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Authors

Avise, JC
Bowen, BW
Lamb, T
[et al.](#)

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Mitochondrial DNA Evolution at a Turtle's Pace: Evidence for Low Genetic Variability and Reduced Microevolutionary Rate in the Testudines¹

John C. Avise,* Brian W. Bowen,* Trip Lamb,† Anne B. Meylan,‡ and Eldredge Bermingham§

*Department of Genetics, University of Georgia; †Department of Biology, East Carolina University; ‡Florida Marine Research Institute; and §Smithsonian Tropical Research Institute

Evidence is compiled suggesting a slowdown in mean microevolutionary rate for turtle mitochondrial DNA (mtDNA). Within each of six species or species complexes of Testudines, representing six genera and three taxonomic families, sequence divergence estimates derived from restriction assays are consistently lower than expectations based on either (a) the dates of particular geographic barriers with which significant mtDNA genetic clades appear associated or (b) the magnitudes of sequence divergence between mtDNA clades in nonturtle species that otherwise exhibit striking phylogeographic concordance with the genetic partitions in turtles. Magnitudes of the inferred rate slowdowns average eightfold relative to the "conventional" mtDNA clock calibration of 2%/Myr sequence divergence between higher animal lineages. Reasons for the postulated deceleration remain unknown, but two intriguing correlates are (a) the exceptionally long generation length of most turtles and (b) turtles' low metabolic rate. Both factors have been suspected of influencing evolutionary rates in the DNA sequences of some other vertebrate groups. Uncertainties about the dates of cladogenetic events in these Testudines leave room for alternatives to the slowdown interpretation, but consistency in the direction of the inferred pattern, across several turtle species and evolutionary settings, suggests the need for caution in acceptance of a universal mtDNA-clock calibration for higher animals.

Introduction

Evolutionary rates in DNA sequences exhibit heterogeneities of several types (see reviews in Nei 1987; Wilson et al. 1987; Li and Graur 1991). Variable substitution rates are apparent (a) across nucleotide positions within a codon (e.g., in protein-coding regions, mean rates for synonymous substitutions and introns are normally approximately five times higher than those for nonsynonymous changes), (b) among nonhomologous genes within a lineage (nonsynonymous rates can vary by orders of magnitude, as between the slowly evolving histones and the rapidly evolving apolipoproteins), (c) among classes of DNA within a genome (e.g., introns and pseudogenes evolve more rapidly than tRNA and rRNA loci), and (d) among genomes within an organismal lineage [e.g., synonymous substitution rates in the nuclear genomes of plants are roughly fourfold higher than those in chloroplast DNA (cpDNA)]. Under

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Address for correspondence and reprints: John C. Avise, Department of Genetics, University of Georgia, Athens, Georgia 30602.

the neutral mutation theory (Kimura 1979), such rate heterogeneities across nucleotide sites, genes, and genomes *within* a phylogenetic lineage are interpreted to reflect differential levels of functional constraint across DNA sequences, possibly in conjunction with variation in the underlying rate of mutation (Britten 1986).

A more controversial form of rate heterogeneity (see Wilson et al. 1987) concerns differences in the tempo of homologous DNA sequence evolution *between* organismal lineages. For the dating of cladogenetic events, uniformity in evolutionary rate across lineages clearly would be desirable (Wilson et al. 1977). However, the evidence for a universal and precise "clock" has been challenged frequently. Ayala (1986) summarizes evidence for the "erratic" behavior of particular protein clocks across lineages, as do Britten (1986) and Brunk and Olson (1990) for overall rate of single-copy nuclear DNA (scnDNA) sequence divergence (as gauged by DNA-DNA hybridization) among phylogenetic groups. Some investigators have concluded that mean molecular rates vary in a *predictable* fashion among groups of organisms. For example, molecular evolution appears consistently slower in higher primates (Goodman et al. 1971; Wu and Li 1985; Li et al. 1987; but, for counterarguments, see Easteal 1991). Sibley and colleagues (who formerly supported a "uniform average rate of DNA evolution"; Sibley and Ahlquist 1984) present recent evidence that variation in either generation length or number of cell divisions in the germ line may be correlated with variation in mean rate of DNA evolution among species (Catzeflis et al. 1987; Sibley et al. 1988).

One molecular clock which thus far has remained relatively immune to conclusions of rate heterogeneity across taxa involves mitochondrial DNA (mtDNA) in higher animals. Brown et al. (1979) originally calibrated the mean rate of mtDNA sequence divergence between recently separated primates as being $\sim 2\%/Myr$ (1×10^{-8} substitutions/year/nucleotide position in each evolutionary lineage), and similar rates of initial divergence (averaged over the mtDNA molecule) have been reported in rhinoceroses, rodents, horses, artiodactyls, gallinaceous birds, geese, salmonid fishes, and Hawaiian *Drosophila* (see reviews in Wilson et al. 1985; Shields and Wilson 1987). *Ratios* of mtDNA:scnDNA divergence rates differ between some vertebrates and invertebrates (Powell et al. 1986; Vawter and Brown 1986; Caccone et al. 1988), but the differences have been attributed largely to rate variation in the nuclear genome (perhaps coupled with a more rapid saturation effect on mtDNA sequence divergence in invertebrates; DeSalle et al. 1987). Different nucleotide positions and genes within mtDNA are known to evolve at heterogeneous rates within a lineage (Brown et al. 1982; Gillespie 1986; Moritz et al. 1987), and particular mtDNA genes (such as cytochrome oxidase) also show rate differences as high as fivefold across lineages (Brown and Simpson 1982; Crozier et al. 1989). Nonetheless, in terms of the "conventional" 2%/Myr calibration for mtDNA in higher animals, Wilson et al. (1985, p. 388) concluded that "no major departures from this rate are known for the mtDNA molecule as a whole." Recent studies have challenged this view, with suggestions of significant mtDNA rate heterogeneities across lineages of Hawaiian *Drosophila* (DeSalle and Templeton 1988), mammalian orders (Hasegawa and Kishino 1989), and homeothermic versus heterothermic vertebrates (Kocher et al. 1989; Thomas and Beckenbach 1989).

Here we assemble results from several restriction-site surveys that consistently point to unusually low genetic variability and divergence among mtDNA lineages in several species of marine, freshwater, and terrestrial turtles (order Testudines). Collectively, they provide a case for a significantly slower mtDNA rate in Testudines

relative to other groups of vertebrates. These studies are of further interest because they differ from nearly all previous molecular-rate assessments, by (a) addressing rates of microevolutionary change at the within-species level, (b) including, as an external calibration, concordant phylogeographic patterns from sympatric faunas, and (c) providing independent examples from several separate lineages.

Material and Methods

The present report presents a compilation of information from independent molecular surveys of a variety of turtle species or species complexes (table 1). The original studies were designed to address particular issues of turtle taxonomy, behavior, or biogeography, and the observations on mtDNA rate that are summarized here were an unanticipated by-product of these endeavors.

Each survey involved conventional restriction-site analysis. mtDNAs were isolated by cesium chloride-ethidium bromide gradient procedures and were digested with a battery of type II endonucleases (Lansman et al. 1981). Digestion fragments were end-labeled with radioactive nucleotides, were separated by agarose gel electrophoresis,

Table 1
Turtle Species Examined, and Summary of Evidence
for Slow mtDNA Microevolutionary Rate

Family	Species ^a	Observation	Rationale for Conclusion of Rate Slowdown ^b
Cheloniidae	1, <i>Chelonia mydas</i> (green turtle)	Phylogenetic split, Atlantic-Mediterranean population vs. Indian-Pacific population	Magnitude of p less than anticipated from 3 Mya rise of Isthmus of Panama
Cheloniidae	2, <i>Lepidochelys olivacea</i> (olive ridley) and <i>L. kempi</i> (Kemp's ridley)	Phylogenetic split, between species	Magnitude of p less than anticipated from 3 Mya rise in Isthmus of Panama
Emydidae	3, <i>Malaclemys terrapin</i> (diamondback terrapin)	Phylogenetic split, Atlantic population vs. Gulf of Mexico population	Magnitude of p less than that of five other species with concordant phylogeography
Emydidae	4, <i>Trachemys scripta</i> (slider)	Phylogenetic split, eastern population vs. western population	Magnitude of p less than that of five or six other species with concordant phylogeography
Testudinidae	5, <i>Xerobates agassizi</i> (desert tortoise)	Phylogenetic split, population east of Colorado River vs. population west of Colorado River	Magnitude of p less than anticipated from 5.5 Mya vicariant marine transgression
Emydidae	6, <i>Graptemys geographica</i> (map turtle)	Little geographic differentiation across range	No firm conclusions possible with current data

^a Numbers denote references, as follows: 1 = Bowen et al. (1989, and accepted); 2 = Bowen et al. (1991); 3 = Lamb and Avise (accepted); 4 = E. Bermingham and J. C. Avise (unpublished data); 5 = Lamb et al. (1989); and 6 = T. Lamb (unpublished data).

^b p value is that between indicated phylogeographic clades (after correction for within-region polymorphism; table 2).

and were visualized by autoradiography (Drouin 1980). Differences among digestion profiles usually were attributable to particular restriction-site gains and losses. Some species also exhibited mtDNA size variation and heteroplasmy, but these were disregarded in the calculations of sequence divergence estimates. In these surveys, ~40–90 restriction sites typically were scored per individual, and as many as 226 individuals were assayed per species. Several of the studies involved collections made throughout a species' range (global for some of the marine turtles).

Estimates of sequence divergence and nucleotide diversity were calculated by the "site" approach of Nei and Li (1979). Phylogenies were estimated either by UPGMA clustering of values in distance matrices (Sneath and Sokal 1973, pp. 230–234) or by parsimony analyses of qualitative presence/absence matrices of restriction sites, by using the computer programs PHYLIP (see Felsenstein 1982) or PAUP (Swofford 1990). With respect to the major clusters and clades identified, distance-matrix analyses (by UPGMA) and parsimony analyses (by PAUP) yielded identical branching orders. Except where otherwise mentioned, all clade distinctions described in the present report were supported at the >95% level in networks generated by bootstrap resampling of the data (Felsenstein 1985).

Results

Conclusions about mtDNA rate slowdowns in the various turtles derive from the following lines of evidence (also see summary in table 1).

Chelonia mydas (Green Turtle)

This marine species is distributed pantropically, primarily between the northern and southern 20°C isotherms. An mtDNA survey of ~109 restriction sites in each of 226 specimens from 15 rookeries (Bowen et al. 1989, and accepted) revealed 14 distinct haplotypes that cluster phylogenetically into two distinct groups that correspond exactly to oceanic basin—Atlantic (including Mediterranean) populations versus those in the Indian and Pacific Oceans (fig. 1a). This global genetic structure provides strong evidence that continental barriers between ocean basins have forged the deep-fork topology of the mtDNA gene tree: there are no solid physical obstacles to dispersal within either basin, yet at the present time the Isthmus of Panama probably constitutes a nearly insurmountable barrier to green turtle movement between the Atlantic and Indian-Pacific Oceans.

The Panamanian Isthmus rose ~2.5–3.0 Mya, severing the sea channel that formerly connected the Atlantic and Pacific (Lundelius 1987; Coates et al., accepted). Under a conventional mtDNA-clock calibration (2%/Myr), ~5%–6% sequence divergence between Atlantic and Indian-Pacific clades is thus anticipated, and values of mtDNA divergence (p) near 6% have been reported for several trans-Isthmus geminate pairs of sea urchins (E. Bermingham and H. A. Lessios, unpublished data). However, the two green turtle clades are distinguished by an estimated net sequence divergence of only $p \cong 0.006$ (after correction for within-ocean polymorphism), implying a divergence rate roughly ninefold lower than expected under the conventional mtDNA clock.

Some caveats and counterarguments should be considered. It is possible that the low sequence divergence between Atlantic and Indian-Pacific mtDNA clades could result from interoceanic population contact postdating closure of the Panamanian sea channel. This would necessitate green turtle dispersal around either Cape Horn in South America or, more likely, the Cape of Good Hope in South Africa, routes pre-

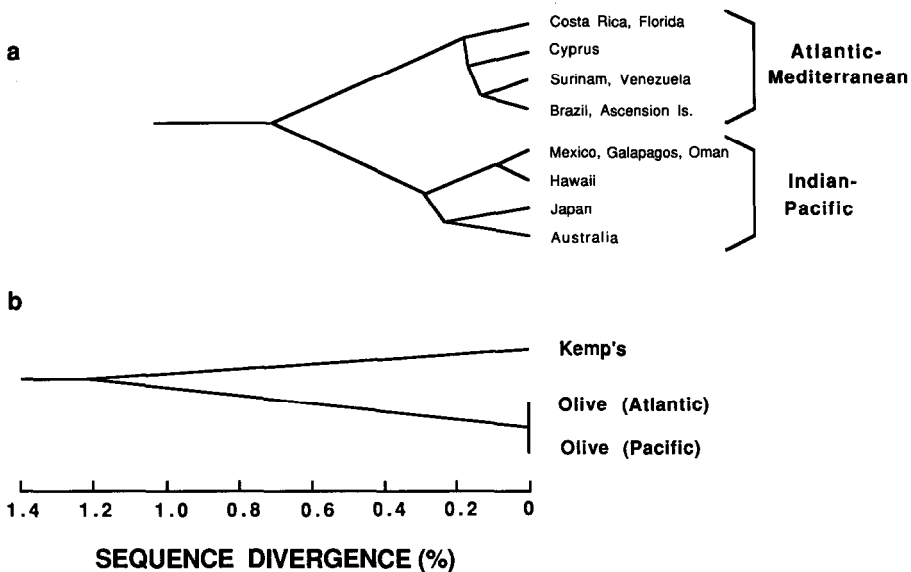


FIG. 1.—Abbreviated phenograms (UPGMA cluster analyses) summarizing matriarchal relationships based on mtDNA genetic distances for (a) global populations of green turtle (Bowen et al., accepted) and (b) ridley complex (Bowen et al. 1991).

sumably constrained at the present time by the stenothermal, warm-water adaptations of green turtles. Much of the Pleistocene was characterized by glacial advances and colder global temperatures (Kennett 1982), making interoceanic gene flow even less likely at that time. (Extremely recent interoceanic migration of assayed female lineages can be ruled out, since it would have destroyed the observed pattern of within-ocean monophyly in the mtDNA phylogeny.) Nonetheless, the possibility of colonization (e.g., of the Atlantic Ocean from an Indian-Pacific ancestor) postdating rise of the Isthmus of Panama cannot be ruled out entirely (Hughes 1972), particularly if demographic contractions within both ocean basins were subsequently severe enough to have led to the current phylogeographic pattern of reciprocal monophyly. If such a post-Isthmus exchange has occurred and if the movement of turtles was around South Africa, then unsurveyed populations from the southern Indian Ocean might prove to be more closely related, in terms of mtDNA, to those in the south Atlantic.

Lepidochelys olivacea and *L. kempfi* (Olive and Kemp's Ridelys)

The olive ridley is distributed worldwide in tropical and subtropical waters, while Kemp's ridley nests almost exclusively along a single stretch of beach in the Gulf of Mexico. The curious geographic distribution of these sister taxa was explained, by Hendrickson (1980), as being due to an original separation of the progenitor of Kemp's ridley from ancestral Pacific ridelys by rise of the Isthmus of Panama, followed by colonization of the Indian and south Atlantic Oceans by *L. olivacea*. [Pritchard (1969) suggested an earlier separation of Kemp's ridley from olive ridley, which would make the following argument for mtDNA rate slowdown even stronger.] Pritchard (1969, pp. 206–215) and Hughes (1972) also emphasized the global uniformity of olive ridelys in terms of morphology, which they attributed to a recent secondary colonization of the Atlantic via the Cape of Good Hope (ridelys appear to be more tolerant of subtropical conditions than are green turtles).

The topology of the mitochondrial phylogeny is in good agreement with these biogeographic scenarios (fig. 1b). In a survey involving a mean of 80 mtDNA restriction sites per individual, Atlantic and Pacific olive ridleys proved indistinguishable, whereas Kemp's ridley and olive ridley differed at $p \cong 0.012$ (Bowen et al. 1991). However, if Hendrickson's (1980) scenario regarding isolation by the Isthmus of Panama is correct, mtDNA sequence divergence between olive ridley and Kemp's ridley should, under a conventional clock calibration, be $p \cong 0.060$. Thus a fivefold departure from the conventional mtDNA rate is implicated.

We now cite some caveats and counterarguments. Evolutionary relationships among populations of ridleys are largely unknown, and scenarios about their biogeographic histories are speculative (see review in Pritchard 1969). Indeed, mtDNA assays provide the only direct test of the genetic distinctiveness of Kemp's ridley and olive ridley currently available (Bowen et al. 1991). Although the mtDNA results are entirely consistent with the phylogenetic uniqueness of Kemp's ridley (relative to the two sampled geographic populations of the olive ridley), there is an element of circularity in using a slow mtDNA clock to bolster Hendrickson's (1980) biogeographic scenario. Thus the possibility remains that separation of Kemp's ridley from olive ridley postdates the rise of the Isthmus of Panama. Under a conventional mtDNA clock, the estimated time of this cladogenetic event would be $\sim 600,000$ years ago. Such a late origin for Kemp's ridley appears unlikely, however, as Zug (accepted) reports Atlantic coast fossils closely resembling Kemp's ridley that date to the early Pliocene.

Malaclemys Terrapin (Diamondback Terrapin)

This brackish-water species is distributed along the Atlantic and Gulf of Mexico, from Maine to Mexico. A survey of 53 specimens from throughout most of this range, assayed for a mean of 74 mtDNA restriction sites per individual, revealed remarkably little genetic variation (Lamb and Avise, accepted). Only six distinct haplotypes (four of which were unique to single individuals) were observed, each differing from nearest relatives by one or two site changes. Nucleotide diversity was 0.0002, the lowest value observed among six comparable geographic surveys of either estuarine species or species complexes in the southeastern United States (table 2).

Two haplotypes (differing by a *Bst*EII site) were present in more than one specimen; their geographic distributions are plotted in figure 2. One genotype was essentially fixed in populations north of Cape Canaveral, and the other was essentially fixed in samples to the south and through the Gulf of Mexico. This geographic pattern is remarkably concordant with significant mtDNA phylogenetic partitions within five other species in the region (fig. 2; see review in Avise, accepted). Yet the net sequence divergence between Atlantic and Gulf of Mexico terrapins, $p \cong 0.001$, is one or two orders of magnitude lower than values for these other five taxa (table 2). If, as a standard for comparison, we adopt the mean mtDNA divergence ($p \cong 0.014$) between regional populations of these nonturtle species (excluding the toadfish, because of uncertainties of taxonomy; table 2), and if the phylogeographic subdivisions between Atlantic and Gulf of Mexico populations were molded by similar historical biogeographic events (as is strongly suggested by the concordant phylogeographic patterns), then the pace of mtDNA evolution in the diamondback terrapin, relative to that of the other species, is slowed by an average of 14-fold.

Some caveats and counterarguments should be cited. The putative phylogenetic distinction between Gulf of Mexico and most Atlantic populations of the diamondback terrapin was not statistically significant by bootstrapping criteria (because of the low

Table 2

Comparative mtDNA Genetic Diversities Within and Between Regional Populations of Each of Six Coastal or Marine Species That Exhibit Geographically Concordant Phylogenetic Partitions Distinguishing Atlantic versus Gulf of Mexico Areas

SPECIES (sample size) ^a	NO. OF DIFFERENT mtDNA CLONES	NO. OF RESTRICTION SITES SCORED PER INDIVIDUAL	MEAN WITHIN- REGION DIVERSITY		NET p BETWEEN REGIONAL POPULATIONS ^b
			Genotypic	Nucleotide	
Toadfish ^c (60)	13	51	0.68	0.0022	0.096
American oyster (212)	82	65	0.68	0.0020	0.022
Horseshoe crab (99)	10	40	0.52	0.0016	0.016
Seaside sparrow (40)	11	89	0.42	0.0004	0.010
Black sea bass (29)	5	61	0.22	0.0003	0.007
Diamondback terrapin (53)	6	74	0.14	0.0002	0.001

^a Taxa are arranged in rank order according to mean sequence divergence between mtDNA clades of Atlantic and Gulf provinces. The turtle is in the last row. References are as follows: toadfish, Avise et al. (1987b); American oyster, Reeb and Avise (1990); horseshoe crab, Saunders et al. (1986); seaside sparrow, Avise and Nelson (1989); black sea bass, Bowen and Avise (1990); and diamondback terrapin, Lamb and Avise (accepted).

^b After correction for within-region polymorphism: $p_{corr} = p_{xy} - 0.5(p_x + p_y)$, where p_{xy} is the mean pairwise genetic distance between individuals in populations x and y and where p_x and p_y are nucleotide diversities (mean genetic distances) within regions.

^c An uncertain comparison, because the Atlantic and Gulf forms are currently considered two distinct species and may not be sister taxa.

level of mtDNA differentiation involved). It is the shared phylogeographic pattern with other species that indicates the influence of a common vicariant history. Nonetheless, the possibility remains that diamondback terrapins have been in more recent evolutionary contact than have the other species and that this accounts for the limited mtDNA sequence divergence. For example, perhaps the terrapin populations were isolated by a later Pleistocene glacial episode whereas populations of the other species were isolated by earlier episodes. If terrapin mtDNA evolves at the conventional vertebrate rate, then the split in its matriarchal genealogy may have occurred ~50,000 years ago, whereas under the same assumption the intraspecific mtDNA splits for other species listed in table 2 (excluding the toadfish) would be 350,000–1,100,000 years ago.

Trachemys scripta (Slider)

Only two mtDNA haplotypes, differing at three restriction sites, were observed among a total of 55 sliders collected from six southeastern states (table 3). Nonetheless, the geographic distribution of these haplotypes shows good agreement with the major mtDNA phylogeographic subdivisions within each of six freshwater fish species similarly sampled throughout the region (fig. 3). Within-region nucleotide diversity in the slider was much lower than that observed in any of the fishes, and net sequence divergence between regional populations was also lower than values for five of the six surveyed fish taxa (table 3). If we again adopt, as a standard for comparison, the mean mtDNA divergence between regional populations of these nonturtle species ($p \cong 0.052$), then the magnitude of the east/west dichotomy in the slider is, on average, about one-ninth that of the other species. If it is assumed that the phylogeographic subdivisions between eastern and western populations of all of these species were molded by similar historical biogeographic events (as is suggested by the concordant

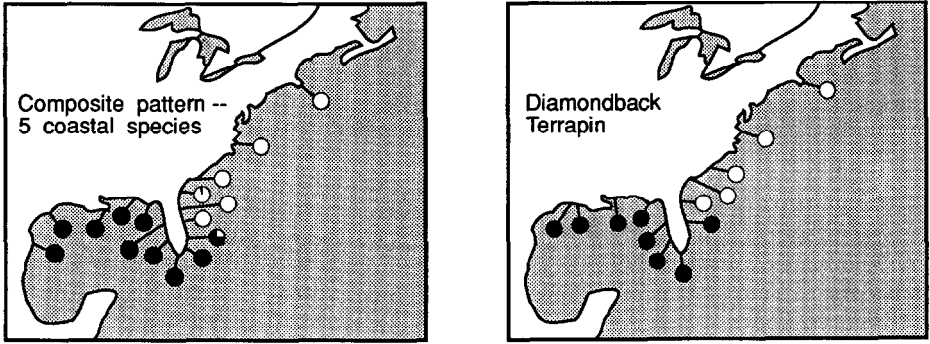


FIG. 2.—*Right*, Pie diagrams showing geographic distributions of two mtDNA clone types in diamondback terrapin. *Left*, Pie diagrams summarizing distributions of two major mtDNA phylads within five other species of coastal vertebrates and invertebrates similarly assayed (see review in Avise, accepted). In this latter representation, each species collected at a given locale was afforded equal weight in generating this overall summary of phylogeographic patterns.

phylogeographic patterns), then slider mtDNA appears to have diverged at a slower pace than that observed in mtDNA of five of the six fish species; it is notable that the sixth species is a primitive Holostean fish with unusually long generation time (see below).

Let us here consider some caveats and counterarguments. Because the phylogeographic patterns in the sliders and in each of six surveyed species (or species complexes) of fishes are similar, we suspect that shared vicariant histories have an overriding influence on intraspecific population structure (Bermingham and Avise 1986; Avise, accepted). Nonetheless, the magnitudes of mtDNA clade separation differ considerably across taxa, and in one assayed fish species (the bowfin) the east-west mtDNA divergence is as low as that observed in the pond sliders (table 3). Thus, as in the diamondback terrapin, a possibility remains that eastern and western populations of sliders (and bowfins) have been in contact more recently than have populations of most of the other species and that this accounts for the limited mtDNA sequence divergences. If the mtDNAs of the slider and bowfin evolve at the conventional vertebrate rate, then the splits in these matriarchal genealogies may have occurred $\sim 300,000$ years ago, whereas under the same assumption the fundamental intraspecific mtDNA splits for remaining species listed in table 3 would average ~ 3 Mya.

Xerobates agassizi (Desert Tortoise)

Only five different mtDNA haplotypes were observed among 56 specimens of this tortoise collected throughout its range in southwest deserts of North America (Lamb et al. 1989). By contrast, comparable mtDNA surveys of two other reptile species in the same region (Lamb et al., accepted) revealed 18 mtDNA haplotypes among 36 desert iguanas (*Dipsosaurus dorsalis*) and revealed 30 haplotypes among 51 chuckwallas (*Sauromalus obesus*). Genotypic diversities (Nei 1987) for the desert iguana, chuckwalla, and desert tortoise were 0.96, and 0.98, and 0.72, respectively.

In terms of mtDNA phylogeny, most of the desert tortoises (with the exception of three specimens from extreme southwest Sonora) grouped into two distinct assemblages—one east and the other west of the Colorado River. One reasonable explanation for this phylogeographic split involves the vicariant influence of a Pliocene marine

Table 3

Comparative mtDNA Genetic Diversities Within and Between Regional Populations of Each of Seven Freshwater Species in Southeastern United States That Exhibit Geographically Concordant Phylogenetic Partitions Distinguishing Eastern versus Western Drainages

SPECIES ^a (sample size)	NO. OF DIFFERENT mtDNA CLONES	NO. OF RESTRICTION SITES SCORED PER INDIVIDUAL	MEAN WITHIN-REGION DIVERSITY		NET <i>p</i> BETWEEN REGIONAL POPULATIONS ^b
			Genotypic	Nucleotide	
Bluegill sunfish ^c (. . .)	0.085
Redear sunfish (77)	7	48	0.49	0.0026	0.082
Warmouth sunfish (74)	32	50	0.95	0.0074	0.056
Spotted sunfish (79)	17	41	0.67	0.0110	0.044
Mosquitofish ^d (173)	22	49	0.56	0.0013	0.037
Bowfin (75)	13	54	0.70	0.0028	0.006
Slider (55)	2	41	0.48	0.0000	0.006

^a Taxa are arranged in rank order according to mean sequence divergence between mtDNA clades of these regions. The turtle is in the last row. References are as follows: bluegill sunfish, Avise et al. (1984); all sunfish species and bowfin, Bermingham and Avise (1986); mosquitofish, K. T. Scribner and J. C. Avise (unpublished data); and slider, E. Bermingham and J. C. Avise (unpublished data).

^b See footnote b to table 2.

^c Variability within regions was not estimated for this species because only selected markers were used in the geographic survey. Hence the between-region sequence divergence is also uncorrected.

^d Two species (*Gambusia affinis* and *G. holbrooki*) currently are recognized; formerly they were considered subspecies.

transgression that extended from the Gulf of California as far north as the current Lake Mohave in southern Nevada (Lamb et al. 1989). This incursion, known as the Bouse embayment, dates to ~5.5 Mya, on the basis of potassium-argon decay estimates (Shafiqullah et al. 1980). However, under the conventional mtDNA clock, the magnitude of sequence divergence distinguishing these desert tortoise clades ($p \cong 0.053$) predicts a divergence time of only 2.6 Mya. Thus, either these mtDNA clades postdate the Bouse formation or the mtDNA rate in the desert tortoise is reduced.

Some discrepancy also exists between mtDNA and paleontological divergence estimates for the desert tortoise and other closely related (by some accounts congeneric) North American tortoise species. A fairly complete fossil record indicates that, by the early middle Miocene (~15 Mya), the desert tortoise lineage (*agassizi*) was distinguishable from a second group (*polyphemus*) (Bramble 1982). Yet mtDNA comparison of these two groups (mean $p = 0.112$; Lamb et al. 1989) suggests a late Miocene-early Pliocene lineage separation (=6 Mya). Thus, inter- as well as intraspecific comparisons point to a twofold mtDNA rate slowdown in the desert tortoise.

Some caveats and counterarguments should be cited. Among the turtles considered thus far, the desert tortoise provides the weakest case for a slowed mtDNA rate. mtDNA clonal diversity is certainly low compared with that in other assayed reptiles in the American Southwest, but concordant phylogeographic support for the possible vicariant influence of the Bouse inundation is lacking (i.e., populations of the chuckwalla and desert iguana show no evidence, from mtDNA, for significant historical separations associated with the Colorado River or the Bouse embayment; Lamb et al., accepted). Thus the particular dating of the mtDNA genealogical split in the desert tortoise certainly remains open to question.

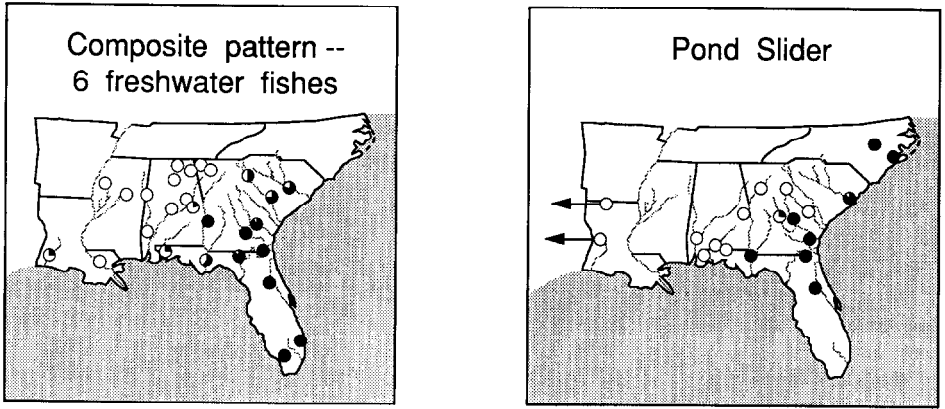


FIG. 3.—*Right*, Pie diagrams showing geographic distributions of two mtDNA phylads in slider (arrows on two westernmost pies indicate that samples came from central Texas). *Left*, Pie diagrams summarizing distributions of two major mtDNA phylads within six species of freshwater fishes similarly assayed (see review in Avise, accepted). In this latter representation, each species collected at a given locale was afforded equal weight in generating this overall summary of phylogeographic patterns.

Graptemys geographica (Map Turtle)

This aquatic species ranges widely, from the central U.S. (Mississippi valley) through the Great Lakes region, with a disjunct population in the Susquehanna drainage of Pennsylvania. An mtDNA survey of 12 individuals from the four corners of the species' range (including the Susquehanna) involved 59 restriction sites (T. Lamb, unpublished data). No restriction-site variation was detected either within or among populations (an unusual finding, particularly over such a large geographic scale; Avise et al. 1987a). Although no direct clock interpretations are available for these data, the pattern of low intraspecific mtDNA variability in Testudines certainly is maintained.

Some caveats should be considered. A substantial portion (one-third to one-half) of the species' range was subject to Pleistocene glacial advances, and northern populations likely represent post-Pleistocene (Holocene) dispersal. Thus the lack of stronger geographic differentiation (though not the low level of haplotype diversity per se) might be explained in terms of a recent range expansion.

Discussion

Low mtDNA Variability and Evolutionary Rate

The mtDNA phylogenies within several species of assayed turtles exhibit considerably lower levels of variability and differentiation than do most other vertebrate and invertebrate species assayed with comparable methods and effort (Avise et al. 1987a). Furthermore, the particular phylogeographic patterns observed indicate that mean microevolutionary rates in mtDNAs of assayed turtles are probably lower, by several fold, than the "standard" mtDNA clock thought to characterize many other higher animals (Wilson et al. 1985). Table 1 summarizes rationales for the conclusion of a mtDNA rate slowdown, and table 4 compiles provisional estimates of the apparent magnitudes of the rate reductions; across the turtle species examined, these reductions are 2–14-fold.

Several points should be made about these inferences. First, although a general slowdown is strongly implicated, we hesitate to propose an exact clock calibration(s)

Table 4

Estimated Magnitudes of Slowdown in Microevolutionary Rate for Turtle mtDNA

Comparison	Expected Time of Divergence ^a (Mya)	Observed Sequence Divergence ^b (%)	Inferred Slowdown in mtDNA Evolutionary Rate ^c
Atlantic green turtle vs. Pacific green turtle	3.0	0.6	10-fold
Olive ridley vs. Kemp's ridley	3.0	1.2	5-fold
Atlantic diamondback terrapin vs. Gulf of Mexico diamondback terrapin ..	0.7	0.1	14-fold
Eastern slider vs. western slider	3.0	0.6	10-fold
Eastern desert tortoise vs. western desert tortoise	5.5	5.3	2-fold

^a From geologic evidence or from comparisons with concordant phylogeographic patterns in nonturtle species.

^b Corrected for within-region polymorphism, as in footnote b of table 2.

^c Relative to 2%/Myr, the "conventional" mtDNA-clock calibration of divergence between a pair of lineages.

for turtle mtDNA, in part because of uncertainties about external evidence for dating of absolute divergence times (see the caveats and counterarguments mentioned above). However, a working hypothesis for rate of mtDNA evolution in Testudines, taken from the mean sequence divergence between pairs of lineages in table 4 is $\sim 0.25\%$ /Myr (compared with the "conventional" vertebrate rate of 2%/Myr). Second, although the evidence for slower mtDNA divergence rates comes from species representing three (of 12) living families of turtles, studies of additional taxa may be desirable to establish the generality of the proposed rate retardation in Testudines. Third, we do not imply that mtDNAs of the examined nonturtle species (or of the various turtle species) necessarily have evolved at either a uniform or a standard pace—indeed, a face-value interpretation of sequence-divergence estimates suggests some heterogeneity within these groups as well (e.g., see both the contrast between the bowfin and the sunfish, in table 3, and the contrasts between the various turtles, in table 4). Fourth, as discussed below, our inferences stem primarily from observations on microevolutionary phenomena in turtles. In the future, it will be of interest to test these rate inferences by further mtDNA comparisons between higher taxa of Testudines for which paleontological or other estimates of relative and absolute divergence times are available.

Why Might Turtle mtDNA Evolve Slowly?

It is not likely the inferred mtDNA evolutionary rates are due to biases in the assay methods employed—since the reported surveys of both turtle and nonturtle species were conducted in the same laboratory, using essentially identical techniques as applied to comparable amounts of genetic information (typically ~ 300 nucleotide positions in recognition sequence per individual, or $\sim 2\%$ of the mtDNA genome). It is unlikely that the trend toward low mtDNA divergence is due to a taxonomic "oversplitting" of turtles relative to other vertebrates, because both the absolute and the relative time calibrations employed derive either from geologic evidence or from comparative phylogeographic patterns across numerous taxa. Thus, even if other taxonomic conventions (e.g., different species or subspecies limits) had been applied to these turtles, the conclusions about mtDNA rate would remain unaltered. Nor is the trend toward low mtDNA differentiation in turtles likely attributable to historical

demographic factors alone. Under neutrality theory, within-population mtDNA variability is expected to reflect the evolutionary effective size (N_e) of the female population (Avise et al. 1988). While low within-region mtDNA polymorphism in turtles (e.g., see tables 2 and 3) could in principle be due to low N_e , the net sequence divergence (after correction for within-population variability) between lineages in isolated populations probably cannot. The most critical lines of evidence for low mtDNA nucleotide substitution rates involve net sequence divergence between allopatric clades.

It is unlikely that the rate slowdown is due to the "saturation effect" that in other vertebrates causes a plateau in mtDNA divergence levels between taxa separated for more than about 15–20 Myr (Brown et al. 1979). All separations reported in the present paper are well within the initial portion of the divergence curve, where sequence differences are expected to be linearly related to time. Finally, the appearance of a slowdown is not due to the possibility that splits in the mtDNA gene genealogy significantly predate the population separations (Tajima 1983; Neigel and Avise 1986; Nei 1987; Pamilo and Nei 1988). Any bias stemming from failure to recognize this phenomenon would operate counter to proposals of a rate slowdown (by generating larger rather than smaller genetic distances between extant forms).

What, then, might account for a comparative mtDNA rate slowdown in the Testudines? In general, the following two classes of explanation have been advanced for the normal *high* rate of evolution in other vertebrate mtDNA (Wilson et al. 1985): (1) relaxation of functional constraints that is due to a postulated tolerance of diminished accuracy in the transcription or translation apparatus and (2) inefficiency of mtDNA repair mechanisms. As to the first possibility, it is not obvious why turtle mtDNA might be more tightly constrained in terms of function, particularly since turtles have a comparatively low metabolic rate (Gibbons 1987). One possibility is that the fraction of mtDNA sites free to experience a high rate of substitution is, for some reason, much lower in turtles, such that the plateau at which the molecule becomes saturated with nucleotide substitutions is reached more quickly than it is in other vertebrates. Such an explanation has been suggested for the low mtDNA distances observed in Hawaiian *Drosophila*, where only ~8% of all sites (rather than the 20% in mammals) evolve rapidly (perhaps because of a compositional constraint associated with low G-C content; DeSalle et al. 1987). However, we doubt that a similar phenomenon could itself account for observations reported in the present paper, because the sequence-divergence estimates for populations of Testudines considered were well below even this plateau.

The second class of explanation involves efficiency of mtDNA repair mechanisms. It is conceivable that turtles might have more efficient enzymes for mtDNA replication and error correction, resulting in a slower rate of sequence evolution. This possibility has been suggested to account for the low rate of sequence evolution in plant mtDNA relative to animal mtDNA (Palmer and Herbon 1988). However, such hypotheses for turtles (and plants) are, at present, entirely *ad hoc*.

One intriguing correlate of the postulated slow rate of turtle mtDNA evolution is the long generation length of most species of Testudines (Gibbons 1987). For example, the age of first reproduction in most marine turtles is probably at ≥ 20 –30 years (Zug et al. 1986), which is among the oldest known for any vertebrate. A longstanding debate in the neutrality theory of molecular evolution concerns whether evolutionary rates in nuclear DNA are influenced by a "generation-time effect" (Kohne 1970; Wilson et al. 1977). In organisms with short generations, there are probably more

germ-line DNA replications per year—and hence more opportunities for mutational errors in sidereal time. Sibley and Ahlquist (1987) review evidence that delayed maturity is indeed negatively correlated with average rate of genomic evolution in mammals and birds. Nonetheless, the mode of mtDNA replication in germ cells differs from that of nuclear DNA, and it need not be coincident with cell divisions (Rabinowitz and Swift 1970). Little is known about numbers of mtDNA replications in the germ lines of organisms with differing generation lengths, but such information might prove particularly relevant to these arguments. In any event, the generation-time effect cannot be the only factor influencing rates of mtDNA and scnDNA evolution, because varying ratios of mtDNA/scnDNA substitution rates apply to different animal groups (Powell et al. 1986; Vawter and Brown 1986; Caccone et al. 1988).

A second intriguing correlate of the postulated slow rate of mtDNA evolution in Testudines is the low metabolic rate of turtle species. A. P. Martin and S. R. Palumbi (personal communication) recently uncovered empirical evidence for a strong positive correlation between pace of metabolism and DNA substitution rate (both nuclear and mitochondrial), across selected vertebrates. They note both that sharks with low metabolism show a 5–10-fold slowdown in mtDNA evolutionary rate relative to that in primates (thus the mtDNA rates for sharks and turtles appear roughly similar) and that rates of mammalian mtDNA evolution are themselves correlated with metabolic pace. These results may be due to an association between levels of oxygen utilization and frequency of DNA damage caused by oxygen radicals (Richter et al. 1988).

Some other molecular traits also appear to evolve slowly in turtles. Chromosome characters are remarkably conservative in Testudines (Bickham and Baker 1979; Bickham 1984), and in some taxa chromosome-arm homologies inferred from colinear G-band patterns have been conserved over >200 Myr (Bickham 1981)! In terms of allozyme evolution, Bonhomme et al. (1987) concluded from a global survey of green turtle rookeries that “the biochemical rates of evolution in *C. mydas* are intrinsically low.” [Other reports nonetheless show, for various Testudines, allozyme-variability estimates that are close to the average vertebrate values (Smith et al. 1977; Scribner et al. 1986)]. Many turtle groups are also conservative in morphological evolution (see Bickham 1984). Although it might be tempting to hypothesize some causal connections between evolutionary rates at these various levels, the known mosaic nature of chromosomal, molecular, and organismal evolutionary rates among other vertebrate groups (e.g., see Wilson 1976) is a sobering reminder of the danger of overly facile conclusions. Evolutionary forces as well as molecular mechanisms underlying rates of evolution in traits as diverse as mtDNA, karyotype, and morphology surely must differ.

Clearly, we cannot yet explain the apparent trend toward a reduced microevolutionary rate for mtDNA in turtles. As stressed by Moritz et al. (1987), although a standard calibration for vertebrate mtDNA is often employed to attach provisional dates to separation events, the evidence for a uniform rate actually is quite limited, both because of uncertainties in external dating information and because of experimental errors in the sequence-divergence estimates themselves. Some of these same concerns apply to the current synopsis, yet we find the consistency in the evidence from a wide variety of turtle species and evolutionary settings especially noteworthy. Perhaps these observations will contribute to the ultimate goal of understanding the processes governing lineage-specific differences in rates of genetic evolution.

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