The Relative Rate of DNA Evolution in Primates

Simon Easteal
Human Genetics Group, John Curtin School of Medical Research,
The Australian National University

In 73 relative-rate tests involving the sequences of 17 genes between humans and six nonhuman primate taxa, there is only one significant \((P < 0.01)\) difference in evolutionary rate—i.e., that between human and Old World–monkey \(\psi\eta\)-globin genes. No evolutionary rate difference between humans and Old World monkeys is evident from analysis of 18 other genes with a total length of 6 kb. This and the comparison, between humans and other primate taxa, of new extended \(\psi\eta\)-globin sequences suggest that earlier observations of evolutionary-rate differences between humans and other primates were based on differences that are peculiar to \(\psi\eta\)-globin and that are not representative of the whole genome, which appears to be evolving at a stochastically uniform rate. This is supported by whole-genome single-copy DNA and mitochondrial DNA comparisons, neither of which shows any evidence of evolutionary-rate variation among primate taxa. Uniformity in the evolutionary rate of the DNA of primate and other mammalian taxa is inconsistent with current mammalian fossil-record interpretation. Either there has been a general slowing down in rate across lineages or the fossil record has been misinterpreted.

Introduction

DNA and protein differences between humans and other apes are less than those expected for the accepted mammalian divergence times when a constant rate of molecular evolution is assumed. Two alternative explanations have been proposed. Either, as first suggested by Goodman (1961), the rate of molecular evolution is slower in primates—and in hominoids in particular—than in other mammals, or, as originally argued by Sarich and Wilson (1967), the fossil record has been misinterpreted in deriving divergence times. The former explanation has been the more widely accepted in recent years (Goodman 1985; Britten 1986; Li and Tanimura 1987; Li et al. 1987; Sibley and Ahlquist 1987), although the latter has retained some support (Hasegawa et al. 1987; Wilson et al. 1987; Easteal 1988, 1990).

The issue can only be resolved by testing for rate variation in a way that does not depend on fossil-record interpretation. One approach is the relative-rate test of Wu and Li (1985), in which the evolutionary rates of two species are compared relative to a third, more distantly related reference species. With this approach some knowledge of phylogeny is necessary because a reference species must be chosen that is the most distantly related species of the three being compared. Beyond this, however, no assumptions need be made about species divergence times.

The relative-rate approach can readily be applied to primates, among which the branching order of strepsirhines, New World monkeys, Old World monkeys, and then

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Address for correspondence and reprints: Simon Easteal, Human Genetics Group, Division of Clinical Sciences, J.C.S.M.R., A.N.U., G.P.O. Box 334, Canberra ACT 2601, Australia.

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the apes branching in the order of gibbons, orangutans, and finally the African apes is now well established.

The branching order of the African apes remains controversial, but a branching of gorillas before humans and chimpanzees is now indicated by whole-genome, non-repetitive DNA-DNA hybridization studies (Sibley and Ahlquist 1984, 1987; Felsenstein 1987; Caccone and Powell 1989), two-dimensional protein electrophoresis (Goldman et al. 1987), and DNA sequence data (Fitch et al. 1988; Holmquist et al. 1988; Miyamoto et al. 1988; Goodman et al. 1989; Koop et al. 1989; Williams and Goodman 1989).

In the present paper I apply the principle of the relative-rate test to nuclear and mitochondrial DNA sequences differences among primate taxa and also test for evidence of rate variation in the whole-genome DNA-DNA hybridization data. In so doing I demonstrate that, with two exceptions, there is among primate lineages no evidence of variation in rate of DNA evolution—and thus no evidence of a general slowing down of rate in the lineage leading to humans.

Material and Methods

DNA sequences were obtained either from the EMBL database (1989, release 19) or directly from the published literature. Formal relative-rate tests were performed by the method of Wu and Li (1985). Alignment of noncoding sequences was by an iterative multiway procedure using the computer program ALIGN supplied by D. Smith (Australian National University), except where published multiway sequence alignments were already available. Rates of synonymous and nonsynonymous sites were estimated by the method of Li et al. (1985) by using a computer program supplied by W.-H. Li (University of Texas). Correction for multiple substitutions in noncoding sequences was made by assuming a Poisson distribution (Jukes and Cantor 1969).

Evolutionary-rate variation for whole-genome single-copy DNA was assessed using the F-ratio test (Felsenstein 1984). This test compares the sum of squares (SS) of the phylogenetic trees with the least-squares topology constructed with and without the constraint that branch lengths are equal. Least-squares topologies were obtained by using the algorithms FITCH (branch lengths unconstrained) and KITCH (branch lengths constrained) from the phylogenetic reconstruction package PHYLIP (J. Felsenstein, University of Washington).

Results

Nuclear DNA Sequences

Table 1 shows the results of relative-rate tests for both the sequences analyzed by Li et al. (1987) and for strepsirhine sequences and other sequences, including the expanded region of the \( \gamma\)-globin gene (Fitch et al. 1988; Miyamoto et al. 1988; Goodman et al. 1989), published since Li et al.'s analysis. Additional genes were included in the analysis only if a suitable reference sequence—i.e., an orthologue of a more distantly related primate species in human-ape comparisons or an orthologue from another order of eutherian mammal in human—Old World—monkey comparisons—was available. For alcohol dehydrogenase a closely related human paralogue was used. The involucrin gene sequences (Djian and Green 1989a, 1989b; Teumer and Green 1989; Tseng and Green 1989) were not included because of perceived alignment problems resulting from the repetitive nature of the gene sequence. Minor discrepancies between the results in table 1 and the results of comparisons of the same genes between the same taxa reported by Li et al. (1987) arise either from differences
in reference-species selection or sequence alignment procedures or from expansion of
the length of sequence or inclusion of sequence gaps in the analysis. In only one
comparison is such a discrepancy significant. Li et al. (1987) reported that between
humans and New World monkeys there was a significant difference in the rate of \( \psi \eta \)-globin gene evolution. In the present analysis, which is based directly on the results
reported by Fitch et al. (1988), no such difference is apparent.

A total of 73 comparisons involving 17 genes and six nonhuman primate taxa
were made. In only one of these comparisons is there a significant \((P < 0.01)\) rate
difference between humans and the other compared species. This comparison is that
between human and Old World–monkey \( \psi \eta \)-globins. This comparison involves nearly
8 kb of sequence, in addition to the 2 kb for which Li et al. (1987) also reported a
significant \((P < 0.01)\) difference in rate. For the orangutan, gorilla, and chimpanzee
\( \psi \eta \)-globin comparisons, the same 8 kb of additional sequence is also available (Mi-
yamoto et al. 1988). In the comparisons of these extended sequences the differences
in rate are all substantially less than those obtained from the shorter sequences (Li et
al. 1987). The orangutan and human rates are now identical. In the human-gorilla
comparison, where no other additional data were included, the difference for the \( \psi \eta \-
globin comparison changes the result for the comparison of all genes combined. Where
previously there appeared to be a slower rate of evolution in the human lineage,
significant at the 0.01 level, there is now no evidence of any overall rate difference
between humans and gorillas.

In the rate difference among apes the reduction associated with the increased
length of \( \psi \eta \)-globin sequence suggests that the original observation of significant rate
differences may be due to sampling error and that the difference is confined to the
8 kb region originally analyzed. For the human—Old World–monkey comparison, when
the \( \psi \eta \)-globin sequence, which comprises \( \sim 63\% \) of the compared nucleotides, is re-
moved, although the value of \( K_{13} - K_{23} \) is still positive there is no significant difference
in rate for the remaining 18 genes, which have a total length of 6 kb \((K_{12} = 8.0; K_{13} -
K_{23} = 0.8 \pm 0.5)\).

Overall \( K_{13} - K_{23} \) has a positive value, indicating a slower rate of evolution in
humans in only 52% of all comparisons. In the remaining 48% of comparisons \( K_{13} -
K_{23} \) has either a negative or zero value. There is thus no overall indication of a slowdown
in evolutionary rate in the human lineage.

DNA Thermostability Comparisons

Nuclear nonrepetitive-DNA thermostability data are consistent with the nuclear-
DNA sequence data in showing an absence of rate variation among lineages. Table
shows \( \Delta T_{50}H \) (Sibley and Ahlquist 1987) and \( \Delta T_{m}R \) (Benveniste 1985) values above
the diagonal and \( \Delta T_{m} \) values (Caccone and Powell 1989) below the diagonal.

For the \( \Delta T_{50}H \) data Felsenstein (1987) found no evidence of rate variation among
lineages. Caccone and Powell (1989) noted that this appears also to be the case for the
\( \Delta T_{m} \) data. Application of the "F-ratio test" (Felsenstein 1984) to the \( \Delta T_{m} \) data
confirms this to be the case \((SS FITCH = 0.307; SS KITCH = 0.392; F-ratio = 0.595;
F_{0.05(12,22)} = 2.4)\).

The more complete data sets of Sibley and Ahlquist (1987) and Caccone and
Powell (1989) are restricted to apes and Old World monkeys. The \( \Delta T_{m}R \) values ob-
tained by Benveniste (1985) for strepsirhine—New World–monkey comparisons, al-
though less complete, show no evidence of rate variation between human and either
Old World–monkey or New World–monkey lineages. Old World monkeys and humans
Table 1
Differences in the Numbers of Substitutions/100 Nucleotides, between Human and Other Primate Genes

<table>
<thead>
<tr>
<th>GENE A**</th>
<th>LEMUR</th>
<th>NEW WORLD MONKEY</th>
<th>OLD WORLD MONKEY</th>
<th>PONGO</th>
<th>GORILI</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>K12</td>
<td>K12-K23</td>
<td>K12</td>
<td>K12</td>
<td>K12-K23</td>
</tr>
<tr>
<td>η Pseudogene</td>
<td>28.9</td>
<td>6.0±2.9</td>
<td>11.1</td>
<td>7.9</td>
<td>1.5±0.4**</td>
</tr>
<tr>
<td>Exon silent sites:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Globin (106)</td>
<td></td>
<td></td>
<td></td>
<td>6.1</td>
<td>1.1±3.4</td>
</tr>
<tr>
<td>β-Globin (114)</td>
<td></td>
<td></td>
<td></td>
<td>23.0</td>
<td>-1.0±8.3</td>
</tr>
<tr>
<td>γ-Globin (106/74)</td>
<td>37.8</td>
<td>-3.0±7.8</td>
<td>8.9</td>
<td>2.8±5.6</td>
<td></td>
</tr>
<tr>
<td>δ-Globin (105)</td>
<td></td>
<td></td>
<td></td>
<td>22.1</td>
<td>-1.1±5.8</td>
</tr>
<tr>
<td>ε-Globin (99)</td>
<td>28.5</td>
<td>-11.2±8.8</td>
<td>11.5</td>
<td>3.1</td>
<td>0.0±2.4</td>
</tr>
<tr>
<td>γ-Globin (100)</td>
<td>33.2</td>
<td>16.9±11.0</td>
<td>12.3</td>
<td>3.6</td>
<td>0.6±2.1</td>
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<tr>
<td>Insulin (82)</td>
<td>25.0</td>
<td>-10.6±8.0</td>
<td>18.6</td>
<td>3.1</td>
<td></td>
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<tr>
<td>TGF (276)</td>
<td>8.2</td>
<td>-0.1±3.3</td>
<td></td>
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<tr>
<td>ADH (260)</td>
<td>6.8</td>
<td>2.0±1.8</td>
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<td></td>
<td></td>
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<tr>
<td>Ig (309)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apo A1 (158)</td>
<td>7.9</td>
<td>5.3±4.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apo E (226)</td>
<td>10.6</td>
<td>5.1±3.2</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>EPO (145)</td>
<td>11.2</td>
<td>5.1±5.9</td>
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<td></td>
</tr>
<tr>
<td>Pep (263)</td>
<td>10.5</td>
<td>0.0±3.3</td>
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<td></td>
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<tr>
<td>α1 AT (140)</td>
<td>10.9</td>
<td>6.7±6.8</td>
<td></td>
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<td></td>
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<tr>
<td>POMC (180)</td>
<td>15.6</td>
<td>-6.2±4.2</td>
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<td>Introns:</td>
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<td></td>
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<tr>
<td>α-Globin (237)</td>
<td>4.5</td>
<td>-4.5±2.1</td>
<td>3.1</td>
<td>0.4±1.1</td>
<td></td>
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<tr>
<td>β-Globin (190)</td>
<td>8.2</td>
<td>3.2±3.5</td>
<td>3.7</td>
<td>0.6±1.3</td>
<td></td>
</tr>
<tr>
<td>β-Globin (655/960)</td>
<td>29.7</td>
<td>-5.0±3.1</td>
<td>7.4</td>
<td>2.1±1.2</td>
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</tr>
<tr>
<td>δ-Globin (863)</td>
<td>10.9</td>
<td>1.6±1.7</td>
<td>9.8</td>
<td>-0.4±1.1</td>
<td></td>
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<tr>
<td>ε-Globin (941)</td>
<td>24.4</td>
<td>-2.9±2.2</td>
<td></td>
<td>4.0</td>
<td>0.4±0.8</td>
</tr>
<tr>
<td>Protein</td>
<td>Length (aa)</td>
<td>Rate (K)</td>
<td>Standard Error (SE)</td>
<td>Rate of Substitution (K1-K2)</td>
<td>Standard Error (SE)</td>
</tr>
<tr>
<td>------------</td>
<td>-------------</td>
<td>------------</td>
<td>---------------------</td>
<td>------------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>γ-Globin</td>
<td>974</td>
<td>33.6</td>
<td>±1.4 ± 2.9</td>
<td>13.5 ± 1.6</td>
<td>7 ± 0.7</td>
</tr>
<tr>
<td>Insulin</td>
<td>360</td>
<td>18.8</td>
<td>±6.7 ± 3.8</td>
<td>3.2 ± 1.2</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>IgE</td>
<td>340</td>
<td>2.9</td>
<td>±1.6 ± 1.0</td>
<td>3.0 ± 0.7</td>
<td>0.7 ± 0.6</td>
</tr>
<tr>
<td>Flanking sequences:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Globin</td>
<td>195</td>
<td>5.0</td>
<td>±2.4 ± 2.3</td>
<td>5.0 ± 1.8</td>
<td>0.6 ± 1.5</td>
</tr>
<tr>
<td>β-Globin</td>
<td>400</td>
<td>10.2</td>
<td>±3.2 ± 3.5</td>
<td>3.5 ± 1.5</td>
<td>1.7 ± 1.8</td>
</tr>
<tr>
<td>δ-Globin</td>
<td>288/146</td>
<td>19.4</td>
<td>±5.3 ± 3.5</td>
<td>3.5 ± 1.5</td>
<td>1.7 ± 1.8</td>
</tr>
<tr>
<td>ε-Globin</td>
<td>649</td>
<td>9.0</td>
<td>±2.6 ± 2.6</td>
<td>6.3 ± 1.0</td>
<td>1.0 ± 1.0</td>
</tr>
<tr>
<td>γ-Globin</td>
<td>343</td>
<td>24.1</td>
<td>±6.1 ± 2.7</td>
<td>2.9 ± 0.9</td>
<td>0.2 ± 0.9</td>
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<tr>
<td>Insulin</td>
<td>790</td>
<td>18.6</td>
<td>±3.9 ± 2.5</td>
<td>3.6 ± 1.4</td>
<td>1.7 ± 1.4</td>
</tr>
<tr>
<td>TGF</td>
<td>312</td>
<td>8.1</td>
<td>±1.3 ± 2.2</td>
<td>8.1 ± 1.3</td>
<td>1.3 ± 3.9</td>
</tr>
<tr>
<td>POMC</td>
<td>230</td>
<td>15.6</td>
<td>±4.7 ± 3.9</td>
<td>0.6 ± 0.6</td>
<td>0.1 ± 0.6</td>
</tr>
<tr>
<td>IgE</td>
<td>539</td>
<td>2.7</td>
<td>±0.4 ± 0.7</td>
<td>2.7 ± 0.4</td>
<td>0.1 ± 0.7</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>27.1</td>
<td>±1.3 ± 1.1</td>
<td>13.0 ± 1.4</td>
<td>7.9 ± 0.3**</td>
</tr>
</tbody>
</table>

**NOTE.** The relative-rate test involves a comparison (K1-K2) of the rate of substitution (K) between sequence 1 and a reference sequence (3) and between sequence 2 and the reference sequence. In all comparisons the human sequence is sequence 2; thus negative values of K1-K2 indicate a relatively faster rate of substitution in humans. The reference genes were those used by Li et al. (1987), except for the following: rabbit for all lemur comparisons; human β-globin/rabbit β2-globin for New World–monkey β-globin; lemur for New World–monkey γ-globin; dog/mouse for New World–monkey insulin; rabbit for Old World–monkey α- and δ-globins; lemur for Old World–monkey δ-globin; New World monkey for Old World–monkey γ-globin and insulin; mouse for Old World–monkey TGF (transforming growth factor); Old World monkey for orangutan α- and β-globins; lemur for orangutan ε-globin; New World monkey for orangutan γ-globin; rabbit for Old World–monkey Apo E (apolipoprotein E); human γ ADH (alcohol dehydrogenase) for Old World–monkey β ADH, and pig for Old World–monkey Pep (pepsinogen). In all cases these are the most closely related available sequences. General data sources were GenBank (release 48), EMBL (release 12), Wu and Li (1985), Li and Tanimura (1987), Li et al. (1987), and Easteal (1988). For specific genes, data sources were as follows: Old World–monkey and orangutan α- and β-globin, Shaw et al. (1987); New World–monkey γ-globin, Spritz and Giebel (1988); New World–monkey and Old World–monkey TGF, Sharples et al. (1987); human β-globin, Hsu et al. (1988); Old World–monkey POMC, Patel et al. (1988); human POMC, Takahashi et al. (1981); cow POMC, Nakanishi et al. (1979); human Apo A, Sogawa et al. (1983); Old World–monkey Apo A, Evers et al. (1988); pig Pep A, Tsukagoshi et al. (1988); human ADH, Ikutat et al. (1986); Old World–monkey ADH, Trezise et al. (1989); human Apo E, Breslow et al. (1982); Old World–monkey Apo E, Marotti et al. (1989); and rabbit Apo E, Hao et al. (1987). The results for the gorilla and chimpanzee comparisons, except for γγ-globin, were obtained directly from Li et al. (1987). Estimates of the evolutionary rates of the γγ-globin genes were obtained from Fitch et al. (1988), Miyamoto et al. (1987), and Goodman et al. (1989). The approximate lengths of the γγ-globin sequences compared were as follows: human–chimpanzee, 10,150; human–gorilla, 10,080; human–orangutan, 9,927; human–Old World monkey, 9,417; human–New World monkey, 1,827; and human–strepsirhine, 724.

*Approximate number of sites compared.

** P < 0.01.
Table 2
Thermostability Differences between Primate Taxa

<table>
<thead>
<tr>
<th>Homo</th>
<th>Pan</th>
<th>Gorilla</th>
<th>Pongo</th>
<th>Hylobates</th>
<th>Old World Monkey</th>
<th>New World Monkey</th>
<th>Strepsirhine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homo</td>
<td>1.63</td>
<td>2.27</td>
<td>3.60</td>
<td>4.76</td>
<td>7.34</td>
<td>13.1</td>
<td>24.1</td>
</tr>
<tr>
<td>Pan</td>
<td>1.59</td>
<td>2.23</td>
<td>3.57</td>
<td>4.83</td>
<td>7.21</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Gorilla</td>
<td>2.50</td>
<td>2.55</td>
<td>3.55</td>
<td>4.69</td>
<td>7.18</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Pongo</td>
<td>3.49</td>
<td>3.52</td>
<td>3.57</td>
<td>4.83</td>
<td>7.43</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Hylobates</td>
<td>5.04</td>
<td>4.66</td>
<td>5.15</td>
<td>4.83</td>
<td>7.05</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Old World Monkey</td>
<td>6.78</td>
<td>7.01</td>
<td>7.12</td>
<td>7.33</td>
<td>6.98</td>
<td>13.1</td>
<td>24.5</td>
</tr>
<tr>
<td>New World Monkey</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

NOTE.—Except for those involving New World monkeys and strepsirhine, which are from Benveniste (1985) and have differences estimated as \( \Delta T_{\text{mR}} \), comparisons above the diagonal are from table 5 of Sibley and Ahlquist (1987) and have differences estimated as \( T_{\text{mH}} \). \( T_{\text{mH}} \) values in comparisons with Pan are weighted average values for \( P. \text{paniscus} \) and \( P. \text{troglodytes} \). \( \Delta T_{\text{mR}} \) values are average values for humans compared with six species of New World monkeys and with two species of strepsirhine and for one species of New World monkey compared with two species of Old World monkey. Comparisons below the diagonal are the \( \Delta T_{\text{m}} \) values from Caccone and Powell (1989), with average values for \( P. \text{paniscus} \) and \( P. \text{troglodytes} \) and for \( H. \text{lar} \) and \( H. \text{syndactylus} \).

have identical \( \Delta T_{\text{mR}} \) values (13.1) when compared with New World monkeys. The values for these three taxa (24.5, 24.1, and 24.0, respectively) are extremely similar when compared with those for prosimians, with the human value being intermediate between the other two.

Mitochondrial DNA

The sequence of a 0.9-kb segment of the mitochondrial genome containing three tRNA genes and parts of two protein coding genes has been determined for 12 primate species (Brown et al. 1982; Hasegawa et al. 1987; Hayasaka et al. 1988). Because of the relatively high rate of transition substitutions in mtDNA, it is necessary to separate transitions from transversions in estimating relative rates of evolution (Hasegawa et al. 1987). If this is not done, the saturating effect of the more rapidly occurring transitions might mask among-lineages rate differences that would be evident for transversions. The among-taxa numbers of transversion substitutions, derived from the combined data of Hasagawa et al. (1987) and Hayasaka et al. (1988), are shown in table 3. There is no indication of any rate differences between any of the lineages; the values down each column of the table are extremely similar. In only one case (\( \text{Homo} \) and \( \text{Pan} \) compared with \( \text{Gorilla} \)) is the number of transitions in the human comparison the lowest. In the comparison of \( \text{Homo}, \text{Pan}, \) and \( \text{Gorilla} \) with \( \text{Pongo} \) the human rate is the highest, and in all other comparisons the human rate is within the range of those for other lineages.

Comparison of the human and Old World–monkey mitochondrial cytochrome C oxidase subunit II genes (681 bases) and the cow gene (Ramharack and Deeley 1987) shows a nonsignificantly higher number of silent transversion substitutions in the Old World–monkey comparison (58) than in the human comparison (55)—but the same number (39) of replacement-site transversions. These two regions of mtDNA are thus consistent with the nuclear DNA in showing no evidence of evolutionary-rate variation among primate lineages.
Table 3
Numbers of Transversion-Type Differences between Mitochondrial DNA Sequences of Primate Taxa

<table>
<thead>
<tr>
<th></th>
<th>Pan</th>
<th>Gorilla</th>
<th>Pongo</th>
<th>Hylobates</th>
<th>Old World Monkey</th>
<th>New World Monkey</th>
<th>Strepsirhine* and Tarsiform</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homo</td>
<td>0.55</td>
<td>0.90</td>
<td>3.92</td>
<td>5.03</td>
<td>8.30</td>
<td>10.97</td>
<td>15.51</td>
</tr>
<tr>
<td>Pan</td>
<td>1.01</td>
<td>3.80</td>
<td>4.91</td>
<td>8.18</td>
<td>11.07</td>
<td>15.52</td>
<td></td>
</tr>
<tr>
<td>Gorilla</td>
<td>3.67</td>
<td>5.03</td>
<td>8.07</td>
<td>10.75</td>
<td>15.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pongo</td>
<td>5.83</td>
<td>8.47</td>
<td>12.40</td>
<td>14.49</td>
<td>15.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hylobates</td>
<td></td>
<td>8.63</td>
<td>11.66</td>
<td>14.98</td>
<td>15.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Old World Monkey*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11.44</td>
<td>14.57</td>
<td></td>
</tr>
<tr>
<td>New World Monkey</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15.89</td>
<td></td>
</tr>
</tbody>
</table>

NOTE.—Data are from Brown et al. (1982) and Hayasaka et al. (1988).
* Values are the average values for lemurs and tarsiers.
Values for comparisons involving Old World monkeys are the average for four species of *Macaca*.

Discussion

The results of the present study show that, when no assumption other than their relative order is made about species divergence times, the rates of DNA evolution between humans and other primates appear generally to be stochastically uniform. Since the view that there is a slowing down of the rate of molecular evolution in the human lineage has become so widely accepted, it is necessary to consider other recent studies to examine how this contrary conclusion has been reached.

Some investigators, such as Britten (1986) and Goodman et al. (1982, 1983), have arrived at the conclusion that primate evolutionary rates are variable by making species-divergence-time assumptions on the basis of fossil-record interpretation. In studies of this kind the validity of the fossil-record interpretation is not being tested; it is assumed, and the validity of a finding of rate variation is only as good as this assumption.

There is ample reason for thinking that the mammalian fossil record is not always correctly interpreted. Thus, for example, the presumed polychotomous divergence of eutherian orders is inconsistent with their hierarchical phylogeny indicated by DNA sequence comparisons (Fastao 1988, and accepted); and, more pertinent, the taxonomic status of the fossils *Sivapithecus* and *Aegyptopithecus* has recently been revised, with a resulting dramatic change in the estimated time of divergence of humans from the other African apes (Wilson et al. 1987).

More interesting than fossil record–based studies have been those that have used the relative-rate approach (Li and Tanimura 1987; Li et al. 1987). Li et al. (1987), in an analysis of the relative evolutionary rates of the sequences of 11 primate genes, found that one, the ψη-globin gene and its flanking sequences, has evolved more slowly in humans than in other primates. In 10 other nuclear genes no significant differences were observed. However, the human rate was (nonsignificantly) lower than that of the compared species in 28 of the 36 comparisons. In addition, in comparisons of humans with gorillas, Old World monkeys, and New World monkeys, the rate differences were significant when all genes (including ψη-globin, which constituted ~60% of the compared nucleotides) were combined. The interpretation that these results demonstrate a slower rate of molecular evolution in humans than in other primates depends very much on the difference observed in a single gene, ψη-globin.
With one exception, the apparent human slowdown is not evident in the present analysis for comparisons involving more extensive sequence data for the region surrounding \( \psi \) globin \( (Fitch \ et \ al. \ 1988; \ Miyamoto \ et \ al. \ 1988; \ Goodman \ et \ al. \ 1989) \) or in a total of 68 other sequence comparisons.

Hasegawa et al. \( (1987) \) demonstrated by relative-rate tests that the rate of evolution of a segment of mitochondrial DNA does not vary among the human, chimpanzee, gorilla, orangutan, gibbon, and cow lineages. Hayasaka et al. \( (1988) \) analyzed, in addition to these, the homologous mitochondrial DNA sequences from several Old World monkeys, a New World monkey, and two strepsirhines. They showed by relative-rate test that there is no rate variation among the taxa; however, they concluded that the rate had slowed down in the human lineage. This anomalous conclusion was based on consideration of fossil record-derived divergence times. Analysis of the entire data set presented here, as well as of the cytochrome C oxidase subunit II gene, shows that there is no evidence of rate variation in the evolution of mitochondrial DNA.

This result based on analysis of transversion substitutions evident from DNA sequence data would appear to contradict the earlier finding of Templeton \( (1983a, \ 1983b) \). From analysis of restriction-map data, he concluded that the rate of mitochondrial DNA evolution has been slower in the human lineage than in the combined gorilla-chimpanzee lineage. Nei and Tajima \( (1985) \), however, showed that assumptions underlying Templeton's test for rate variation were invalid and that the molecular clock hypothesis could not be rejected by the data he analyzed. Furthermore, Templeton's analysis was based on a phylogeny that placed chimpanzees and gorillas in a monophyletic group relative to humans, which in the light of the analysis of nucleotide sequence data now seems highly improbable.

Sibley and Ahlquist's \( (1984) \) study of DNA-DNA hybridization is unusual in that it led to the conclusion that the rate of evolution based on fossil record-derived divergence times did not vary among lineages. The reason for this is that different divergence times were assumed. Specifically, an orangutan divergence of 16 Mya was assumed, based on the assumption that Sivapithecus is uniquely ancestral to orangutans. They support their rate-constancy argument by consideration of the previously estimated evolutionary rates of bird DNA, which were assumed to be the same as the presumed primate rates. However, aside from the fact, recognized by Sibley and Ahlquist \( (1987) \), that the bird and mammal rates may be different, the way in which the bird rates were obtained has been questioned \( (Hounde \ 1986, \ 1987) \).

In the analysis of an expanded data set, Sibley and Ahlquist \( (1987) \) conclude that rate does vary among lineages in proportion to age at first breeding. This conclusion, however, is not arrived at through any formal analysis of the data, and Felsenstein \( (1987) \) was unable to detect any rate variation among lineages by analysis of the same data set. Caccone and Powell \( (1989) \) found no evidence of rate variation in their DNA-DNA hybridization study, and that result is confirmed here.

The one exception to the apparent overall pattern of rate uniformity presented here is the \( \psi \) globin gene, which has evolved relatively faster in the Old World-monkey lineage than in the human lineage. This rate difference was observed for a 2-kb sequence by Li et al. \( (1987) \). It is still apparent for the 10-kb sequence now available, indicating that the difference is real and not due to sampling error. The demonstration of a real difference for the \( \psi \) globin region does not, however, mean that a difference exists for the genome generally. There are two reasons for thinking that it does not.

First, there is no evidence of any rate difference between the human and Old World–monkey lineages in the comparison of 6 kb of sequence from 18 genes other
than the $\gamma$-globin gene. Although this is a smaller number of nucleotides than that compared at the $\gamma$-globin gene, it is three times as many nucleotides as were originally sequenced at the $\gamma$-globin gene and which showed a significant rate difference. If that rate difference occurred generally throughout the genome, it would be apparent from comparison of 6 kb of sequence. A sample of 6 kb from 18 genes is a better indicator of overall evolutionary rate than is a sample of 10 kb for one gene. Second, the $\Delta T_{\text{R}}$ values (Benveniste 1985) for the human and Old World–monkey lineages compared with New World monkeys are identical. While further sequence data are needed to resolve the issue conclusively, it appears from the data presently available that $\gamma$-globin is unusual in showing a rate difference and that generally there is rate uniformity between the two lineages. It demonstrates the need for caution in interpreting results obtained from the comparison of sequences in the region of a single gene.

A rate difference has also been demonstrated between two other nonhuman primate lineages. The noncoding sequences of the $\epsilon$, $\gamma$, and $\psi$-globin genes have evolved faster in the galago lineage than in the lemur lineage (Koop et al. 1989). Since this rate difference has been observed at three separate genes and is consistent with the DNA-DNA hybridization results (Bonner et al. 1980), it would appear to be a general property of the single-copy genome. It demonstrates that, even if they are not the general rule, evolutionary-rate differences between lineages do exist.

Two important conclusions can be drawn from the finding that molecular evolutionary rates are generally uniform both among primates as shown here and between primates and other mammalian orders (Easteal 1988, 1990). First, the favored explanation for the presumed slower evolutionary rate in humans is that the rate of molecular evolution is dependent on cell generation time, which is relatively greater in the human lineage (Goodman 1985; Li and Tanimura 1987; Li et al. 1987). The absence of lineage-specific differences in evolutionary rate means that the rate of DNA evolution is not cell-generation-time dependent. As pointed out by Sarich and Wilson (1973), this would suggest that mutations arise predominantly by processes that are independent of DNA replication.

Second, since the discrepancy between the observed and expected degree of nucleotide change between apes and other primates is not explained by substitution-rate differences, it follows that either there has been a general slowing down of molecular evolutionary rate in the different mammalian lineages during the Cenozoic, as proposed by Goodman (1985) and Gingerich (1986), or the divergence times of at least some primate and/or other mammalian taxa are different from those currently proposed; it also follows that the fossil record has been misinterpreted in some way.

The first possibility cannot be ruled out; however, it is difficult to understand how such a slowdown could have occurred. A number of factors have been identified that could cause variation in molecular evolutionary rate; these include natural selection, variation in cell-generation time (Wu and Li 1985; Li et al. 1987), varying efficiency of DNA-repair mechanisms (Britten 1986), and differential exposure to environmental mutagens (Goodman 1985). It seems highly unlikely, however, that any of these could account for a gradual DNA-evolutionary-rate slowdown that affected all lineages equally. Goodman et al. (1975) and Gingerich (1986) have suggested that natural selection might do this with respect to particular genes. An interlineage slowdown, to be consistent with both the relative-rate analysis presented here and that of genes in different mammalian orders (Easteal 1988, 1990), must have affected many
genes as well as noncoding sequences and mitochondrial DNA. Natural selection could not account for such a generalized effect.

Cell-generation time varies among lineages and has been put forward as an explanation for apparent interlineage rate variation. It could not account for a uniform rate change among lineages. Variation in the efficiency of DNA-repair mechanisms also has been put forward to explain apparent evolutionary-rate differences among lineages. It is unlikely that the enzymes involved in DNA repair have independently evolved to be more efficient to the same degree in diverse lineages. Similarly, it is difficult to conceive of an environmental mutagen whose effects could have been uniform on organisms with different life histories and living in different environments and in different parts of the world. The effects of a reduction in incident ultraviolet radiation, for example, would vary with latitude, habitat, morphology, and reproductive behavior and physiology.

Although a uniform interlineage slowdown might be hard to envisage, it may nevertheless have occurred. Distinguishing between it and fossil-record misinterpretation will require more extensive investigation of evolutionary rates in different taxonomic groups by using a combination of the relative-rate approach and fossil record-derived divergence times.

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LITERATURE CITED


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