Patterns of sexual size dimorphism in Chelonia: revisiting Kinosternidae

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Rensch's rule, a macroevolutionary pattern in which sexual size dimorphism (SSD) increases with body size in male-biased SSD species, or decreases with female-biased SSD species, has been investigated in many vertebrates because it indicates whether SSD is being driven by sexual selection or a different force (i.e. fecundity or natural selection). Evidence in turtles has shown some conflicting results, which may be explained by the different phylogenies used in the analyses. Because the newly available well-resolved phylogeny of family Kinosternidae provides evidence for the ancient monophyly of Staurotypidae and Kinosternidae and their recognition as separate families (previously Staurotypidae was considered as a subfamily within Kinosternidae) and introduced the genus *Cryptochelys* for the monophyletic *leucostomum* clade, we revisit the pattern of SSD and body size in Kinosternidae. By contrast to what had been proposed, we found that the Kinosternidae as formerly recognized (i.e. including *Staurotypus* and *Claudius*) and the restricted Kinosternidae both follow a pattern consistent with Rensch's rule. Our analysis with published body size data did not change our results, confirming the importance of the phylogeny used in macroevolutionary studies. © 2014 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2014, **111**, 806–809.

ADDITIONAL KEYWORDS: allometry – body size evolution – comparative method – Rensch's rule – reptiles – selection – turtles.

INTRODUCTION

Understanding general patterns of phenotypic evolution is important because it allows us to recognize potential evolutionary drivers, such as sexual selection or fecundity selection. Rensch's rule, a pattern in which sexual size dimorphism (SSD) increases with body size in male-biased SSD species (Rensch, 1950), or decreases with female-biased SSD species (Rensch, 1960), has been investigated in many vertebrates (Abouheif & Fairbairn, 1997; Szekely, Freckleton & Reynolds, 2004; Dale *et al*., 2007; Lindenfors, Gittleman & Jones, 2007; Frydlova & Frynta, 2010; Starostova, Kubicka & Kratochvil, 2010). Several studies have investigated this pattern in turtles during the last 23 years with different methods and different results. The first studies did not take the

phylogenetic relationships into account. Nevertheless, some supported Rensch's rule in groups such as Kinosternidae (Berry & Shine, 1980; Iverson, 1985; Cox, Butler & John-Alder, 2007), although not at the level of the Order Testudines (Gibbons & Lovich, 1990) or in the families Emydidae, Geoemydidae, and Testudinidae (Cox *et al*., 2007). Later studies included phylogenies in their analyses and concluded that the Testudines and the Testudinidae follow Rensch's rule (Ceballos *et al*., 2013; Halámková, Schulte & Langen, 2013), although the Cheloniidae and Kinosternidae were found to be isometric (no allometry between SSD and body size) (Ceballos *et al*., 2013; Halámková *et al*., 2013). The Podocnemididae and the genus *Graptemys* were found to follow a pattern converse to Renschs's rule (i.e. SSD decreases with body size in male-biased SSD species, or increases with body size in female-biased SSD species) (Lindeman, 2008; Ceballos *et al*., 2013). Finally, the Geoemydidae and

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Emydidae show evidence in favour and against Rensch's rule (Ceballos *et al.*, 2013; Halámková *et al.*, 2013). These contrasting results suggest that the phylogeny used is fundamental when analyzing macroevolutionary patterns, and all advances on resolving relationships among species should help our understanding of such patterns.

In this sense, we emphasize the recent resolution of the phylogenetic history of the 25 species of the family Kinosternidae (Iverson, Le & Ingram, 2013). To reconstruct the phylogenetic history of this group, Iverson *et al.* (2013) used three regions of the mitochondrial genome and three nuclear fragments to build a well-resolved phylogeny, which was also calibrated using fossil records. Their study has important implications because it provided evidence for the ancient monophyly of Staurotypidae and Kinosternidae and their recognition as separate families (previously considered as a subfamily within Kinosternidae), and introduced the genus *Cryptochelys* for the monophyletic and tropical *leucostomum* clade.

Because the aforementioned studies used kinosternid phylogenies with very different topologies and reached different conclusions on whether SSD evolution follows an isometric (Ceballos *et al.*, 2013; Halámková *et al.*, 2013) or allometric pattern with body size (Rensch's rule) as had been proposed previously (Berry & Shine, 1980; Iverson, 1985; Cox *et al.*, 2007), in the present study, we revisit this issue using the newly available well-resolved phylogeny of the Kinosternidae.

MATERIAL AND METHODS

Data on mean and maximum body size (linear carapace length; cm) were collected for all 25 species that comprise the families Kinosternidae and Staurotypidae (*sensu* Iverson *et al.*, 2013), plus two subspecies of two genera, for a total of 27 taxa (see Supporting information, Table S1). These data were log transformed and male body size (on the *y*-axis) was correlated with female body size (on the *x*-axis) using phylogenetic major axis regression (PRMA) (Ceballos *et al.*, 2013). Phylogenetic regressions used the phylogeny in Iverson *et al.*, 2013 (Fig. 1), and were conducted using library 'phytools' (Revell, 2011) in R, version 3.0.1 (R Core Team, 2013). Analyses were performed at different phylogenetic levels: (1) family level: all species formerly in Kinosternidae (Kinosternidae + Staurotypidae in Iverson *et al.*, 2013); (2) all taxa excluding *Staurotypus* and *Claudius* (restricted Kinosternidae in Iverson *et al.*, 2013); and (3) the three monophyletic clades: *Kinosternon*, *Sternotherus*, and *Cryptochelys* in the restricted Kinosternidae.
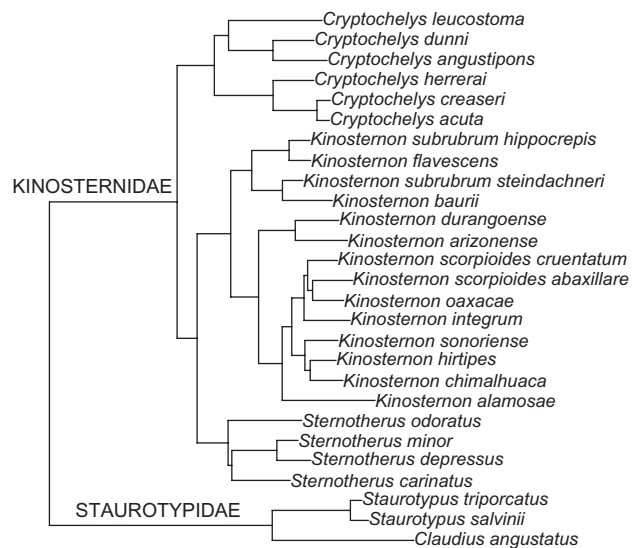

Figure 1. Phylogeny of kinosternid and staurotypid taxa used in the present study (*sensu* Iverson *et al.*, 2013).

For interpretation of the results: if the slope (*b*) was larger than 1 (*b* > 1), and its 95% confidence interval (CI) > 1, it would indicate that males drive body size evolution, a pattern consistent with Rensch's rule, and sexual selection would be its main evolutionary driver. If the 95% CI included 1 (regardless of any value of *b*), it would indicate that neither sex is driving body size evolution (or that both sexes are influencing it almost equally), an isometric pattern. Finally, if *b* < 1 and its 95% CI < 1, then it would indicate a pattern contrary to Rensch's rule (i.e. that females drive body size evolution) (Ceballos *et al.*, 2013).

We assessed the appropriateness of using mean versus maximum body size data and found that maximum data exaggerate SSD values and make its variation much higher when compared to mean data. This is reflected in their standard deviations: $SD_{mean\ males} = 3.31$, $SD_{mean\ females} = 3.4$, $SD_{max\ males} = 5.26$, and $SD_{max\ females} = 5.6$. For this reason, we report the results using mean body size data only and suggest that future studies use mean data.

Finally, to test how robust our results were with respect to the available body size data, we repeated our analysis using the body size data from Halámková *et al.* (2013) with the phylogeny from Iverson *et al.* (2013). In addition to PRMA, we also employed a second method used in similar comparative studies (Lindeman, 2008; Halámková *et al.*, 2013), namely phylogenetic independent contrasts (PICs) (Felsenstein, 1985) using library 'ape' (Paradis, Claude & Strimmer, 2004) in R software. We calculated the absolute values of the male and female PICs, which were regressed to obtain the slope interpreted as explained above.

Table 1. Results from the phylogenetic major axis regression (PRMA) and phylogenetic independent contrasts (PICs) on mean body size of of kinosternid and staurotypid taxa used in the present study, in accordance with the phylogeny in Iverson *et al*. (2013)

PRMA

Taxa	N	R^2	Intercept	Slope	Slope 95% confidence interval	Pattern
(Kinosternidae + Staurotypidae)	27	0.83	−0.35	1.15	1.02, 1.29	RR
Kinosternidae	24	0.84	−1.28	1.55	1.38, 1.76	RR
Cryptochelys clade	6	0.98	−1.50	1.62	1.50, 1.76	RR
Kinosternon clade	14	0.43	−1.25	1.54	1.06, 2.40	RR
Sternotherus clade	4	0.96	−0.79	1.36	1.14, 1.63	RR

PICs

Taxa	N	F	d.f.	Slope	P	Pattern
(Kinosternidae + Staurotypidae)	27	122.90	1, 25	1.05	3.85E-11	RR
Kinosternidae	24	112.80	1, 22	1.42	3.99E-10	RR
Cryptochelys clade	6	250.00	1, 4	1.61	9.35E-05	RR
Kinosternon clade	14	8.99	1, 12	1.01	1.11E-02	RR
Sternotherus clade	4	48.46	1, 2	1.33	2.00E-02	RR

RESULTS

Most kinosternid taxa exhibit a male-biased SSD (15 of 24), whereas only one of three staurotypid taxa do so (see Supporting information, Table S1). Whether PRMA analyses were conducted for Kinosternidae as formerly recognized (i.e. including Staurotypus and Claudius), or for the restricted Kinosternidae, we found the slope of male size plotted against female size (Fig. 2) to be significantly larger than 1 ($b > 1$), with 95% CI > 1 in both cases (Table 1). This indicates that SSD increases with body size, a pattern consistent with Rensch's rule. When the PRMA analyses were conducted separately for the *Cryptochelys*, *Kinosternon*, and *Sternotherus* clades, the results were identical ($b > 1$ and 95% CI > 1 for all three cases) (Table 1). These results did not change when the second method, PICs, was used (Table 1).

DISCUSSION

By contrast to the results reported in other recent studies (Ceballos *et al*., 2013; Halámková *et al*., 2013), our findings suggest that SSD follows an allometric pattern consistent with Rensch's rule in the Kinosternidae, with or without the inclusion of *Claudius* and *Staurotypus*. Thus, the available data now support the hypothesis that males drive body size evolution in this family, with sexual selection as its main driver.
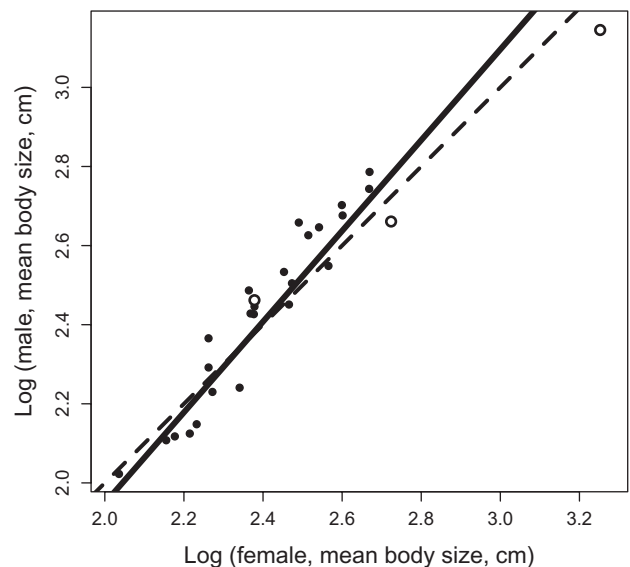

Figure 2. Relationship of male body size with female body size (natural log of mean linear carapace length; cm) of kinosternid (black circles, $N = 24$) and staurotypid (white circles, $N = 3$) taxa used in the present study. The dashed line represents isometry; the solid line represents the PRMA model.

Furthermore, when we repeated the analysis using body size data from Halámková *et al*. (2013) the conclusion that Kinosternidae follows Rensch's rule was confirmed ($N = 21$, $b = 1.40$, 95% CI = 1.18–1.69).

This latter result indicates that the contrasting results reported in Ceballos *et al*. (2013) and Halámková *et al*. (2013) are explained by the use of different phylogenies. This conclusion highlights the importance of phylogenetic studies for studying macroevolutionary patterns such as Rensch's rule, although, in some cases, nonphylogenetic and phylogenetic informed studies may provide similar results (Cox *et al*., 2007).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Body size data (linear carapace length; cm) of kinosternid + staurotypid taxa discriminated by sex used in the present study.