REPTILES & AMPHIBIANS

Plastral Asymmetries in Young European Pond Turtles, *Emys orbicularis* (Linnaeus 1758)

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During ontogeny, developmental changes can result in asymmetries (deviations of organisms or parts of organisms from bilateral symmetry) (Daloso 2014). Two types of bilateral asymmetry have been described (Cocilovo et al. 2006), fluctuating asymmetry (FA), when differences in the degree of symmetry are minor (Davis and Grosse 2008), and directional asymmetry (DA), when one side of a bilateral trait always develops more than the other (Laia et al. 2015). FA is a sensitive indicator of developmental stress and is a widely used parameter to evaluate instability and plasticity caused by stressful conditions during the development of organisms. Rather than being genetically determined, FA is influenced mostly by environmental characteristics (Costa et al. 2015). Although genetic factors may modulate its expression (Stige et al. 2006; Palmer et al. 2010; Klingenberg 2015), factors that have been implicated in FA include diseases, pollutants, habitat fragmentation, and nutritional stress (Davis and Grosse 2008; Buică and Cogălniceanu 2013; McCall 2014). Phenotypic plasticity of organisms under stress can have great evolutionary importance if stress responses become permanent and fixed by natural selection (Daloso 2014).

Scutes of turtle shells are typically arranged in longitudinal rows with strict bilateral symmetry (Cherepanov 2014). Although some asymmetries have been described (Davis and Grosse 2008; Rivera and Claude 2008; Băncilă et al. 2012; Buică and Cogălniceanu 2013; Cherepanov 2014; McCall 2014; Malashichev 2016), relatively few studies address asymmetries in reptiles (Laia et al. 2015).

The European Pond Turtle (*Emys orbicularis*) (Fig. 1) is widely distributed from northwestern Africa (where it occupies the Mediterranean region that includes island populations) to Lithuania in the north and Iran in the east. In Spain, it has a fragmented distribution. These turtles are increasingly stressed by habitat alterations, industrial and agricultural pollution, marsh drainage, aquifer water extraction, fisheries bycatch, and to a lesser degree by collecting for consumption or pets (Rodríguez-Rodríguez and Escrivà-Colomar 2016). The species is listed as near-threatened (NT) on the IUCN Red List (Tortoise & Freshwater Turtle Specialist Group 1996) and among "animal and plant species of community interest in need of strict protection" by the Council of Europe (1992). Herein we describe for the first time variations in FA and DA patterns in plastra of young European Pond Turtles, providing a framework for future examinations of asymmetry in turtle shells.

Fig. 1. A young European Pond Turtle (*Emys orbicularis*). Photograph by Albert Martínez-Silvestre.

Methods

Using a Nikon D70 digital camera (image resolution of 2,240 x 1,488 pixels) equipped with a Nikon AF Nikkor 28–200 mm telephoto lens, we photographed the plastra of 141 young turtles (carapace length 20.0–76.9 mm) that died of unknown causes in the recuperation center of "Canal Vell" in Ebre Natural Park in southwestern Catalonia, Spain. None showed signs of injury or accessory scutes. The camera was placed on a tripod so the focal axis of the camera was horizontal and centered on the midventral aspect of each animal. We did not determine the sexes of specimens as they were too young. Detailed information on the studied animals can be requested from the senior author.

We identified 19 landmarks (Fig. 2) that were digitized using tpsDig2 software version 1.40 (Rohlf 2015) and saved to a

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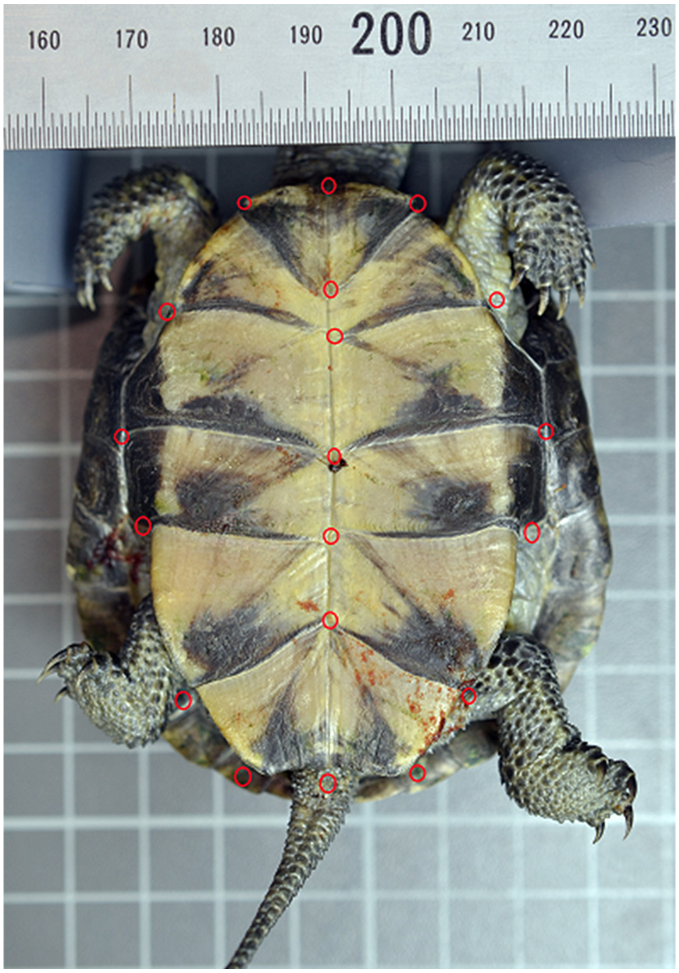

Fig. 2. Ventral view of a young European Pond Turtle (*Emys orbicularis*) with 19 landmarks scored in plastral junctions and used for morphometric analysis. We examined six pairs of plastral scutes in each specimen (gular, humeral, pectoral, abdominal, femoral, and anal) as well as seven medial landmarks.

TPS file. We examined six pairs of plastral scutes (gular, humeral, pectoral, abdominal, femoral, and anal) and seven median landmarks. The selection of landmarks was based on those used in previous studies (Davis and Grosse 2008; Băncilă et al. 2012; Barros et al. 2012). We determined the straight-line length of the carapace using digital measurements of each plastron and the accompanying scale (Regis and Meik 2017). Each plastron was digitized twice to determine if the technique produced repeatable estimates. Measurement error was estimated from a Procrustes ANOVA by considering individual as the main source of variation and residuals representing variation in digitized replicates as the second source of error (Cocilovo et al. 2006).

We superimposed landmark configurations using a Generalized Procrustes Analysis, which removes the spatial variation that does not correspond to form. This analysis also provides information on size and shape through centroid size (CS) and Procrustes distance (PD) estimates. CS was calculated as the square root of the sum of squared distances of each landmark from the centroid of the landmark configuration (Mitteroecker and Gunz 2009). For allometry, shape (Procrustes coordinates) was plotted against CS.

We performed a Procrustes analysis of variance (ANOVA) to study the asymmetric component of shape. The "individual*side" variation stood for FA and the "side" variation for DA (Cocilovo et al. 2006). Finally, we conducted a Principal Component Analysis (PCA) from regression residuals.

For all statistical analyses, we used MorphoJ software version 1.07a (Klingenberg 2011) with a = 0.05.

Results

Variation attributable to digitizing error represented only 2.4% of total variance. Procrustes ANOVA indicated that both DA and FA were significant ($P < 0.01$; Table 1), with DA accounting for 37.9% of variation and FA for 5.6%. The regression of asymmetric components against log-transformed CS revealed that asymmetry increased significantly during ontogeny ($P = 0.0020$) and that size explained only 1.42% of the asymmetric shape changes for asymmetric components. The PCA indicated that PC1 and PC2 accounted for 22.04% and 15.91% of the variation (PC1 + PC2 = 37.95%), respectively. Along the first axis, changes involved mainly the more anterior scutes (gular, humeral, pectoral), whereas along the second axis, changes were mainly on abdominal scutes. Collectively, changes were right-biased.

Table 1. Procrustes analysis of variance of plastral shape asymmetry. DA = directional asymmetry; FA = fluctuating asymmetry. DA accounted for substantially more variation than FA (37.9 versus 5.6%, respectively). Variation attributable to digitizing error represented only 2.4% of total variance. Mean squares (MS) indicate the amount of variation one level higher in the hierarchy. The F value represents the comparison of each MS to one level lower of MS, which could be a source of error. d.f. = degrees of freedom.

Effect	Sum of Squares	Mean Squares	d.f.	F	P
Individual	0.5549702	0.000233180	2380	9.60	<0.0001
Side (DA)	0.0027822	0.000163661	17	6.74	<0.0001
Individual*Side (FA)	0.0578137	0.000024291	2380	2.31	<0.0001
Error	0.0503445	0.000010501	4794		

Discussion

We used landmark-based geometric morphometric analysis to detect significant fluctuating and directional plastral asymmetry in young Mediterranean Pond Turtles, with DA accounting for substantially more variation than FA. Low FA might indicate that environmental stress factors affecting the Ebre National Park population are still negligible. One possible explanation for high DA is that this might be a product of lateralization of locomotor function (McCall 2014).

Size accounted for only a low percentage of asymmetric shape changes, although regression was significant. This confirmed a slight detectable asymmetric change during growth and the presence of asymmetry from early stages of development. If paired plastral scutes grow by forming new tissue partly along the central suture line, the growth of one can impinge on the other; so, in older turtles, the central plastral suture line tends to deviate (to the right in our sample) from a straight line (Rivera et al. 2006; Davis and Grosse 2008). However, such a deviation must not alter the elliptical shape of the plastron in order to not affect its hydrodynamics (Claude et al. 2003). Otherwise, turtles with a deformed plastron are likely to swim poorly and would be quickly eliminated by natural selection.

As studies of DA in reptiles are scarce, more research is needed, with future studies, for example, focusing on a possible relationship of DA and sexual dimorphism in order to reveal if asymmetries occur equally in both sexes. An unexpected conclusion is that in biometrical studies of turtle shells, bilateral characters should not be examined on only one side.

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