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Sexual dimorphism in the claws of the European pond turtle (*Emys orbicularis*): potential implications for the reproductive fitness of the species

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There are many biometric differences between the males and females of the European pond turtle (*Emys orbicularis*) as regards their size, colouring, the shape of the plastron, tail, etc. The males use their claws to grasp the female during copulation and hence sexual selection should favour the males that have larger claws, which allow them to grasp the female better. Here, we address this type of sexual dimorphism in a comparative analysis of indices obtained from claw length, the length of the carapace and the locality where individuals were sampled. The results show that the curvature of the claws differs between the two sexes, being longer in males and increasing with age, size, and hence, the state of sexual maturity, than in females. Greater claw length could confer advantages for males when grasping the carapace of females, and hence, improve their reproductive fitness. Importance in the reproductive success that might have this feature in males could originate future studies that will relate the shape, thickness, length and other measures of the claws in males with their reproductive success in different populations, genetic variety, and most importantly, viability of populations.

Key words: claw; dimorphism; *Emys orbicularis*; Spain; mate selection; terrapin.

Darwin (1859) developed the concept of sexual selection in "The Origin of Species", indicating its importance for natural selection and explaining the differences between the sexes of a given species. In sexual selection, characters that favour certain individuals during copulation, aimed at achieving better reproductive fitness, are selected positively. In most species of animals, sexual differences between the sexes lead females to produce the maximum number of offspring, while males optimize copulation (Dussenbury, 2000).

In chelonians, Bonnet *et al.* (2010) reported that the greater size of the females and the difference in carapace characteristics between the sexes are due to mate selection in males, and fecundity in females. However, in most species this selection allows them to be differentiated visually.

When reaching sexual maturity, the European pond turtle (*Emys orbicularis*)

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exhibits several traits that differ between males and females. On average, females are larger than males (CHEYLAN, 1992; GARIBOLDI & ZUFFI, 1994; MAZZOTTI, 1995; ZUFFI & GARIBOLDI, 1995a; ZUFFI *et al.*, 1999; ALARCOS *et al.*, 2008; D´ANGELO *et al.*, 2008; FATTIZZO, 2008; SANCHO & RAMIA, 2008), possibly so that they can produce greater clutch sizes (ZUFFI *et al.*, 1999), and develop a higher annual reproductive frequency (FORSMAN & SHINE, 1995). By contrast, the smaller mean size of the males would be due to the need to maximize encounters with females, and fecundate as many of them as possible (BONNET *et al.*, 2010). Similar arguments were invoked by VALDEÓN (2007) to account for this sexual dimorphism in *Emys orbicularis'* populations in the north of Spain.

Other secondary sexual characteristics are the marked dark pigmentation in males (FATTIZZO, 2008), the different colour of the iris (FATTIZZO, 2008; AYRES & CORDERO, 2001), their shorter forelegs (FATTIZZO, 2008; RAMOS *et al.*, 2009), and different biometric indices and measurements of the carapace (ZUFFI & GARIBOLDI, 1995b; D´ANGELO *et al.*, 2008; FATTIZZO, 2008). Despite this, there are several practical ways of sexing individuals, related to copulation, and reproductive fitness: the concavity of the plastron of males (ANDREU & LÓPEZ-JURADO, 1998), which facilitates coupling with the convex back of females, and the longer tail length and wider tail base in males (FATTIZZO, 2008). The more external emplacement of the cloaca in males (CHEYLAN, 1992; RAMOS *et al.*, 2009) would allow the tail to move more freely and to join the cloacae in both sexes.

Some authors have reported that the forelegs of males are slightly shorter than those of females (FATTIZZO, 2008; RAMOS *et al.*, 2009) in order to allow a stronger grasp and better balance for the males when mounting the females. In several species of terrestrial tortoises, there is also sexual dimorphism in claw curvature. In the box turtle (*Terrapene carolina*) the claws of the hind legs are longer and more curved than in the females, and they are used by the males to hook themselves over the carapace of the female to facilitate copulation (EVANS, 1953). In the sea turtle *Caretta caretta*, it has been observed that the males have longer and more curved claws on their forelegs than the females (WIBBELS *et al.*, 1991). These examples in different groups of chelonians, both aquatic and terrestrial, possibly suggest that curved claws have been selected in males to improve their grasping power during copulation. In the sea turtle *Chelonia midas*, LIMPUS (1993) described the importance of secondary sexual characters for correct positioning during copulation, which is of importance since there are usually satellite males waiting nearby. In this sense, JESSOP *et al.* (1999) reported that any carelessness or decline in androgens in males during copulation (grasping), due to aggression by satellite males, could result in the copulating male becoming detached from the female. In *E. orbicularis*, the species studied here, it is common to see, in the field, these attempts by satellite males to displace copulating males (personal observation). In *E. orbicularis*, the curved shape of the claw could also enhance the capacity of males for remaining on the back of females for longer time during copulation, bearing in mind that this may last several days (ROVERO *et al.,* 1999). The males that last longest on the female, and have the best grasp (large curved claws) will have better reproductive fitness, and the possibility of transmitting this trait to the offspring. It is important to note that reproductive fitness in females is correlated positively to the height of the carapace (ZUFFI *et al.,* 1999). Hence, it would be reasonable to think that traits in males leading to longer and stable copulas with bulged-carapace females will have larger offspring. Nail curvature, as such reproductive trait, might thus enhance fitness in males.

The aim of the present work is to show that the greater curvature of the claws in males could be a dimorphic sexual character, possibly related to reproductive fitness in view of their direct relationship with attachment, and the time spent grasping the female during copulation.

MATERIALS AND METHODS

The biometric measurements were taken on individuals from four populations from southwest Zamora (Almeida: QF47; Fornillos: QF28; Piñuel: TL58), and Northwest Salamanca (Trabanca: QK16) provinces on the central-western part of Spain (Region of Castile & Leon), where the species still has abundant populations (ALARCOS *et al.,* 2013).

We selected populations living in streams forming pools that tend to persist throughout the year. In all these localities, the surroundings are Mediterranean pastures and Dehesas (*Quercus ilex* and *Fraxinus excelsior*) with extensive livestock.

We measured the straight carapace length (Lc) of all individuals to determine the possible existence of relationship between Lc and the shape of the claws. Also, we divided the individuals into adults (Ad), males (M), females (F) and juveniles (J) (Fig. 1), in order to observe possible differences between different sexual developments, and sex with respect to the type of claws.

Only individuals whose sex was correctly defined were included in the statistical model (Sex), bearing in mind the secondary sexual characters usually employed in the literature, and in other samplings of the same population (ALARCOS *et al.,* 2008); males have a more curved plastron, have longer and thicker tails at the basis, are very often darker or with a blackish head colouration, and have a wider carapace at the hind legs (ANDREU, 1982; AYRES & CORDERO, 2001 *in* AYRES, 2014). Thus, we considered juveniles those individuals with undefined CSS (Sexual Secondary Character) and Lc smaller than 80 mm. Subadults (Sb) were defined as individuals with Lc ranging from 80 mm -135 mm and prominent CSS. Those individuals with Lc within this range but CSS not well defined, were considered as females. In the end, all used adult individuals had a Lc larger than 135 mm, and prominent CSS.

To work and compare the measurements of the claws, we have developed a Claw Index based on the length of the first (UG), and third (UM) claws of the forelegs. The biometric measurements involved the curved length of the claw along its external perimeter, from the base to the tip, using a flexible metric tape (UGM for the first claw, and UMM for the third

Figure 1: Claws of adult *Emys orbicularis*. (a) Male. (b) Female.

claw). We also measured the straight length from the claw base to the tip, this time using a digital calliper (UGC for the first claw, and UMC for the third claw).

Curvature was calculated as a Claw Index (CLI = [(L-l) / (L+l)]*100), that employed the longest (L; in this case UGM and UMM), and shortest (l; in this case UGC and UMC) length of the claw. This index reflects how much longer the curve is compared to the straight-line measurement, as a percentage. Accordingly, the higher the value of this index, the greater the curvature of the claw. CL1 corresponds to the index for the first claw and CL2 for the third claw.

All the techniques used in this research were subjected to ethic parameters, and none resulted in injuries or death of the animals.

Statistical analyses

We fitted Generalized Linear Models (GLM) to claw curvature for both the first and the third digits of the turtles, to test the hypothesis that the claws in males are more curved than in females. We included Sex and Lc, as well as the sampling location (Loc) as predictors of claw curvature. We assumed a gamma distribution of error, and a log link function to deal with the lack of normality and homogeneity of variance in the residuals. The model thus, takes the form:

$$\mathrm{CLI} = \exp\left(\sum \beta + \alpha i * Xi\right)$$

where CLI is the claw curvature index, β is the intercept parameter in the fixed effects term, Xi is the set of predictor variables, and αi is the set of associated parameters in the linear term.

The full model included Sex and Lc as well as the respective pair-wise interaction plus the factor 'locality'. We included this factor in the fixed term since only three categories is too low to be included as a random term in a mixed model (Zuur *et al.*, 2009). The full model included all the potential combinations of Sex and Lc, as well as the respective pair-wise interactions plus the qualitative variable of locality acting as intercepts. We conducted a backward selection of predictor variables, removing each one at a time. To test for the effects of each associated parameter, we analyzed the delta Akaike Information Criterion corrected for small samples (AICc). When the elimination of any parameter determines an increment of AICc higher than 4 units (Burnham & Anderson, 2004), this parameter will be supported in the model. If the opposite is true, then the associated term will be removed from the supported model.

We calculated a pseudo-R^2 based on the residual deviance of the supported model, and the deviance of a null model, which included only an intercept parameter. The equation takes the form: $R^2 = [(Dev.null - Dev.res)/Dev.null]*100$, where Dev.null is the residual deviance of the null model and Dev.res is the residual deviance of the fitted model. All analyses were conducted using the R v3.02 software (R DEVELOPMENT CORE TEAM, 2008).

RESULTS

The mean biometric measurements for 197 individuals were recorded; 144 were adults, 74 were females and 70 were males; 51 were subadults, 20 were females and 31 were males. And two were juveniles not sexed so not included in the analysis (Tables S1, S2).

The value of the Akaike Information Criterion (AIC) for the CLI index supported the choice of two models that included the Sex and Lc variables, their interaction, and - in one of the models - the "locality" factor (Loc) (Table 1). In the case of the curvature of the third claw (CLI2), the best model was the one that included both predictors plus the interaction between them. For CLI2, the choice only supported the model that included Sex and Lc, together with the interaction between them. In this case, the locality was not found to have a significant effect on the curvature of the claws. The explanatory power of both models was relatively high, greater than 40% (CLI1) and 50% (CLI2) respectively (see R^2 in Table 2).

Since the inclusion of locality in the model for NI1 was not relevant for the AICc, henceforth we shall only describe the results for the models that included Sex and Lc and the Sex*Lc interaction. As a categorical variable, the inclusion of "Loc" as a factor would essentially involve similar curvature patterns with respect to Sex and Lc, but with slightly higher values in some of the localities in particular.

The models selected suggest that claw curvature in males and females is different and changes with size (Tables 2, S2). Anyway, claw curvature in males tends to be always higher than in females. Claw curvature in males increased with the size of the individuals (towards aged males), while it decreased in females (Fig. 2). Therefore, similar curvature values observed between sexes in the early ontogenetic stages disappeared with increasing size of the specimens and a greater age.

DISCUSSION

The analyses carried out here show that the claws of males are more curved than those of females. Similar findings have been reported for other chelonians such as *Chrysemys picta* (ERNST *et al.*, 1994). In this species, the males have very long straight claws, and these are used during courtship to smack the snout and the neck of females, and not so much for grasping during copula (ERNST *et al.*, 1994).

Other chelonians also display similar courtship behaviour; in several genera, such as *Chrysemys, Graptemys, Malaclemys, Pseudemys and Trachemys*, the males also vibrate their claws in different ways in front of the female (FRITZ, 1999). This has been explained more in depth in several other turtle species (ERNST & LOVICH, 2009; LAHANAS, 1982; GIBBONS & LOVICH, 1990; SELMAN, 2012).

In this sense, JESSOP *et al.* (1999) reported that any carelessness or decline in

Table 1: Estimation of parameters for the models selected. Shape - dispersion parameter of the gamma distribution (a) model for CLI1, and (b) model for CLI2 . Both CLI1 and CLI2 = $\exp^{(\text{intercept+SxM+Lc+SxM*Lc+Shape})}$. SxM stands for male invividuals.

(a) Param.	**Estimate**	**Std.Error**
(Intercept)	1.26	0.36
SxM	-0.39	0.52
Lc	-0.007	0.002
SxM:Lc	0.008	0.003
Shape	0.22	
(b) Param.	**Estimate**	**Std.Error**
(Intercept)	2.654008	0.329803
SxM	-1.405198	0.475656
Lc	-0.010065	0.00234
SxM:Lc	0.017706	0.003443
Shape	0.1792668	

androgens in males during copulation (grasping) due to aggression by satellite males, could result in the copulating male becoming detached from the female. With respect to the species examined here, in *E. orbicularis* it is common to see these attempts by satellite males to displace copulating males in the field. In this species, the curved shape of the claw could also be linked to the possibility of the male to remain longer on the back of the female during copulation, bearing in mind that this may last several days (ROVERO *et al.*, 1999). The males that last longer on the female, and have the best grasp (large curved claws), will have better reproductive fitness and the possibility of transmitting this trait to the offspring.

Likewise, the individuals who remain longer on top of the female will, to a large extent, prevent multipaternity, which has been seen in different chelonians for the same clutch (ROQUES *et al.*, 2006), since a female may copulate with more than one male during a given breeding season (ROVERO *et al.*, 1999). For females, multipaternity is advantageous, since it allows greater genetic variability for the offspring (ROQUES *et al.*, 2006), but not for the male because it reduces the number of direct descendants. Spending more time on the female's back eliminates, or at least reduces, the possibility of access by other males, which could account for the low rates of multipaternity in *E. orbicularis* (ROQUES *et al.*, 2006).

In the present study, differences in claw curvature between males and females appear early at the subadult stage. Therefore, this trait could be useful for sexing individuals, thus complementing other commonly used characters (see AYRES, 2014). However, it would be necessary to test this pattern in other locations. Specifically, it is uncertain how ubiquitous is this pattern across all the geographical distribution range of *E. orbicularis*. Sexual dimorphic characters have been shown to change across the species' distribution ranges (IVERSON, 1995; GIBBONS & LOVICH,

Figure 2: Simulation of the claw curvature values for both indices (CLI1 and CLI2) according to the models chosen. SxF – females and SxM – males; Lc – carapace length.

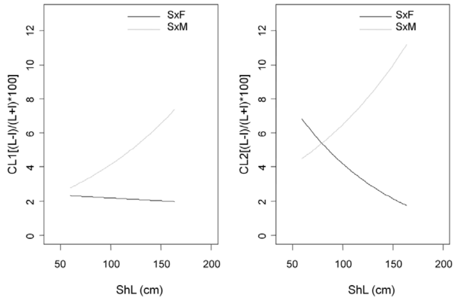

Table 2: Selection of models for (a) CLI1, and (b) CLI2 using the corrected Akaike Information Criterion (AICc) for small samples. Dev.res - residual deviation of the fitted model; Dev.fit - deviation explained by the fitted model; R^2 - coefficient of determination based on the residual deviation of the fitted model, and the deviation of the null model.

(a) Term (CLI1)	**Parameters**	**AICc**	**ΔAICc**	**Dev.res**	**Dev.fit**	**R^2**
Loc+Sex*Lc	**8**	**846.3**	**0**	**43.981**	**33.705**	**43.38**
Sex*Lc	**4**	**847.4**	**1.1**	**46.146**	**31.54**	**40.59**
Loc+Sex+Lc	7	848.9	2.6			
Loc+Sex	4	849.9	3.6			
Sex	2	850.2	3.9			
Sex+Lc	3	850.5	4.2			
Lc	2	950.8	104.5			
Loc	4	913.8	67.5			
Loc+Lc	4	914.8	68.5			
Null	1	949	102.7	77.686		
(b) Term (CLI2)	**Parameters**	**AICc**	**ΔAICc**	**Dev.res**	**Dev.fit**	**R^2**
Sex*Lc	**4**	**927.4**	**0**	**40.3**	**53.5**	**57.0**
Loc+Sex*Lc	8	932.2	4.8			
Sex+Lc	3	948.1	20.7			
Loc+Sex+Lc	7	948.7	21.4			
Loc+Sex	4	947.8	20.4			
Loc+Lc	4	1048.4	120.9			
Sex	2	948.4	21.0			
Loc	4	1046.3	118.9			
Null	1	1096.2	168.8	93.8		
Lc	2	1097.5	170.1			

1990; LOVICH *et al.*, 2010; SELMAN, 2012). Interestingly, this trait might be accounted as a potential predictor of differences in multipaternity among populations. For instance, ROQUES *et al.* (2006) reported low percentage of multipaternity in south-west Spain, as compared to observations in Italian populations (ROVERO *et al.*, 1999). In this sense, and for future work, it would be interesting to consider the curvature of the nail as a variable for rating or discarding the possible role of multipaternity differences observed among populations.

So, and finally, the results of this study suggest the possibility of future investigations such as checking the reproductive fitness of larger males and/or males with more curved claws in the field. Other traits, such as claw diameter, hardness, etc could be used to determine the state of health of an individual and/or population, especially in central and southern Spain, where climate change is affecting aquatic habitats and reptiles alarmingly (ARAÚJO *et al.*, 2006; ARAÚJO *et al.*, 2011).

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