Evol Biol (2013) 40:194–208
DOI 10.1007/s11692-012-9199-y

RESEARCH ARTICLE

Phylogenetic Patterns of Sexual Size Dimorphism in Turtles and Their Implications for Rensch's Rule

Claudia P. Ceballos · Dean C. Adams · John B. Iverson · Nicole Valenzuela

Received: 14 May 2012 / Accepted: 7 August 2012 / Published online: 28 August 2012
© Springer Science+Business Media, LLC 2012

Abstract Sexual size dimorphism (SSD) is widespread in nature and may result from selection operating differentially on males and females. Rensch's rule, the increase of SSD with body size in male-biased-SSD species (or decrease in female-biased-SSD species), is documented in invertebrates and vertebrates. In turtles, evidence for Rensch's rule is inconclusive and thus the forces underlying body size evolution remain obscure. Using a phylogenetic approach on 138 turtle species from 9 families, we found that turtles overall and three families follow Rensch's rule, five families display isometry of SSD with body size, while Podocnemididae potentially follows a pattern opposite to Rensch's rule. Furthermore, male size evolves at faster rates than female size. Female-biased-SSD appears ancestral in turtles while male-biased-SSD evolved in every polytypic family at least once. Body size follows an Ornstein–Uhlenbeck evolutionary model in both sexes and SSD types, ruling out drift as a driving process. We explored whether habitat type or sex determination might be general drivers of turtle body size evolution using a phylogenetic context. We found that males are proportionally larger in terrestrial habitats and smaller in more aquatic habitats, while the sex-determining mechanism had no influence on body size evolution. Together, our data indicate that Rensch's rule is not ubiquitous across vertebrates, but rather is prevalent in some lineages and not driven by a single force. Instead, our findings are consistent with the hypotheses that fecundity-selection might operate on females and ecological-selection on males; and that SSD and sex-determining mechanism evolve independently in these long-lived vertebrates.

Keywords Sexual selection · Sexual size dimorphism · Evolution · Fecundity selection · Ecological selection · Adaptation · Comparative method · Reptiles · Turtles

Introduction

Body size is one of the most important morphological traits of an organism, as it influences fitness by its effects on survival and reproduction (Fairbairn et al. 2007). Males and females of many species are characterized by different adult sizes (Andersson 1994; Fairbairn et al. 2007). The direction of this sexual size dimorphism (SSD) varies among taxa (particularly turtles), with some species displaying female-biased SSD (larger females) while others possess male-biased SSD (larger males) (Pritchard and Trebbau 1984; Cox et al. 2007; Ernst et al. 2007; Fairbairn et al. 2007; Stillwell et al. 2010). These contrasting SSD patterns may be generated from multiple forces acting on body size of males and females in distinct ways. Thus, it is paramount to discern the relative role of these forces to understand the evolution of body size and its consequences. For example, sexual selection may favor larger males in

Electronic supplementary material The online version of this article (doi:10.1007/s11692-012-9199-y) contains supplementary material, which is available to authorized users.

C. P. Ceballos
Escuela de Medicina Veterinaria, Facultad de Ciencias Agrarias, Universidad de Antioquia, 1226 Medellín, Colombia

C. P. Ceballos · D. C. Adams · N. Valenzuela (✉)
Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA 50010, USA
e-mail: nvalenzu@iastate.edu

J. B. Iverson
Department of Biology, Earlham College, Richmond, IN 47374, USA

species exhibiting male to male combat to compete for mates (Rensch 1950; Berry and Shine 1980), or it may favor smaller males if lower size enhances male mobility and translates into better access to females (Berry and Shine 1980; Szekely et al. 2004). On the other hand, fecundity selection may favor larger females (Valenzuela 2001; Stephens and Wiens 2009), or natural selection may favor larger body size of one sex or the other depending on the ecological context (Bonner 2006). Sexual selection favoring larger body size of males in male-biased SSD species has been proposed as the primary force behind the macro evolutionary pattern commonly known as Rensch's rule. Rensch's rule describes the pattern where SSD increases as the average body size increases across related species that exhibit male-biased SSD (Rensch 1950). Subsequently, Rensch noted that "the opposite correlation applies" to species with female-biased SSD (Rensch 1960). That is, SSD decreases as the average body size increases (Fairbairn 1990, 1997; Fairbairn and Preziosi 1994).

Over recent decades Rensch's rule has been documented across species in many animal groups. Some studies have documented patterns of Rensch's rule at the intraspecific level in: water striders (Fairbairn 2005), grasshoppers (Wolak 2008), multiple fish (Young 2005; Lengkeek et al. 2008; Walker and McCormick 2009), and mud turtles *Kinosternon hirtipes* (Iverson 1985). Rensch's rule has also been reported in primates (Clutton-Brock et al. 1977), some birds (Dale et al. 2007), varanid lizards (Frydlova and Frynta 2010), chameleons (Stuart-Fox 2009) and other reptiles (Cox et al. 2007). On the other hand, Rensch's rule is not observed in female-biased SSD birds (Webb and Freckleton 2007) and several mammalian Orders (Lindenfors et al. 2007). Overall, support for Rensch's rule derives mainly from male-biased SSD clades, and a few female-biased clades (Szekely et al. 2004; Fairbairn 2005; Stuart-Fox 2009).

Understanding the proximate and ultimate causes of such large-scale patterns helps reveal the forces behind the origins and maintenance of sexual dimorphism (Ceballos and Valenzuela 2011). If related taxa follow Rensch's rule, it would suggest that the species' average body size could explain the degree of SSD observed across taxa, and that perhaps a single underlying physiological or evolutionary force may be responsible for generating such a trend (Abouheif and Fairbairn 1997). For groups that follow Rensch's rule, the sex with the larger body size variation (males) is purportedly the driver of size divergence, while body size of the less variable sex (females) co-varies passively with that of males (Fairbairn 1997; Herczeg et al. 2010). In birds, the mating strategy mediates SSD, such that under polygyny, sexual selection on males results in a SSD pattern concordant with Rensch's rule, while under polyandry, sexual selection on females results in a SSD pattern opposite to Rensch's rule (Dale et al. 2007). Importantly, non-adaptive explanations for the observed variability in SSD, such as phylogenetic conservatism (Felsenstein 1985; Harvey and Pagel 1991), may explain the maintenance of specific SSD patterns. For instance, half of the observed SSD variation in primates could be explained by phylogenetic history, 36 % by differences in size or scaling, and 14 % by variation in habitat, mating system and diet (Cheverud et al. 1985).

Studies on Rensch's rule in turtles are not conclusive, due in part to our poor knowledge about the biology and reproductive life history of many species, and because phylogenetic relationships of many species are still being elucidated (Bickham et al. 2007). Evidence of Rensch's rule in turtles is mixed, with some data supporting it while other data do not. For example, SSD reportedly increased with mean body size in kinosternid turtles, supporting Rensch's rule (Berry and Shine 1980; Iverson 1991). In contrast, SSD and carapace length were not associated across 63 turtles from 8 families, suggesting that turtles do not follow Rensch's rule, and that previous reports likely reflected low sample size or other errors (Gibbons and Lovich 1990). However, a comparative analysis of allometry and SSD across turtles and other vertebrates and invertebrates found that overall "Rensch's rule is general and highly significant" (Abouheif and Fairbairn 1997). Others found support for Rensch's rule in Geoemydidae and Kinosternidae, and isometry in Testudinidae and Emydidae (Cox et al. 2007). Finally, a recent phylogenetic study of SSD in emydid turtles reported that only lineages with male-biased SSD followed Rensch's rule, but not those with female-biased SSD (Stephens and Wiens 2009). Another recent study explored the evolution of turtle body size in a phylogenetic context but did not address SSD (Eastman et al. 2011). These conflicting results reveal that the macroevolutionary relationship of SSD and body size may not be generalizable in turtles. Yet, assessing this pattern is crucial to decipher which sex is the main driver of the evolution of SSD in turtles, and to shed light on the potential forces generating such a pattern. Namely, if male body size evolves faster than females, sexual selection would be a likely dominant evolutionary force underlying SSD evolution; but if female size evolves faster than males, fecundity selection may be more prevalent in turtles. Alternatively, if changes in male and female body size were comparable, it would suggest that mixed and equipotential evolutionary forces might be acting simultaneously in this group. Beyond sexual and fecundity selection, some extrinsic forces have been hypothesized to influence turtle body size, yet they have not been examined in a phylogenetic context. Namely, habitat use (aquatic to terrestrial) has been proposed as a mediator of body size evolution in turtles, where larger male size in terrestrial habitats results from predation pressure, mate searching needs, or desiccation avoidance,

and smaller male size in aquatic habitats results from selection to reduce energy expenditure during mate searching (Lindeman 2008). Other studies detected a relationship between habitat type, mating strategy, and SSD. For example: in terrestrial species that exhibit male combat, males are as large or larger than females; in semiaquatic or bottom-walker species that exhibit forced insemination, males are also larger or as large as females; and in aquatic species that exhibit female choice, males are smaller than females (Berry and Shine 1980). Finally, the mechanism of sex determination has also been proposed to co-evolve with body size, with temperature-dependent sex determination (TSD) being favored in species with SSD to permit the production of the larger sex at optimal temperatures for growth enhancement and genotypic sex determination (GSD) in less dimorphic species (Head et al. 1987; Webb et al. 1987; Deeming and Ferguson 1988; Ewert and Nelson 1991).

Here we examine the trends in the evolution of male and female body size in turtles and how they co-vary with SSD through evolutionary time at the Order and Family levels. We use phylogenetic comparative methods and an expanded taxonomic coverage from that used in previous studies of chelonians to better understand the pattern of evolution of SSD in turtles and to reveal potential alternative forces driving these patterns. We first tested whether or not males drive macroevolutionary patterns in a manner consistent with Rensch's rule in turtles, and whether selective or neutral scenarios best explain the macroevolutionary patterns of male and female body size evolution. The following predictions were made, and are depicted in Fig. 1. (1) If males are the main driver of SSD evolution, because they change body size (increase or decrease) disproportionally faster than females through evolutionary time (Rensch 1950, 1960; Abouheif and Fairbairn 1997), then SSD should increase with the species average body size in male-biased SSD species, or SSD should decrease with size in female-biased SSD species (scenarios 1a and 1b in Fig. 1). This pattern follows Rensch's rule and may be explained by sexual selection. (2) In contrast, if females are the main driver of SSD evolution, then SSD should increase with the species average body size in female-biased SSD species, or SSD should decrease with body size in species with male-biased SSD species (scenarios 2a and 2b in Fig. 1). This pattern is the converse of Rensch's rule and may be explained by fecundity selection. (3) Alternatively, if body size changes in males and females at the same rate such that both sexes influence the evolution of SSD equally, then SSD should remain isometric overall as species average body size increases or decreases, regardless of the SSD pattern (scenarios 3a and 3b in Fig. 1). This third scenario also runs counter to Rensch's rule, and may be explained by the action of multiple evolutionary forces including sexual selection, fecundity selection, or natural selection on the evolution of SSD in particular turtle species, but with no overall general trend. (4) Fourth, body size of males or of females may evolve neutrally in no particular direction due to drift, a scenario that would also be inconsistent with Rensch's rule. A noticeable difference among these last two scenarios is that under alternative 3, males and females may evolve toward optimal body sizes as reflected in an Ornstein–Uhlenbeck (OU) model of evolution, whereas under alternative 4, neutral evolution of body size may follow a Brownian Motion (BM) model of evolution (Butler and King, 2004). (5) Finally, if sex determination co-evolves with SSD or if extrinsic forces such as habitat use mediate the evolution of body size we should observe an association between the sex-determining

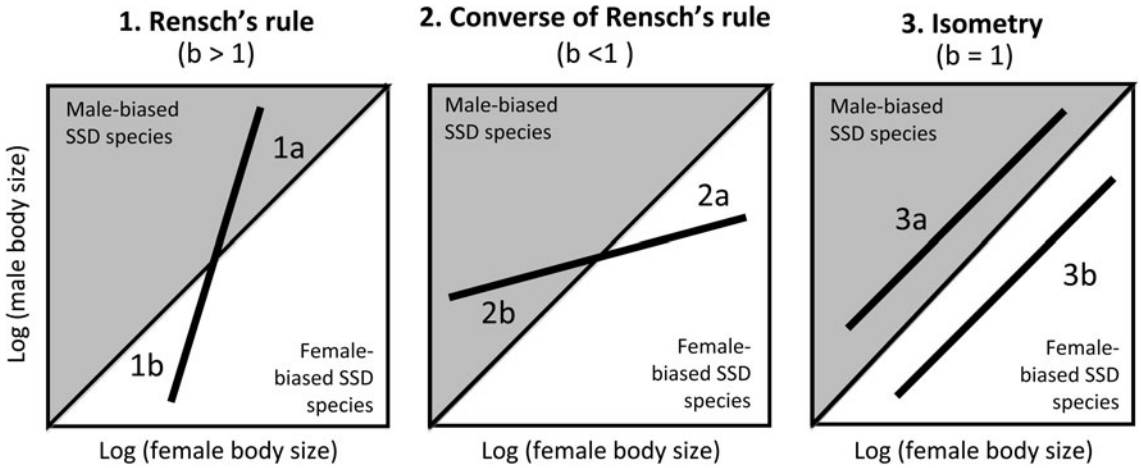


Fig. 1 Potential relationship between sexual size dimorphism (SSD) and body size of males and females. Scenarios *1a* and *1b* represent a pattern consistent with Rensch's rule; scenarios *2a* and *2b* represent the converse pattern of Rensch's rule; and scenarios *3a* and *3b* represent a pattern of isometry between SSD and body size, which is also counter to Rensch's rule. *Gray areas* encompass species with male-biased SSD, and white areas encompass species with female-biased SSD (modified from Fairbairn 1997; 2005)

mechanism and SSD or between the level of aquatic/terrestrial habitat use and SSD, respectively.

These predictions were tested using a phylogenetic comparative approach in which the correlation of body size of males and females across extant species was calculated while accounting for the phylogenetic non-independence of the taxa under examination. Additionally, we reconstructed ancestral body size values of males and females to estimate how body size of each sex varied through evolutionary time, and contrasted the fit of BM and OU models to the data on sex-specific body size evolution.

Materials and Methods

Data Collection

Data on average, minimum and maximum (largest recorded) body size data [linear carapace length, LCL (cm)] for males and females were compiled from over 450 published papers (and references cited therein when appropriate), and included information for 138 out of 322 (43 %) extant turtle species, belonging to 10 out of the 14 (71 %) chelonian families (van Dijk et al. 2011). Table 1 summarizes the average, minimum, and maximum body size of the means for males and females by species per family, and Online Resource 1 lists the raw data for each sex and species. For studies that reported the minimum and maximum LCL only we calculated the average of the two. Studies reporting only the maximum or only the minimum values were excluded from the calculation of average body size, as were body size data for juveniles or unsexed individuals. When data for the same species were found in multiple studies, all values were used to calculate a single average body size for each sex.

We followed the species scientific names suggested by the Turtle Taxonomy Specialist Group (van Dijk et al. 2011) for taxa whose names are still debated (i.e., *Phrynops zuliae* can be found as *Batrachemys zuliae*). Body size data were natural-log transformed, and a SSD index was calculated according to Lovich and Gibbons (1992) and Stephens and Wiens (2009) as follows:

$1 \times [(\text{larger sex}/\text{smaller sex}) - 1]$ if females are larger than males or,

$-1 \times [(\text{larger sex}/\text{smaller sex}) - 1]$ if males are larger than females.

In this way, positive values indicate female-biased SSD, negative values indicate male-biased SSD, and zero denotes isomorphism.

Phylogenetic relationships were based on a recent turtle supertree of all recognized living chelonians (Iverson et al. 2007). In this supertree the phylogenetic relationships of most turtle families were well resolved and supported, except for Platysternidae and Chelydridae. Within families, the relationships among most genera were also well-resolved (Iverson et al. 2007). The supertree was dated by fossil calibration using 21 divergence times among major lineages obtained from a fossil-calibrated turtle molecular phylogeny (Near et al. 2005) as described in (Valenzuela and Adams 2011). The supertree was then pruned to include only the species for which body size data were collected in this study. Tree pruning was performed using

Table 1 Summary statistics of body size (average linear carapace length, cm) of males and females of extant turtle species collected from published literature

Family	*n* (*N*)	Male size (cm)			Female size (mm)		
		Avg	Min	Max	Avg	Min	Max
Carettochelyidae	1 (1)	30.2	30.2	30.2	37.1	37.1	37.1
Chelidae	20 (56)	21.3	11.7	36.2	25.2	14.5	41.3
Cheloniidae	5 (6)	77.8	60.5	92.9	80.1	58.5	96.3
Chelydridae	3 (4)	34.5	27.9	46.9	30.2	25.7	39.0
Emydidae	32 (54)	13.7	8.9	27.2	19.5	10.7	33.1
Geoemydidae	24 (66)	19.1	9.8	43.8	23.9	11.7	50.0
Kinosternidae	13 (25)	11.9	7.4	17.5	11.4	8.4	15.3
Podocnemididae	7 (8)	27.1	17.3	38.4	35.1	20.7	63.3
Testudinidae	25 (45)	24.1	7.5	85.2	24.4	8.2	74.9
Trionychidae	8 (27)	32.1	14.9	86.5	37.2	13.5	91.3
Total/average	138 (292)	29.2	19.6	50.5	32.4	20.9	54.1

Body size data by species and bibliographic references are included in Table S1

N total number of species per clade, *n* number of species per clade included in this study, *Avg* average body size of species means, *Min* minimum body size among species means, *Max* maximum body size among species means

library "ape" (Paradis 2006) in R software version 2.11.1. Alternative analyses of character reconstruction were also conducted using the entire supertree and including missing data as detailed below.

Rensch's Rule

To test if SSD increases, decreases or remains isometric with species average body size (Fig. 1), data on average body size of males and females were correlated using phylogenetic major axis regression (PRMA) (Revell 2011). This analysis accounts for the shared evolutionary history of species which otherwise may result in false correlations between traits (Felsenstein 1985; Harvey and Pagel 1991; Revell 2010). We chose this approach over standard regression because there was no a priori reason to use one or the other sex as the dependent variable (Fairbairn 1997); a decision that phylogenetic generalized least squares (PGLS) requires. Indeed, initial investigation of our data using PGLS found that results were overly sensitive to the arbitrary choice of which sex was used as the dependent variable (results not shown), and since this choice cannot be made on biological grounds, PRMA is preferred in this circumstance. Additionally, phylogenetically independent contrasts (Felsenstein 1985) have been used in the past in several studies of SSD in vertebrates including turtles (Tubaro and Bertelli 2003; Gosnell et al. 2009; Stephens and Wiens 2009), but was not used here as it is a special case of PGLS (Garland and Ives 2000; Rohlf 2001). To facilitate comparison with previous studies (Fairbairn 1997, 2005; Frydlova and Frynta 2010; Herczeg et al. 2010; Polak and Frynta 2010; Remes and Székely 2010), we used males as the dependent variable in PRMA.

Because Rensch's rule was initially proposed for "closely-related species" (Rensch 1950), and given that global analyses can hide significant patterns in smaller groupings (Stephens and Wiens 2009), PRMA was performed at two taxonomic levels, across the entire order Testudines and within families. Importantly, for under-sampled families we provide only a qualitative pattern evaluation. PRMA models were performed using library "phytools" (Revell 2011) in R. To determine how SSD varies with body size we examined the slope of the regression of body size of males on females and interpreted the results as follows. (1) If the slope is greater than one ($b > 1$), then SSD evolves as predicted by Rensch's rule (scenario 1 in Fig. 1). (2) If the slope is less than 1 ($b < 1$), then SSD evolves opposite to Rensch's rule (scenario 2 in Fig. 1). (3) If the slope is not different than 1 ($b = 1$), SSD evolves in overall isometry with body size, also counter to Rensch's rule (scenario 3 in Fig. 1). We also ran the same analyses using data collected on maximum LCL (Online Resource 1), and most results were identical to those obtained using average body size. However, while maximum body size may be closer to the asymptotic limit for a species, estimates of average body size are less susceptible to sampling errors and are therefore a better descriptor of the population's typical body size. For this reason, results using maximum body size should be treated with caution. Furthermore, Rensch's rule pattern refers to SSD changes as a function of average and not maximum body size (Rensch 1950).

Ancestral Body Size and Evolutionary Rates

We estimated ancestral body size values for both males and females for each node of the phylogeny using maximum likelihood (Schluter et al. 1997; Iverson et al. 2007), and the "ace" function in the "Geiger" package in R (Harmon et al. 2008). Values were calculated for the entire turtle clade and for different subclades. These were then used to test several hypotheses concerning the directionality of body size change (i.e., if male-biased is ancestral or derived compared to female-biased SSD), to discern whether the evolutionary patterns in different subclades are concordant, and to determine which sex drives the evolution of SSD (by comparing body size changes of males and females through evolutionary time). Specifically, ancestral states of body size were used to test the above hypotheses in the following way. (1) If a lineage follows a pattern consistent with Rensch's rule (from the PRMA analyses above), then body size of males should evolve relatively faster than that of females through evolutionary time. (2) If a lineage follows a pattern opposite to Rensch's rule, then body size of females should evolve relatively faster than that of males. (3) If a lineage follows a pattern of isometry, then there should be no trend in the direction of body size changes of any sex. To test the robustness of our approach, a complementary ancestral reconstruction of male and female size and of SSD using the full turtle phylogeny with missing data for taxa with unavailable information was also carried out in Mesquite 2.74 (Maddison and Maddison 2011) using squared change parsimony. Results did not differ using this approach and thus, will not be reported hereafter.

To test whether selective pressures or neutral forces might be operating pervasively across the monophyletic turtle clade or within families, we tested whether a Brownian motion (BM) (Felsenstein 1973, 1985) or an Ornstein–Uhlenbeck (OU) model of evolution (Hansen 1997; Butler and King 2004) better fit the data of body size of males and females. Models were compared using a likelihood ratio test. If the BM model fits the data better it would indicate that body size evolves neutrally by drift with

no particular direction. If instead, the OU model fits the data better, then it would indicate that body size evolves adaptively toward one or more body size optima as a result of selection. Additionally, to determine whether body size evolution of males was faster or slower than body size evolution of females, we calculated the evolutionary rate for each sex in a phylogenetic context using the Brownian motion rate parameter from the optimal model of evolution as determined in the previous analysis [sensu (O'Meara et al. 2006)]. Rate estimates were obtained for the entire turtle clade and different subclades using the function "fit.continuous" in "Geiger" (Harmon et al. 2008).

Finally, we performed two additional novel analyses to explore in a phylogenetic context the potential effects of two other forces purported to influence turtle body size. First, we tested the association between body size and habitat type (aquatic to terrestrial) (Lindeman 2008) using PGLS. Here, the SSD index was calculated as described above (Lovich and Gibbons 1992; Stephens and Wiens 2009). Habitat type data were obtained for most species ($n = 134$) (Ernst et al. 2007), and coded 1–6 as described previously (Joyce and Gauthier 2004) to obtain a variable for habitat use: 1 = terrestrial, 2 = mainly terrestrial and rarely in water, 3 = terrestrial but often in water, 4 = stagnant or flooded small areas, 5 = slow and fast waters, 6 = large rivers or marine. Second, we used phylogenetic ANOVA (Garland et al. 1993) to test for an association between SSD and sex determining mechanism (Head et al. 1987; Webb et al. 1987; Deeming and Ferguson 1988; Ewert and Nelson 1991). Two tests were carried out for this second analysis: one in which the sex determining mechanism was classified as TSD or GSD (Valenzuela et al. 2003); and a second test in which the type of TSD or GSD system was taken into account, namely, sex determination was classified as TSDIa, TSDII, XY, ZW, or homomorphic sex chromosomes (Valenzuela and Lance 2004). These tests were performed on the subset of 57 species for which data on broad or detailed sex determination was available (Online Resource 1).

Results

Concordance to Rensch's Rule Predicted Patterns

Most of the extant turtle species exhibited a female-biased SSD, and only 28 % of the species ($n = 38$) exhibited male-biased SSD (Fig. 2). Figure 3 summarizes the data on body size for each sex and SSD in Chelonians. The correlation between sample size per family and effect size (slope estimate) was not significant ($R^2 = 0.214$, $F = 1.938$, $p = 0.21$), indicating that sample size itself may not introduce a bias in the pattern estimation of SSD to

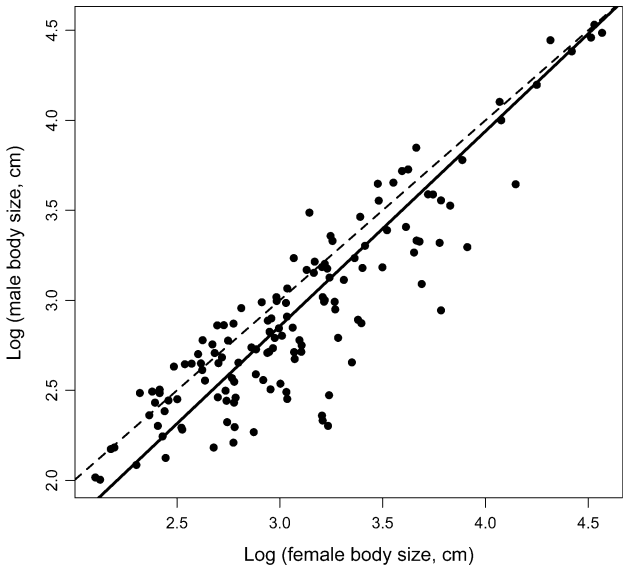


Fig. 2 Relationship of male body size on female body size (natural log of average linear carapace length measured in cm) of turtle species ($n = 138$) collected from published studies. The *dashed line* indicates isometry and the *solid line* represents the PRMA model fitted to the data. The entire turtle order follows Rensch's rule after accounting for the phylogenetic relationships of the species (see statistics in Table 2)

body size. However, it should be noted that while families with few species that were almost fully sampled provide significant biological insight (e.g., Podocnemididae), results from undersampled, but speciose, families such as Kinosternidae and Trionychidae should be treated with caution until data for additional taxa are obtained.

We found support for a pattern consistent with Rensch's rule at the level of the entire order Testudines, as the correlation of average male body size with female body size of the 138 turtle species compiled in this study had a slope greater than one (scenario 1 in Fig. 1, Table 2). Likewise, at the family level we found that Emydidae, Testudinidae, and Chelydridae followed Rensch's rule pattern (Table 2). In contrast, the families Chelidae, Cheloniidae, Geoemydidae, Kinosternidae, and Trionychidae each had a slope not different from one, thus following a pattern of isometry (scenario 3 in Fig. 1, Table 2). Of these, only Geoemydidae and Chelidae have a sample size of at least 20 species and thus these results are statistically more reliable. Notably, the family Podocnemididae had a slope less than one, a pattern that is the converse to Rensch's rule (scenario 2a in Fig. 1). While

Fig. 3 Sexual size dimorphism (SSD) of extant taxa ($n = 138$) and reconstructed ancestral dimorphism in chelonians (see section "Materials and Methods"). Phylogenetic relationships from the turtle supertree (Iverson et al. 2007) dated by fossil calibration as described in Valenzuela and Adams (2011) and in the text. *MS* male size, *FS* female size ▶

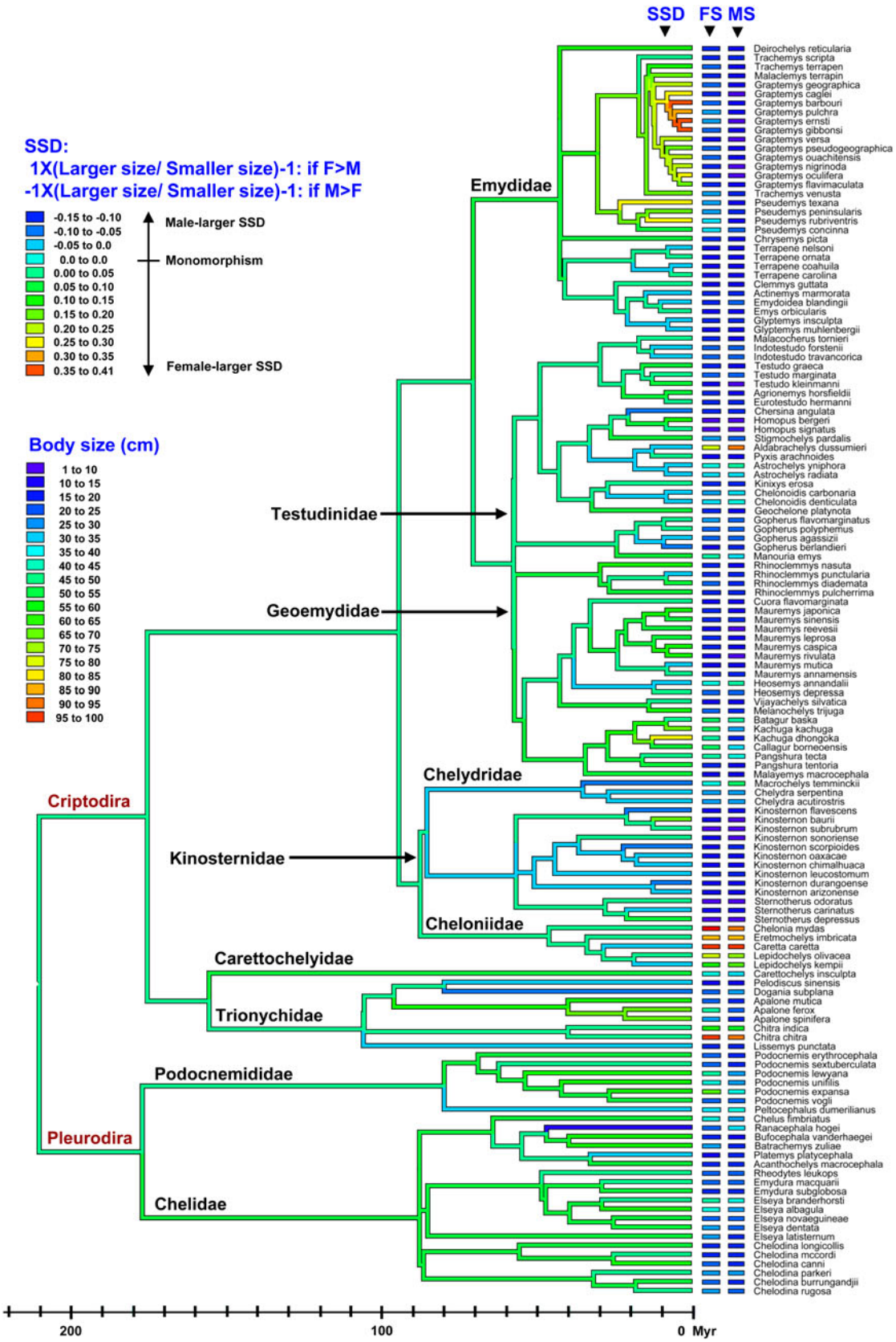

SSD FS MS
SSD:
1X(Larger size/ Smaller size)-1: if F>M
-1X(Larger size/ Smaller size)-1: if M>F
-0.15 to -0.10
-0.10 to -0.05
-0.05 to 0.0
0.0 to 0.0
0.00 to 0.05
0.05 to 0.10
0.10 to 0.15
0.15 to 0.20
0.20 to 0.25
0.25 to 0.30
0.30 to 0.35
0.35 to 0.41
Male-larger SSD
Monomorphism
Female-larger SSD
Body size (cm)
1 to 10
10 to 15
15 to 20
20 to 25
25 to 30
30 to 35
35 to 40
40 to 45
45 to 50
50 to 55
55 to 60
60 to 65
65 to 70
70 to 75
75 to 80
80 to 85
85 to 90
90 to 95
95 to 100
Emydidae
Testudinidae
Geoemydidae
Chelydridae
Kinosternidae
Cheloniidae
Criptodira
Carettochelyidae
Trionychidae
Podocnemididae
Pleurodira
Chelidae
Deirochelys reticularia
Trachemys scripta
Trachemys terrapen
Malaclemys terrapin
Graptemys geographica
Graptemys caglei
Graptemys barbouri
Graptemys pulchra
Graptemys ernsti
Graptemys gibbonsi
Graptemys versa
Graptemys pseudogeographica
Graptemys ouachitensis
Graptemys nigrinoda
Graptemys oculifera
Graptemys flavimaculata
Trachemys venusta
Pseudemys texana
Pseudemys peninsularis
Pseudemys rubriventris
Pseudemys concinna
Chrysemys picta
Terrapene nelsoni
Terrapene ornata
Terrapene coahuila
Terrapene carolina
Clemmys guttata
Actinemys marmorata
Emydoidea blandingii
Emys orbicularis
Glyptemys insculpta
Glyptemys muhlenbergii
Malacochersus tornieri
Indotestudo forstenii
Indotestudo travancorica
Testudo graeca
Testudo marginata
Testudo kleinmanni
Agrionemys horsfieldii
Eurotestudo hermanni
Chersina angulata
Homopus bergeri
Homopus signatus
Stigmochelys pardalis
Aldabrachelys dussumieri
Pyxis arachnoides
Astrochelys yniphora
Astrochelys radiata
Kinixys erosa
Chelonoidis carbonaria
Chelonoidis denticulata
Geochelone platynota
Gopherus flavomarginatus
Gopherus polyphemus
Gopherus agassizii
Gopherus berlandieri
Manouria emys
Rhinoclemmys nasuta
Rhinoclemmys punctularia
Rhinoclemmys diademata
Rhinoclemmys pulcherrima
Cuora flavomarginata
Mauremys japonica
Mauremys sinensis
Mauremys reevesii
Mauremys leprosa
Mauremys caspica
Mauremys rivulata
Mauremys mutica
Mauremys annamensis
Heosemys annandalii
Heosemys depressa
Vijayachelys silvatica
Melanochelys trijuga
Batagur baska
Kachuga kachuga
Kachuga dhongoka
Callagur borneoensis
Pangshura tecta
Pangshura tentoria
Malayemys macrocephala
Macrochelys temminckii
Chelydra serpentina
Chelydra acutirostris
Kinosternon flavescens
Kinosternon baurii
Kinosternon subrubrum
Kinosternon sonoriense
Kinosternon scorpioides
Kinosternon oaxacae
Kinosternon chimalhuaca
Kinosternon leucostomum
Kinosternon durangoense
Kinosternon arizonense
Sternotherus odoratus
Sternotherus carinatus
Sternotherus depressus
Chelonia mydas
Eretmochelys imbricata
Caretta caretta
Lepidochelys olivacea
Lepidochelys kempii
Carettochelys insculpta
Pelodiscus sinensis
Dogania subplana
Apalone mutica
Apalone ferox
Apalone spinifera
Chitra indica
Chitra chitra
Lissemys punctata
Podocnemis erythrocephala
Podocnemis sextuberculata
Podocnemis lewyana
Podocnemis unifilis
Podocnemis expansa
Podocnemis vogli
Peltocephalus dumerilianus
Chelus fimbriatus
Ranacephala hogei
Bufocephala vanderhaegei
Batrachemys zuliae
Platemys platycephala
Acanthochelys macrocephala
Rheodytes leukops
Emydura macquarii
Emydura subglobosa
Elseya branderhorsti
Elseya albagula
Elseya novaeguineae
Elseya dentata
Elseya latisternum
Chelodina longicollis
Chelodina mccordi
Chelodina canni
Chelodina parkeri
Chelodina burrungandjii
Chelodina rugosa
200
100
0 Myr

Table 2 Results from the phylogenetic major axis regression (PRMA) of average male body size on female body size of turtles at different taxonomic levels

Taxa	*n*	Intercept	Slope	Slope 95 % confidence interval	Pattern
Turtle clade	138	−0.3921	1.0829	1.0238, 1.1456	RR
Chelidae	20	−0.7908	1.1959	0.8796, 1.6551	Isometric
Cheloniidae	5	0.4825	0.8821	0.7268, 1.0659	Isometric
Chelydridae	3	−0.6498	1.2279	1.0913, 1.3857	RR
Emydidae	32	−0.9255	1.2399	1.0462, 1.4789	RR
Geoemydidae	24	−0.6907	1.1643	0.9255, 1.4770	Isometric
Kinosternidae	13	−0.7813	1.3361	0.9071, 2.0654	Isometric
Podocnemididae	7	0.8818	0.7029	0.5699, 0.8549	Converse RR
Testudinidae	25	−0.3801	1.0998	1.0440, 1.1589	RR
Trionychidae	8	0.0156	0.9665	0.7735, 1.2057	Isometric

Classification of the pattern between SSD and body size as follows: Rensch's rule (RR) = follows RR ($b > 1$), the converse of RR ($b < 1$), or isometry ($b = 1$) as depicted in Fig. 1

NS not significant at alpha = 0.05

Podocnemididae has a relatively small sample size, these results constitute an almost complete representation for the family ($n = 7$ out of 8 extant species) and thus may be biologically meaningful. Finally, results for Podocnemididae were identical when using the alternative topology presented in a recent phylogeny for this family (Vargas-Ramirez et al. 2008) in these two analyses.

Interestingly, all extant Chelydridae species exhibit a male-biased SSD pattern, and SSD was greater in the larger species *Macrochelys temminckii*, while SSD was lesser in the smaller *Chelydra* spp. (see reconstruction of ancestral values in section below, Fig. S1). Furthermore, the rate of body size evolution in males was twice as high as the rate of body size evolution in females (see evolutionary rates of

Table 3 Brownian model (BM) and Ornstein–Uhlenbeck (OU) models of evolution fitted to body size data of males and females at different taxonomic levels

Clade	Sex	BM	OU	LRT	*P* value	Best model
Turtle clade ($n = 138$)	Male	6,023.29	9,852.0	48.26	<0.001	OU
	Female	5,678.98	9,046.2	43.71	<0.001	OU
Chelidae ($n = 20$)	Male	222.632	629.0	10.34	0.001	OU
	Female	190.78	389.2	4.52	0.033	OU
Cheloniidae ($n = 5$)	Male	230.41	772.1	1.01	0.314	BM
	Female	284.77	900.1	0.83	0.360	BM
Chelydridae ($n = 3$)	Male	65.94	65.9	$1.58\ e^{-08}$	0.999	BM
	Female	33.03	33.0	$-5.49\ e^{-08}$	0.999	BM
Emydidae ($n = 32$)	Male	134.86	306.5	20.62	<0.001	OU
	Female	84.71	123.6	4.26	0.039	OU
Geoemydidae ($n = 24$)	Male	712.01	1678.4	13.61	<0.001	OU
	Female	504.74	801.8	5.64	0.017	OU
Kinosternidae ($n = 13$)	Male	23.73	42.8	2.09	0.149	BM
	Female	12.75	26.4	3.37	0.066	BM
Podocnemididae ($n = 7$)	Male	179.21	7,543.2	4.77	0.029	OU
	Female	729.89	21,911.2	6.63	0.010	OU
Testudinidae ($n = 25$)	Male	3,112.3	24,354.4	21.78	<0.001	OU
	Female	2,363.16	22,026.5	21.24	<0.001	OU
Trionychidae ($n = 8$)	Male	794.24	1,228.1	0.46	0.499	BM
	Female	891.00	1,453.6	0.52	0.469	BM

LRT likelihood ratio test

body size in section below, Table 3). However, the small sample size in Chelydridae ($n = 3$ out of 4 extant taxa) precludes any statistically supported description of its SSD pattern as a function of body size, and thus will not be discussed further. The same is true for Cheloniidae.

Ancestral Body Size Values

When ancestral values of body size of males and females were calculated for the entire turtle order, a female-biased SSD was reconstructed as the ancestral state (Fig. 3). Furthermore, female-biased SSD was reconstructed as ancestral in 6 families: Chelidae, Cheloniidae, Emydidae, Geoemydidae, Testudinidae, and Trionychidae, with the SSD being very slight in Trionychidae. Male-biased SSD appears to be derived and to have evolved multiple times, with some reversals to have also occurred. Indeed, the data indicate that the male-biased SSD pattern has evolved in every turtle family at least once, and to be ancestral in Chelydridae, Kinosternidae, and Podocnemididae. The only exception is the family Carettochelyidae whose single living species (*Carettochelys insculpta*) is female-biased, and where the newly discovered fossil of a relative from the Eocene (*Allaeochelys crassesculpta*) (Joyce et al. 2012) suggests that this pattern is ancestral to this family. Reversals from male- to female-biased SSD are observed in four families: Kinosternidae, Geoemydidae, Testudinidae, and Emydidae (Fig. 3). Results were robust to using the entire turtle phylogeny and treating all taxa for which body size data was unavailable as displaying missing character states.

An inspection of the reconstructed ancestral states of body size of males and females reveals that in the families Chelidae, Cheloniidae, Emydidae, Geoemydidae, Kinosternidae, Testudinidae, and Trionychidae (Fig. 4, S2–S8), the size of males and females increased and decreased in

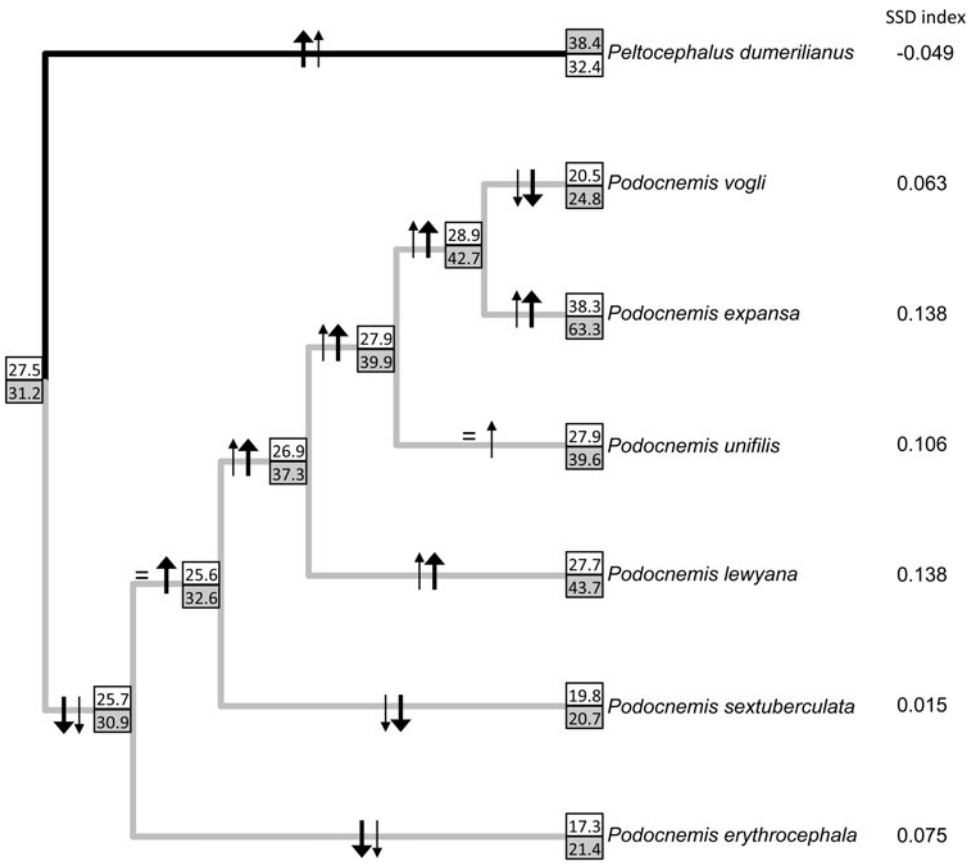


Fig. 4 Estimated body size (cm) and sexual size dimorphism of extant Podocnemididae turtles and reconstructed ancestral values. Log-likelihood for ancestral reconstruction of male body size = −33.87, and of female body size = −42.32. *Numbers* in *upper boxes* indicate mean body size of males and in *lower boxes* indicate mean body size of females (carapace length, cm). *Black branches* denote male-biased SSD, and *gray branches* female-biased SSD. *Arrows* indicate increases (pointing upwards) or decreases (pointing downwards) in body size between nodes for males (*left-hand arrows*) and females (*right-hand arrows*). The equal sign indicates no change in body size between nodes for males (*left-hand sign*) and females (*right-hand sign*). *Thicker arrows* denote a relatively faster evolutionary rate of body size of one sex compared to the other. Male-biased SSD of extant species is denoted by a *negative sign*; otherwise SSD is female-biased

Table 4 Evolutionary rates of body size for male and female turtles at different taxonomic levels

Clade	Evolutionary rate of males body size	Evolutionary rate of females body size	Percentage of change of males in relation to females	PRMA (average body size in this study)
Turtle clade ($n = 138$)	9,852	9,046	9 % faster	RR
Chelidae ($n = 20$)	629	389	62 % faster	Isometric
Cheloniidae ($n = 5$)	230	285	19 % slower	Isometric
Chelydridae ($n = 3$)	66	33	100 % faster	RR
Emydidae ($n = 32$)	306	124	148 % faster	RR
Geoemydidae ($n = 24$)	1,678	802	109 % faster	Isometric
Kinosternidae ($n = 13$)	24	13	86 % faster	Isometric
Podocnemididae ($n = 7$)	7,543	21,911	66 % slower	Converse RR
Testudinidae ($n = 25$)	24,354	22,026	11 % faster	RR
Trionychidae ($n = 8$)	794	891	16 % slower	Isometric

BM log-likelihood of Brownian motion model, *OU* log-likelihood of Ornstein–Uhlenbeck model, *LRT* log ratio test

magnitude through evolutionary time instead of the size of one sex changing disproportionally more in a single direction compared to the other. Thus, SSD diverged independently of body size and these changes balance out to generate a pattern of overall isometry in Chelidae, Cheloniidae, Geoemydidae, Kinosternidae and Trionychidae. On the other hand, in Emydidae and Testudinidae, that exhibit male-biased SSD derived from an ancestral female-biased SSD, the size of males and females changed in magnitude through evolutionary time in similar proportion (S4, S5), yet they exhibit a pattern consistent with Rensch's rule. Finally, in Podocnemididae in which most species are female-biased (6 out of 7), females exhibited a greater magnitude of change in body size overall compared to males (S7) and SSD was more accentuated in larger species as expected in a pattern opposite to Rensch's rule. Ancestral values of male and female body size in Podocnemididae also reveal an increase in SSD through evolutionary time, and this increment was associated with a greater enlargement of females compared to males.

Tempo and Mode of Body Size Evolution of Males and Females

When the BM and OU models of evolution of males and females were compared across the entire chelonian order, the OU model provided a significantly improved fit to the data (Table 3). At the family level, in only the two least speciose families (Chelydridae and Cheloniidae) and the two most underrepresented families (Kinosternidae and Trionychidae) did the BM model provide a better fit, likely due to scarcity of extant species or under-sampling. In the remaining families the OU model provided a better fit than the BM model. Results did not change for Podocnemididae when using an alternative topology (Vargas-Ramirez et al. 2008).

The rates of evolution of body size of males and females calculated across all turtles and for each chelonian family (Table 4) were somewhat consistent with the observations on the magnitude of change in body size of males and females as described above. Namely, body size evolution in females displayed a rate almost 3x faster than body size evolution in males in Podocnemididae, and appeared even faster when using the phylogenetic topology from Vargas-Ramirez et al. (2008). Accordingly, the magnitude of change observed in Podocnemididae females was larger than in males as described above. In contrast, some families followed a pattern of isometry, yet body size evolved faster in females than males or vice versa. In these cases isometry resulted because the evolutionary changes in body size within those families did not occur in a single direction. Namely, in some lineages, body size of males evolved at faster rates than in females, while in other lineages within the same clade the opposite occurred. Overall, this pattern resulted in males exhibiting faster evolutionary rates compared to females in Chelidae, Chelydridae, Emydidae, Geoemydidae, Kinosternidae, and Testudinidae, suggesting that males may be driving the evolution of SSD in those families. In contrast, in Cheloniidae, Podocnemididae, and Trionychidae, females may be driving the evolution of SSD. Interestingly, across all turtles males evolved at a faster rate (Table 4).

Finally, the PGLS of SSD on habitat type was significant ($F_{1,132} = 3.87$, $p = 0.05$) revealing a broad trend in turtles where males tend to be proportionally larger in terrestrial habitats and relatively smaller in more aquatic habitats (Fig. 5). In agreement with our findings, the recently discovered fossil of a carettochelyid turtle, *Allaeochelys crassesculpta* was described as a highly aquatic species with female-larger SSD (Joyce et al. 2012). On the other hand, phylogenetic ANOVA of SSD by sex determination

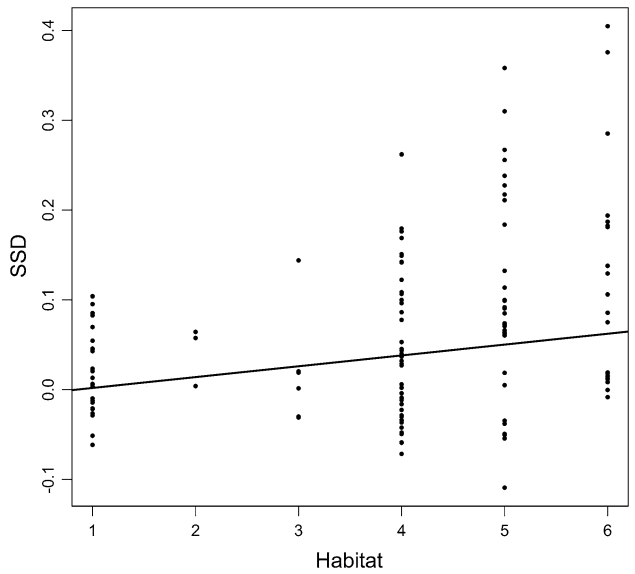


Fig. 5 Relationship between SSD index and habitat type (1 = terrestrial, 2 = mainly terrestrial and rarely in water, 3 = terrestrial but often in water, 4 = stagnant or flooded small areas, 5 = slow and fast waters, 6 = large rivers or marine) (Joyce and Gauthier 2004). The *solid line* indicates the phylogenetic generalized least square (PGLS) model fitted to the data

was not significant for the TSD versus GSD comparison once the phylogenetic relationships were taken into account ($F_{1,62} = 0.0193$, $p = 0.96$), nor for the comparison using the detailed sex-determining system information ($F_{4,57} = 3.1728$, $p = 0.19$).

Discussion

Understanding the causes of sexually dimorphic phenotypes in nature has been a pervasive question in evolutionary biology, and one that drove Darwin to postulate the principles of sexual selection (Darwin 1871). Body size is among the most conspicuous difference between males and females in many animals (Andersson 1994). Importantly, body size is highly variable, can be linked to individual fitness by its effect on reproduction and survival, and may respond to various evolutionary forces in a sex-specific manner. The noticeable large-scale pattern known as Rensch's rule (1950, 1960) (Fig. 1.1), implies that males increase body size at a faster evolutionary rate than females, suggesting that males are the main drivers of the evolution of SSD, likely as the result of sexual selection (reviewed in Fairbairn 2005). In this study we examined the covariation of SSD and body size across turtles to test (a) if SSD gets accentuated, diminishes or remains constant as body size increases among taxa (patterns consistent with Rensch's rule, opposite to Rensch's rule or isometric, respectively), (b) if one or the other sex drives the evolution of SSD, (c) whether selection or neutral evolution are pervasive forces behind SSD evolution, and (d) whether other factors such as habitat use or the link to sex determining mechanism may explain the evolution of male and female body size in these long-lived vertebrates. We found that the chelonian order in general and three families in particular follow a pattern consistent with Rensch's rule (scenario 1 in Fig. 1) whereas most other families exhibit an isometric pattern between SSD and body size (scenarios 3a and 3b in Fig. 1). Thus, we found evidence that turtles follow Rensch's rule overall and in some lineages, but not ubiquitously. Notably, our results indicate that the evolution of body size in chelonians is more complex than previously anticipated (as discussed below).

While our results are consistent with some previous studies on Rensch's rule in turtles, they run counter to others. For instance, one study examined the relationship between SSD and body size of 63 turtle species from 8 different families and concluded that turtles do not follow any pattern in particular (Gibbons and Lovich 1990). Although that study did not account for the phylogenetic non-independence of taxa, the authors suggested that chelydrids and testudinids were major exceptions to this overall pattern (Gibbons and Lovich 1990). Indeed, our results are in agreement with their conclusion that those two families do follow Rensch's rule (although the pattern for Chelydridae is only qualitative). On the other hand, earlier studies found support for Rensch's rule in kinosternid turtles (Berry and Shine 1980, 11 species; Iverson 1991, 25 species; Iverson 1985, within *Kinosternon hirtipes*). Here the 95 % confidence interval of the PRMA model fit to this group indicated that the slope was not significant and thus the data failed to support Rensch's rule (Table 2). Similarly, our findings counter the recent report of Rensch's rule in Geoemydidae and Kinosternidae (which we found to follow an isometric pattern), and the isometry found in Testudinidae and Emydidae (Cox et al. 2007) (which we found to follow Rensch's rule). These differences are likely due to the lack of phylogenetic correction, which is noted by Cox et al. (2007). A more recent study using a phylogenetic context tested several hypotheses related to the evolution of SSD in emydid turtles, including that described by Rensch's rule (Stephens and Wiens 2009), concluded that emydids show a pattern consistent with Rensch's rule, particularly those species with male-biased SSD (the subfamily Emydinae). Our results support their observations in that we found support for Rensch's rule pattern between SSD and body size in the family Emydidae. However, at the subfamily level, Emydinae ($n = 10$) shows overall male-biased SSD but this SSD is isometric with body size ($b = 1.11$, 95 % CI = 0.995, 1.241), while Deirochelyinae ($n = 22$) exhibits female-biased SSD but follows Rensch's rule ($b = 1.628$, 95 % CI = 1.118, 2.565).

Macroevolutionary Significance

Identifying an overall support for Rensch's rule in turtles but not across all families has several important macroevolutionary implications. Generally, the presence of Rensch's rule implies that there is greater divergence in male size than in female size during the evolutionary history of a given clade (Fairbairn 2005). We did detect evidence for Rensch's rule across all turtles as well as a higher evolutionary rate for male body size as compared to females across chelonians and in 6 out of 9 families (Table 4). Interestingly however, some of the families in which male size evolved faster than female size did not follow Rensch's rule but exhibited an isometric pattern between SSD and body size. Furthermore, our analysis of the direction and magnitude of separate changes in size of males and females from the common ancestor to the extant taxa revealed that both sexes increase and decrease size through time, and importantly, that males did so more drastically than females (Table 4), albeit with no particular directionality. This observation is in agreement with the prediction derived from Rensch's rule, that if greater changes in male body size compared to females drive the evolution of SSD, then SSD should be accentuated as species body size increases in male-biased SSD species, or SSD should decrease with size in female-biased SSD species (scenarios 1a and 1b in Fig. 1) (Fairbairn and Preziosi 1994). But this concordance is restricted to the overall chelonian Order and three families (Emydidae, Testudinidae, and Podocnemididae). Our other findings counter the alternative prediction that an isometric relationship of SSD and body size, as observed here for several families, would be the result of neither sex changing body size disproportionally faster than the other through evolutionary time, a scenario counter to Rensch's rule. Instead, the isometric pattern in Chelidae, Geoemydidae and Kinosternidae (Table 4) can be explained by the fact that changes in male body size in these lineages occurred without any consistent directionality (Figs. S2, S5, S6).

What forces may be acting to generate the patterns observed here? First, the predominance of female-biased SSD in chelonians (72 %) suggests that fecundity selection may be the most important selective force acting on turtles in general, while the greater and seemingly non-directional evolution of male body size suggests that perhaps male size has drifted neutrally overall. Interestingly however, across all turtles the OU model of evolution provided a better fit to the data. Namely, body size of males and females overall predominately followed the OU model of evolution (Table 3). These findings suggest that deviant body sizes are being "pulled" back to an optimum value for each sex, which would explain why male and female body sizes change over time with no particular directionality. These results support the notion that fecundity selection might be operating on female body size in turtles, and rule out drift as the driver of male body size. Instead, finding that male body size evolution follows an OU model, irrespective of the direction of SSD, implies that selection is also acting to maintain an optimal body size in males. Consistently, a PRMA analysis in which the dataset was divided in two subgroups by whether the taxa exhibited male- or female-biased SSD, indicated that male-biased SSD turtles followed Rensch's rule while female-biased taxa exhibit an isometric relationship of body size to SSD (results not shown), as would be expected if selection is acting on body size via sex-specific forces. We hypothesize that the greater magnitude of changes of males compared to females may reflect lesser constraints on male body size evolution (perhaps because fecundity selection on females is stronger than selection on males), which would permit greater departures from an optimum before they are corrected via selection. Interestingly, the significant PGLS of SSD on habitat type suggests that male size is proportionally larger in terrestrial habitats and relatively smaller in more aquatic habitats (Fig. 5). This result is concordant with that reported for the Emydidae: where predation pressure, mate searching needs, or desiccation avoidance, might drive larger male size in terrestrial habitats, and the reduction of energy expenditure during mate searching might drive smaller male size in aquatic habitats (Lindeman 2008). Finally, we found no evidence for any association between SSD and the sex-determining mechanism employed by different taxa once phylogenetic relationships were taken into account. This finding suggests that what appears to be an intriguing co-evolutionary link between two important fitness related traits (Ewert and Nelson 1991) might be a similarity generated from shared evolutionary history rather than sex determination facilitating the evolution of SSD patterns.

Second, in terms of Rensch's rule, it should be noted that the most widely accepted force behind Rensch's rule is sexual selection favoring larger body size of males (Fairbairn and Preziosi 1994; Abouheif and Fairbairn 1997; Szekely et al. 2004), yet the pattern described by Rensch's rule has been found in taxonomic groups in which SSD is not correlated with sexual selection, and sexual selection may act in organisms that do not follow Rensch's rule. For instance, varanid lizards exhibit a pattern consistent with Rensch's rule, but SSD is not related to male–male combat, nor to clutch size or habitat type (Frydlova and Frynta 2010). Likewise, not finding a pattern consistent with Rensch's rule in some turtle families does not rule out the possibility that sexual selection may still favor larger body size in males in some of these species via male–male combat or female choice (Berry and Shine 1980) or smaller body size for greater mobility in others (Berry and Shine 1980; Lindeman 2008; Bonnet et al. 2010). In fact, SSD in

various turtles has been found to vary with reproductive strategy (male–male combat, forced insemination), as well as with habitat type, fecundity traits (clutch and egg size), and sexual differences in the diet (Berry and Shine 1980; Gibbons and Lovich 1990; Gosnell et al. 2009; Stephens and Wiens 2009). Thus, our results on habitat type and evidence from other studies indicate that the evolution of male size in turtles may be driven by a variety of ecological and sexual selective forces.

Third, Rensch's rule has been supported mainly in male-biased SSD clades, and in very few female-biased clades (Szekely et al. 2004; Fairbairn 2005; Stuart-Fox 2009). Since turtles are a clade with an overall female-biased SSD pattern, our results support the hypothesis that female-biased species may follow Rensch's rule even if not generally. Importantly, our findings do not agree with previous suggestions that Rensch's rule is predominant in vertebrates, including turtles (Abouheif and Fairbairn 1997; Fairbairn 1997, 2005), but instead that Rensch's rule is prevalent in some lineages (e.g., 3 out of 9 chelonian families in our study) and absent in others. This is not surprising if different forces beyond sexual selection drive SSD evolution, which may be the case in chelonians as described above (Berry and Shine 1980; Lindeman 2008; Gosnell et al. 2009; Stephens and Wiens 2009).

In conclusion, we have found evidence that SSD and body size in turtles follow a pattern consistent with Rensch's rule overall and in a several families, and a pattern of isometry in which SSD does not consistently change with body size in other families, with only one family that appears to exhibit a pattern counter to Rensch's rule. Our evidence suggests that neither sex is driving the evolution of body size across all turtles, but instead, that selection likely operates more strongly in one sex in some lineages while it operates more strongly on the other sex (and in distinct directions) in other lineages. Our data underscore that although it is widely accepted that sexual selection on males is the main drivers of Rensch's rule, this is not always the case. Namely, observing that sexual selection on males drives SSD and body size evolution does not imply that the clade follows Rensch's rule. We also found evidence that males and females in turtles in general followed an OU model of evolution, suggesting that selection and not drift underlies body size evolution in these long-lived vertebrates. Our results provide strong support for the broad influence of habitat use on the evolution of body size in chelonians, and of fecundity selection as a major force operating on female body size in turtles. Finally, we found no support for the coevolution of sex-determining mechanism and SSD in turtles.

Acknowledgments We thank the undergraduate students from the Iowa Turtle Army at N.V. lab who helped during the literature review and data compilation. Funding was provided from grants: P.E.O. International Peace Scholarship to C.C., National Science Foundation (NSF) Doctoral Dissertation Improvement Grant DEB-0808047 to N.V. and C.C., NSF IOS 0743284 and associated RET and REU supplements to N.V., and support to C.C. from the Ecology, Evolution and Organismal Biology Department at Iowa State University. The Joseph Moore Museum of Natural History supported the research of JBI. The authors declare no conflict of interest.

References

Abouheif, E., & Fairbairn, D. J. (1997). A comparative analysis of allometry for sexual size dimorphism: Assessing Rensch's rule. *American Naturalist, 149*(3), 540–562.

Andersson, M. (1994). *Sexual selection*. New Jersey: Princeton University Press.

Berry, J. F., & Shine, R. (1980). Sexual size dimorphism and sexual selection in turtles (order Testudines). *Oecologia, 44*(2), 185–191.

Bickham, J. W., Iverson, J. B., Parham, J. F., Philippen, H. D., Rhodin, A. G. J., Shaffer, H. B., et al. (2007). An annotated list of modern turtle terminal taxa with comments on areas of taxonomic instability and recent change. *Chelonian Research Monographs, 4*, 173–199.

Bonner, J. T. (2006). *Why size matters*. Princeton, NJ: Princeton University Press.

Bonnet, X., Delmas, V., El-Mouden, H., Slimani, T., Sterijovski, B., & Kuchling, G. (2010). Is sexual body shape dimorphism consistent in aquatic and terrestrial chelonians? *Zoology, 113*(4), 213–220.

Butler, M. A., & King, A. A. (2004). Phylogenetic comparative analysis: A modeling approach for adaptive evolution. *American Naturalist, 164*(6), 683–695.

Ceballos, C. P., & Valenzuela, N. (2011). The role of sex-specific plasticity in shaping sexual dimorphism in a long-lived vertebrate, the snapping turtle *Chelydra serpentina*. *Evolutionary Biology, 38*(2), 163–181.

Cheverud, J. M., Dow, M. M., & Leutenegger, W. (1985). The quantitative assessment of phylogenetic constraints in comparative analyses: Sexual dimorphism in body weight among primates. *Evolution, 39*(6), 1335–1351.

Clutton-Brock, T. H., Harvey, P. H., & Rudder, B. (1977). Sexual dimorphism, socioeconomic sex ratio and body weight in primates. *Nature, 269*(5631), 797–800.

Cox, R. M., Butler, M. A., & John-Alder, H. B. (2007). Chapter 4: The evolution of sexual size dimorphism in reptiles. In D. J. Fairbairn, W. U. Blanckenhorn, & T. Szekely (Eds.), *Sex, size and gender roles: Evolutionary studies of sexual size dimorphism*. Oxford, UK: Oxford University Press.

Dale, J., Dunn, P. O., Figuerola, J., Lislevand, T., Szekely, T., & Whittingham, L. A. (2007). Sexual selection explains Rensch's rule of allometry for sexual size dimorphism. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 274*(1628), 2971–2979.

Darwin, C. (1871). *The descent of man, and selection in relation to sex*. London: John Murray.

Deeming D. C, & Ferguson M. W. J. (1988). Environmental regulation of sex determination in reptiles. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 322*(1208),19.

Eastman, J. M., Alfaro, M. E., Joyce, P., Hipp, A. L., & Harmon, L. J. (2011). A novel comparative method for identifying shifts in the rate of character evolution on trees. *Evolution, 65*(12), 3578–3589.

Ernst, C. H., Altenburg, R. G. M., & Barbour, R. W. (2007). Turtles of the world. Available at http://wbd.etibioinformatics.nl/bis/turtles.php?menuentry=inleiding.

Ewert, M. A., & Nelson, C. E. (1991). Sex determination in turtles—diverse patterns and some possible adaptive values. *Copeia, 1991*(1), 50–69.

Fairbairn, D. J. (1990). Factors influencing sexual size dimorphism in temperate waterstriders. *American Naturalist, 136*(1), 61–86.

Fairbairn, D. J. (1997). Allometry for sexual size dimorphism: Pattern and process in the coevolution of body size in males and females. *Annual Review of Ecology and Systematics, 28*, 659–687.

Fairbairn, D. J. (2005). Allometry for sexual size dimorphism: Testing two hypotheses for Rensch's rule in the water strider *Aquarius remigis*. *American Naturalist, 166*(4), S69–S84.

Fairbairn, D. J., Blanckenhorn, W. U., & Szekely, T. (2007). *Sex, size and gender roles. Evolutionary studies of sexual size dimorphism*. New York: Oxford University Press.

Fairbairn, D. J., & Preziosi, R. F. (1994). Sexual selection and the evolution of allometry for sexual size dimorphism in the water strider, *aquarius remigis*. *American Naturalist, 144*(1), 101–118.

Felsenstein, J. (1973). Maximum likelihood estimation of evolutionary trees from continuous characters. *American Journal of Human Genetics, 25*(5), 471–492.

Felsenstein, J. (1985). Phylogenies and the comparative method. *American Naturalist, 125*(1), 1–15.

Frydlova, P., & Frynta, D. (2010). A test of Rensch's rule in varanid lizards. *Biological Journal of the Linnean Society, 100*(2), 293–306.

Garland, T., Dickerman, A. W., Janis, C. M., & Jones, J. A. (1993). Phylogenetic analysis of covariance by computer simulation. *Systematic Biology, 42*(3), 265–292.

Garland, T., & Ives, A. R. (2000). Using the past to predict the present: Confidence intervals for regression equations in phylogenetic comparative methods. *American Naturalist, 155*(3), 346–364.

Gibbons, J. W., & Lovich, J. E. (1990). Sexual dimorphism in turtles with emphasis on the slider turtle (*Trachemys scripta*). *Herpetological Monographs, 4*, 1–29.

Gosnell, J. S., Rivera, G., & Blob, R. W. (2009). A phylogenetic analysis of sexual size dimorphism in turtles. *Herpetologica, 65*(1), 70–81.

Hansen, T. F. (1997). Stabilizing selection and the comparative analysis of adaptation. *Evolution, 51*(5), 1341–1351.

Harmon, L. J., Weir, J. T., Brock, C. D., Glor, R. E., & Challenger, W. (2008). GEIGER: investigating evolutionary radiations. *Bioinformatics, 24*(1), 129–131.

Harvey, P. H., & Pagel, M. D. (1991). *The comparative method in evolutionary biology*. Oxford, England; New York, USA: Oxford University Press, Inc.

Head, G., May, R. M., & Pendleton, L. (1987). Environmental determination of sex in the reptiles. *Nature, 329*(6136), 198–199.

Herczeg, G., Gonda, A., & Merila, J. (2010). Rensch's rule inverted-female-driven gigantism in nine-spined stickleback *Pungitius pungitius*. *Journal of Animal Ecology, 79*(3), 581–588.

Iverson, J. B. (1985). Geographic variation in sexual dimorphism in the mud turtle *Kinosternon hirtipes*. *Copeia, 1985*(2), 388–393.

Iverson, J. B. (1991). Phylogenetic hypotheses for the evolution of modern kinosternine turtles. *Herpetological Monographs, 5*, 1–27.

Iverson, J. B., Brown, R. M., Akre, T. S., Near, T. J., Le, M., Thomson, R. C., et al. (2007). In search of the tree of life for turtles. *Chelonian Research Monographs*, *4*, 85–106.

Joyce, W. G., & Gauthier, J. A. (2004). Palaeoecology of Triassic stem turtles sheds new light on turtle origins. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 271*(1534), 1–5.

Joyce, W. G., Micklich, N., Schaal, S. F. K., & Scheyer, T. M. (2012). Caught in the act: The first record of copulating fossil vertebrates. *Biology Letters,*. doi:10.1098/rsbl.2012.0361.

Lengkeek, W., Didderen, K., Cote, I. M., van der Zee, E. M., Snoek, R. C., & Reynolds, J. D. (2008). Plasticity in sexual size dimorphism and Rensch's rule in Mediterranean blennies (Blenniidae). *Canadian Journal of Zoology, 86*(10), 1173–1178.

Lindeman, P. V. (2008). Evolution of body size in the map turtles and sawbacks (Emydidae: Deirochelyinae: Graptemys). *Herpetologica, 64*(1), 32–46.

Lindenfors, P., Gittleman, J. L., & Jones, K. E. (2007). Sexual size dimorphism in mammals. In: D. J. Fairbairn, W. U. Blanckenhorn & T. Szekely (Eds.), *Sex, size and gender roles: Evolutionary studies of sexual size dimorphism* (pp. 16–26). Oxford: Oxford University Press.

Lovich, J. E., & Gibbons, J. W. (1992). A review of techniques for quantifying sexual size dimorphism. *Growth, Development, and Aging, 56*(4), 269–281.

Maddison, W. P., & Maddison, D. R. (2011). *Mesquite: A modular system for evolutionary analysis*. Available at http://mesquiteproject.org.

Near, T. J., Meylan, P. A., & Shaffer, H. B. (2005). Assessing concordance of fossil calibration points in molecular clock studies: An example using turtles. *American Naturalist, 165*(2), 137–146.

O'Meara, B. C., Ane, C., Sanderson, M. J., & Wainwright, P. C. (2006). Testing for different rates of continuous trait evolution using likelihood. *Evolution, 60*(5), 922–933.

Paradis, E. (2006). *Analysis of phylogenetics and evolution with R*. New York: Springer.

Polak, J., & Frynta, D. (2010). Patterns of sexual size dimorphism in cattle breeds support Rensch's rule. *Evolutionary Ecology, 24*(5), 1255–1266.

Pritchard, P. C. H., & Trebbau, P. (1984). *The turtles of Venezuela: Society for the study of amphibians and reptiles*. Athens, OH.

Remes, V., & Székely, T. (2010). Domestic chickens defy Rensch's rule: Sexual size dimorphism in chicken breeds. *Journal of Evolutionary Biology, 23*(12), 2754–2759.

Rensch, B. (1950). Die abhangigkeit der relativen Sexualdifferenz von der Korpergrosse. *Bonner Zoologische Beitraege, 1*, 58–69.

Rensch, B. (1960). *Evolution above the species level*. New York: Columbia University Press.

Revell, L. J. (2010). Phylogenetic signal and linear regression on species data. *Methods in Ecology and Evolution, 1*(4), 319–329.

Revell, L. J. (2011). Phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution, 3*(2), 217–223.

Rohlf, F. J. (2001). Comparative methods for the analysis of continuous variables: Geometric interpretations. *Evolution, 55*(11), 2143–2160.

Schluter, D., Price, T., Mooers, A. O., & Ludwig, D. (1997). Likelihood of ancestor states in adaptive radiation. *Evolution, 51*(6), 1699–1711.

Stephens, P. R., & Wiens, J. J. (2009). Evolution of sexual size dimorphisms in emydid turtles: Ecological dimorphism, Rensch's rule, and sympatric divergence. *Evolution, 63*(4), 910–925.

Stillwell, R. C., Blanckenhorn, W. U., Teder, T., Davidowitz, G., & Fox, C. W. (2010). Sex differences in phenotypic plasticity affect variation in sexual size dimorphism in insects: From physiology to evolution. *Annual Review of Entomology, 55*(1), 227–245.

Stuart-Fox, D. (2009). A test of Rensch's rule in dwarf chameleons (*Bradypodion* spp.), a group with female-biased sexual size dimorphism. *Evolutionary Ecology, 23*(3), 425–433.

Szekely, T., Freckleton, R. P., & Reynolds, J. D. (2004). Sexual selection explains Rensch's rule of size dimorphism in shorebirds. *Proceedings of the National academy of Sciences of the United States of America, 101*(33), 12224–12227.

Tubaro, P. L., & Bertelli, S. (2003). Female-biased sexual size dimorphism in tinamous: A comparative test fails to support Rensch's rule. *Biological Journal of the Linnean Society, 80*(3), 519–527.

Valenzuela, N. (2001). Maternal effects on life-history traits in the Amazonian giant river turtle *Podocnemis expansa*. *Journal of Herpetology, 35*(3), 368–378.

Valenzuela, N., & Adams, D. C. (2011). Chromosome number and sex determination coevolve in turtles. *Evolution, 65*(6), 1808–1813.

Valenzuela, N., Adams, D. C., & Janzen, F. J. (2003). Pattern does not equal process: Exactly when is sex environmentally determined? *American Naturalist, 161*(4), 676–683.

Valenzuela, N., & Lance, V. A. (2004). *Temperature dependent sex determination in vertebrates*. Washington, DC: Smithsonian Books.

van Dijk, P. P., Iverson, J. B., Shaffer, H. B., Bour, R., Rhodin, A. G. J., & Turtle Taxonomy Working Group. (2011). Turtles of the world, 2011 update: Annotated checklist of taxonomy, synonymy, distribution, and conservation status. En: Chelonian research monographs. In A. G. J. Rhodin, P. C. H. Pritchard, P. P. van Dijk, R. A. Saumure, K. A. Buhlmann, J. B. Iverson, & R. A. Mittermeier (Eds.), *Conservation biology of freshwater turtles and tortoises: A compilation project of the IUCN/SSC tortoise and freshwater turtle specialist group* (pp. 000.165–000.241). Lunenburg, MA: Chelonian Research Foundation.

Vargas-Ramirez, M., Castaño-Mora, O. V., & Fritz, U. (2008). Molecular phylogeny and divergence times of ancient South American and Malagasy river turtles (Testudines: Pleurodira: Podocnemididae). *Organisms Diversity & Evolution, 8*(5), 388–398.

Walker, S. P. W., & McCormick, M. I. (2009). Sexual selection explains sex-specific growth plasticity and positive allometry for sexual size dimorphism in a reef fish. *Proceedings of the Royal Society B-Biological Sciences, 276*(1671), 3335–3343.

Webb, G. J. W., Beal, A. M., Manolis, S. C., & Dempsey, K. E. (1987). The effects of incubation temperature on sex determination and embryonic development rate in *Crocodylus johnstoni* and *C. porosus*. In G. J. M. Webb, S. C. Manolis, & P. J. Whitehead (Eds.), *Wildlife management: Crocodiles and alligators* (pp. 507–531). Chipping Norton, NSW: Surrey Beatty & Sons.

Webb, T. J., & Freckleton, R. P. (2007). Only half right: Species with female-biased sexual size dimorphism consistently break Rensch's rule. *PLoS ONE, 2*(9).

Wolak, M. E. (2008). Rensch's rule applies to clinal variation of body size in the argentine grasshopper *Dichroplus pratensis*: Correction of Bidau and Marti. *Annals of the Entomological Society of America, 101*(5), 801.

Young, K. A. (2005). Life-history variation and allometry for sexual size dimorphism in Pacific salmon and trout. *Proceedings of the Royal Society B-Biological Sciences, 272*(1559), 167–172.