Delayed trait development and the convergent evolution of shell kinesis in turtles

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Abstract
Developmental processes are foundational to clarifying the causes of convergent evolution. Here, we show how a key convergently evolving trait is slowly "acquired" in growing turtles. Adaptive morphological change tends to originate late in turtle ontogeny, owing to design constraints imposed by the shell. We investigated this trend by examining derived patterns of shell formation associated with the multiple (≥ 8) origins of shell closure (kinesis) in small-bodied turtles. Using box turtles as a model, we demonstrate that the flexible hinge joint required for shell kinesis differentiates gradually and via extensive repatterning of shell tissue. Disproportionate changes in shell shape and size substantiate that this transformation is a delayed ontogenetic response (3-5 years post-hatching) to structural alterations that arise in embryogenesis. These findings exemplify that the translation of genotype to phenotype may reach far beyond embryonic life stages. Thus, the temporal scope for developmental origins of adaptive morphological change might be broader than generally understood. We propose that delayed trait differentiation via tissue repatterning might facilitate phenotypic diversification and innovation that otherwise would not arise due to developmental constraints.

Disciplines
Animal Sciences | Ecology and Evolutionary Biology | Genetics | Population Biology

Comments
Convergent phenotypes develop slowly in turtles with shell kinesis

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**Summary**

Developmental processes are foundational to clarifying the causes of convergent evolution. Here, we show how a key convergently evolving trait is slowly “acquired” in growing turtles. Adaptive morphological change tends to originate late in turtle ontogeny, owing to design constraints imposed by the shell. We investigated this trend by examining derived patterns of shell formation associated with the multiple (≥ 8) origins of shell closure (kinesis) in small-bodied turtles. Using box turtles as a model, we demonstrate that the flexible hinge joint required for shell kinesis differentiates gradually and via extensive repatterning of shell tissue. Disproportionate changes in shell shape and size substantiate that this transformation is a delayed ontogenetic response (3-5 years post-hatching) to structural alterations that arise in embryogenesis. These findings exemplify that the translation of genotype to phenotype may reach far beyond embryonic life stages. Thus, the temporal scope for developmental origins of adaptive morphological change might be broader than generally understood. We propose that delayed trait differentiation via tissue repatterning might facilitate phenotypic diversification and innovation that otherwise would not arise due to developmental constraints.
1. Introduction

Similar environmental selective pressures often lead to similar adaptive phenotypes in species that do not share a recent common ancestor, i.e. convergent evolution [1-4]. This fascinating trend provides an opportunity to evaluate the extent to which evolution is predictable and repeatable [3, 4]. Indeed, major advances in formulating a theoretical framework for the study of convergent evolution were achieved in recent years [5, 6]. However, hypotheses targeting the role of developmental processes are rarely addressed (but see [7-10]).

Although the primacy of natural selection is unequivocal in explaining convergent evolution, developmental processes may ultimately limit the number of evolvable character states [3]. For instance, the likelihood of trait convergence might be higher in species groups that exhibit morphological stasis owing to design limitations imposed by underlying developmental processes [11, 12]. The atypical turtle body plan is an outstanding model to examine such a phylogenetic pattern [13, 14].

The turtle’s shell forms via changes in the skeletal architecture of tetrapods that have profoundly influenced turtle diversification over the last 210 million years [15, 16]. As a consequence of shell development, turtles are the only tetrapod to feature bone sutures in the thoracic region analogous to the ones commonly found in the cranium [17]. These sutures have been co-opted repeatedly to give rise to an assortment of functional shell adaptions [15, 18-20]. Beginning in the early Cretaceous, hinge sutures that enable shell closure via muscle-induced movement of the shell (i.e. kinesis) have evolved independently in multiple lineages [15, 21-29]. Adaptive hypotheses on this convergence generally invoke enhancement of anti-predator defense in terrestrial habitats or in shallow water (Fig. 1) [23, 29-34], as well as avoidance of dehydration while in terrestrial burrows [35].
Shell kinesis comprises a suite of musculoskeletal traits that develop in a taxon-specific manner [16, 23-25, 27, 36]. Even so, gradual differentiation of one or two hinge joints during post-embryonic stages is common to all kinetic-shelled lineages [37-39]. To explain delayed hinge differentiation, morphologists proposed that boundaries of ectodermal plates (i.e. scutes) and underlying bones must align as these shell elements grow and shift their positions (Fig. 1) [23, 40, 41]. Furthermore, mechanical strain exerted by muscles during shell closure might prevent sutural fusion of adjacent bones, leading to the formation of elastic connective tissue [23, 41]. This complex transformation, however, has not been examined. In general, post-embryonic tissue changes are challenging to describe in long-lived organisms, e.g. humans [42].

We investigated developmental processes underlying the convergence of shell hinges in turtles with shell kinesis. We first quantified evolutionary origins of kinesis and tested the hypothesis that small-bodied turtles with terrestrial habits are more likely to feature kinetic hinges [34, 43, 44]. Then, using box turtles (Terrapene) as a model, we tested the prediction that fusion of a precursor hinge suture is delayed and elastic tissue forms as kinesis becomes functional in juveniles [23], hereafter ‘heterochronic’ model. Alternatively, this suture might fuse and undergo repatterning in conjunction with elastic tissue formation, hereafter ‘repatterning’ model. In either case, we expected associated shell structures (scutes and buttresses) to also undergo repatterning [23, 37]. Lastly, we tested whether kinetic shells exhibit disproportionate size and shape changes during ontogeny [29, 43].

2. Materials and methods

(a) Comparative phylogenetic analyses

We performed comparative analyses in the phytools R package [45]. Using stochastic character mapping [46], we reconstructed ancestral character states of shell kinesis on the
most recent molecular phylogeny of turtles ($N = 292$ species) [47]. We simulated character state histories 1,000 times (along branches and on nodes) with sampling conditioned on an equal-rates model (Markov k-state 1) for state transitions. We then computed simulated averages for absent-to-present and present-to-absent transitions to evaluate hypothetical independent origins of shell kinesis, as well as potential reversals.

We tested the hypothesis that body size, i.e. carapace length (CL), is shorter in kinetic-shelled species with a simulation-based phylogenetic analysis of variance (pANOVA) [48]. We evaluated the correlation of habitat type (aquatic/terrestrial) and shell type (akinetic/kinetic) with Pagel’s test for discrete characters [49], assuming that transitions in one character depended on the other, and vice versa, and that all rates for state transitions were different. To do so, we optimized model fitting with the geiger ‘fitDiscrete’ function and ran two separate Pagel’s tests: one based on the standard aquatic/terrestrial turtle dichotomy [44], and another that included semiaquatic Kinosternon as terrestrial (following refs. [50-52]). All character state data appear in Table S1 of the electronic supplementary material.

(b) Shell tissue preparation

We prepared samples following standard histological protocols for bone tissue [53]. We first fixed hatchling carcasses, obtained from a previous study [16], in 10% buffered formalin before dehydrating them in an increasing ethanol series. We stained tissue of representative lineages with the most common forms of plastral (ventral shell) kinesis in alizarin red to confirm the absence of hinges. We then focused on emydid lineages with plastral kinesis—Terrapene ornata and Emys (Emydoidea) blandingii [16]—and akinetic-shelled Chrysemys picta and Glyptemys insculpta. See the electronic supplementary material for further details on specimens used.
We skeletonized preserved adult *C. picta*, *E. blandingii*, and *T. ornata* in the ISU herpetological collection to be scanned with a NextEngine 3D surface scanner to compare 3D models of shell morphology. We also dissected formalin-fixed plastron tissue and stained it in hematoxylin and eosin solution to examine immature sutures in hatchling *T. ornata*. We further decalcified adult plastron tissue in 5% formic acid and stained it with Verhoeff-van Gieson solution to compare elastic fiber variation in sutures of *T. ornata* versus *G. insculpta*.

(c) Shell morphometric analyses

To ascertain whether disproportionate plastron growth occurs in kinetic-shelled species, we examined log-transformed measurements of plastron length (PL) and CL in live-trapped adults and juveniles of *C. picta*, *E. blandingii*, and *T. ornata* from Thomson, Illinois [54]. Using digital calipers, we recorded shell measurements in embryos (stages 17-22) and hatchlings preserved in previous studies [16, 55]. We evaluated PL variation in relation to CL with a general linear model, which included a species by PL interaction term to account for interspecific variation in PL growth rates. The plastron may occlude the carapace once the plastral hinge is functional [23], thus we expected a 1:1 PL-to-CL relationship in adults of kinetic-shelled species. In emydid turtles, PL/CL generally approaches one (i.e. isometry) [56], thus an ANOVA was suitable to test mean differences in this ratio [57].

Following Myers et al. [58], we quantified post-hatching plastron shape by digitizing 12 fixed homologous landmarks in a subsample of museum specimens. We placed landmarks in the same position in all photographed specimens (with a ruler for scaling) to minimize distortion using tpsDig [59]. We then performed generalized Procrustes analysis to remove non-shape variation using tpsRelw [60]. For comparison, we first superimposed landmarks and translated them to a shared origin, followed by rescaling to units of centroid size. We subsequently rotated them to minimize the sums-of-squares differences among all landmark
configurations [61]. After orthogonal projection into a linear tangent space, aligned Procrustes shape coordinates depicted shape variation, which we represented as thin-plate spline deformation grids using tpsSpline [62].

We explored shape variation with a principal component analysis and regressed the first PC axis against log PL to test whether plastron deformation increased disproportionately during post-hatching growth in kinetic-shelled emydids. We evaluated interspecific differences in shape deformation, represented by the first PC axis, with a general linear model (as above). We conducted these statistical analyses using base functions of R [63].

3. Results

(a) Multiple origins of shell kinesis

On average 9.30 shell kinesis absent-to-present transitions arose in *Pelusios*, *Lissemys*, Kinosternidae, *Emys*, *Terrapene*, *Kinixys*, *Cyclemys*, and *Cuora* (95% HPD = 8-11; 1,000 simulations) (Fig. 1d; Fig. S1). In contrast, only 0.77 present-to-absent transitions were indicated (95% HPD = 0-3) (Fig. S1). The presence of shell kinesis was positively correlated with habitat terrestriality in a model that included semiaquatic *Kinosternon* as terrestrial (Pagel’s correlation test: $P = 0.0009$, likelihood ratio = 18.6), otherwise the correlation was not supported ($P = 0.324$; likelihood ratio = 4.66) (Fig. 1d). Further, species with shell kinesis were smaller (CL: 206.5 mm ± 9.07 SE, $N = 68$) than akinetic-shelled species (CL: 441.8 mm ± 23.7 SE, $N = 223$) (pANOVA: $F = 63.4$, $P = 0.008$; permutations = 1,000) (Fig. 1d-e).

(b) Repatterning of shell tissue

By hatching, a plastral hinge is absent in lineages representing diverse types of plastral kinesis (Emydidae, Kinosternidae, Pelomedusidae; Fig. S2). In *T. ornata*, a proper hyoplastral-hypoplastral bone suture forms by 3 yr post-hatching in the location of the
incipient hinge joint (Fig. 2a-b). However, this suture is progressively repatterned as the plastral hinge becomes functional 3-5 yr post-hatching: interdigitating bony processes are reduced and replaced with fibrous connective tissue (Fig. 2c-e; Movie S1).

In adult *T. ornata*, reduced plastral buttresses permit movement of the anterior and posterior plastral lobes. In *E. blandingii*, plastral buttresses are partially reduced and thus kinesis is limited to the anterior lobe. By contrast, adult akinetic-shelled *C. picta* features fully formed buttresses. This trend is mirrored in hatchlings, though buttress reduction occurs via bone remodeling and connective tissue forms as kinetic-shelled *T. ornata* grows (Fig. 3a-d). Hinge tissue is highly fibrous, collagen-rich, and covered by a cornified tissue layer (Fig. 3e).

(c) Shell growth and shape

Plastron growth rates varied among species (CL x species interaction: $F_{2, 1953} = 74, P < 0.0001$). *Terrapene ornata* exhibited the highest plastron growth rate (slope = 1.04, 95% CI = 1.02-1.05; $N = 221$), resulting in a 1:1 PL/CL ratio in adults (1.00 ± 0.004 SE; $N = 156$) (Fig. 4a-b). Kinetic-shelled *E. blandingii* featured a similar growth rate (slope = 0.94, 95% CI = 0.93-0.95; $N = 136$) as akinetic-shelled *C. picta* (slope = 0.95, 95% CI = 0.94-0.95; $N = 1,602$), but the adult PL/CL ratio (0.94 ± 0.001 SE; $N = 1,365$) was higher than in *C. picta* (0.92 ± 0.009 SE; $N = 70$) (Tukey HSD test: $P = 0.0002$).

Plastron shape shifted with PL during post-hatching growth (ANOVA: $F_{1, 58} = 336, P < 0.0001$), though in a species-specific manner (PL x species: $F_{2, 58} = 18.6, P < 0.0001$) (Fig. 4c). In *T. ornata* ($N = 15$), plastron shape deformation was greater than in *E. blandingii* (Tukey HSD test: $P < 0.0001; N = 24$) and *C. picta* (Tukey HSD test: $P = 0.001; N = 26$). The plastron in *E. blandingii* and *C. picta* displayed similar patterns of moderate shape deformation (Fig. 4d-e). By contrast, *T. ornata* exhibited extensive shape deformation related to a broadening of the anterior plastral lobe (Fig. 4e).
4. Discussion

How the turtle’s shell is patterned in developing embryos has recently come to light [64, 65], yet many ecologically relevant shell phenotypes emerge long after development in the egg is over [66, 67]. Perhaps this is not surprising as shell development is incomplete in hatchling turtles [68-70]. Nonetheless, documenting progressive changes in the skeletal architecture of such long-lived organisms is challenging. Here, by examining both museum specimens and live-trapped turtles in the wild, we link the convergent evolution of shell kinesis with developmental processes that span both embryonic and post-embryonic life stages.

(a) At least eight independent origins of shell kinesis in extant turtles

Shell kinesis has evolved repeatedly across many fossil and extant turtles since the early Cretaceous, ca. 112 Ma ago [15, 21-29]. Our analyses initially indicated that this complex trait arose independently nine times in extant turtles. However, a more conservative interpretation is that it arose at least eight times. Kinesis is a classic diagnostic feature of Cyclemys (Geoemydidae) [39], though the phylogeny of Pereira et al. [47] proposes that C. fusca shares a recent common ancestor with akinetic Heosemys and Notochelys. This arrangement would have increased absent-to-present (shell kinesis) transitions by one. Future studies are needed to resolve this discrepancy, but also to clarify the likelihood of reversals to akinesis in geoemydids and emydids. These clades diversified over the last 20 million years and might be predisposed to develop kinesis [71, 72], thus reversals cannot be entirely ruled out [73]. Even so, present-to-absent transitions occurred on average less than once in our character state simulations, suggesting that reversals are rare.
Is shell kinesis a common solution to a common ecological problem? Turtles are notably susceptible to predator attacks during juvenile life stages [31, 74], particularly in terrestrial habitats [30]. Thus, predator-driven selection may favor the evolution of shell morphologies that enhance survival [75]. Indeed, kinetic-shelled turtles frequently occupy terrestrial habitats, where the majority of their predators reside, for purposes other than egg laying [37, 50-52, 76, 77]. Although our phylogenetic correlations did not strongly support this well-known trend, we demonstrated that kinetic-shelled species tend to be smaller. These results are congruent with the complex co-evolutionary history of shell morphology and habitat preference in turtles [15, 34, 44, 78, 79]. For instance, some species retained shell kinesis after undergoing terrestrial-to-aquatic reversals [80], and the adaptive value of kinesis may not be entirely related to shell closure in highly aquatic lineages with reduced plastron size, e.g. *Sternotherus* [81]. Furthermore, the degree of shell closure enabled by kinesis might be associated with the extent to which terrestrial habitats are used [23]. A promising approach to disentangling these intriguing relationships is to integrate ecological, developmental, and phylogenetic studies across diverse kinetic-shelled turtles.

(b) Gradual tissue repatterning underpins the convergent evolution of shell kinesis

The iconic ‘hinge’ joint that defines the anatomy of shell kinesis develops gradually [37-39]. Based on previous observations [23, 40, 41], we expected this extraordinary transformation to unfold via two plausible sequences of events, summarized as: (i) a ‘heterochronic’ model of delayed sutural fusion followed by elastic tissue formation in the incipient hinge region or (ii) a ‘repatterning’ model predicting normal sutural fusion followed by reorganization with elastic tissue formation. Our histological and morphometric analyses were in agreement with this ‘repatterning’ scenario.
Ectodermal plates (i.e. scutes) that cover the shell must align with bone sutures to permit hinge movement. Because this key configuration was absent in hatchlings, scutes must undergo positional rearrangement as turtles grow. We showed that abdominal-pectoral scute boundaries aligned with the hypoplastral-hyoplastral suture as kinesis was activated in juvenile (~ 3 yr) box turtles (*T. ornata*). Though partially fused, this transient hinge suture was highly flexible. Flexibility increased as the suture was broken down and filled with dense collagen fiber (> 5 yr), corroborating the hypothesis that mechanical strain exerted by muscles during shell closure prevents sutural fusion and leads to the emergence of elastic hinge tissue [23, 41]. Consistent with comparisons of hatchling and adult emydids [43], we demonstrated morphological variation related to hinge differentiation in *T. ornata*: the plastron underwent substantial shape deformation, plastron length increased disproportionately, and bony buttresses that structurally bridge the plastron and carapace were repatterned. These changes were less extensive in *E. blandingii*, consistent with its less derived form of kinesis. Still, buttresses were also reduced and lined with elastic tissue. Akinetic-shelled *C. picta* did not exhibit shell tissue repatterning.

Shell repatterning in emydid turtles may be generalized to other kinetic-shelled species, particularly geoemydids that also feature a hypoplastral-hyoplastral hinge, i.e. *Cyclemys* and *Cuora* [26]. The hinge is situated distal to the shell buttresses of all other species. Thus, repatterning is likely confined to the developing hinge region of *Kinosternon* and *Pelusios*, similarly to lineages that lack scutes (*Lissemys*) or that feature carapacial kinesis (*Kinixys*). Despite structural shell differences, cellular mechanisms that govern sutural fusion and elastic tissue production are probably common to all turtles, as they are shared by all vertebrates [82]. That such mechanisms are employed gradually in ontogeny and long after embryo life stages is noteworthy.
(c) Hinge development is a delayed response to structural alterations in embryos

Fibrous joint development often requires mechanical strain provided by skeletal muscle contraction [82, 83]. In kinetic-shelled turtles, novel neck-shoulder-plastron muscle connections established during embryo life stages exert strain on the developing plastron [23-25]. Hence, hinge differentiation in juveniles is a delayed response to embryonic alterations that transform the shell tissue microenvironment. Remarkably, such musculoskeletal mechanical linkages that enable the development and function of kinesis vary in a lineage-specific manner, rendering the convergent evolution of shell kinesis a fascinating example of many-to-one mapping, i.e. similar function achieved by diverse structures [84]. Similarly, cranial sutures respond to mechanical cues from diverse musculoskeletal sources [85], potentially explaining the convergence of cranial kinesis in a wide variety of vertebrates [86]. Thus, delayed trait differentiation via strain-induced repatterning is crucial to phenotypic diversification.

(d) Evo-devo implications of delayed trait ‘acquisition’ via tissue repatterning

Kinetic shell hinges are not merely ‘acquired’, at least not in a Lamarckian sense [87], by slowly-growing turtles. Instead, delayed trait differentiation is a function-induced developmental transformation (e.g. [88]), which initially requires heritable genetic change in embryonic traits. Crucially, form-to-function effects on the shell will only manifest as individuals mature. This key developmental process promotes novel phenotypic variants that may otherwise not arise in embryogenesis owing to developmental constraints. Although these plastic properties of developing skeletal tissue were first described in the 19th century [89], how they facilitate macroevolutionary change has only been emphasized in recent decades [90-94]. Likewise, our study highlights that the genotype-to-phenotype translation is
not always a one-to-one relationship, as it may often involve tinkering of intricately interrelated traits over the course of multiple life stages.

**Ethics.** Procedures were approved by the ISU Institutional Animal Care and Use committee (protocol # 2-11-7091-J).

**Data accessibility.** Data are available as electronic supplementary material.

**Competing interests.** We declare none.

**Authors’ contributions.** G.A.C. designed the study, wrote the paper, and performed phylogenetic analyses; G.A.C. and K.Q. conducted histological assays and morphometric analyses; G.A.C. and F.J. conducted field sampling; F.J. provided logistical support and advice.

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Figure Legends

Figure 1. Shell kinesis via hinge rotation in box turtles (illustration credit: Jessica Gassman; a). Pectoral (Pec) and abdominal (Ab) ectodermal scutes are aligned with the suture of hyoplastron (Hyo) hypoplastron (Hyp) bones (b; modified from [95]), unlike in akinetic-shelled species (b; modified from [96]). Kinesis enhances predator defense on land: an eastern box turtle (Terrapene carolina) survives an attack by activating its plastral hinge (c; from Life in Cold Blood with permission from the British Broadcasting Company). Shell kinesis (absent = blue; present = red) has evolved at least 8 times, probably in conjunction with evolutionary transitions from aquatic (cyan) to terrestrial (yellow) habitats in small-bodied turtles (CL = carapace length in mm) (d-e); time-calibrated phylogeny from [47].

Figure 2. The immature junction (inlet: H & E sagittal section) of the pectoral (Pec) and abdominal (Ab) scutes in hatchling Terrapene ornata does not feature a plastral hinge (a). Ossification centers of the hyoplastral (Hyo) and hypoplastral (Hyp) bones (in alizarin red) expand and begin to fuse near the Pec-Ab scute junction a year after hatching in T. ornata (b). These bones are sutured three years post-hatching (e). By year five, the suture is re-patterned and replaced by fibrous connective tissue, particularly on the external surface of the plastron (d). Fibrous connective tissue proliferates externally and the serrated edges on the interior plastral surface are further reduced in fully-grown adults (e). Silhouettes are based on images from [77].

Figure 3. Plastral buttresses (axillary = Ax; inguinal = In) are fully developed in Chrysemys picta (a), partially reduced in Emys blandingii (b), and fully reduced in Terrapene ornata (c). This key difference is pre-patterned in hatchlings (left panel) and corresponds to akinesis in C. picta, as well as varying degrees of kinesis (arrows) in adult E. blandingii versus T. ornata
Progressive breakdown of bone and scute tissue and replacement with fibrous connective tissue near the axillary plastral buttress in growing *T. ornata* (d). The hyoplastral-hypoplastral suture (VVG sagittal section) displays an interdigitating pattern in akinetic turtles (left: *Glyptemys insculpta*; scale bar = 10 µm), in contrast to the cornified (yellow) and collagen-rich (red) fibrous connective tissue comprising the hinge of kinetic-shelled species (right: *T. ornata*; scale bar = 50 µm) (e).

**Figure 4.** Scaling of the plastron during embryonic and post-hatching stages varied among species (a), as *T. ornata* (TO) displayed a higher growth rate and thus attained a larger adult plastron-to-carapace length ratio compared to *C. picta* (CP) and *E. blandingii* (EB) (b). Plastron shape deformation during post-hatching growth was also greater in *T. ornata* (c). A principal component (PC) plot supports that *T. ornata* undergoes substantial shape deformation (d), as the anterior plastral lobe broadens during post-hatching growth (e).
Figure 2

a  Hatchling

b  1 year

C

Hyo

Hyp

3 years

D

Hyo

Hyp

5 years

E

Hyo

Hyp

External

Internal

20+ years
Figure 4

(a) Log plastron length vs. Log carapace length for C. picta, E. blandingii, and T. ornata.

(b) Plastron-carapace ratio for CP, EB, and TO species.

(c) Plastron shape vs. Log plastron length for CP, EB, and TO species.

(d) PC1 vs. PC2 scatter plot for CP, EB, and TO species.

(e) Anterior and Posterior views of C. picta, E. blandingii, and T. ornata.
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1. Supplementary materials and methods

(a) Character state data

Character data (Fig. S1; Table S1) were obtained from anatomical appraisals of shell kinesis [1-4]. Putative modes of shell kinesis not associated with musculoskeletal mechanical linkages that enable shell closure were excluded, for example shell kinesis related to egg-laying in females [5]. Habitat type data for 262 species were obtained from the comprehensive review of Mota-Rodrigues and Felizola Diniz-Filho [6], which followed a binary (aquatic versus terrestrial) classification scheme [7]. Habitat type data for an additional 30 species was obtained from other sources [8-19]. Maximum carapace length (CL) was obtained for 277 species from the most recent global analysis of turtle body size evolution [20]. Additional data for 11 species were obtained from other reviews [21, 22], as well as the primary literature for three species [23-25].

(b) Museum specimens used in this study

Hatchlings of Pelusios castaneus were purchased from a private breeder, otherwise all other museum-preserved hatchlings (see Fig. S2) originated from eggs collected in the wild and incubated in moist vermiculite (-150 kPa water potential) in an environmental chamber set to a constant 27 °C or 30 °C [see ref. 26]. We measured embryos of Chrysemys picta (N = 74), Emys blandingii (N = 43), and Terrapene ornata (N = 38) also previously sampled by Cordero and Quinteros [26]. In addition, hatchlings from their study were measured: Chrysemys picta (N = 33), Emys blandingii (N = 16), and Terrapene ornata (N = 6).

We examined adults specimens of E. blandingii, Glyptemys insculpta, and T. carolina donated to the Iowa State University herpetological collection by the Iowa Department of Natural Resources. Fluid-preserved specimens of T. ornata and C. picta collected in Thomson, Illinois and deposited in this collection were also examined. In addition, we sampled road-killed T. ornata collected in Grant and Hooker counties, Nebraska. Specimens of juvenile T. ornata from eastern Iowa were obtained from the teaching collection of Cornell College (Mount Vernon, Iowa). We estimated the age of juvenile T. ornata by counting primary growth rings, following the technique of Legler [27].

Sampling was conducted with permits from: Arkansas Game & Fish Commission #020520132; Illinois Dept. of Natural Resources #NH13.0073; Iowa Dept. of Natural Resources #14; Nebraska Game & Parks Commission #310. Representative specimens examined in this study were transferred to the herpetological collections of the University of Kansas and Carnegie Museum of Natural History.
2. Supplementary results

Figure S1.
Shell kinesis has evolved at least eight times in extant turtle lineages, as supported by stochastic character mapping on the time-calibrated phylogeny of Pereira et al. [28]. Shell kinesis: blue = absent; red = present. Posterior probabilities (colour gradients) were estimated on 1,000 stochastic character maps. Ancestral state reconstructions followed an equal rates model (Markov k-state 1) of state transitions (rate = 0.001).
Figure S2. Plastron development is incomplete in hatchling turtles, such that some akinetic (a = Chrysemys picta; b = Chelydra serpentina) and kinetic-shelled species feature similar ossification patterns (alizarin red stain; scale bars = 2 mm). Locations of incipient plastral hinges (arrows) may vary according to lineage: Hyoplastron-hypoplastron (Hyo-Hyp) suture in emydids *Emys blandingii* (c) and *Terrapene ornata* (d); two hinges in Kinosternidae (e; *Kinosternon subrubrum*) will differentiate at the Epi (epiplastron)-Hyo and Hyp-Xip (xiphiplastron) sutures; in Pelomedusidae (f; *Pelusios castaneus*) a hinge eventually differentiates at the Hyo-mesoplastron (Me) suture.
3. Supplementary references


