mechanisms combined with the locations of all known events in the area. It suggests that the triangular area between the plate boundary, the reactivated fracture and a fault to the north on which an earthquake of magnitude ($M_s = 7.0$) occurred in 1982 (refs. 9,13; Fig. 3*b*) is being 'squeezed' towards the north and west. This triangular feature is clearly seen in a map of the anomaly in free-air gravity[14].

Finally, the Macquarie Ridge earthquake is the clearest example I know of termination of an oceanic fault rupture by a 'geometric barrier'[15]. The aftershocks on the main fault end very abruptly in the south in a cluster, which is located at the point where the bathymetry shows a clear break in the Macquarie Ridge. The ridge is offset to the west by more than 20 km here and the trend changes from north-northeast to the north of this break to nearly north-south to the south of this break. □

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Rates of mitochondrial DNA evolution in sharks are slow compared with mammals

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THE rate of mitochondrial DNA (mtDNA) evolution has been carefully calibrated only in primates[1]. Similarity between the primate calibration and rates estimated for other vertebrates[2-4] has led to widespread assumption of a constant molecular clock in vertebrates even though this has never been rigorously tested[5]. We report here the examination of mtDNA sequence variation for 13 species of sharks from two orders that are well represented in the fossil record to test the constancy hypothesis. Nucleotide substitution rates in the cytochrome *b* and cytochrome oxidase I genes in sharks are seven- to eightfold slower than in primates or ungulates. This difference in substitution rate cannot be explained by nucleotide composition bias, codon-usage bias, selection, or choice of genes sequenced, and was confirmed by comparing species recently separated by the rise of the Isthmus of Panama. Such differences in mtDNA substitution rates among taxa indicate that it is inappropriate to use a calibration for one group to estimate divergence times or demographic parameters for another group. High-resolution studies of molecular evolutionary rates require taxon-specific calibrations.

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TABLE 1 Regression analysis of the number of transversions per site against origination and first appearance time for lineages of sharks and primates

Group	Rate ($\times 10^{-10}$ yr^{-1})	r^2	95% CI ($\times 10^{-10}$ yr^{-1})	Rate through the origin ($\times 10^{-10}$ yr^{-1})
Origination times				
Carcharhinoids only*	6.3	0.86	2.8, 9.9	7.2
Lamnoids only†	8.2	0.84	3.2, 13.3	8.1
All sharks‡	7.1	0.86	5.0, 9.1	7.8
First appearance times (from Fig. 3*a*)				
All sharks*	7.0	0.68	3.6, 10.0	9.2
Primates†	64.7	0.90	25.4, 100.0	50.1

Primate analysis was based on the ND4 and ND5 genes[1] using the same methods as for sharks (see Fig. 2 legend). Macaque was used to root the least-squares topology for primates. Origination and first appearance times are given in Fig. 1 for sharks and in Fig. 3*a* legend for primates.
† $P < 0.05$; * $P < 0.01$; ‡ $P < 0.001$.

Using the polymerase chain reaction (PCR), sections of 855-1,551 base pairs (bp) from the cytochrome *b* and cytochrome oxidase I protein-coding genes were amplified and sequenced from 13 species of sharks representing 12 distinct lineages. To minimize *a priori* the influence of selection in measurements of molecular differences, we focused on substitutions at 4-fold degenerate sites. We first considered only transversion substitutions because they accumulate linearly over time[4] and thus are relatively free from saturation effects[6,7].

First appearance times of each lineage were compiled from the known fossil record (Fig. 1). From these data, we estimated evolutionary rates in two ways. First we used the first appearance of a taxon in the fossil record as the minimum time for evolutionary change along that lineage. Second, we overlaid the inferred topology of relationships from phylogenetic analysis of mtDNA sequence data on the stratigraphic record of first appearance times (Fig. 1). Origination and divergence time of taxa were taken to be the oldest fossil occurrence of either lineage. The inferred phylogeny and the available fossil record are largely concordant.

We estimated rates of nucleotide substitution in two ways. First, we estimated the number of transversion changes along each lineage (Fig. 2) and plotted these values against first appearance in the fossil record for that lineage (Fig. 3*a*) and against origination times. Substitution rates estimated from first appearance and origination times are similar for the two orders of sharks and are at least five times slower in sharks than in mammals (Table 1). Second, we plotted the average corrected number of transversion differences between species against divergence times (Fig. 3*b*). Divergence rates estimated in this way are about six- to sevenfold slower in sharks than in ungulates or primates.

To evaluate whether transitions also show a slower rate of accumulation in sharks, we compared complete cytochrome *b* sequences from two individuals of populations of the bonnethead shark (*Sphyrna tiburo*) separated by the Isthmus of Panama. Migration of these warm-water marine sharks between the Pacific and Atlantic is unlikely either around the tip of South America or through the freshwater Panama canal. Thus, the two populations were probably separated about 3.5 Myr ago[8]. The bonnetheads are 8% different (corrected for multiple hits[9]) at fourfold sites, suggesting a maximum divergence rate of 2.3% per million years. By comparison, chimpanzee and human mtDNAs differ by 27% at silent sites after about 5 Myr divergence, giving a divergence rate of 5.4% per million years.

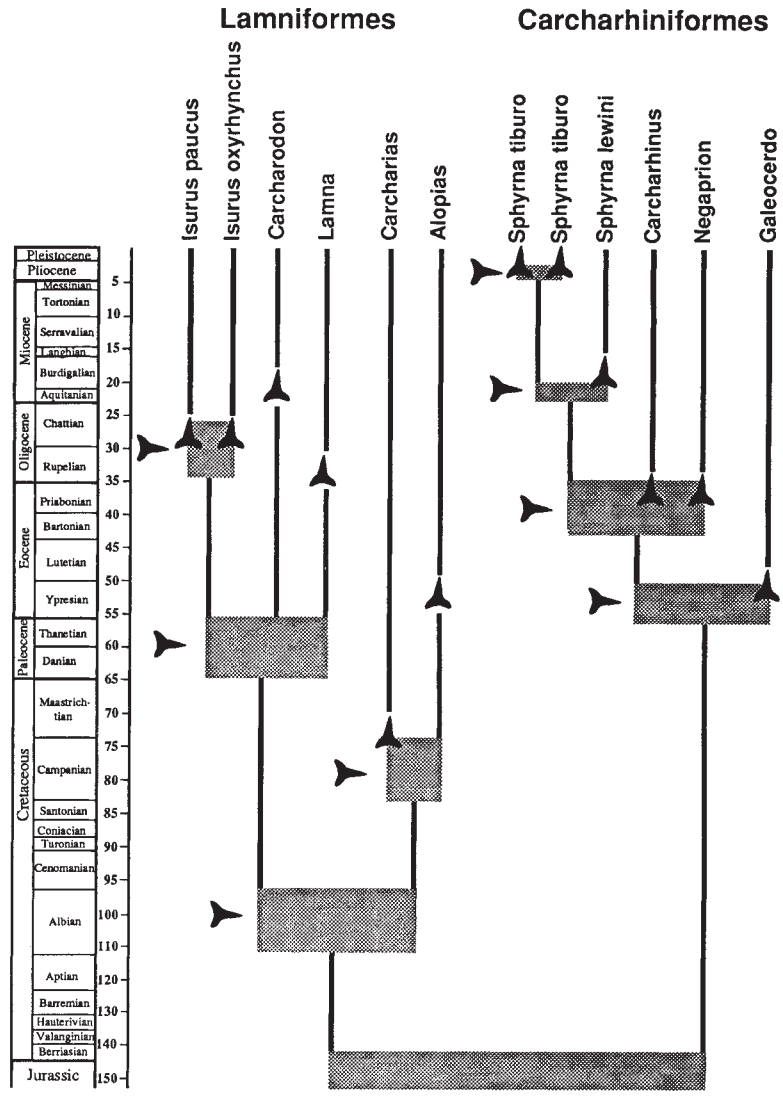

FIG. 1 Phylogenetic relationships among extant sharks and the stratigraphic record of first appearance times based on the occurrence of distinctive fossil teeth. Represented taxa are *Carcharhinus porosus* (smalltail shark), *Negaprion brevirostris* (lemon shark), *Galeocerdo cuvieri* (tiger shark), *Sphyrna lewini* (scallopped hammerhead), *S. tiburo* (bonnethead), *Isurus oxyrhynchus* (shortfin mako), *I. paucus* (longfin mako), *Carcharodon carcharias* (white shark), *Lamna nasus* (porbeagle), *Alopias superciliosus* and *A. vulpinus* (bigeye and common thresher, respectively), and *Carcharias tausus* (sand tiger shark). Phylogenetic relationship among species in each order was established using maximum-likelihood analysis and least-squares cluster analysis of the transversion differences between taxa (see Fig. 2) using the computer program PHYLIP[30]. On the basis of maximum-likelihood analysis, branch lengths not much different from zero were collapsed. The inferred topologies of relationships in each group are corroborated by previous hypotheses based on analysis of morphology[23,24] and isozymes[25]. Upright shark teeth identify the first appearance of lineages in the fossil record. Sideways shark teeth identify minimum origination and divergence times based on the inferred phylogenetic relationships. First appearance times (in millions of years ago) and references are as follows: *Isurus oxyrhynchus* and *I. paucus*, ⩾30 (ref. 26); *Carcharodon*, 20–23 (ref. 27) (however, the *Carcharodon* lineage may have originated from *Paleocarcharodon* in the Paleocene[27,28]); *Lamna*, 30–35 (ref. 27) (however, *Lamna* may have originated from *Cretolamna* sometime in the Late Cretaceous–Early Paleocene[27,28]); *Alopias*, 50–56 (refs 26, 28) (Alopiidae is also known from the Cenomanian, 90–97 Myr, although this date is uncertain[27]); *Carcharias* (=*Odontaspis*), 74–83 (ref. 27); *Sphyrna*, 20–23 (ref. 27); *Carcharhinus*, Middle Eocene 38–50 (ref. 27); *Negaprion*, Middle Eocene 38–50 (ref. 27); *Galeocerdo*, 50–56 (ref. 27). The divergence between *Sphyrna*, *Carcharhinus*, and *Negaprion* occurred by Late Eocene time[10] (38 Myr). Origination times for the two lineages of *Sphyrna tiburo* assume that the divergence of these geminate taxa coincided with the rise of the Isthmus of Panama in the Pliocene[8]. The divergence time between *Alopias* and *Carcharias* is based on the first appearance date for *Carcharias*, although the divergence may be more ancient than this.

A similar rate is obtained for ungulates based on comparison of sheep and goat. These data confirm that sharks have a slower mtDNA substitution rate than primates and ungulates.

The reduced rate of mtDNA evolution in sharks could be an artefact of an incomplete or misinterpreted fossil record. First occurrences of a taxon in the fossil record will only underestimate its age; therefore Fig. 3*a* provides a maximum rate of change consistent with the data. Yet this maximum is still substantially lower than in mammals. To bring our data into line with the mammalian rate would require that first appearance times for 12 distinct lineages from two orders of sharks are overestimated by five to seven times. Because the fossil record of shark teeth is abundant and reasonably continuous, such systematic bias is unlikely[10]. Voluminous fossil, biochemical and molecular data[11] suggest that the primate data are also correct.

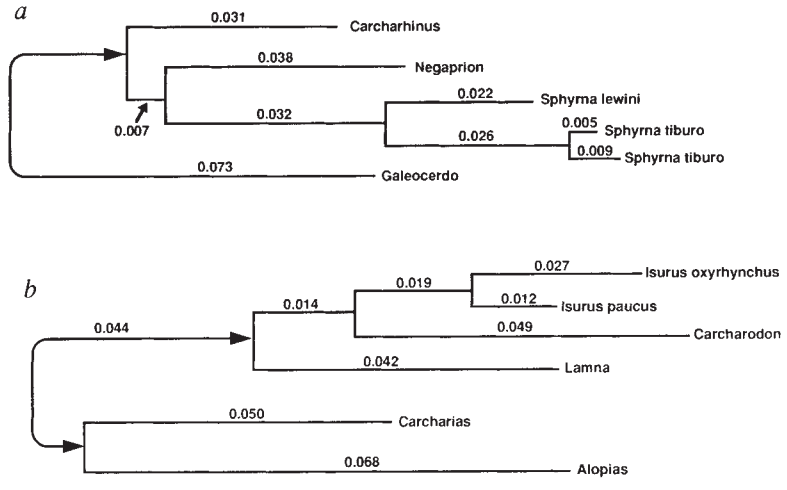

FIG. 2 Topologies of the inferred relationships among carcharhinoid (*a*) and lamnoid (*b*) sharks. The topologies were constructed from matrices of uncorrected transversion differences per 4-fold degenerate site using the FITCH algorithm in PHYLIP[30]. Branch lengths are given. (Similar branch lengths were obtained when we used the neighbour-joining algorithm.) Topologies for each order were rooted using species from the other order as an outgroup, but branch lengths were determined for each order independently of the other. The branch length for the *Alopias* lineage is the median of two species, *Alopias superciliosus* and *A. vulpinus*. Primer sequences and protocols for the extraction, amplification and sequencing of DNA are available in ref. 31. Sequences and EMBL accession numbers available from A.P.M.

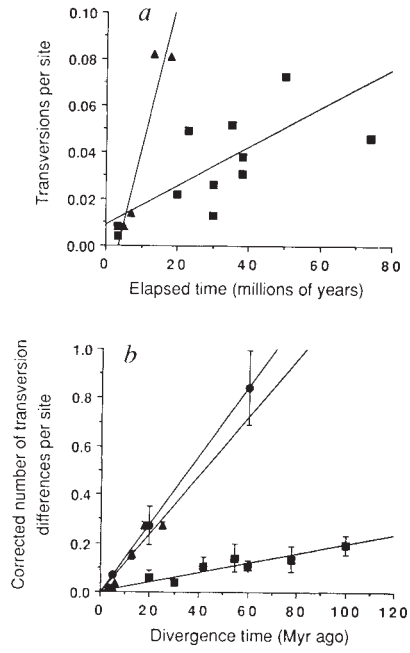

FIG. 3 *a*, Number of transversions per 4-fold degenerate site in independent shark (■) and primate (▲) lineages plotted against first appearance times of the lineages. Number of transversions per site per lineage estimated using least-squares analysis (see Fig. 2 and legend). First appearance times for sharks are from Fig. 1. Regression statistics are given in Table 1. All primate data are from ref. 1 and using first appearance and divergence times of 5 Myr, chimp against human; 6 Myr, gorilla against chimp/human; 13 Myr, orangutan against hominoids; 18 Myr, gibbon against great apes; 25 Myr, macaque against higher primates. *b*, Graph of the average corrected number of transversion differences per 4-fold degenerate site in pairwise comparison against divergence time (divergence times for sharks from Fig. 1). Errors bars are ±2 s.d. of the mean from all pairwise comparisons. ●, Ungulates; ▲, primates; ■, sharks. Corrected numbers of transversions per site were estimated using the equation, $K = 0.5 \ln(1/1-2Q))$ (ref. 9), where Q is the proportion of transversions. Ungulates are represented by the divergences between sheep and goat (5 Myr), cow versus deer/sheep/goat (20 Myr), and Suidae versus Ruminantia (60 Myr)[4]. The regressions are through the origin and yielded average evolutionary rates of 70, 60 and 10×10^{-10} transversions per 4-fold degenerate site per million years for ungulates, primates and sharks, respectively.

Molecular divergence in sharks could be slow, despite a high substitution rate, if constraints on nucleotide composition are more severe in sharks than in mammals[12]. Nucleotide bias can be estimated as $B = \sum |0.25 - f_i|/1.5$, where f_i is the frequency of the ith base at 4-fold degenerate sites. Sharks and primates have similar biases: average (±1 s.d.) B values are 0.42 ± 0.07 and 0.41 ± 0.04, respectively, suggesting that the reduced rate in sharks is not due to enhanced compositional constraints. Codon usage patterns are also similar, with an average of 3.7 different codons used across all 4-fold degenerate codons in the sequences from sharks and mammals. Furthermore, because we are sampling 4-fold degenerate sites, it is unlikely that the rate differences are the result of differences in selection or are an artefact of the genes sequenced and compared[13].

Various hypotheses have been proposed to explain rate heterogeneity in nuclear genes, including differences in generation time[14], DNA repair efficiency[15] and exposure to mutagens[16]. Sharks[17] and primates have similar generation times, indicating that this hypothesis may not extend to mitochondrial genomes. If rate heterogeneity reflects differential repair efficiency, then perhaps mammals have lost a major repair pathway or sharks possess replication machinery with remarkable fidelity. Finally, mtDNA damage *in vivo* is proportional to metabolic rate[18] because oxygen radicals are potent intracellular mutagens[19]. The evolutionary implication of these measurements is that taxa with low weight-specific metabolisms might show slower mtDNA evolution. The metabolic rate in shark tissues is 5–10 times lower than in mammals of similar body size[20]. Reduced mtDNA evolution suggested for other poikilothermic taxa[21,22] provides additional, emerging support for a link between metabolic physiology and mtDNA evolution.

The rate discrepancy between sharks and mammals has important implications for the comparative use of molecular data in systematics, population biology and molecular evolution. First, estimates of divergence times based on molecular differences and an inappropriate calibration will be wrong. Here the error would be almost 10-fold. Second, estimates of genetically effective population size are based on a comparison of observed genetic diversity and the expected rate of change. If the wrong calibration is used, estimates of effective population size and inferences of the demographic history of populations could be severely in error. Third, efforts to identify physiological, life-history or environmental effects on evolutionary rates must be based on careful calibration of these rates in different taxa. Our demonstration of a rate difference in sharks can be considered as a first step in the elucidation of such effects on rates and patterns of vertebrate mtDNA evolution. □

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