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**SYMPOSIUM**

# Performance Surface Analysis Identifies Consistent Functional Patterns across 10 Morphologically Divergent Terrestrial Turtle Lineages

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**Synopsis** Newly-developed methods for utilizing performance surfaces—multivariate representations of the relationship between phenotype and functional performance—allow researchers to test hypotheses about adaptive landscapes and evolutionary diversification with explicit attention to functional factors. Here, information from performance surfaces of three turtle shell functions—shell strength, hydrodynamics, and self-righting—is used to test the hypothesis that turtle lineages transitioning from aquatic to terrestrial habitats show patterns of shell shape evolution consistent with decreased importance of hydrodynamic performance. Turtle shells are excellent model systems for evolutionary functional analysis. The evolution of terrestriality is an interesting test case for the efficacy of these methods because terrestrial turtles do not show a straightforward pattern of morphological convergence in shell shape: many terrestrial lineages show increased shell height, typically assumed to decrease hydrodynamic performance, but there are also several lineages where the evolution of terrestriality was accompanied by shell flattening. Performance surface analyses allow exploration of these complex patterns and explicit quantitative analysis of the functional implications of changes in shell shape. Ten lineages were examined. Nearly all terrestrial lineages, including those which experienced decreased shell height, are associated with morphological changes consistent with a decrease in the importance of shell hydrodynamics. This implies a common selective pattern across lineages showing divergent morphological patterns. Performance studies such as these hold great potential for integrating adaptive and performance data in macroevolutionary studies.

## Introduction

Evolutionary researchers have begun incorporating quantitative analyses of adaptive landscape principals into studies on evolutionary diversification (Stayton 2011a, 2019; Ingram and Mahler 2013; Slater 2013; Uyeda and Harmon 2014; Polly et al. 2016; Benson et al. 2018). Such landscapes—multivariate summaries of the relationships of genotype or phenotype to fitness—have a long history in evolutionary research, going back at least to the modern synthesis (Wright 1932), but their use as quantitative tools in research has been limited (though see Arnold 2003). Current uses of landscape concepts in evolutionary research generally proceed according to one of two frameworks. The first and by far most common involves fitting models inspired by adaptive landscapes to phylogenies and phenotypic data sets, using the observed distributions of species to reconstruct the number and in some cases the locations of adaptive "peaks." Models utilizing Ornstein-Uhlenbeck (OU) models are likely the most familiar of these (Butler and King 2004; Ingram and Mahler 2013; Slater 2013; Uyeda and Harmon 2014; Benson et al. 2018) although others are possible (Boucher et al. 2018).

These models have been used successfully in previous studies; however, they possess some limitations, particularly in multivariate data sets. First, while the methods can produce robust results with regard to the number of adaptive peaks which they

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reconstruct, the locations of peaks can be far more sensitive to slight changes in the input data (including rigid rotations of the original data; Adams and Collyer 2018). In addition, these models will often reconstruct peaks in locations of phenotypic space which are biologically impossible or highly unreasonable (Uyeda and Harmon 2014; Stayton 2018). More generally, these methods are not tied to any biologically-relevant models of phenotypic development or functional performance—results are sometimes interpreted in these terms, but they do not enter into the analyses themselves.

A second set of methods, inspired by the landscape work of Arnold (1983, 2003; Arnold et al. 2001) and drawing from theoretical morphology (Raup and Graus 1972; Niklas and Kerchner 1984; Niklas 1986, 1994, 1997; McGhee 1999), have been developed which more directly integrate performance information. These methods involve the construction and manipulation of performance surfaces—multivariate summaries of the relationships between phenotype and functional performances. Rather than using data on observed species to reconstruct the locations of adaptive peaks, performance surface methods assess performance for multiple functions on a range of theoretical phenotypes, usually evenly-spaced across occupied areas of phenotypic space, and use those performance data to predict which regions of shape space produce the optimal compromises in performance across multiple functions. The phenotypes of actual species are then used to assess the degree to which performance in one or a few functions can explain phenotypic evolution. These methods do not make use of phylogenetic information during the discovery of optima, and they have not yet been the subject of as much theoretical exploration as have the model-fitting methods. However, they have the advantage of grounding their searches in information about functional performance, meaning that all optima discovered can be interpreted in ecologically-relevant terms and ensuring that optima will never be reconstructed in regions associated with biologically-impossible phenotypes. Notably, these methods easily accommodate simultaneous analysis of performance in multiple functions; as the studies in this volume illustrate, many and perhaps most organismal structures perform multiple functional roles. In addition, these methods are not limited to the reconstruction of adaptive peaks—many topological features of adaptive landscapes, from ridges and valleys to plateaus and calderas, can be explored using these methods.

Turtle shells have proven to be exceptionally useful structures for such studies (Stayton 2011a, 2019; Polly et al. 2016), given morphologies that are straightforward to characterize and a well-understood set of functions which have been subject to quantitative modeling and validation. In particular, previous studies have made use of information on the known relationships between shell shape and shell strength (Polly et al. 2016; Stayton 2011a, 2018; Williams and Stayton 2019), hydrodynamic efficiency (Watson and Granger 1998; Rivera 2008; Jones et al. 2011; Stayton 2019), or the ability of turtles to right themselves after being overturned (Domokos and Várkonyi 2008; Golubović et al. 2015).

In addition, a number of ecomorphological patterns have been documented in turtles (Claude et al. 2003; Rivera 2008; Angielczyk et al. 2011; Stayton 2011a; Golubović et al. 2015; Berlant and Stayton 2017; McLaughlin and Stayton 2016; Wise and Stayton 2017; Stayton et al. 2018), providing a basis for functional hypothesis testing. One such pattern has been studied within a quantitative performance surface context—Stayton (2011) and later Polly et al. (2016) explored the tendency of terrestrial turtles to differ in shell shape from their aquatic relatives. Terrestrial turtles tend to have taller shells than those of aquatic species, with more domed carapaces (the dorsal parts of the shell) and broader plastra (the ventral parts of the shell), although this pattern is not universal (Claude et al. 2003; Stayton et al. 2018). Polly et al. (2016) demonstrated that these differences likely result from trade-offs between shell strength and hydrodynamic efficiency. Taller shells are usually stronger, but generate more drag, than flatter shells, so it is reasonable that terrestrial turtles, released from selection pressure for efficient swimming, should evolve taller and thus stronger shells. However, this trade-off between strength and hydrodynamics is not the only potential explanation for the observed differences. For example, tall shells can also facilitate self-righting when a turtle has been turned onto its back, which may be of greater or more frequent importance in terrestrial species relative to aquatic ones. Finally, taller, more hemispherical shells will also possess lower surface area to volume ratios than flatter shells, which in turn will lead to slower rates of heat exchange with the environment in terrestrial species (Boyer 1965). This has been suggested as advantageous in terrestrial taxa, which may not have access to water for thermoregulation.

These results and interpretations, however, remain preliminary—earlier studies had limited coverage of turtle diversity, lacked phylogenetic or rigorous functional context (Claude et al. 2003) or only inspected a single clade (the Emydidae). None used validated measures of shell performance. More extensive exploration of these trends has been hampered by complications regarding both the relationship between ecology and morphology in turtle shells and the relationship between morphology and performance. First, although the shell shapes of terrestrial and aquatic species differ in some general ways, there is a great deal of morphological disparity within each of the two groups (Stayton et al. 2018). Some aquatic species show relatively highly-domed shells with broad plastra, while many terrestrial species possess shells which differ from the typical tall and domed morphology characteristic of tortoises (Testudinidae) or box turtles (*Cuora* and *Terrapene*). A number of terrestrial species are characterized by robust shells which are nevertheless flat (*Geoemyda*, *Heosemys depressus* and *H. spinosa*, *Vijayachelys silvatica*; see Claude et al. 2003). These species are known to hide under leaf litter, a lifestyle for which a flattened shell is appropriate. These species are sometimes even flatter than highly aquatic close relatives. A typical interpretation of these patterns (Stayton et al. 2018) is that these species have abandoned the strong shell shapes of terrestrial turtles in favor of an alternative strategy: evading predator attacks rather than surviving them, or reducing drag when moving through leaf litter.

This interpretation, however, makes a number of assumptions about patterns that have also been shown to be more complicated than previously thought (Stayton et al. 2018; Stayton 2018). First, moving from an aquatic to terrestrial habitat does not require an increase in selective pressure for shell strength (aquatic species have predators too, sometimes very strong ones!; Heithaus et al. 2008), nor an increase in selective pressure for self-righting. Instead, the evolution of a terrestrial lifestyle simply implies a decrease in the relative importance of hydrodynamic efficiency as a selective pressure; the importance of any one other factor may or may not increase. Second, even if selection pressure for strength or self-righting performance increases, these can be accommodated by a range of shell shape changes. An increase in shell strength does not require an increase in carapace height—this is one mechanism by which strength can increase, but an increase in bridge length (providing an increase in the amount of bone which connects the lateral edges of the carapace and plastron) or plastron width can also accomplish the same goal (Stayton 2018). It is possible that the shells of certain flat terrestrial turtles are stronger than those of equally- or even less-flat aquatic relatives. Such a pattern would be important for understanding the potential flexibility of terrestrial turtle responses to different selective pressures; however, this has not been considered in previous publications.

Here, performance surface analyses are used to test the hypothesis that turtle lineages which have undergone an evolutionary transition from aquatic to terrestrial habitats have also experienced evolutionary changes in shell shape, which are consistent with a decreased importance of selection for hydrodynamic efficiency. These changes can coincide with an increased importance of strength, self-righting, or both, as selective factors. Such changes can also be consistent with the evolution of flatter shells, so long as other changes in shell shape (e.g., an increase in bridge length) are also consistent with an increased importance of shell strength or self-righting ability.

## Materials and methods

### Phenotypic data

Most details of the morphological data can be found in Stayton et al. (2018). Briefly, 53 3D landmarks, distributed over the surface of the turtle shell, were digitized on one side of all specimens. In most cases, specimens were placed in a box of sand and landmark locations were captured using a 3DX portable digitizer (Immersion Corporation, San Jose, CA). A few large specimens were photographed in dorsal, lateral, and ventral view. Landmarks were digitized using tpsDig 2.1 (Rohlf 2006), and assembled using a custom MATLAB routine (Stayton 2011a). A few additional specimens, representing rare or inaccessible species, were also digitized this way using published images (Broadley 1981; Cann 1997; Branch 2007; Fritz et al. 2008; Murphy et al. 2011). All specimens represented individuals with fully ossified shells. Whenever possible, five females and five males were included for each species. Wild-caught specimens were preferred over captive specimens. The present study encompasses 2731 specimens, representing 281 species (92% of all hard-shelled species, and all genera; Supplementary Material File 01). All known terrestrial turtle species are represented in this data set.

Data were subjected to a generalized Procrustes fit (Zelditch et al. 2004). Fitted data were subjected to a principal components (PCs) analysis using the "plotTangentSpace" command in the R package geomorph (Adams et al. 2018). Mean PC scores were used in subsequent analyses (Fig. 1A).

The construction of performance surfaces (see below) required the creation of a "mesh" of shapes spanning shape space. Here, as in Stayton (2019), surfaces were constructed across the observed range

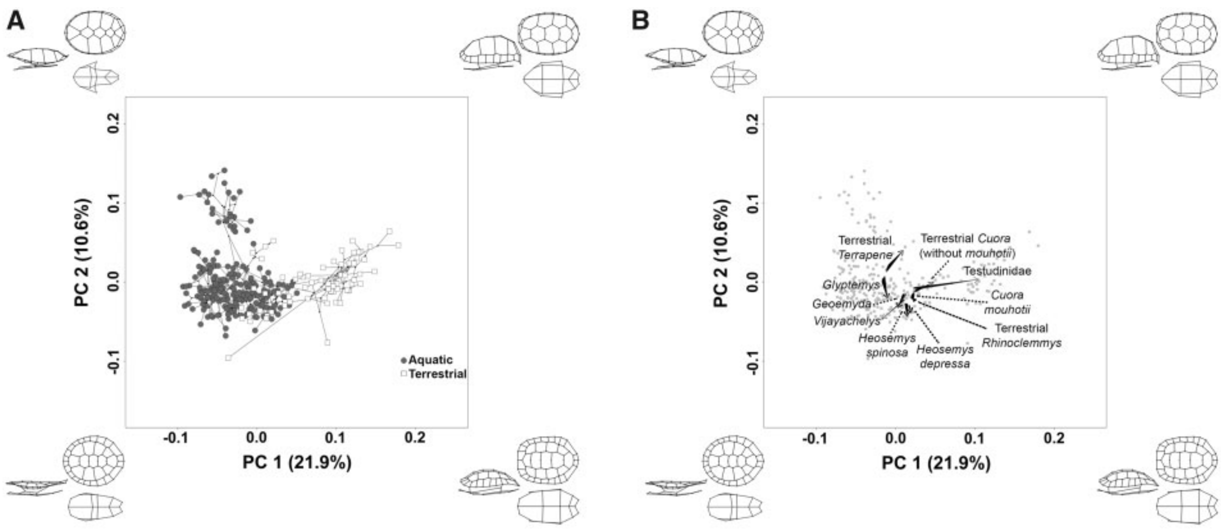

**Fig. 1** (**A**) Phylomorphospace for all species examined in this study. Large symbols indicate species means. Lines indicate evolutionary lineages. Points at the intersection of lineages represent reconstructed ancestors. (**B**) Morphospace showing all species (grey dots) as well as evolutionary vectors (arrows) pointing from aquatic progenitors to terrestrial descendants for each terrestrial lineage. Vectors reconstructed for all 1000 phylogenies are shown. Images in the corners of each diagram illustrate shapes associated with those regions of shape space (top—dorsal view of carapace; middle, left—lateral view of whole shell; bottom—ventral view of plastron; anterior is the left).

of species values on PCs 1 and 2—these axes are the only ones correlated with important ecological variation, including the aquatic-terrestrial gradient (Claude et al. 2003; Stayton 2011a). The loadings of all landmark coordinates on the PC axes were used to extract a series of shapes (255 for strength and self-righting, 20 for hydrodynamics) spanning PC axes 1 and 2, which were then used in performance analyses.

## Performance data

Details of the analysis of performance for turtle shell strength, hydrodynamics, and stability regarding self-righting ability are given in Stayton (2019). Briefly, shell strength was assessed by building finite element (FE) models for a series of shapes, representing all combinations of a set of 17 points along PC1 and 15 points along PC2, each spanning the observed range of species on those axes and spaced 0.025 units apart. Models were built by warping an initial model of a bog turtle (*Glyptemys muhlenbergii*) using the procedure of Stayton (2009). All models were scaled to the same surface area in order to eliminate variation in performance due to size (Dumont et al. 2009)—this resulted in a set of shells all approximately 10 cm in carapace length (relatively small for the species sampled here). FE analysis was then conducted on all models, using the same material properties and the same 12 load cases (restraints and loads) on each model. Load cases, each representing a point load as might be caused by a predator's tooth, were distributed across the surface of the shell. The maximum von Mises stress observed for each load case was recorded for each model and then averaged, providing an estimate of the strength of each model (with higher stresses being associated with weaker shells). This procedure has proven successful in predicting failure in actual turtle shells (Stayton 2018).

Hydrodynamic performance was assessed by direct measurement. A set of 20 shapes, distributed across the observed values of PCs 1 and 2, were selected, and models of those shapes (with closed anterior and posterior apertures) were printed in plastic. Each model was placed in a wind tunnel, drag was measured for a range of wind speeds, and the drag coefficient (a unitless measure that describes the relationship between fluid velocity and drag) was calculated for each shape. This procedure provides accurate measures of the drag coefficients of turtles moving at observed speeds in water (see Stayton (2019) and references therein).

Finally, self-righting performance was assessed using the *F*-index, a unitless measure that describes the height of an object relative to length and width and which has been shown to predict self-righting performance in tortoises (Golubović et al. 2015). The *F*-index was extracted for each of the 255 shapes used for strength testing.

## Performance surface analysis

A cubic spline was applied to the set of performance data for each function to create a finer mesh of points (spanning observed values of PCs 1 and 2 and spaced 0.005 units apart), representing the performance surfaces. These surfaces were then analyzed using a procedure from Stayton (2019) (modified from Polly et al. (2016)): All surfaces were scaled to unit height, then each surface was assigned a weight from 0 to 1, ensuring that the weights from all three surfaces summed to 1. Under a certain set of assumptions about the relationship between performance and fitness (e.g., that the relationship is linear for each function and that effects are additive between functions; Polly et al. 2016), these weights represent the relative importance of selection for all three functions. The values of performance for each surface were multiplied by their respective weights, and then the surfaces were summed (i.e., the rescaled, weighted performance values for all three functions were summed for each point in shape space), to create an overall performance surface. The location of optimum performance on this combined surface represents an adaptive "peak" for that particular combination of weights (see Figure 3 in Stayton (2019) for more details).

This procedure was conducted for all combinations of relative weights for all functions, in intervals of 0.01, and the location of optimum performance for each combination was recorded. This set of optima represents all possible adaptive "peaks" for species experiencing any possible combination of relative weights for the functions: those for which shell strength is the only relevant function (weight for strength = 1.00, weight for the other functions = 0.00), those for which performance on all functions is equally important (weights for all functions = 0.33), those for which strength and hydrodynamic performance are twice as important as self-righting performance (weight for strength and hydrodynamic performance = 0.40, weight for self-righting performance = 0.20), and so on. The distribution of actual turtle species matched the distribution of these peaks fairly closely, indicating that these three functions can be used to predict a large amount of ecologically-relevant variation in turtle shell shape (Stayton 2019).

Although all performance surfaces were constructed using validated measures, it is likely that performance values were not estimated with perfect accuracy. To accommodate this possibility, two more searches were conducted which, besides recording the locations of optimal performance for all combinations of relative weights, also recorded the locations of points in shape space where overall performance came close to optimal for each combination of relative weights. Specifically, the preceding procedures were repeated twice, once recording the locations of all points (as well as associated weights) where overall performance came within 3% of the optimum observed value, and once recording the locations of all points where overall performance came within 5% of the optimum observed value. These expanded searches necessarily produced a broader set of optima (Fig. 2) and may represent more realistically the set of adaptive peaks upon which species actually evolve.

Size was not considered. Of the measures of performance used here, only stress over a given set of loads varies with size; the drag coefficient and *F*-index are scale-free and will not vary with size. Since stress for a given set of loads decreases as size increases, if size were incorporated as a third phenotypic dimension, then optimal performance for any combination of weights (where the weight for strength was not 0) would always be found at the greatest modeled size, and any observed size variation would be uninformative. Future studies could benefit from incorporating size into estimates of hydrodynamic or self-righting performance.

## Hypothesis testing

The hypothesis for this study—lineages transitioning from terrestrial to aquatic habitats show evidence of a decreased importance of hydrodynamic performance—was tested as follows. First, the primary literature was used to identify terrestrial taxa (see Stayton et al. (2018) for details). Ultimately, 69 species, representing 10 evolutionary independent invasions of land, were identified: all Testudinidae (tortoises; 47 species in the data set), terrestrial *Terrapene* (North American box turtles, 4 species), all *Glyptemys* (2 species), *Cuora mouhotii*, all other terrestrial *Cuora* (East Asian box turtles, 5 species), all terrestrial *Rhinoclemmys* (4 species), all *Geoemyda* (2 species), *H. depressa*, *H. spinosa*, and *V. silvatica*. It was not necessary to differentiate between "terrestrial" and "semiterrestrial" species. Some terrestrial species will often move in and even feed in water (Stayton 2011b; Natchev et al. 2015), but so long as they are known to spend significantly more time on land than their relatives and show non–shell evidence for adaptation to life on land (e.g., a lack of webbing between the digits), such species are sufficient.

Next, a set of 1000 phylogenies was pulled from the posterior distribution of the analysis conducted by McLaughlin and Stayton (2016). Additional species, not included in that original study, were added to the trees based on information from an additional

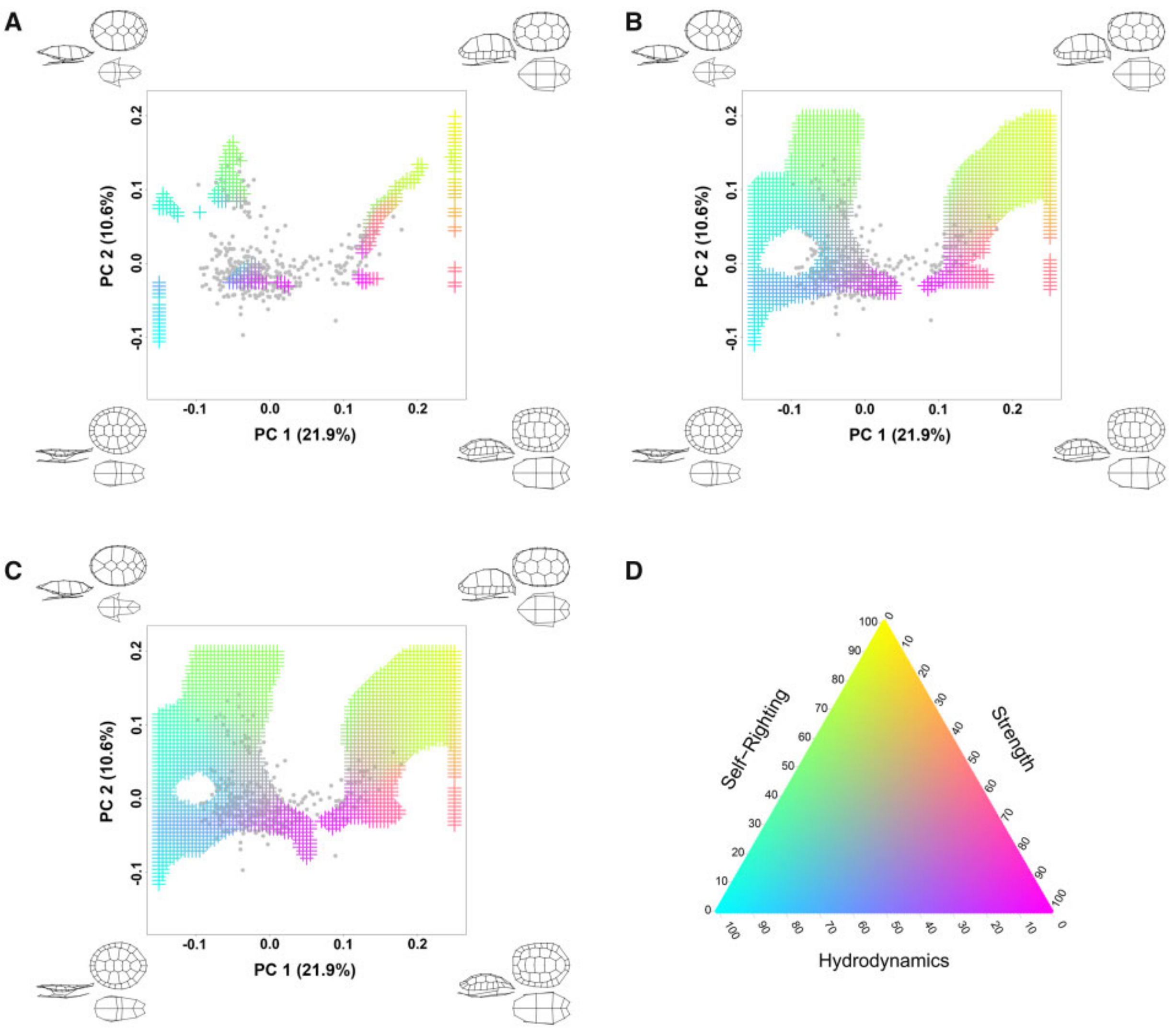

**Fig. 2** (**A**–**C**) Diagrams of all performance optima. Grey dots represent species means (as seen in Fig. 1); crosses represent the location of reconstructed optima. Colors of crosses represent the relative weights for three functions associated with each optimum. Images in the corners of (A)–(C) illustrate shapes associated with those regions of shape space (top—dorsal view of carapace; middle, left—lateral view of whole shell; bottom—ventral view of plastron; anterior is the left). (A) Maximum performance only, (B) Maximum performance and all points within 3% of maximum performance, (C) Maximum performance and all points within 5% of maximum performance. (**D**) Ternary diagram illustrating the color coding for optima in (A)–(C).

phylogeny (Thomson and Shaffer 2010), or were simply added into a polytomy at the base of their genera (e.g., all genera described in Turtle Taxonomy Working Group (2017) were assumed to be monophyletic). Although all trees differed in branch lengths and some aspects of the topology, all were in agreement about the patterns of evolution leading to each terrestrial clade. Thus, it was possible in each case to identify, using simple parsimony, the locations at which the transitions to the terrestrial habitat occurred.

After phylogenies were developed, ancestral state reconstruction of PC1 and 2 scores, implemented using the "fastAnc" command in the R package phytools (Revell 2012), was conducted for all nodes on each phylogeny. The branches along which the aquatic-terrestrial transitions occurred were identified, and the reconstructed PC1 and 2 scores for the initial nodes of those lineages—in other words, the most recent aquatic ancestors of each terrestrial clade, hereafter referred to as the "progenitors" of terrestrial clades—were recorded. The observed average PC1 and 2 scores for all species within each terrestrial lineage were also recorded, and averaged. Thus, each terrestrial lineage produces two sets of PC1 and 2 scores: the reconstructed scores of the aquatic progenitors for each lineage, which represent the "starting" points for morphological and functional evolution in response to terrestrial environments, and the mean scores of all extant species in each lineage, which represent the "terminal" points. Cases where terrestrial clades included only a single species used that species' PC scores as terminal values and reconstructed scores of its immediate ancestor as progenitor values.

The performance optima that fell nearest to each of these scores were identified—for example, for the Testudinidae, the location of the performance optimum nearest the reconstructed PC1 and 2 scores of the tortoise progenitor, as well as the location of the performance optimum nearest the mean PC1 and 2 scores across all tortoise species, were found—and the relative weights associated with those optima were recorded. This study predicts that the weights for hydrodynamic performance will be higher in the optima nearest the locations of the progenitors than in the terrestrial descendants. To quantify this, the weights for hydrodynamic performance for the progenitors of each terrestrial clade were subtracted from the weights for hydrodynamic performance in the terrestrial descendants—the hypothesis predicts negative values for this measure. In addition, an overall measure was also generated by averaging the progenitor weights for all terrestrial lineages and subtracting those from the mean descendant weights for all terrestrial lineages.

This procedure was repeated for each of the phylogenies and for each set of expanded performance optima (within 3% and within 5%). Each repetition produced 11 measures—one for each independently-derived clade of terrestrial turtles, and one for all terrestrial turtles taken together.

Significance was assessed through simulation. Significance was only assessed on 100 of the phylogenies used in this study (due to computational limitations). Evolution was simulated 1000 times along each phylogeny, using a Brownian-motion model of evolution with variances and covariances derived from the observed data. For each simulation, the procedures used to extract differences in weights for aquatic performance between progenitors and descendants were repeated—scores on PCs 1 and 2 were derived for each progenitor, the locations of nearest performance optima were found for all progenitors and descendants for each of the 10 independently-derived terrestrial lineages, and the differences in hydrodynamic weights associated with those optima were recorded. Data were averaged across all simulations on each phylogeny, and the number of times that the simulated differences were lower than the observed differences (i.e., the number of times that the weight associated with hydrodynamic performance decreased more than what was observed) was used to calculate a *P*-value. In addition, the differences in aquatic weights were summed across all terrestrial lineages, in both the observed and simulated data, and the proportion of times that the simulated sums of differences were lower than the observed differences was used to calculate a *P*-value.

## Results

Terrestrial turtles were in general associated with taller, more domed carapaces and larger plastra, although some terrestrial species showed relatively flat shells, similar to those of aquatic species (Fig. 1A). Vectors pointing from the progenitors of each terrestrial clade to the mean of their descendants show a variety of directions; there is no obvious signal of morphological convergence among terrestrial species (Fig. 1B). The set of performance optima matched the observed distribution of species fairly closely, though there do appear to be some "unoccupied" optima at high PC1 and 2 scores, and low PC1 and 2 scores (Fig. 2; Stayton 2019).

When the search for performance optima was expanded to points that came within a certain percentage of optimum performance, the map of optima obviously expanded (Fig. 2). This expansion was heaviest at low PC1 scores or a combination of high PC1 and PC2 scores, but it also increased the overlap between optima and the observed distribution of turtle shells.

Despite the lack of any obvious morphological convergence, the evolution of terrestrial clades is very consistently associated with a decrease in the relative importance of hydrodynamics as a selective factor (Table 1). This consistency increases as the set of performance optima is expanded to allow points within a range of the observed optima. The differences were also typically significant (Table 1)—terrestrial clades consistently showed a greater decrease in the weight of hydrodynamic performance than would be expected if evolution were not influenced by any adaptive peaks (i.e., evolution according to a Brownian Motion model). This consistency increased as the set of performance optima was expanded. *Vijayachelys* was very consistently associated with an increase in the importance of hydrodynamic performance; this is the only lineage which never showed a significant decrease in inferred weight for hydrodynamics, and thus evidence against the hypothesis.

## Discussion

Lineages of terrestrial turtles, although they might evolve highly divergent morphologies, show strong evidence of evolution toward regions of shape space associated with a lower importance of selection for hydrodynamic efficiency. This is consistent with theoretical expectations on the importance of various functions in turtles with different ecologies, as well as the divergent lifestyles and habitats of terrestrial species.

Thus, the hypothesis of this study was corroborated for all terrestrial lineages taken together, and for individual terrestrial lineages. The strongest results were found among the Testudinidae, *Glyptemys*, and *Heosemys*; consistent but weaker results were found among the box turtles (*Terrapene* and *Cuora*), *Rhinoclemmys*, and *Geoemyda*.

In some cases, interpretation is straightforward. Tortoises, for example, are both the oldest clade of terrestrial turtles and the most consistently terrestrial (with no known reversions to aquatic habitats, as is inferred in *Terrapene* and *Rhinoclemmys*)—it is thus unsurprising that this clade shows strong evidence of a change in selective pressures during the transition to terrestriality.

The box turtles (*Terrapene* and both *Cuora* lineages) also represent relatively old lineages (∼10–14 myr in both cases; McLaughlin and Stayton 2016) with relatively tall shells, but they are more weakly associated with decreases in weights associated with hydrodynamic performance. Here, an explanation might be found among the set of optima—box turtles show evidence of phenotypic evolution toward regions of shape space not associated with any optima. Thus, both aquatic progenitors and terrestrial descendants might be located closest to adjacent or even identical optima, implying very little change in the importance of any functional factors. Why are these taxa evolving away from regions where performance optima are located? Likely because of additional functional demands (i.e., the requirement that the head and limbs must be able to be fully withdrawn into the shell while it is closed up) not considered in the analysis. It is also possible that the mechanical analyses used here are not entirely appropriate for box turtles—the carapace and plastron of these species are connected by ligament, not bone, and there is a ligamentous hinge across the plastron as well. These connections can resist compression as well as bone, but not tension, so some FE results may mischaracterize shell performance. Future studies are planned, which will examine patterns of morphological and mechanical evolution in the carapace only, to determine whether box turtles morphologies are really so unexpected as they appear here.

The weak (but still significant) evidence for a decrease in the relative importance of hydrodynamic performance for *Rhinoclemmys* and *Geoemyda* has no similarly simple explanation. Neither lineage is particularly young, so both should have had sufficient time to evolve toward optima more consistent with a terrestrial habitat.

Notably, some terrestrial lineages indicated a decrease in the relative importance of hydrodynamic selective factors during the transition to terrestriality, despite the fact that these lineages also experienced a decrease in shell height. Both *Heosemys* lineages and *Geoemyda* encompass flat species which in many cases are flatter than their aquatic relatives. However, *Heosemys* and *Geoemyda* also possess shells characterized by large plastra and extensive bridges between the carapace and plastron. These bridges, which are longitudinally much larger than those of related aquatic species, provide additional cross-sectional area to resist loads and also likely prevent stresses due to bending at the anterior and posterior shell apertures (Vega and Stayton 2011; Wise and Stayton 2017, Williams and Stayton 2019). Thus, these species are able to maintain strong shells while keeping shell height low enough to allow them to hide under terrestrial leaf litter. Their aquatic relatives, in contrast, probably cannot maintain extensive bridges because these might hinder the large anterior–posterior limb excursions necessary for effective swimming (Pace et al. 2001).

Given the fact that other flat, cryptic terrestrial lineages show evidence for a decrease in the importance of hydrodynamic performance, it is puzzling that *Vijayachelys* reliably showed a pattern of morphological evolution consistent with an increase in the importance of hydrodynamic performance. This may be due to inaccurate representation of the species' shell shape—the lineage is represented by only four individuals (although no abnormalities were noted in the specimens). If shell shape in *Vijayachelys* is accurately represented, its ecology might not be. The life history of this species is poorly known. It is consistently reported as terrestrial (Whitaker and Vijaya 2009; Smart et al. 2014), but numerous individuals have also been found with extensive algae on the shells (Whitaker and Vijaya 2009). This could indicate significant aquatic activity, though it is still doubtful whether this species could be characterized as being under stronger selective

**Table 1** Differences in reconstructed weights for aquatic performance in terrestrial clades and their aquatic progenitors, along with results of significance tests for those differences. *P*-values are in parentheses. Negative numbers indicate a decrease in the reconstructed importance of aquatic performance during the transition to terrestriality. Results are presented for sets of optima reconstructed using optimal values only, as well as all points within either 3% or 5% of the optimum

| Lineage | Difference in weights (optima only) | Difference in weights (optima and points within 3%) | Difference in weights (optima and points within 5%) |
|---|---|---|---|
| Testudinidae | −0.095 ± 0.080 | −0.121 ± 0.105 | −0.191 ± 0.118 |
| | (0.980) | (0.03) | (<0.001) |
| *Glyptemys* | −0.302 ± 0.138 | −0.302 ± 0.131 | −0.283 ± 0.134 |
| | (<0.001) | (<0.001) | (<0.001) |
| *Terrapene* | −0.032 ± 0.042 | −0.035 ± 0.023 | −0.034 ± 0.048 |
| | (0.053) | (0.003) | (<0.001) |
| *Cuora (-mouhotii)* | −0.026 ± 0.059 | −0.091 ± 0.103 | −0.032 ± 0.065 |
| | (0.056) | (<0.001) | (<0.001) |
| *Cuora mouhotii* | −0.105 ± 0.017 | −0.130 ± 0.018 | −0.104 ± 0.019 |
| | (<0.001) | (<0.001) | (<0.001) |
| *Rhinoclemmys* | −0.068 ± 0.056 | −0.029 ± 0.032 | −0.045 ± 0.062 |
| | (0.058) | (0.002) | (<0.001) |
| *Vijayachelys* | 0.558 ± 0.069 | 0.806 ± 0.049 | 0.547 ± 0.065 |
| | (1.000) | (1.000) | (1.000) |
| *Heosemys depressa* | −0.228 ± 0.000 | −0.224 ± 0.005 | −0.227 ± 0.005 |
| | (<0.001) | (<0.001) | (<0.001) |
| *Heosemys spinosa* | −0.297 ± 0.065 | −0.274 ± 0.043 | −0.301 ± 0.061 |
| | (<0.001) | (<0.001) | (<0.001) |
| *Geoemyda* | −0.026 ± 0.063 | −0.087 ± 0.090 | −0.049 ± 0.092 |
| | (0.390) | (<0.001) | (<0.001) |
| All lineages | −0.151 ± 0.104 | −0.197 ± 0.247 | −0.631 ± 0.237 |
| | (<0.001) | (<0.001) | (<0.001) |

pressure for aquatic performance than its aquatic relatives (the algae could also simply reflect high humidity in this species' habitat; Deepak et al. 2014). Moreover, a primarily terrestrial habitat is more consistent with the lack of webbing between the digits of this species, as well as the presence of a concavity in the plastron of males (believed to increase stability during mating, an important selective factor in terrestrial males). The pattern of coloration on the dorsal surface of the shell is also consistent with specializing to live under leaf litter. This species is often found in tree holes or termite mounds (Deepak et al. 2014)—could their shape reflect adaptation for a burrowing habit, which might benefit from increased streamlining? Perhaps, but the burrowing *Gopherus polyphemus* does not show a similar shape relative to other tortoises, and neither species is particularly narrow. Thus, the reasons for the unusual patterns of shell evolution in *Vijayachelys* remain speculative.

In general, expansion of optima to include points also representing near-optimal performance increased the reconstructed changes in importance of hydrodynamic performance for terrestrial lineages and improved their significance. These changes may reflect the distribution of optima. When only optimal combined performance is considered, the distribution of optima is restricted and patchy. The nearest optima for progenitors and descendants both may be located close together or even be identical (as was the case for *Terrapene*), implying only limited changes in the relative importance of performance for various functions. However, as a less strict criterion for optimality is implemented and the range of optima expands, it becomes easier for progenitors and descendants to be located nearer to different optima which imply different weights for various functions. This likely also provides a more realistic view of the ways performance and selective regimes change; future studies may benefit from utilizing a (slightly) relaxed criterion for extracting adaptive optima.

Future studies could also incorporate body size more fully into analyses. First, ways to incorporate size into hydrodynamic and self-righting performance could be explored. Once these are quantified, studies could examine the ways in which size and shape evolution interact—certain species may offset relatively weak shell shapes by an increase in shell size, for example. It is unlikely that this effect is biasing this particular study's results—with the possible exception of tortoises, all terrestrial lineages are the same size as or smaller than their closest aquatic relatives, so changes in the relative importance of shell strength are not being "masked" by increases in size—but given the massive range of sizes seen in turtles, this is clearly an axis of considerable importance.

A previous analysis of a large subset of the present data (Stayton 2019) also incorporated an OU model-fitting analysis, which can serve as a useful comparison. That study conducted an l1ou (Khabbazian et al. 2016) analysis of the PC1 and PC2 scores used here. This model-fitting analysis was not conducted with terrestrial species in mind, but given that model-fitting approaches typically do not incorporate ecological information during their search for their adaptive peaks, this does not affect the relevance of the results. That model reconstructed 35 peaks in PCs 1 and 2. Nine were associated with terrestrial taxa. Most of these were also exclusive to terrestrial taxa: tortoises were associated with five peaks, and three terrestrial *Cuora* species were associated with another. All *Terrapene* species, including the aquatic *T. coahuila*, were associated with a single peak. However, additional peaks were shared among terrestrial and aquatic taxa—*Glyptemys*, *Geoemyda*, both *Heosemys*, terrestrial *Rhinoclemmys*, and the remaining *Cuora* were all associated with peaks shared with large numbers of aquatic taxa. The reconstructed locations of these peaks are reasonable as well, occurring within or near the observed range of actual turtle PCs 1 and 2 scores. Thus, while this analysis does not imply unrealistic adaptive peaks for terrestrial taxa, it does not provide useful information about common patterns of evolution among terrestrial lineages, and of course it does not provide any information about the functional or performance implications of these patterns. OU-based analyses can be useful, but performance surface methods outperform them for analyses which seek to interpret evolution in functional terms.

In conclusion, performance surface analyses reveal a consistent pattern of functional change among morphologically-divergent terrestrial turtle lineages. Conventional adaptive landscape reconstruction methods (i.e., OU models) miss this pattern. These methods can be applied to any structure with known relationships between morphology and performance, particularly structures that must perform two or more functions (this may well be the majority of structures). Performance surface analyses can illustrate surprising context-dependent trade-offs or reveal unexpectedly optimal phenotypes as well as acceptable-seeming morphologies which nonetheless fail to optimize performance for any combination of selective factors. This ability to explain unoccupied as well as occupied regions of phenotypic space, to explore the implications of known evolutionary trajectories as well as paths not taken, and to do so in an explicit quantitative framework, represents strength of performance surface methods for researchers working on any multi-function structure.

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## Supplementary data

Supplementary data available at *ICB* online.

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