

Geometric morphometrics offer insight on the intersexual differences in allometric coefficients of bite force in the northern map turtle (*Graptemys geographica*)

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ABSTRACT

Research has shown that northern map turtles exhibit significant female biased trophic size dimorphism. This dimorphism results in females having a greater bite force than males. However, recent research has demonstrated that males achieve higher allometric coefficients of bite force than females. This intersexual difference in bite performance relative to size could possibly be explained by a divergence of skull morphology.

We tested map turtles for intersexual differences in skull morphology by performing geometric morphometric analyses on landmarked skull radiographs. We used painted turtles, a species with no documented trophic size dimorphism, as a control. A principal component analysis (PCA) of landmark data revealed that important intersexual separation occurs along the first principal component axis in map turtles but not in painted turtles. Further analysis of PCA results indicated that male map turtles differed from females by their elongated jaw abductor muscle chamber and smaller temporal fossa. Results from a discriminant function analysis on these same data indicate that intersexual differences in skull shape are significant for map turtles but not for painted turtles.

Our results indicate that there exist intersexual differences in skull morphology in the northern map turtle. These differences occur in an area of the skull – the jaw abductor muscle chamber – which has previously been documented to affect bite performance. Because we didn't observe similar differences in our omnivorous non-dimorphic control species, the observed divergence in skull morphology could possibly be an adaptation by male map turtles to sustain themselves on a molluscivorous diet despite constraints on the size of their heads.

INTRODUCTION

Research has shown that the northern map turtle, *Graptemys geographica*, displays significant female-biased sexual size dimorphism of the head (Lindeman, 2000; Bulté *et al.*, 2008a). This dimorphism reflects intersexual dietary differences (Lindeman, 2000, 2006) and therefore exemplifies trophic morphology dimorphism. In turtles, bite force is positively correlated with head dimensions (Herrel *et al.*, 2002). This is also observed in northern map turtles (Bulté *et al.*, 2008a). Therefore, having larger heads allows map turtles to eat harder molluscs (Bulté *et al.*, 2008a). This is regarded as an adaptation to durophagy (Lindeman & Sharkey, 2001).

Interestingly, even though bite force scales positively with head width in both sexes in map turtles, the allometric coefficient relating head width to bite force is significantly higher in males. (Bulté *et al.*, 2008a). This means that bite force increases faster with head width in males than in females. The ultimate causation for this difference is unclear at the moment but it suggests that trophic dimorphism in morphology occurs together with trophic size dimorphism in this species. Indeed, the difference between male and female bite performance could be explained by variation in the morphology of their skulls. Herrel and associates (2007) demonstrated such a relationship in their examination of the skulls of male and female *Anolis carolinensis* lizards. Lappin and associates (2006) also demonstrated this in a similar study on *Sauromalus ater*. Differences in skull morphology have also been shown to alter bite performance interspecifically between turtle species (Herrel *et al.*, 2002) as well as between lizard (Herrel *et al.*, 2001) and bird (Van der Meij & Bout, 2008) species.

There are numerous variations in head shape that can increase bite force. Numerous studies have found that animals with proportionately higher heads have

stronger bites (Herrel *et al.*, 2001, 2002; Herrel & O'Reilly, 2005; Lappin *et al.*, 2006). Having a higher head can improve bite performance by positioning the jaw abductor muscles at a more perpendicular angle relative to the lower jaw and/or by providing a larger attachment area for the jaw abductors (Herrel *et al.*, 2002). Similarly, Herrel's work on *Anolis* lizards (2007) determined that an enlargement of the jaw abductor muscle chamber was the main variable accounting for the difference in bite force between males and females. Variations in the configuration of the jaw lever system can also increase bite force. Generally, force generation is increased by short outlevers (Herrel *et al.*, 2001; Schenk & Wainwright, 2001). In finches for example, a rearward shift of the quadrate increased the lever arm of jaw abductors and was associated with stronger bite forces (Van der Meij *et al.*, 2008).

The object of this study is to locate differences between the head shapes of male and female northern map turtles. This will be done by performing a geometric morphometric comparison of male and female map turtle skulls. Geometric morphometrics uses multiple measurements of an object to determine its shape. Multivariate analyses can then be employed to calculate if there is a significant difference between the shapes of objects. Geometric morphometrics is an appropriate approach to examine differences in skull shape because it aims to describe configurations while allowing size and shape to be considered separately (Claude *et al.*, 2004). Since the skulls of female map turtles are generally much larger than those of the males, it is paramount for this study to compare their geometry independently from size. Examining skulls will not only detect differences in skull shape but will also allow inferences about the configuration and size of head muscles which can also affect bite force (Herrel *et al.*, 2007).

To determine whether the differences in skull shape observed in northern map turtles are linked to this species trophic size dimorphism and durophageous diet, we also needed to test a control group of a non-sexually dimorphic and non-durophageous turtle species using geometric morphometrics. Our control species is the painted turtle (*Chrysemys picta*). It is an omnivorous turtle (Cooley *et al.*, 2003) with no documented trophic dimorphism. If the male/female variation we expect to find in map turtles is a mechanism to compensate for trophic size dimorphism, we should not observe as much between-sex variation in a non-sexually dimorphic species such as the painted turtle. If this is the case, this study will have found a potential compensation mechanism for sexually dimorphic male northern map turtles.

MATERIALS AND METHODS

Map turtle and painted turtle specimens were provided by the Canadian Museum of Nature (CMN). These specimens have been collected by the museum over more than a century and come from a variety of locations of each species' range across North America. The CMN had an insufficient number of female specimens of map turtles. Therefore, additional map turtle specimens had to be ordered from the Royal Ontario Museum to complete our sample. Sex was mainly determined by visual inspection of the location of the vent. Turtle size and the length of anterior claws were also used as indicators of sex in map turtles and painted turtles, respectively. The head width and the plastron length were measured for each specimen using callipers. Head width is a measure of trophic size while plastron length is a measure of body size.

We needed to confirm our assumption of the existence of trophic size dimorphism in map turtles and the absence of it in painted turtles. To do this, we

calculated the relative head width of each specimen as the dividend of head width over plastron length. We pooled these data by sex and species. We tested if there was variation between sexes/species using a model type I multi-way ANOVA. Since we assume trophic size dimorphism to occur only in map turtles, we expected a significant effect of the sex/species interaction on relative head width. We found that this interaction is indeed significant ($p=2.2 \text{ E-}7$) and accounts for 32% of the variation of relative head width. Because we are primarily concerned with intraspecific differences between each gender, we performed a t-test on the relative head width of each species, pooling the data by sex. As expected, there was a significant difference between the relative head size of male and female map turtles ($p= 1,28\text{E-}06$). Male painted turtles had a higher mean relative head width than females but this difference was non significant ($p= 0,231$). This confirms our assumption that trophic size dimorphism occurs in map turtles but not in painted turtles.

Specimens' heads were radiographed from a dorsal view using the SDS X-Ray Imaging System. Voltage, amperage and exposure time were controlled by Kevex X-Ray Source Control Interface 4.1.4 software. Because specimen size ranged greatly, these settings varied between specimens. Before scanning the turtles, we ensured that the top surface of their heads was at level with the horizontal to ensure that we were scanning all skulls from the same angle. This is necessary to ensure that variations observed in our landmarks were not the result of variability in head positioning. We used laboratory clamps and elastic bands to hold turtles in place. Images were transferred from phosphor plates placed under the turtles via an Orex PcCR812 HS scanner and read using VideoRen software to export the image to a TIF format.

ImageJ software (Rasband, 2008) was used to adjust different sections of single photographs in order to maximize the visibility of skull structures. We created morphometric data files for each species by landmarking the skull photos using the tpsutil (Rohlf, 2008) and tpsDig programs (Rohlf, 2006). We chose landmarks in order to obtain coverage of the entire length and width of the skull. Our selection of landmarks was restricted to structures that were easily identifiable and repeatable between photographs. Eight landmarks were chosen according to these criteria and marked on one side of the skull for each photograph (Fig. 1): 1) the tip of the premaxilla, 2) the back of the nasal cavity, 3) the outside maxima curvature of the eye socket, 4) the inside maxima curvature of the inferior temporal fossa, 5) the anterior maxima curvature of inferior temporal fossa, 6) the anterior maxima curvature of the inner ear cavity, 7) the tip of the squamosal bone and 8) the tip of the supraoccipital bone.

All manipulations and analyses of the morphometric data were done using MorphoJ software (Klingenberg, 2008). Separate data sets were created for each species. A Procrustes fit by principal axes was performed on the data to remove variations in translation, rotation and scaling in the landmark configurations. Covariance matrices of the Procrustes coordinates were generated for each data set. Within group covariance was pooled by sex.

Two methods were used to test for the presence of sexual dimorphism in our species. First, we performed principal component analyses (PCA) of each species' covariance matrix. We plotted the axes of the PCA to look for evidence of dimorphism between the sexes. Following this, we interpreted the principal component coefficients and plotted the principal component shape changes to describe the shape variation between the sexes. For the second analysis, we

performed a discriminant function analysis (DFA) on the covariance matrices for each species, with sex as the grouping criterion. DFA calculates the linear combination of variables that maximizes the separation between predefined groups (Herrel *et al.*, 2007). The T-square statistic and associated p-value indicate whether the shape difference between the sexes of each species is significant. We used the Mahalanobis distance for each species to compare the extent to which skull shape differs between the sexes. Finally, we referred to the classification/misclassification tables to examine the degree to which the sexes can be identified by skull shape for each species.

RESULTS

Results of the PCA indicate a clear separation of male and female map turtle along the first principal component's (PC1) axis (Fig. 2A), accounting for 26.5% of the sample variance. PC2 accounts for 22.2% of the sample variance. However, there is no clear separation of sexes along this axis. Thus, PC2 and subsequent principal components were not interpreted as axes of differentiation between the sexes. The PC1 axis separates males, which score low and negative, from females, which score high and positive, with very little overlap. Such a separation is not observed for the painted turtle (Fig. 2B), even though the PC1 axis accounts for 36.1% of the variance in that sample.

Examining the principal component coefficients of PC1 for the map turtle (Table 1), we find that landmarks 3, 4 and 5 show the greatest variation. A thin-plate spline transformation grid was generated for the map turtle (Fig. 3) to assist with the interpretation of the coefficients. This grid indicates that much of the variation along PC1 involves the shape of the jaw abductor muscle chamber (landmarks 4, 5,

6), which is located between the squamosal, parietal, supraoccipital and quadrate bones (Wyneken, 2001). A positive change along PC1, as illustrated in Figure 3, results in a longitudinal compression of the jaw abductor muscle chamber. Since females score positive and males score negative on the PC1 axis, the transformation grid indicates that males have longer abductor muscle chamber than females. In association with the variation of the jaw abductor muscle chamber, the grid also shows important variation in the shape of the temporal fossa. In this case, a positive change along PC1 leads to a longitudinal expansion of the temporal fossa. This indicates that males have shorter temporal fossa than females.

The results of the DFA on the covariance matrices for each species demonstrate a significant difference in head shape between male and female map turtles ($P=0.0082$) but not between male and female painted turtles ($P=0.28$). The Mahalanobis distance between male and female is greater for map turtles (3.0806) than for painted turtles (1.8796). Classification of the specimens to the group mean to which they were the less distant (Mahalanobis d^2) yielded a higher percentage of correct assignment for map turtles than painted turtles (Table 2). Cross validation classification also yielded a higher percentage of correct assignment for map turtles than painted turtles (Table 2).

DISCUSSION

Our results indicate that intersexual differences in skull morphology exist in the northern map turtle. We found that male map turtles have proportionately longer jaw abductor muscle chambers and smaller temporal fossa than females. A larger jaw abductor muscle chamber has previously been linked to stronger bite force in male reptiles (Herrel, *et al.*, 2007). In that case, the size of the muscle chamber was

directly positively correlated to measurements of the jaw abductor muscles. We did not take any measurement of the jaw abductor muscles. However, the principal jaw abductor muscle in turtles, the abductor mandibulae, is attached to the parietal, supraoccipital and quadrate bone (Wyneken, 2001). These bones, along with the squamosal bone, form the abductor muscle chamber in which the abductor mandibulae resides. Therefore we can infer that the elongation of the jaw abductor muscle chamber we observed in male map turtles results in an elongation of the abductor mandibulae. Jaw muscle metrics have been positively correlated with bite force in numerous species (Van der Meij& Bout, 2004; Van Wassenbergh, *et al.*, 2005; Herrel, *et al.*, 2007). Hence, the differences in skull morphology we documented could explain how male map turtles achieve higher allometric coefficients of bite force than females.

Our data suggest that there might be a lateral component to the variation of the dimensions of the jaw abductor muscle chamber and temporal fossa in the map turtle. However, our landmarks are poorly positioned to describe such variation. Our choice of landmarks was fairly limited due to the low repeatability of many skull structures from one radiograph to the next. Therefore, our quantitative description of skull shape could be improved by strategically placing a few more landmarks in these areas. Moreover, our analysis did not include any vertical component of the skull because it was impractical for us to radiograph skulls from the sides with the equipment we had. A similar analysis from this angle would be very insightful as it would allow us to describe skulls in three dimensions and verify the intersexual variation of head height and the jaw lever systems.

We predicted that, if the intersexual differences in skull shape we observed were linked to map turtles' trophic size dimorphism, such differences should not be

observed in a control species that is not sexually dimorphic. Results from our geometric morphometric analysis of the painted turtle's skull shape were consistent with this prediction. Indeed, we found that intersexual differences in skull shape in this species were non-significant and less important than in map turtles. These results suggest that the intersexual differences in skull shape we observed in the map turtles are adaptations to its durophagous diet and resulting trophic size dimorphism.

Interestingly, our results indicate that male and female map turtles have different morphological adaptations of their skulls and jaw muscles. These are concordant with the intersexually different coefficients of bite force previously reported for this species. Our results therefore show that each sex achieves higher bite performance through different mechanisms. All species of genus *Graptemys* exhibit female biased trophic size dimorphism, which is more pronounced in molluscivorous species (Lindeman, 2000). This suggests that developmental constraints may prevent males from growing heads as large as females'. Therefore, males may have evolved larger jaw abductor muscles to overcome the limits imposed on their bite performance by their head size. It has been suggested that testosterone might limit head growth in male map turtles (Bulté *et al.*, 2008a). Indeed, such an interaction between testosterone and head growth has been demonstrated in male garter snakes (Shine & Crews, 1988). Moreover, testosterone has been found to limit overall body growth in other reptiles (Cox & John-Adler, 2005; Cox *et al.*, 2005). Since testosterone has previously been demonstrated to limit growth in other reptiles, it is possible that it operates similarly in turtles. This hypothesis could be tested by comparing the head growth of castrated male map turtles to that of non-castrated males or through some other hormonal treatment. Our study provides new evidence

that the existence of developmental constraints in one sex of a species can lead to the evolution of intersexually different traits in response to the same selective pressure.

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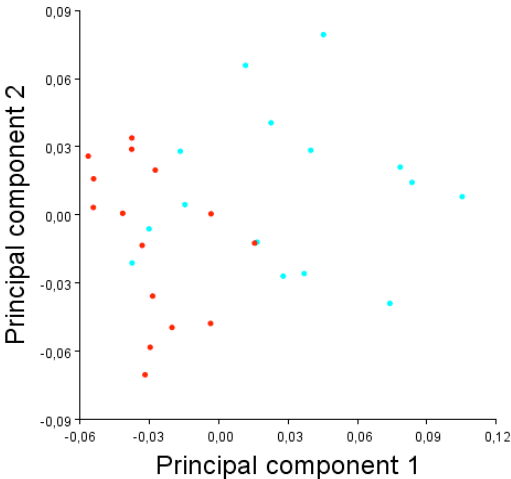
FIGURES AND TABLES

Figure 1.



Figure 2.

A.



B.

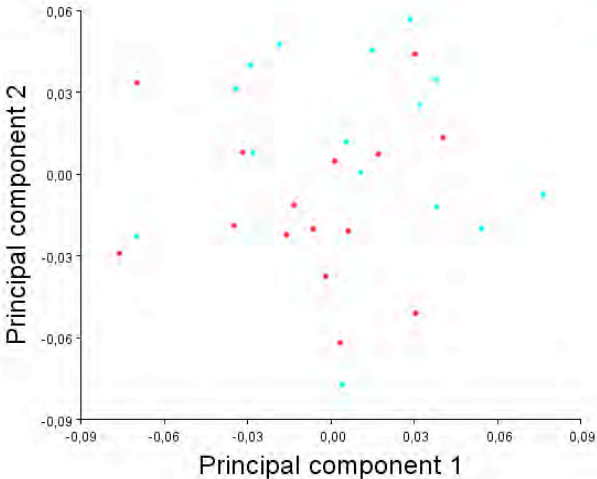


Figure 3.

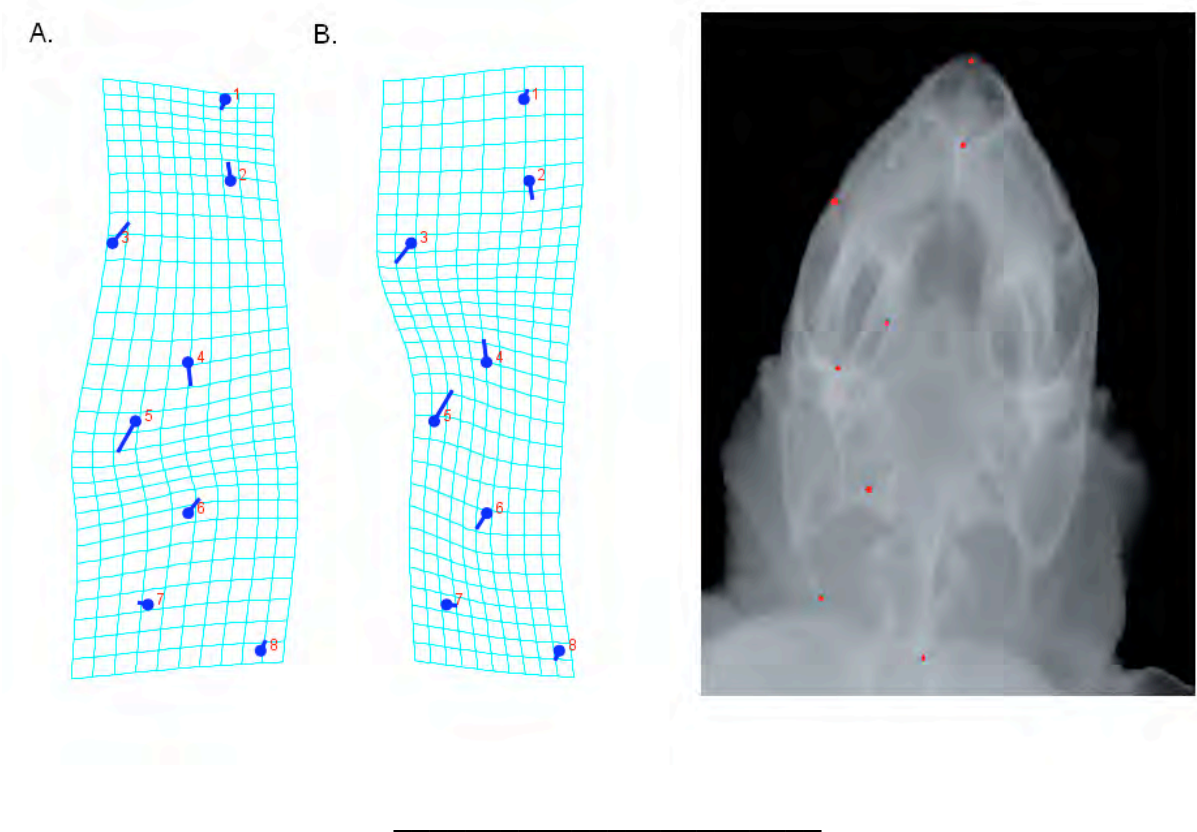


Table 1.

Landmark	Axis	PC1	Net PC1
1	x1	-0,05	0,16
	y1	-0,16	
2	x2	-0,05	0,31
	y2	0,30	
3	x3	0,28	0,44
	y3	0,34	
4	x4	0,04	0,40
	y4	-0,40	
5	x5	-0,32	0,61
	y5	-0,53	
6	x6	0,18	0,31
	y6	0,25	
7	x7	-0,15	0,15
	y7	0,03	
8	x8	0,07	0,17
	y8	0,15	

Net PC1 is the absolute coefficient for a landmark, combining its x and y component. It is calculated using the Pythagorean theorem.

Table 2.

Classification	Species	True Group	Allocated to		Total	% properly classified
			Female	Male		
Discriminant function	<i>Graptemys geographica</i>	Female	12	3	15	80,0%
		Male	0	15	15	100,0%
		Total	12	18	30	90,0%
	<i>Chrysemis picta</i>	Female	13	2	15	86,7%
		Male	3	12	15	80,0%
		Total	16	14	30	83,3%
Cross validation	<i>Graptemys geographica</i>	Female	12	3	15	80,0%
		Male	3	12	15	80,0%
		Total	15	15	30	80,0%
	<i>Chrysemis picta</i>	Female	9	6	15	60,0%
		Male	9	6	15	40,0%
		Total	18	12	30	50,0%

CAPTIONS

Figure 1. Radiograph of the head of a male *Chrysemis picta* in dorsal view. Landmarks used to quantify head shape are illustrated (1-8). These same landmarks were used on all skull radiographs for both species.

Figure 2. Scatter plot of the PC scores on the first two axes obtained from a PCA on the skull shape data for (A) *Graptemys geographica* and (B) *Chrysemis picta*. Data points for females are in blue while data points for males are in red.

Figure 3. Thin-plate spline transformation grids for PC1 of *Graptemys geographica*. The dots represent the Procrustes coordinates of the landmarks. The length and direction of the lines indicate the movement of the landmarks that corresponds to 0.1 (A) and -0.1 (B) Procrustes distance in the direction of PC1.

Table 1. Principal component coefficients of landmarks 1-8 for *Graptemys geographica*.

Table 2. Principal component coefficients of landmarks 1-8 for *Graptemys geographica*.

APPENDIX

Canadian Museum of Nature and Royal Ontario Museum (*) catalogue numbers of the specimens used to quantify skull shape for this report.

Graptemys geographica

10231	19210 (R-203-66)	19929	27450	3304 (2)	9718*
1089*	19347*	20929*	3239	3304	975
1092	19350	21157-1	3240	3304 (3)	5096*
1517	19415	21157-2	32971	35163	7220
19210 (R-201-66)	19612	22641	3304 (1)	9506	7221

Chrysemis picta

16659	17158-6	19923	21978-1	26050	28442-2
16786	1789	19931	21978-2	26444	2966
17158-3	19646	21555	23336	26461	3895
17518-4	19661	2159	23343	2673	3937
17158-5	19709	2161	23946	28442-1	9435