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Plastral pigmentation symmetries in Western Hermann's tortoise (*Testudo hermannihermanni*)

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ABSTRACT

Shape is a fundamental morphological descriptor, one method of its estimation being from digitally processed images. Elliptic Fourier method is an outline-based morphometrics that has some advantages: it does not require either landmarks or previous knowledge about the shape variation of the objects under study; it can visualize the contour of information and reconstruct the original shape; it can be mathematically normalized to size, rotation and starting point of the contour trace; and it can be conducted automatically using computer software. Hence, the variation can be decomposed into several mutually independent quantitative characteristics. In this manner, unacceptable errors based only on human visual judgment of shape, which is frequently deceptive and misled by size factors, can be effectively eliminated. In this study, we applied an elliptic Fourier analysis (EFA) to assess pigmentation pattern symmetries of plastron (ventral shell) of 52 western Hermann's tortoises (Testudohermannihermanni) studied from private breeders, and belonging to two different Mediterranean populations: Albera (Catalonia, NE Iberian Peninsula, n=14) and Balearic Islands (n=38). Low coefficients of regression appeared between carapace length and symmetrical and asymmetrical components. Based on this sample, it was shown that plastral pigmentation design does not present differences for either the symmetrical or the asymmetrical component. These results provide a baseline for further comparisons in variation of plastral pigmentation pattern based on EFA.

Keywords: image analysis, outline fitting functions, plastral pigmentation pattern, *Testudininidae*

INTRODUCTION

Hermann's tortoise (*Testudo hermanni*GMELIN, 1789) is one of five tortoise species traditionally placed in the genus *Testudo*, the others being the marginated tortoise (*T. marginata*), Greek tortoise (*T. graeca*), Russian tortoise (*T. horsfieldii*), and Kleinmann's or Egyptian tortoise (*T. kleinmanni*).

*T. hermanni*is widely distributed in western and southern Europe, from Catalonia to the Balkans, up to European Turkey [1]. Most populations in the western part of the distribution range (e.g. Spain, France and Italy) have been severely reduced, whilst the species is still abundant in eastern areas (i.e. the Balkans). Two subspecies are recognized, *T. hermannihermanni*and *T. hermanniboettgeri*, inhabiting the western (Spain, France, and Italy) and the eastern (all the other countries) parts of the distribution range, respectively [2]. The subspecies *T. h. hermanni* includes the former subspecies *T. h. robertmertensi* and has a number of local forms. It has a highly arched shell with an intensive coloration, with its yellow coloration making a strong contrast to the dark patches. The colours wash out somewhat in older animals, but that intense yellow is often maintained. The underside has two connected

bilateral black bands along the central seam. The coloration of the head ranges from dark green to yellowish, with isolated dark patches. A particular characteristic is the yellow fleck on the cheek found in most specimens, although not in all; *T. h. robertmertensi* is the name of a morph with very prominent cheek spots. Generally, the forelegs have no black pigmentation pattern on their undersides. The base of the claws is often lightly coloured. The tail in males is larger than in females and possesses a spike. Generally, the shell protecting the tail is divided. Pending more definitive data, and with no impact on the subject of our research, we felt justified in employing a conservative species-level taxonomy that retains these two 'classic' subspecies of *Testudo*, all of which are well-recognized, uncontroversial, monophyletic groups.

Varied and efficient methods have been developed to describe and quantify natural objects from images. The most common ones use superimposition techniques (e.g. Procrustes methods), decomposition into harmonics (Fourier series and functions, wavelets), analysis of spiral functions, and combinations of parameters from elementary geometry (e.g. circularity index, lengthening). Since the shape of the plastral pigmentation design is an irregular curved structure, the use of the conventional metrical approach, consisting of distances, angles and ratios, was difficult to apply effectively. This shape is too poorly suited for recognition with landmarks and, therefore, the Procrustes-type methods cannot be used. Nevertheless, its morphologic variation can be quantified from 2D image analysis of their outlines using an alternative procedure, the Elliptical Fourier Function (EFF). EFF, developed by Kuhl and Giardina[3], transforms the data from a spatial domain into a frequency domain. They are defined as a parametric formulation in the form: $x = f(t)$, $y = f(t)$, allowing the separation of the boundary contour into separate x- and y- components.

EFF allows the analysis of contours that cannot be simply represented as single-valued functions. Another attractive property of Fourier Descriptors (FDs) is that they not only allow the precise reconstruction of the biological outline, but also permit the re-creation, at any time, of the form from the EF coefficients, in the absence of the group specimen. Elliptical Fourier Analysis(EFA) has been successfully applied to a number of morphological structures (see [4], for a review).

Research on asymmetries does not deal with extreme deviations in shape, but instead with small, subtle, departures from perfect bilateral symmetry. In this research, we present symmetries of plastral pigmentation (black areas of the ventral shell) from two populations of *T. h. hermanni* in W Mediterranean based on EFA. To the authors' knowledge, nothing similar has been applied to the study of symmetries in tortoises.

MATERIAL AND METHODS

Specimens

In order to analyse plastral pigmentation pattern in western Hermann's tortoise (*T. hermannihermanni)*, 52 domestic pure animals randomly collected from authorized private breeders were selected. Their age, according to declaration of keepers, ranged from 6 to 60 months. Thirty-eight (22 females and 16 males) tortoises were descendants of animals from Balearic Islands and the rest (14, 8 females and 6 males) from an Iberian continental area (Albera, NE Iberian Peninsula), both native populations being geographically separated. No differentiation between Balearic Islands was performed, as for most specimens the exact insular group (Majorca and Minorca) was unknown. Selected animals were sexed and measured (midline straight carapace length, from the front of the gularscute, to the rear of the carapace).

Extraction of the outlines

Ventral image capture for each animal was performed with a Nikon (Tokyo, Japan) D70 digital camera (image resolution of 2,240 x 1,488 pixels) equipped with a Nikon AF Nikkor® 28-80 mm telephoto lens. The focal axis of the camera was parallel to the horizontal plane and centred on the plastron (ventral shell). Images included a scale (10 mm x 20 mm) (Figure 1). It should be stated that this type of photographic materials can be used with good reliability as plastrum is very flat, so one does not expect important image distortions. The photographs were directly input into computer as GIF files and, subsequently, contour was hand-extracted using a graphics software package (Adobe Photoshop version 14.2.1.CC®). Finally, they were transformed to BMP-256 colour files. Second author (Miralles) was responsible for all this "field" part of the research.

Elliptic FDs

Image capture was carried out using the SHAPE® software package (available on lne at http://lbm.ab.a.u-tokyo.ac.jp/~iwata/shape/), which identified the outline of the pattern and generated an elliptical Fourier description. Briefly, the procedure was as follows: images were binarized (i.e. transformed into white for the bone outline and black for the background, in pixels), so the outlines of each continuous contour (interface between the black and the white pixels) were automatically obtained and digitalized. The coefficients of the elliptic Fourier a_n, b_n, c_n and d_n descriptors, which describe each harmonic, were calculated by the discrete Fourier transformation of the chain-coded contour, the position of the first point being on the standardized outline for both right and left plastral pigmentation contours. Since the coefficients are computed separately for each outline, the outlines need not have the same number of points. Shape was approximated by the first 20 harmonics (H). Each harmonic corresponded to the four coefficients defining the ellipse in the *xy*-plane. Coefficients were subsequently normalized for size and aligned by the longest radius [3].

The 80 coefficients were classified into two groups related to symmetrical and asymmetrical variations. The coefficients a_n and d_n represent symmetrical, and b_n and c_n represent asymmetrical variations. That is, the contour reconstructed only by the former coefficients is completely symmetrical, and the latter represent the remaining, i.e. asymmetrical, variation of the contour. The component of symmetrical variation can be understood as the differences among the individual averages over all transformed copies and represents the variation among individuals. The component of asymmetry can be extracted from the differences among the transformed copies of each individual [5]. Thus, from 80 coefficients initially calculated, we classified them into twogroups related to symmetrical variation (40 coefficients) and asymmetrical variation (40 coefficients).

Analysis of plastral pigmentation symmetry and asymmetry

Given the enormous number of normalized coefficients of EFDs obtained from the above technique, it is still impossible to openly interpret these coefficients as shape descriptors and the morphological correlation for each coefficient is difficult to explain discretely. Hence, Principal Components Analysis (PCA), based on a variance and covariance matrix, was carried out in order to summarize the information of the variations stored in the Fourier descriptor coefficients. PCA was carried out on the total sample in the variance-covariance matrices of symmetrical and asymmetrical coefficients of harmonics. The PC explaining variance >1% were subjected to two separate two-way multivariate analyses of variance (Non-Parametric-Multivariate-Analysis-Of-VAriance, NPMANOVA) using Euclidean distance and sex and geographical group as nominal variables ("factors" or "main effects"). As this analysis consisted of two-way ANOVA without replication, the interaction was not tested. Finally, a regression was conducted, using length (log transformed) as independent, and coefficients as dependent variables. In practice, one uses a number of harmonics less than infinity for the summation. Therefore, one tries a range of harmonics and settles on a manageable number that adequately represents the original curve, so additional harmonics do not increase the accuracy of the reconstruction. The statistic treatment was undertaken with the PAST® package [6]. The significance level was established at 5%.

RESULTS

We conducted EFA, including all the materials. Firstly, on the total sample of 52 tortoises, the variance-covariance matrices of symmetrical and asymmetrical coefficients were analyzed by PCA, respectively, and five and four PCs, which explained 95.3% and 97.8% of the total variance observed, respectively, were extracted to provide data reduction (Table 1). For symmetrical and asymmetrical coefficients, the EFA-PCs were subjected to NPMANOVA to test how the differences in shape between geographical groups and sex contributed to the total variability. Comparing the results of these analyses, it was found that the differences in plastral pigmentation shape were not significant for either the symmetrical or the asymmetrical components, either for the geographical groups or for sexes (Tables 2 and 3). Low coefficients of regression appeared between carapace length and symmetrical and asymmetrical components (R^2=0.02 and 0.01, respectively).

DISCUSSION

The outlines employed in the present study turned out to be an example of apparently very complex data that are very well-suited for a particular analysis, in this case bilateral shape. Because EFA identified the longest radius of the best-fitting ellipse to achieve the requisite standardizations, the fact that the plastral pigmentation shape is not circular for the outlines made the use of such standardizations very tenable. Moreover, the variables retained here,

being defined independently of the size, allowed the study independently of a possible allometry – although no regression appeared between length and symmetrical component. Our results indicate that neither the symmetrical nor the asymmetrical components of plastral pigmentation patterns in captive *Testudo hermanni* are the source of shape symmetrical differences, either at individual level or between sexes or geographical groups.

Figure 1. Example of a ventral image capture. Images included a scale (10 mm x 20 mm, bottom).

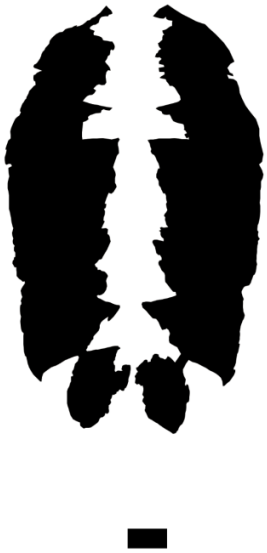

Table 1. Eigenvalues and contributions of the first 20 principal components (PC) of the elliptic Fourier descriptors estimated for the symmetrical (Symm) and asymmetrical (Asymm) data pigmentation pattern symmetries of plastron (ventral shell) of 52 western Hermann's tortoises (*T. hermannihermanni*). The variance-covariance matrices of symmetrical and asymmetrical coefficients explained 95.3% and 97.8% of the total variance observed, respectively, for PC explaining variance >1% (values in bold).

	Symm			Asymm		
PC	Eigenvalue	% variance	% cumulative variance	Eigenvalue	% variance	% cumulative variance
1	5.31E+12	62.806	**62.806**	2.12E+13	83.274	**83.274**
2	2.10E+12	24.860	**87.666**	2.24E+12	8.786	**92.060**
3	3.20E+11	3.780	**91.446**	1.04E+12	4.059	**96.118**
4	2.24E+11	2.644	**94.090**	4.47E+11	1.751	**97.869**
5	1.04E+11	1.227	**95.318**	1.63E+11	0.639	98.508
6	7.39E+10	0.874	96.192	6.63E+10	0.260	98.768
7	4.19E+10	0.496	96.687	5.32E+10	0.209	98.977
8	3.99E+10	0.471	97.158	3.86E+10	0.151	99.128
9	3.57E+10	0.421	97.580	3.73E+10	0.146	99.274
10	3.16E+10	0.374	97.954	2.73E+10	0.107	99.381
11	2.39E+10	0.282	98.236	2.38E+10	0.093	99.475
12	2.01E+10	0.237	98.473	1.76E+10	0.069	99.544
13	1.86E+10	0.220	98.692	1.56E+10	0.061	99.605
14	1.63E+10	0.193	98.885	1.38E+10	0.054	99.659
15	1.45E+10	0.172	99.057	1.14E+10	0.045	99.703
16	1.30E+10	0.154	99.210	1.05E+10	0.041	99.744
17	1.02E+10	0.120	99.331	8.43E+09	0.033	99.777
18	8.62E+09	0.102	99.433	7.96E+09	0.031	99.809
19	6.84E+09	0.081	99.513	6.66E+09	0.026	99.835
20	6.39E+09	0.076	99.589	6.41E+09	0.025	99.860

Table 2. Non-Parametric-Multivariate-Analysis-Of-VAriance, NPMANOVA, for the symmetrical components for pigmentation pattern symmetries of plastron (ventral shell) of 52 western Hermann's tortoises (*T. hermannihermanni*)with 4 Principal Components. The significance level was established at 5%.

Source	Sum of squares	Degree of freedom	Mean square	F	p
Group	5.54E+12	1	5.54E+12	0.470	0.573
Sex	4.14E+12	1	4.14E+12	0.351	0.671
Residual	5.66E+14	48	1.18E+13		
Total	4.12E+14	51			

Table 3. Non-Parametric-Multivariate-Analysis-Of-VAriance, NPMANOVA, for the asymmetrical components for pigmentation pattern symmetries of plastron (ventral shell) of 52 western Hermann's tortoises (*T. hermannihermanni*)with 5 Principal Components. The significance level was established at 5%.

Source	Sum of squares	Degree of freedom	Mean square	F	p
Group	4.69E+12	1	4.69E+12	0.166	0.784
Sex	3.83E+13	1	3.83E+13	1.356	0.182
Residual	1.36E+15	48	2.82E+13		
Total	1.27E+15	51			

Symmetry is a basic property of shapes and structures. Growing organisms are likely to encounter conditions or processes that interfere with developmental precision, resulting in an unexpected asymmetrical appearance, so in biological and physical systems this seems to imply stability and natural development[7]. Accordingly, if symmetrical individuals have greater developmental stability, they will usually exhibit greater reproductive success, and better survival rates than asymmetrical individuals [8]. The term “stress” is a common, but inconsistently defined, term that can be considered to represent processes or stimuli "that are challenging or threatening to the survival, health and reproductive success of animals" [9]. We cannot therefore either accept or reject that developmental mechanisms or environmental conditions can affect symmetry in plastral pigmentation for captive *T. hermanni*. What can be stated with certainty is that plastral pigmentation patterns in this species present a high symmetry level, with similarity between sexes and geographical groups, thus indicating the existence of a tendency towards no bilateral phenodeviant individuals, at least for captive animals of Balearic and Albera origins. The lack of asymmetry between sexes detected in this research is especially interesting, as its potential role as a signal of reproductive quality. A female may also be an individual who can successfully produce more offspring [10]. Møller[11] has documented a number of cases in which fecundity appears to have a negative correlation with individual asymmetry.

In conclusion, the results here are considered encouraging and suggest the need to initiate further research to perform more geometrical studies of symmetrical patterns in *Testudo hermanni*.

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