Convergent evolution provides evidence of similar radiations in shell shape in the turtle families Emydidae and Geoemydidae

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Abstract

This study investigates phenotypic diversification in the species-rich and ecologically-diverse turtle families Emydidae and Geoemydidae. In particular, we are interested in whether these groups, with many ecologically and morphologically similar species, show similar patterns of evolutionary radiation. We focused on directions of evolution and evolutionary allometry; we also quantitatively investigated whether two supposed morphological analogs shared within the two groups (e.g., “box turtles” and “wood turtles”) show evidence of convergence. A set of 53 three-dimensional landmarks were digitized on 1029 turtle shells representing 50 emydid species and 62 geoemydid species. These data were analyzed using standard geometric morphometric techniques. Evolutionary patterns were assessed using tests for phylogenetic signal, and the relationship between size and shell shape was determined via phylogenetic regression. Significant phylogenetic signal was detected for shell shape, while tests for allometry also showed significance. Three hypothesis testing methods were applied in order to determine whether the supposed morphological analogs in the emydid and geoemydid families exhibited convergence. While there was no evidence of shared adaptive peaks among either box turtles or wood turtles, it was determined that both of these groups have evolved to be more similar to one another relative to their ancestors than would be expected by chance; they have, indeed, converged. Although each family shows some unique patterns of diversification, overall there is an impressive array of similarities between the Emydidae and Geoemydidae radiations.

Box Turtles

Wood Turtles

Geoemydidae

Materials and Methods

Original data

- Primary data consisted of 3D landmark coordinates digitized on 1029 adult turtle specimens¹.
- 50 emydid species (94% of Emydidae) and 62 geoemydid species (90% of Geoemydidae) were included.
- 53 landmarks were shared among all specimens (Figure 1).
- Data were collected from one side of the shell only.

(Figure 1) Landmarks used for 3D coordinates of digitized Emydidae and Geoemydidae shells.

Geometric morphometric analyses

- Data were aligned using a Generalized Procrustes fit.
- Principal components analysis (PCA) was conducted on fitted coordinates².

Phylogeny

- Cytochrome b sequences for a total of 253 specimens (83.8% of hard-shelled species) were acquired from GenBank.
- The sequences were aligned in Clustal X and then by hand in Mesquite^{3,4}.
- The best-supported topology and branch lengths were inferred using an analysis in BEAST⁵.
- The resulting tree was pruned to only include members of the Emydidae, Geoemydidae, and Platysternidae.

Evolutionary analyses

- Tests for phylogenetic signal were conducted on species' average shapes using the multivariate K statistic⁶.
- Phylomorphospaces were generated for each species' mean shape.
- Tests for allometry were conducted by running regression analysis on phylogenetic independent contrasts of PC scores versus centroid size.

Hypothesis testing

- Pairwise Procrustes distances were calculated among all pairs of species mean shape and used to check whether the nearest noncongeneric neighbor of each morphotype was a member of the same morphotype from the other family.
- Morphospace plots were inspected to determine if putative morphotypes occurred in the same regions of shape space.
- An Ornstein-Uhlenbeck model-fitting procedure was employed in SURFACE in order to determine if clades were evolving in response to adaptive peaks⁷.
- Convergence analysis was implemented in the R package *convevol* to quantify the amount of convergent evolution between two morphotypes and to evaluate whether the two morphotypes had converged more than expected by chance⁸.

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(Figure 2) Morphospace of PCs 1 and 2 for all Emydidae and Geoemydidae specimens categorized by genus. Images at the corners show shapes representative of maximum and minimum scores on each PC axis, with all other PC scores set to zero.

(Figure 3) Phylomorphospace of PCs 1 and 2 for all Emydidae and Geoemydidae species categorized by genus. Images at the corners show shapes representative of maximum and minimum scores on each PC axis, with all other PC scores set to zero.

Results

- The first 10 PCs together accounted for 67.3% of the total variation in the data set.
- Neither box nor wood turtles appear to be each other's closest neighbors in shape space.
- Significant phylogenetic signal (Bonferroni corrected; $\alpha = 0.00417$) was found for a number of individual and combined PC axes (Table 1).
- Tests for allometry also showed significance for numerous aspects of shape (Table 2).
- There were only three cases in which the nearest noncongeneric neighbor of a species in a given morphotype was a member of the same morphotype from another family.
- SURFACE analysis provided no evidence for shared adaptive peaks among either box or wood turtles.
- Convergence analysis showed that box and wood turtles had evolved to be more similar to one another than would be expected by chance ($P < 0.001$); they were 16.5% and 9% more similar compared to their ancestors, respectively.

PCs	K/K_{mult}	P -value
All	0.07247163	< 0.001
1	1.257155	< 0.001
2	1.533575	< 0.001
3	0.8694399	< 0.001
4	0.4801966	0.010
5	1.085205	< 0.001
6	0.5028149	0.015
7	0.8925919	< 0.001
8	0.4400297	0.034
9	0.604372	< 0.001
10	0.5648089	0.006
1:10	0.1050381	< 0.001

(Table 1) Results of tests for phylogenetic signal within all Emydidae and Geoemydidae species. Significant results are shown in bold.

PCs	Slope/ λ	P -value
All	75.37	< 0.001
1	-1.01E-05	0.086
2	5.51E-05	< 0.001
3	-3.37E-05	0.066
4	-1.86E-05	0.008
5	5.47E-05	< 0.001
6	-3.61E-05	0.212
7	-3.20E-05	0.216
8	3.76E-05	0.555
9	-1.72E-06	0.009
10	3.02E-06	0.164
1:10	0.507	< 0.001

(Table 2) Results of tests for allometry within all Emydidae and Geoemydidae species. Significant results are shown in bold.

Discussion

Evolutionary analyses

- Both quantitative and qualitative analyses showed significant amounts of phylogenetic structure in both families.
- Since box turtles from both families tend to be smaller, characterized by moderate to low PC2 scores, allometry may be one component in the determination of their shell shape.
- Allometric trends provide less explanatory power for wood turtles.

Hypothesis testing

- The first two tests for convergent evolution provided evidence against convergence in both morphotypes: neither box turtles nor wood turtles were generally each other's nearest neighbors in shape space and neither morphotype showed evidence of a common adaptive peak.
- However, the *convevol* method showed that box and wood turtles have evolved to be more similar to one another relative to their ancestors than would be expected by chance.
- While the third test might appear to contradict the results of the first two, it is important to keep in mind that there is more than one way for convergence to occur, such as with multiple adaptive peaks, provided that each of the peaks is located in a similar region of shape space.
- Alternatively, these results could be explained by a more complicated adaptive landscape than what is modeled by SURFACE, as the method assumes the presence of distinct adaptive peaks, but it is also possible that the adaptive landscape could take the form of a uniform slope, adaptive ridges, or adaptive plateaus.

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