Molecular Phylogeny and Evolution of Primate Mitochondrial DNA[1]

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We determined nucleotide sequences of homologous 0.9-kb fragments of mitochondrial DNAs (mtDNAs) derived from four species of old-world monkeys, one species of new-world monkeys, and two species of prosimians. With these nucleotide sequences and homologous sequences for five species of hominoids, we constructed a phylogenetic tree for the four groups of primates. The phylogeny obtained is generally consistent with evolutionary trees constructed in previous studies. Our results also suggest that the rate of nucleotide substitution for mtDNAs in hominines (human, chimpanzee, and gorilla) may have slowed down compared with that for old-world monkeys. This evolutionary feature of mitochondrial genes is similar to one found in nuclear genes.

Introduction

In determining the evolutionary course of primates, phylogenetic relationships and divergence times between species have been of particular interest to anthropologists and evolutionary biologists. In the past 20 years, the genetic differences among species of primates have been studied using a variety of molecular methods, such as immunological methods (Dene et al. 1976; Sarich and Cronin 1976), amino acid sequencing of proteins (Goodman 1976), analyses of blood protein polymorphisms (King and Wilson 1975; Nozawa et al. 1977), DNA-DNA hybridization analyses (Bonner et al. 1980; Sibley and Ahlquist 1984, 1987), restriction-enzyme analyses of mitochondrial DNAs (mtDNAs) (Ferris et al. 1981; Hayasaka et al. 1988), and nucleotide sequencing of genes (Brown et al. 1982; Hixson and Brown 1986; Koop et al. 1986; Miyamoto et al. 1987). To estimate divergence times by using nucleotide sequence comparisons, the rate of nucleotide substitution among primate species must be calibrated. However, previous molecular analyses have provided two contradicting views of the rate of molecular evolution in primates, one suggesting that the rate of molecular evolution has slowed down in hominoids (Dene et al. 1976; Goodman 1976; Koop et al. 1986; Sibley and Ahlquist 1987) and the other suggesting that the rate in hominoids has been approximately the same as that in other lineages of primates (Sarich and Cronin 1976). Nucleotide sequence comparisons of nuclear genes support the former view (Koop et al. 1986; Li and Tanimura 1987).

Because mitochondrial genes have higher rates of nucleotide substitution than do nuclear genes (Brown et al. 1979; Brown 1983), restriction-enzyme analyses of mtDNAs have often been used to investigate both the genetic relationships among closely related species and the genetic variability within primate species (Ferris et al.

1. Key words: primates, mitochondrial DNA, phylogeny, molecular evolution, rate retardation.

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Mol. Biol. Evol. 5(6):626–644. 1988.

0737-4038/88/0506-0002$02.00

1981; Hayasaka et al. 1986, 1988; Horai et al. 1986; Cann et al. 1987). However, the rate of nucleotide substitution in primate mtDNAs has only been estimated for hominoids (Anderson et al. 1981; Brown et al. 1982). To estimate rates of nucleotide substitution for mtDNAs in other groups of primates, we determined the nucleotide sequences of homologous 0.9-kb mtDNA fragments from seven species of primates (four old-world monkeys, a new-world monkey, and two prosimians). We then constructed a phylogenetic tree by comparing these sequences with the 0.9-kb mtDNA fragments from five hominoid species (Anderson et al. 1981; Brown et al. 1982). The phylogenetic relationships among primate groups shown by our analyses are generally consistent with results of previous studies (Clark 1963; Simons 1972; Dene et al. 1976; Goodman 1976; Sarich and Cronin 1976; Dutrillaux 1979; Delson 1980; Ferris et al. 1981; Gingerich 1984; Pilbeam 1984; Sibley and Ahlquist 1984, 1987; Andrews 1986; Koop et al. 1986). Our results also revealed that the rate of nucleotide substitution for mtDNAs may have slowed down in hominines (human, chimpanzee, and gorilla) compared with that for macaques. Evolutionary implications of these findings will be discussed.

Material and Methods

Closed circular forms of mtDNAs were purified from liver samples (Hayasaka et al. 1986) from seven species of primates, including Japanese macaque (*Macaca fuscata*), rhesus macaque of Indian origin (*M. mulatta*), crab-eating macaque of Philippine origin (*M. fascicularis*), Barbary macaque (*M. sylvanus*), common squirrel monkey (*Saimiri sciureus*), Philippine tarsier (*Tarsius syrichta*), and ring-tailed lemur (*Lemur catta*). The macaque species belong to old-world monkeys, whereas the squirrel monkey is a new-world monkey and the tarsier and lemur are prosimians.

The 0.9-kb fragments contain genes for three tRNAs ($tRNA^{His}$, $tRNA^{Ser}$, and $tRNA^{Leu}$), a 3′ region of the NADH-dehydrogenase subunit 4 (ND4), and a 5′ region of the ND5. To identify mtDNA fragments that contained the sequence homologous to the 0.9-kb *Hin*dIII fragments of hominoids (Anderson et al. 1981; Brown et al. 1982) for each of the seven species, we used the human 0.9-kb *Hin*dIII fragments labeled with α^{32}P-dCTP (Rigby et al. 1977) as a probe for Southern blot analysis (Southern 1975). This probe detected 0.9-kb *Hin*dIII fragments for Japanese macaque, rhesus macaque, crab-eating macaque, and squirrel monkey; 0.4-kb and 0.5-kb *Hin*dIII fragments for the Barbary macaque; a 1.6-kb *Hin*dIII fragment for the tarsier; and a 2.2-kb *Hin*dIII/*Xba*I fragment for the lemur. The fragments of the tarsier and lemur were inserted into pUC19 plasmid DNAs (Yanisch-Perron et al. 1985), and those of the other species were inserted into M13 mp10 phage DNAs (Messing 1983). To sequence tarsier and lemur mtDNAs, we isolated fragments from pUC19 clones, digested them with restriction enzymes or exonucleases (Henikoff 1984; Yanisch-Perron et al. 1985), and inserted them into M13 mp10. Nucleotide sequences of fragments inserted into M13 mp10 were determined by the dideoxy-chain termination method (Sanger et al. 1977). Most of the sequences were determined for more than one clone or for both strands. Enzymes and reagents used in cloning and sequencing were purchased from Takara Shuzo (Kyoto), Nippon Gene (Toyama, Japan), and Amersham (Buckinghamshire, England).

We aligned the nucleotide sequences of the homologous 0.9-kb fragments of the seven species with those of five hominoid species (Anderson et al. 1981; Brown et al. 1982) and estimated the number of nucleotide substitutions per site between species by using the six-parameter method of nucleotide substitutions (Gojobori et al. 1982).

On the basis of the estimated numbers, a phylogenetic tree was constructed using the neighbor-joining (NJ) method (Saitou and Nei 1987). The numbers of synonymous and nonsynonymous nucleotide substitutions were estimated by the method of Nei and Gojobori (1986). Significance of the rate difference of nucleotide substitution was tested according to the method of Wu and Li (1985).

Results and Discussion

1. Characteristics of Nucleotide Substitutions

Nucleotide sequences for the 12 species are shown in figure 1. Sequence similarity ranges from 67.7% between the human and tarsier to 96.4% between Japanese and rhesus macaques (table 1). Average similarity is 75.3% between hominoids and old-world monkeys, 71.9% between catarrhines (hominoids and old-world monkeys) and the new-world monkey, and 69.6% between anthropoids and prosimians. The latter two values are as low as those between hominoids and mice or cows (Anderson et al. 1981, 1982; Bibb et al. 1981; Brown et al. 1982).

The ratio of the number of transitions to the total number of nucleotide substitutions becomes smaller as the divergence time between the species compared increases (table 1), as noted in previous studies (Brown et al. 1982; Brown 1983). The largest transition ratios were observed among macaque mtDNAs (94.8%), followed by those among hominines (91.8%). The smallest transition ratios were observed between anthropoid and prosimian mtDNAs (50.8%) and between tarsier and lemur mtDNAs (45.3%). These ratios are slightly larger than the corresponding values between hominoids and mice or cows (Brown et al. 1982). Both nucleotide divergence and transition ratios indicate that divergence of >30 Myr is associated with a high level of hidden variation (see below).

2. tRNA Genes

In addition to substitutions, several insertions/deletions were observed in the three tRNA genes contained in the 0.9-kb region. All but one insertion/deletion occurred in the TψC, D—and in extra loops rather than in stem regions where nucleotide changes are more likely to cause structural changes. As has been observed in all mammalian mtDNAs so far sequenced (Anderson et al. 1981, 1982; Bibb et al. 1981; Brown et al. 1982), all of these primate mtDNAs lack the D-arm of $tRNA^{Ser}$. The remaining one insertion/deletion occurred at the boundary between $tRNA^{Ser}$ and $tRNA^{Leu}$ in the tarsier. A deletion at this site was also observed in the mouse tRNA (Bibb et al. 1981).

The anticodons of the three tRNAs (marked by asterisks in fig. 1) were conserved in all species. Moreover, the stem structures (marked by plus signs in fig. 1) were also maintained by more than 10 pairs of compensating nucleotide substitutions. (A pair of nucleotide substitutions are called *compensating* when they do not change an intrastrand base pairing in a stem region of an rRNA of a tRNA.) Thus, the function of the tRNAs seems to have been maintained, in spite of a substantial number of insertions/deletions and nucleotide substitutions (fig. 1).

3. Polypeptide Coding Regions

Figure 2 shows amino acid sequences of the carboxy-terminal region of ND4 and amino-terminal region of ND5 for the 12 species, as deduced from the nucleotide sequences. In general, amino acid sequences of ND4 are better conserved than those of ND5 (table 2).

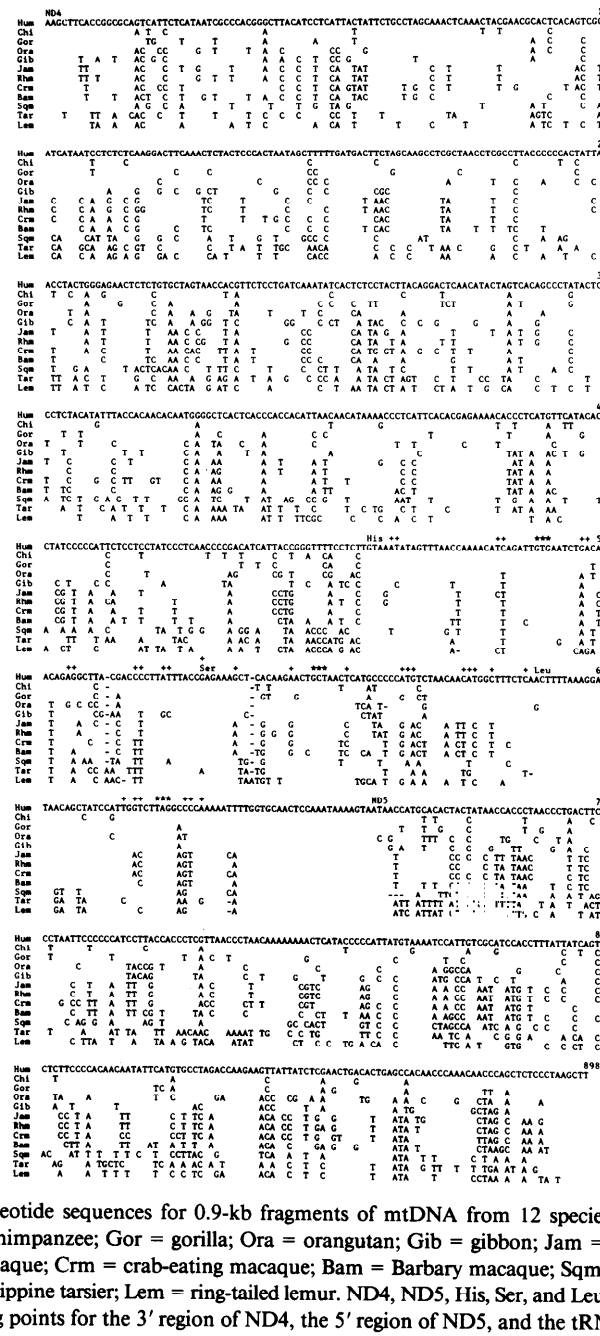

FIG. 1.—Nucleotide sequences for 0.9-kb fragments of mtDNA from 12 species of primates; Hum = human; Chi = chimpanzee; Gor = gorilla; Ora = orangutan; Gib = gibbon; Jam = Japanese macaque; Rhm = rhesus macaque; Crm = crab-eating macaque; Bam = Barbary macaque; Sqm = common squirrel monkey; Tar = Philippine tarsier; Lem = ring-tailed lemur. ND4, ND5, His, Ser, and Leu above the sequences indicate the starting points for the 3′ region of ND4, the 5′ region of ND5, and the tRNA^{His}, tRNA^{Ser}, and tRNA^{Leu} genes, respectively. The nucleotide sequence for the human is derived from Anderson et al. (1981). Those for the four species of apes are derived from Brown et al. (1982). The whole nucleotide sequence for the human is shown on the top. For the other species, only nucleotides different from those in the human sequence are shown. A gap is denoted by a dash (-). Triple asterisks (***) represent anticodons of the three tRNAs. Plus signs (+) indicate the sites where compensating nucleotide substitutions are observed.

Table 1

Percent Similarity of Nucleotide Sequences (above the Diagonal) and Proportion of Transitional Nucleotide Substitution (below the Diagonal) between Species

	Species Compared											
Species Compared	Human	Chimpanzee	Gorilla	Orangutan	Gibbon	Japanese Macaque	Rhesus Macaque	Crab-eating Macaque	Barbary Macaque	Squirrel Monkey	Tarsier	Lemur
Human		91.2	89.7	84.0	81.9	76.8	76.7	75.1	74.4	72.7	67.7	69.2
Chimpanzee	93.7		89.4	82.9	81.1	75.8	75.0	73.3	75.2	71.7	67.9	69.2
Gorilla	91.3	90.5		83.4	81.1	76.5	76.6	73.9	75.7	73.0	68.7	70.8
Orangutan	75.5	77.8	77.9		81.2	75.6	75.4	74.0	76.1	72.0	69.8	71.1
Gibbon	72.2	74.0	73.4	69.0		75.4	76.2	74.4	75.9	73.2	69.2	70.5
Japanese macaque	64.4	66.4	65.9	65.6	65.0		96.4	91.6	87.6	71.1	68.6	71.8
Rhesus macaque	64.1	67.0	65.2	65.5	63.4	96.9		90.7	88.1	70.7	68.3	71.1
Crab-eating macaque	66.8	69.5	69.2	67.8	66.4	94.7	96.4		87.7	71.3	68.9	70.2
Barbary macaque	67.7	67.1	67.0	64.0	64.4	92.8	93.5	94.5		71.3	68.1	71.3
Squirrel monkey	59.8	60.9	60.2	55.6	56.5	60.5	60.7	60.2	60.2		67.8	71.5
Tarsier	53.3	52.6	53.2	51.9	52.7	52.7	52.7	52.9	52.6	48.4		74.8
Lemur	48.4	48.7	47.1	50.0	47.9	50.0	50.8	52.6	50.0	46.9	45.3	

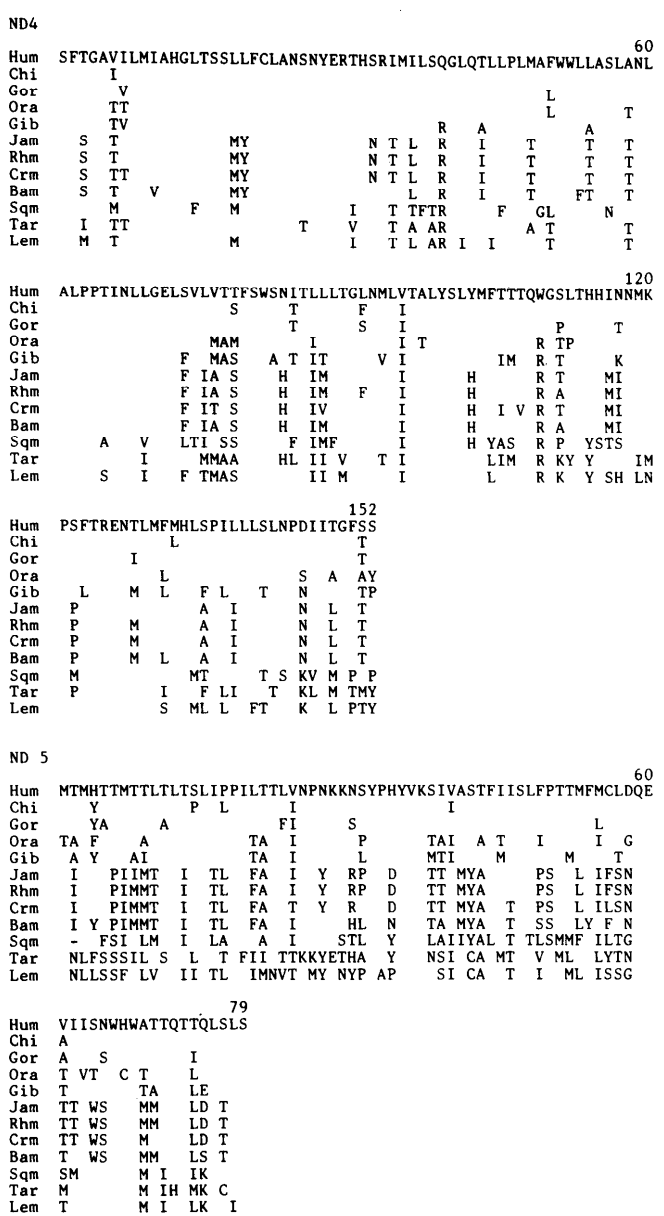

FIG. 2.—Amino acid sequences for the carboxy-terminal region of ND4 and amino-terminal region of ND5, corresponding to nucleotides 2–457 and 660–896 (fig. 1), respectively, from the 12 species deduced from the nucleotide sequences. Amino acids are translated according to the genetic code table of Anderson et al. (1981) and represented by single letters. Abbreviations for the species are the same as those in fig. 1. The whole amino acid sequence for the human is shown on the top. For the other species, only amino acids different from those in the human sequence are shown. A gap is denoted by a dash (-).

In the ND5 gene of squirrel monkey, three bases corresponding to one amino acid have been deleted (figs. 1, 2). The deletion occurred at the second codon of the gene. The 5′-terminal regions of the ND5 gene in starfish (Himeno et al. 1987) and sea urchin (Cantatore et al. 1987) are ~70 bases longer and the same region of *Dro-*

Table 2
Percent Similarity of Amino Acid Sequences of ND4 (above the Diagonal) and ND5 (below the Diagonal) between Species

	Species Compared											
Species Compared	Human	Chimpanzee	Gorilla	Orangutan	Gibbon	Japanese Macaque	Rhesus Macaque	Crab-eating Macaque	Barbary Macaque	Squirrel Monkey	Tarsier	Lemur
Human		95.4	94.1	88.2	80.9	79.6	78.3	77.0	78.3	70.4	70.4	72.4
Chimpanzee	92.4		94.1	86.8	82.2	79.6	79.6	77.0	78.3	69.7	69.7	72.4
Gorilla	87.3	87.3		86.8	82.2	77.6	77.6	76.3	77.0	69.7	69.1	71.1
Orangutan	72.2	72.2	67.1		82.9	78.9	77.0	77.6	78.3	68.4	75.7	75.7
Gibbon	75.9	77.2	70.9	75.9		77.6	77.0	78.3	78.3	67.8	72.4	73.7
Japanese macaque	53.2	51.9	50.6	55.7	54.4		98.0	96.1	95.4	69.7	71.1	75.0
Rhesus macaque	53.2	51.9	50.6	55.7	54.4	98.7		95.4	96.1	68.4	69.7	72.7
Crab-eating macaque	54.4	51.9	50.6	53.2	51.9	92.4	93.7		92.8	67.1	71.1	72.4
Barbary macaque	54.4	55.7	53.2	55.7	57.0	82.3	83.5	81.0		67.1	69.1	73.0
Squirrel monkey	50.6	50.6	48.1	50.6	51.9	53.2	53.2	55.7	54.4		64.5	71.1
Tarsier	43.0	44.3	43.0	41.8	45.6	36.7	35.4	39.2	36.7	45.6		76.3
Lemur	46.8	49.4	44.3	49.4	46.8	50.6	50.6	53.2	46.8	53.2	54.4	

sophila (Clary et al. 1984) is 78 bases shorter than their counterparts in mammals (Anderson et al. 1981, 1982; Bibb et al. 1981; Brown et al. 1982; present study).

Table 3 reveals that the rates of synonymous nucleotide substitutions are approximately the same among these 12 species for both ND4 and ND5. Moreover, the rate of the nonsynonymous nucleotide substitutions is much larger in ND5 than in ND4 (table 3). These features agree well with the neutral theory of molecular evolution (Kimura 1968, 1983), since synonymous nucleotide substitutions are considered to be nearly neutral to selection while nonsynonymous nucleotide substitutions are under selective constraints. Therefore, the data in table 3 suggest that, while both genes have about the same rate of neutral nucleotide substitution, they are under different degrees of selective constraints. From these observations, it can probably be said that the 5′-terminal region of the ND5 gene is under relaxed constraints.

4. Phylogenetic Relationships among Primates

The number of nucleotide substitutions for a given pair of species was calculated by the six-parameter method (Gojobori et al. 1982) (table 4). Using the calculated numbers, we constructed a phylogenetic tree by the NJ method (Saitou and Nei 1987) (fig. 3). Although we also used the distance Wagner (DW) method (Farris 1972) and unweighted pair grouping (UPG) method (Sneath and Sokal 1973; Nei 1975), the branching patterns of the trees obtained were unchanged (data not shown). Both the NJ and DW methods are based on the principle of minimum change. On the other hand, the UPG method is based on the assumption that the rate of nucleotide substitution is the same for all lineages. Moreover, the algorithms for constructing phylogenetic trees are different from each other. Because these three different methods give phylogenetic trees with the same topology, the phylogenetic relationships derived from these mtDNA sequence comparisons (fig. 3) appear reliable.

The maximum-parsimony method has often been used to construct phylogenetic trees from comparisons of nucleotide sequences (e.g., see Koop et al. 1986). However, Sourdis and Nei (1988) suggest that the distance-matrix methods, such as the NJ method, are generally superior to the maximum-parsimony method in constructing phylogenetic trees. We therefore did not use the maximum-parsimony method.

The topology of the tree, except for the position of the tarsier, is generally in agreement with the widely accepted classification of primates (Clark 1963; Simons 1972) that is based on fossil records (Delson 1980; Gingerich 1984; Pilbeam 1984; Andrews 1986) and other molecular analyses (Dene et al. 1976; Goodman 1976; Sarich and Cronin 1976; Dutrillaux 1979; Bonner et al. 1980; Ferris et al. 1981; Brown et al. 1982; Sibley and Ahlquist 1984, 1987; Koop et al. 1986). Although it previously has been reported (Dene et al. 1976; Goodman 1976; Martin 1978; Bonner et al. 1980) that the tarsier is more closely related to anthropoids than to prosimians, these mtDNA sequence comparisons show that the tarsier is more closely related to the lemur than to anthropoids.

Among the macaque species, the Barbary macaque (from North Africa) appears to be distantly related to the other three types of Asian macaques. Moreover, the Japanese and rhesus macaques are shown as the most closely related among Asian macaques. This view is compatible not only with morphological (Fooden 1980) and paleontological data (Delson 1980) but with the results of restriction-enzyme analyses for mtDNAs (Hayasaka et al. 1988), although it differs from conclusions based on blood protein polymorphisms (Nozawa et al. 1977).

Table 3
Synonymous and Nonsynonymous Nucleotide Substitutions in ND4 and ND5 Genes

Species Compared	Synonymous Nucleotide Substitutions					Nonsynonymous Nucleotide Substitutions				
	No. of Sites	No. of Substitutions[a]	Ratio (%)	Distance[b]	No. of Substitutions/Site/Year/Lineage[c] ($\times 10^{-8}$)	No. of Sites	No. of Substitutions[a]	Ratio (%)	Distance	No. of Substitutions/Site/Year/Lineage[c] ($\times 10^{-8}$)
ND4:										
Among hominines[d]	115.22	42.67	37.03	0.511	5.11–2.55	340.78	8.67	2.54	0.026	0.26–0.13
Hominines-orangutan	115.44	50.00	43.31	0.646	2.49–1.79	340.56	22.00	6.46	0.068	0.26–0.19
Great apes[e]-gibbon	115.17	57.63	50.04	0.825	2.75–2.06	340.83	32.38	9.50	0.102	0.34–0.25
Among Asian macaques	111.22	30.00	26.97	0.334	...[f]	344.78	6.67	1.93	0.020	...[f]
Asian-Barbary macaques	111.61	48.00	43.01	0.639	10.65–5.32	344.39	8.33	2.42	0.025	0.41–0.21
Hominoids-macaques	113.34	63.18	55.74	1.020	2.55–1.70	342.66	41.83	12.21	0.133	0.33–0.22
Catarrhines[g]-squirrel monkey	111.94	72.65	64.90	1.503	2.15–1.67	340.06	61.24	18.01	0.203	0.29–0.23
Anthropoids[h]-prosimians[i]	112.37	78.35	69.72	1.991	1.99–1.53	343.63	61.95	18.03	0.206	0.21–0.16
Tarsier-lemur	111.50	65.50	58.74	1.147	...[f]	344.50	51.50	14.95	0.167	...[f]
ND5:										
Among hominines	54.33	18.00	33.12	0.437	4.37–2.19	182.67	8.67	4.74	0.049	0.49–0.25
Hominines-orangutan	55.83	27.72	49.65	0.814	3.13–2.26	181.17	29.28	16.16	0.182	0.70–0.51
Great apes-gibbon	54.21	31.42	57.96	1.111	3.70–2.78	182.79	24.58	13.49	0.148	0.49–0.37
Among Asian macaques	52.89	12.00	22.69	0.270	...[f]	184.11	4.00	2.17	0.022	...[f]
Asian-Barbary macaques	51.78	21.17	40.88	0.591	9.85–4.92	185.22	15.17	8.19	0.087	1.45–0.72
Hominoids-macaques	53.53	32.73	61.14	1.266	3.17–2.11	183.47	47.72	26.00	0.319	0.80–0.53
Catarrhines-squirrel monkey	52.81	29.30	55.47	1.009	1.44–1.12	181.19	52.04	28.72	0.362	0.52–0.40
Anthropoids-prosimians	52.67	35.12	67.20	1.697	1.70–1.31	184.43	59.43	32.22	0.421	0.42–0.32
Tarsier-lemur	51.00	36.08	70.75	2.153	...[f]	186.00	46.92	25.22	0.307	...[f]

[a] Each number of synonymous and nonsynonymous substitutions represents the average over all pairs of species compared.

[b] Number of nucleotide substitutions per site, as estimated by the method of Jukes and Cantor (1969).

[c] Calculated using the following divergence times estimated from paleontological data (Delson 1980; Pilbeam 1984; Andrews 1986): 5–10 Myr within hominines, 13–18 Myr for hominines and orangutan, 15–20 Myr for great apes and gibbon, 3–6 Myr for Asian and Barbary macaques, 20–30 Myr for hominoids and macaques, 35–45 Myr for catarrhines and squirrel monkey, and 50–65 for anthropoids and prosimians.

[d] Human, chimpanzee, and gorilla.

[e] Hominines and orangutan.

[f] Not calculated because reliable estimates for the divergence times between the species compared are not available.

[g] Hominoids and macaques.

[h] Catarrhines and squirrel monkey.

[i] Tarsier and lemur.

Table 4
Number of Nucleotide Substitutions per Site between Species of Primates

Species Compared	Species Compared											
	Human	Chimpanzee	Gorilla	Orangutan	Gibbon	Japanese Macaque	Rhesus Macaque	Crab-eating Macaque	Barbary Macaque	Squirrel Monkey	Tarsier	Lemur
Human		0.094	0.110	0.179	0.205	0.268	0.271	0.292	0.304	0.329	0.405	0.372
Chimpanzee			0.113	0.192	0.214	0.285	0.298	0.324	0.292	0.348	0.401	0.372
Gorilla				0.189	0.215	0.274	0.271	0.315	0.285	0.329	0.391	0.347
Orangutan					0.211	0.289	0.292	0.317	0.279	0.339	0.369	0.344
Gibbon						0.293	0.280	0.308	0.286	0.322	0.385	0.354
Japanese macaque							0.037	0.088	0.137	0.360	0.392	0.336
Rhesus macaque								0.099	0.130	0.367	0.397	0.347
Crab-eating macaque									0.135	0.354	0.388	0.361
Barbary macaque										0.357	0.404	0.343
Squirrel monkey											0.409	0.344
Tarsier												0.290
Lemur												

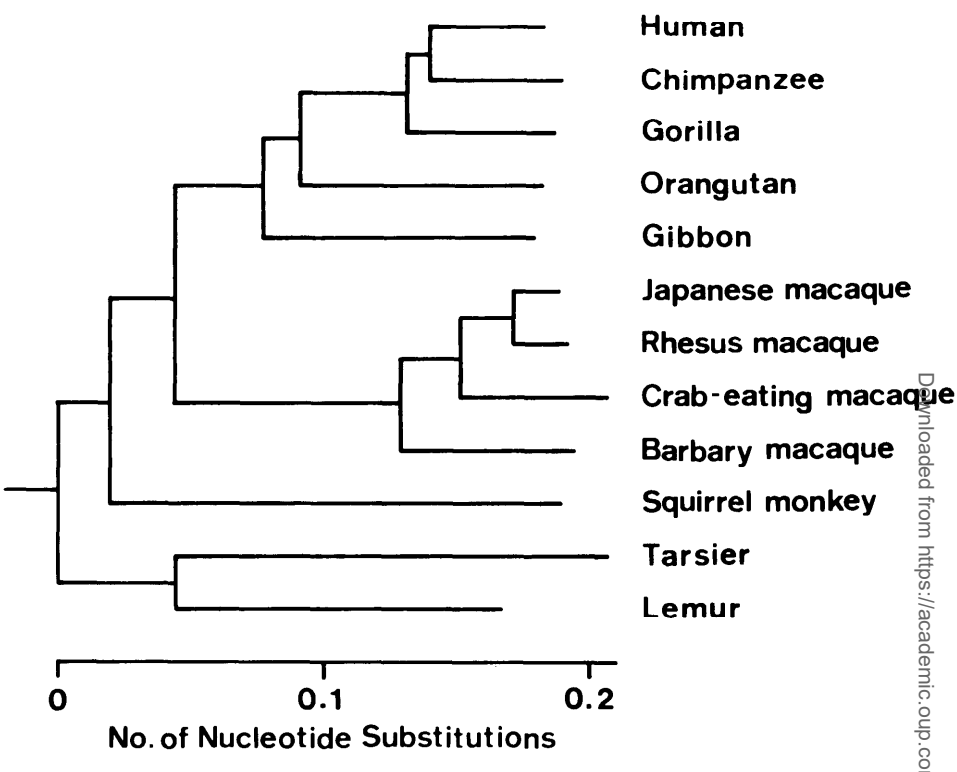

FIG. 3.—A phylogenetic tree for 12 species of primates, as constructed by the NJ method. The root of the tree is taken as the midpoint of the longest path, which is between the crab-eating macaque and tarsier.

5. Accuracy of mtDNA Phylogeny

Among other species phylogenetic relationships inferred from mtDNA analyses sometimes conflict with those inferred from nuclear genes (Lansman et al. 1983; DeSalle and Giddings 1986). Since mammalian mtDNAs are inherited maternally (Giles et al. 1980), unlike nuclear genes, these conflicting features are often attributed to differences in modes of inheritance (Lansman et al. 1983; DeSalle and Giddings 1986; Hayasaka et al. 1988).

Among the macaque species, phylogenetic relationships inferred from mtDNA analyses may reflect the ancient processes of speciation more accurately than do those inferred from analyses of nuclear genes. This expectation is based on the fact that macaque females usually stay in their natal populations for life, whereas males move between populations (Sugiyama 1976). Thus, the phylogenetic relationships inferred from maternally inherited mtDNAs will not be disturbed by hybridization between different species of macaques; interspecific hybridization may only be caused by migration of male macaques between populations. In contrast, phylogenetic relationships inferred from nuclear genes may be affected by this hybridization after speciation. Such interspecific hybrids among macaques have been observed in nature (Fooden 1964; Eudey 1980), and those macaques born in captivity are known to be fertile (Bernstein and Gordon 1980).

The discrepancy in phylogenetic relationships of the tarsier with other primate species may result from differences in modes of inheritance between mitochondrial and nuclear genes. The phylogenetic relationship between the tarsier and other primate

groups has long been controversial. Clark (1963) and Simons (1972) classified tarsier as a prosimian, while Hill (1955) classified it with anthropoids as a haplorhine. Furthermore, previous molecular studies (Dene et al. 1976; Sarich and Cronin 1976; Bonner et al. 1980) are inconclusive with respect to the phylogenetic position of the tarsier. For example, Dene et al. (1976) concluded, using an immunological approach, that the tarsier was more closely related to anthropoids than to prosimians. Using a similar method, however, Sarich and Cronin (1976) concluded that the tarsier, anthropoids, and prosimians were equally related. Bonner et al. (1980) concluded, using a DNA-DNA hybridization analysis, that the tarsier was more closely related to anthropoids than to prosimians. However, the degree of greater proximity to anthropoids than to prosimians was so low as to prevent a definite conclusion on the phylogenetic position of the tarsier.

Our results also suffer from ambiguity. Because the higher rate of nucleotide substitution for mtDNA is likely to cause parallel and backward mutations, the number of nucleotide substitutions is inevitably underestimated, particularly between distantly related species, even after making corrections against these multiple mutations by using the six-parameter method (Gojobori et al. 1982). Therefore, phylogenetic relationships between distantly related species are less reliable than those between closely related species. Indeed, when we used the nucleotide sequences of mouse and cow in the phylogenetic analysis, the primate species were not monophyletic (data not shown). For this reason, we did not include these two sequences in the present analysis. Underestimation of the number of nucleotide substitutions can be seen even within the primate species. In figure 4, a linear relationship between the divergence times of species and the numbers of nucleotide substitutions does not hold for divergence times >30 Myr. This implies that anthropoids, tarsier, and lemur are too distantly related to determine conclusively their phylogenetic relationships from the analysis of nucleotide sequences of mtDNAs.

6. Rate Retardation for Hominine mtDNAs

To estimate the rate of nucleotide substitution for mtDNAs, we examined the relationship between between-species number of nucleotide substitutions and divergence time (fig. 4, table 5). Figure 4 shows that the rate of nucleotide substitution for the sequenced region of mtDNAs is higher in macaques than in hominines (compare a and b in fig. 4). Previous paleontological studies (Delson 1980; Pilbeam 1984; Andrews 1986) have suggested the following divergence times: ~6 Myr ago (Mya) between macaques and other old-world monkeys, 3–6 Mya between Barbary and Asian macaques, and 5–10 Mya within hominine species (table 3, fn.). Using these divergence times and the number of nucleotide substitution estimated from our data, we estimated the rate of nucleotide substitution to be $(1.12–2.24) \times 10^{-8}$/site/year/lineage between Barbary and Asian macaques and $(0.53–1.05) \times 10^{-8}$/site/year/lineage among hominines (table 5). Thus, the rate of nucleotide substitution in macaques has been approximately twice as high as that in hominines. The rate of nucleotide substitution for mtDNAs has been estimated to be $(1–2) \times 10^{-8}$/site/year/lineage among species of mice (Ferris et al. 1983) and between sheep and goats (Brown et al. 1979). (Because these authors used the rate of nucleotide substitution/site/year/2 lineages, while we use the rate/site/year/lineage, we adjusted their rates to correspond to ours.) Moreover, table 3 reveals that the rates of both synonymous and nonsynonymous nucleotide substitutions in ND4 and ND5 genes are higher in macaques than in hominines. Thus,

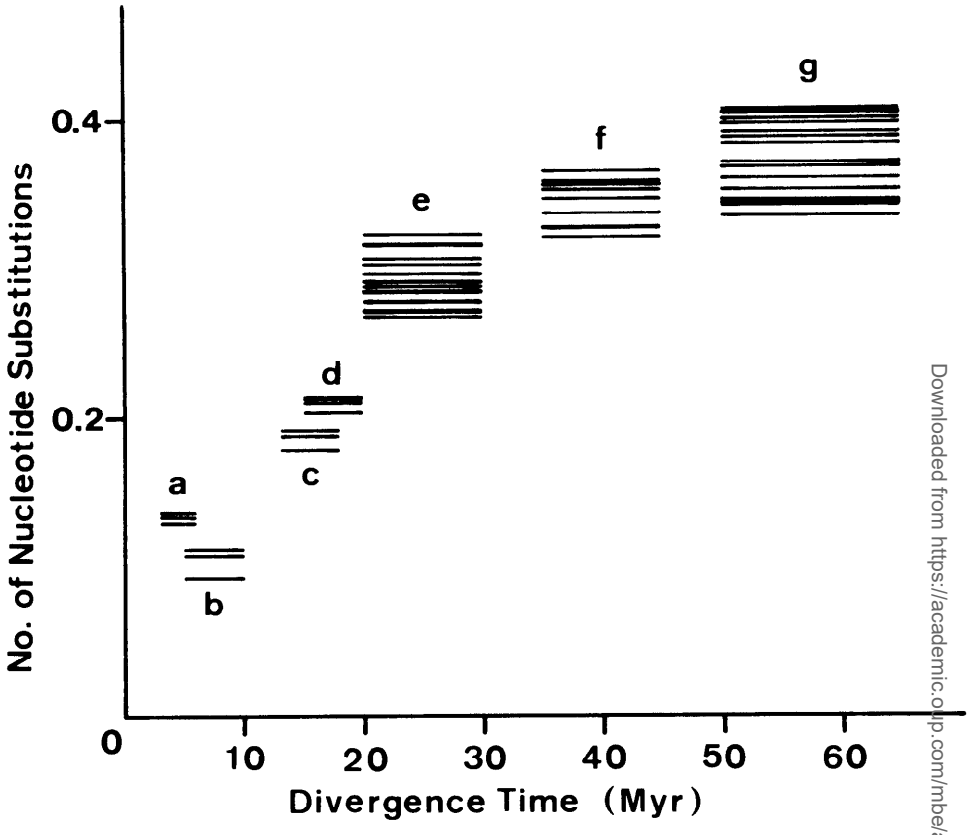

FIG. 4.—The relationship between the number of nucleotide substitutions per site between species and divergence time. a, Divergence between Barbary macaque and Asian macaques; b, divergence among hominines; c, divergence between hominines and orangutan; d, divergence between great apes and gibbon; e, divergence between hominoids and old-world monkeys; f, divergence between catarrhines and new-world monkey; and g, divergence between anthropoids and prosimians. For the divergence times, see fn. to table 3.

the rate of nucleotide substitution for mtDNAs in hominines has probably slowed down compared with that for other mammalian species.

The retardation in the rate of nucleotide substitution for hominines is also suggested in figure 3. The lengths of branches from the Barbary and Asian macaques to their branching point are longer than those from hominines to the branching point among hominines, while the divergence time of the macaques is considered to be shorter than that of the hominines.

To compare the rates of molecular evolution between two lineages, a relative-rate test (Wilson et al 1977; Nei 1987) has often been used. In this test, a reference species is necessary. The reference species should be chosen so that the numbers of nucleotide substitutions between the reference species and both of the two species compared are larger than that between the two species. Then, the lineage with the smaller number of nucleotide substitutions between the reference species and the other two species is considered to have a lower rate of nucleotide substitution than the other lineage.

Table 6 shows the results of the relative-rate test between macaques and hominoids

Table 5
Rate of Nucleotide Substitution for mtDNA within Primate Lineages

Species Compared	Position in Figure 4	Average Between-Pair No. of Nucleotide Substitutions/Site	Divergence Time (Myr)	No. of Nucleotide Substitutions/Site/Year/Lineage ($\times 10^{-8}$)
Asian-Barbary macaques ...	a	0.134	3–6	2.24–1.12 (1.68)
Among hominines	b	0.105	5–10	1.05–0.53 (0.79)
Hominines-orangutan	c	0.187	13–18	0.72–0.52 (0.62)
Great apes-gibbon	d	0.211	15–20	0.70–0.53 (0.62)
Hominoids-macaques	e	0.291	20–30	0.73–0.49 (0.61)

NOTE.—Numbers in parentheses are median of upper and lower values.

when using the squirrel monkey as a reference species. In this table the number of nucleotide substitutions is calculated by the two-parameter method of Kimura (1980). Using the method of Wu and Li (1985), we examined the statistical significance of the difference between the number of nucleotide substitutions accumulated in macaque

Table 6
Relative-Rate Test between Apes and Macaques, Using the Squirrel Monkey as a Reference Species (Species A)

	SPECIES C					
SPECIES B	Human	Chimpanzee	Gorilla	Orangutan	Gibbon	Mean
Japanese macaque:						
1	0.156	0.156	0.162	0.161	0.173	0.162
2	0.129	0.146	0.129	0.143	0.134	0.136
3	0.027	0.009	0.033	0.018	0.039	0.025
Rhesus macaque:						
1	0.161	0.166	0.165	0.167	0.170	0.166
2	0.126	0.149	0.124	0.141	0.123	0.133
3	0.035	0.018	0.041	0.026	0.047	0.033
Crab-eating macaque:						
1	0.168	0.175	0.182	0.174	0.179	0.176
2	0.145	0.170	0.153	0.159	0.145	0.155
3	0.023	0.005	0.029	0.014	0.034	0.021
Barbary macaque:						
1	0.174	0.159	0.167	0.155	0.167	0.164
2	0.151	0.153	0.138	0.141	0.132	0.143
3	0.023	0.005	0.029	0.014	0.034	0.021
Mean:						
1	0.165	0.164	0.169	0.164	0.172	0.167
2	0.138	0.155	0.136	0.146	0.134	0.142
3	0.027	0.009	0.033	0.018	0.039	0.025

NOTE.—1, 2, and 3 represent the values for d_{OB}, d_{OC}, and d_{OB}–d_{OC}, respectively. d_{XY} represents the number of nucleotide substitutions between X and Y, where X and Y denote species or their branching point. $d_{OB} = (d_{AB} + d_{BC}-d_{AC})/2$, and $d_{OC} = (d_{AC} + d_{BC}-d_{AB})/2$, where A–C represent species compared and O represents the branching point between species B and C. The number of nucleotide substitutions was calculated by the method of Kimura (1980). According to the significance test of Wu and Li (1985), all differences 3 are not statistically significant.

and hominoid lineages. Table 6 reveals that, for each comparison, nucleotide substitutions have occurred in the macaque lineage more frequently than in the hominoid lineage, though the differences are not statistically significant. These observations also suggest that the rate of nucleotide substitution may have been higher in macaques than in hominoids.

When we use either of the two prosimian species as a reference species, however, the differences in the number of nucleotide substitutions between the two lineages disappear. As mentioned above, the number of nucleotide substitutions between anthropoids and prosimians is probably underestimated severely and therefore unreliable. Thus, the relative-rate test may not be valid if the two prosimian species are used as reference species.

In spite of the foregoing observations and discussion, our conclusion of retardation in the rate of nucleotide substitution still depends heavily on the estimates of divergence times. Paleontological data (Delson 1980) and analyses of blood protein polymorphisms (Nozawa et al. 1977; Cronin et al. 1980) support the validity of our estimates of the divergence times within macaques. From the analysis of blood protein polymorphisms, Nozawa et al. (1977) suggested that Japanese, rhesus, and crab-eating macaques diverged within the past 0.5 Myr. On the other hand, Cronin et al. (1980) analyzed plasma protein polymorphisms and suggested that these three diverged 2 Mya and that Barbary and Asian macaques diverged 3 Mya. On the basis of paleontological data, Delson (1980) suggested that Barbary and Asian macaques diverged 5 Mya, that the crab-eating macaque and the other two Asian macaques diverged 1 Mya, and that the Japanese and rhesus macaques diverged 0.3 Mya. On the other hand, it is widely accepted that the divergence of hominines occurred 5–10 Mya (Pilbeam 1984; Andrews 1986; Koop et al. 1986). Thus, our estimates for the divergence times seem reliable.

This retardation in rate of nucleotide substitution among hominines as compared with other primates has also been observed in nuclear gene comparisons (Britten 1986; Koop et al. 1986; Li and Tanimura 1987). Li and Tanimura (1987) reported that the rate of synonymous nucleotide substitution for nuclear genes was about twice as high in old-world monkeys as in hominines, which is compatible with our results for mtDNAs. While the causes of this similar retardation in both nuclear and mitochondrial sequence divergence are unknown, difference in generation time among these species and coordinate replication of mtDNA and nuclear DNA represent possible explanations.

7. Conclusions and Prospects

Our analysis of the nucleotide sequences of mtDNAs has provided new insights into evolutionary relationships among primates, particularly the phylogenetic position of the tarsier and the phylogenetic relationships among species of macaques. In addition, we have shown that the rate of nucleotide substitution for mtDNAs may have slowed down in hominines compared with that in macaques, as was observed for nuclear genes. To obtain a more precise view of phylogenetic relationships among primate species, we need to accumulate more data on nucleotide sequences of both mitochondrial and nuclear genes. Moreover, to clarify the extent and cause of rate retardation in the divergence of hominine mitochondrial and nuclear gene sequences, we must extend such comparisons to a variety of taxonomic groups.

Acknowledgments

We thank Dr. T. Tanaka (Shizuoka Laboratory Animal Center) for a liver specimen of a crab-eating macaque, Drs. S. Kodera and H. Inagaki (Japan Monkey Centre) for the Barbary macaque and tarsier livers, and Drs. O. Takenaka, T. Ishida, and J. Suzuki (Primate Research Institute, Kyoto University) for the livers of a lemur and a rhesus macaque. We also thank Drs. T. Inoue and S. Aota (National Institute of Genetics) for technical advice and Dr. N. Saitou (University of Tokyo) for a computer program. Finally, stimulating discussions and encouragements during this study are due to Prof. K. Nozawa (Primate Research Institute, Kyoto University) and Prof. E. Matsunaga (National Institute of Genetics). This work was supported by Grants-in-aids for Scientific Research on Priority Areas of "Bioenergetics" and "Development of Evolutionary and Population Genetics Incorporating Newer Molecular Findings" to S.H. and T.G. from the Ministry of Education, Science and Culture, Japan.

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MASATOSHI NEI, reviewing editor

Received March 22, 1988; revision received June 20, 1988