

Mitochondrial 16S rRNA Sequence Diversity of Hominoids

R. Noda, C. G. Kim, O. Takenaka, R. E. Ferrell, T. Tanoue, I. Hayasaka, S. Ueda, T. Ishida, and N. Saitou

We determined nucleotide sequences of the 16S rRNA gene of mitochondrial DNA (mtDNA) (about 1.6 kb) for 35 chimpanzee, 13 bonobo, 10 gorilla, 16 orangutan, and 23 gibbon individuals. We compared those data with published sequences and estimated nucleotide diversity for each species. All the ape species showed higher diversity than human. We also constructed phylogenetic trees and networks. The two orangutan subspecies were clearly separated from each other, and Sumatran orangutans showed much higher nucleotide diversity than Bornean orangutans. Some gibbon species did not form monophyletic clusters, and variation within species was not much different from that among species in the subgenus *Hylobates*.

Superfamily Hominoidea of Primates is divided into two families, Hominidae that consists of human, chimpanzee, gorilla, and orangutan, and Hylobatidae containing gibbons and siamang (Glover 1997). The phylogenetic relationship of Hominiidae species is now established (e.g., Chen and Li 2001; Horai et al. 1995; Ruvolo 1997; Saitou 1991; Sibley and Ahlquist 1987). In this respect, nucleotide sequences of various regions of mitochondrial DNA (mtDNA) have been frequently used for elucidating the phylogenetic relationship of hominoids (e.g., Arnason et al. 1996; Brown et al. 1982; Hixson and Brown 1986; Horai et al. 1992, 1995; Hayashi et al. 1995; Roos and Geissmann 2001; Ruvolo et al. 1994; Xu and Arnason 1996).

It is important to estimate genetic diversity of apes not only from the natural history viewpoint, but also for comparison with human. In recent years mtDNA sequence data have been used to examine the genetic diversity of apes following the great wave of human mtDNA diversity studies (e.g., Gagneux et al. 1999; Garner and Ryder 1996; Goldberg and Ruvolo 1997; Morin et al. 1994; Muir et al. 2000; Ruvolo et al. 1994; Saltonstall et al. 1998; Warren et al. 2001; Wise et al. 1997; Zhi et al. 1996). However, even those already published mtDNA sequence data seem to be insufficient for estimating the genetic diversity of apes, in particular, orangutans and gibbons. As for gibbons, the phylogenetic relationship of species in the family Hylobatidae is not yet clarified.

We collected ape genomic DNA to study

the intraspecific variation of ape species as a part of the ape genome project Silver (<http://sayer.lab.nig.ac.jp/~silver/>). As the initial step, we examined mtDNA 16S rRNA diversity of chimpanzee, bonobo, gorilla, orangutan, and eight gibbon species. We particularly focused on the diversity of the two orangutan subspecies and on the phylogenetic relationship of the gibbon species.

Materials and Methods

Genomic DNA samples for 35 chimpanzee (*Pan troglodytes*), 14 bonobo (*Pan paniscus*), 10 gorilla (*Gorilla gorilla*), 16 orangutan (*Pongo pygmaeus*), and 23 gibbon (genus *Hylobates*) individuals were used (see Table 1). Polymerase chain reaction (PCR) was carried out using specific primers based on primate mitochondrial sequences: SN-MtSd, 5'-CACACCGCCCGTCACYCYCYTCAA-3', and SN-MtSr, 5'-ACCGGGCTC TGCCATCTTAACSN-3' for PCR. This region includes part of the 12S rRNA, valine transfer RNA, and most of the 16S rRNA gene. Each PCR mixture contained 160 mM each of dATP, dTTP, dCTP, and dGTP, 150 mM MgCl₂, reaction buffer containing no Mg²⁺ ion, 1 mM of each primer, and 2 units *Taq* polymerase (TOYOBO and NIPPON GENE). About 200 ng of genomic DNA was used as template. The PCR program consisted of 20–45 cycles of 1-min denaturation at 94°C followed by 1-min primer annealing at 65°C or 68°C, and a 2-min extension at 72°C (PE GeneAmp PCR system 2400 and 9700). Immediately preced-

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Table 1. Ape DNA samples used in this study

Species individual ID	Accession numbers
Chimpanzee chimp_#1-#35	AB062513-AB062547
Bonobo bonobo_#1-#5 bonobo_#6-#13	AB050147-AB050151 AB065137-AB065144
Gorilla gorilla_#1-#4 gorilla_#5-#10	AB050152-AB050155 AB064490-AB064495
Orangutan orang_#1-#16	AB050156-AB050172
<i>H. agilis</i> (subgenus <i>Hylobates</i>) <i>H. agilis</i> _#1-#3	AB050172-AB050174
<i>H. lar</i> (subgenus <i>Hylobates</i>) <i>H. lar</i> _#1-#3	AB050175-AB050177
<i>H. muelleri</i> (subgenus <i>Hylobates</i>) <i>H. muelleri</i> _#1-#2	AB050178-AB050179
<i>H. klossii</i> (subgenus <i>Hylobates</i>) <i>H. klossii</i> _#1-#3	AB050180-AB050182
<i>H. pileatus</i> (subgenus <i>Hylobates</i>) <i>H. pileatus</i> _#1-#3	AB050183-AB050185
<i>H. moloch</i> (subgenus <i>Hylobates</i>) <i>H. moloch</i> _#1-#2	AB050186-AB050187
<i>H. syndactylus</i> (subgenus <i>Symphalangus</i>) <i>H. syndactylus</i> _#1-#5	AB050188-AB050192
<i>H. concolor</i> (subgenus <i>Nomascus</i>) <i>H. concolor</i> _#1	AB050193

ing and following these 20–45 cycles, a 9-min hot-start step at 94°C and a 60-min extension step at 72°C were included.

PCR products were confirmed by 1.5%

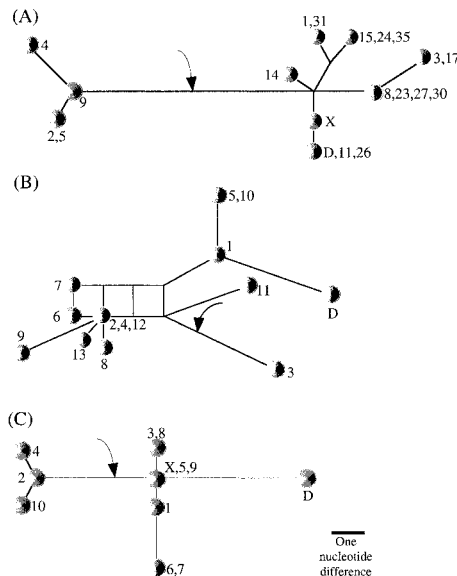


Figure 1. Phylogenetic networks of the mtDNA 16S rRNA gene region for (A) chimpanzee, (B) bonobo, and (C) gorilla. Numbers designate individuals of Table 2. D and X designate sequences determined by Horai et al. (1995) and Xu and Arnason (1996), respectively. Edge lengths are proportional to the number of nucleotide differences. Arrows indicate the edges in which roots are probably located.

agarose gel electrophoresis and were purified using Micro Spin Columns (Pharmacia Biotech). The purified PCR products were sequenced by the dideoxy chain-termination method using fluorescent ddNTPs, sequenase enzyme, and buffer from the ThermoSequenase kit (PE Biosystems). Samples were electropho-

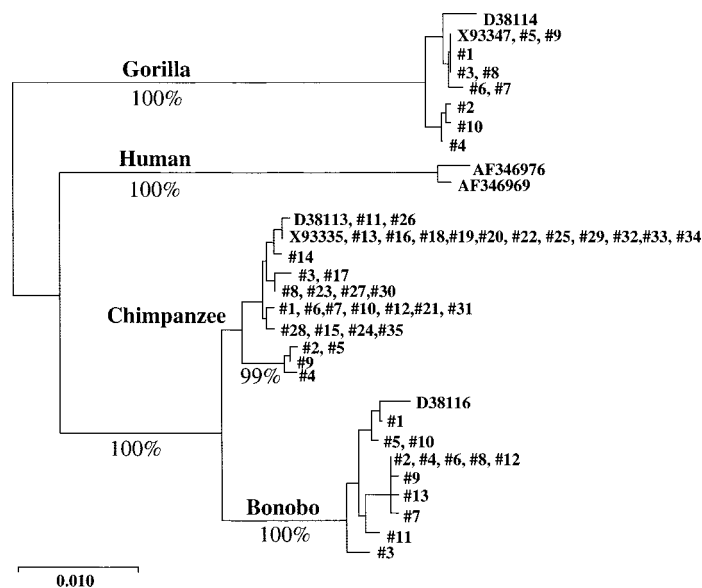


Figure 2. Phylogenetic tree of the mtDNA 16S rRNA gene region for chimpanzee, bonobo, and gorilla. The tree was rooted by using the midpoint rooting method.

resed in the automated sequencer ABI PRISM 377 (PE Biosystems). Both the PCR primers were used for sequencing. In addition, two other primers were used: SN-MtSi1, 5'-CAATTAAGAAAGCGTTCAAGCTC-3', and SN-MtSi2, 5'-TGCTAGAGGTGATGT TTTTGG-3'.

CLUSTAL W version 1.6 (Thompson et al. 1994) was used for multiple alignment and phylogenetic tree construction. Phylogenetic trees were constructed using the neighbor-joining method (Saitou and Nei 1987). Phylogenetic networks (Bandelt 1994; Saitou and Yamamoto 1997) were also constructed. MEGA2 (Kumar et al. 2000) was used for computation of nucleotide diversity and UPGMA tree construction. Nucleotide sites containing missing data and gaps were excluded from constructing trees and networks.

Results

Because there were considerable nucleotide differences among hominoid mtDNA 16S rRNA sequences, we divided hominoids into three groups: African apes, orangutan, and gibbons.

African Apes

We determined mtDNA sequences for 35 chimpanzee, 13 bonobo, and 10 gorilla. The following sequence data retrieved from the DDBJ/EMBL/GenBank International Sequence Database were also used for comparison: two chimpanzee sequences (D38113 and X93335), one bonobo sequence (D38116), and two gorilla se-

Table 2A. Variant sites of chimpanzee

	Site Position
	----- 111111 -----
	111235666667888999123347
	1137971234561015268383477
Sequence	2413759447525696729370790
X93335 ^a	CTTCTCATTATTGGCACTCAAATGA
D38113C.....G
Chimp_#1 ^bC...A.T.....
Chimp_#2 ^c	TCCT.T..GC..AT..TGG..A.
Chimp_#3 ^dGC.....A...C...C..
Chimp_#4	TCCTCT..GC..AT.T..GG..A.
Chimp_#8 ^eC....A.....C..
Chimp_#9	TCCT.T..GC..AT...GG..A.
Chimp_#11C.....
Chimp_#14A.....G..
Chimp_#15 ^fC...AA.....
Chimp_#26C.....*
Chimp_#31	***.....C...A.T.....
Chimp_#32 ^g	***.....
Chimp_#35	***.....C...AA.....

^a Chimps 13, 16, 18, 19, 20, 22, 25, and 29 were identical with this sequence.

^b Chimps 6, 7, 10, 12, and 21 were identical with this sequence.

^c Chimp 5 was identical with this sequence.

^d Chimp 17 was identical with this sequence.

^e Chimps 23, 27, and 30 were identical with this sequence.

^f Chimps 24 and 28 were identical with this sequence.

^g Chimps 33 and 34 were identical with this sequence.

quences (D38114 and X93347). Nucleotide sequences with accession numbers starting with D were determined by Horai et al. (1995), while those with accession numbers starting with X were determined by Xu and Arnason (1996). Those sequence data were multiply aligned for each species, and variant nucleotide sites are presented in Table 2A, B, and C for chimpanzee, bonobo, and gorilla, respectively.

Phylogenetic networks for each species were constructed based on the multiply aligned sequence data (Figure 1). Four chimpanzee individuals (nos. 2, 4, 5, and 9) were clearly separated from the remaining individuals. Arrows indicate the position of the roots based on the phylogenetic tree of four hominid species shown in Figure 2. Networks for chimpanzee (Figure 1A) and for gorilla (Figure 1C) happened to be unrooted trees, for all the nucleotide sites were compatible with each other. One rectangle in the bonobo network (Figure 1B) denotes the existence of incompatible nucleotide configurations; the configuration at site 1788 is incompatible with those at sites 225, 687, and 1368. Subspe-

Table 2B. Variant sites of bonobo

	Site Position
	----- 111111111111 -----
	11111222334567889900333778888
	22458266383280686636036680234
Sequence	45704525560270927887808685607
Consensus	GTTATGGGCCCTTCCAATCCATCGTTCAG
D38116	TA.....T..C.GGTA...CT.C....
Bonobo_#1	**.....T..C.....CT.C...*
Bonobo_#2	**.....T..C.....CT.C...*
Bonobo_#3	**.....TCCT.....C...G..T
Bonobo_#4T..C.....A..CTAC....
Bonobo_#5T..C.....A..CTAC....
Bonobo_#6	**...T.....
Bonobo_#7	**...T.....
Bonobo_#8	***.....A..*
Bonobo_#9	**ACG.T.....*
Bonobo_#10	**.....T..C.....A..CTAC..**
Bonobo_#11	**.....T..C.....TGC.....G*
Bonobo_#12	***.....**
Bonobo_#13	***.....T.....*

cies identification is not clear for all the chimpanzees used in this study, but most of them seem to be *Pan troglodytes verus* according to their mtDNA control region sequences (Shinoda K, personal communication). Therefore it is possible that the small cluster with four chimpanzees may correspond to another subspecies, probably *Pan troglodytes troglodytes*.

We also constructed a phylogenetic tree for human, chimpanzee, bonobo, and gorilla (Figure 2). Two human sequences that showed the greatest divergence out of the 53 complete mitochondrial genomes (Ingman et al. 2001) were included in this tree. The tree was rooted by using the midpoint rooting method. The phylogenetic relationship of the four species is compatible with that based on the complete mtDNA sequences (Horai et al.

Table 2C. Variant sites of gorilla

	Site Position
	----- 11111 -----
	1111123446778900144
	12233429478188937811
Sequence	03734975889075945778
X93347	ACCTATGCCCACTTACAGTT
D38114	T.....C.TGGCC
gorilla_#1	*****C.....
gorilla_#2	***GT...T..TC.G....
gorilla_#3	*****A.....
gorilla_#4	*****T..TC.G..A..
gorilla_#5	*****
gorilla_#6	***..C.A..G.....
gorilla_#7	***..C.A..G.....
gorilla_#8	***..A.....
gorilla_#9	*TT.....
gorilla_#10	***.....TT.TC.G....

1995). Phylogenetic relationships of the haplotypes belonging to the same species were more or less compatible with the phylogenetic networks shown in Figure 1.

The nucleotide diversity within species was calculated using the Jukes–Cantor correction, and the result is shown in Table 3. The nucleotide diversities of chimpanzee, bonobo, and gorilla (0.0027 ± 0.0007 , 0.0033 ± 0.0008 , and 0.0024 ± 0.0007 , respectively) were greater than that for human (0.0016 ± 0.0004), in which sequence data of the 53 complete mitochondrial genomes (Ingman et al. 2001) were used. It should be noted that individuals sampled from the global scale were used for human.

Orangutan

We determined mtDNA sequences for 16 orangutans. Orangutan sequence data retrieved from the DDBJ/EMBL/GenBank International Sequence Database were also

Table 3. Nucleotide diversity (π) in 14 hominoid species

Species	No. of sequences ^a	$\pi \pm$ S.E. ^b	No. of sites (bp) ^c
<i>Homo sapien</i>	53 (53)	0.0016 \pm 0.0004	1669
<i>Pan troglodytes</i>	37 (2)	0.0027 \pm 0.0007	1546
<i>Pan paniscus</i>	14 (1)	0.0033 \pm 0.0008	1676
<i>Gorilla gorilla</i>	12 (2)	0.0024 \pm 0.0007	1704
<i>Pongo pygmaeus pygmaeus</i>	9 (1)	0.0034 \pm 0.0008	1574
<i>Pongo pygmaeus abelii</i>	9 (1)	0.0066 \pm 0.0013	1667
<i>Hylobates lar</i>	4 (1)	0.0028 \pm 0.0011	1530
<i>Hylobates agilis</i>	3 (0)	0.0319 \pm 0.0037	1661
<i>Hylobates muelleri</i>	2 (0)	0.0065 \pm 0.0020	1541
<i>Hylobates klossii</i>	3 (0)	0.0347 \pm 0.0041	1537
<i>Hylobates pileatus</i>	3 (0)	0.0405 \pm 0.0042	1423
<i>Hylobates moloch</i>	2 (0)	0.0203 \pm 0.0035	1601
<i>Hylobates syndactylus</i>	6 (0)	0.0058 \pm 0.0014	1715

^a The numbers in parentheses show the sequences retrieved from the DDBJ/EMBL/GenBank database.

^b Standard error (S.E.) was calculated by the bootstrap method of MEGA2.

^c Number of nucleotide sites compared.

Table 4A. Variant sites of the Bornean orangutan

	Site Position
	----- 111111111111111111
	1112222345555668801277777777777777
	88999999990000228165577481399001677777888899
Sequence	78012567890125278238934636809252802489037813
D38115	GGACTCTAACTAAGTATAGTACTTAGTAATCCAAACAG
ora#001	CAGG.ACCTACT.....TATA.GCCT..G..A.T....CACA
ora#002	**AC.....TATA..CC..CGA.A.T*****
ora#003	****TCCTAACT....GTATA..CC...G..A.T....****
ora#004	*****GNNT..TATA..CC...G.....****
ora#006A.TATAC.CC.C.G.....*
ora#007	*****...A.TATAC.CC.C.G.CCCGGTCT****
ora#014	*****CCTACT.....TATA..CCN..G.....*
ora#015	**GGGACCTACT....A.TATAC.CC.C.G.....*

used for comparison: D38115 determined by Horai et al. (1995) for a Bornean orangutan (*P. p. pygmaeus*) and X97707 determined by Xu and Arnason (1996) for a Sumatran orangutan (*Pongo p. abelii*). Sequences for ora#001, ora#002, ora#003, ora#004, ora#006, ora#007, ora#014, and ora#015 determined in the present study were closely related with the Bornean orangutan sequence, while those for

ora#005, ora#008, ora#009, ora#010, ora#011, ora#012, ora#013, and ora#016 were similar to the Sumatran orangutan. These sequence features were compatible with the source of the individuals.

The nucleotide difference between these two groups is substantial. There are 54 nucleotide differences in the 1.7 kb region that contains the complete 16S rRNA gene. We therefore concluded that those

Table 4B. Variant sites of the Sumatran orangutan

	Site Position
	----- 1111111111
	11111222223444577888999990245577777
	89999999900115777778567534059168899835547888
Sequence	501256789015040245673020249634248910475637134
X97707	CACTCTAACTACAACCCTACCTGCACAGTTGTCCCTCACTCAA
ora#005	T.....T....C...TT.ACCA.....T..C....
ora#008	*T.....C..T...C...TT.ACCA.....T..C.ACT
ora#009	*****...T....C...TTCACCA...N.T..C....
ora#010	*****C.TTTACATC.T...A.CA.TT.CT.....
ora#011	*****...T....TC.T...A.CA.TTNCTTN.G...
ora#012	*****.....-.....C..N.....
ora#013	*****CCTACTC..T.T...TCATG...A.CA.TTNCT.....
ora#016	*GGGTCCTACTAC.T.T...TCATG...A.CA.TTNCT.....

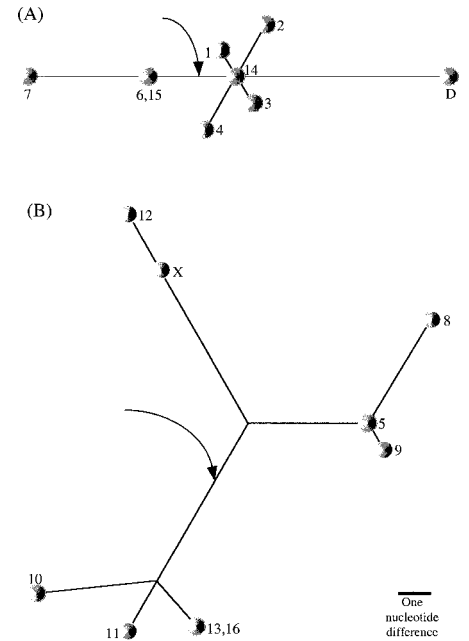


Figure 3. Phylogenetic networks of the mtDNA 16S rRNA gene region for (A) Sumatran orangutan and (B) Bornean orangutan. Numbers designate individuals of Table 4. D and X designate sequences determined by Horai et al. (1995) and Xu and Arnason (1996), respectively. Edge lengths are proportional to the number of nucleotide differences. Arrows indicate the edges in which roots are probably located.

two sequence groups belong to two subspecies of orangutan. These two groups of sequences were multiply aligned, as shown in Table 4. Phylogenetic networks for each subspecies were constructed based on the multiply aligned sequence data (Figure 3). Both networks turned out to be unrooted trees because of no reticulation. We also constructed a phylogenetic tree for the two orangutan subspecies (Figure 4). As expected, individuals belonging to the two subspecies formed clear monophyletic groups, separated by a branch with 100% bootstrap probability.

Nucleotide diversities within subspecies are 0.0034 ± 0.0008 and 0.0066 ± 0.0013 for Bornean and Sumatran orangutans, respectively (see Table 3). Therefore Sumatran orangutan was shown to have about two times higher mtDNA diversity than Bornean orangutan. It is interesting to note that nucleotide diversities for those two subspecies are greater than those for African great apes and for human, even if we treated the two subspecies separately.

Gibbons

We determined the mtDNA 16S rRNA sequences for 22 individuals of gibbons. Because the number of individuals examined was small for most of the species (see Table 1), we present variant sites only for

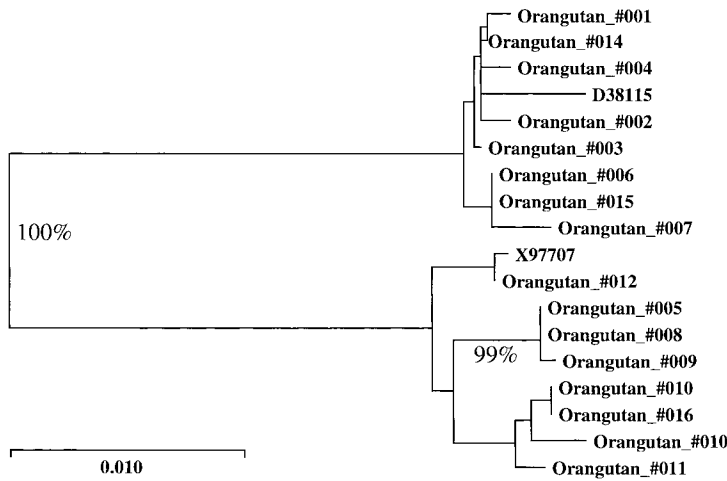


Figure 4. Phylogenetic tree of the mtDNA 16S rRNA gene region for Sumatran and Bornean orangutan. The tree was rooted by using the midpoint rooting.

siamangs (Table 5). Figure 5 shows the phylogenetic network for those five siamang sequences. We observed one pair of incompatible configurations: one for site 1199 and the other for sites 191 and 668. This is why there is one rectangle.

Figure 6 shows the phylogenetic tree of the 22 gibbon sequences and a *Hylobates lar* sequence determined by Xu and Arnason (1996; DDBJ/EMBL/GenBank accession number X99256). The root was determined by the outgroup sequences (human, chimpanzee, bonobo, gorilla, and orangutan). We covered three subgenera of gibbons in this study (see Table 1), and the subgenus *Symphalangus* made a clear monophyletic cluster. Subgenus *Hylobates* also formed a monophyletic group, except for one individual (no. 3) of *H. pileatus*. This *H. pileatus* individual was very close to a *H. concolor* individual (see Figure 6). Because *H. concolor* belongs to the subgenus *Nomascus* (Glover 1997), this clustering was unexpected. This kind of species intermingling was also observed within the subgenus *Hylobates*. *H. agilis* individual no. 1 clustered with *H. klossi* no. 2, while *H. agilis* no. 3 clustered with two *H. muelleri* individuals. *H. klossi* also showed nonmonophyly.

We therefore estimated net nucleotide substitutions or evolutionary distance between species by subtracting within-species variation (Nei 1987). UPGMA was used for reconstructing the species phylogeny. This is because the branch lengths of a species tree should be proportional to evolutionary time. Figure 7A shows a UPGMA tree for eight gibbon species. Because one individual of *H. pileatus* clustered with a *H. concolor* individual in the phylogenetic tree of mtDNA genes (see

Figure 6), we also constructed a UPGMA tree after eliminating this *H. pileatus* individual (tree in Figure 7B). Only the position of *H. pileatus* differs between the two trees.

Discussion

Higher Nucleotide Diversity of Apes Than Human

All the ape species used in the present study, except for *H. concolor* in which only one individual sample was available, showed higher nucleotide diversity than for human. Although a limited number of individuals were used in the present study, this finding is consistent with that of previous studies for mtDNA (e.g., Gagneux et al. 1999; Goldberg and Ruvolo 1997; Wise et al. 1997). This tendency toward higher nucleotide diversity in apes than human was also observed for nuclear DNA (Kaessmann et al. 1999; Kitano et al. 2000; Sumiyama et al. 2000). This is indirect support for the recent expansion model on modern humans. We compared the same set of 53 human individuals for the whole D-loop region of mtDNA of about 1.1 kb using Ingman et al.'s (2001) data. Nucleotide diversity for the control region was estimated to be 0.0158 ± 0.0017 , which is 10 times higher than that (0.0016 ± 0.0004) for the 16S rRNA region. Because the same set of individuals was used for these two computations, the source of the difference comes from that of the evolutionary rate. If we assume that the same relationship holds for ape mtDNA sequence evolution, mtDNA D-loop diversity values for our sample are expected to be about 10 times those shown in Table 3.

Table 5. Variant sites of siamang (*Hylobates syndactylus*)

Sequence	Site position
	11111
	11122346688911257
	1289905075612369082
	1234783951978447868069703
Consensus	GTTATAATTCCCTAAATACTAATTA
<i>H. syndactylus</i> .#1	***...GC...CG.G..TCG....
<i>H. syndactylus</i> .#2	GTTA.....TT....C.....
<i>H. syndactylus</i> .#3	***...GC.....GT....C.
<i>H. syndactylus</i> .#4	TA...C..CT.T..G.....GC.G
<i>H. syndactylus</i> .#5	*.ATC.....TT....C....G...

Relationship of Bornean and Sumatran Orangutan

The two orangutan subspecies (*Pongo pygmaeus pygmaeus* and *P. p. abelii*) differ cytogenetically by a pericentric inversion in chromosome 2, a specific inversion in chromosome Y, and Sumatran Y carries a distally located nuclear organizing region that is absent in Bornean orangutan, but they interbreed in captivity and produce fertile offspring (Xu and Arnason 1996). Genetic differences between and within Bornean and Sumatran orangutans have been studied by various authors. Zhi et al. (1996) examined mtDNA restriction fragment length polymorphisms (RFLPs), partial sequences of the 16S rRNA gene, and nuclear minisatellite polymorphism. Bornean orangutan (and *P. p. pygmaeus*) clearly separated from Sumatran orangutan (*P. p. abelii*) in the mtDNA gene genealogy. This dichotomous pattern was also observed by Warren et al. (2000), who used mtDNA control region sequences. We also observed two distinct lineages in orangutan (Figure 4). However, Muir et al. (2000) presented a somewhat different picture using NADH dehydrogenase subunit 3 and cytochrome *b* gene sequences. Bornean

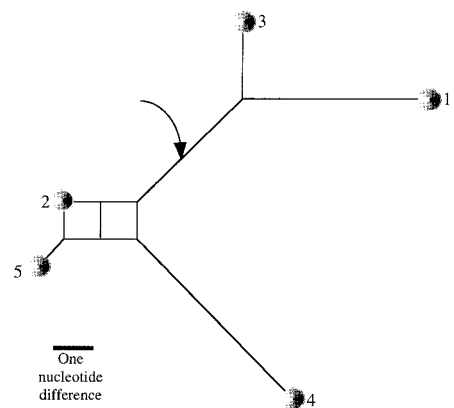


Figure 5. Phylogenetic networks of the mtDNA 16S rRNA gene region for *H. syndactylus* (siamang). Edge lengths are proportional to the number of nucleotide differences. Arrows indicate the edges in which roots are probably located.

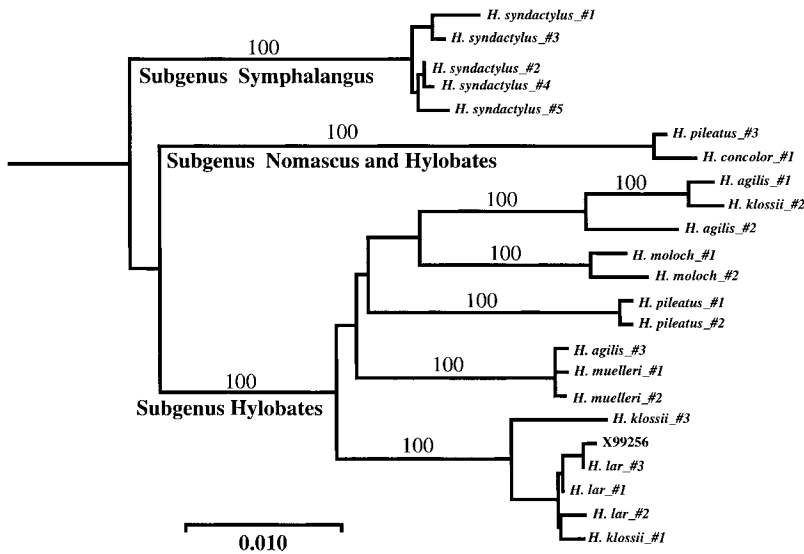


Figure 6. Phylogenetic tree of the mtDNA 16S rRNA gene region for gibbons.

haplotypes formed a compact cluster, while Sumatran haplotypes showed much larger heterogeneity, and the root of the orangutan gene tree is within the Sumatran group (Figure 4 of Muir et al. 2000). Because sampled individuals differ from study to study, in the future it may be necessary to study more individuals as well as nuclear sequence data to clarify the relationship between Bornean and Sumatran orangutans.

The same problem applies to within-subspecies sequence variation. Warren et al. (2001) studied many Bornean orangutan individuals and found a considerable amount of mtDNA variation in Bornean orangutan. On the other hand, Muir et al. (2000) found much greater genetic variation among Sumatran orangutan than Bornean orangutan. Our present results are intermediate between the results of Warren et al. (2000) and those of Muir et al.

(2000); nucleotide diversity (0.0066 ± 0.0013) for Sumatran orangutan was about twice as high as that (0.0034 ± 0.0008) for Bornean orangutan. Zhi et al. (1996) estimated nucleotide diversity based on their mtDNA RFLP data, and Sumatran orangutan showed more than five times higher diversity (0.0175) than Bornean orangutan (0.0033). However, their mtDNA 16S rRNA sequence data seem to show higher variation for Bornean orangutan than Sumatran orangutan. A slightly higher heterozygosity was observed for Bornean orangutan from DNA fingerprint data (Zhi et al. 1996).

Phylogenetic Relationship of Gibbon Species

Hayashi et al. (1995) used a single individual for six gibbon species and estimated the phylogenetic tree of those gibbon species. The five species of subgenus *Hylobates* (*H. lar*, *H. klossii*, *H. agilis*, *H. moloch*, and *H. pileatus*) formed a clear monophyletic cluster in their tree. We also observed a clear clustering of the *Hylobates* species subgenus, except for one individual of *H. pileatus* (Figure 6). However, the relationship within this subgenus is unclear. *H. lar* and *H. klossii* formed a monophyletic group with 100 bootstrap probability in Hayashi et al. (1995). Two individuals of *H. klossii* clustered with *H. lar* in our results, but one *H. klossii* individual clustered with two *H. agilis* individuals (Figure 6). There are several competing hypotheses on the phylogenetic relationship of species within subgenus *Hylobates* (Geissmann 1995). Because these gibbon species are closely related, hybridization is possible, and this may be the source of incongruent phylogenetic relationships, depending on the individuals used from the same species. Another problem is species identification. It is not so easy to identify gibbon species (Geissmann 1995).

It is known that a mating of siamang and common gibbon produced a viable offspring (Myers and Shafer 1979). Therefore there is a possibility of introgression, even between subgenera. However, a close similarity between one individual of *H. pileatus* and *H. concolor* is problematic, for the geographic distributions of *H. concolor* and *H. pileatus* does not overlap (Geissmann 1995). Further study will be necessary on this matter.

The phylogenetic relationship of the subgenera of Hylobatidae is also not yet established. *H. concolor* of *Nomascus* was located as basal in Hayashi et al. (1995),

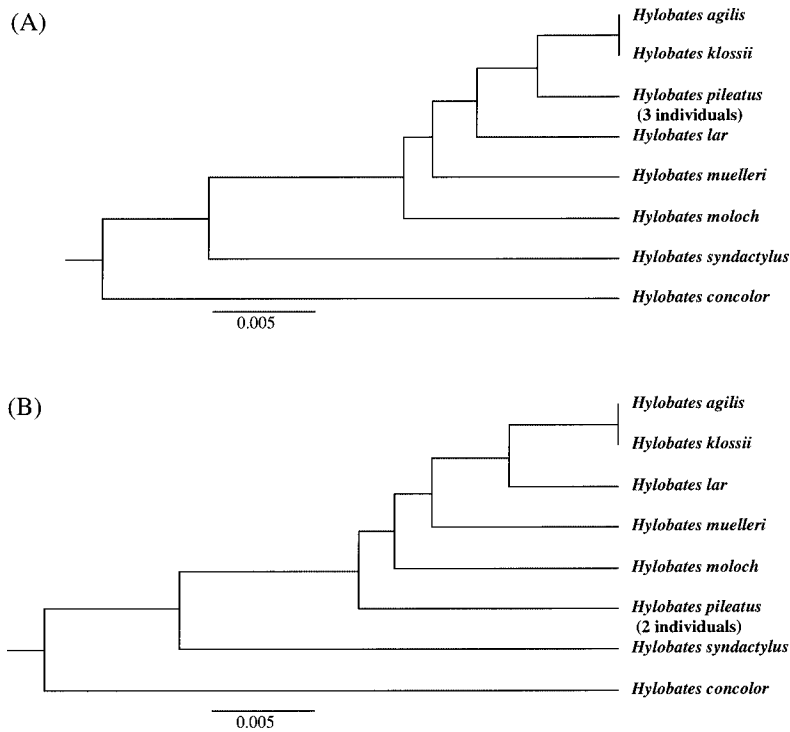


Figure 7. Phylogenetic tree of the eight gibbon species. UPGMA was used. (A) When all three *H. pileatus* individuals were included. (B) When *H. pileatus* no. 3 which was closely related to *H. concolor* was eliminated from the comparison.

and *H. gabriellae* and *H. leucogenys*, both belonging to subgenus *Nomascus*, were also basal in Roos and Geissmann (2001). However, our gene tree for gibbons (Figure 6) showed that *H. syndactylus*, belonging to subgenus *Symphalangus*, was basal. Because of the shorter branch lengths for the *H. syndactylus* lineage, *H. concolor* became basal in the species tree estimated by using UPGMA (Figure 7). More sequence data from nuclear DNA as well as mtDNA are necessary to clarify this higher order phylogenetic relationship of gibbons.

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