doi:10.1111/j.1558-5646.2010.01118.x

# ADAPTIVE EVOLUTION OF PLASTRON SHAPE IN EMYDINE TURTLES

**Kenneth D. Angielczyk,[1,2] Chris R. Feldman,[3,4] and Gretchen R. Miller[5,6]**

[1]*Department of Geology, The Field Museum, 1400 South Lake Shore Drive, Chicago, Illinois 60605*

[2]*E-mail: kangielczyk@fieldmuseum.org*

[3]*Department of Natural Resources and Environmental Science, University of Nevada Reno, 1664 North Virginia Street, Reno, Nevada 89557*

[4]*E-mail: ophis@cabnr.unr.edu*

[5]*Shelburne Community School, 345 Harbor Road, Shelburne, Vermont 05482*

[6]*E-mail: gretchen.rebecca@gmail.com*

Received November 9, 2009
Accepted August 18, 2010

**Morphology reflects ecological pressures, phylogeny, and genetic and biophysical constraints. Disentangling their influence is fundamental to understanding selection and trait evolution. Here, we assess the contributions of function, phylogeny, and habitat to patterns of plastron (ventral shell) shape variation in emydine turtles. We quantify shape variation using geometric morphometrics, and determine the influence of several variables on shape using path analysis. Factors influencing plastron shape variation are similar between emydine turtles and the more inclusive Testudinoidea. We evaluate the fit of various evolutionary models to the shape data to investigate the selective landscape responsible for the observed morphological patterns. The presence of a hinge on the plastron accounts for most morphological variance, but phylogeny and habitat also correlate with shape. The distribution of shape variance across emydine phylogeny is most consistent with an evolutionary model containing two adaptive zones—one for turtles with kinetic plastra, and one for turtles with rigid plastra. Models with more complex adaptive landscapes often fit the data only as well as the null model (purely stochastic evolution). The adaptive landscape of plastron shape in Emydinae may be relatively simple because plastral kinesis imposes overriding mechanical constraints on the evolution of form.**

**KEY WORDS: Brownian motion, morphological evolution, Ornstein–Uhlenbeck model, phylogenetic scale, shell kinesis, Testudinoidea.**

Understanding how and why phenotypes evolve requires knowledge of the heritable and environmental components underlying traits, as well as the roles of various and often competing evolutionary forces (Falconer and Mackay 1996; Futuyma 1998). Stochastic processes such as drift and mutation, deterministic forces such as sexual and viability selection, and evolutionary constraints such as trade-offs, genetic covariances, developmental "rules," and biophysical limits, all influence the rate and trajectory of morphological evolution (Maynard Smith et al. 1985; Wake 1991; Arnold 1992; Schluter 1996; Futuyma 1998; DePristo et al. 2005; Brakefield 2007; Roff and Fairbairn 2007). Assessing the forces responsible for producing morphological patterns in natural systems can be difficult because the selection pressures, genetic architecture, and ontogenies of traits are rarely known (Feder and Mitchell-Olds 2003; Ellegren and Sheldon 2008; Stinchcombe and Hoekstra 2008). One solution to this obstacle is to use techniques that can characterize patterns of morphological variation and relate this variation to potential causal variables. Geometric morphometrics (e.g., Bookstein 1991, 1996; 1998; Rohlf and Marcus 1993; Rohlf 1998; Adams et al. 2004; Zelditch et al. 2004) is especially useful in this regard because it can quantify the shapes of organisms or their parts, and also provides a set

of statistical tools for examining patterns of covariance between shape and other variables. Thus, geometric morphometrics is ideally suited for elucidating the factors that influence morphological evolution (e.g., Myers et al. 1996; Monteiro and Abe 1999; Adams and Rohlf 2000; Caumul and Polly 2005; Webster and Zelditch 2005; Goswami 2006; Myers et al. 2006; Stayton 2006; Pierce et al. 2008, 2009; Polly 2008).

The turtle shell is one of the most recognizable evolutionary novelties among tetrapods. As a fundamental component of the turtle phenotype, the shell is influenced by different and potentially competing selective pressures. For example, selection promoting a light, hydrodynamic shell in semi-aquatic turtles may directly oppose selective forces favoring a robust, domed shell suitable for protection or self-righting behavior (Domokos and Várkonyi 2008). There is a broad correspondence between shell morphology and functional and environmental factors that spans most of turtle diversity (e.g., Romer 1956; Zangerl 1969; Claude et al. 2003; Depecker et al. 2006; Renous et al. 2008), and this pattern implies the existence of morphological adaptive zones in which particular shell shapes are optimal. However, the correlations are not exact (Pritchard 2008), suggesting that not all turtle species respond to selective pressures in the same way, or that different combinations of selective pressures and evolutionary constraints can produce disparate results. Given these observations, a pertinent question to ask is whether we can characterize the adaptive landscape for shell shape in specific groups of turtles.

A particularly dramatic specialization of the turtle shell is the development of a mechanical hinge on the ventral shell (plastron). This trait, known as plastral kinesis, is relatively rare among living chelonians. In most turtles the plastron is rigid and immovable (Fig. 1) but turtles with advanced plastral kinesis can pull the bottom shell up towards the top shell (carapace) to more securely retract the extremities and in some cases completely seal the turtle from the outside (e.g., Bramble 1974; Pritchard 2008) (Fig. 1). Hence, plastral kinesis is widely viewed as an anti-predator adaptation. Plastral kinesis has evolved repeatedly among living turtles: once within pleurodires (Bramble and Hutchison 1981), twice within kinosternids (Bramble et al. 1984), and as many as six times in testudinoids, in which it has appeared at least twice in testudinids (tortoises; see Ernst and Barbour 1989), two times in geoemydids (old world pond turtles; Bramble 1974; Claude 2006), and probably two times in emydids (new world pond turtles; Feldman and Parham 2002).

Recently, there has been increasing interest in using geometric morphometric techniques to investigate factors that affect turtle shell shape (Magwene 2001; Claude et al. 2003; Valenzuela et al. 2004; Claude 2006; Myers et al. 2006, 2007; Rivera 2008; Rivera and Claude 2008; Stayton 2009). Of particular relevance to the present study, Claude et al. (2003) and Claude (2006) used

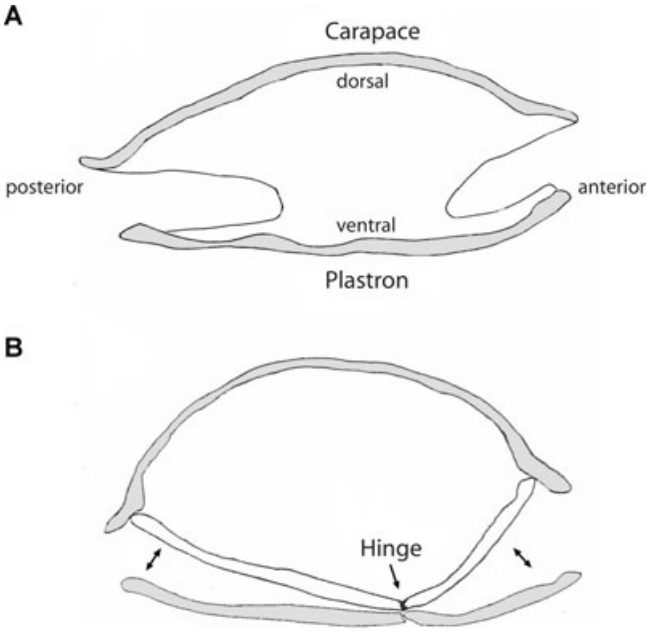


**Figure 1. Sagittal view of turtle shells. (A) Typical turtle shell lacking plastral kinesis; the rigid plastron is firmly attached to the carapace by a solid bony bridge (modified from Meylan and Gaffney 1989). (B) Turtle shell exhibiting advanced plastral kinesis; the hinged plastron, shown in both open and closed positions, is loosely attached to the carapace (modified from Bramble 1974).**

geometric morphometrics to investigate the effects of habitat preference, phylogeny, and plastral kinesis on shell morphology in the Testudinoidea (*sensu lato*) (i.e., not including the Platysternidae; Parham et al. 2006). Claude and colleagues determined that all three factors significantly influenced shell shape, but the presence or absence of a hinge accounted for the largest proportion of plastron shape variation, followed by habitat preference and phylogeny. However, phylogenetic data available for testudinoids at the time did not allow for a detailed treatment of phylogeny in their analyses, and their sample sizes for some species were small (in some cases, one or two specimens), potentially resulting in inaccurate mean shapes for species (e.g., Polly 2003a, 2005).

Given these results, an important question to ask is whether the patterns observed when considering all testudinoids remain the same when specific testudinoid lineages are examined individually. In other words, is shape variation is structured in a comparable way at different phylogenetic scales, particularly when detailed phylogenetic information is included and species means are estimated from large samples of specimens? If turtle evolution is highly restricted by mechanical and genetic constraints, then we might expect to find similar relationships between shell shape and factors such as habitat preference or plastral kinesis regardless of the phylogenetic scale. Alternatively, if different turtle lineages possess some flexibility in their response when faced with common environmental or functional problems, then

the relationship between shell shape and factors such as kinesis could vary from group to group. Under such a scenario, the composite pattern that emerges from a comparison of shape variation in turtles across clades might be different than the pattern if only members of a single clade were examined, potentially masking complexity in the relationship between shape and other factors of interest. Previous investigations of morphological variation at different phylogenetic scales in other taxa have shown that patterns apparent at one scale do not always hold when examined at another (Ashton 2001; Blackburn and Ruggeiro 2001; Cruz et al. 2005; Hone and Benton 2007; Finarelli 2008; Novack-Gottshall and Lanier 2009).

We use geometric morphometrics to investigate patterns of plastron shape variation in emydine turtles. Emydinae is a small clade of testudinoid turtles consisting of at least 10 extant species (possibly 11; Fritz et al. 2005), and a handful of extinct species (Mylnarski 1956; Holman 1995, 2002; Holman and Fritz 2001, 2005). Although the clade inhabits a broad geographic range, and is morphologically and ecologically diverse, its monophyly is well supported by morphological and molecular data (Gaffney and Meylan 1988; Stephens and Wiens 2008; Spinks et al. 2009). Emydines are particularly relevant to the question of whether the relationships between shell shape, plastral kinesis, habitat preference, and phylogeny show constancy across phylogenetic scales because their evolutionary history parallels larger patterns of testudinoid evolution, with different emydines adapting to terrestrial or aquatic habitats (Stephens and Wiens 2003), and independent acquisitions of plastral kinesis (Feldman and Parham 2002).

Here, we first address the question of whether scale matters. Specifically, we investigate whether the effects on plastron shape of the causal variables habitat preference, phylogeny, and plastral kinesis are similar in Emydinae and its more inclusive parent clade, the Testudinoidea. To do this, we use geometric morphometrics to document the main patterns of plastron shape variation in emydines, and then use path analysis to examine the relationship between shell shape, habitat preference, phylogeny, and plastral kinesis. Understanding the main components of emydine plastron shape variation and their relationship to likely causal variables is also important because it provides a framework for addressing additional questions about the evolution of the turtle shell (e.g., Angielczyk and Parham 2005; Angielczyk 2007; Angielczyk and Sheets 2007; Stayton 2009). Second, we attempt to explicitly model the role of selection in shell shape evolution. We use the modeling approach of Hansen (1997), as applied by Butler and King (2004), to determine whether the observed patterns of shell shape variation in emydines follow a null model of stochastic evolution, or are more consistent with various models of adaptive evolution (i.e., global stabilizing selection versus divergent selective regimes).

**Table 1. Sample size, habitat type, and presence or absence of plastral kinesis for the emydine turtles and two outgroup species.**

| Lineage species | Specimens sampled *n* | Habitat | Plastron |
|---|---|---|---|
| Emydinae | | | |
| *Clemmys guttata* | 301 | aquatic | akinetic |
| *Emys blandingii* | 103 | aquatic | kinetic |
| *Emys marmorata* | 769 | aquatic | akinetic |
| *Emys orbicularis* | 73 | aquatic | kinetic |
| *Glyptemys insculpta* | 98 | terrestrial | akinetic |
| *Glyptemys muhlenbergii* | 81 | aquatic | akinetic |
| *Terrapene carolina* | — | terrestrial | kinetic |
| *Terrapene coahuila* | 26 | aquatic | kinetic |
| *Terrapene nelsoni* | — | terrestrial | kinetic |
| *Terrapene ornata* | 105 | terrestrial | kinetic |
| Deirochelyinae | | | |
| *Chrysemys picta* | 115 | aquatic | akinetic |
| *Deirochelys reticularia* | — | aquatic | akinetic |

# Materials and Methods

## MATERIALS

We collected morphometric data from 1671 specimens; 1556 specimens belonged to eight extant emydine species, and 115 specimens belonged to the deirochelyine outgroup species *Chrysemys picta* (Appendix S1). We did not collect morphometric data for *Terrapene carolina* because of the considerable morphological differences between the subspecies, which may actually represent a paraphyletic assemblage of lineages (Spinks et al. 2009), or for *T. nelsoni* because of a paucity of material in the collections we visited. However, our sample included *T. coahuila* and *T. ornata*, the most dedicated aquatic and terrestrial members of the genus, respectively. Our sampling of the species differed greatly (Table 1) because of specimen availability and the fact that some data were used in previous analyses (e.g., Angielczyk and Sheets 2007). Samples for all species include a mixture of males and females, and represent growth series ranging from hatchlings to large adults. The majority of specimens in the dataset are alcohol-preserved, although some dried or skeletonized specimens and live individuals also were used. We did not notice any differences in the size or shape of turtle shells due to method of preservation.

## PHYLOGENETIC ANALYSES AND ANCESTRAL STATE RECONSTRUCTION

Comparative analyses require phylogenetic information to accommodate similarity due to common ancestry and to reconstruct patterns of trait change (Felsenstein 1985; Garland et al. 2005). Although the systematics of emydine turtles has been extensively

studied (Gaffney and Meylan 1988; Bickham et al. 1996; Burke et al. 1996; Feldman and Parham 2002; Stephens and Wiens 2003, 2008; Spinks and Shaffer 2009; Spinks et al. 2009), the model-based approach we employed (Butler and King 2004) required particular parameter estimates. Specifically, we needed branching order, divergence times, and ancestral state reconstructions (ASR), to properly model trait change over time due to selection, drift, and changes in the selective regime (see subsequently). Therefore, we used two previously published molecular datasets to infer emydine relationships, and then conducted ASR and estimated evolutionary distances. We analyzed the mitochondrial dataset (two markers, 2.1 Kb) of Feldman and Parham (2002) and the nuclear dataset (seven markers, 5.8 Kb) of Spinks et al. (2009). Because these datasets included different numbers of terminal taxa sampled from different locations, we pruned the more extensive nuclear matrix to match the mitochondrial data, retaining single terminal taxa from the same geographic regions in both datasets (Appendix S2). However, we kept two representatives of *Emys marmorata* and *E. orbicularis* in the nuclear dataset because these taxa display substantial geographic variation (Spinks and Shaffer 2005; Fritz et al. 2007) and removing this variation lead to dubious topologies in preliminary analyses. In addition, we added nuclear data (molecular methods reported in Spinks et al. 2009) for *Terrapene nelsoni* (GenBank numbers HQ266660-HQ266665), so that both mitochondrial and nuclear datasets possessed all 10 extant emydines and the same two deirochelyine outgroup species. We analyzed the datasets separately because each likely contains unique historical information; in fact, an ancient reticulation between *Emys* species may be responsible for possible discordant signal between the two genomes (Spinks and Shaffer 2009).

To infer phylogenetic relationships among emydines and simultaneously estimate divergence times, we constructed "chronophylogenetic" trees in BEAST 1.4.8 (Drummond and Rambaut 2007). A chronophylogenetic tree is simply a phylogenetic hypothesis in which branch lengths represent units of time separating taxa. We conducted Bayesian chronophylogenetic analyses on both mtDNA and nucDNA datasets under a relaxed molecular clock model, which allows substitution rates to vary across branches according to an uncorrelated lognormal distribution (Drummond et al. 2006; Drummond and Rambaut 2007). The fossil record for emydines may be problematic (Holman 2002; Parham and Irmis 2008), so rather than using fossil calibrations points we simply set the mean of branch rates to 1 (ucld.mean). Here, time is measured in arbitrary units such that one unit of time is the mean time required for one substitution to exist per site (Drummond et al. 2006; Drummond and Rambaut 2007). This results in agnostic time values, and we simply scaled the root of the phylogeny to 1, providing a relative measure of divergence times. We performed Bayesian phylogenetic analyses in BEAST under the GTR + I + Γ substitution model (Rodriguez et al. 1990; Yang 1994; Gu et al. 1995), the best fit substitution model as determined by the Akaike Information Criterion (AIC) in Modeltest 3.7 (Posada and Crandall, 1998). We ran analyses for 10 million generations and sampled trees every 1000 generations. We then computed the maximum credibility tree in TreeAnnotator 1.4.8 (Drummond and Rambaut 2007), after excluding trees sampled prior to the stable equilibrium.

We also estimated the amount of evolutionary change separating taxa using simple pairwise sequence divergences. We calculated ML-corrected (GTR + I + Γ) sequence distances between taxa in PAUP * 4.0b10 (Swofford 2002).

To understand the evolution of shell morphology in emydines as it pertains to plastral kinesis and habitat preference, we reconstructed the pattern of character changes for both kinesis and habitat on our ML phylogenetic hypotheses. We scored each OTU as either akinetic or kinetic, and as either aquatic or terrestrial, based on descriptions from the literature (Bramble 1974; Ernst and Barbour 1989; Ernst and Lovich 2009). Our treatment of these variables closely follows that of Claude et al. (2003) and Claude (2006) to maintain consistency with those studies. Bramble (1974) carefully detailed kinesis in emydines and noted minor differences in the hinging mechanisms among kinetic species of *Emys* and *Terrapene*. However, this variation is relatively trivial compared to the major differences between kinetic and akinetic species. Therefore we coded shell kinesis as a simple binary character: akinetic or kinetic (see Claude 2006) (Table 1). We also treated habitat preference as a two state trait: aquatic or terrestrial. We recognize that the boundary between these states is somewhat indistinct because most emydines are proficient in water and on land, and a number of species split time between aquatic and terrestrial habitats (e.g., *E. marmorata*, *G. insculpta*) depending on the location, season, or even their age (Ernst et al. 1994; Ernst and Lovich 2009). To simplify this complex trait, we followed Claude et al. (2003) and coded species as either aquatic or terrestrial depending on presence or absence of webbing between the digits (Table 1). We then conducted ASR by tracing characters on the maximum credibility Bayesian trees in Mesquite 2.71 (Maddison and Maddison 2009). We used the maximum parsimony method of ASR (Schluter et al. 1997) and formally considered character transitions to be unordered (Fitch parsimony), although the latter point is trivial because both characters are binary. One character or the other was assigned to a node if it created fewer steps, otherwise the node was considered equivocal. We then used these reconstructions to test various models of adaptive evolution (see subsequently).

### MORPHOMETRIC DATA COLLECTION AND SUPERIMPOSITION

We quantified plastron shape using two-dimensional, landmark-based geometric morphometrics. Although the turtle plastron is

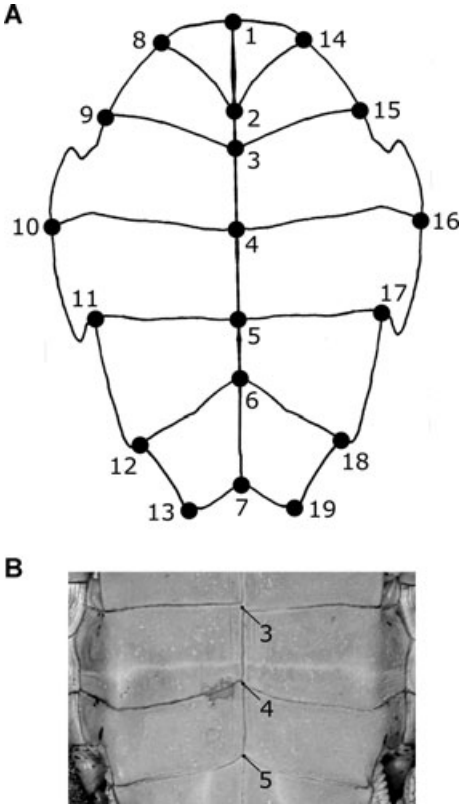


**Figure 2.** **Images of an emydine plastron (bottom shell). (A) Drawing showing the arrangement of keratinous scales on the ventral surface of the plastron of *Emys marmorata*, and the positions of the 19 landmarks used in this study (anterior to the top). (B) Close-up of the plastron of an *E. marmorata* specimen (CAS 50473) demonstrating protocol for landmark placement along the midline when the junctions between scales were not completely square. In such cases, the landmark was placed at the midpoint of the seam separating the two most strongly offset scales.**

not perfectly flat (e.g., males of most of species displayed some concavity along the posterior midline of the plastron), the amount of deformation introduced by minor topography is relatively negligible when projecting the three-dimensional object into a two-dimensional plane, and should not bias our analyses. We photographed each specimen in ventral view, with the specimen oriented so that the plane formed by the surface of the plastron was parallel to the camera lens, and included a ruler in each photograph to record scale. We digitized a total of 19 landmarks (Fig. 2) on the digital images using TpsDig 2.04 (Rohlf 2005). Seventeen of these landmarks are endpoints of or intersections between the sulci that delineate the keratinous plastral scutes (the enlarged, specialized scales that cover the shell). The remaining two landmarks represent extremal points on the margins of the anal scutes. Twelve of the landmarks are bilaterally symmetric. To avoid inflating degrees of freedom in the statistical analyses, among other problems (Kingenberg et al. 2002), we reflected symmetric landmarks from one side of each specimen onto the other and calculated the average position for each pair of landmarks. We carried out subsequent analyses on these "half" specimens. In some cases, damage or incompleteness prevented digitization of both members of a symmetric pair of landmarks. In those instances, we used the coordinates of the undamaged member of the pair instead of an average.

Following data collection, we superimposed the configurations of landmarks for all specimens using generalized Procrustes analysis (Rohlf 1990; Bookstein 1991) in the program CoordGen 6d (Sheets 2002) to remove the effects of position, orientation, and scale from the dataset. We next derived partial warp and uniform component scores (e.g., Bookstein 1989, 1991, 1996, 1998; Rohlf 1998; Zelditch et al. 2004) for the specimens. Partial warp and uniform component scores describe shape differences between specimens and a reference shape, in this case, the shape of the mean specimen in the dataset. The two uniform components describe variation that represents uniform (or affine) transformations of shape that affect all parts of a specimen equally, whereas partial warp scores describe progressively more localized, nonuniform shape variation.

## PATH ANALYSIS OF FACTORS AFFECTING PLASTRON SHAPE

Claude et al. (2003) considered the effects of phylogeny and habitat preference in their analysis of shell shape among testudinoids, and Claude (2006) added plastral kinesis to the list of factors under consideration. The results of these analyses, particularly those of Claude (2006), indicated that plastral kinesis was the major determinant of plastron shape in testudinoids, consistently explaining more variance than habitat preference or phylogeny within the major testudinoid groups. As mentioned previously, we wanted to test whether analyzing equivalent data at a different phylogenetic scale (i.e., within Emydinae as opposed to across Testudinoidea) would alter our interpretation of the results. However, we also wanted to include phylogeny in a more sophisticated manner than the categorical approach used by Claude et al. (2003) and Claude (2006). Here we incorporate actual phylogenetic data (molecular genetic data) to more explicitly treat phylogeny.

We used path analysis (e.g., Wright 1921, 1968; Sokal and Rohlf 1995), a type of structural equation modeling (e.g., Ullman in Tabachnik and Fidell 2007), to quantitatively assess the relationships between plastron shape and phylogeny, habitat preference, plastral kinesis, and maximum adult size. Path analysis allows the variation in one or more criterion variables (in this case plastron shape variation) to be partitioned among a set of discrete and/or continuous predictor variables. Although the method assumes that causal relationships between the predictor and criterion variables can be described using an additive linear model, path analysis differs from techniques like multiple regression because

it does not require the predictor variables to be independent of one another. The latter assumption is useful when dealing with biological data because nonindependence due to common descent (Cheverud et al. 1985; Felsenstein 1985) can influence shape directly (e.g., shared genetic covariances), as well as indirectly through interactions with other factors such as habitat preference or adult body size. The assumption of a linear relationship between predictor and criterion variables is perhaps more problematic for biological data, but unless the relationship is strongly nonlinear, violation of this assumption will primarily result in underestimation of the strength of the effect of a given predictor variable (Wright 1968). Path analysis has a long history of use in biometric studies (e.g., Wright 1968; Crespi and Bookstein 1989), and has been applied to geometric morphometric data (Caumul and Polly 2005).

Because plastral morphologies associated with kinesis are usually most strongly expressed in adult individuals (e.g., the plastral hinge of *T. ornata* does not become functional until about 4 years of age; Ernst et al. 1994), our path analysis focused on large adult plastron shapes for the eight sampled emydine species. Instead of conducting the path analysis on the plastron shape of the largest specimen of each species or the mean shape of a subset of large individuals, however, we instead chose to use the mean shape of all of the specimens of a given species after their shapes had been standardized to that of a large adult of that species. To accomplish this standardization, we regressed the Procrustes superimposed landmark coordinates for all sampled specimens of a given species against the natural logarithm of centroid size, and recorded the residuals for each specimen. We then added the residuals for the specimens to the shape predicted by the regression at the size of the largest individual of the species in the dataset, essentially modeling the shape of each individual at that (large) size (Zelditch et al. 2003, 2004). The standardization procedure assumes a linear relationship between size and shape, and we have not identified evidence to suggest that this assumption is violated. The regression fit the shape data well for each species (Table S1). In addition, we conducted a principal components (PC) analysis of the specimens assigned to each species, and then examined the relationship between the scores on each PC axis and the natural logarithm of centroid size. For each species, PC1 showed a strong linear relationship with centroid size, but PC scores on the higher axes did not show a relationship (linear or otherwise), a result consistent with multivariate linearity (Zelditch et al., 2004). It is important to note that because of this standardization procedure, our results are only applicable to adult plastron shapes, and do not consider whether the effects of kinesis and the other causal variables change over ontogeny.

The shape variables used in the path analysis as criterion variables were PC scores for the shape-standardized mean specimens of the eight emydine species under consideration. To generate these scores, we first Procrustes superimposed all of the shape-standardized individuals belonging to a given species, and calculated the mean shape for that species. We then superimposed the resulting eight species means, ran a PCA on the covariance matrix of the partial warp and uniform component scores of the eight mean specimens, and recorded the scores of the means on the first seven PC axes. Together, these seven PCs described 98.3% of the shape variation in the dataset. Following Polly (2003a, b) and Caumul and Polly (2005), we standardized the PC scores to a mean of zero and a variance equal to the proportion of shape variation described by each PC axis.

We used four predictor variables in the path analysis: plastral kinesis, habitat preference, adult body size, and phylogeny. We coded plastral kinesis and habitat preference as binary categorical variables (see previously mentioned), and we treated body size and phylogeny as continuous variables. For maximum body size, we used the natural logarithm of the centroid size of the largest specimen in each species (i.e., the centroid size used in the shape standardization process for each species). The second continuous variable was genetic divergence, which we used as a proxy for phylogeny. Specifically, we used GTR + I + $\Gamma$ corrected pairwise distances between the eight taxa. Because of our separate treatment of nuclear and mitochondrial data in the phylogenetic analyses, we calculated a set of distances for both datasets. The two path diagrams (one including phylogenetic distances based on nuclear DNA and the other mitochondrial DNA) are shown in Figure 3.

The set of simultaneous equations describing the path diagram is relatively simple in this case, thus we calculated the path coefficients (see Fig. 3) by solving the sets of equations by hand. Doing this requires the correlations between the various predictor variables, and between the predictor variables and the criterion variables. We computed the correlations between phylogeny and size, phylogeny and shape, and phylogeny and kinesis as matrix correlations. We converted the size differences between the species, and the differences between the PC scores for the mean specimens of the eight species on each of the seven PC axes into Euclidean distance matrices so we could determine a matrix correlation. To compare phylogeny and the discrete binary character plastral kinesis, we used the method described by Burgman (1987) and Legendre and Fortin (1989) for transforming a discrete character into a distance matrix (by coding cells in the matrix as 1 when taxa share the same state and 0 when they possess differing states) for use in a Mantel test of matrix correlation. We calculated correlations between plastral kinesis and shape, and habitat preference and shape, as the square-root of the multiple coefficient of determination from an analysis of variance (ANOVA). We calculated the correlation between size and shape as the square-root of the multiple coefficient of determination from a multivariate regression of shape on the natural

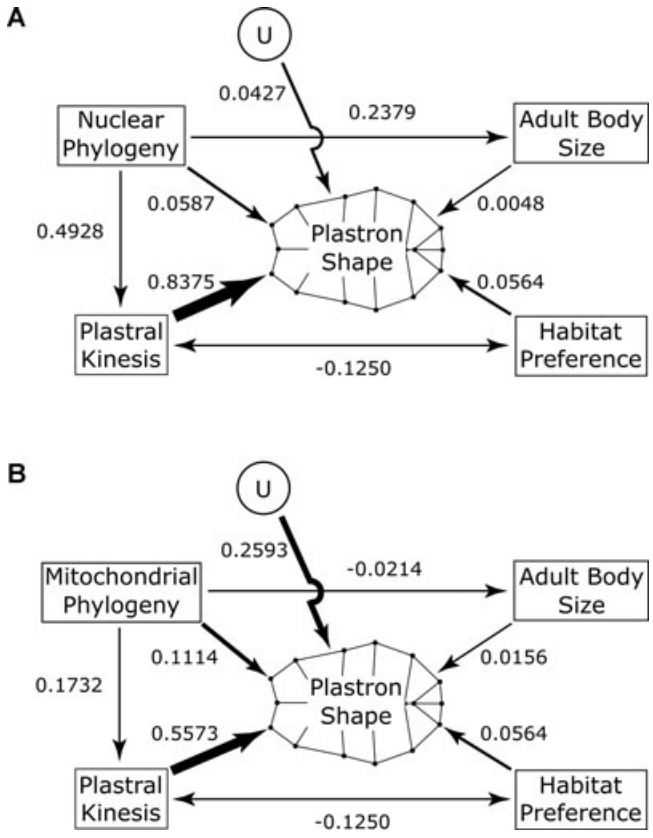


**Figure 3. Diagram showing the path model used in the analysis, as well as the contributions of phylogeny, body size, habitat preference, and kinesis (predictor variables) to plastron shape variance (criterion variable). Path analyses were conducted separately using phylogenetic distances based on the nucDNA (A) and mtDNA (B); see Materials and Methods for details on correlations and pairwise associations used for the path model. The thickness of the paths leading to plastral kinesis are scaled to reflect the relative strengths of their effects; single-headed arrows represent relationships assumed to be causal in the model, whereas double-headed arrows indicate correlation relationships. Values on paths are partial correlation path coefficients. Coefficients on paths leading to plastron shape are summed across all seven principal components used to describe shape. U represents unexplained variance, as a proportion of 1.**

logarithm of centroid size. Finally, we determined the correlation between the categorical variables habitat preference and plastral kinesis using a matrix correlation between the habitat and kinesis distance matrices described above. Our use of multiple kinds of correlations (e.g., matrix correlations, square roots of coefficients of determination) should not unduly bias the analysis because the use of multiple correlation types or a mix of correlations and covariances is not uncommon in path analysis and structural equation modeling (e.g., Crespi and Bookstein 1989; Caumul and Polly 2005; Ullman in Tabachnik and Fidell 2007). The coefficients for the four paths leading to plastron shape were calculated as the square-root of the sum of the squared path coefficients for each PC, weighted by the proportion of shape variance explained by each axis. Finally, we calculated the unexplained variance term ($U$) as one minus the sum of the squared coefficients for the four paths leading to plastron shape.

## MODEL-BASED ANALYSIS OF PLASTRON SHAPE EVOLUTION

Given that Claude et al. (2003) and Claude (2006), along with our path analyses, demonstrate that the predictor variables influence plastron shape (see Results), a logical next question is to consider how this trait has evolved: in a manner consistent with random evolutionary change, or one suggestive of adaptive evolution. To test these alternative hypotheses of plastron shape evolution in emydines, we used the approach of Hansen (1997), as implemented in the R package, ouch (Butler and King 2004). Hansen's (1997) method is based on using an Ornstein–Uhlenbeck (OU) process to model the movement of fitness optima on an adaptive landscape in response to known selective forces as well as background factors that might prevent the optima realized in particular species from reaching a theoretically ideal value (also see Hansen et al. 2008; Labra et al. 2009). The fit of the OU model can be compared to a null model of Brownian motion to determine whether an adaptive scenario is a more likely explanation of the observed data than undirected stochastic change. Model selection statistics (e.g., AIC) are then used to compare the fit of the various models. Butler and King's (2004) implementation of Hansen's method in ouch also provides maximum likelihood estimates of several evolutionary parameters: $\sigma$ (the standard deviation of random changes, essentially the strength of random fluctuations in the evolutionary process assumed to be caused the interaction of selective forces not explicitly included in the model), $\alpha$ (the rate of adaptation toward an optimum), $\theta_k$ (optimal value of a character in selective regime $k$), and $\theta_0$ (value of a character at the root node of the phylogeny).

The shape variables used in the modeling were PC scores for the shape-standardized mean specimens of the eight emydine species under consideration, plus the outgroup species *Chrysemys picta*. To generate these scores, we first Procrustes superimposed all of the shape-standardized individuals belonging to a given species, and calculated the mean shape for that species. We then superimposed the resulting nine species means, ran a PCA on the covariance matrix of the partial warp and uniform component scores of the eight mean specimens, and recorded the scores of the means on the first three PC axes. Together, these axes explain approximately 84% of the variance in this dataset, and are also the axes that explain significantly more variation than succeeding axes based on the test of Anderson (1958; also see Morrison 1990 and Zelditch et al. 2004). Scores on PC axis one are of particular interest because this axis captures much of the shape

variation associated with plastral kinesis (see subsequently), and thereby can provide insight specifically into the evolution of the component of shape variation correlated with plastral kinesis.

Hansen's (1997) model requires a phylogenetic topology and branch lengths representing evolutionary time. The latter are significant because the units of σ and α are related to time (Butler and King, 2004). Given the topological differences between our nuclear and mitochondrial phylogenies, we ran the model-based analyses using both topologies. In both cases, we used the branch lengths produced by the relaxed molecular clock tree searches (BEAST analyses) for analyses in ouch.

We examined the fit of our data to 10 evolutionary models for each combination of topology/branch length/shape metric. The first model was the null hypothesis of Brownian motion (BM), which can represent several evolutionary scenarios, including random evolution, evolution in which the optimum is varying in a random way over time, or a case in which the optimum is influenced collectively by a wide range of factors that are not explicitly included in the model. The second model included a single optimum (OU.1), implying that separate optima do not exist for terrestrial and aquatic turtles, or kinetic and akinetic turtles. The third and fourth models included two optima: one for kinetic turtles and one for akinetic turtles. The models differed in whether plastral kinesis was reconstructed as having evolved once (OU.2) or twice (OU.2a) within emydines because our phylogenetic hypotheses are consistent with either option (also see Feldman and Parham 2002). The fifth and sixth models included two optima (one for aquatic turtles and one for terrestrial turtles), and differed in whether the common ancestor of *Terrapene* was considered to be aquatic (OU.3) or terrestrial (OU.3a) because our phylogenetic hypotheses are consistent with either option. The remaining four models more finely subdivided the optima, so that different optima existed for aquatic kinetic, aquatic akinetic, terrestrial kinetic, and terrestrial akinetic turtles. The models differed in the reconstruction of the histories of kinesis and habitat as follows: single origin of kinesis and aquatic ancestral habitat for *Terrapene* (OU.4), dual origin of kinesis and aquatic ancestral habitat for *Terrapene* aquatic (OU.4a), single origin of kinesis and terrestrial ancestral habitat for *Terrapene* (OU.5), dual origin of kinesis and terrestrial ancestral habitat for *Terrapene* (OU.5a).

We calculated three model selection statistics to help assess the fit of the ten models to our data and to choose a preferred model for each combination of topology, branch lengths, and shape metrics: deviance ($-2\log L$), the Akaike Information Criterion (AIC), and the Schwartz (or Bayes) Information Criterion (SIC) (see Burnham and Anderson 2002). We used the AIC and SIC as the primarily determinants of our preferred model, and the likelihood ratio test (LRT) to test whether the selected model fit the data significantly better than the null (BM).

# Results

## PHYLOGENETIC ANALYSES AND CHARACTER EVOLUTION

We conducted phylogenetic analyses to perform ancestral state reconstructions and to estimate relative divergence times among taxa. Our simple Bayesian analyses of previously published mitochondrial (Feldman and Parham 2002) and nuclear sequence data (Spinks et al. 2009) recover trees consistent with our expectations based on more complex treatments of these same data (see Feldman and Parham 2002; Spinks and Shaffer 2009; Spinks et al. 2009). Briefly, emydine turtles are monophyletic, as are the polytypic genera, and disagreement between mitochondrial and nuclear topologies exists in the arrangement of the *Emys* species and the placement of *C. guttata* (Fig. 4).

Optimizing plastral kinesis onto the two phylogenetic hypotheses indicates either multiple gains (in *Emys* and *Terrapene*) or multiple losses (in *E. marmorata* and *C. guttata*) of this trait are equally parsimonious, regardless of the topology (Fig. 4). Mapping habitat preference onto the topologies indicates two independent gains of a terrestrial lifestyle (in *G. insculpta* and at least once *Terrapene*).

## MORPHOMETRIC ANALYSES

In addition to serving as variables in the path analysis, the PCA results when only specimens of the eight emydine species ($n = 1556$) were included provide qualitative insight into major patterns of variation in the data (Fig. 5). The addition of outgroup specimens (*C. picta*) has relatively minor effects (Fig. 6), which we discuss subsequently (see Discussion). The first four eigenvalues of the PCA were distinct from each other and from the other eigenvalues according to Anderson's test (Anderson 1958; also see Morrison 1990 and Zelditch et al. 2004). Together, the first four PC axes explain ~68% of the shape variance in the data. Thus, our interpretation of the PCA focuses on these four factors.

The first PC axis primarily describes shape differences in the bridge region and posterior lobe of the plastron, as well as differences in the relative lengths of the plastral scutes near the midline of the plastron. The kinetic and akinetic species are well differentiated on this axis, and the shape differences correspond to modifications related to plastral kinesis (Bramble 1974). The second PC axis also primarily describes shape differences in the bridge, posterior lobe, and along the midline. However, the distribution of species on this axis does not show a clear relationship to kinesis, phylogeny, or habitat preference. Therefore, this shape variation may reflect the influence of other factors that not considered here, or idiosyncratic differences among species that lack a clear causal factor. Shape differences described by the third and fourth PC axes mainly concern landmark positions along the midline of the plastron, as well as differences in the shape of the

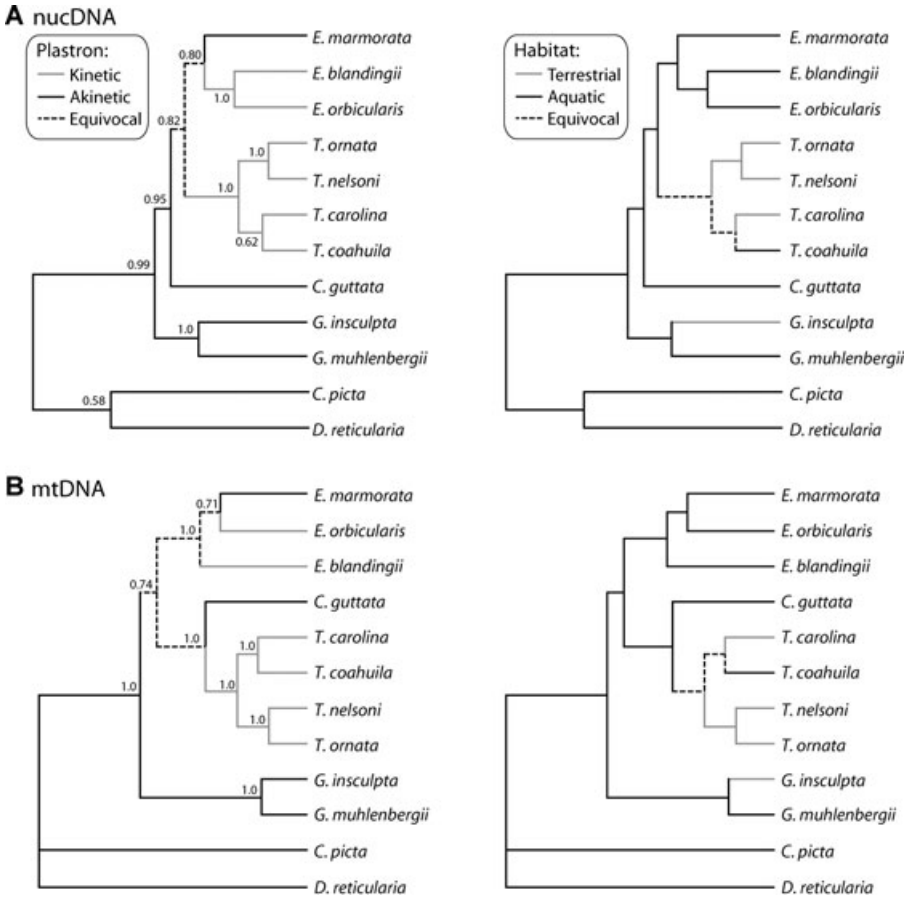


**Figure 4. Phylogenetic hypotheses for emydine turtles based Bayesian relaxed-clock analyses of (A) nucDNA and (B) mtDNA data. Branch lengths drawn proportional to evolutionary divergence estimates; numbers adjacent to clades are nodal support values in Bayesian posterior probabilities. Also shown is the most parsimonious reconstruction of plastral kinesis and habitat preference evolution in emydines. Lineages reconstructed with shell kinesis illustrated with grey lines, those without kinesis with black lines, and those of uncertain ancestral states with dashed lines; lineages reconstructed as chiefly aquatic illustrated with black lines, those with terrestrial habitats with grey lines, and those of uncertain ancestral states with dashed lines.**

anterior and posterior lobe margins. PC axis three also does not show a clear relationship with kinesis, phylogeny, or habitat preference, with considerable overlap in the positions of kinetic and akinetic species, as well as species in different clades and those inhabiting different environments. Finally, PC axis four partly describes shape differences associated with kinesis and habitat type. Kinetic and akinetic aquatic species differentiate along axis four; kinetic species possess higher scores than akinetic species. Likewise, the two most highly terrestrial species, *Glyptemys insculpta* and *Terrapene ornata*, share the lowest scores on PC four.

## PATH ANALYSIS OF FACTORS AFFECTING PLASTRON SHAPE

The path analysis (Fig. 3) demonstrated that plastral kinesis, habitat preference, phylogeny, and body size, explain a substantial amount of the observed shape variation (Table 2); ∼74% of the morphological variation is explained in the analysis with mitochondrial DNA distances, and ∼95% for the analysis with nuclear DNA distances. Among the predictor variables, plastral kinesis has the strongest direct effect on plastron shape, explaining nearly 53% and 86% of shape variation in the mitochondrial and nuclear analyses, respectively. Phylogeny has the second strongest direct effect on plastron shape, but explains considerably less variance than plastral kinesis; ∼11% for the analysis with mitochondrial DNA distances, and ∼6% for the analysis including nuclear DNA. Habitat preference explains only slightly less variance than phylogeny (∼6% for both analyses). Finally, adult body size had a very weak effect on plastron shape, explaining only ∼1% of the variance in the data for both analyses.

## MODEL-BASED ANALYSES OF PLASTRON SHAPE EVOLUTION

We used a model-based approach to compare the fit of our plastron shape data to a null hypothesis of random evolution to various adaptive models. In the former, variation in the trait over time and through branching events is predicted to follow a path of Brownian

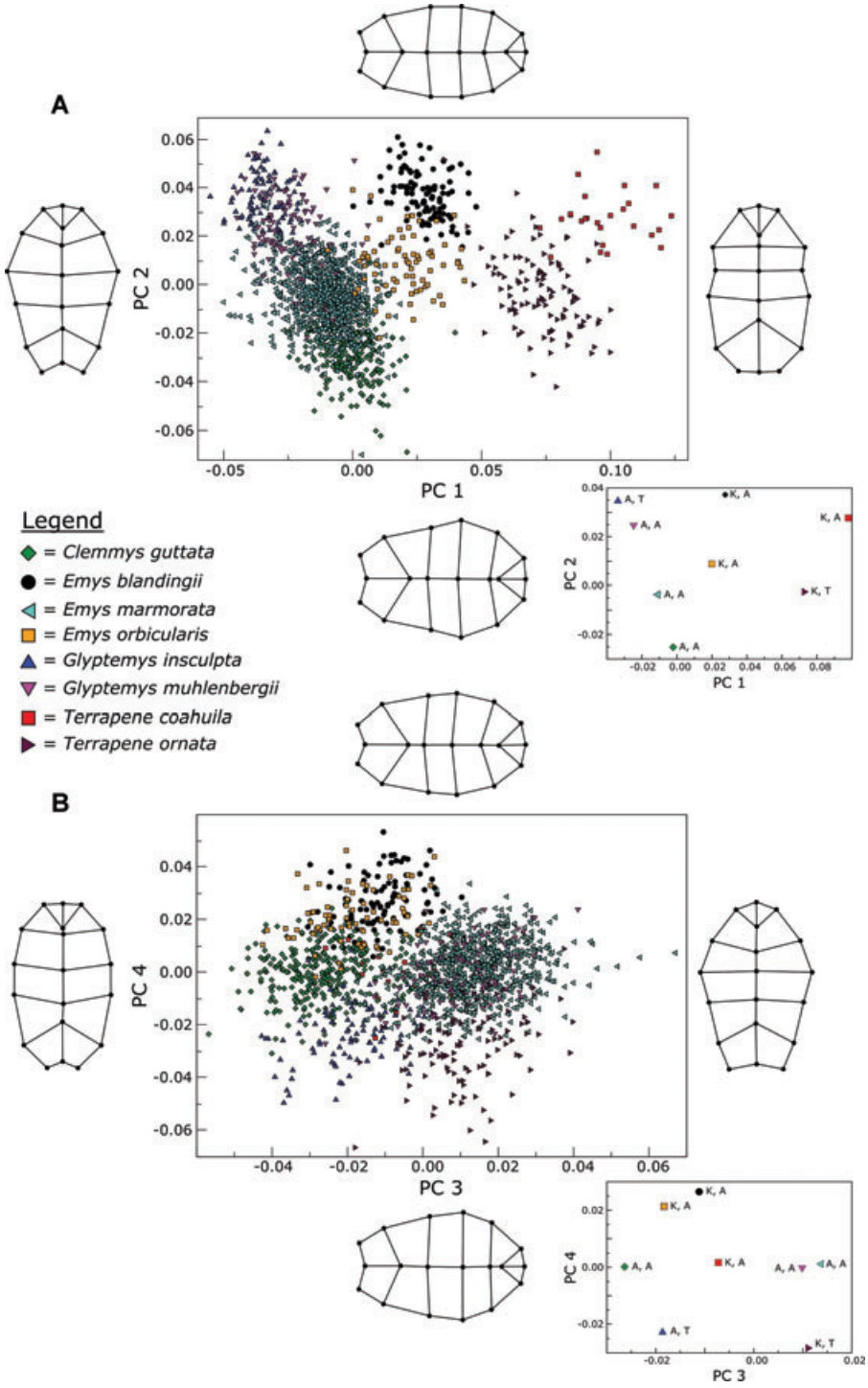


**Figure 5.** Principal components plots summarizing shape variation among the 1556 shape-standardized emydine specimens included in the morphometric dataset. (A) PCs 1 and 2. (B) PCs 3 and 4. Stylized plastra to the left and right of each plot depict the shapes represented at the positive and negative endpoints of the x-axis, respectively. Stylized plastra above and below each plot depict the shapes represented at the positive and negative endpoints of the y-axis, respectively. Inset plots show the positions of the size-standardized mean specimens of each species on the PC axes. Codes for inset plots refer to state of kinesis and habitat preference of the species (K, A: kinetic aquatic; K, T: kinetic, terrestrial; A, A: akinetic, aquatic; A, T: akinetic, terrestrial).

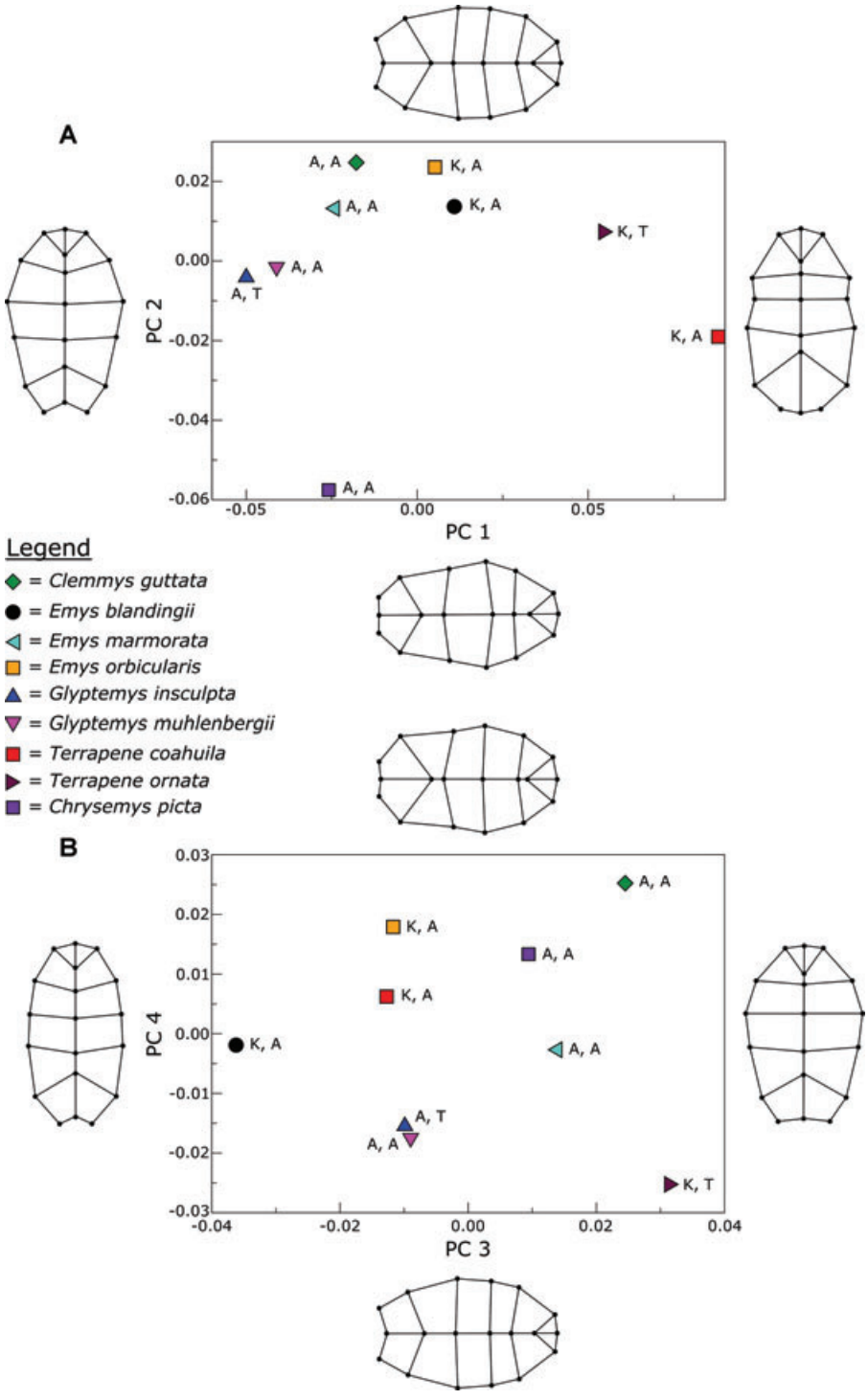


**Figure 6.** Principal components plots summarizing shape variation among mean specimens of the eight size-standardized emydine species and the outgroup *Chrysemys picta*. (A) PCs 1 and 2. (B) PCs 3 and 4. Principal component scores from the first three axes of this analysis are the shape variables used in the evolutionary models. Stylized plastra to the left and right of each plot depict the shapes represented at the positive and negative endpoints of the x-axis, respectively. Stylized plastra above and below each plot depict the shapes represented at the positive and negative endpoints of the y-axis, respectively. Codes for inset plots refer to state of kinesis and habitat preference of the species (K, A: kinetic aquatic; K, T: kinetic, terrestrial; A, A: akinetic, aquatic; A, T: akinetic, terrestrial).

**Table 2. Percent of morphological variance ($R^2$) explained by the four paths leading directly to plastron shape. Variance calculated for both mitochondrial (mtDNA) and nuclear phylogenetic hypotheses (nucDNA) using ML-corrected, pairwise genetic distances; $R^2$ calculated as the square of the path coefficients multiplied by 100.**

| Path | mtDNA $R^2$ (%) | nucDNA $R^2$ (%) |
|---|---|---|
| Plastral kinesis | 55.73 | 83.75 |
| Phylogeny | 11.14 | 5.64 |
| Habitat preference | 5.64 | 5.87 |
| Body size | 1.56 | 0.47 |
| Total explained | 74.07 | 95.73 |
| Unexplained | 25.93 | 4.27 |

motion (BM), whereas in the latter, the distribution of variation should conform to patterns expected under a process of adaptive hill-climbing (Hansen 1997; Butler and King 2004). We compared the fit of our plastron data, measured here as scores on PC axes 1, 2, and 3, across two competing phylogenetic hypotheses (mtDNA and nucDNA trees) and with differing ASRs, to these models (Table 3). For PC1 scores, model OU.2a (optima for kinetic and akinetic turtles, dual origin of kinesis) was preferred regardless of phylogenetic topology used in the model, and the LRT showed that this model fit the data significantly better than BM in both cases ($P < 0.01$). The α values for these models (1.172 for the mtDNA tree and 2.451 for the nuDNA tree; Table S2) imply relatively strong selection. Hansen (1997) noted that the phylogenetic half-life, or the time that it takes before adaptation to a new selective regime (optimum) is more important than constraints from the ancestral state, is equal to ln(2)/α, and the phylogenetic half-life for PC 1 scores on the mtDNA and nuDNA trees appears rapid relative to the branch lengths. However, because we did not calibrate our branch lengths to absolute time, we cannot offer a temporaly-calibrated phylogenetic half-life. For scores on PC2, we could not reject the null model of Brownian motion for either the mtDNA or nuDNA topologies. A four optimum model (OU.5a, separate optima for aquatic akinetic, aquatic kinetic, terrestrial akinetic, and terrestrial kinetic turtles, with dual origin of plastral kinesis) best fits the scores on PC3 for the mtDNA and nuDNA topologies, and the LRT showed that the OU.5a model fit the data significantly better than the BM null in both cases ($P < 0.01$). The α values for these models (7.752 for the mtDNA tree and 6.780 for the nuDNA tree; Table S2) imply strong selection and correspond to short phylogenetic half-lifes.

## Discussion

The plastron (bottom shell) is one of the fundamental components of the turtle bauplan and possibly evolved earlier in chelonian history than the carapace (upper shell) (Li et al. 2008; Nagashima et al. 2009). It is integral in forming the overall shape of the turtle, protecting the ventral surface, and functionally orienting the limbs (Pritchard 2008). The plastron shows considerable morphological diversity as well as interesting functional variation, with some species possessing a mechanical hinge along the midsection (plastral kinesis), and others possessing immovable plastra (akinetic) (see Bramble 1974; Ernst and Barbour 1989). Despite the functional significance of the plastron, relatively little work has been conducted to understand the factors mediating its evolution, or whether the plastron appears to be under selection in a way consistent with hypothesized roles such as protection or drag reduction (e.g., Domokos and Várkonyi 2008; Rivera 2008).

Our analyses demonstrate that plastron shape in emydine turtles is related to plastral kinesis, phylogeny, and habitat preference, but not to adult body size. Further, these factors do not explain equal amounts of shape variation. Instead, plastral kinesis explains the majority of shape variation. The dominating effect of plastral kinesis is not surprising because this trait is accompanied by highly distinctive and relatively stereotyped modifications in plastron architecture and shape (Bramble 1974; Claude 2006). Changes in the plastron are required to form the plastral hinge, to accommodate the ligamentous connection between the plastron and carapace, to create greater fit between plastral lobes and ventral edges of the carapace, and to accommodate pectoral and pelvic modifications associated with shell closing (Bramble 1974; Claude 2006). Accordingly, we found dramatic differences between the plastra of akinetic and kinetic taxa. Kinetic emydines possess longer, wider anterior and posterior plastral lobes and narrower bridges than their akinetic relatives (much of this variation was captured by the first PC axis). Shape differences in the bridge reflect the loss of a bony connection between the carapace and plastron, whereas the observed widening, particularly of the posterior lobe (most extreme in *Terrapene*), allows the plastron to form a more complete seal with the margin of the shell. These patterns are congruent with those seen in Testudinoidea as a whole (Claude 2006).

Phylogeny and habitat preference each account for only one fifth to less than one tenth of the variance in plastron shape compared to kinesis. Interestingly, phylogeny and habitat preference explain approximately equal amounts of morphological variation, implying that common descent exerts as much influence on shape as habitat (at least in the very generalized sense that we treated the latter here). Nevertheless, we did detect some shape variation consistent with differences in habitat preference; compared to their aquatic relatives, the terrestrial species show a shortening and widening of the plastron, and a lengthening of the abdominal scute (PC axis four weakly differentiates terrestrial and aquatic species in the analysis excluding *C. picta*).

**Table 3.** Performance of alternative models of plastron shape evolution across both nuclear (nucDNA) and mitochondrial (mtDNA) derived phylogenies. Plastron shape is quantified as scores of mean specimens of the eight emydines and *Chrysemys picta* on PC1, PC 2, and PC 3. Likelihood ratio tests (LRTs) were conducted for each model against the Brownian motion null model.

| | BM | OU.1 | OU.2 | OU.2a | OU.3 | OU.3a | OU.4 | OU.4a | OU.5 | OU.5a |
|---|---|---|---|---|---|---|---|---|---|---|
| Principal component 1 on nucDNA phylogeny | | | | | | | | | | |
| −2logL | −32.00 | −32.19 | −41.94 | **−43.15** | −32.39 | −32.55 | −42.79 | −43.96 | −44.28 | −45.11 |
| AIC | −28.00 | −26.19 | −33.94 | **−35.15** | −24.39 | −24.55 | −30.79 | −31.96 | −32.28 | −33.11 |
| SIC | −27.61 | −25.60 | −33.15 | **−34.36** | −23.60 | −23.76 | −29.61 | −30.78 | −31.10 | −31.92 |
| LR | – | 0.19 | 9.94 | **11.15** | 0.39 | 0.55 | 10.79 | 11.96 | 12.28 | 13.11 |
| *P*-value | – | 0.661 | 0.007 | **0.004** | 0.823 | 0.761 | 0.029 | 0.018 | 0.016 | 0.011 |
| Principal Component 2 on nucDNA phylogeny | | | | | | | | | | |
| −2logL | **−44.30** | −42.75 | −42.87 | −42.77 | −42.82 | −42.74 | −43.44 | −43.48 | −42.90 | −42.81 |
| AIC | **−40.30** | −36.75 | −34.87 | −34.77 | −34.82 | −34.75 | −31.44 | −31.48 | −30.90 | −30.81 |
| SIC | **−39.91** | −36.16 | −34.09 | −33.98 | −34.04 | −33.96 | −30.26 | −30.30 | −29.71 | −29.63 |
| LR | – | 1.55 | 1.43 | 1.53 | 1.48 | 1.55 | 0.86 | 0.82 | 1.40 | 1.49 |
| *P*-value | – | 0.213 | 0.490 | 0.466 | 0.478 | 0.461 | 0.930 | 0.936 | 0.843 | 0.829 |
| Principal Component 3 on nucDNA phylogeny | | | | | | | | | | |
| −2logL | −40.00 | −44.70 | −45.69 | −45.69 | −45.48 | −45.48 | −56.67 | −56.67 | −57.26 | **−57.36** |
| AIC | −36.00 | −38.70 | −37.69 | −37.69 | −37.48 | −37.48 | −44.67 | −44.67 | −45.26 | **−45.36** |
| SIC | −35.60 | −38.11 | −36.90 | −36.90 | −36.69 | −36.69 | −43.48 | −43.48 | −44.08 | **−44.18** |
| LR | – | 4.71 | 5.69 | 5.69 | 5.48 | 5.48 | 16.67 | 16.67 | 17.27 | **17.37** |
| *P*-value | – | 0.030 | 0.058 | 0.058 | 0.064 | 0.064 | 0.002 | 0.002 | 0.002 | **0.002** |
| Principal Component 1 on mtDNA phylogeny | | | | | | | | | | |
| −2logL | −32.33 | −32.35 | −42.46 | **−46.46** | −32.35 | −33.16 | −42.93 | −48.05 | −44.28 | −47.22 |
| AIC | −28.33 | −26.35 | −34.46 | **−38.46** | −24.36 | −25.16 | −30.93 | −36.05 | −32.28 | −35.22 |
| SIC | −27.94 | −25.75 | −33.67 | **−37.67** | −23.56 | −24.37 | −29.74 | −34.86 | −31.09 | −34.04 |
| LR | – | 0.02 | 10.13 | **14.13** | 0.02 | 0.83 | 10.60 | 15.72 | 11.95 | 14.89 |
| *P*-value | – | 0.889 | 0.006 | **0.009** | 0.988 | 0.661 | 0.031 | 0.003 | 0.018 | 0.005 |
| Principal Component 2 on mtDNA phylogeny | | | | | | | | | | |
| −2logL | **−44.58** | −42.86 | −43.25 | −42.93 | −43.02 | −42.86 | −43.63 | −43.50 | −43.27 | −42.96 |
| AIC | **−40.58** | −36.86 | −35.25 | −34.93 | −35.02 | −34.86 | −31.63 | −31.50 | −31.27 | −30.96 |
| SIC | **−40.18** | −36.27 | −34.46 | −34.14 | −34.23 | −34.07 | −30.44 | −30.31 | −30.09 | −29.77 |
| LR | – | 1.72 | 1.33 | 1.65 | 1.56 | 1.72 | 0.95 | 1.08 | 1.31 | 1.62 |
| *P*-value | – | 0.190 | 0.515 | 0.438 | 0.459 | 0.424 | 0.917 | 0.898 | 0.860 | 0.805 |
| Principal Component 3 on mtDNA phylogeny | | | | | | | | | | |
| −2logL | −40.60 | −44.70 | −45.69 | −45.69 | −45.50 | −45.60 | −56.67 | −56.67 | −56.94 | **−56.99** |
| AIC | −36.60 | −38.70 | −37.69 | −37.69 | −37.50 | −37.60 | −44.67 | −44.67 | −44.94 | **−44.99** |
| SIC | −36.20 | −38.11 | −36.90 | −36.90 | −36.71 | −36.81 | −43.48 | −43.48 | −43.76 | **−43.81** |
| LR | – | 4.11 | 5.09 | 5.09 | 4.91 | 5.00 | 16.07 | 16.07 | 16.35 | **16.40** |
| *P*-value | – | 0.043 | 0.079 | 0.079 | 0.086 | 0.082 | 0.003 | 0.003 | 0.003 | **0.003** |
| d.f. | – | 1 | 2 | 2 | 2 | 2 | 4 | 4 | 4 | 4 |

**Parameters for preferred model in each case are shown in bold. Abbreviations: AIC=Akaike Information Criterion; SIC=Schwartz Information Criterion; LR=Likelihood Ratio; d.f.=degrees of freedom; BM=Brownian motion; OU.1=Ornstein–Uhlenbeck model with a single selective optimum; OU.2=Ornstein–Uhlenbeck model with selective optima for akinetic and kinetic turtles and a single origin of plastral kinesis; OU.2a=Ornstein–Uhlenbeck model with selective optima for akinetic and kinetic turtles and two origins of plastral kinesis; OU.3=Ornstein–Uhlenbeck model with selective optima for terrestrial and aquatic turtles and an aquatic ancestor for *Terrapene*; OU.3a=Ornstein–Uhlenbeck model with selective optima for terrestrial and aquatic turtles and a terrestrial ancestor of *Terrapene*; OU.4=Ornstein–Uhlenbeck model with selective optima for aquatic akinetic, aquatic kinetic, terrestrial akinetic, and terrestrial kinetic turtles, a single origin of plastral kinesis, and an aquatic ancestor for *Terrapene*; OU.4a=Ornstein–Uhlenbeck model with selective optima for aquatic akinetic, aquatic kinetic, terrestrial akinetic, and terrestrial kinetic turtles, two origins of plastral kinesis, and an aquatic ancestor for *Terrapene*; OU.5=Ornstein-Uhlenbeck model with selective optima for aquatic akinetic, aquatic kinetic, terrestrial akinetic, and terrestrial kinetic turtles, a single origin of plastral kinesis, and a terrestrial ancestor for *Terrapene*; OU.5a=Ornstein–Uhlenbeck model with selective optima for aquatic akinetic, aquatic kinetic, terrestrial akinetic, and terrestrial kinetic turtles, two origins of plastral kinesis, and a terrestrial ancestor for *Terrapene*.**

Adult body size explains a negligible amount of interspecific shape variation in our path analysis, indicating that changes in body size among emydine species are not accompanied by strong changes in adult plastron shape. This may imply that the pattern of shape change over ontogeny is relatively consistent among emydines regardless of body size (i.e., little heterochrony). However, a more detailed examination of their ontogenies is needed to test this hypothesis.

Alternatively, the relatively weak effects of habitat preference and body size may indicate that the relationship between these variables and plastron shape is not linear, leading the path model to underestimate their influence. However, we think this is unlikely. In the case of habitat preference, the plastron shape differences between the terrestrial and aquatic emydines we sampled are relatively minor, particularly when compared to differences related to kinesis (e.g., the plastron of aquatic *T. coahuila* is much more similar to its terrestrial, kinetic relative *T. ornata* than it is to aquatic, akinetic *C. guttata* or *G. muhlenbergii*). The dramatic, stereotyped differences seen in when comparing the carapaces of terrestrial testudinids and aquatic emydids (e.g., Claude et al. 2003) simply do not exist in our sample, so it is logical for habitat preference to have a weak effect. Likewise, preliminary data suggest that the smallest emydine species (*G. muhlenbergii*) possesses an adult shape that is similar to its much larger sister species (*G. inscultpa*) despite their disparity in size (Angielczyk 2007), a result consistent with adult body size having a minor influence on plastron shape.

Our results for emydines closely mirror those for testudinoids as a whole, in that plastral kinesis explains the majority of plastron shape variation, whereas phylogeny and habitat preference account for much less variance (Claude 2006). Here, the influence of phylogeny is equal to or greater than habitat preference in emydines, whereas the reverse appears to be the case when testudinoidea is considered as a whole (Claude et al. 2003; Claude 2006). We may see greater "phylogenetic signal" (sensu Blomberg and Garland 2002) at this narrower phylogenetic scale if the genetic architecture underlying the plastron is more similar in emydines simply due to recent common ancestry (less time since divergence). For example, the genetic variance–covariance matrix (G matrix) is expected to be highly constrained by pleiotropy (Cheverud 1984), and to decrease in similarity with increasing phylogenetic distance (Steppan et al. 2002). Because the body elements of the turtle shell are primarily controlled by conserved morphogens (e.g., *Hox* and *Lbx* genes; Kawashima-Ohya et al. 2005; Nagashima et al. 2009), known to be profoundly pleiotropic, it seems plausible that closely allied taxa may display greater evidence of a genetic constraint. One caveat, however, is that some of the disparity in the variance attributed to phylogeny between emydines and all testudinoids could also reflect analytical differences between studies. Regardless, the general relationship between plastron shape and plastral kinesis, phylogeny, and habitat preference, appears roughly consistent, despite the drastic differences in phylogenetic scale and different methods used to incorporate phylogeny into the analysis. Such consistency implies that the same deterministic forces have influenced morphological evolution across turtle lineages, emphasizing the importance of testing whether observed patterns of shape variation are consistent with an adaptive model of evolution.

The observed patterns of plastron shape variation on PC1 and PC3 (when *C. picta* is included; Fig. 6) are more consistent with an adaptive model of evolution (OU) than with a purely stochastic model (BM), regardless of the phylogenetic hypothesis (mtDNA versus nucDNA), whereas the variation on PC2 best fits a stochastic model for both trees. The optimal model for PC 1 was relatively simple (OU.2a; optima for kinetic and akinetic turtles, dual origin of kinesis), whereas that for PC 3 was more complex (OU.5a; separate optima for aquatic akinetic, aquatic kinetic, terrestrial akinetic, and terrestrial kinetic turtles, with dual origin of plastral kinesis). These results seem consistent with the distribution of taxa on the PC axes (Fig. 6), and the shape differences captured by the axes. PC1 shows a clear relationship with plastral kinesis: akinetic species fall towards the low end of the axis whereas kinetic species are located on the moderate to high end of the axis, and the captured shape differences correspond to changes in the bridge region associated with the development of the hinge and the ligamentous connection between the plastron and carapace, as well as the shape of the anterior and posterior lobes of the plastron. Given this dichotomy, a model with two adaptive zones is not surprising. The distribution of species on PC2, and the shape differences described by PC2, do not show a clear relationship to kinesis, habitat preference or phylogeny, and may reflect individual differences between the species that could reasonably result from drift. The distribution of species on PC3 in part emphasizes similarities between kinetic and akinetic taxa, and taxa inhabiting different environments. Given that this axis seems to capture information related to both factors, it is not surprising that a model with separate optima for the various combinations of kinesis and habitat preference is preferred.

The preferred models for PC1 and PC3 also imply strong selection and relatively short phylogenetic half-lifes. Although this conclusion might seem somewhat surprising, it is consistent with some aspects of emydine history. For example, some of the oldest emydine fossils known already are recognizable as members of *Glyptemys* and *Terrapene*, with the *Terrepene* specimens showing many modifications of the plastron for kinesis (Holman and Fritz 2005). Similarly, branch lengths within the major emydine clades are notably longer than the branches between the clades, particularly for the nucDNA data.

We find it intriguing that a model with just two adaptive zones (one for akinetic species and one for kinetic species) provides

the best fit for the major axis of variation in our data (PC1), with more highly parameterized models offering little improvement in fit. Although the caveat that our relatively small sample of species makes it more difficult for highly parameterized models to be well-supported must be taken into consideration, these results are intuitively pleasing because they are congruent with the results from path analyses demonstrating that kinesis explained by far the greatest amount of plastron shape variance. Taken together, these results suggest that plastron shape evolution in emydine turtles is not rigidly constrained, but at the same time not highly flexible, because the adaptive landscape appears quite simple. In other words, although a landscape with a single optimum plastron shape is clearly too simple, only one additional zone is needed to adequately explain nearly a third of the observed variation. The fact that the differing demands of aquatic and terrestrial environments did not strongly modify the adaptive landscape of emydines may partly stem from the fact that most members of the clade are not true habitat specialists, but instead spend time in both aquatic and terrestrial environments. Additionally, a relatively simple adaptive landscape would be expected if only a small set of solutions can remedy the morphological problems introduced by a hinging plastron. As mentioned previously, plastral kinesis requires significant compensatory changes to plastron shape and internal anatomy. Therefore, the modifications producing plastral kinesis in emydines may have occurred in a stereotyped way because the adaptive landscape of this trait is unimodal, limiting the types of shape change that could exist once kinesis had evolved. Interestingly, a number of other turtle taxa display plastral kinesis. The further phylogenetically removed these taxa are from emydines, the more distinct their kinetic modifications (e.g., geoemydids, kinosternids, pelomedusids; Bramble 1974; Bramble and Hutchison 1981; Bramble et al. 1984). Thus, whereas extensive morphological alterations always appear necessary to accommodate plastral kinesis, indicating the trait produces an overriding affect on plastron shape regardless of environment or phylogeny, a number of unique solutions have evolved across diverse kinetic taxa. When combined with our results for Emydinae, these observations suggest the global adaptive landscape for plastral kinesis in turtles may be complex, but independent lineages may be confined to the same few adaptive zones.

## ACKNOWLEDGMENTS

For access to emydine specimens we thank: J. Vindum and R. Drewes (CAS); A. Resetar (FMNH); R. Feeney (LACM); C. Austin (LSUMNS); S. Sweet (MSE); J. McGuire and C. Conroy (MVZ); A. Wynn (NMNH); P. Collins (SBMNH); B. Hollingsworth (SDMNH); P. Holroyd (UCMP); R. Symonds (UMZC); J. Buskirk. We are grateful to J. Parham and S. Sweet for field assistance and the California Department of Fish and Game for permits. We thank P. Spinks for aid with sequences. We appreciate helpful discussions and comments on the manuscript from A. Aronowsky, J. Buskirk, G. Hunt, M. Matocq, J. Parham, A. Horsnby, A. de Queiroz, D. Polly, P. Roopnarine, D. Swiderski, M. Zelditch, and three anonymous reviewers. Data collection funded by NSF DBI-0306158 (to KDA) and G. Miller's work on the project made possible by NSF REU DBI-0353797 (to R. Mooi of CAS).

## LITERATURE CITED

Adams, D. C., and F. J. Rohlf. 2000. Ecological character displacement in *Plethodon*: biomechanical differences found from a geometric morphometric study. Proc. Natl. Acad. Sci. USA 97:4106–4111.

Adams, D. C., F. J. Rohlf, and D. E. Slice. 2004. Geometric morphometrics: ten years of progress following the "revolution." Ital. J. Zool. 71:5–16.

Anderson, T. W. 1958. An introduction to multivariate analysis. John Wiley and Sons, New York.

Angielczyk, K. D. 2007. How to be a miniature turtle: comparisons of ontogeny in the Emydinae using geometric morphometrics. Integr. Comp. Biol. 46(Suppl.):e3.

Angielczyk, K. D., and J. F. Parham. 2005. Geometric morphometric analysis of plastron shape in the western pond turtle (*Emys marmorata*): implications for conservation and paleontology. Integr. Comp. Biol. 45:957.

Angielczyk, K. D., and H. D. Sheets. 2007. Investigation of simulated tectonic deformation in fossils using geometric morphometrics. Paleobiology 33:125–148.

Arnold, S. J. 1992. Constraints on phenotypic evolution. Am. Nat. 140:S85–S107.

Ashton, K. G. 2001. Body size variation among mainland populations of the western rattlesnake *Crotalus viridis*. Evolution 55:2523–2533.

Bickham, J. W., T. Lamb, P. Minx, and J. C. Patton. 1996. Molecular systematics of the genus *Clemmys* and the intergeneric relationships of emydid turtles. Herpetologica 52:89–97.

Blackburn, T. M., and A. Ruggeiro. 2001. Latitude, elevation, and body mass variation in Andean passerine birds. Glob. Ecol. Biogeogr. 10:245–259.

Blomberg, S. P., and T. Garland, Jr. 2002. Tempo and mode in evolution: phylogenetic inertia, adaptation and comparative methods. J. Evol. Biol. 15:899–910.

Bookstein, F. L. 1989. Principal warps: thin-plate splines and the decompositions of deformations. IEEE Trans. Pattern Anal. Mach. Intell. 11:567–585.

———. 1991. Morphometric tools for landmark data. Cambridge Univ. Press, Cambridge.

———. 1996. Biometrics, biomathematics, and the morphometric synthesis. Bull. Math. Biol. 58:313–365.

———. 1998. A hundred years of morphometrics. Acta Zool. Acad. Sci. Hung. 44:7–59.

Brakefield, P. M. 2007. Evo-devo and constraints on selection. Trends Ecol. Evol. 21:361–368.

Bramble, D. M. 1974. Emydid shell kinesis: biomechanics and evolution. Copeia :707–727.

Bramble, D. M., and J. H. Hutchison. 1981. A reevaluation of plastral kinesis in African turtles of the genus *Pelusios*. Herpetologica 37:205–212.

Bramble, D. M., J. H. Hutchison, and J. M. Legler. 1984. Kinosternid shell kinesis: structure function and evolution. Copeia:456–475.

Butler, M. A., and A. A. King. 2004. Phylogenetic comparative analysis: a modeling approach for adaptive evolution. Am. Nat. 164:683–695.

Burgman, M. A. 1987. An analysis of the distribution of plants on granite outcrops in southern Western Australia using Mantel tests. Vegetatio 71:79–86.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and inference: a practical information-theoretic approach. 2nd ed. Springer, New York.

Burke, R. L., T. E. Leuteritz, and A. J. Wolf. 1996. Phylogenetic relationships of emydine turtles. Herpetologica 52:572–584.

Caumul, R., and P. D. Polly. 2005. Phylogenetic and environmental components of morphological variation: skull mandible, and molar shape in marmots (*Marmota*, Rodentia). Evolution 59:2460–2472.

Cheverud, J., M. Dow, and W. Leutenegger. 1985. The quantitative assessment of phylogenetic constraints in comparative analyses: sexual dimorphism in body weight among primates. Evolution 39:1335–1351.

Cheverud, J. M. 1984. Quantitative genetics and developmental constraints on evolution by selection. J. Theor. Biol. 110:155–171.

Claude, J. 2006. Convergence induced by plastral kinesis and phylogenetic constraints in Testudinoidea: a geometric morphometric assessment. Fossil Turtle Research, Volume 1. Russ. J. Herpetol. 13(Suppl.): 34–45.

Claude, J., E. Paradis, H. Tong, and J. C. Auffray. 2003. A geometric morphometric assessment of the effects of environment and cladogenesis on the evolution of the turtle shell. Biol. J. Linn. Soc. 79:485–501.

Crespi, B. J., and B. L. Bookstein. 1989. A path-analytic model for the measurement of selection on morphology. Evolution 43:18–28.

Cruz, F. B., L. A. Fitzgerald, R. E. Espinoza, and J. A. Schulte. 2005. The importance of phylogenetic scale in tests of Bergmann's and Rapoport's rules: lessons from a clade of South America lizards. J. Evol. Biol. 18:1559–1574.

Depecker, M., C. Berge, X. Penin, and S. Renous. 2006. Geometric morphometrics of the shoulder girdle in extant turtles (Chelonii). J. Anat. 208:35–45.

DePristo, M. A., D. M. Weinreich, and D. L. Hartl. 2005. Missense meanderings in sequence space: a biophysical view of protein evolution. Nat. Rev. Genet. 6:678–687.

Domokos, G., and P. L. Várkonyi. 2008. Geometry and self-righting of turtles. Proc. R. Soc. Biol. Sci. Ser. B 275:11–17.

Drummond, A. J., and A. Rambaut. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7:214.1–214.8.

Drummond, A. J., S. Ho, M. Phillips, and A. Rambaut. 2006. Relaxed phylogenetics and dating with confidence. PLOS Biology 4:e88.

Ellegren, H., and B. C. Sheldon. 2008. Genetic basis of fitness differences in natural populations. Nature 452:169–175.

Ernst, C. H., and R. W. Barbour. 1989. Turtles of the world. Smithsonian Institution Press, Washington, DC.

Ernst, C. H., and J. E. Lovich. 2009. Turtles of the United States and Canada. 2nd ed. Johns Hopkins Univ. Press, Baltimore.

Ernst, C. H., J. E. Lovich, and R. W. Barbour. 1994. Turtles of the United States and Canada. Smithsonian Institution Press, Washington, DC.

Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. 4th ed. Addison-Wesley Longman, Harlow, UK.

Feder, M. E., and T. Mitchell-Olds. 2003. Evolutionary and ecological functional genomics. Nat. Rev. Genet. 4:649–655.

Feldman, C. R., and J. F. Parham. 2002. Molecular phylogenetics of emydine turtles: taxonomic revision and the evolution of shell kinesis. Mol. Phylogenet. Evol. 22:388–398.

Felsenstein, J. 1985. Phylogenies and the comparative method. Am. Nat. 125: 1–15.

Finarelli, J. A. 2008. Hierarchy and the reconstruction of evolutionary trends: evidence for constraints on the evolution of body size in terrestrial caniform carnivorans (Mammalia). Paleobiology 34:553–562.

Fritz, U., T. Fattizzo, D. Guicking, S. Tripepi, M. G. Pennisi, P. Lenk, U. Joger, and M. Wink. 2005. A new cryptic species of pond turtle from southern Italy, the hottest spot in the range of the genus *Emys* (Reptilia, Testudines, Emydidae). Zool. Scr. 34:351–371.

Fritz, U., S. Guiking, H. Kami, M. Arakelyanm, M. Auer, D. Ayaz, S. A. Fernández, A. G. Bakiev, A. Celani, A. Džukić, et al. 2007. Mitochondrial phylogeography of European pond turtles (*Emys orbicularis*, *Emys trinacris*): an update. Amphib-Reptilia 28:418–426.

Futuyma, D. J. 1998. Evolutionary biology. 3rd ed. Sinauer Associates, Sunderland, USA.

Gaffney, E. S., and P. A. Meylan. 1988. A phylogeny of turtles. Pp. 157–219 *in* M. J. Benton, ed. The phylogeny and classification of tetrapods. Clarendon Press, Oxford.

Garland, T., Jr., A. F. Bennett, and E. L. Rezende. 2005. Phylogenetic approaches in comparative physiology. J. Exp. Biol. 208:3015–3035.

Goswami, A. 2006. Morphological integration in the carnivoran skull. Evolution 60:169–183.

Gu, X., Y. Fu, and W. H. Li. 1995. Maximum likelihood estimation of the heterogeneity of substitution rate among nucleotide sites. Mol. Biol. Evol. 12:546–557.

Hansen, T. F. 1997. Stabilizing selection and the comparative analysis of adaptation. Evolution 51:1341–1351.

Hansen, T. F., J. Pienaar, and S. H. Orzack. 2008. A comparative method for studying adaptation to a randomly evolving environment. Evolution 62:1965–1977.

Holman, J. A. 1995. A new species of *Emydoidea* (Reptilia: Testudines) from the late Barstovian (medial Miocene) of Cherry County, Nebraska. J. Herpetol. 29:548–553.

———. 2002. Additional specimens of the Miocene turtle *Emydoidea hutchisoni* Holman 1995—new temporal occurrences, taxonomic characters, and phylogenetic inferences. J. Herpetol. 36:436–446.

Holman, J. A., and U. Fritz. 2001. A new emydine species from the Medial Miocene (Barstovian of Nebraska, USA with a new generic arrangement for the species of *Clemmys* sensu McDowell (1964) (Reptilia: Testudines: Emydidae). Zoo. Abh. Mus. Tierkde. Dresden 51:321–344.

———. 2005. The box turtle genus *Terrapene* (Testudines: Emydidae) in the Miocene of the USA. Herpetol. J. 15:81–90.

Hone, D. W. E., and M. J. Benton. 2007. Cope's Rule in the Pterosauria, and differing perceptions of Cope's Rule at different taxonomic levels. J. Evol. Biol. 20:1164–1170.

Kawashima-Ohya, Y., S. Kuraku, and S. Kuratani. 2005. *Hox* code in embryos of Chinese soft-shelled turtle *Pelodiscus sinensis* correlates with the evolutionary innovation in the turtle. J. Exp. Zool. 304B:107–118.

Kingenberg, C. P., M. Barluenga, and A. Meyer. 2002. Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. Evolution 56:1909–1920.

Labra, A. J. Pienaar, J., and T. F. Hansen. 2009. Evolution of thermal physiology in *Liolaemus* lizards: adaptation, phylogenetic inertia, and niche tracking. Am. Nat. 174:204–220.

Legendre, P., and M. J. Fortin. 1989. Spatial pattern and ecological analysis. Vegetatio 80:107–138.

Li, C., X.-C. Wu, O. Rieppel, L.-T Wang, and L.-J. Zhao. 2008. An ancestral turtle from the Late Triassic of southwestern China. Nature 456:497–501.

Maddison, D. R., and W. P. Maddison. 2009. Mesquite: a modular system for evolutionary analysis. Version 2.71. http://mesquiteproject.org.

Magwene, P. M. 2001. Comparing ontogenetic trajectories using growth process data. Syst. Biol. 50:640–656.

Maynard Smith, J., R. Burian, S. Kauffman, P. Alberch, J. Campbell, B. Goodwin, R. Lande, D. Raup, and L. Wolpert. 1985. Developmental constraints and evolution. Q. Rev. Biol. 60:265–287.

Meylan, P. A., and E. S. Gaffney. 1989. The skeletal morphology of the Cretaceous cryptodiran turtle, *Adocus*, and the relationships of the Trionychoidea. Am. Mus. Novit. 2941:1–60.

Monteiro, L. R., and A. S. Abe. 1999. Function and historical determinants of shape in the scapula of xenarthran mammals: evolution of a complex morphological structure. J. Morphol. 241:251–263.

Morrison, D. F. 1990. Multivariate statistical methods. 3rd. ed. McGraw-Hill, New York.

Myers, E. M., F. J. Janzen, D. C. Adams, and J. K. Tucker. 2006. Quantitative genetics of plastron shape in slider turtles (*Trachemys scripta*). Evolution 60:563–572.

Myers, E. M., J. K. Tucker, and C. H. Chandler. 2007. Experimental analysis of body size and shape during critical life-history events of hatchling slider turtles, *Trachemys scripta elegans*. Funct. Ecol. 21:1106–1114.

Myers, P., B. L. Lundrigan, B. W. Gillespie, and M. L. Zelditch. 1996. Phenotypic plasticity in skull and dental morphology in the Prairie Deer Mouse (*Peromyscus maniculatus bairdii*). J. Morphol. 229:229–237.

Mył narski, M. 1956. On a new species of emydid tortoise from the Pliocene of Poland. Study on the Tertiary bone—breccia fauna from Węże near Działoszyn in Poland. Acta Palaeontol. Pol. 1:153–164.

Nagashima, H., F. Sugahara, M. Takechi, R. Ericsson, Y. Kawashima-Ohya, Y. Narita, S. Kuratani. 2009. Evolution of the turtle body plan by the folding and creation of new muscle connections. Science 325:193–196.

Novack-Gottshall, P. M., and M. A. Lanier. 2009. Scale-dependence of Cope's rule in body size evolution of Paleozoic brachiopods. Proc. Natl. Acad. Sci. USA 105:5430–5434.

Parham, J. F., J. L. Boore, and C. R. Feldman. 2006. The complete mitochondrial genome of the enigmatic bigheaded turtle (*Platysternon*): Description of unusual genomic features and the reconciliation of phylogenetic hypotheses based on mitochondrial and nuclear DNA. BMC Evol. Biol. 6:11.1–11.11.

Parham, J. F., and R. B. Irmis. 2008. Caveats on the use of fossil calibrations for molecular dating: a comment on Near et al. Am. Nat. 171:132–136.

Pierce, S. E., K. D. Angielczyk, and E. J. Rayfield. 2008. Patterns of morphospace occupation and mechanical performance in extant crocodilian skulls: a combine geometric morphometric and finite element modeling analysis. J. Morphol. 269:840–864.

———. 2009. Shape and mechanics in thalattosuchian (Crocodylomorpha) skulls: implications for feeding behaviour and niche partitioning. J. Anat. 215:555–576.

Polly, P. D. 2003a. Paleophylogeography: the tempo of geographic differentiation in marmots (*Marmota*). J. Mammal. 84:369–384.

———. 2003b. Paleophylogeography of *Sorex araneus*: molar shape as a morphological marker for fossil shrews. Mammalia 68:233–243.

———. 2005. Development and phenotypic correlations: the evolution of tooth shape in *Sorex araneus*. Evol. Develop. 7:29–41.

———. 2008. Developmental dynamic and G-matrices: can morphometric spaces be used to model phenotypic evolution? Evol. Biol. 35:83–96.

Posada, D., and K. A. Crandall. 1998. Modeltest: testing the model of DNA substistution. Bioinformatics 14:817–818.

Pritchard, P. C. H. 2008. Evolution and structure of the turtle shell. Pp. 45–83 *in* J. Wyneken, M. H. Godfrey, and V. Bels, eds. Biology of turtles. CRC Press, Boca Raton, FL.

Renous, S., F. de Lapparent de Broin, M. Depecker, J. Davenport, and V. Bels. 2008. Evolution of locomotion in aquatic turtles. Pp. 97–138 *in* J. Wyneken, M. H. Godfrey, and V. Bels, eds. Biology of turtles. CRC Press, Boca Raton, FL.

Rivera, G. 2008. Ecomorphological variation in shell shape of the freshwater turtle *Pseudemys concinna* inhabiting different aquatic flow regimes. Integr. Comp. Biol. 48:769–787.

Rivera, G., and J. Claude. 2008. Environmental media and shape asymmetry: a case study on turtle shells. Biol. J. Linn. Soc. 94:483–489.

Rodriguez, F., J. L. Oliver, A. Marin, and J. R. Medina. 1990. The general stochastic model of nucleotide substitution. J. Theor. Biol. 142:485–501.

Roff, D. A., and D. J. Fairbairn. 2007. The evolution of trade-offs: where are we? J. Evol. Biol. 20:433–447.

Romer, A. S. 1956. Osteology of the Reptiles. Univ. of Chicago Press, Chicago.

Rohlf, F. J. 1990. Rotational fit (Procrustes) method. Pp. 227–236 *in* F. J. Rohlf and F. R. Bookstein, eds. Proceedings of the Michigan morphometrics workshop. Museum of Zoology Special Publication 2. University of Michigan, Ann Arbor, MI.

———. 1998. On applications of geometric morphometrics to studies of ontogeny and phylogeny. Syst. Biol. 47:147–158.

———. 2005. tpsDig 2.04. Department of Ecology and Evolution, State University of New York, Stony Brook, NY.

Rohlf, F. J., and F. L. Marcus. 1993. A revolution in morphometrics. Trends Ecol. Evol. 8:129–132.

Schluter, D. 1996. Adaptive radiation along genetic lines of least resistance. Evolution 50:1766–1774.

Schluter, D., T. Price, A. O. Mooers, and D. Ludwig. 1997. Likelihood of ancestor states in adaptive radiation. Evolution 51:1699–1711.

Sheets, H. D. 2002. CoordGen 6d. http://www3.canisius.edu/~sheets/morphsoft.html.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry. 3rd ed. W. H. Freeman and Company, New York.

Spinks, P. Q., and H. B. Shaffer. 2005. Range-wide molecular analysis of the western pond turtle (*Emys marmorata*): cryptic variation, isolation by distance, and their conservation implications. Mol. Ecol. 14:2047–2064.

———. 2009. Conflicting mitochondrial and nuclear phylogeneies for the widely disjunct *Emys* (Testudines: Emydidae) species complex, and what they tell us about biogeography and hybridization. Syst. Biol. 58: 1–20.

Spinks, P. Q., R. C. Thomson, G. A. Lovely, and H. B. Shaffer. 2009. Assessing what is needed to resolve a molecular phylogeny: simulations and empirical data from emydid turtles. BMC Evol. Biol. 9:56.1–56.17.

Stayton, C. T. 2006. Testing hypotheses of convergence with multivariate data: morphological and functional convergence among herbivorous lizards. Evolution 60:824–841.

———. 2009. Applicaton of thin plate spline transformations to finite element models, or, how to turn a bog turtle into a spotted turtle to analyze both. Evolution 63:1348–1355.

Steppan, S. J., P. C. Phillips, and D. Houle. 2002. Comparative quantitative genetics: evolution of the G matrix. Trends Ecol. Evol. 17:320–327.

Stephens, P. R., and J. J. Wiens. 2003. Ecological diversification and phylogeny of emydid turtles. Biol. J. Linn. Soc. 79:677–610.

———. 2008. Testing for evolutionary trade-offs in a phylogenetic context: ecological diversification and evolution of locomotor performance in emydid turtles. J. Evol. Biol. 21:77–87.

Stinchcombe, J. R., and H. E. Hoekstra. 2008. Combining population genomics and quantitative genetics: finding the genes underlying ecologically important traits. Heredity 100:158–170.

Swofford, D. L. 2002. PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods). Sinauer Associates, Sunderland, MA.

Tabachnik, B. G., and L. S. Fidell. 2007. Using multivariate statistics. Allyn and Bacon, Boston, MA.

Valenzuela, N., D. C. Adams, R. M. Bowden, and A. C. Gauger. 2004. Geometric morphometric sex estimation for hatchling turtles: a powerful alternative for detecting subtle sexual shape dimorphism. Copeia :735–742.

Wake, D. B. 1991. Homoplasy: the result of natural selection, or evidence of design limitations? Am. Nat. 138:543–567.

Webster, M., and M. L. Zelditch. 2005. Evolutionary modifications of ontogeny: heterochrony and beyond. Paleobiology 31:354–372.

Wright, S. 1921. Correlation and causation. J. Agric. Res. 20:557–585.

———. 1968. Evolution and the genetics of populations. Vol. 1. Genetic and biometric foundations. Univ. of Chicago Press, Chicago.

Yang, Z. 1994. Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. J. Mol. Evol. 39:306–314.

Zangerl, R. F. 1969. The turtle shell. Pp. 311–339 *in* C. Gans, A. D. Bellairs, and T. S. Parsons, eds. The biology of the Reptilia. Vol. 1. C. Academic Press, Lond.

Zelditch, M. L., H. D. Sheets, and W. L. Fink. 2003. The ontogenetic dynamics of shape disparity. Paleobiology 29:139–156.

Zelditch, M. L., D. L. Swiderski, H. D. Sheets, and W. L. Fink. 2004. Geometric morphometrics for biologists: a primer. Elsevier, Amsterdam, The Netherlands.

Associate Editor: G. Hunt

## *Supporting Information*

The following supporting information is available for this article:

**Appendix S1.** Abbreviations follow Leviton et al. (1985) except for: ASDM: Arizona Sonoran Desert Museum; GPZ: Gladys Porter Zoo; JRB: James R. Buskirk; KDA: Kenneth D. Angielczyk; MSE: Museum of Systematics and Ecology (University of California, Santa Barbara); UCMP: University of California Museum of Paleontology; UMZC University Museum of Zoology (University of Cambridge); WCS: Wildlife Conservation Society (Bronx Zoo).

**Appendix S2.** Specimens used and GenBank accession numbers for nuclear (nucDNA) and mitochondrial (mtDNA) sequence datasets.

**Table S1.** Results of multivariate regressions of partial warp and uniform component scores (shape) against the natural logarithm of centroid size.

**Table S2.** Parameters estimated for preferred models of plastron shape evolution.

Supporting Information may be found in the online version of this article.

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.