Evolution, 60(3), 2006, pp. 563–572

QUANTITATIVE GENETICS OF PLASTRON SHAPE IN SLIDER TURTLES (*TRACHEMYS SCRIPTA*)

ERIN M. MYERS,[1,2] FREDRIC J. JANZEN,[1,3] DEAN C. ADAMS,[1,4,5] AND JOHN K. TUCKER[6,7]
[1]*Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, Iowa 50011*
[2]*E-mail: emyers1@iastate.edu*
[3]*E-mail: fjanzen@iastate.edu*
[4]*Department of Statistics, Iowa State University, Ames, Iowa 50011*
[5]*E-mail: dcadams@iastate.edu*
[6]*Illinois Natural History Survey, 8450 Montclair Avenue, Brighton, Illinois 62012*
[7]*E-mail: jktucker@inhs.uiuc.edu*

Abstract.—Shape variation is widespread in nature and embodies both a response to and a source for evolution and natural selection. To detect patterns of shape evolution, one must assess the quantitative genetic underpinnings of shape variation as well as the selective environment that the organisms have experienced. Here we used geometric morphometrics to assess variation in plastron shell shape in 1314 neonatal slider turtles (*Trachemys scripta*) from 162 clutches of laboratory-incubated eggs from two nesting areas. Multivariate analysis of variance indicated that nesting area has a limited role in describing plastron shape variation among clutches, whereas differences between individual clutches were highly significant, suggesting a prominent clutch effect. The covariation between plastron shape and several possible maternal effect variables (yolk hormone levels and egg dimensions) was assessed for a subset of clutches and found to be negligible. We subsequently employed several recently proposed methods for estimating heritability from shape variables, and generalized a univariate approach to accommodate unequal sample sizes. Univariate estimates of shape heritability based on Procrustes distances yielded large values for both nesting populations ($h^2 \approx 0.86$), and multivariate estimates of maximal additive heritability were also large for both nesting populations ($h^2_{\max} \approx 0.57$). We also estimated the dominant trend in heritable shape change for each nesting population and found that the direction of shape evolution was not the same for the two sites. Therefore, although the magnitude of shape evolution was similar between nesting populations, the manner in which plastron shape is evolving is not. We conclude that the univariate approach for assessing quantitative genetic parameters from geometric morphometric data has limited utility, because it is unable to accurately describe how shape is evolving.

Key words.—Additive genetic covariance matrix, clutch effects, geometric morphometrics, Procrustes distance, quantitative genetics.

Received November 14, 2005. Accepted January 12, 2006.

To understand morphological evolution, one must first document patterns of variation and then examine the relative influence that environmental and genetic factors play in the development and maintenance of these patterns. Although natural and sexual selection are important forces that shape proximate patterns of morphology, for these patterns to be realized through evolutionary time there must be heritable genetic variation of the morphological traits under selection (Lande 1979; Lande and Arnold 1983; Janzen and Stern 1998). A fruitful way to examine this process, characterizing both genetic and phenotypic variation, is to integrate the tools of evolutionary quantitative genetics and morphometrics.

The field of evolutionary quantitative genetics was developed to characterize, quantify, and predict the ways in which in traits can and do respond to natural and sexual selection (Roff 1997; Lynch and Walsh 1998). Univariate quantitative genetics has been used to determine the degree of heritability for a trait, as well as predict how traits will respond to selection. To examine such a trait however, one must first accurately quantify its phenotype, whether in the form of size, color, and/or shape. Shape, in particular, is important to examine because it is likely a major aspect of phenotype upon which natural selection can act. However, summarizing shape alone can be challenging because such analyses are also influence by other aspects of phenotype such as size. The tools of geometric morphometrics can be used to quantify shape variation and assess covariation in shape with other variables (Bookstein 1989; Rohlf and Marcus 1993; Adams et al. 2004; see Materials and Methods section below). In particular, these methods allow shape to be quantified after the potentially confounding effects of size, position, and orientation inherent in biological data, have been held mathematically constant (Rohlf and Slice 1990). Recently, several methods have been proposed to combine geometric morphometric data with the powerful tools of evolutionary quantitative genetics. One approach is a univariate approximation based on Procrustes distance (Monteiro et al. 2002: see also Klingenberg and Monteiro 2005), while a second is a multivariate generalization of the standard breeders' equation (Klingenberg and Leamy 2001; Klingenberg 2003).

The slider turtle, *Trachemys scripta*, is an ideal species in which to study shape variation in offspring at several ecological levels. Clutch sizes are substantive ($X \approx 13$, 6–27 for the focal populations in this study; Tucker et al. 1998a) and female shell morphology differs among local populations (Tucker et al. 1998b). Moreover, females are philopatric, returning to the same nesting area year after year, presenting an opportunity for genetic differentiation and differential selection on both mother and offspring between nesting sites (J. K. Tucker and F. J. Janzen, unpubl. data). Furthermore, body size of young sliders experiences strong selection from avian predators (Janzen et al. 2000a,b).

The turtle shell, comprised of a dorsal portion (carapace) and ventral portion (plastron), constitutes a highly novel mor-

© 2006 The Society for the Study of Evolution. All rights reserved.

phological trait (Gilbert et al. 2001). The carapace and plastron are of neural crest origin (Clark et al. 2001), deriving from vertebral and rib elements and lying superficial to both limb girdles (Burke 1989). The shell provides a significant measure of protection from predation (Greene 1988), particularly in adult turtles, and also can strongly impact locomotor performance (e.g., Zani and Claussen 1995). A number of genes are involved in guiding development of the shell (Loredo et al. 2001; Vincent et al. 2003; Kuraku et al. 2005), potentially providing ample genetic substrate for evolutionary modification of this unique trait. In this study, we chose to examine shape of the plastron because variation in this trait may contribute to fitness variation within a population. Shell shape could influence individual survival and fitness, particularly in the early life-history stages where avian predation is often intense and locomotor performance on land or in water is critical (e.g., Janzen et al. 2000a,b). The plastron also possesses ideal characteristics for morphometric analysis, with unambiguous landmark locations and relatively flat shape.

Based on this knowledge of the plastron and of our study organism, we generated two related predictions about plastron shape: (1) plastron shape should be variable among hatchling turtles at the level of the individual, clutch, and nesting area, as a result of potential differential selection and inheritance, and (2) that the variation has a heritable genetic component. To address these predictions, we used recent advances in the fields of evolutionary quantitative genetics and geometric morphometrics. We quantified the shape of hatchling plastrons to address potential variation, examined environmental and genetic influences on shape variation, and estimated the degree of heritability for plastron shape.

Materials and Methods

Specimens

A total of 1314 slider hatchling turtles (*Trachemys scripta*) from 162 clutches (families) was used in this study. These individuals were obtained from one nesting area in Calhoun County, Illinois (Swan Lake: 735 individuals from 92 families) and one nesting area in Jersey County, Illinois (Dabbs Road: 579 individuals from 70 families), which are <5 km apart on the Illinois River (Tucker 1997). Samples were obtained in 1996 and 2003. For the 1996 sample, 22 gravid females were hand captured in the field, and from them 267 eggs were obtained. From each nesting female, a subset of eggs was used to assess levels of yolk testosterone. The remaining 195 eggs were incubated in the laboratory under approximately constant temperature and hydric conditions to control for environmental effects (for details of incubation regime see Janzen et al. 1998). Upon hatching, neonates were maintained for 2–3 months in individual containers, and were then euthanized and preserved as voucher specimens (see Janzen et al. 1998). For the 2003 sample, 1119 individuals from 140 families were obtained and incubated under controlled conditions similar to those used in 1996. After hatching, each individual was photographed for morphological quantification (see below), and was then returned to its natal site for release in the wild.

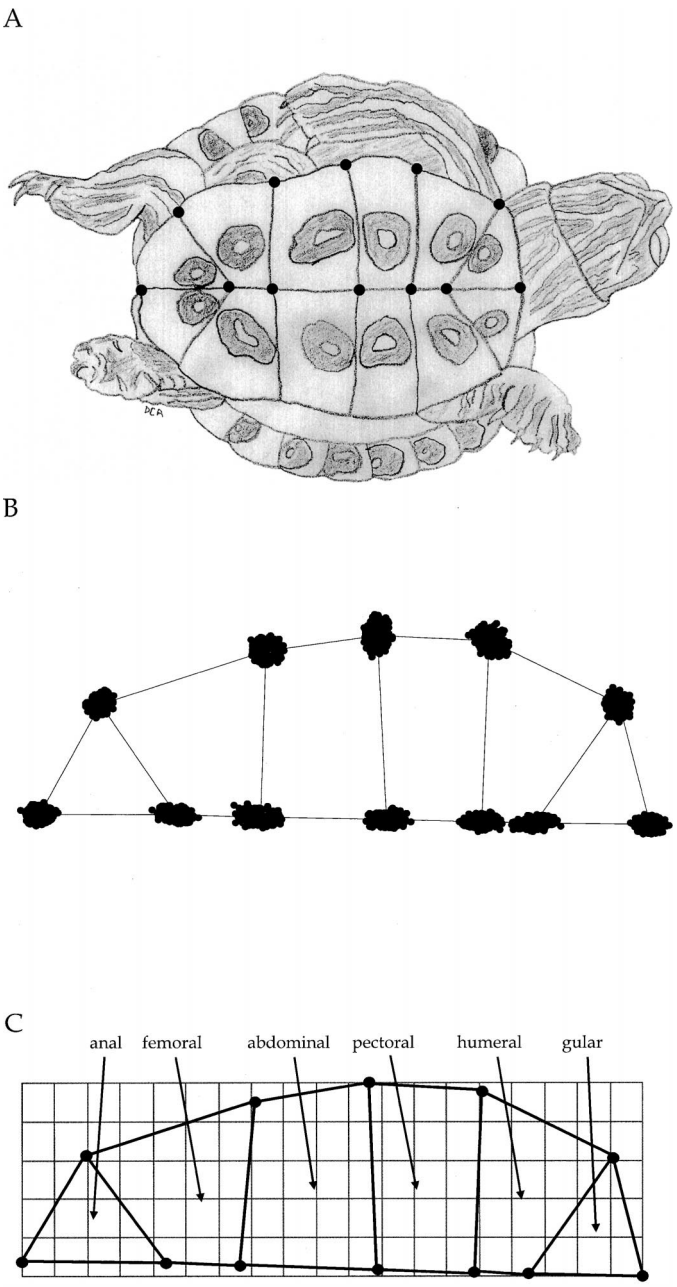


FIG. 1. (A) Ventral view of hatchling slider turtles (*Trachemys scripta*) showing the landmarks used in this study. (B) Generalized Procrustes superimposition of 1314 hatchling turtles showing the total variation at each landmark. (C) Average hatchling shape shown as a thin-plate spline deformation grid (specific scutes are noted).

Morphological Data Collection

Plastron shape was quantified using landmark-based geometric morphometric methods (Rohlf and Marcus 1993; Adams et al. 2004). These methods allow a rigorous quantification of shape, after the effects of nonshape variation have been mathematically held constant. First, digital images of the plastron of each specimen were obtained using a Nikon DXM-1200 high-resolution digital camera. From each image, the x, y coordinates of 12 anatomical landmarks (Fig. 1A)

were then recorded using TpsDig (Rohlf 2002a). Landmarks included the intersections of the lines delineating the gular, humeral, pectoral, abdominal, femoral, and anal scutes, and are biologically repeatable (i.e., operationally homologous) anatomical points. Using the set of *x, y* coordinates, a generalized Procrustes analysis (GPA) was performed to superimpose the specimens to a common coordinate system, and mathematically remove the effects of digitizing position, orientation, and scale (Rohlf and Slice 1990). From the aligned specimens (Fig. 1B), the average specimen was calculated (Fig. 1C), and shape variables were then generated as partial warp scores from the thin-plate spline (Bookstein 1989; 1991) and the two standard uniform components (Bookstein 1996; Rohlf and Bookstein 2003). Together, these variables (called the weight matrix) capture the linear and non-linear aspects of shape variation, and can be used to test hypotheses of shape variation and covariation within and among groups using standard multivariate statistical procedures (see e.g., Adams and Rohlf 2000; Rüber and Adams 2001; Adams 2004). All superimposition and thin-plate spline computations for generating morphometric shape variables were performed in TPSRelw (Rohlf 2002b).

Statistical Analyses

To assess variation in plastron shape at the nesting area levels, we performed a nested multivariate analysis of variance (MANOVA) on the weight matrix, where the beach effect was tested against the clutch effect. Clutch variation was also assessed using a MANOVA. We then examined patterns of shape variation using a principal components analysis (PCA), where individual hatchlings were coded by area. For assessing whether plastron shape variation exhibited a heritable component, we performed several analyses. First, we examined whether plastron shape covaried with possible nongenetic maternal effects, including egg dimensions and levels of yolk testosterone, using data obtained from the 1996 sample. For the egg dimensions (length and width), a two-block partial least squares analysis was performed (Rohlf and Corti 2000), which assessed the degree of association between plastron shape and egg dimensions. For levels of yolk testosterone, a different procedure was used because values of yolk testosterone levels were obtained for each *clutch*, rather than for each hatchling (see Janzen et al. 1998). Consequently, here we were obliged to calculate the mean plastron shape for each clutch and then determine the association between plastron shape and yolk testosterone level using a Mantel test of matrix correlation (Mantel 1967). Analyses were performed in NTSYSpc (Rohlf 2000), JMP (SAS Institute Inc. 2002), and TPSPLS (Rohlf 2002c).

Second, we investigated whether variation in plastron shape among clutches had a genetically heritable component. We used several recent protocols that have been proposed for estimating heritability of shape variation derived from landmark data. The first approach is a univariate approximation of shape heritability based on Procrustes distance (Monteiro et al. 2002), the measure of shape difference used in geometric morphometric analyses (Bookstein 1991). A second method is a modification of this approach (Klingenberg and Monteiro 2005), while a third technique (Klingenberg and Leamy 2001; Klingenberg 2003) is based on the multivariate generalization of the breeders' equation (Lande 1979), and thus investigates shape heritability using the original multivariate data set (the weight matrix). Because prior fieldwork revealed that these two nesting areas are allopatric with respect to female nesting and reproductive behavior (J. K. Tucker, unpubl. data), heritability estimates were obtained for each nesting area separately, and were subsequently compared to one another. We explicitly justify the reliability of these heritability estimates in the Discussion based on the large sample sizes, common-garden design, likelihood of high incidence of multiple paternity, tests for maternal effects, and low probability of dominance effects.

One significant concern with the univariate approach proposed by Monteiro et al. (2002) is that it is only appropriate when sample sizes among families are equal. Because this constraint is unrealistic even for controlled breeding experiments, we have formulated a generalization of their method that is suitable for designs with equal or unequal sample sizes. For this approach, we first superimposed all specimens using GPA, and calculated the mean for each family (clutch), as well as the overall mean. The partial Procrustes distances between each specimen and its family mean, as well as each family mean versus the overall mean, were then calculated as:

$$d_{\text{Proc}}(X_1, X_2) = \sqrt{\sum_{i=1,j=1}^{p,k} (X_{1ij} - X_{2ij})^2} \tag{1}$$

where X_1 and X_2 are the aligned *x, y* coordinates of two specimens, k is the number of dimensions of the landmark coordinates (here $k = 2$), and p is the number of landmarks. From these, estimates of variation among and within families were calculated as:

$$MS_{\text{among}} = \frac{\sum_{j=1}^{M} L_j d^2_{\text{Proc}}(\overline{X_j}, \overline{\overline{X}})}{(M - 1)m} \tag{2}$$

$$MS_{\text{pooledwithin}} = s_e^2 = \frac{\sum_{i=1}^{L_i} \sum_{j=1}^{M} d^2_{\text{Proc}}(X_{ij}, \overline{X_j})}{\left(\sum_{j=1}^{M} L_j - M\right)m} \tag{3}$$

where m is the dimensionality of shape space (i.e., the number of shape variables), M is the number of families, and L_j is the number of individuals in the j^{th} family. The variance component among families was then calculated as:

$$s_a^2 = \frac{MS_{\text{among}} - MS_{\text{pooledwithin}}}{L} \tag{4}$$

where L is the harmonic mean of the family sample sizes. The intraclass correlation, expressing the proportion of among-family variance relative to total variance, was calculated as $t = s_a^2/(s_a^2 + s_e^2)$ (see eq. (5) of Monteiro et al. 2002). Because our data could be modeled as a full-sibling design, heritability (h^2) was then found as $t/2$ (see Falconer and Mackay 1996; Lynch and Walsh 1998). Note that this approach is conservative, because a large fraction of turtle clutches exhibits multiple paternity (e.g., >30% in the paint-

ed turtle, a close relative of *T. scripta*; Pearse et al. 2002). The significance of this estimate was assessed using a permutation test, where individuals were randomly assigned to clutches and h^2 was recalculated. This procedure was repeated 9999 times to generate a theoretical distribution of possible h^2 for comparison to the observed h^2. Additionally, the difference in observed heritability estimates for the two nesting populations was calculated ($D = h_1^2 - h_2^2$), and was compared to a distribution of possible difference scores obtained from the permutation procedure. This approach allowed us to determine whether the observed h^2 estimates for the two nesting populations differed significantly from one another (for an analogous procedure see e.g., Adams 2004).

Our equations for MS_{among} and $MS_{\text{pooledwithin}}$ (eqs. 2 and 3 above) differ from the estimates derived from the *F*-ratio of Monteiro et al. (2002: eq. 3) in two important respects. First, *m* was not present in their original equation, because it canceled out of the numerator and denominator in their *F*-ratio. For accurate estimation of MS_{among} and $MS_{\text{pooledwithin}}$ however, *m* must be included (L. Monteiro pers. comm.). Second, the sample size term, *L* of their approach is only useful when sample sizes among families are equal. When this is not the case, *L* in their equation has no meaning, as it differs among families. To account for this, we have adapted a generalized MS_{among} formula based on those used in analysis of variance with unequal sample sizes (see Sokal and Rohlf 1995, p. 208). This formulation includes L_j for each family, and thus correctly accounts for unequal sample sizes. A similar adjustment is required for $MS_{\text{pooledwithin}}$, where the denominator is based on the L_j for each family, rather than on the harmonic mean (*L*). These alterations do not affect estimates for data with equal sample sizes among families, but are generalizations of the original procedure that permit the analysis of data with unequal sample sizes among families.

One advantage of the univariate approach of Monteiro et al. (2002) is that it is intuitive, and relatively straightforward to implement. However, by representing shape differences among specimens by their Procrustes distance, it necessarily makes important simplifying assumptions. For instance, whereas Procrustes distance may reflect the magnitude of shape difference between two objects, it does not describe how the two objects differ (i.e., the direction of shape difference in shape space: see Klingenberg 2003). Thus, the univariate approach is able to identify whether two groups have similar levels of shape heritability, but is unable to detect whether the shape features themselves are evolving in similar ways in the two groups (for a discussion see Klingenberg 2003). Additionally, simplifying the multivariate breeders' equation to a univariate estimate is only possible by imposing the restrictive assumption that shape variation follows an isotropic model, in which variation at each landmark is equal and independent of other landmarks (Goodall 1991). Although it is difficult to assess the assumption of isotropic error and the resulting covariance among landmarks (Rohlf and Slice 1990; Adams et al. 2004), it is widely accepted that this is an unrealistic assumption for biological datasets (see e.g., Klingenberg 2003).

As a result of these shortcomings and the restrictive assumptions required to implement the univariate approach, an alternative has been proposed that examines shape heritability using a multivariate framework (Klingenberg and Leamy 2001; Klingenberg 2003). Because shape can vary by the relative shifting of landmarks, treating shape as a multivariate dataset throughout the analysis is particularly appropriate (Klingenberg 2003). For this approach, we first superimposed all specimens using GPA, and calculated the weight matrix (the set of shape variables for all specimens). We then used restricted maximum likelihood (REML) to estimate the genetic and phenotypic variance components for each nesting population (e.g., Lynch and Walsh 1998, ch. 26; Milner et al. 2000; Klingenberg and Leamy 2001). This approach makes fewer assumptions than traditional regression methods (Milner et al. 2000), and can better accommodate the unbalanced designs typically found in experiments performed on wild populations. We implemented a full-sib 'animal' model design using the software package VCE5 (Neumaier and Groeneveld 1998; Kovač and Groeneveld 2003). From this, the additive genetic (**G**) and phenotypic (**P**) covariance matrices of shape were obtained. Using the multivariate generalization of the breeders' equation: $\mathbf{\Delta\bar{z}} = \mathbf{GP}^{-1}\mathbf{s}$ (Lande 1979; Lande and Arnold 1983), we obtained $\mathbf{GP}^{-1}$, which can be thought of as the multivariate version of heritability. If selection is treated as approximately constant, the left dominant eigenvector of $\mathbf{GP}^{-1}$ describes the direction in the phenotype space that will yield the maximal response to selection (Klingenberg and Leamy 2001). The dominant eigenvalue associated with this direction describes the maximum additive heritability (Efimov et al. 2005) of a set of phenotypic traits (h^2_{max}). This value is also the multivariate equivalent of realized heritability (e.g., Falconer and Mackay 1996, p. 197), and allows one to predict the maximum phenotypic response to the selection differential (see Klingenberg and Leamy 2001).

Because the multivariate approach to shape evolution captures both the magnitude and direction of heritable shape change, statistically assessing both of these components of shape change is possible. To examine the magnitude of shape heritability, we calculated the dominant eigenvector and h^2_{max} for each nesting population. We then assessed the statistical significance of each h^2_{max} estimate using a permutation procedure, in which individuals were randomly assigned to clutches, the **G** and **P** matrices recalculated in VCE5, and h^2_{max} obtained. Because the calculations of the variance components using REML were computationally intensive, 999 permutations were used to generate a theoretical distribution of possible h^2_{max} for comparison to the observed h^2_{max} estimates. We also calculated the difference in h^2_{max} estimates ($D = h_1^2 - h_2^2$) to determine whether the observed h^2_{max} estimates for the two nesting populations differed significantly from one another. We also assessed differences in heritability between the two nesting populations ($D = h_1^2 - h_2^2$) using heritability estimates computed as: $h^2 = tr(\mathbf{G})/tr(\mathbf{P})$ (Klingenberg and Monteiro 2005). This approach estimates heritability as the total genetic variation relative to the total phenotypic variation, although genetic and phenotypic covariation is ignored. As before, the difference in heritability estimates between populations obtained from this approach was compared to a distribution of possible difference scores obtained from the permutation procedure.

To examine the direction of shape evolution, we calculated

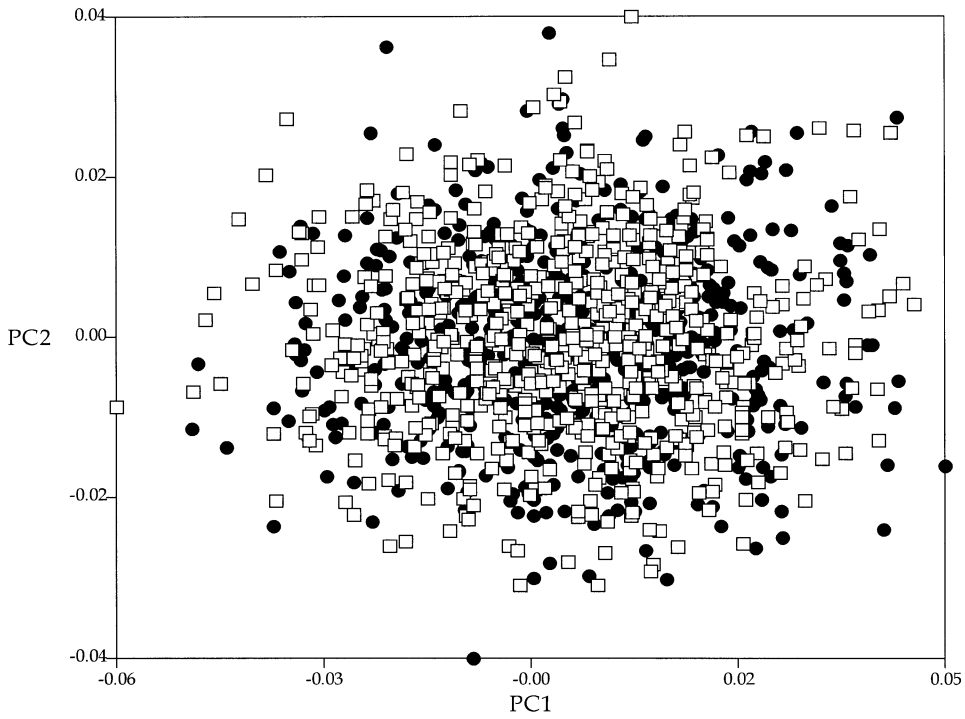


FIG. 2. Plot of first two dimensions of a principal components analysis of plastron shape for 1314 *Trachemys scripta* hatchling turtles. The first two dimensions explain 36% of the total variation in shape. Closed circles represent individuals from Dabbs Road, and open squares represent individuals from Swan Lake.

the correlation between the dominant eigenvectors for each of the two nesting areas as the cosine of the angle between the two vectors. If shape variation had evolved in a similar manner within each population (i.e., the direction of inheritance was the same), the correlation between these two vectors should be larger than expected by chance. To statistically assess this possibility, we compared the observed correlation between dominant eigenvectors to a set of random values, which were obtained from iterations of the permutation procedure above. This approach was used to determine whether the observed correlation was different from what is expected under random chance (see e.g., Klingenberg and Leamy 2001; M. L. Collyer and D. C Adams, unpubl. ms.). The average of the random angles, as well as the smallest angle between vectors obtained through the simulation, were also observed. Finally, to anatomically describe the shape feature most likely to respond to selection, we projected the shape variables for each specimen onto the left dominant eigenvector of $\mathbf{GP}^{-1}$ to generate thin-plate spline deformation grids for specimens from both the negative and positive sides of this vector. This procedure was performed for both nesting populations, and the resulting graphics of shape deformation along this direction were qualitatively compared.

RESULTS

Patterns of Shape Variation

Using a nested MANOVA, we found no significant differences in plastron shape between the two nesting areas (Wilks' $\Lambda = 0.854$, $F = 1.2$, $P =$ ns). Examining patterns of shape variation among nesting locations using a PCA revealed this pattern, as there was substantial overlap in plastron shape between Swan Lake and Dabbs Road turtles (Fig. 2). However, there was significant shape variation among clutches (Wilks' $\Lambda = 0.000043$, $F = 4.635$, $P \ll 0.0001$). Thus, significant variation in plastron shape was observed in these data, and appears due to differences between individual clutches.

Association of Shape with Other Factors

We examined possible nongenetic maternal effects by assessing the degree of association between plastron shape and several additional variables on a subset of clutches ($n = 22$). Using a Mantel test of matrix correlation (Mantel 1967), we found no relationship between plastron shape and yolk testosterone levels ($r_M = 0.20$, $P > 0.05$). Thus, hormone allocation to egg yolks by nesting females was not a predictor of hatchling plastron shape. We did find a significant association between plastron shape and egg dimensions ($r = 0.435$, $P_{Rand} = 0.001$) using a two-block partial least squares analysis, and an ANOVA on PLS scores revealed differences among clutches (Dabbs: $F = 23.85$, df = 8,73, $P < 0.0001$; Swan: $F = 18.34$, df = 12,100, $P < 0.0001$). The egg dimension axis described a contrast between relatively longer and wider eggs, whereas the plastron shape axis described a contrast between relatively longer, skinnier turtles and relatively shorter, wider turtles. Therefore, this analysis revealed that relatively longer turtles were hatched from elongate eggs, whereas more rounded turtles were hatched from more circular eggs (Fig. 3). This pattern is not surprising, given the constraints of egg dimensions on hatchling size and shape.

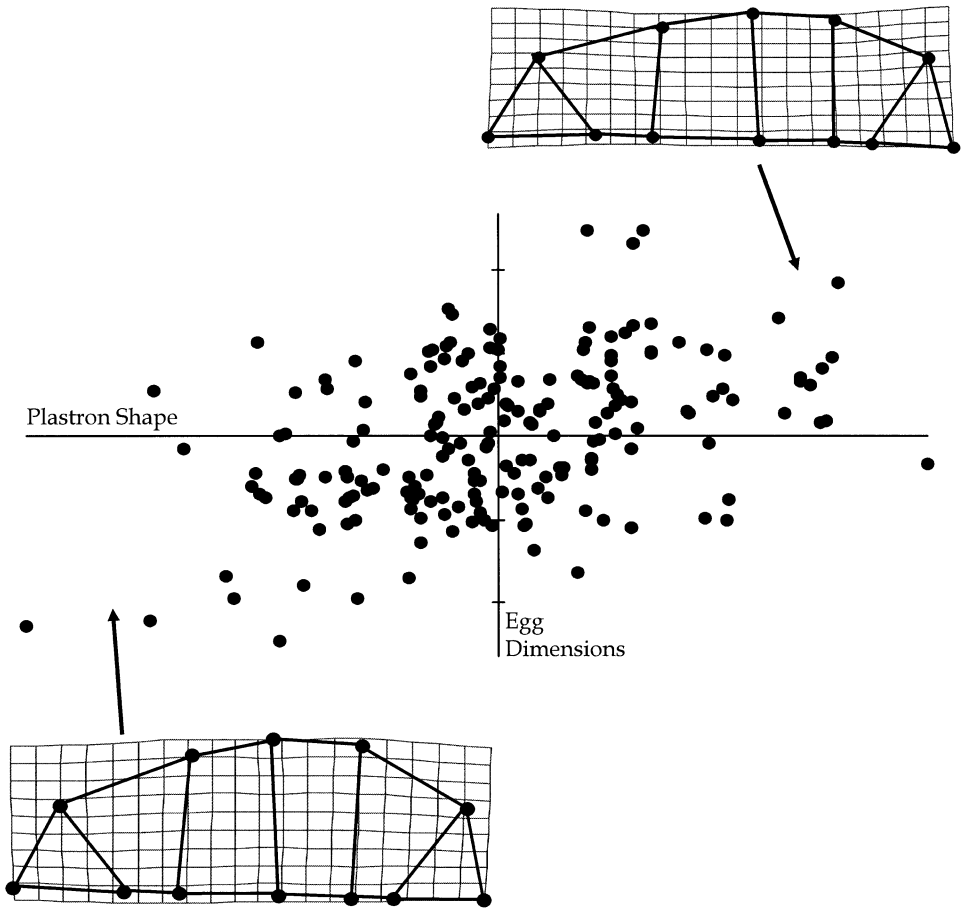


FIG. 3. Results from a two-block partial least squares analysis showing the association between plastron shape (X) and egg dimensions (Y) for *Trachemys scripta*. Thin-plate spline deformation grids are shown along the negative and positive sides of the plastron shape axis.

However, it is unlikely that females directly influence this relationship through nongenetic maternal effects, unless they provision their offspring by manipulating the relative shape of their eggs (see Discussion).

Assessment of Plastron Shape Heritability and Predicted Response to Selection

Using the univariate estimate of shape heritability based on Procrustes distance (Monteiro et al. 2002), we found a large and significant heritable component of plastron shape variation for both nesting areas (Dabbs Road: $h^2 = 0.858$, $P_{R\text{and}} = 0.0001$, N = 70 families; Swan Lake: $h^2 = 0.859$, $P_{R\text{and}} = 0.0001$, N = 92 families: Table 1). Further, these values were not statistically distinguishable from one another, implying that the amount of shape heritability was equivalent for the two nesting populations ($h_1^2 - h_2^2 = 0.00077$, $P_{R\text{and}} = 0.273$). Using the multivariate approach (Klingenberg and Leamy 2001; Klingenberg 2003), we found large and significant levels of $h^2_{\max}$ for both nesting areas (Dabbs Road: $h^2_{\max} = 0.575$, $P_{R\text{and}} = 0.022$; Swan Lake: $h^2_{\max} = 0.519$, $P_{R\text{and}} = 0.030$), and like the univariate analyses, these values were not statistically distinguishable ($h_1^2 - h_2^2 = 0.056285$, $P_{R\text{and}} = 0.320$). Similarly, the proportional variance approach (Klingenberg and Monteiro 2005) revealed that the magnitude of shape heritability was not distinguishable between populations ($h_1^2 - h_2^2 = 0.002557$, $P_{R\text{and}} = 0.112$). Therefore, regardless of which approach is used to estimate heritability, plastron shape appears to have a substantive heritable genetic component, whose magnitude is similar for the two nesting areas.

From the multivariate heritability matrix ($\mathbf{GP^{-1}}$) we calculated the dominant eigenvector for each nesting population and estimated the shape feature with the most potential to respond evolutionarily to linear selection. We found that the

TABLE 1. Procrustes estimates of shape variance components, intraclass correlation, and heritability for hatchling *Trachemys scripta* plastrons from two nesting areas. The significance level for heritability was based on a permutation test with 9999 iterations.

Source	MS	Variance component	Intraclass correlation	h^2	P_{Rand}
(A) Nest analysis for Dabbs Road (n = 70 families)					
Clutch	0.004807	0.000567	0.42948	0.8589	0.0001
Error	0.000754	0.000753			
(B) Nest analysis for Swan Lake (n = 92 families)					
Clutch	0.004555	0.000561	0.42987	0.8597	0.0001
Error	0.000744	0.000744			

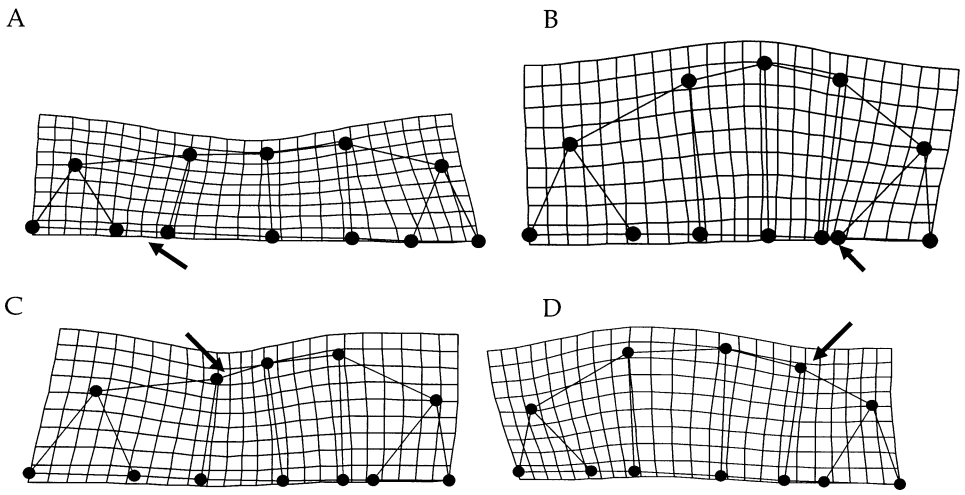


FIG. 4. The shape feature exhibiting the maximal evolutionary response to linear selection, as calculated by the dominant eigenvector of $\mathbf{GP}^{-1}$. Figure represents shapes along this vector in both the negative and positive directions for (A and B) Dabbs Road and (C and D) Swan Lake populations of *Trachemys scripta* (deformation grids are displayed at 3X the range of the observed shape variation in the dataset). Arrows are used to draw attention to those areas of increased shape deformation.

dominant eigenvectors for the two nesting populations were not significantly correlated with one another ($r = 0.195$, $P_{R\text{and}} = 0.631$). This lack of correlation of vectors corresponds to a difference of 78° in the direction of the two vectors in shape space. Further, the smallest angle between vectors generated by randomly assigning specimens to families was 40°. Taken together, these results suggest that although the level (magnitude) of shape heritability in the two nesting populations was concordant, the direction of shape evolution was not.

This difference was evident when the shape features described by each vector were depicted graphically (Fig. 4). Interestingly, in both nesting populations this feature generally described contrasts in shape between laterally expanded and laterally compressed plastrons (i.e., long-and-skinny vs. short-and-stout shapes). However, the manner in which these general features are achieved was different in both populations. For the Dabbs Road nesting area, laterally compressed shapes were achieved by a relative anterior-posterior compression of the femoral and humeral scutes along the midline of the plastron (Fig. 4a and 4b), whereas the same overall shape was achieved in the Swan Lake nesting area more through an anterior-posterior compression of the abdominal and pectoral scutes along the perimeter of the plastron (Fig. 4c and 4d). Therefore, if the selection differential is proportional to this vector (Klingenberg and Leamy 2001), it would likely drive the evolution of length and width of hatchling plastrons similarly in both populations, but would accomplish this overall morphological convergence through differential changes in central scute size and shape in each.

DISCUSSION

In this study we used geometric morphometric methods to examine patterns of plastron shape variation in hatchling turtles from 162 familial clutches from two aquatically contiguous nesting areas. We detected significant differences in plastron shape between the two nesting areas, as well as among clutches. We also evaluated and discounted the potential impact of two nongenetic maternal effects on these differences and proceeded to apply several recent methods for estimating heritability from shape variables. Our analyses revealed substantial heritability of plastron shape in both nesting areas, but that the direction of shape evolution was strikingly different in each. We explore the evolutionary implications of these findings below.

The relationship between plastron shape and several nongenetic maternal effects was examined, revealing that levels of yolk testosterone did not predict hatchling shape. This observation is important for our subsequent heritability analyses, because yolk testosterone correlates with egg size in a related turtle, *Chrysemys picta* (Bowden et al. 2004). In *C. picta*, smaller eggs are more testosterone-laden and tend to be laid by younger (although not necessarily smaller) females. In *T. scripta*, no such correlation exists (Janzen et al. 1998). Egg dimensions were correlated with hatchling shape in our study, and these egg dimensions differed among clutches. However, these findings are not unexpected, given the constraints imposed on hatchling size and shape through egg size. Differences in general egg dimensions could conceivably be due to variation in size of the maternal pelvic aperture during oviposition, although such constraints are more likely to exist only in species of turtles smaller than *T. scripta* (Tucker et al. 1978; Congdon and Gibbons 1987; Clark et al. 2001). Although there can be differences in egg dimensions due to relative position in the order of egg laying (Tucker and Janzen 1998), it is likely that these effects are minimized because all eggs from each clutch were analyzed in our study. In sum, there is no evidence in these turtles to suggest that females manipulate egg dimensions prior to oviposition so as to subsequently alter plastron shape of the neonates and thereby serve as a nongenetic maternal effect underlying the patterns we noted.

Quantitative genetic analyses of wild animals are fraught with difficulties. The required time, effort, and financial cost to generate good quantitative genetic estimates has meant

such studies constitute only a small fraction of the literature, yet it is precisely these data that provide us with the necessary genetic insight to fully understand microevolution in the wild. We designed our study of a free-ranging turtle to minimize confounding variables and thereby to obtain reasonable estimates of quantitative genetic parameters: (1) the large sample size (162 families averaging over eight offspring each) improves the accuracy of the estimates; (2) the common-garden experimental design minimized the otherwise inflationary contribution of environmental variance to heritability estimates; (3) assuming the offspring in each clutch are full siblings renders our analyses conservative because multiple paternity (i.e., half sibs) is high in turtle clutches (e.g., >30% in *C. picta*; Pearse et al. 2002); (4) statistical analyses of a subset of clutches assessed two of the most likely nongenetic maternal effects and largely discounted any substantive inflationary impact of those factors on the heritability estimates; and (5) dominance effects are generally small for morphological traits of free-ranging organisms (Crnokrak and Roff 1995), leading to similar heritability estimates from full sibling and parent-offspring designs (Mousseau and Roff 1987). Consequently, we have strong confidence in our heritability estimates and their comparability to other quantitative genetic estimates from breeding designs.

Possible genetic effects on plastron shape were examined using several recent methods for landmark data. With these univariate and multivariate approaches, a significant heritable component was revealed, and estimates were statistically the same for each population ($h^2 \approx 0.86$ and $h^2_{max} \approx 0.57$, respectively). The concordance of these estimates indicates that, for this dataset, the general magnitude of shape heritability is the same for the two populations. These relatively high heritability estimates are in accord with, although somewhat higher than, typical values for morphological characters (reviewed in Roff 1997). It should be noted, though, that the individuals used in our experiment derived from eggs reared under constant thermal and hydric conditions in the laboratory. This artificial incubation environment could reduce the environmental variance associated with plastron shape under natural conditions and, hence, artificially influence the heritability estimates. However, an experiment designed to test this hypothesis in common snapping turtles detected no differences in phenotypic variances between hatchlings from eggs reared in the laboratory compared to their siblings deriving from the natal nests (J. R. St. Juliana and F. J. Janzen, unpubl. data), therefore these environmental effects may, in fact, be negligible. After having accounted for possible confounding nongenetic maternal effects, our quantitative genetic analyses thus suggest that plastron shape possesses significant potential for microevolutionary response to linear selection.

We adopted a novel approach for estimating the shape feature with the most potential to respond evolutionarily to linear selection, using the dominant eigenvector of the heritability matrix (Klingenberg and Leamy 2001). We found that the general features of this morphological change were similar between the two nesting populations, describing a lateral expansion or compression of the plastron. However, the manner in which this was accomplished anatomically differed considerably between the two populations. Previous studies have shown that other aspects of morphology (e.g. linear size and mass) of hatchling turtles exert a significant impact on performance and survival in nature (Janzen et al. 2000a, b). Although not included in these studies, body shape may also have important consequences for hatchling performance and survival (e.g., swimming ability or avoidance of avian predation). The relatively high heritability of plastron shape implies an ability to rapidly respond to selection pressures in a changing environment, such as variable water levels, predator populations, and weather conditions along the Mississippi River (Tucker 1997; Janzen et al. 2000b; Filoramo and Janzen 2002). Alternatively, this high heritability might suggest that selection acting on plastron shape is generally weak, compared with size variation, but is still a contributing factor. Measurements of the plastron and carapace are tightly correlated ($r = 0.77$ to 0.88) in other species of turtles, suggesting that carapace shape may also be genetically correlated with plastron shape and thus exhibit an indirect evolutionary response to selection on plastron shape (Martin and Layne 1987). Further studies are directly examining hatchling fitness in association with shape, rather than size alone, to determine what role hatchling performance and survivorship play in shaping the plastron (Myers and Tucker, unpubl. data).

The integration of evolutionary quantitative genetic and geometric morphometric approaches in our study produced results that also have significant implications for systematics and phylogenetics. Despite the growing use of molecular characters in systematic and phylogenetic studies, morphological traits remain a significant component of these important evolutionary analyses, particularly those involving fossil material (e.g., Shaffer et al. 1997). Shell morphology has played a substantial role in this regard for turtles (reviewed for plastron morphology in Lovich and Ernst 1989; Lovich et al. 1991), including sliders and their relatives, which have had a contentious history regarding their systematics (Ernst 1990; Legler 1990; Seidel and Jackson 1990). To the extent that the plastron shape features are retained into adulthood, our study implies that, in concert with substantial heritable variation, selection on plastron width in particular (as well as abdominal, pectoral, femoral, and humeral scute morphology) should readily yield substantial morphological evolution. In addition to genetic constraints on plastron shape evolution, other morphometric studies examining shell shape have shown that environmental shifts can contribute to plastral shape change (Claude et al. 2003). The potential ease with which taxonomically important characters like plastron shape can evolve should give pause to systematic studies of these turtles that involve fossil material or only focus on morphology.

In sum, we extended the univariate method of assessing heritability from geometric morphometric shape variables to encompass analyses with unequal sample sizes. Using this generalized approach and a proportional variance technique we have shown that there is a great deal of heritable variation in plastron shape. When compared to the multivariate approach, we found that all methods yielded similar results, and the same was true when comparing heritability estimates between both nesting populations. However, using the multivariate approach we found that the *direction* of shape her-

itability differed significantly between nesting populations. This finding is an empirical demonstration of the concerns raised by Klingenberg (2003), who argued that univariate approaches are unable to detect important aspects of how, and in what direction, shape is evolving. For this reason, we advocate the use of the multivariate approach in morphometric heritability studies. Interestingly, in our example, the shape feature most likely to respond to linear selection was concordant between the two nesting populations, describing a general contrast between elongated and stout plastron shapes. However, the specific manner by which these shapes were achieved was different in each nesting population. Thus, the microevolutionary trajectory of plastron shape likely differs between the two populations. Our research suggests that further studies on the relationship between plastron shape and fitness of turtles would be useful to test the evolutionary hypotheses generated herein.

Acknowledgments

Turtles and eggs were collected under the Illinois Department of Conservation Scientific Permit A93.0202 and extensions. We thank M. Wilson and S. Ford for help with egg and yolk hormone measurements. C. Klingenberg and an anonymous reviewer provided valuable comments on earlier versions of the manuscript. The research was conducted under Iowa State University Animal Care Protocol number 6-6-3258-1-J. This work was supported in part by National Science Foundation grants DEB-0122281 and DEB-0446758 to DCA and National Science Foundation grants DEB-9629529 and DEB-0089680 to FJJ. EMM was supported by a U.S. Department of Agriculture, Initiative for Future Agricultural and Food Systems Multidisciplinary Graduate Education Training Grant (2001-52100-11506). This is contribution 10 of the National Great Rivers Research and Education Center.

Literature Cited

Adams, D. C. 2004. Character displacement via aggressive interference in Appalachian salamanders. Ecology 85:2664–2670.

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

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

———. 1991. Morphometric tools for landmark data: geometry and biology. Cambridge Univ. Press, Cambridge, U.K.

———. 1996. A standard formula for the uniform shape component in landmark data. Pp. 153–168 *in* L. F. Marcus, M. Corti, A. Loy, G. J. P. Naylor, and D. E. Slice, eds. Advances in morphometrics. NATO ASI series A: life sciences, Vol. 284. Plenum Press, New York.

Bowden, R. M., H. K. Harms, R. T. Paitz, and F. J. Janzen. 2004. Does optimal egg size vary with demographic stage because of a physiological constraint? Funct. Ecol. 18:522–529.

Burke, A. C. 1989. Development of the turtle carapace: implications for the evolution of a novel bauplan. J. Morphol. 199:363–378.

Clark, K., G. Bender, B. P. Murray, K. Panfilio, S. Cook, R. Davis, K. Murnen, R. S. Tuan, and S. F. Gilbert. 2001. Evidence for the neural crest origin of turtle plastron bones. Genesis 31: 111–117.

Clark, P. J., M. A. Ewert, and C. E. Nelson. 2001. Physical apertures as constraints on egg size and shape in the common musk turtle, *Sternotherus odoratus*. Funct. Ecol. 15:70–77.

Claude, J., E. Paradis, H. Tong, and J. 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.

Congdon, J. D., and J. W. Gibbons. 1987. Morphological constraint on egg size: a challenge to optimal egg size theory? Proc. Natl. Acad. Sci. USA 84:4145–4147.

Crnokrak, P., and D. A. Roff. 1995. Dominance variance: associations with selection and fitness. Heredity 75:530–540.

Efimov, V. M., V. Y. Kovaleva, and A. L. Markel. 2005. A new approach to the study of genetic variability of complex characters. Heredity 94:101–107.

Ernst, C. H. 1990. Systematics, taxonomy, variation, and geographic distribution of the slider turtle. Pp. 57–67 *in* J. W. Gibbons, ed. Life history and ecology of the slider turtle. Smithsonian Institution Press, Washington, DC.

Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. 4th ed. Longman Group, Harlow, U.K.

Filoramo, N. I., and F. J. Janzen. 2002. An experimental study of the influence of embryonic water availability, body size, and clutch on survivorship of neonatal red-eared sliders, *Trachemys scripta elegans*. Herpetologica 58:67–74.

Gilbert, S. F., G. A. Loredo, A. Brukman, and A. C. Burke. 2001. Morphogenesis of the turtle shell: the development of novel structure in tetrapod evolution. Evol. Dev. 3:47–58.

Goodall, C. R. 1991. Procrustes methods in the statistical analysis of shape. J. R. Stat. Soc. B. 53:285–339.

Greene, H. W. 1988. Antipredator mechanisms in reptiles. Pp. 1–152 in C. Gans and R. B. Huey, eds. Biology of the Reptilia, Vol. 16. Alan R. Liss, New York.

Janzen, F. J., and H. S. Stern. 1998. Logistic regression for empirical studies of multivariate selection. Evolution 52:1564–1571.

Janzen, F. J., M. E. Wilson, J. K. Tucker, and S. P. Ford. 1998. Endogenous yolk steroid hormones in turtles with different sex-determining mechanisms. Gen. Comp. Endocrinol. 111: 306–317.

Janzen, F. J., J. K. Tucker, and G. L. Paukstis. 2000a. Experimental analysis of an early life-history stage: selection on size of hatchling turtles. Ecology 81:2290–2304.

Janzen, F. J., J. K. Tucker, and G. L. Paukstis. 2000b. Experimental analysis of an early life-history stage: avian predation selects for larger body size of hatchling turtles. J. Evol. Biol. 13: 947–954.

Klingenberg, C. P. 2003. Quantitative genetics of geometric shape: Heritability and the pitfalls of the univariate approach. Evolution 57:191–195.

Klingenberg, C. P., and L. J. Leamy. 2001. Quantitative genetics of geometric shape in the mouse mandible. Evolution 55: 2342–2352.

Klingenberg, C. P., and L. R. Monteiro. 2005. Distances and directions in multidimensional shape spaces: implications for morphometric applications. Syst. Biol. 54:678–688.

Kovač, M., and E. Groeneveld. 2003. VCE5. Institute of Animal Science, Federal Agriculture Research Center, Neustadt, Germany.

Kuraku, S., R. Usuda, and S. Kuratani. 2005. Comprehensive survey of carapacial ridge-specific genes in turtle implies co-option of some regulatory genes in carapace evolution. Evol. Dev. 7:3–17.

Lande, R. 1979. Quantitative genetic analysis of multivariate evolution, applied to brain:body size allometry. Evolution 33: 402–416.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution 37:1210–1226.

Legler, J. M. 1990. The genus *Pseudemys* in Mesoamerica: taxonomy, distribution, and origins. Pp. 82–105 *in* J. W. Gibbons, ed. Life history and ecology of the slider turtle. Smithsonian Institution Press, Washington, DC.

Loredo, G. A., A. Brukman, M. P. Harris, D. Kagle, E. E. Leclair, R. Gutman, E. Denney, E. Henkelman, B. P. Murray, J. F. Fallon, R. S. Tuan, and S. F. Gilbert. 2001. Development of an evo-

lutionarily novel structure: fibroblast growth factor expression in the carapacial ridge of turtle embryos. J. Exp. Zool. 291: 274–281.
Lovich, J. E., and C. H. Ernst. 1989. Variation in the plastral formulae of selected turtles with comments on taxonomic utility. Copeia 1989:304–318.
Lovich, J. E., A. F. Laemmerzahl, C. H. Ernst, and J. F. McBreen. 1991. Relationships among turtles of the genus *Clemmys* (Reptilia, Testudines, Emydidae) as suggested by plastron scute morphology. Zool. Script. 20:425–429.
Lynch, M., and B. Walsh. 1998. Genetics and analysis of quantitative traits. Sinauer, Sunderland, MA.
Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 27:209–220.
Martin, P. L., and J. N. Layne. 1987. Relationship of gopher tortoise body size to burrow size in a southcentral Florida USA population. Fla. Sci. 50:264–267.
Milner, J. M., J. M. Pemberton, J. M. Brotherstone, and S. D. Albon. 2000. Estimating variance components and heritabilities in the wild: a case study using the ''animal model'' approach. J. Evol. Biol. 13:804–813.
Monteiro, L. R., J. A. F. Diniz-Filho, S. F. dos Reis, and E. D. Araújo. 2002. Geometric estimates of heritability in biological shape. Evolution 56:563–572.
Mousseau, T. A., and D. A. Roff. 1987. Natural selection and the heritability of fitness components. Heredity 59:181–198.
Neumaier, A., and E. Groeneveld. 1998. Restricted maximum likelihood estimation of covariances in sparse linear models. Genet. Sel. Evol. 30:3–26.
Pearse, D. E., F. J. Janzen, and J. C. Avise. 2002. Multiple paternity, sperm storage, and reproductive success of female and male painted turtles (*Chrysemys picta*) in nature. Behav. Ecol. Sociobiol. 51:164–171.
Roff, D. A. 1997. Evolutionary quantitative genetics. Chapman and Hall, New York.
Rohlf, F. J. 2000. NTSYS-pc: Numerical taxonomy and multivariate analysis system. Ver. 2.10p. Exeter Software, Setauket, NY.
———. 2002a. TPSDig. Ver. 1.36. Department of Ecology and Evolution, State University of New York, Stony Brook, NY.
———. 2002b. TPSRelw. Ver. 1.30. Department of Ecology and Evolution, State University of New York, Stony Brook, NY.
———. 2002c. TPSpls. Ver. 1.13c. Department of Ecology and Evolution, State University of New York, Stony Brook, NY.
Rohlf, F. J., and F. L. Bookstein. 2003. Computing the uniform component of shape variation. Syst. Biol. 52:66–69.
Rohlf, F. J., and M. Corti. 2000. Use of two-block partial least squares to study covariation in shape. Syst. Biol. 49:740–753.
Rohlf, F. J., and L. F. Marcus. 1993. A revolution in morphometrics. Trends Ecol. Evol. 8:129–132.
Rohlf, F. J., and D. E. Slice. 1990. Extensions of the Procrustes method for the optimal superimposition of landmarks. Syst. Zool. 39:40–59.
Rüber, L., and D. C. Adams. 2001. Evolutionary convergence of body shape and trophic morphology in cichlids from Lake Tanganyika. J. Evol. Biol. 14:325–332.
SAS Institute Inc. 2002. JMP. Ver. 5. Cary, NC.
Seidel, M. E., and D. R. Jackson. 1990. Evolution and fossil relationships of slider turtles. Pp. 68–73 *in* J. W. Gibbons, ed. Life history and ecology of the slider turtle. Smithsonian Institution Press, Washington, DC.
Shaffer, H. B., P. Meylan, and M. L. McKnight. 1997. Tests of turtle phylogeny: molecular, morphological, and paleontological approaches. Syst. Biol. 46:235–268.
Sokal, R. R., and F. J. Rohlf. 1995. Biometry. 3rd ed. W. H. Freeman and Company, New York.
Tucker, J. K. 1997. Natural history notes on nesting, nests, and hatchling emergence in the red-eared slider turtle, *Trachemys scripta elegans*, in west-central Illinois. Ill. Nat. Hist. Surv. Biol. Notes 140:1–13.
Tucker, J. K., and F. J. Janzen. 1998. Order of oviposition and egg size in the red-eared slider turtle (*Trachemys scripta elegans*). Can. J. Zool. 76:377–380.
Tucker, J. K., R. S. Funk, and G. L. Paukstis. 1978. The adaptive significance of egg morphology in two turtles (*Chrysemys picta* and *Terrapene carolina*). Bull. Md. Herpetol. Soc. 14:10–22.
Tucker, J. K., G. L. Paukstis, and F. J. Janzen. 1998a. Annual and local variation in reproduction in the red-eared slider, *Trachemys scripta elegans*. J. Herpetol. 32:515–526.
Tucker, J. K., F. J. Janzen, and G. L. Paukstis. 1998b. Variation in carapace morphology and reproduction in the red-eared slider *Trachemys scripta elegans*. J. Herpetol. 32:294–298.
Vincent, C., M. Bontoux, N. M. Le Douarin, C. Pieau, and A.-H. Monsoro-Burq. 2003. *Msx* genes are expressed in the carapacial ridge of turtle shell: a study of the European pond turtle, *Emys orbicularis*. Dev. Genes Evol. 213:464–469.
Zani, P. A., and D. L. Claussen. 1995. Effects of extrinsic load on locomotion in painted turtles (*Chrysemys picta*). Copeia 1995: 735–738.

Corresponding Editor: C. Fenster