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A geometric morphometric assessment of the effects of environment and cladogenesis on the evolution of the turtle shell

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In the largest group of extant turtles, the Testudinoidea, the acquisition of an aquatic or terrestrial way of life has occurred within two clades, allowing the study of homoplasy linked to environment (commonly named convergence). Here we appraise the respective importance of two sources of morphological variation: a major cladogenetic event and a major environmental shift (aquatic vs. terrestrial). The repeatability of the same evolutionary process (environmental change) allows an assessment of the weights of both natural selection and phylogenetic constraints on several morphological features of the shell. These sources of morphological variance on the complex shell structure were studied using geometric morphometrics. We depict the morphological variation of three parts of the turtle shell: epidermal carapace, bony carapace, and plastron. In the three structures, we found that both phylogeny and environment were significant sources of morphological variation, and geometric morphometrics allowed the pattern of morphological variation due to each effect to be assessed. The assessment of the homoplasy due to environment and of the pattern of morphological variability suggests that the carapace has undergone similar morphological changes between aquatic and terrestrial environments within the two clades. The radiation of the Testudinoidea is interpreted as an adaptive radiation.

ADDITIONAL KEYWORDS: adaptive radiation – geometric morphometrics – morphological evolution – natural selection – phyletic constraints – Testudinoidea – turtles.

INTRODUCTION

Although the phenotype of a given species results from selective processes, it is also shaped by a set of constraints best viewed from the context of its phylogenetic history (Maynard-Smith *et al.*, 1985). Gould & Lewontin (1979) claimed that underestimation of historical, developmental and biomechanical constraints would involve false ideas on the current use of certain features of organisms as well as on the role of selection in their evolution. It may be difficult to categorize the different constraining factors in evolution. Raff (1996) assigned them to three categories: physical, genetic, and phyletic constraints. The third class involves the effects of internal organization and ontogenetic rules that may constrain the evolution of related species in a shared and restricted number of ways. The different elements of organisms do not evolve independently and it has been shown that covariation among characters can bias an evolutionary pathway away from the direction of greatest increase in fitness (Lande & Arnold, 1983; Schluter, 2000). Assessing the respective roles of both phyletic constraints and selective forces on the evolution of multicharacter phenotypes requires taking their covariation into account.

Comparing a set of morphological characters across species is a complex issue in evolutionary biology, and

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several attempts have been made to interpret morphological variation in the simplest way. This may explain the use of certain integrative concepts, such as heterochrony and/or approaches such as geometric morphometrics. Geometric morphometrics allows morphological variation to be depicted in a more realistic and integrative way than do traditional multivariate methods (Rohlf, 2000). Such approaches have recently beeen used to analyze morphological variation and covariation among a large number of species at high taxonomic levels (Schaefer & Lauder, 1996; Marcus, Hingst-Zaher & Zaher, 2000; Marroig & Cheverud, 2001).

The aim of this paper is to assess the respective roles of natural selection and phyletic constraints on the variation of shell morphology in the largest extant group of turtles, the Testudinoidea. As compared to the classic body plan of tetrapods, the shell of turtles represents an evolutionary novelty (Burke, 1989). Turtles share common ontogenetic rules that probably constrain further evolution of this external structure. The Testudinoidea superfamily includes two major and monophyletic clades: the New World Testudinoidea (Emydidae), and the Old World ones (Geoemydidae (or Bataguridae according to some authors) and Testudinidae). Each clade contains both aquatic and terrestrial species (Hirayama, 1984; Gaffney & Meylan, 1988; Lamb & Lydeard, 1994; Bickham *et al.*, 1996; Shaffer, Meylan & Mcknight, 1997; Wu, Zhou & Yang, 1999). The phylogenetic relationships within these clades, especially among geoemydid turtles, are still debated, indicating that homoplasy of morphology or radiation events may impede the reconstruction of the evolutionary history of this group (McCord *et al.,* 2000). Some studies have considered Geoemydidae to be paraphyletic (Hirayama, 1984; Gaffney & Meylan, 1988): Testudinidae were thought to be related to some geoemydid species. However, the recent phylogenetic analysis performed on molecular data by Honda *et al*. (2002), McCord *et al*. (2000) and Shaffer *et al*. (1997) suggested that Testudinidae should be considered as the sister taxa of all other Geoemydidae. This phylogenetic scenario seems not to be refuted by other phylogenetic studies (Wu *et al*., 1999), and we will consider it as a valid hypothesis. For simplicity, we will follow the proposition of Shaffer *et al*. (1997) and refer to Testudinoidae for the clade containing both testudinids and geoemydids (Fig. 1). The systematics of the emydids seems to be more consistent and this group may be divided in two monophyletic clades: Emydinae and Deirochelyinae (Bickham *et al*., 1996) (Fig. 1).

The fossil record attests the appearance of aquatic geoemydid species in the early Eocene with the fossil *Echmatemys* and some European and Asiatic species (Hutchison, 1998; Holroyd & Hutchison, 2000; de Broin, 2001; Holroyd, Hutchison & Strait, 2001). Testudinids appeared at the same time with the terrestrial genus *Hadrianus* (Hutchison, 1998). These fossils demonstrate the existence of two distinct environmental forms in the early Eocene. The first Emydidae, *'Graptemys' inornata,* is reported from the late Eocene (Clark, 1937). However Holroyd *et al*. (2001) state that 'Emydid P first appears shortly after *Echmatemys* [Wasachtian] and appears to be related to Emydidae sensu stricto', which confirmed at least an early Eocene radiation for Emydidae and Geoemydidae + Testudinidae. There is evidence of some terrestrial emydid forms at least from the middle Miocene (with the first occurrence of the genus *Terrapene*), which may indicate that evolution toward terrestriality evolved later in the Emydidae (Holman, 1987). Some extant genera of the Geoemydidae contain both terrestrial and aquatic species (*Cuora, Melanochelys, Heosemys*), indicating that the acquisition of a new environmental way of life has occurred several times in this clade. Similarly the recent molecular phylogenetic analysis of Feldman & Parham (2002) indicates that the acquisition of a terrestrial or aquatic ecology occurred at least twice in the Emydinae. On the other hand, some clades are conservative with respect to their environment: for instance, all Testudinidae species are terrestrial, and Deirochelyinae species are all aquatic. The change of habitat is not irreversible: some aquatic forms, for example *Terrapene cohauila*, are thought to have a terrestrial ancestor (Minx, 1996) (although Feldman & Parham (2002) give an alternative interpretation). The opposite is the case in several genera such as *Melanochelys* or *Cuora* (Hirayama, 1984; Gaffney & Meylan, 1988; Wu, Zhou & Yang, 1998; Lenk, Fritz & Wink, 1999; Wu *et al*., 1999; McCord *et al*., 2000; Honda *et al*., 2002).

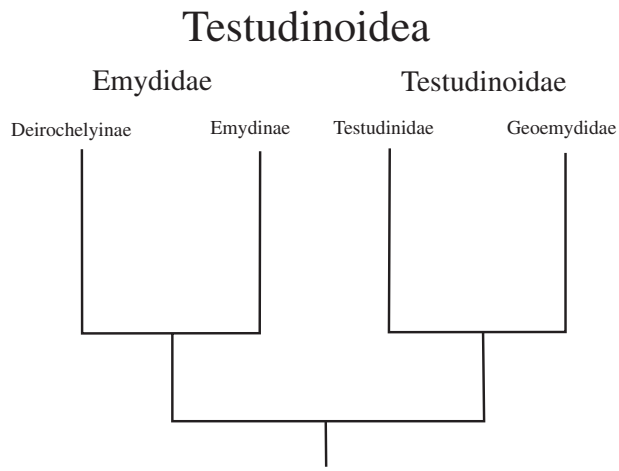

Figure 1. General phylogenetic relationships among the Testudinoidea.

At least on the shell, the discrete morphological characters used for phylogenetic reconstruction seem to be highly liable to homoplasy, precluding the diagnosis of the two clades on the basis of these characters (McDowell, 1964; Hirayama, 1984). This may explain why morphological phylogenies are far from being congruent with molecular phylogenetic reconstructions (Hirayama, 1984; Gaffney & Meylan, 1988; Wu *et al*., 1998; Lenk *et al*., 1999; Wu *et al*., 1999; McCord *et al*., 2000; Honda *et al*., 2002; Van-der-Kuyl *et al.*, 2002). Although the idea has been formulated that the shell shape displays convergent evolution and is strongly related to environment (Romer, 1967), this hypothesis has been neither demonstrated nor clearly refuted (Schubert-Sondern, 1962; Staesche, 1964).

Nevertheless, for several reasons the shell of turtles is a reliable structure for studying the morphological evolution of multiple characters: homology between the elements of the shell is easy to identify in a given taxonomic group. Since the number of epidermal scutes and bony plates has remained constant during the evolution of the Testudinoidea, the shell provides numerous landmarks for depicting morphological variation in a realistic way. As reported above, a change in main habitat (aquatic vs. terrestrial) has occurred several times within the two main clades of the Testudinoidea. Thus, this group constitutes a relevant frame which may allow the appraisal of selection and phylogenetic constraints acting on the shell shape. By focusing on both a major cladogenetic event and main environmental shifts, we address the question of the relative efficiency of phyletic constraints related to each clade, and of selective pressures related to the two environments, to account for the morphological variation within this group. Moreover, the use of an integrative morphometric method allowed us to determine whether the effects of each source of variation (cladogenesis vs. environment) concerned similar features of the shell, and whether the morphological changes between environments were similar among clades. These methods allowed us to decompose the shape variability associated with each effect, and allowed a rigorous assessment of the morphological patterns associated with cladogenesis and environmental shift. Finally, our design also allowed us to test the classic assumption that environmental shift potentially generates similar morphologies (homoplasy) among clades.

MATERIAL AND METHODS

All specimens used in this analysis were adults without any detectable abnormality (such as injuries by predators, or unusual additional scutes or plates). On these animals, the three-dimensional morphometric approach was applied to three different structures: epidermal carapace, bony carapace and plastron. These structures were studied independently for practical and statistical reasons.

For the study of epidermal and bony carapaces, 250 individuals of both sexes belonging to 117 species were measured, representing 85% of the specific diversity of the Testudinoidea. The Testudinoidae were represented by 44 aquatic species and 43 terrestrial ones. Among the Emydidae, 27 were aquatic and three were terrestrial. The study of the plastron was conducted on 263 individuals of both sexes belonging to 120 species, covering 87% of the total number of species of the Testudinoidea. Testudinoidae and emydids were represented, respectively, by 43 and 29 aquatic species, and 45 and 3 terrestrial ones.

Most of the missing taxa were very rare species. The list of measured individuals is given in the Appendix.

Superimposition procedure

Forty-six landmarks were digitized on the whole bony carapace, 32 on the epidermal carapace, and 42 on the plastron (Fig. 2). These landmarks were digitized in three dimensions using the MicroScribe-3D digitizing system (version 3D; accuracy: 0.38 mm). These landmarks correspond to the intersections between bony plates or epidermal scutes, and thus are of type 1 or 2 according to Bookstein's nomenclature (Bookstein, 1991). No landmarks corresponded to the interneural plate sutures (the number and position of neural plates are often variable within a species (Staesche, 1961; Pritchard, 1988)).

For the plastron, some type 3 landmarks were added to the types 1 and 2 landmarks: both axillar and inguinal notches, and xiphiplastral extremities. All sets of landmarks, also called configurations (two replicates for each individual) were superimposed (scaled, translated, and rotated) following the Procrustes method of generalized least squares superimposition (GLS) (details of this method are given by Rohlf (1990) and Bookstein (1991)). Like most geometric morphometric methods, the Procrustes procedure allows treatment of size and shape as two independent components. Since the Procrustes distance between two individuals may be defined by an angle (in the so-called Kendall shape space), it is not strictly similar to the Euclidean distance obtained by the projection of individuals on the Kendall tangent space. The GLS was performed with the TPSSMALL software (Rohlf, 1998b) which appraises the correlation between the Procrustes and the Kendall tangent space distances. It is required to assess this correlation to ensure that the amount of shape variation in a data set is small enough to permit statistical analyses to be performed in the linear tangent space, approximating the Kendall shape space which is non-linear (see Rohlf

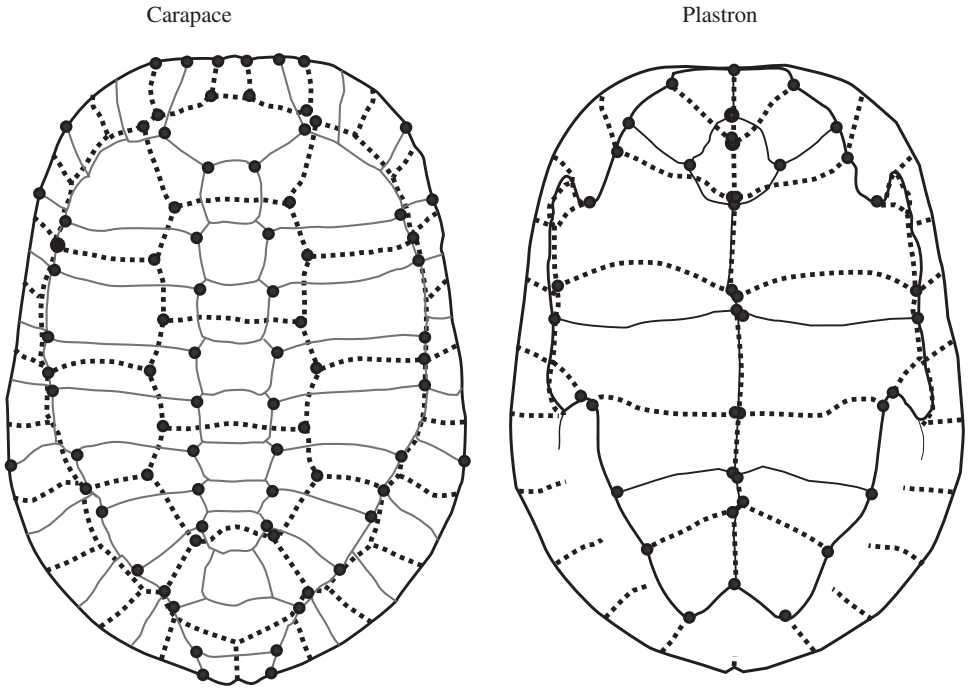

Figure 2. Locations of landmarks on the carapace in the dorsal view and on the plastron in the ventral view. Dotted lines indicates groove sulci of epidermal scutes, and sutures between bony plates are indicated by solid lines. This specimen belongs to *Mauremys leprosa*, an aquatic Geoemydid.

(1998a), Rohlf (1998b) and Marcus *et al*. (2000) for further details). This correlation was almost perfect for the three structures ($r > 0.99$ in all three cases). The coordinates of the newly superimposed configurations were then considered as raw data for further statistical analyses. Only the coordinates on the right hand side were taken into account for further analyses in order to reduce redundancy in variables. This method took into account all morphological variability except antisymmetry, directional and fluctuating asymmetries which were not thought to occur or be prevalent in our data. Size was assessed by the centroid size, which is defined as the square root of the sum of squared distances from each landmark to the centroid of the configuration of landmarks for a specimen (Bookstein, 1991).

Statistical procedures

Further statistical procedures were performed with R 1.3.0 (Ihaka & Gentelman, 1996). For each structure, the size measurement error was estimated using one-way analysis of variance (ANOVA) of the centroid size of all replicates, considering individuals as the source of variation. The shape measurement error was estimated from all replicates with one-way Procrustes ANOVAs considering individuals as the source of variation. This type of analysis was proposed by Klingenberg & McIntyre (1998). It implies that the mean squares were calculated from the sum of the sums of squares of each coordinate divided by the relevant degrees of freedom (d.f.) for each effect (i.e. the conventional d.f. multiplied by the number of coordinates minus 7 d.f.). The percentage of measurement error was computed following Yezerinac, Lougheed & Handford (1992).

As interindividual variation (including the potential effect of sexual dimorphism) may be high in turtle shell, we checked intraspecific variation of both size and shape. The size intraspecific variation was compared with the size interspecific variation using a one-way ANOVA taking the factor species into account. The shape intraspecific variation was compared with interspecific variation with a one-way multivariate analysis of variance (MANOVA) on the coordinates of individuals, taking the factor species into account. For a given species, a consensus (mean configuration) was computed from the superimposed replicates of individuals belonging to this species. The interspecific morphological variability was quantified with a principal component analysis (PCA) on the coordinates of the consensus configurations. The PCA was performed on the variance-covariance (VCV) matrix of the species consensus coordinates. This PCA allowed us to take

into account the correlation among coordinates of landmarks, which may be used to depict morphological variability as landmark displacements. Since the correlation between size and shape may bias evolutionary interpretations (by evolutionary allometry, (Klingenberg, 1996)) the relationship between centroid size and morphological variation was tested with a multiple regression of the consensus configurations coordinates on centroid size.

As mentioned above, the phylogenetic features, i.e. branch lengths, and phylogenetic position of taxa within the clades considered here are poorly known and still under debate (Hirayama, 1984; Gaffney & Meylan, 1988; Bickham *et al*., 1996; Wu *et al*., 1998; Lenk *et al*., 1999; Wu *et al*., 1999; McCord *et al*., 2000; Feldman & Parham, 2002). It is thus impossible to use current phylogenetic comparative methods to analyse our data (Felsenstein, 1985; Harvey & Pagel, 1991; Donoghue & Ackerly, 1996; Martins, 2000). Our purpose here is to compare environmental and phylogenetic constraints among two clades, rather than to assess the correlation between shape and environment at the interspecific level. We used ANOVA methods taking into account variation between groups of species, comparable to those dealing with convergence in ecological communities (Schluter, 1986; Schluter & Ricklefs, 1993).

In order to assess the effects of cladogenesis and environment on the morphology, we used a two-way ANOVA for size and a MANOVA involving environment and clade as sources of variation. The configurations (coordinates of landmarks) were taken as the multiple responses in the MANOVAs. As our goal was not to appraise every characteristic of the ecology for each species (which may be difficult as the ecological characteristics of many species need more studies), the ecological context was chosen to assess a main environmental shift (aquatic vs. terrestrial). Since most of the Testudinoidea are able to both swim and walk, the modalities defined for environmental effect corresponded to the preferred habitat as defined for each species in the literature (Bourret, 1941; Pritchard, 1979; Ernst & Barbour, 1989; Ernst, Lovich & Barbour, 1994; Bonin, Devaux & Dupré, 1996). Attribution of modality was also based on development of webbing between toes and presence or absence of cloacal bursae, possibly used as balasts for hydrostatism in most aquatic testudinoids (Jackson, 1969).

Following the recommendation of Venables (1998), we chose a sequential ANOVA model rather one based on type III sums of squares (see also Nelder, 1999). In the sequential ANOVA model, the second factor, the interaction and the error were computed after having taken into account the first one. In our case, environment and clade effects were alternatively considered as the first or second factor. In order to test for the significance of each factor, only the second factor was tested, the interaction and error estimation being of course independent from the order of entry of the factors. It must be kept in mind that cladogenesis may be associated with shift in environment; the procedure described above assesses the phylogenetic constraint as the variance explained by clade, removing any effect of environment, and it assesses the environmental effect as the variance explained by environment, removing the effect of the main cladogenesis between Emydidae and Testudinoidae.

The morphological variance associated with each effect was depicted using the eigenvectors of their appropriate VCV matrices.

The interaction term can be interpreted as follows: the greater the interaction term, the more different the morphological changes between environments within each clade. If the interaction between environment and clade is not a significant source of variation, but environment is, we may conclude that similar morphological transformations occurred in the two clades.

Homoplasy due to environment may hide the effects of cladogenetic events on complex structures, in the following scenario: first, selection may act on the characters which allow discrimination between the clades; second, the morphological variance due to environmental shift may exceed the interclade morphological variance. In such cases, it should be evaluated whether the environmental shift has produced either convergent or parallel evolution. We tested whether cladogenesis and environmental shift acted similarly on the shape variation using a Monte Carlo procedure (Klingenberg & McIntyre, 1998; Debat *et al.*, 2000). We first computed four consensus mean shapes for each environment within each clade; we then computed the mean consensus shape for each clade across environments, and the same for each environment across each clade. We computed the VCV matrix between the mean Emydidae and the mean Testudinoidae values, and the VCV matrix between mean aquatic and terrestrial forms. The single eigenvectors of these two VCV matrices were extracted and the angles between them calculated with their cosine. This angle was compared to a random distribution of angles formed by pairs of random vectors (using a Monte Carlo procedure with 10 000 replicates).

In order to assess the relative importance of environment and cladogenesis, we compared the variance due to environment and the variance due to clade, using results from paired sequential ANOVA or MANOVA. The variation along each effect was estimated as the sum of variance of each coordinate, which is comparable to a Procrustes ANOVA (see above).

The variance for each combination of environment and clade was assessed as the sum of the sums of

squares for each landmark divided by the d.f.s of the Procrustes ANOVA. The significance of the difference in variance between environments and clades was tested with an *F*-test (the d.f.s being calculated as in the Procrustes ANOVA). This last procedure allowed testing of whether the difference between environments or between clades may change the magnitude of morphological variation.

RESULTS

Concerning size, the measurement error did not contribute to more than 0.01% of the total variance for the three structures considered. For shape, the measurement error was larger but still negligible, being not greater than 1.51% for any of the three structures. Size and shape intraspecific variation (Tables 1, 2) were significantly lower than was interspecific variation, indicating that sexual dimorphism or other sources of intraspecific variability did not impede further investigations.

Size was significantly correlated with shape, suggesting an evolutionary allometry (bony carapace: multiple $R^2 = 0.80$, $F_{[69,47]} = 2.80$, $P < 0.001$; epidermal carapace: multiple $R^2 = 0.67$, $F_{[48,68]} = 2.91$, $P < 0.001$; plastron: multiple $R^2 = 0.82$, $F_{[69,50]} = 3.28$, $P < 0.001$).

There was no significant difference in mean size between environmental groups and between clades (Table 3). This means that there was no pattern of size convergence associated with environments between Emydidae and Testudinoidae.

For all the skeletal and epidermal structures considered, both cladogenesis and environmental shift were highly significant sources of morphological variation (Table 4). The effect of environment on the mean morphologies was illustrated by the species plots of the first principal component (PC) (which explained 34% of the total variance) for the bony carapace

Table 1. Intraspecific and interspecific variation in size

	d.f.	Mean squares	*F*	*P*
Bony carapace				
Species	116	65375.2	9.38	$<10^{-15}$
Residuals (intraspecific)	134	6966.5		
Epidermal carapace				
Species	116	38695.2	9.04	$<10^{-15}$
Residuals (intraspecific)	134	4278.8		
Plastron				
Species	120	46973.2	9.28	$<10^{-15}$
Residuals (intraspecific)	135	5056.8		

Size intra- and interspecific variation were compared using a one-way analysis of variance (ANOVA) taking the factor species into account.

Table 2. Intraspecific and interspecific variation in shape

	d.f.	Wilks λ	*F*	d.f. num	d.f. den	*P*
Bony carapace						
Species	116	$<10^{-40}$	2.52	7935	5353.6	$<10^{-15}$
Residuals	134					
Epidermal carapace						
Species	116	$<10^{-29}$	2.98	5520	4684	$<10^{-15}$
Residuals	134					
Plastron						
Species	120	$<10^{-41}$	1.82	8211	5895.1	$<10^{-15}$
Residuals	139					

Shape intra- and interspecific variation were compared with a one-way multivariate analysis of variance (MANOVA) taking the factor species into account. num = numerator, den = denominator.

Table 3. Sequential two-way analysis of variance (ANOVA) of interspecific size variation

Structure/effect	Mean squares	d.f.	*F*	*P*
Bony carapace				
Environment (2nd factor)	10 784	1	0.324	0.571
Clade (2nd factor)	44 458	1	1.334	0.251
Clade × environment	14 110	1	0.423	0.517
Residuals	33 330	113		
Epidermal carapace				
Environment (2nd factor)	10 129	1	0.513	0.475
Clade (2nd factor)	22 497	1	0.139	0.288
Clade × environment	7 492	1	0.379	0.539
Residuals	19 742	113		
Plastron				
Environment (2nd factor)	8 797	1	0.379	0.539
Clade (2nd factor)	12 188	1	0.526	0.470
Clade × environment	24 913	1	1.075	0.302
Residuals	23 174	116		

Table 4. Sequential two-way mutivariate analysis of variance (MANOVA) and Procrustes analysis of variance (ANOVA) of interspecific shape variation

	MANOVA					Procrustes ANOVA	
Structure/effect	Wilks λ	*F*	d.f. num	d.f. den	Pr (>*F*)	d.f.	Mean squares
Bony carapace							
Environment (2nd factor)	0.0767	7.85	69	45	<0.0001	62	1.16×10^{-3}
Clade (2nd factor)	0.0529	11.68	69	45	<0.0001	62	4.83×10^{-4}
Clade × environment	0.3409	1.26	69	45	0.205	62	1.13×10^{-4}
Residuals						7006	5.67×10^{-5}
Epidermal carapace							
Environment (2nd factor)	0.1620	7.11	48	66	<0.0001	41	1.24×10^{-3}
Clade (2nd factor)	0.1736	6.54	48	66	<0.0001	41	5.40×10^{-4}
Clade × environment	0.5051	1.35	48	66	0.130	41	1.29×10^{-4}
Residuals						4633	8.28×10^{-5}
Plastron							
Environment (2nd factor)	0.0880	7.21	69	48	<0.0001	62	1.06×10^{-3}
Clade (2nd factor)	0.0666	9.74	69	48	<0.0001	62	8.40×10^{-4}
Clade × environment	0.2871	1.72	69	48	0.023	62	5.00×10^{-4}
Residuals						7192	7.20×10^{-4}

(Fig. 3), the first PC (25%) for the epidermal carapace (Fig. 4), and the second PC (16%) for the plastron (Fig. 5).

The morphological differences between environments involved, in terrestrial forms: for the plastron, a shortening and a widening of the plastron, and longer abdominal scutes; for the carapace, a general doming of the carapace, with the fifth vertebral scute, pygal and nuchal plates having more internal bending, correlated with a shortening of the carapace, longer first marginal scutes, larger vertebral scutes, the impair costal tended to be shorter on the periphery providing an alternative pattern of costal plates, and a backward migration of the centre of gravity, with the highest point of the shell in the middle (Fig. 6).

The differences between clades were easily detectable by the examination of the species plots on the second PC (17% of the total variance) for the bony

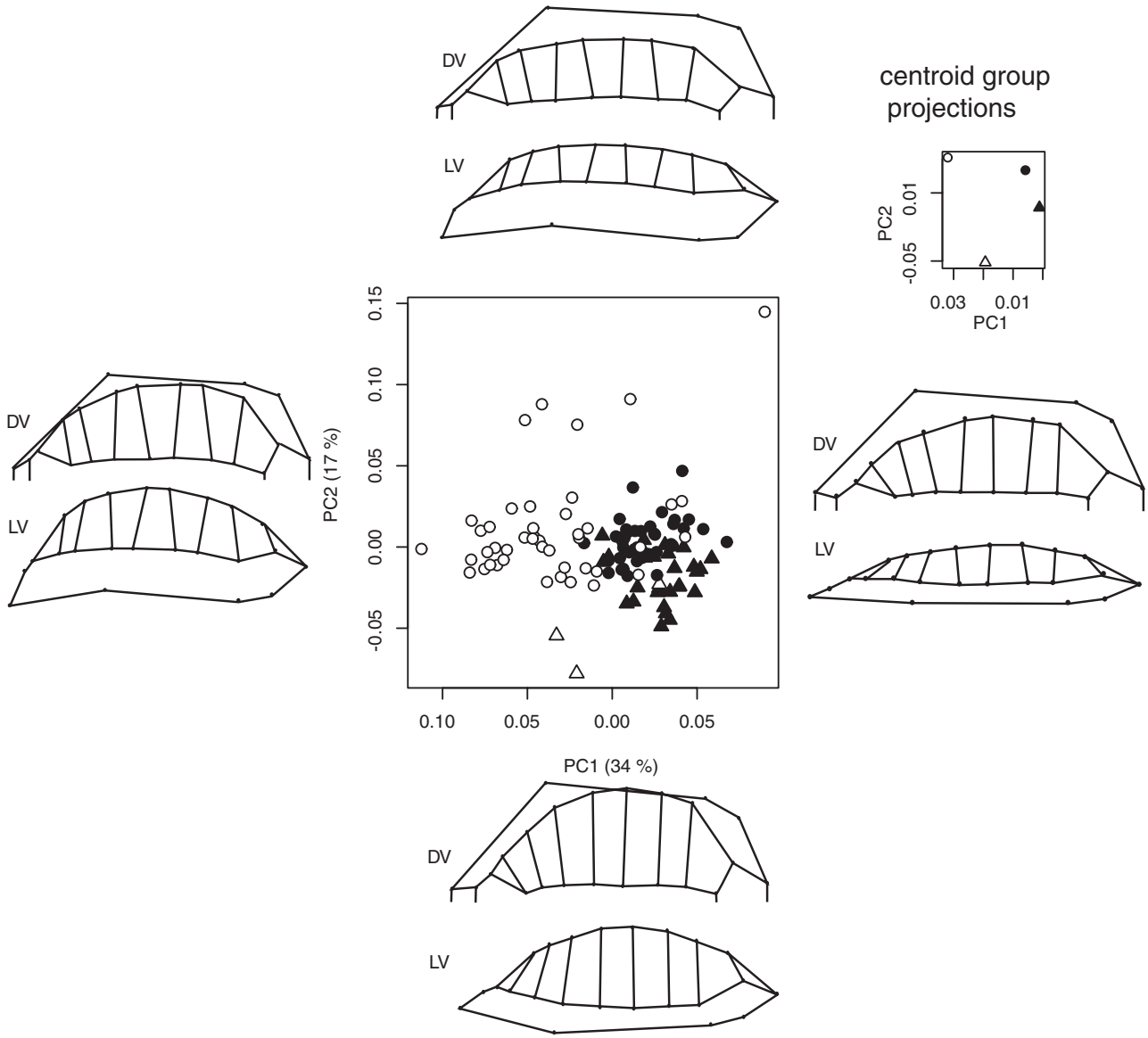

Figure 3. Principal component analysis on the species coordinates for the bony carapace. Diagrams around the graph are amplified shape, in dorsal (DV) and lateral (LV) views, derived from the eigenvectors along the first two principal components (PC1 and PC2). Filled symbols are aquatic species and empty ones are terrestrial species. Circles are Testudinoidae and triangles are Emydidae. The graph in the upper right shows the projections of the mean for each group.

carapace (Fig. 3), on the third (13%) and fourth (9%) PCs for the epidermal carapace (not shown), and on the first PC (33%) for the plastron (Fig. 5), with species clearly grouped more by clade than by environment.

The morphological differences between clades involved, for the plastron, a longer bridge, smaller gular and anal scutes (narrower and shorter) in the Testudinoidae, but more rounded lobes in the emydids. For the carapace, the Testudinoidae had a narrower pleural contour, a wider first marginal scute, a longer fifth vertebral scute, a longer sixth costal plate, an anteriorly wider first vertebral scute, a medially longer first costal plate, wider posterior neural plates, laterally less developed costal plates, a more elongated and rectangular contour, an alternative pattern of costal plates with the third, fifth, and seventh being shorter on the peripheral series, more posterior third peripheral plate, a shorter pygal plate, and a shorter nuchal plate (Fig. 7).

For the epidermal and bony carapaces, the interaction between clade and environment was not signi-

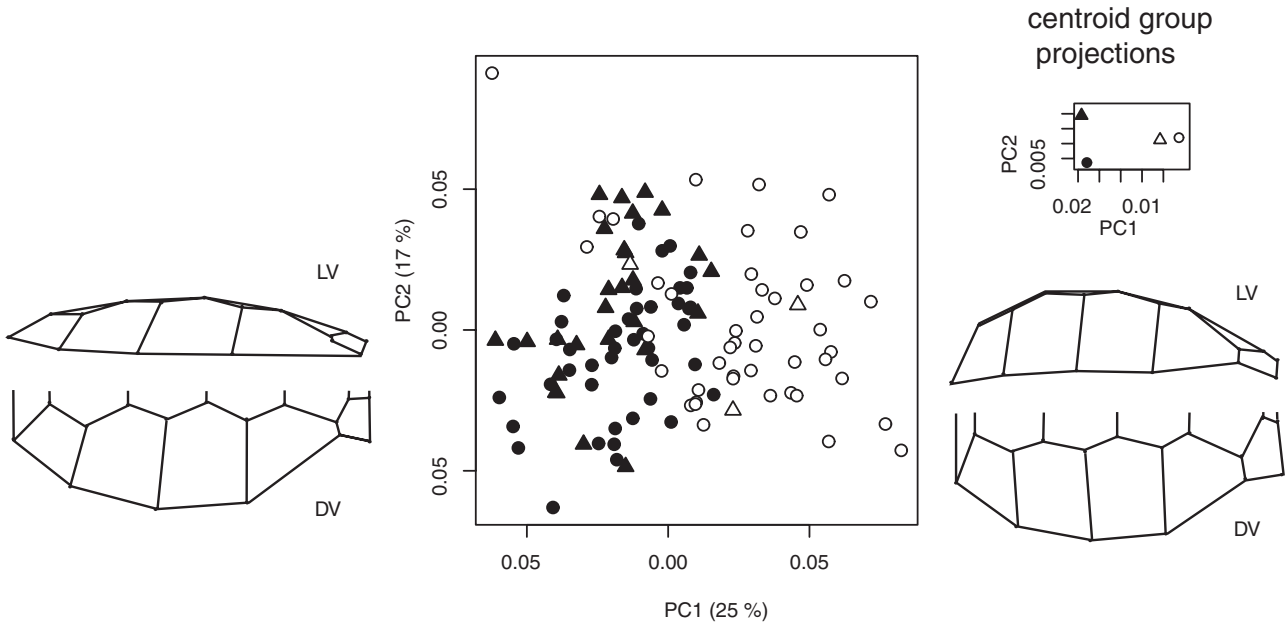

Figure 4. Principal component analysis on the species coordinates for the epidermal carapace. Diagrams around the graph are amplified shape, in dorsal (DV) and lateral (LV) views, derived from the eigenvectors along the first two principal components (PC1 and PC2). Filled symbols are aquatic species and empty ones are terrestrial species. Circles are Testudinoidae and triangles are Emydidae. The graph in the upper right shows the projections of the mean for each group.

ficant (Table 4). This result attests to the morphological changes in the carapace between environments being similar in both clades. As no pattern of size convergence was found between the two clades, this suggests that evolutionary allometry is not involved in the similarity of shape change between environments, within each clade.

The interaction term between clade and environment was significant ($P = 0.02$) only for the plastron. The transformation between environments in each clade was then dissimilar for this structure. Note, however, that, as compared with the level of significance of the main factors (all $P < 0.0001$), the significance of this interaction may be considered as marginal. Moreover, the first PC differentiates hinged forms, i.e. box turtles which occur in both clades and both environments, from the other species. This underlines the fact that the morphological changes characterizing box turtles are most important and particularly involved the plastron (as suggested by Bramble (1974)). This fact, combined with the overrepresentation of box turtles ($N = 2$) in the smallest group considered here, the terrestrial emydids ($N = 3$), likely hid the common pattern of plastral shape changes of both clades between environments. The examination of the material suggested that some particular traits of the plastron showed a homogeneous pattern of modification between the two environments across clades, for example plastral or abdominal scute lengths.

The environmental shift and the clade membership involved different shape components for the bony and epidermal carapaces: the cosines between the eigenvectors attached at each effect were, respectively, 0.006 and 0.035, underlying the fact that the VCV matrices were clearly independent (Monte Carlo test: $P = 0.96$ for the bony shell and $P = 0.79$ for the epidermal shell). For the plastron, the two sources of morphological variation were significantly correlated (cosine = –0.406, Monte Carlo test: $P < 0.001$).

The sequential MANOVA allowed assessment of the relative part of phylogenetic constraints (i.e. how phenotypes are identical within a clade) and the selection due to environment (assessed by the amount of convergence between clades). The comparisons of the mean squares between each pair of sequential MANOVAs showed that the effects of environment and cladogenesis on the three morphological structures were of the same order of magnitude (Table 4). Nonetheless, these comparisons showed that the interenvironment variance was the most important source of variance for each of the three structures (up to twice for bony and epidermal carapaces).

The terrestrial species of the Testudinoidae showed the largest variation in size, and the species of Emydidae the lowest (Table 5). This relationship was

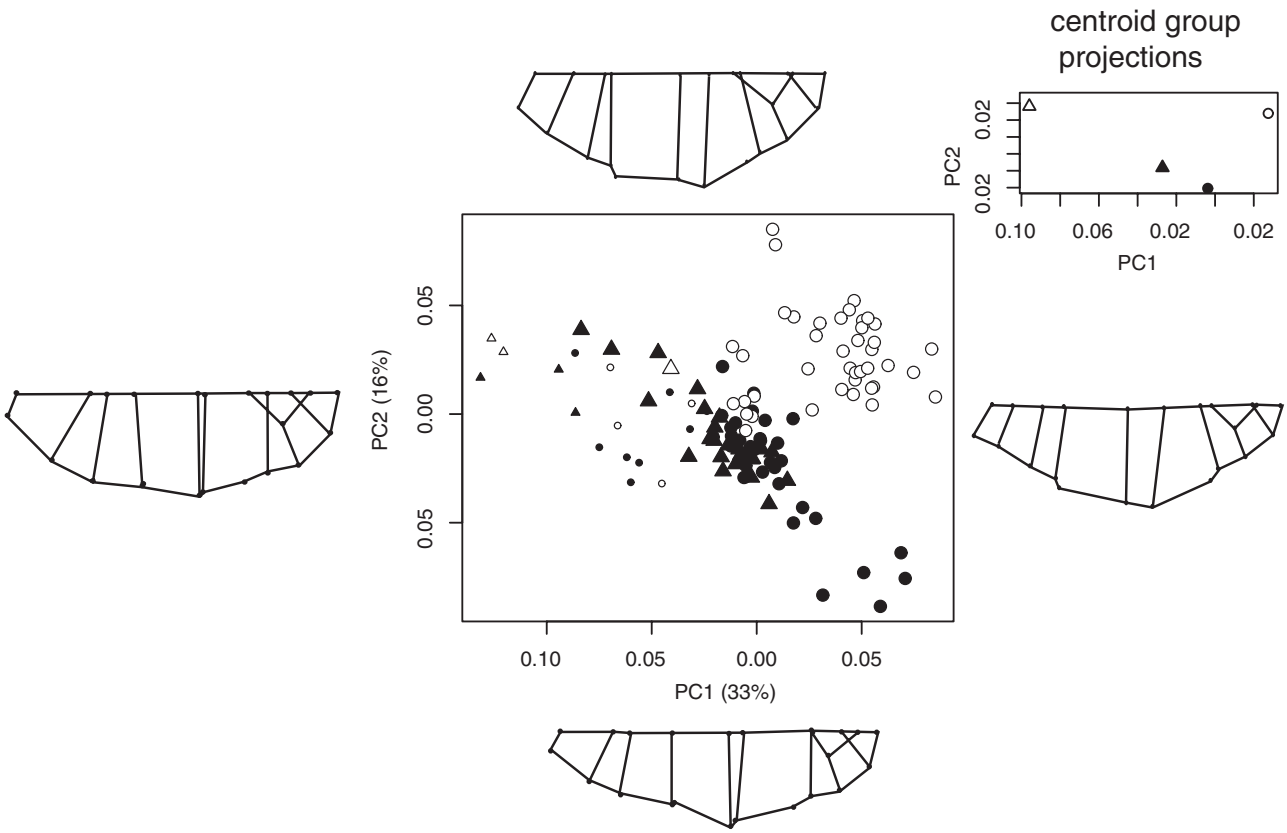

Figure 5. Principal component analysis on the species coordinates for the plastron. Diagrams around the graph are amplified hemiplastral shape (ventral view, VV), derived from the eigenvectors along the first two principal components (PC1 and PC2). Filled symbols are aquatic species and empty ones are terrestrial species. Circles are Testudinoidae and triangles are Emydidae. Small symbols are box turtles (turtles with a hinged plastron). The graph in the upper right shows the projections of the mean for each group.

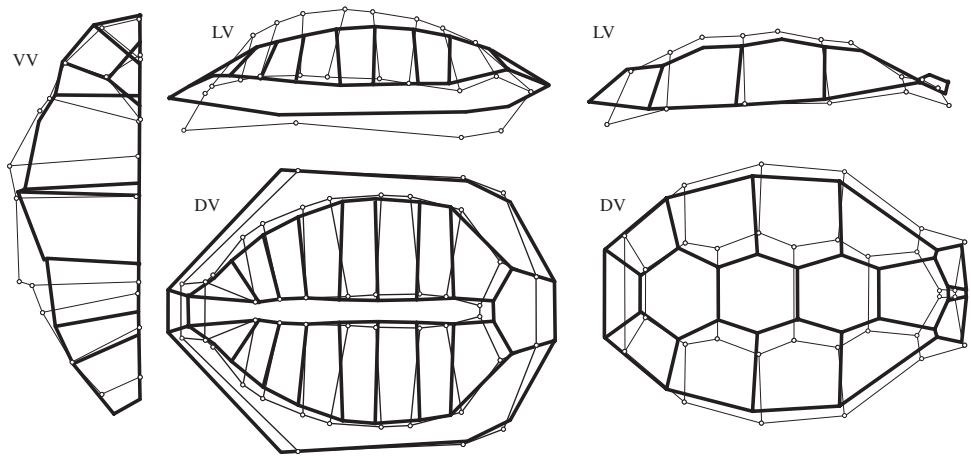

Figure 6. Morphological variation related to the environment effect. Differences were magnified (×1.2) and computed from the variance-covariance matrix of the factor environment, taking into account firstly the factor of clade. The terrestrial form is shown in normal lines and the aquatic form in bold lines. From left to right: plastron, bony carapace and epidermal carapace. DV = dorsal view, LV = lateral view, VV = ventral view.

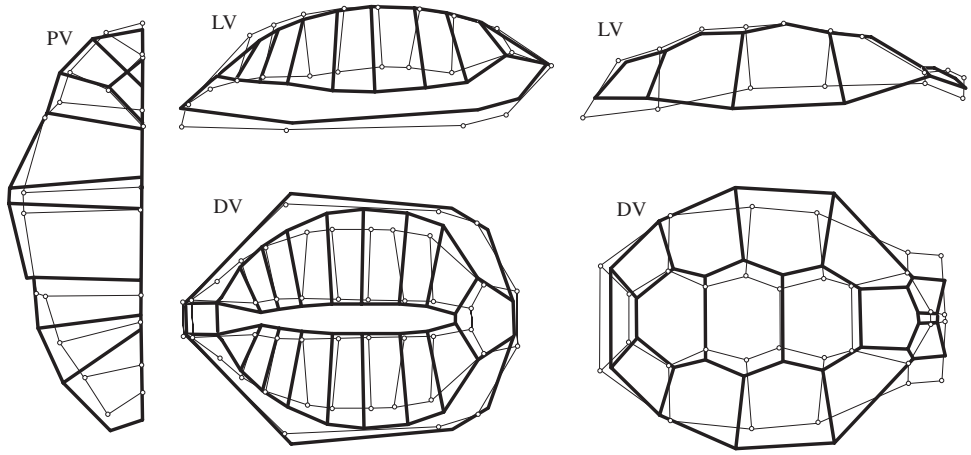

Figure 7. Morphological variation related to the clade effect. Differences were magnified (about ×1.5) and computed from the variance-covariance matrix of the factor of clade, taking into account firstly the factor of environment. The Testudinoidae form is shown in normal lines and the Emydidae form in bold lines. From left to right: plastron, bony carapace and epidermal carapace. DV = dorsal view, LV = lateral view, VV = ventral view.

Table 5. Shape and size interspecific variance for clade and environmental groups

			Shape		Size	
Structure	Clade	Environment	Variance	d.f.	Variance	d.f.
Bony carapace	Emydididae	Aquatic	3.74×10^{-5}	1612	978.44	26
		Terrestrial	7.58×10^{-5}	124	244.33	2
	Testudinoidae	Aquatic	3.55×10^{-5}	2666	745.88	43
		Terrestrial	8.87×10^{-5}	2604	1053.64	42
Epidermal carapace	Emydididae	Aquatic	6.69×10^{-5}	1066	963.48	26
		Terrestrial	8.99×10^{-5}	82	256	2
	Testudinoidae	Aquatic	7.63×10^{-5}	1763	691.05	43
		Terrestrial	9.85×10^{-5}	1722	1046.38	42
Plastron	Emydididae	Aquatic	5.51×10^{-5}	1736	1027.68	28
		Terrestrial	8.16×10^{-5}	124	432.33	2
	Testudinoidae	Aquatic	7.11×10^{-5}	2666	702.89	43
		Terrestrial	8.42×10^{-5}	2666	1059.47	43

significant only for the bony and epidermal carapaces (respectively, $F_{[42,2]} = 4.3$, $P = 0.043$; $F_{[42,2]} = 4.1$, $P = 0.046$). The terrestrial emydids were significantly less variable than the terrestrial species of the Testudinoidae for bony carapace size variation ($F_{[26,2]} = 4.0$, $P = 0.048$). There were no other significant differences in size variation for the other pairwise comparisons.

For the three structures, both clades presented a significant increase in morphological variance towards terrestrial environment ($1.18 < F_{[82 < \text{d.f.1} < 2666,1066 < \text{d.f.2} < 2666]} < 2.45$; $0 < P < 0.026$). This suggests that environment involves similar changes in morphological disparity in both clades. The levels of morphological variation were similar in terrestrial forms for the three structures ($1.03 < F_{[1722 < \text{d.f.1} < 2666,82 < \text{d.f.2} < 124]} < 1.17$; $0.12 < P < 0.43$); the aquatic Testudinoidae were slightly more variable than the Emydidae for plastron and epidermal carapace ($1.14 < F_{[1763 < \text{d.f.1} < 2666,1066 < \text{d.f.2} < 1736]} < 1.29$; $0 < P < 0.008$), but variation in these two groups was similar for bony carapace in aquatic species ($F_{[1612,\ 2666]} = 1.03$, $P = 0.24$). Cladogenesis did not appear to produce more substantial morphological diversity among the turtle groups considered here than did environmental shift (Table 5).

DISCUSSION

Our results clearly show that whereas both cladogenesis and environment may be considered as significant sources of shape variation in testudinoids, they do not appear to affect size. More surprisingly, even if size is commonly thought to have an adaptive significance at the intraspecific level, the mean size of species did not differ significantly between terrestrial and aquatic environments. It must be remembered that neither large sea turtles, belonging to the Chelonioidea, nor any other fossil marine turtles were involved in this study. Although larger size may be associated with marine habitats in turtles (Pritchard & Trebbau, 1984), size does not appear to be an adaptive trait associated with the two major non-marine environments considered here.

We have shown that cladogenesis and environmental shift imply significant changes which, moreover, concern distinct morphological components of the carapace. Environment acts mostly on shell height and on the architecture of costal plates, terrestrial species having a more rounded and shorter shell, and a distinctive alternative pattern of costal plates. Clades differed by their shell contour, emydids being posteriorly wider, and by more localized differences, as in the relative extension of scutes or bones. Even if there were no or few diagnostic discrete characters that may allow easy separation of the two clades, our study shows clearly that the shell is a phylogenetically informative biological structure. The divergence between environments concerns different shape components than those inherited by the members of each clade. This indicates that, in Testudinoidea, the environmental shift has not generated homoplasy which would have potentially hidden clade recognition. Thus, each clade exhibits a recognizable morphology which is not much altered by environmental shift, even though each environment significantly modified this morphology.

Despite the slightly particular pattern for the plastron due to the convergence between box turtles, the two clades exhibited fairly similar adaptive patterns in each environment. The turtle shell is a highly specific structure. It is likely that, despite the existence of two clades, the ontogenetic rules related to the carapace are shared by most of these species. These constraints combined with biomechanical limitations should globally restrain the adaptive designs adopted by species under diverging selective pressures in these two contrasted environments. This would then account for the convergent and/or parallel evolutionary patterns depicted in our study. However, whatever the constraints that can affect evolutionary changes, selective pressures are a likely source of morphological variation among the Testudinoidea. Several types of evidence may suggest an important role of selection in shaping the testudinoid carapace. These are the morphofunctional interpretations of phenotypes, the existence of a convergent adaptation (the box turtles) among the testudinoids, and the reduction of interspecific variation among aquatic turtles (see below).

Based on the features commonly shared by the two clades within each environment, it is possible to propose a morphofunctional hypothesis of the role of natural selection in shaping the carapace in both environments. The flat aquatic shell may be thought to enhance hydrodynamics. The forward displacement of the centre of gravity could be functionally related to swimming by allowing better control of displacement in water. By comparison, the centre of gravity in marine turtles is quite anterior (Davenport, Munks & Oxford, 1984). Understanding functional correlates of terrestrial shapes seems to be more difficult (Schubert-Sondern, 1962; Mlynarski, 1966). As mentioned above, terrestrial species exhibited an important shell morphological variation. Few of these species are flat (*Geoemyda, Malacochersus*) and inhabit a unique terrestrial environment (living under dead leaves or in rocky crevasses). Most of them, however, display domed shells, a mid-positioned centre of gravity, and a shortening of the body. These features may be thought to enhance the stability of these animals with inarticulate trunks in terrestrial environments. The pattern of alternate costal plates might be interpreted as strengthening the shell (Auffenberg, 1974), in terms of architecture, it is undoubtedly related to neural pattern (see Pritchard (1988) for a review). These morphofunctional hypotheses require additional experiments to be clearly demonstrated (Schluter, 2000).

The external skeleton of turtles being an integrated structure, one may expect some consistency in the morphological changes among the different parts of which it is composed. Although the elements of the carapace exhibit congruent morphological changes between the two environments for both clades, this was not the case for the plastron. For this structure, the significance of the interaction term of the MANOVA between environment and clade indicates that shape changes related to environment were different in the two clades. As already stated in our results, we consider this discrepancy as being marginal and potentially due to the presence of hinged forms (box turtles) in our samples. Box turtles exist in both clades (Bramble, 1974). Such morphology may be thought to be adaptive and to provide turtles with more complete protection (Feldman & Parham, 2002). The plastral hinged condition particularly involved plastral modifications, leading these forms to remain closely located in the morphospace defined for this structure. Again, some constraints could have chan-

nelled the evolution towards this adaptive phenotype. In that case, however, the plastrons of box turtles have not kept any morphological features that would allow recognition of their clade of origin. This suggests that, for reasons of natural selection or architectural constraints, the acquisition of a plastral hinge has led to a more achieved pattern of convergence than those observed for the adaptation to terrestrial or aquatic ways of life.

The interspecific shape variance for terrestrial turtles was about twice that of aquatic species. Previous studies have shown that environmental change may involve changes in morphological variability. For example, Schluter (1986) showed analogous change in morphological variation between environments among different finch communities. Several non-exclusive hypotheses could be stated. First, the turtle shell morphology may be less functionally constrained in terrestrial environments than it is in aquatic ones. Second, the adaptive landscape (Schluter, 2000) in terrestrial environments might be more complex than it is in aquatic ones, providing multiple niches and multiple adaptive peaks in the former. As a matter of fact, aquatic turtles, except the presence of box turtles, present a homogeneous morphology. On the other hand, terrestrial ones involve at least three phenotypic types: the commonly domed shelled turtles, box turtles (with hinged plastron), and leaf turtles (with strongly serrated carapace which live cryptically under dead leaves). Conversely, there was no clear difference in variance among clades, even if aquatic Testudinoidae were found to be significantly more variable than were aquatic emydids for two of the three structures. It thus seems that similar patterns of disparity are observed between the two groups. The conservation of the same evolutionary flexibility (*sensu* Lovette, Bermingham & Ricklefs, 2002) may explain why the two groups radiated in a similar way, both within and between environments.

The Testudinoidea are the most taxonomically and ecologically diversified group of turtles, which includes about half of the current turtle diversity. Paradoxically, the three current families composing this clade appeared later than did any other turtle family. Their presence has been recorded only since the early Eocene, whereas most other modern turtle families appeared in the fossil record during the Cretaceous. As early as the early Eocene, terrestrial and aquatic species seemed to occur in the two clades. Since this period, environmental shifts have probably occurred several times in the lineages composing both clades. As such, the radiation of the testudinoids meets all the criteria to be considered as adaptive, i.e. a common ancestry, phenotype–environment correlation, trait utility and rapid speciation (Schluter, 2000). Similar shifts in environment (aquatic/terrestrial) have also occurred in other organisms and have been reported recently in Jamaican crabs (Schubart, Diesel & Blair-Hedges, 1998), and gastropods (Rosenberg, 1996), in both of which a relationship between morphology and environment has been established. Concerning our study, we speculate that the difference in diversification rates with other clades, such as the Chelonioidea, Pelomedusoidea or Trionychoidea, suggests that the Testudinoidea acquired a high evolutionary flexibility (*sensu* Lovette *et al*., 2002), allowing them to spread in widely different environments, and thus to become the most diversified clade of turtles. Paradoxically, this group shows a great stability in the number of scutes and bony plates, and species in both clades may share relatively similar ontogenetic trajectories. It may be concluded that morphological flexibility is not necessarily correlated with developmental reprogramming. This hypothesis should be easy to test in the future, considering the ontogenetic trajectory of different species belonging to both clades and inhabiting both environments, and comparing the morphological variance of the Testudinoidea against other superfamilies.

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APPENDIX

List of measured specimens

Abbreviations

PCHP: Chelonian Research Institute Collection, Oviedo
C: Marc Cheylan collection, EPHE Montpellier
H: Haiyan Tong collection, Paris
MNHN: Museum National d'Histoire Naturelle, Paris
JC: personal collection
*Specimen in which only bony and epidermal carapace were measured
†Specimen in which only plastron was measured
un: unnumbered specimen

Aquatic Emydidae

Graptemys barbouri: PCHP1070, PCHP1452, PCHP1457, PCHP1587†, PCHP26† PCHP1704*, PCHP27, PCHP2815, PCHP286, PCHP4823, PCHP3992, PCHP3682*, PCHP4823†
Graptemys geographica: PCHP1176, PCHP3343
Graptemys flamaculata: PCHP1556, PCHP806
Graptemys versa: PCHP2264*, PCHP2164†
Graptemys kohni: PCHP4486
Graptemys ernsti: PCHP85
Graptemys pulchra: PCHP29
Graptemys gibbonsi: PCHP3651†
Graptemys nigrinoda: PCHP*, PCHP3699†
Pseudemys floridana: PCHP1147, PCHP4143, PCHP4694*, PCHP5694†, PCHP4130†
Pseudemys nelsoni: PCHP1139, PCHP298, PCHP4140, PCHP3774

Pseudemys concinna: PCHP1559*, PCHP*, PCHP1560†
Pseudemys rubriventris: PCHP4721, PCHP4722†
Pseudemys alabamensis: PCHP99†
Deirochelys reticularia: PCHP1178, PCHP1431
Malaclemmys terrapin: PCHP5225, PCHP3699, PCHP1224, PCHP1555
Trachemys stejneri: PCHP1318, PCHP, 3187
Trachemys terrapen: PCHP6060*, PCHP1319
Trachemys scripta: PCHP2736, PCHP299*, PCHP301, JC11, JC12, JC1, JC3, JC4, H un
Trachemys dorbigny: PCHP3181, PCHP2954
Trachemys grayi: PCHP350
Trachemys decorata: PCHP334, PCHP un†
Chrysemys picta: PCHP2534, PCHP333, MNHN un, MNHN un†, MNHN 1887790
Clemmys guttata: PCHP2854, PCHP2855, PCHP2856*, PCHP2857, PCHP2853, MNHN 1874491, MNHN 1887795, MNHN 1887791, MNHN un*, MNHN 1887800, PCHP2852†
Glyptemys mulhenberghii: PCHP6245
Emys marmorata: PCHPun*, PCHP64†, PCHP3672†
Emys blandinghii: PCHP307, PCHP1180†
Emys orbicularis: C un, C un, C un, C un, C un*, C un*, MNHN 1888729, MNHN 1887793†
Terrapene coahuila: PCHP2692, PCHP3333†

Terrestrial Emydidae
Glyptemys insculpta: PCHP2013, PCHP4716
Terrapen carolina: PCHP2011, PCHP2529, PCHP2665*, PCHP1485, PCHP6059†
Terrapen ornata: PCHP2365*, PCHP2356†

Aquatic Testudinoidae
Siebenrockiella crassicolis: PCHP2509, H5, MNHN 187588†
Morenia petersi: PCHP2679, PCHP2680*, PCHP4160*, PCHP2735†
Morenia ocellata: PCHP4680, PCHP4167†
Orlitia borneoensis: PCHP2632, PCHP3366
Kachuga kachuga: PCHP2742
Kachuga dhongokha: PCHP3943, PCHP3458
Kachuga tentoria: PCHP2941, PCHP2939
Kachuga tecta: PCHP2370, PCHP2371
Kachuga smithii: PCHP2934, PCHP2936
Hieremys annandalii: PCHP3804
Hardella thurgi: PCHP2730, PCHP3220, PCHP3221
Chinemys megalocephala: PCHP2039
Chinemys nigricans: PCHP4626*, PCHP4655, PCHP4073
Chinemys reevesi: PCHP5240, PCHP6064
Ocadia sinensis: PCHP6190, H4†
Callagur borneoensis: PCHP4994, PCHP4191†, PCHP3941†
Batagur baska: PCHP4617
Geoclemmys hamiltoni: PCHP487, PCHP4630†
Malayemys subtrijuga: PCHP3442, PCHP3445
Sacalia beali: PCHP2619, PCHP3950, PCHP3447†
Mauremys mutica: PCHP2666, PCHP3194, PCHP2314
Mauremys caspica: PCHP2957, PCHP4651, PCHP6068
Mauremys japonica: PCHP4006, PCHP5505, PCHP5477
Mauremys pritchardi: PCHP4214, PCHP3441, PCHP3435, PCHP3438†
Mauremys iversoni: PCHP4628, PCHP3279, PCHP3278, PCHP3365, PCHP3280†, PCHP4658†
Mauremys leprosa: PCHP4738, MNHN 1886206, MNHN 188820*, MNHN 1927231*, Jc2, C un
Melanochelys trijuga: PCHP4994, PCHP5498, PCHP3330*
Notochelys platynota: PCHP4961, PCHP1222*, PCHP3650†
Cuora amboinensis: PCHP3460*, PCHP3704, PCHP5078
Cuora zhaoi: PCHP4193
Cuora aurocapitata: PCHP3993*, PCHP4420†
Cuora mccordi: PCHP5071
Cuora pani: PCHP3925*, PCHP3243†
Cuora trifasciata: PCHP3823*, PCHP5443
Heosemys grandis: PCHP4709
Cyclemys dentata: PCHP5529
Cyclemys tcheponensis: PCHP5244
Cyclemys oldhamii: PCHP3952
Rhinoclemmys melanosterna: PCHP2434
Rhinoclemmys nasuta: PCHP2443
Rhinoclemmys punctularia: PCHP2871
Rhinoclemmys diademata: PCHP3656
Rhinoclemmys funerea: PCHP4380
Annamemys annamensis: PCHP4071

Terrestrial Testudinoidae
Rhinoclemmys areolata: PCHP2963
Rhinoclemmys pulcherrima: PCHP1444, H32
Rhinoclemmys rubida: PCHP1234*
Rhinoclemmys annulata: PCHP1222
Pyxidea mouhotii: PCHP2960, TH10
Geoemyda spengleri: PCHP2527
Geoemyda sylvatica: PCHP2725
Geoemyda yuwonoi: PCHP4984
Cistoclemmys flavomarginata: PCHP2023*, PCHP3818, PCHP3819†, H3
Cistoclemmys galbinifrons: PCHP3242
Heosemys spinosa: PCHP5402, PCHP67
Heosemys depressa: PCHP5481†
Gopherus agassizii: PCHP134
Gopherus berlandieri: PCHP4481, PCHP4353
Gopherus polyphemus: PCHP6298, PCHP3600
Indotestudo elongata: PCHP1787, H29
Indotestudo forsteni: PCHP2119, PCHP2121

Indotestudo travancorica: PCHP2837
Chelonoidis chilensis: PCHP1834, PCHP1833
Chelonoidis denticulata: PCHP2489, PCHP284
Chelonoidis nigra: PCHP2302
Chelonoidis carbonaria: PCHP5252, PCHP3192
Geochelone pardalis: PCHP4476, PCHP4821
Geochelone elegans: PCHP4702
Geochelone sulcata: PCHP4355, PCHP5340*, PCHP5352
Asterochely radiata: PCHP5340*, PCHP5347*, PCHP1304
Aldabrachelys elephantina: PCHP2601, PCHP2604
Malacochersus tornieri: PCHP1300
Testudo marginata: PCHP2211
Testudo weissingeri: PCHP4010
Testudo horsfieldi: PCHP2929, PCHP4633, C un, C un
Testudo graeca: C un, C un, C un, C un, C un, C un, C un*, C un*, C un*
Testudo hermanni: C un, C un, C un, C un, C un, C un, C un, C un†, C un†, C un†, C un†, JC7, JC8
Testudo kleinmani: PCHP4346, PCHP4348
Pyxis arachnoides: PCHP5439
Chersina angulata: PCHP2569, PCHP2574
Homopus areolatus: PCHP2605, PCHP2612
Psammobates oculifer: PCHP4064, PCHP4204
Psammobates tentorius: PCHP2619, PCHP3387
Psammobates geometricus: PCHP3388
Kinixys belliana: PCHP485, PCHP3945
Kinixys erosa: PCHP2307
Kinixys homeana: PCHP2864, PCHP2893, Hun
Manouria impressa: PCHP2900, PCHP4683
Manouria emys: PCHP2780, PCHP2781, PCHP2783