

COMPARATIVE ANALYSES OF FUNCTIONAL RELATIONSHIPS IN THE EVOLUTION OF TROPHIC MORPHOLOGY IN THE MAP TURTLES (EMYDIDAE: GRAPTEMYS)

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ABSTRACT: Variation in relative width of the head and alveolar surfaces of the jaws in map turtles (*Graptemys*) is associated with the degree of molluscivory in adult females. Adult males are substantially smaller in body size and are primarily insectivorous, and they take few mollusks regardless of their relative head width or alveolar width. We assessed functional relationships within and between the sexes using comparative analyses (independent contrasts and squared-change parsimony) of the residuals of regressions of head width on plastron length and alveolar width on head width. Residuals of head width and alveolar width were significantly correlated within females but not within males in all comparative analyses. Residuals of each regression were significantly correlated between males and females for a subset of analyses. Our results suggest that evolutionary changes in relative head width have been correlated with changes in relative alveolar width in females of *Graptemys* due to a functional relationship that is related to the tendency to feed on mollusks. Males have exhibited changes in the two characters similar to changes in females over evolutionary time, probably as a result of genetic covariation, but due to males' primarily insectivorous nature, changes in the two characters have not shown significant correlation with one another.

Key words: *Graptemys*; Emydidae; Diet; Ecomorphology; Comparative methods

GRAPTEMYS is a diverse genus of aquatic North American turtles characterized by extreme sexual size dimorphism, with females being larger (Gibbons and Lovich, 1990) and more variable in trophic morphology and diet than males (Lindeman, 2000). Among females of *Graptemys*, Lindeman (2000) recognized four non-overlapping phylogenetic and ecological groupings on the basis of width of the head relative to body size and width of the alveolar surfaces (crushing surfaces of the jaws) relative to head width: (1) four species of the *pulchra* clade (sensu Lamb et al., 1994) had extremely broad heads (megacephaly) and alveolar surfaces; (2) three populations of the monotypic *geographica* clade had moderately broad heads (mesocephaly) and extremely broad alveolar surfaces; (3) three species (six total populations) of the *pseudogeographica* clade were mesocephalic with moderately broad alveolar surfaces; and (4) four species (seven total populations) of the *pseudogeographica* clade had relatively narrow heads (microcephaly) and alveolar surfaces. Among males, groupings based on relative head

and alveolar width were less evident (Lindeman, 2000). Megacephalic adult females have been reported to feed almost exclusively on mollusks whereas mesocephalic adult females tend to feed heavily, but not exclusively, on mollusks, and microcephalic adult females consume few mollusks (reviewed in Lindeman, 2000). In contrast, adult males of all species are primarily insectivorous, exhibit less overall variation in head and alveolar width, and have only rarely been reported to feed heavily on mollusks (Lindeman, 2000).

A functional correlation between relative head and alveolar width has not been demonstrated for *Graptemys*, although it has long been assumed without support from modern comparative analyses (Garman, 1890; Hay, 1892; Lindeman, 2000; Vogt, 1981). We used comparative analyses to assess whether a functional relationship exists between these characters in *Graptemys*. Comparative methods can be used to analyze correlation in species' characters within a phylogenetic framework through recognition of the nonindependence of data points taken from species

comprising a clade (Felsenstein, 1985; Harvey and Purvis, 1991; Huey and Bennett, 1987; Maddison, 1991). These methods have made it possible to study ecomorphological adaptation in an historical context (Losos and Miles, 1994).

We also investigated the possibility that sexual differences exist in the relationship between relative head and alveolar width. In species of *Graptemys* with meso- or megacephalic females, molluscivory has been assumed to be the selective force responsible for the putative functional correlation, yet because males of all species take few mollusks, sexual differences may exist in the relationship of head width to alveolar width.

METHODS

Measurements of midline plastron length (PL), head width at the upper jaw (HW), and maximum alveolar width (AW) of the upper jaw were taken by the senior author on 2278 specimens representing all recognized species and subspecies of *Graptemys* (Ernst et al., 1994). We analyzed both HW/PL and AW/HW relationships for each sex and each species or population using simple linear regression analysis after transformation of variables logarithmically to correct for allometry (Lindeman, 2000). We included juveniles of unknown sex in analyses for both males and females to represent individuals over a full range of body sizes.

Allometric regression analyses yielded slope and intercept values representing the relationship between variables over a range of body sizes. To represent each relationship in a single number for the purpose of comparative analyses, we used two predicted values for both HW at 0.86·(maximum measured PL) and AW at 0.86·(maximum measured HW): (A) the value predicted from the allometric regression equation for the sex and species or population in question (Lindeman, 2000), and (B) the value predicted from an average equation for the variable and sex in question, derived by averaging the slope and intercept values for relationships of HW/PL or AW/HW over all 20 data sets. Residuals were calculated as A - B.

We used values at 86% of maximum PL or HW to represent an approximation of median body size of adults; on average, female turtles mature at 72% of maximum PL (Shine and Iverson, 1995), and values for males of *Graptemys* are similar (P. Lindeman, unpublished data). Due to small sample size ($n = 2$), means of PL and HW were used in lieu of the above calculations for males of *G. geographica* from Alabama.

Although phylogenetic comparative analyses (see below) were used, we chose not to compute residuals via phylogenetic comparative methods (see Martins and Garland, 1991:555) because of a confounding relationship between body size (PL) and categorization of a species as micro-, meso-, or megacephalic. Although categorizations were based on relative head width, megacephalic females have the largest body sizes and microcephalic females have the smallest body sizes, exaggerating the differences in absolute head width (Lindeman, 2000). For the purposes of simple illustration, the problem this introduces into residual analysis is best outlined by consideration of a nonphylogenetic analysis using residuals (Fig. 1). If simple linear regression were used on the relationship between \log_{10} (predicted HW) and \log_{10} (0.86·maximum PL) in females, the regression line used for calculation of residuals would have a slope (1.85) markedly different from the 20 individual regressions (range of slopes 0.62–1.03; Lindeman, 2000). If the 20 slopes and 20 intercepts are averaged, then the line used for calculation of residuals has a slope (0.78) intermediate to slopes of the 20 individual regressions. A similar problem would be expected to occur in phylogenetic generation of residuals. Hence, the averaging method outlined above, while not phylogenetic, retained a biologically-realistic relationship between the variables in generating residual values which could then be analyzed for correlation phylogenetically (see below).

The phylogeny used in the present analysis (Fig. 2) was a modified version of a phylogeny of *Graptemys* based on analysis of mtDNA (Lamb et al., 1994). We re-

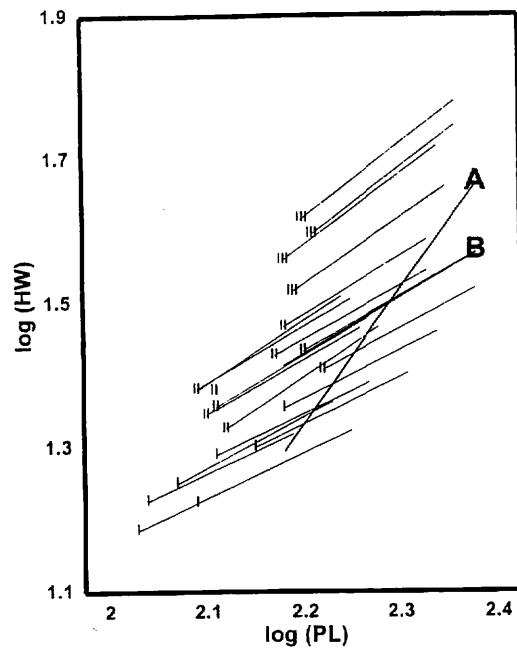


FIG. 1.—Twenty allometric regressions of head width (HW) on plastron length (PL) for data on adult females (defined as ranging in PL from 72–100% of maximum measured PL) of species or populations of *Graptemys*. Labels to the left of each line indicate whether females of the species are classified as microcephalic (I), mesocephalic (II), or megacephalic (III; Lindeman, 2000). Also shown are lines representing two potential methods for deriving a set of residuals: (A) regressing estimated $\log_{10}(\text{HW})$ on $\log_{10}(0.86 \cdot (\text{maximum PL}))$, and (B) averaging slopes and intercepts of the 20 regression equations. Note that the slope of line A (1.85) is much steeper than slopes of individual data sets (range 0.62–1.03, mean = 0.78; see text).

solved a trichotomy involving *G. nigrinoda* by placing it in as close a relationship as possible with *G. flavimaculata* and *G. oculifera*. Such a solution is suggested by anatomical similarities among these three “sawbacked” species (Cagle, 1954; Dobie, 1981; Mount, 1975) and their occurrence in adjacent river drainages (Ernst et al., 1994). Branch lengths were the number of character changes in Lamb et al. (1994: their Fig. 6). We inserted branches with a length of 1.0 into the tree to separate multiple populations of some taxa. Placement of inserted branches for taxa with three or four populations was based on subspecies status and geographical proximity.

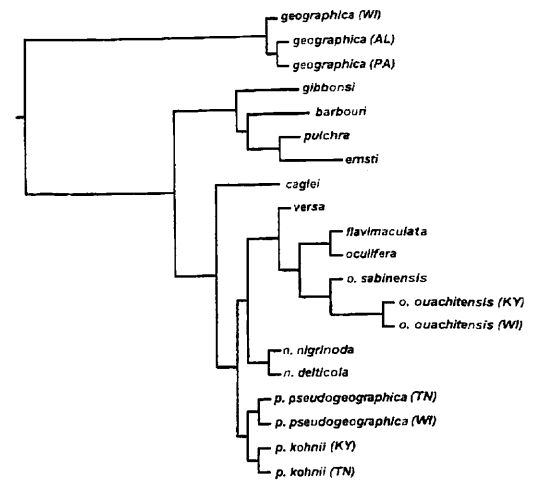


FIG. 2.—Phylogeny of species and populations of *Graptemys* used in the present study, modified from Lamb et al. (1994) to include multiple populations of some species (see text).

We used residual values in both independent contrasts (Felsenstein, 1985) and squared-change parsimony (Huey and Bennett, 1987; Maddison, 1991) to analyze the correlation of four relationships in residuals: (1) female HW versus female AW; (2) male HW versus male AW; (3) female HW versus male HW; and (4) female AW versus male AW. We conducted analyses in the programs CONTRAST and PARSCONT in COMPARE 2.0 (distributed by E. Martins via the WWW at <http://evolution.uoregon.edu/~emartins/EE/COMPARE> or via anonymous ftp to [work.uoregon.edu](ftp://work.uoregon.edu), Department of Biology, University of Oregon, Eugene, Oregon, U.S.A.). In these programs, all comparisons are nondirectional (sensu Harvey and Purvis, 1991); i.e., values generated for correlation analyses were computed as the difference between immediately-adjacent nodes.

Two types of analysis were conducted for each data set in both independent contrasts and squared-change parsimony. Branch lengths as modified from Lamb et al. (1994) were used in “gradualist” analyses and were set equal to 1.0 in “punctuational” analyses (Martins and Garland, 1991). For comparative purposes, simple Pearson correlation coefficients (“TIPS”

TABLE 1.—Allometric regression equations describing the log-log relationships of head width to plastron length and alveolar width to head width, derived by averaging slopes and intercepts of allometric regression equations for 20 populations of *Graptemys*.

Sex	Dependent variable	Slope	Intercept
Female	Head width	0.780	-0.288
Female	Alveolar width	1.109	-0.775
Male	Head width	0.511	0.148
Male	Alveolar width	1.113	-0.779

analyses: Martins and Garland, 1991) were also computed for each relationship. Within each method of analysis, each of the four data sets was used twice, hence alpha was interpreted at 0.025.

RESULTS

Mean slope and intercept values used to generate genus-wide regressions and residual values are given in Table 1. Correlation between HW and AW residuals in females was significant in all four comparative analyses, whereas correlation was not significant in any of the four analyses for males (Table 2). Correlation between the sexes in HW residuals was significant in both independent-contrasts analyses and in the punctuational squared-change parsimony analysis. Correlation between the sexes in AW residuals was highly significant in both squared-change parsimony analyses, but not significant in either independent-contrasts analysis.

In general, choice of analysis by independent contrasts versus squared-change parsimony or use of gradualist versus punctuational models made little difference in the correlation coefficients of with-

in-sex analyses, whereas correlation coefficients varied substantially according to the comparative method employed in between-sex analyses for both HW and AW residuals (Table 2). TIPS analysis resulted in substantially higher correlation coefficients for all four analyzed relationships, except when compared with independent-contrasts analyses of the correlation in HW residuals between the sexes.

DISCUSSION

Comparative analyses revealed overall weaker correlation among variables representing the trophic morphology of *Graptemys* than was shown through simple TIPS analyses. Martins and Garland (1991) also found weaker correlation when using comparative analyses as compared to TIPS, and they strongly cautioned against use of nonphylogenetic correlation analyses. Our findings demonstrate phylogenetic signal in the data, as would be expected given the finding that micro-, meso-, and megacephaly are highly clustered within the phylogeny of *Graptemys*, with few transitions from one character state to another (Lindeman, 2000).

Correlation coefficients were similar regardless of whether or not branch lengths from Lamb et al. (1994) were used or all set to 1.0, and for within-sex analyses, little difference was noted in correlation coefficients between independent contrasts and squared-change parsimony. Harvey and Purvis (1991) previously contrasted results of these two methods, using an example in which *P*-values changed from significance under one method to nonsignificance under the other. In computer sim-

TABLE 2.—Correlation of residual values of the relationships of head width (HW) to plastron length and alveolar width (AW) to HW using five different methods of analysis. TIPS = simple Pearson correlation coefficient. In gradualist analyses, heterogeneous branch lengths were used, while in punctuational analyses, all branch lengths were set to 1.0.

Variables	TIPS	Independent contrasts, gradualist	Independent contrasts, punctuational	Squared-change parsimony, gradualist	Squared-change parsimony, punctuational
Female HW/AW	0.80**	0.55*	0.59**	0.62**	0.59**
Male HW/AW	0.63**	0.34	0.33	0.38	0.31
Female HW/Male HW	0.78**	0.76**	0.75**	0.43	0.49*
Female AW/Male AW	0.92**	0.31	0.44	0.81**	0.82**

* *P* < 0.025; ** *P* < 0.01.

ulations, Martins and Garland (1991) found that independent contrasts outperformed squared-change parsimony and that gradualist models outperformed punctuational models, although differences were not dramatic in either case.

All comparative analyses revealed a strong functional relationship between head width and alveolar width in females of *Graptemys*. The relationship undoubtedly is strongly influenced by selective pressures involving the biomechanical forces required for crushing mollusk shells in meso- and megacephalic females. As relative head width in females increases, the proportion of the roof of the mouth occupied by alveolar surfaces also increases (Lindeman, 2000), to the point that females with the broadest heads have alveolar surfaces that meet along their median edges in the anterior half of the roof of the mouth (P. Lindeman, personal observation). While the extent to which relative width of the head and alveolar surfaces is genetically versus environmentally determined has not been studied in *Graptemys*, differences in these aspects of trophic morphology between sympatric species are evident in hatchlings (Lindeman, 2000), suggesting partial if not complete genetic determination.

In contrast to the results for females, evolutionary increases or decreases in male head width do not appear to have been associated with corresponding increases or decreases in alveolar width. We interpret the lack of significant correlation in males to be the result of the general paucity of mollusks in male diets (reviewed in Lindeman, 2000). Males mature several years earlier than females (Lindeman, 1999) and are much smaller than females as adults (Gibbons and Lovich, 1990). Broadening of the head and alveolar surfaces in such small turtles may be constrained from reaching a threshold that would enable them to be significant molluscivores. Correlation in trophic morphology between the sexes may result largely from genetic covariance.

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