Similarities and differences in adult tortoises: a morphological approach and its implication for reproduction and mobility between species

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Abstract. Sexes in Chelonia display marked differences. Sexual size dimorphism (SSD) is important in evolutionary biology. Different sexual strategies result in species specific selection. Biometric variation in male and female tortoises of two species is studied. Eighteen biometrics were measured in 75 museum specimens (20 *Testudo graeca*; 55 *T. hermanni*). Nine of 18 parameters in *T. hermanni* and two of 18 in *T. graeca* were sexually dimorphic. Multivariate analyses (principal component analysis) highlighted two components, with bridge length the first and anal divergence the second component. The bridge length can be used to separate sexes and species. Males of both species were most different, whereas females of two species overlapped in body shape measurements. We hypothesise that female similarity could be a by-product of reproductive biology and sexual selection that optimise individual fitness.

Keywords.   *Testudo hermanni*, *Testudo graeca*, biometry, morphological model, reproductive strategies.

INTRODUCTION

The evolution of the complex structures that define extinct and living chelonian species was studied by Lee (1996). A progression of anatomical patterns appeared in the evolution of Chelonia. Physical structures range from complete ossification with a broadly developed carapace (i.e. *Testudo*, *Geochelone*) to reduction in bony shell (i.e. *Chelydra*, *Apalone*), or even to a poorly ossified carapace (i.e. *Trionyx*; Ernst et al., 1994).

Sexual size dimorphism (SSD) (Darwin, 1874) is a very often debated aspect in reptilian species, particularly in recent years (Berry and Shine, 1980; Bonnet et al., 2001; Olsson et al., 2002; Rubolini et al., 2006; Zuffi et al., 2006). Sexes in Chelonia often display marked morphological differences, with the largest dimensions often occurring in females (Berry and Shine, 1980; Ernst and Barbour, 1989; Ernst et al., 1994). Several hypotheses

have been advanced to explain the patterns of carapacial, scute or colour variation in turtle sexes. Habitat, diet, and habitus provide important correlations with carapace, head and skull shape (e.g. Claude et al., 2003, 2004; Zuffi et al., 2007), while different degrees of sexual dimorphism were recently found in the European species of the *Testudo* (i.e. *T. graeca*, *T. hermanni* and *T. marginata*) by Willemsen and Hailey (2003).

Despite the wide interest in the evolution, selection and adaptive forces that mold carapacial morphology of chelonian species, few papers have dealt with morphology and function (Bonnet et al., 2001; Claude et al., 2003, 2004; Willemsen and Hailey, 2003; Zuffi et al., 2007). Broad patterns have become clearer, but more detailed comparisons at the specific level are needed.

As data set for a preliminary comparison to examine details of interspecific sexual dimorphism in tortoises, we selected two similar, Palaearctic species of *Testudo* (but see Lapparent de Broin et al., 2006 and their proposal to change genus into *Eurotestudo*), *T. graeca* and *T. hermanni*. In both these species, there are pronounced sexual differences in body size dimensions, and their courtship patterns have been described on average (Buskirk et al., 2001; Cheylan, 2001), but no real inspection of relationships between morphology and function has been yet performed (but see Bonnet et al., 2001 for the *Testudo horsfieldii*).

We present a brief study on the sexual dimorphism in *T. hermanni* and *T. graeca*, and we examine SSD within a species and between species. Our main idea is to test if morphological differences between sexes and between species may be helpful in explaining different evolutionary and reproductive strategies for each species (i.e. reproductive systems, movement). We wish to emphasize that this is a preliminary study and that further analyses might need to be conducted when possible.

MATERIALS AND METHODS

We examined 71 museum specimens (9 males and 9 females of *T. graeca*; 26 males and 27 females of *T. hermanni*) in the Museum of Zoology "La Specola" of the University of Florence, Italy and at the Civic Museum of Natural Science "Doria" of Genoa, Italy. We made 18 measurements of shell (Soldani, 2000), of the limbs and of the head and tail. Measurements were made with a calliper (accuracy ± 0.1 mm) or with a tape measure (all the measurements in millimetres). Weights were taken with an analogic dynamometre (accuracy: ± 25 g).

For each we record: taxon, sex, age class (adult or juvenile, according to external morphology of plastron and tail length, Cheylan, 2001), season, year, locality and region of capture and/or death, and preservation matter (i.e. in liquid, mounted), matter of collecting (alive; dead). Animals born and dead in captivity were not considered. Abbreviations for morphometrical parameters are: *carlen* (carapace length from nucal to sopracaudal scute), *carwid* (carapace width between 6th and 7th marginal scute), *plalengmin* (minimum length between jugal and anal scute), *plalengmax* (maximum length between giugal scute and an estreme margin of the anal scute), *plawid* (piastron width between abdominal and pectoral scutes), *brilen* (bridge length), *carhei* (carapace height between plastron base and the 3rd vertebral plate), *anadiv* (length of anal divergence), *latcirc* (latitudinal circumference), *loncirc* (longitudinal circumference), *fore* (forelimb length), *hind* (hindleg length), *bmass* (body mass in grams), *healen* (head length), *heawid* (head width), *heahei* (head height), *tailen* (total tail length), *cloaca* (distance between cloaca and tip of the tail). We excluded one juvenile that we could not determine its sex (Cheylan, 2001).

Statistic analyses were performed with SPSS 13.0 statistical package for Windows. All parameters were tested for normality. Parameters that were not normally distributed were log transformed prior to analyses.

For each of selected morphological variables, we used an analysis of covariance (ANCOVA) to examine dependent variables for the influence of sex, species and their interactions, using the log transformed carapace length as covariate. We, thus, tested the differences between sexes for each species, the differences between males of the two species, and finally between the females of the two species.

As all selected variables were highly intercorrelated, we followed up this univariate analysis with a principal component analysis (PCA) in order to identify the categorical variables responsible for the variation we measured (see Fowler and Cohen, 1993).

RESULTS

Nine parameters out of 18 in *T. hermanni* and two out of 18 in *T. graeca* were sexually dimorphic (see Table 1 for details). In particular, males had relatively shorter bridges at any particular carapace length than did females (Fig. 1A, B). Comparisons of sexes by species were significant (*T. hermanni*: ANOVA, $F_1 = 194.653$, $P < 0.0001$, ANCOVA interaction sex × lncarapace length: $F_{2,49} = 281.87$, $P < 0.0001$, $R_{adj} = 0.917$; and *T. graeca*: ANOVA, $F_1 = 74.751$, $P < 0.0001$; ANCOVA, $F = 43.802$, $P < 0.0001$, interaction sex × lncarapace length $F_{2,15} = 46.538$, $P < 0.001$, $R_{adj} = 0.843$). Both species had a marked sexual dimorphism.

Two parameters were identified by PCA as the most important morphometric features of carapace: the bridge length (first component) and the anal divergence (second component, Table 2). While females widely overlapped, males were markedly different in morphological space (Fig. 2).

DISCUSSION

Sexual size dimorphism is very evident in *T. hermanni* (Fig. 2a), but less evident for our much smaller sample of *T. graeca* (Fig. 2b). Nonetheless, males attain smaller body sizes than do females. Furthermore, we found that the bridge length, a parameter not previously recognized as a sexually dimorphic one (Bonnet et al., 2001; Claude et al., 2003, 2004; Willemsen and Hailey, 2003), plays an important role in separating sexes and species. Bridge length should be carefully considered in future comparative studies.

Males of the Hermann's tortoises have a more marked anal divergence and smaller carapace and plastron than do females. In males of the Moorish tortoise we observed a marked anal divergence as well, but a larger carapace than females.

It is interesting to underline how the bridge length appears to work in defining both sexual and interspecific differences. The bridge length is inversely proportional to the degree of mobility (Soldani, 2000). Mobile species (i.e. aquatic turtles such as *Emys*, *Trachemys*, *Lyssemys*, *Pelomedusa* and others) have smaller bridges when compared with terrestrial taxa (Soldani, 2000; Zuffi, unpubl.). Besides, within a particular taxon (i.e. genus

Table 1. Mean values (± standard deviation) of the 18 morphological variables measured in both sexes of the *Testudo hermanni* and *T. graeca* (ANCOVA, see methods for details) (in bold significant values).

Variable	Testudo hermanni					Testudo graeca				
	males	females	df	F	P	males	females	df	F	P
carlen	129.5±21.3	145.9±34.7	1,51	4.28	**0.043**	155.09±24.22	142.67±23.89	1,16	1.2	0.29
carwid	100.6±14.8	109.9±24.3	1,51	2.782	0.101	113.67±19.72	104.67±17.02	1,16	1.074	0.315
plalengmin	89.81±14.91	119.27±22.77	1,50	14.442	**<0.0001**	129.76±23.74	119.56±17.46	1,14	0.904	0.358
plalengmax	110.18±17.52	129.99±26.91	1,50	9.720	**0.001**	141.98±25.46	135.51±27.81	1,14	0.234	0.636
plawid	90.68±13.28	97.47±16.65	1,51	2.613	0.112	94.72±15.08	90.19±13.79	1,16	0.443	0.515
brilen	49.52±6.30	64.04±11.93	1,50	29.415	**<0.0001**	67.99±11.81	65.94±8.89	1,16	0.172	0.684
carhei	67.62±10.33	76.51±14.21	1,50	6.631	**0.013**	78.32±11.65	75.63±7.65	1,15	0.309	0.586
anadiv	39.29±9.16	29.42±7.00	1,50	19.238	**<0.0001**	35.44±8.52	25.78±4.54	1,14	7.303	**0.017**
latcirc	333.04±53.28	366.44±70.36	1,50	3.68	0.061	389.83±61.19	347.51±48.84	1,14	2.23	0.158
loncirc	275.12±41.39	300.41±56.00	1,50	3.384	0.072	313.00±49.95	284.57±32.58	1,14	1.692	0.214
fore	42.12±7.74	44.15±9.87	1,28	0.391	0.537	46.07±8.24	43.04±6.52	1,14	0.632	0.44
hind	44.31±8.41	42.11±12.90	1,20	0.235	0.633	45.11±6.82	43.01±11.24	1,14	0.215	0.65
bmass	468.91±248.25	704.80±440.71	1,46	5.096	**0.029**	679.44±559.51	312.86±157.16	1,14	2.793	0.117
healen	28.11±6.57	28.77±5.86	1,36	0.108	0.744	28.73±2.71	26.44±4.47	1,12	1.34	0.27
heawid	19.35±3.02	20.47±2.58	1,36	1.467	0.234	19.47±1.5	17.69±1.71	1,12	4.337	0.059
heahei	18.63±3.56	19.90±2.89	1,35	1.385	0.247	16.81±2.83	14.66±2.64	1,12	2.184	0.165
tailen	49.55±11.38	31.91±10.11	1,47	33.021	**<0.0001**	40.20±11.26	24.57±4.04	1,9	6.904	**0.027**
cloaca	25.61±6.77	14.10±5.65	1,34	30.867	**<0.0001**	---	---	---	---	---

(a)

(b)
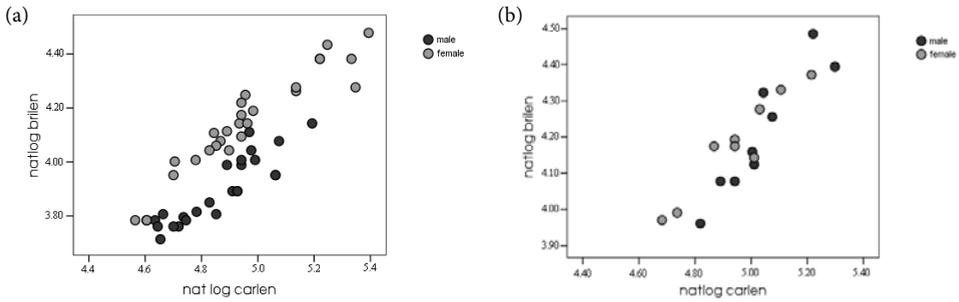
Fig. 1. Morphometric differences in carapace length versus bridge length (log transformed variables) in males and females: (a) in *T. hermanni*, (b) in *T. graeca*.

Table 2. Principal Component analysis in *Testudo hermanni* and *T. graeca* (in bold the most informative variables for each component).

Variable	PC1	PC2
brilen	**0.982**	$6.719*10^{-2}$
plalengmin	0.967	0.215
plalengmax	0.936	0.281
carhei	0.876	0.384
latcirc	0.867	0.485
loncirc	0.861	0.495
carlen	0.856	0.467
carwid	0.763	0.547
plawid	0.746	0.581
anadiv	0.162	**0.973**

or family), a bigger and longer bridge is typical of females. Females have less space for their limbs than do males (Lebboroni and Chelazzi, 1991; Andreu et al., 2000; Corti and Zuffi, 2003).

In terrestrial tortoises, males must be able to quickly right themselves during male-male interactions for access to the females (Bonnet et al., 2001). The righting response is is inversely related to bridge length and positively correlated with space for the legs (Soldani, 2000; Corti and Zuffi, 2003; Zuffi, unpubl.). However, a short bridge may also be less effective in protecting males from predation. Females apparently sacrifice mobility for protection, thus their relatively long bridges.

However, we also found interspecific variation in these sexually dimorphic traits. Such differences may be related to differences in species specific courtship behaviours. For instance, male *T. hermanni* chases the female, bites her hind legs and back of the carapace. He mounts directly on the female carapace, performing such an action for long time before copulation. In contrast, male *T. graeca* usually courts females with strong
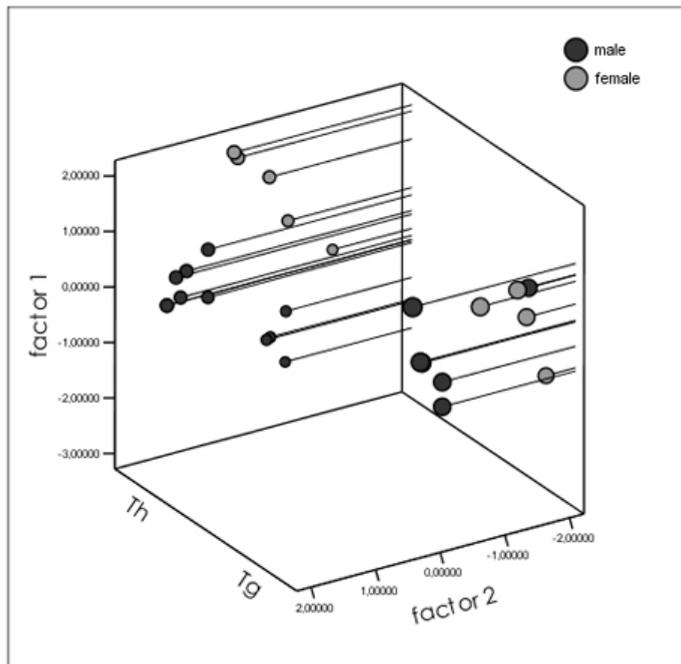
Fig. 2. Principal component analysis of carapacial and sexual differences in *T. hermanni* and *T. graeca*. Small circles, *T. hermanni*; large circles, *T. graeca*; black circles, males; grey circles, females.

blows from the gular shields on the posterior side of female carapace and with a pre-mounting behaviour before actual copulation. After a typical general scheme, similar to the *T. graeca* one, the *T. hermanni* male climbes on the female's carapace and gives her repeated blows with the great spur of his tail tip. This happens whether the female is stationary or moving. We hypothesize that *T. hermanni* males need to be more mobile than *T. graeca* males.

Our study is a preliminary one and only experimental studies will serve to elucidate the interaction of behavioural and morphological patterns. Nonetheless, our finding that sexual dimorphic characters vary interspecifically is important. Mobility is a critical skill in tortoises and we show that females have effectively sacrificed this when compared to males. The selection producing such an outcome is little understood. Further studies should experimentally examine interspecific variation in these traits by comparing degrees of mobility between males and females and between species of tortoises.

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