

Repeatability of Habitat-Associated Divergence in Shell Shape of Turtles

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Abstract Repeated patterns of phenotypic divergence between environments across disparate taxa provide strong evidence for the generation of adaptive phenotypes. Flow velocity is an important selective force in aquatic habitats; however, among vertebrates, the study of its effects on morphology has been limited almost exclusively to fully-aquatic bony fishes. We tested whether three confamilial species of semi-aquatic freshwater turtle (family Emydidae: *Graptemys pseudogeographica*, *Graptemys nigrinoda*, and *Pseudemys concinna*) displayed similar patterns of phenotypic divergence in carapace shape between fast- and slow-flowing aquatic environments. We used (1) geometric morphometrics to quantify shell shape, (2) multivariate analysis of variance to test the effects of species, sex, and flow, and (3) phenotypic trajectory analysis to compare

patterns of divergence for six species-sex groups. We found significant effects on shell shape for all factors. In general, ecomorphs from fast-flowing habitats had flatter shells than those from slow-flowing habitats. Furthermore, results of trajectory analysis indicate that the degree to which, as well as the way in which, ecomorphs differed were concordant across all species. Our findings demonstrate that the effects of flow are not limited to fully-aquatic vertebrates, and provide evidence of the ability of flow to drive repeatable phenotypic divergence in tetrapods.

Keywords Carapace · Ecomorph · Emydidae · Flow velocity · Evolution · Turtle

Introduction

Taxa that inhabit multiple environments are often subjected to divergent selection pressures and frequently possess phenotypes correlated with differences in ecological parameters. A major goal of the field of evolutionary morphology is to understand the phenotypic response of disparate taxa to similar selection pressures (Adams 2010). If the dominant force driving phenotypic diversification of a lineage is its unique phylogenetic history, similar environmental parameters are likely to produce disparate phenotypes across multiple lineages (Losos 2011). However, if the dominant force driving phenotypic diversification is natural selection, then similar environmental conditions (i.e., shared selection pressures) are expected to produce similar phenotypes. This repeated evolution across multiple lineages (i.e., parallel evolution) has been demonstrated for a number of taxonomic groups and is considered strong evidence for the production of adaptive phenotypes or ecomorphs (Losos et al. 1998; Adams 2010).

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The velocity of water flow is a critical feature of aquatic environments that has been shown to produce divergent phenotypes in a broad range of taxa. Many flexible plants and macroalgae undergo reconfiguration of shape and size in high flow that allow them to decrease drag. For example, the intertidal alga *Chondrus crispus* undergoes whole-alga realignment at low velocities and changes in shape and size of the crown at higher velocities (Boller and Carrington 2006). Moreover, species inhabiting different flow environments have been found to display different morphotypes. *Nereocystis luetkeana* (giant bull kelp) possess flat, strap-like blades in fast-flowing environments, but ruffled blades in sites protected from high flow (Koehl et al. 2008). The flat blades reduce the interception of light, but can collapse into streamlined bundles that decrease drag in high flow. In contrast, the ruffled blades increase the interception of light, but would incur high drag forces if they were found in high-flow environments (Koehl et al. 2008). Among invertebrates, *Potamopyrgus antipodarum* (New Zealand mudsnail) living in fast-flow environments tend to have smooth shells, whereas those inhabiting slow-flow environments have spiny shells (Holomuzki and Biggs 2006). While smooth-shell morphotypes are more susceptible to predation by fishes, they also avoid the accumulation of suspended algae, which has been shown to increase drag and the potential for flow-induced dislodgment in spiny-shell morphotypes (Holomuzki and Biggs 2006). Sessile invertebrates, which do not have to deal with effects of flow on locomotion, show morphological adaptations associated with the challenges of feeding in high-flow environments. For example, the size and shape of barnacle feeding legs have been found to correlate with flow environment (Marchinko and Palmer 2003). The longer legs and setae found in slow-flow environments provide a larger feeding area, whereas the shorter structures found in high-flow environments are less susceptible to being damaged (Marchinko and Palmer 2003). Among vertebrates, the investigation of patterns of phenotypic variation across different flow regimes has focused primarily on fishes (Langerhans 2008; Haas et al. 2010). Langerhans (2008) demonstrated that in fishes, the shape of the body and caudal fin, as well as muscle architecture differ in a predictable manner between fast- and slow-flowing aquatic environments at both an intra- and inter-specific level. Specifically, fishes inhabiting fast-flow habitats have (1) more streamlined body shapes, which minimize drag during steady swimming, (2) high aspect-ratio caudal fins that optimize thrust production, and (3) a greater proportion of red muscle, which powers continuous swimming.

While the same hydrodynamic forces that have resulted in repeated morphological specializations in fishes are also encountered by other aquatic vertebrates, the effects of

flow on non-piscine vertebrates are less well understood. Moreover, the ability of flow to produce repeated patterns of morphological divergence remains unexamined. By expanding the study of flow-associated morphological divergence beyond just fishes, several novel questions regarding the role of flow in generating repeated patterns of divergence can be examined. First, are flow-associated morphologies limited to fishes or are they found among more-derived vertebrates, such as tetrapods? Second, are such patterns observed in semi-aquatic species, and more specifically, in structures that play an important role in both terrestrial and aquatic environments? Finally, do patterns suggest convergence on forms well suited to specific flow environments?

The shells of freshwater turtles provide an ideal structure to address these questions for several reasons. (1) As tetrapods, turtles represent an unexamined lineage. If such patterns were found among turtles, they would not only indicate how flow impacts turtles, but also provide evidence that the influence of flow on morphology among vertebrates expands beyond fishes. (2) Freshwater turtles are semi-aquatic. While most species spend the majority of time in water, individuals will move across land to search for alternate water sources, find mates, and nest (Ernst and Lovich 2009). In both aquatic and terrestrial environments, a turtle's shell provides protection against predation, and this in itself can be influenced by shape and symmetry: more domed and more symmetrical shells are stronger (Stayton 2009; Rivera and Stayton 2011; Stayton 2011; Vega and Stayton 2011; Rivera and Stayton 2013). However, the shell also serves important habitat-specific roles. Specifically, if a turtle becomes overturned on land, the shape of the shell can have a major impact on the critical ability to right itself: strongly-domed shells of terrestrial species can provide a mechanical benefit during righting (Domokos and Várkonyi, 2008). However, increased shell doming can lead to a considerable decrease in hydrodynamic efficiency (Rivera 2008). Thus, unlike fishes, which in general, only have to adapt to a single environment, the shells of semi-aquatic turtles provide an interesting test of the effects of flow on shape. (3) Patterns of flow-associated intraspecific divergence have been identified in two species of freshwater turtle (Aresco and Dobie 2000; Lubcke and Wilson 2007; Rivera 2008). In particular, Rivera (2008) identified flow-associated differences in 3D shell shape across a broad geographic range in the turtle *Pseudemys concinna*. However, while many species of freshwater turtles inhabit both fast- and slow-flowing aquatic environments, to date, nothing is known regarding the influence of flow on 3D shell shape in other species, thus limiting our ability to assess the repeatability of this response. (4) The study of a rigid shell eliminates the complexities of flow-associated behaviorally-induced

changes to morphology (Stewart and Britton-Simmons 2011) that could compensate for differences in shape. (5) Because propulsion in turtles is exclusively limb-based, functional changes in shell shape should be limited to those that maximize hydrodynamic efficiency. In contrast, studies examining the association between flow velocity and the morphology of fishes have to interpret the complex interactions between modifications of the body and fins that reduce drag and those that increase propulsion (Rivera 2008).

The goal of this study is to assess whether flow velocity is capable of producing repeatable effects on the shell morphology of freshwater turtles. Specifically, we test whether flow velocity affects shell morphology in three species: *Graptemys pseudogeographica*, *Graptemys nigrinoda*, and *Pseudemys concinna*. We then test whether the patterns of morphological divergence between fast- and slow-flow regimes are concordant across the three species, thereby testing the hypothesis that aquatic flow velocity has driven repeated patterns of morphological divergence across multiple species, and providing additional support for the adaptive benefits of shell shape described in Rivera (2008).

Materials and Methods

Graptemys pseudogeographica (False map turtle), *G. nigrinoda* (Black-knobbed map turtle), and *P. concinna* (River cooter) inhabit both fast- and slow-flowing aquatic habitats in the southeastern United States. We collected landmark-based morphometric data from 247 shells of these three species, originating from two (*G. pseudogeographica*) or four sites (*G. nigrinoda* and *P. concinna*); each species was represented by a minimum of one fast-flow and one slow-flow site (Table 1; Fig. 1). In order to facilitate accurate classification of sex via secondary sexual characteristics (elongate foreclaws [*Pseudemys*] and greater precloacal tail length [*Pseudemys* and *Graptemys*]), the minimum size used for each species was determined by the smallest sexually mature individual of the smaller sex (males): 10.7 cm for *G. pseudogeographica*, 7.3 cm for *G. nigrinoda*, and 16.0 cm for *P. concinna* (Fahey 1987; Ernst and Lovich 2009). Data were collected from live specimens (*P. concinna* and *G. nigrinoda*) and fluid-preserved museum specimens (all species). Data from museum specimens of *P. concinna* represent a subset of data analyzed by Rivera (2008), and include sites within the Mobile River basin from which both *G. nigrinoda* and *P. concinna* specimens were available.

To quantify the shape of the shell, 3D coordinate data (x, y, z) were collected for 64 landmarks. These landmarks, located at the junctions of scutes on the carapace

and homologous across the three species, were digitized using a 3D digitizing system (Microscribe G2LX; accuracy of 0.3 mm). As a result of the redundancy associated with the symmetrical structure of the shell, we maximized our sample size by limiting our analyses to one-half of the shell (right side; landmarks, $N = 33$); in cases when the right side was damaged, but the left was intact, a reflected view of the left side was used (Rivera 2008). While all shells displayed slight bilateral asymmetries, based on the specimens with undamaged shells ($N = 230$), left and right sides provided equivalent information regarding differences between specimens (Mantel correlation, $r = 0.95$). Coordinates for all specimens were aligned using a generalized procrustes analysis (GPA; Rohlf and Slice 1990; Zelditch et al. 2004), which removes all non-shape information (translation, scale, rotation) from the data set. A regression of the aligned configurations (i.e., procrustes residuals) with centroid size (calculated during GPA) was then used to correct for the effects of size on shape. Finally, a principal components analysis (PCA) of the regression residuals was used to produce the final shape variables ($N = 92$) used in statistical tests.

A full factorial multivariate analysis of variance (MANOVA) was used to test the effects of species, sex, and flow regime (fast- or slow-flowing) on shell shape. To better understand the response to flow, and test the hypothesis of repeated patterns of divergence in these three species, we then conducted a phenotypic trajectory analysis (PTA; Adams and Collyer 2009; also Collyer and Adams 2007; Adams and Collyer 2007). Specifically, we calculated the magnitude and orientation of the vector (a two-point trajectory) connecting the least-squares means of fast- and slow-flow turtles for each of six species-sex groups. Because the sexes are potentially under different morphological constraints, we then tested for concordance among the three female and three male vectors (i.e., sexes were examined separately) by calculating pair-wise differences in magnitude and direction (i.e., angles) between vectors and testing for statistical significance using a residual randomization procedure (9,999 iterations). In addition to possessing superior statistical power relative to other resampling methods (Anderson and ter Braak 2003), residual randomization procedures are also more robust to power limitations of low sample-size data sets that are inherent to parametric tests (Adams and Collyer 2009). Finally, we used the R package “vegan” (Oksanen et al. 2010) to analyze the multivariate homogeneity of group dispersions (variances) to determine whether a pattern of flow-associated variation was found between flow regimes across the six species-sex groups. All statistical analyses were conducted using R 2.11.1 (R Development Core Team 2010).

Table 1 Specimen data for species \times sex \times flow groups

Species	N	Carapace length (cm)				Site(s)
		Avg.	SD	Min.	Max.	
<i>G. nigrinoda</i>						
Female						
Fast	13	15.6	2.9	13.0	21.8	Cahaba (4), Coosa (1), Tallapoosa (8)
Slow	13	18.3	2.3	14.6	21.8	Mobile/Tensaw Delta
Male						
Fast	20	8.5	0.7	7.3	9.7	Cahaba (16), Coosa (2), Tallapoosa (2)
Slow	15	10.3	0.9	8.2	11.1	Mobile/Tensaw Delta
<i>G. pseudogeographica</i>						
Female						
Fast	25	18.5	1.3	16.3	20.6	Mississippi River
Slow	12	20.7	3.4	15.0	25.5	Reelfoot Lake
Male						
Fast	37	12.2	0.8	10.7	14.2	Mississippi River
Slow	22	12.1	0.7	10.9	13.6	Reelfoot Lake
<i>P. concinna</i>						
Female						
Fast	23	26.4	6.3	16.1	33.9	Cahaba (6), Coosa (2), Tallapoosa (15)
Slow	10	29.9	6.0	16.9	34.5	Mobile/Tensaw Delta
Male						
Fast	22	19.9	2.6	16.0	26.1	Cahaba (9), Coosa (7), Tallapoosa (6)
Slow	35	22.7	3.2	17.9	28.7	Mobile/Tensaw Delta

All sites for *G. nigrinoda* and *P. concinna* are located within the Mobile River basin

Results

Using MANOVA we detected significant effects on carapace shape for all factors (Table 2). Furthermore, PTA on the three female vectors indicated that ecomorphs differed across flow regimes in a concordant manner, both in the degree (i.e., magnitude) of shape change, and orientation in morphospace (Table 3). With the exception of a single significant comparison (i.e., difference between vector magnitudes of *G. nigrinoda* and *P. concinna*), the same pattern was observed for males (Table 3). No consistent pattern was found for the three species regarding the similarity of their response between vector parameters (magnitude or orientation). For example, for females, the magnitude of response was most similar (i.e., smallest difference between magnitudes) between the congeneric species (*G. nigrinoda* and *G. pseudogeographica*: 0.0008),

yet the angle between the vector orientations was smallest for an intergeneric comparison (*G. pseudogeographica* and *P. concinna*: 84.5°). For males, the smallest difference in the magnitude of response between the flow regimes was between *G. pseudogeographica* and *P. concinna* (0.0045), yet these two species showed the largest difference in direction of response (60.6°). Nevertheless, two patterns are apparent in our data. First, females showed smaller differences in vector magnitudes (mean \pm SD, 0.001 \pm 0.0006) than males (mean \pm SD, 0.007 \pm 0.0029). Second, males showed more similar vector orientations (mean \pm SD, 55.7 \pm 4.3°) than females (mean \pm SD, 98.2 \pm 12.1°).

An additional question was whether males and females of the same species respond similarly to the same differences in flow. None of the three intraspecific comparisons displayed statistically significant differences for vector

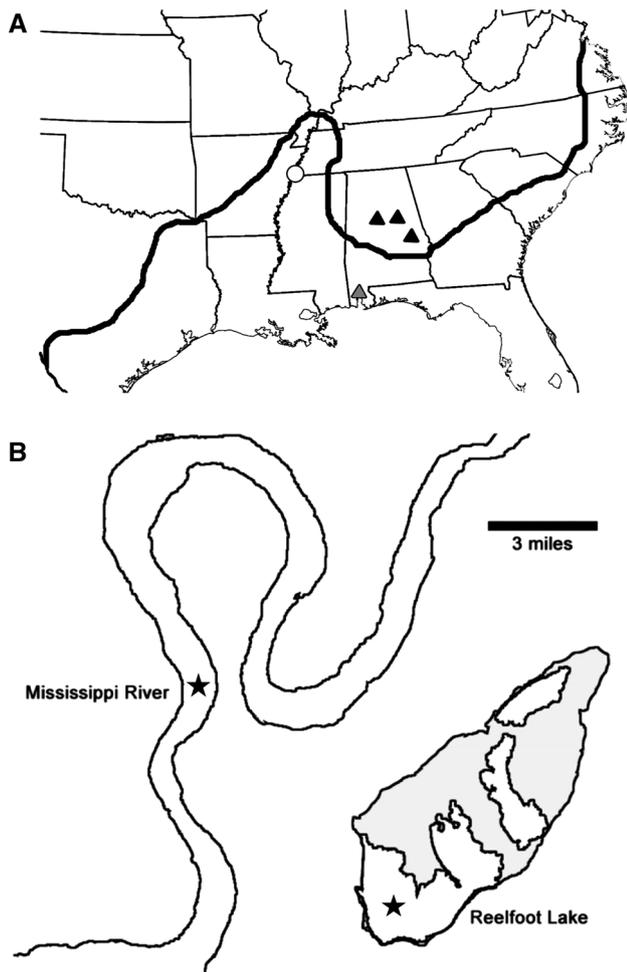


Fig. 1 Map showing the six sites used in this study. **a** Bold line indicates the position of the fall line, which separates the piedmont (above) and the coastal plain (below). Triangles indicate fast-flow (black; Cahaba, Coosa, and Tallapoosa Rivers) and slow-flow (gray; Mobile/Tensaw Delta) sites for *P. concinna* and *G. nigrinoda*. Open circle indicates the Reelfoot Lake and Mississippi River sites for *G. pseudogeographica*. **b** Location of Reelfoot Lake (slow-flow) relative to the Mississippi River (fast-flow). Symbols denote general sampling sites for *G. pseudogeographica* used in this study. Shaded regions within Reelfoot Lake represent cypress swamps, while unshaded regions represent basins (i.e., open areas of water). Modified from Rivera (2008)

Table 2 Results from MANOVA of shell shape for three species across two flow regimes

Factor	df_{Factor}	Pillai's trace	Approx. F	$df_{\text{num}}, df_{\text{den}}$	P
Species	2	1.9310	44.09	184, 290	<0.0001
Sex	1	0.8961	13.50	92, 144	<0.0001
Flow	1	0.7936	6.02	92, 144	<0.0001
Species × sex	2	1.4106	3.77	184, 290	<0.0001
Species × flow	2	1.4145	3.81	184, 290	<0.0001
Sex × flow	1	0.5404	1.84	92, 144	0.0005
Species × sex × flow	2	0.9556	1.44	184, 290	0.0027

orientation ($P > 0.234$; Fig. 2). Likewise, none of the three intraspecific comparisons displayed statistically significant differences ($P < 0.017$) for vector magnitude following adjustment for multiple comparisons; however, the difference between male and female *P. concinna* was marginally significant ($P = 0.042$; Fig. 2) prior to Bonferroni correction. In general, these findings indicate that the amount of morphological evolution between fast and slow ecomorphs was consistent for females and males across all species. For these comparisons, the angle between vectors ranged from 39.4° (*G. nigrinoda*) to 130° (*P. concinna*), and the differences between vector magnitudes ranged from 0.0001 (*G. nigrinoda*) to 0.0089 (*P. concinna*). These results indicate that for intraspecific comparisons, *G. nigrinoda* responded most similarly to divergent flow regimes.

Vector analysis also indicated that the major difference in shape between ecomorphs pertains to shell doming (Fig. 2). PC 1 describes a difference between domed (low scores) and flattened shells (high scores). In contrast, PC 2 depicts variation in the position of maximum shell height. While all three species display the more flattened morphology for both

Table 3 Statistical comparisons of morphological divergence between flow regimes for six species × sex groups

	Vector magnitude			Vector orientation			
	GN	GP	PC	GN	GP	PC	
Female							
GN	–	0.8585	0.7853	GN	–	0.2726	0.7052
GP	0.0008	–	0.6453	GP	102.6	–	0.6359
PC	0.0012	0.0020	–	PC	107.4	84.5	–
Male							
GN	–	0.1451	0.0102	GN	–	0.1566	0.4848
GP	0.0057	–	0.1666	GP	52.4	–	0.4115
PC	0.0101	0.0045	–	PC	54.0	60.6	–

Below diagonal: pairwise differences between vector magnitudes and orientations (angle in $^\circ$). Above diagonal: significance levels of pairwise differences based on 9,999 random permutations. Significant comparisons are shown in bold

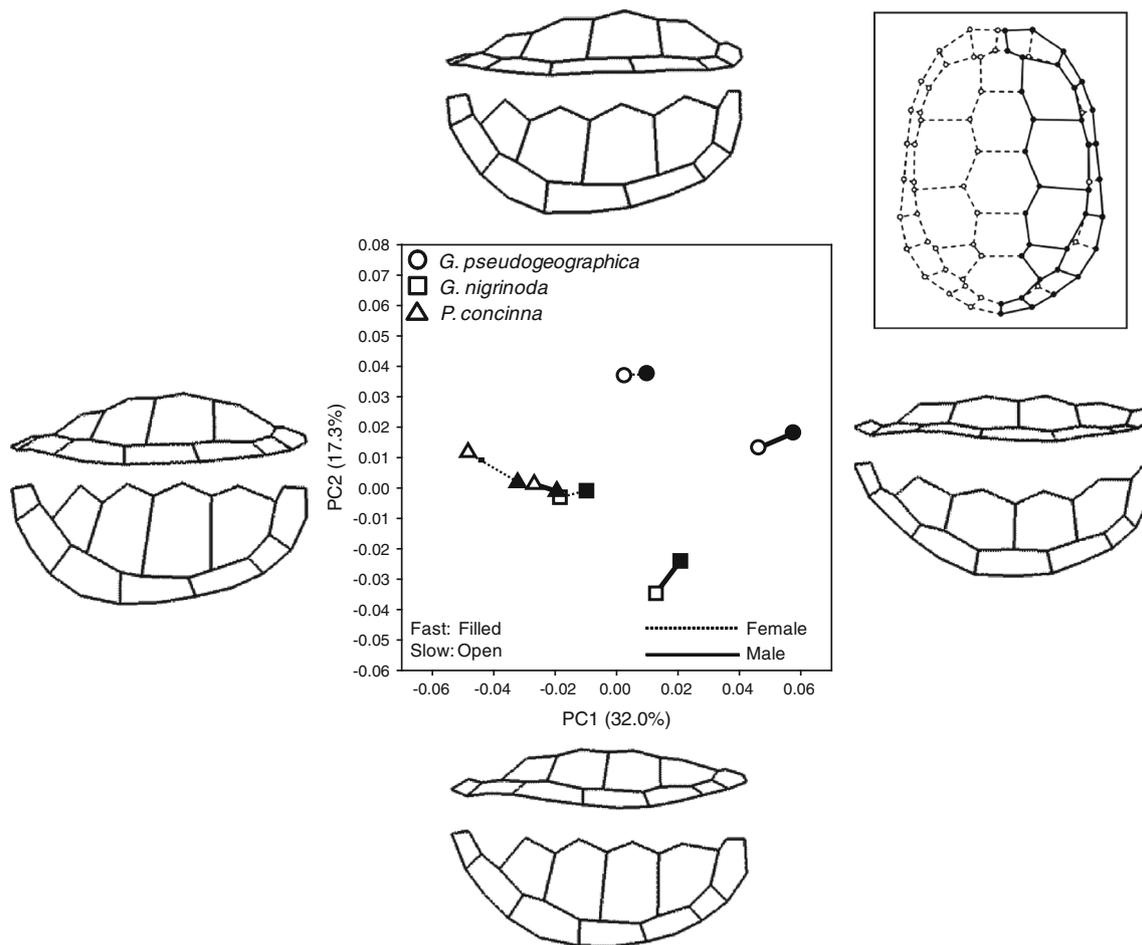


Fig. 2 Effects of flow on carapace shape. The first two principal component axes explain 49.3 % of shape variation across the three species. Symbols represent the mean for each of the 12 species \times sex \times flow groups. Vectors represent the phenotypic divergence between flow regimes for each species \times sex group. Shell illustrations depict the right side (*upper*) and top (*lower*) of the carapace, with anterior end oriented to right. Illustrations highlight

differentiation from domed to flat along PC1. Jagged appearance of flattened shells described by high scores on PC1 is an artifact of visualization; shells in all groups are smooth. Enclosed shell diagram shows the location of landmarks (*circles*). Dashed lines indicate borders between scutes. Closed circles ($N = 33$) indicate landmarks of the *right* side used in GM analysis and are connected by *solid lines*. Anterior end of shell oriented upward

males and females of the fast-flow ecomorphs (i.e., all show similar variation along PC 1), variation along PC 2 is dominated by sexual-shape dimorphism in the two *Graptemys* species and interspecific differences in shape. In contrast, *P. concinna* displays very little variation along PC 2. These descriptions were also confirmed statistically; a factorial ANOVA (species \times sex \times flow) on PC 1 found a highly significant effect of flow ($F = 75.24$, $P < 0.001$), while the same analysis on PC 2 found no effect of flow ($F = 0.1426$, $P = 0.706$).

Finally, our analysis of multivariate group variances indicated that only one of the six species-sex pairs showed a significant difference between flow regimes (*G. nigrinoda* males, $P = 0.006$); furthermore, none of the six species-flow pairs showed a significant difference between the sexes.

Discussion

While previous studies have identified patterns of flow-associated morphological divergence in shell shape in two species of freshwater turtles (Aresco and Dobie 2000; Lubcke and Wilson 2007; Rivera 2008), each of these studies used different morphometric variables and has been limited to a single species. As a result, the level to which chelonian species show parallel responses to divergent flow environments has remained unknown. In this study, we examined morphological divergence among the shells of male and female freshwater turtles of several species. By examining these patterns across three distinct species, our goal was to determine whether similar selective pressures associated with different flow environments were capable of producing repeated effects (i.e., ecomorphs) among the

species. Using PTA, we were able to demonstrate that for both males and females, the vectors of each of the three species showed strong concordance in magnitude and orientation between fast- and slow-flow environments. It is important to note that the three species did not converge on a given form (Adams 2010), rather the general concordance of magnitude and orientation of shape change demonstrates that species-specific shape was modified in the same way in each taxa. Moreover, that these taxa represent separate species demonstrates the independence of these events. Thus, this study is the first to demonstrate that flow can drive repeated patterns of divergence in the shells of turtles.

PTA also demonstrated that the primary difference between fast- and slow-flow ecomorphs is a general flattening of the shell in fast-flow ecomorphs. This is the same pattern found for Rivera's (2008) initial analysis of flow-associated divergence in shell shape for *P. concinna*. Rivera (2008) also tested physical models of fast- and slow-ecomorphs and found that hydrodynamic drag was considerably lower in the former (drag coefficient: fast-flow, 0.27, slow-flow, 0.56). Thus, the patterns found for the two *Graptemys* species, which mirror the morphological differences seen for *P. concinna*, can also be expected to produce more hydrodynamically efficient shell shapes. Moreover, that males display a more consistent response, as indicated by smaller angles between vectors, suggests that females may be more limited in their ability to adapt to flow (i.e., morphological constraints due to reproduction and the space required to hold eggs). This disparity between the sexes means that males may be gaining a greater hydrodynamic advantage. Future tests similar to those conducted by Rivera (2008) could determine the specific hydrodynamic characteristics of these species' shells, as well as test for differences between the sexes.

Flow-associated morphological divergence has now been identified in four species of turtle (*Actinemys marmorata*, see Lubcke and Wilson 2007). In each case, fast-flow ecomorphs possess more flattened (i.e., streamlined) shells, while slow-flow ecomorphs possess domed shells. Rivera (2008) demonstrated that such shape differences had hydrodynamic effects consistent with the theoretical expectation of reduced drag for turtles inhabiting fast-flow environments. Moreover, for the three species included in this study, we are able to show that differences between ecomorphs are statistically concordant, demonstrating that the patterns have been independently repeated across multiple species inhabiting the same (*G. nigrinoda* and *P. concinna*), and different (*G. pseudogeographica*) geographic localities.

Of course, the velocity of water flow is not the only environmental factor that varies between fast and slow flow habitats, and organisms must also contend with other environmental factors for which morphology is important (Rivera and Stayton 2011). In particular, as a major

function of the shell is to serve as a defensive structure, it is important to address whether the observed differences in shape could be explained by differences in predator regimes. Rivera (2008) noted that it was difficult to specifically test this hypothesis for *P. concinna* because the major predator of adults, the American alligator (*Alligator mississippiensis*), is uncommon in fast-flow environments, but sympatric with *P. concinna* in most slow-flow habitats. While Rivera and Stayton (2011) demonstrated that the highly domed shells of *P. concinna* inhabiting slow-flow sites were stronger than the flatter shells of turtles from fast-flow sites, it remains unknown to what extent the observed differences in shell shape would influence the ability to survive a predatory attack from an alligator. Nevertheless, Rivera and Stayton (2011) hypothesized that shape divergence in *P. concinna* could be the result of this functional trade-off in shell shape (i.e., hydrodynamic efficiency vs. strength) with different forces operating in different environments: flatter shells would be favored in fast flow (due to strong hydrodynamic forces, and weak predation pressure) and domed shells would be favored in slow flow (due to weak hydrodynamic forces, and strong predation pressure). While it remains unclear how the risk of predation may influence the degree to which shells are domed in slow-flow habitats, our findings of similar patterns in *G. pseudogeographica* from areas outside of the range of the alligator, as well as those from *A. marmorata* (western pond turtle; Lubcke and Wilson 2007) a species that also lacks a major predator dichotomy between flow environments, clearly indicate a relationship between high-flow environments and flattened shells. Moreover, if selection for hydrodynamic efficiency was not important, a logical expectation would be that the stronger, more domed shells would be favored in both flow regimes; a pattern that is not observed. As previously stated by Rivera (2008), it is clear that future studies should combine data on the forces exerted on the shells of turtles during attacks by alligators, collected from models of adult turtles subjected to alligator bites, and computational methods (e.g., finite element analysis) to examine the ability of shells of different shapes to withstand attacks.

Moreover, adaptive phenotypes can be the result of genetic differentiation and/or phenotypic plasticity (Levins 1968; West-Eberhard 1989). While patterns of flow-induced phenotypic plasticity are well established in fishes (Pakkaasmaa and Piironen 2001; Keeley et al. 2007), no such evidence has yet been presented for turtles. However, a strong heritable component to shell shape has been demonstrated (Myers et al. 2006), providing the foundation necessary for genetic divergence to drive phenotypic differences. Nevertheless, the specific mechanisms responsible for the flow-associated divergence observed in turtles remain unknown and provide a logical direction for future studies.

An additional question regarding the generation of these morphotypes is the direction of morphological change. Did these morphotypes arise from an intermediate ancestor, or did the ancestor already represent one of the observed morphotypes? The answer to this question may in fact differ for each of the species. However, without morphometric data from a large number of populations and a strong understanding of the genetic relationships among populations, neither of which currently exist, this question remains unanswered. Finally, all three species that were examined in this study belong to a single family (Emydidae). While this was necessitated due to our use of landmark-based geometric morphometric methods and the need for homologous landmarks (i.e., shells were required to have the same scute patterns), new techniques that utilize 3D surface meshes (Serb et al. 2011) may provide the opportunity for future interfamilial comparisons.

Beyond our specific findings on turtles, our study also represents the first, to our knowledge, quantitative analysis to demonstrate the repeatable effects of flow-associated morphological divergence among populations of semi-aquatic vertebrates or within the tetrapod clade. To date, such patterns have only been observed in fully-aquatic fishes for which selection for hydrodynamic efficiency is likely maximized. Furthermore, while semi-aquatic tetrapods possess morphological adaptations that improve locomotion through water relative to their terrestrial counterparts (e.g., turtles, frogs, and rodents), small-scale changes associated with flow conditions may also be present. Our findings suggest that the ability of flow to affect morphological divergence may be more pervasive across the animal kingdom than previously thought.

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