^2 = 0.0819$, Wilk' $\lambda = 0.673$, $F_{3,36} = 5.812$, $p = 0.0024$), with negative and similar correlation coefficients for all three colors (r = -0.237, -0.381, -0.228 for red, green and blue colors, respectively, Figure 3). The color decrease was similar in each color ($F = 0.228$, $p = 0.796$).

Table 1. Analysis of total 20 fresh corpses from *Pseudemys concinna*

Source	Sum of Squares	Degres of freedom	Mean square	F-test	p value
Sex	0.21573	1	0.21573	1.8157	0.0680
Replication	-3.11E-15	1	-3.11E-15	-2.62 E-14	0.9278
Interaction	-1.8987	1	-1.8987	-15.98	0.9995
Residual	4.2773	36	0.1188		
Total	2.5943	39			

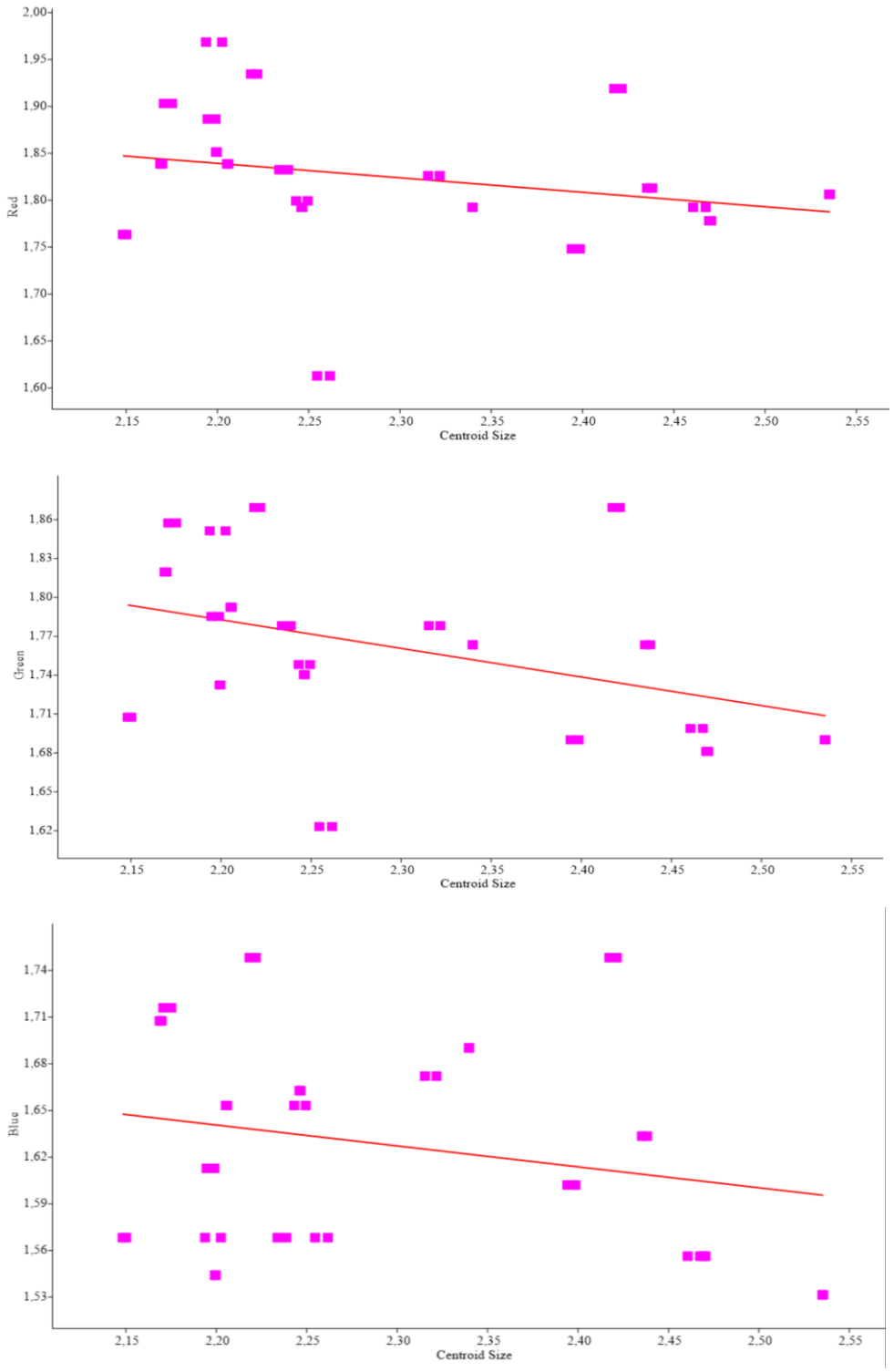

Figure 3. Linear correlations of centroid size and colors for *Pseudemys concinna*

4. Discussion

Animal body coloration is a complex trait resulting from the interplay of multiple mechanisms. There are xanthophores, melanocytes, abundant iridophores, and dermal collagen fibres in *P. concinna,* with differences in the distribution of pigment cell types across body regions[13]. However, over time the number of these pigment-making cells can start to dwindle, and the ones that are left also produce less pigment. In *P. concinna*, a color fading according to size was detected, which can result from age. Although similar findings have been documented for the same species, the employed method is totally novel and easily applicable to similar studies in other species. In the same line, a recent study indicated pigmentation differences between sexes, with females having lighter plastrons than

males. The plastron pigmentation of females decreased with increasing plastron length, whereas males varied by site. In addition, Plastron pigmentation did not correlate with longitude in either sex, indicating no geographical cline in this trait[14]. Another recent study on the relationship between Plastron color and nutrition in Pseudemys nelsoni Carr revealed that Plastron color had no relationship with size or fluctuating asymmetry. In addition, reddish plastron for *P. nelsoni* was highly related to feeding, compared to other external factors, such as age, size, or stress[15]. In wild *P. nelsoni* populations, reddish plastral coloration was related to body size probably due to ontogenetic differences in the diet, as juveniles are omnivorous[15]. The study performed by Steffen et al. [16] aimed to determine if an increase in dietary carotenoids could lead to location-specific changes in painted turtle spots and stripe color. The results indicated that integumentary spot and stripe colors depended on carotenoid access, and increased lutein access led to increased yellow and red chroma, as well as reduced ultra-violet chroma and brightness in male painted turtles[16].

5. Conclusion

The present study opens the door to study if animals in natural habitat present similar plastron pigment production. It can be concluded that colour plastral intensity of *P. concinna* LeConte 1830 tends, effectively, to fade with age. Elucidating the cellular and chemical mediators, and mechanisms, of these slow colour changes will likely require laboratory study.

Declarations

Competing interests

The authors declare no conflicts of interest.

Authors' contribution

Pere M. Parés-Casanova conceived and designed the experiment, analyzed the data, and wrote the first draft of the paper. Albert Martínez-Silvestre contributed to the discussion. Joaquím Soler and Albert Martínez-Silvestre were responsible for corpses collection. All authors read and approved the final version of the manuscript for publishing in the present journal.

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Availability of data and materials

The contents of all supporting data are the sole responsibility of the authors.

Ethical considerations

As there were studied corpses from animals not euthanized for the purpose of this research, it was not required approval from the Ethics Committee.

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