J Mol Evol (1996) 43:431–437

JOURNAL OF MOLECULAR EVOLUTION

The Mitochondrial DNA Molecule of Sumatran Orangutan and a Molecular Proposal for Two (Bornean and Sumatran) Species of Orangutan

Xiufeng Xu, Ulfur Arnason

Division of Evolutionary Molecular Systematics, University of Lund, Sölvegatan 29, S-223 62 Lund, Sweden

Received: 15 May 1996 / Accepted: 21 June 1996

Abstract. The complete mitochondrial DNA (mtDNA) molecule of Sumatran orangutan, plus the complete mitochondrial control region of another Sumatran specimen and the control regions and five protein-coding genes of two specimens of Bornean orangutan were sequenced and compared with a previously reported complete mtDNA of Bornean orangutan. The two orangutans are presently separated at the subspecies level. Comparison with five different species pairs—namely, harbor seal/grey seal, horse/donkey, fin whale/blue whale, common chimpanzee/pygmy chimpanzee, and *Homo*/common chimpanzee—showed that the molecular difference between Sumatran and Bornean orangutan is much greater than that between the seals, and greater than that between the two chimpanzees, but similar to that between the horse and the donkey and the fin and blue whales. Considering their limited morphological distinction the comparison revealed unexpectedly great molecular difference between the two orangutans. The nucleotide difference between the orangutans is about 75% of that between *Homo* and the common chimpanzee, whereas the amino acid difference exceeds that between *Homo* and the common chimpanzee. On the basis of their molecular distinction we propose that the two orangutans should be recognized as different species, *Pongo pygmaeus,* Bornean orangutan, and *P. abelii,* Sumatran orangutan.

Key words: Species recognition — Orangutan — *Pongo* — Hominoids — Species pairs

Correspondence to: U. Arnason

Introduction

The application of molecular methods has added new dimensions to studies in systematics and phylogenetics by making it possible to compare homologous genes of taxa among which other biological comparisons are inadequate or inapplicable. In the same way molecular data can be applied to address evolutionary divergences in cases where the fossil record is fragmentary or absent and to assess systematic designations at different levels.

Bornean and Sumatran orangutans are classified as different subspecies, *Pongo pygmaeus pygmaeus,* and *P. p. abelii,* by recent morphologically based systematics (Koenigswald 1982; Courtenay et al. 1988; Groves 1986; Groves et al. 1992). The orangutans differ cytogenetically by a pericentric inversion in chromosome 2, but they interbreed in captivity and produce fertile offspring (de Boer and Seuanez 1982). The Y chromosomes of Sumatran and Bornean orangutans differ by a pericentric inversion, and the long arm of the Sumatran Y carries a distally located nucleolar organizing region that is absent in Bornean orangutan (Schempp et al. 1993, 1995). The molecular relationship between the two orangutans has been studied previously in analyses based on allelic variation (Bruce and Ayala 1979), restriction mapping of mtDNA (Ferris et al. 1981), nuclear DNA hybridization (Caccone and Powell 1989), two-dimensional protein electrophoresis (Janczewski et al. 1990), and sequence comparisons of the mitochondrial COII gene (Ruvolo et al. 1994). These studies have shown similar, or somewhat greater (Caccone and Powell 1989), difference between the two orangutans than that between the common and the pygmy chimpanzee.

In the present study we address the systematic status of the Sumatran and the Bornean orangutans by quantifying their molecular difference on the basis of comparison of complete mitochondrial DNA (mtDNA) molecules and by comparing this difference with that of all closely related mammalian species pairs currently represented by complete mtDNAs. The use of complete mtDNAs is essential for the comparison because it has been shown (Arnason and Johnsson 1992; Cao et al. 1994) that individual mtDNA genes may provide different answers to phylogenetic questions. The species pairs included in the comparison are harbor seal (Arnason and Johnsson 1992)/grey seal (Arnason et al. 1993); horse (Xu and Arnason 1994)/donkey (Xu et al. 1996); fin whale (Arnason et al. 1991a)/blue whale (Arnason and Gullberg 1993); common chimpanzee (Arnason et al. 1996a)/pygmy chimpanzee (Horai et al. 1995); *Homo* (Arnason et al. 1996a)/common chimpanzee; Bornean orangutan (Horai et al. 1995)/Sumatran orangutan. In addition to the analyses of complete molecules we have also sequenced and compared the complete mtDNA control region of an additional specimen of Sumatran orangutan plus the control regions and the complete NADH1, COII, ATPase6, ATPase8, and Cyt *b* genes of two specimens (''Anna,'' ''Dennis'') of Bornean orangutan. This parallel sequencing of additional specimens was undertaken in order to assess on a wider basis the mtDNA distinction between and among Sumatran and Bornean orangutans.

Materials and Methods

DNA, enriched with respect to mitochondrial DNA, was isolated from frozen kidney tissue of two specimens of Sumatran orangutan (YN93-312 male, YN91-227 female) following a previously described procedure (Arnason et al. 1991a). The samples were generously provided by Dr. Harold M. McClure, Yerkes Regional Primate Research Center, Atlanta, USA.

The mtDNA of the male specimen was sequenced in its entirety. The sequencing was based on 28 unique clones (*Bcl*I, *Bln*I, *Spe*I, *Xba*I, *Hin*dIII) most of which were represented several times in the collection. All regions of the molecule were represented by a minimum of two clones. Sequencing was performed manually applying the dideoxy termination technique (Sanger 1981) with ^{35}SdATP using both universal and numerous specific sequencing primers.

The complete control region of the female specimen was sequenced after PCR amplification and subsequent cloning in M13mp18/19. The six PCR clones sequenced were identical and the same as the male sequence. The same PCR, cloning, and sequencing procedures were applied for the sequencing of the control regions plus five protein-coding genes of two specimens (''Anna,'' ''Dennis'') of Bornean orangutan. This analysis was performed on blood samples collected at Aalborg Zoo for cytogenetic investigation.

The orangutan sequences have been deposited at EMBL with accession numbers X97707–97718 and X98472. Users of the sequences are kindly requested to refer to the present paper and not only to the accessions numbers.

Results

The length of the reported mtDNA molecule of Sumatran orangutan is 16,499 nt (nucleotides) and the nt composition (L-strand) outside the control region is (in percent): A = 30.6; C = 32.5; G = 13.2; T = 23.7. The corresponding values for Bornean orangutan (Horai et al. 1995) are virtually identical: A = 30.7; C = 32.4; G = 13.1; T = 23.8. Outside the control region the two sequences differ overall by 6.3%. The control regions of the two complete sequences, however, differ by a large (80 nucleotide) deletion in the published Bornean sequence relative to the Sumatran. Our sequencing of the control region of the two other specimens of Bornean orangutan has not confirmed this indel difference and, therefore, the alignment of the orangutan control regions in Fig. 1 includes, in addition to the Sumatran sequence, both our sequences (''Anna'' and ''Dennis'') of Bornean orangutan as well as that previously described (Horai et al. 1995). The control region of an additional specimen (female) of Sumatran orangutan was identical to that of the complete (male) sequence. The Sumatran and Bornean (''Dennis'') control regions differ by 11.8%, 82 transitions, 29 transversions, and nine indels (insertions/deletions), counting each indel as one difference irrespective of its length. The control region of ''Anna'' differed by 1.4%, 13 transitions, and one indel, from ''Dennis.'' Apart from indel differences the three Bornean control regions are about equidistantly related to the two identical Sumatran sequences. There are 102 positions, consisting of 70 transitions, 25 transversions, and seven indels (counting each indel as one position irrespective of its length), where the three Bornean sequences of Fig. 1 are identical and different from the Sumatran sequence(s). This number includes all identical positions of ''Anna'' and ''Dennis'' in the region corresponding to the large deletion in the complete Bornean sequence.

The differences between the 13 peptide-coding genes of the two complete mtDNAs were detailed according to gene, codon position, type of substitution (transition or transversion), and aa (amino acid) difference (Table 1). The length of the concatenated alignment of the 13 peptide-coding genes is 11,394 nt. The two sequences differ at 811 positions, 7.1%. Of the 811 differences, 163 are in first, 71 in second, and 577 in third codon position, respectively. The ratio for total nucleotide substitution according to first, second, and third codon position is 2.3:1:8.1. The number of conservative nt substitutions (Irwin et al. 1991), i.e., all nonsynonymous substitutions in first codon position, all substitutions in second codon position, and transversions in third codon position, is 235. The codon position ratio for conservative nucleotide substitutions is 1.6:1:0.7. The inferred aa sequences of the 13 genes differ at 178 positions, 4.7%.

The 12S rRNA genes of the two sequences differ at 29

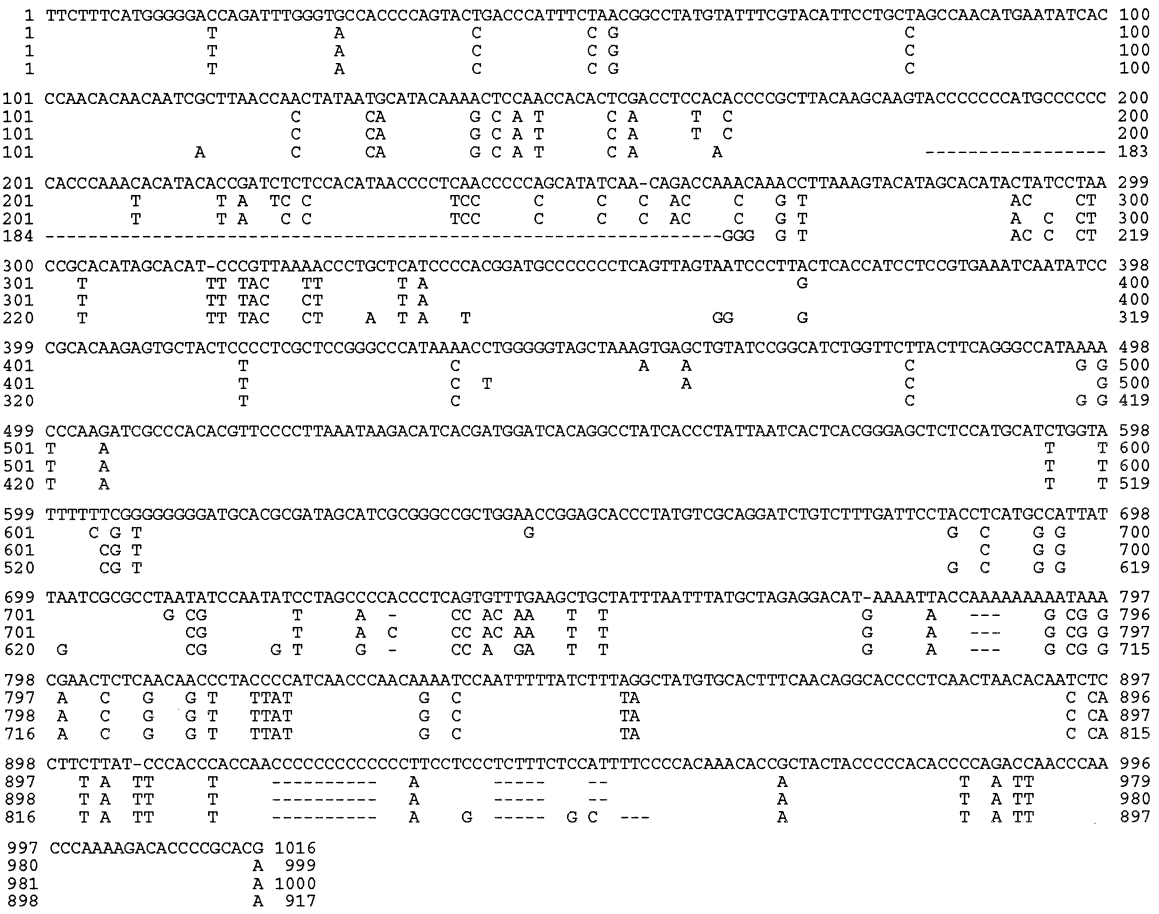

Fig. 1. Alignment between mitochondrial control regions of Sumatran orangutan (top), Bornean orangutan ''Dennis,'' Bornean orangutan ''Anna,'' and previously reported Bornean orangutan (Horai et al. 1995) (bottom). Nucleotide differences relative to Sumatran sequence are shown with *capital letters.* Indels (insertions/deletions) are marked by *dashes* (-). Each row includes 100 positions. *Numbers* refer to actual numbering of the control region of each sequence.

positions (3.0%), 27 transitions, one transversion, and one indel. The 16S rRNA genes differ at 73 positions (4.7%), 59 transitions, ten transversions, and four indels. The 22 tRNA genes (total combined length 1,508 nt) differ at 54 positions (3.6%), 51 transitions, one transversion, and two indels. The greatest difference was registered in tRNA-Asp (seven transitions), tRNA-Lys (six transitions), and tRNA-Ala (five transitions), whereas tRNA-Ile, tRNA-Arg, and tRNA-Leu(CUN) are identical in the two sequences.

In order to address the molecular difference among Bornean orangutans the NADH1, COII, ATPase8, ATPase6, and Cyt *b* genes of ''Anna'' and ''Dennis'' were sequenced and compared with the same genes of the complete Bornean mtDNA. The result is shown in Table 2, which also includes the data of a corresponding comparison between the same genes of the complete Sumatran and Bornean mtDNAs. The comparison shows that there is somewhat less difference between ''Anna'' and ''Dennis'' than between either of these and the complete Bornean sequence. Depending on gene and the mode of comparison the difference among the three specimens is ≈1/10–1/20 of the difference between the Sumatran and Bornean specimens.

The differences between the 12S rRNA genes, the 16S rRNA genes, the t-RNA genes, the 13 peptide-coding genes, and the complete molecules outside the control regions of the complete Sumatran and Bornean mtDNAs were compared with those of five other closely related pairs of species (Table 3). As evident from this comparison, the total nt difference between the two orangutans is much greater than that between the two seals and between the common and the pygmy chimpanzees. The difference is similar to that between horse and donkey and between fin and blue whales. The nt difference between the two orangutans is about 75% of that between *Homo* and the common chimpanzee, but the aa difference between the two orangutans is actually greater than between *Homo* and the common chimpanzee. The common and pygmy chimpanzees and harbor and grey seals are all distinct species, and the molecular difference between the Sumatran and Bornean orangutan is thus decidedly greater than between these two mammalian species pairs, one hominoid and one carnivoran.

Table 1. Nucleotide (detailed according to codon positions 1, 2, and 3) and amino acid differences between the mitochondrial protein-coding genes of Sumatran and Bornean orangutan[a]

Gene	Length (nt)	nt difference		1			2		3				aa difference	
				Ti			Ti	Tv	Ti		Tv			
		No.	(%)	a	b	Tv			a	b	a	b	No.	(%)
NADH1	957	60	(6.3)	4	11	2	5	1	8	26	—	3	18	(5.6)
NADH2	1,044	87	(8.3)	5	17	3	8	1	9	41	—	3	27	(7.8)
COI	1,542	92	(6.0)	7	5	—	1	—	12	60	—	7	7	(1.4)
COII	684	29	(4.2)	1	1	—	1	—	1	22	2	1	2	(0.9)
ATPase8	207	20	(9.7)	3	2	1	6	1	1	6	—	—	10	(14.5)
ATPase6	681	69	(10.1)	6	10	1	7	1	6	31	3	4	20	(8.8)
COIII	783	61	(7.8)	1	4	3	3	1	10	35	—	4	10	(3.8)
NADH3	345	26	(7.5)	1	2	2	1	—	7	10	1	2	5	(4.3)
NADH4L	297	17	(5.7)	2	—	—	—	—	3	12	—	—	—	—
NADH4	1,377	94	(6.8)	6	10	5	8	1	13	47	1	3	23	(5.0)
NADH5	1,812	139	(7.7)	4	21	2	13	3	17	69	2	8	36	(6.0)
NADH6	525	31	(5.9)	2	2	—	3	—	1	23	—	—	5	(2.9)
Cytb	1,140	86	(7.5)	7	9	1	6	—	11	46	5	1	15	(3.9)
Total	11,394	811	(7.1)	49	94	20	62	9	99	428	14	36	178	(4.7)
Cons. diff.					114		71				50			
Total diff.					163		71			577				
Ratio total diff.					2.3		1.0			8.1				
Rato cons. diff.					1.6		1.0			0.7				

[a] Ti: transitions; Tv: transversions; aa: amino acid; a: substitutions involving leucine in both sequences; b: differences other than those involving leucine in both species. The orangutan has ATT (isoleucine) as start codon of the NADH2 and NADH3 genes. The NADH5 gene possibly has a ACA (threonine) start codon, whereas *Homo*, chimpanzees, and gorilla have a methionine start codon in this position. In the orangutan the first methionine codon is in aa position 3 and the protein might thus be two aa shorter at the N-terminus than that of the other species. It is notable that ACA is also the probable start codon of the NADH1 gene of the chimpanzee (Arnason et al. 1996a). Other protein-coding genes of the orangutan mtDNA have a methionine start codon. The COI, COIII, NADH3, NADH4 and Cyt *b* genes are not terminated by complete stop codons.

Table 2. Percentage total nucleotide, conservative nucleotide, and amino acid differences of five mtDNA protein-coding genes in pairwise comparisons of Bornean and Sumatran orangutans[a]

Gene	Length (nt)	Nucleotide				Conservative				Amino acid			
		A/D	A/B	D/B	S/B	A/D	A/B	D/B	S/B	A/D	A/B	D/B	S/B
NADH1	957	0.3	0.8	0.7	6.3	0.1	0.5	0.4	2.3	0.3	1.3	0.9	5.6
COII	684	0.3	0.4	0.4	4.2	0.2	0.2	0	0.7	0.4	0.4	0	0.9
ATPase8	207	0.5	1.5	1.0	9.7	0	1.0	1.0	4.8	0	2.9	2.9	14.5
ATPase6	681	0.4	1.2	0.7	10.1	0.3	0.6	0.3	3.8	0.9	0.9	0	8.8
Cytb	1,140	0.4	0.2	0.6	7.5	0.1	0	0.1	1.9	0.3	0	0.3	3.9
Mean		0.4	0.7	0.7	7.2	0.1	0.3	0.2	2.3	0.4	0.7	0.5	5.3

[a] A: Bornean orangutan "Anna"; D: Bornean orangutan ''Dennis''; B: Bornean orangutan previously reported (Horai et al. 1995); S: Sumatran orangutan presently described. Mean values are based on the combined length of the five sequences

Discussion

The nt difference between the peptide-coding genes of the Sumatran and the Bornean orangutan is 7.1%. The corresponding difference between *Homo* and the common chimpanzee is 9.8%. The aa difference between the orangutans is 4.7%, whereas the corresponding difference between *Homo* and the common chimpanzee is 4.4%. Thus, there is a notable discrepancy between the two modes of comparison. The codon position substitution ratio for conservative nt changes between the two orangutans (1.6:1:0.7) is almost the same as that for the comparison between *Homo* and the common chimpanzee (1.6:1:0.8). The corresponding ratio for all nt substitu-

Table 3. Percent nucleotide (nt) and amino acid (aa) difference in the mtDNAs of six pairs of closely related mammals

	nt difference					aa difference
Species pair	Outside control region	12S rRNA	16S rRNA	t-RNA genes	Peptide-coding genes	
Harbor/grey seal	3.5	2.2	2.6	1.5	4.0	1.6
Horse/donkey	6.9	4.9	3.7	3.4	8.0	1.9
Fin/blue whale	7.5	4.8	5.3	3.3	8.6	3.0
Common/pygmy chimpanzee	3.7	2.1	2.3	2.1	4.2	2.3
Sumatran/Bornean orangutan	6.3	3.0	4.7	3.6	7.1	4.7
Homo/common chimpanzee	8.5	4.5	5.3	4.2	9.8	4.4

tions between the orangutans (2.3:1:8.1), however, differs markedly from that between *Homo* and the common chimpanzee (2.8:1:12.3). The findings suggest that there has been a general increase in the rate of nt substitution in *Pongo* relative to *Homo* and *Pan* and that the substitution has been largely codon position independent. A general increase of this kind would automatically elevate the aa difference because all substitutions in first codon position, except leucine transitions, as well as all substitutions in second codon position, are nonsynonymous.

The analyses of the complete control regions and the NADH1, COII, ATPase8, ATPase6, and Cyt *b* genes of ''Anna'' and ''Dennis'' confirmed the pronounced difference between the complete Sumatran and Bornean sequences (Fig. 1 and Table 1). As evident in Table 1, the difference between Sumatran and Bornean orangutans is particularly pronounced in the ATPase6 and ATPase8 genes. In the ATPase6 gene the two orangutans show 20 aa differences (8.8%), whereas *Homo* and the common chimpanzee differ at only 11 aa positions in the same gene (4.9%). The ATPase6 gene of *Homo* is about equidistant to Sumatran (18.6% aa difference) and any Bornean (16.8% aa difference) orangutan. It is thus apparent that in the *Pongo* lineage, and also after their divergence, nonsynonymous nt substitution has been pronounced in both orangutans. Sumatran and Bornean (''Anna,'' ''Dennis'') orangutans differ at eight aa positions, 11.8%, in the ATPase8 gene. Also in this gene the difference is much greater than that between *Homo* and the common chimpanzee, 5.9%. In contrast to these findings the aa difference (3.9%) between the Cyt *b* gene of the two complete orangutan mtDNAs is much less than that between the same gene of *Homo* and the common chimpanzee, 7.1% (Arnason et al. 1996a). These values exemplify in a clear manner how the molecular evolution of the same gene may differ within the same family.

Irrespective of the mode of comparison (nt or aa) the present comparisons have shown that the molecular differences between the two orangutans are considerably greater than those between acknowledged species of hominoids (common/pygmy chimpanzee) and some other mammals (harbor/grey seals). We propose, therefore, that the two orangutans should be given the rank of separate species, *Pongo abelii,* Sumatran orangutan, and *Pongo pygmaeus,* Bornean orangutan. The two orangutans would not qualify, however, as separate species according to the biological-species concept (Mayr 1940), according to which allopatric forms are included in the same species if they can potentially interbreed. We make the present species status proposal, however, despite the fact that the two orangutans produce fertile offspring in captivity. The reason for this is that we do not consider hybridization incompatibility as an absolute parameter in this context because it has been shown previously that distinct species, such as the fin and the blue whales, which show pronounced molecular differences (Table 3), may still produce fertile offspring in their natural environment (Spilliaert et al. 1991; Arnason et al. 1991b). Among plants the difficulty of using hybridization incompatibility as a parameter for species distinction has been given detailed treatment (Stebbins 1950). The classical examples here are *Platanus occidentalis* and *P. orientalis* and *Catalpa ovata* and *C. bignonioides.* The hybrids of both these crosses are fully fertile despite the clear distinction within each species pair.

The problems associated with species definition were recently addressed by Mallet (1995). In spite of the fact that the proposed genotypic cluster definition primarily applies to forms with some degree of sympatry, the argument of using the option of a single species as a null hypothesis is relevant for the present discussion of the taxonomic distinction between the Sumatran and Bornean orangutans. As mentioned earlier, the common and the pygmy chimpanzees are recognized as separate species. The molecular difference between the two orangutans is greater, however, than that between the two chimpanzees. Therefore, if a single orangutan species is the postulated null hypothesis it necessarily follows that the two presently recognized chimpanzee species (common and pygmy) should be redefined as subspecies. It should be recognized, however, that despite the apparent applicability of the genotypic cluster definition to molecular findings, the use of it for classifying individual specimens has limitations.

The total mitochondrial nt difference between Sumatran and Bornean orangutans is about 75% of that between *Homo* and *Pan.* For this reason the dating of the divergence between Sumatran and Bornean might appear

relatively uncomplicated. This is not so, because the dating of the *Homo*/*Pan* divergence has, in turn, usually been based on a divergence between *Pongo* and the lineage leading to *Gorilla/Pan/Homo.* There is, however, no fossil record that can be related directly to this split, but a sister-group relationship between *Pongo* and *Sivapithecus* has been postulated (Andrews and Cronin 1982; Kappelman et al. 1991). The age of the oldest *Sivapithecus* fossils, 12.5 million years, has generally been applied for dating various hominoid divergences after adding some 0.5–2.5 million years to this paleontological dating. It is quite evident that by such an approach the molecular dating of other divergences, e.g., that of *Homo* and *Pan,* will simply be a direct function of the dating chosen for the divergence between *Sivapithecus* (*Pongo*) and the lineage leading to *Gorilla/Pan/Homo,* irrespective of the sophistication of the calculations in other respects.

The application of a nonprimate reference, the evolutionary divergence between artiodactyls and cetaceans dated at 60 MYBP (million years before present) (Arnason and Gullberg 1996), has yielded datings of hominoid divergences that differ radically from those based on the *Sivapithecus/Pongo* reference (Arnason et al. 1996b). According to the artiodactyl/cetacean 60 MYBP reference the divergence between Sumatran and Bornean orangutans took place ≈10 MYBP. Irrespective of this dating the molecular difference between the two orangutans (⩾75% of that between *Homo* and *Pan*) is highly unexpected considering the fact that the evolutionary separation of Sumatran and Bornean orangutans has not resulted in any osteomorphological differentiation that has permitted their recognition as different species. Therefore no paleontological findings of *Pongo* have the potential to resolve the evolutionary separation of Sumatran and Bornean orangutans. The distinction between *Sivapithecus* and the *Gorilla/Pan/Homo* lineage is based on paleontologically definable characters. It is, therefore, quite possible that the morphological distinction between *Sivapithecus* and *Pongo,* which are separated at the generic level, is the result of a much longer evolutionary separation than generally recognized, and that the divergence between the *Sivapithecus/Pongo* and *Gorilla/Pan/Homo* lineages took place much earlier than inferred from the paleontological record. If this is so the use of the evolutionary separation between *Pongo* and the lineage leading to *Gorilla/Pan/Homo* as a reference set at 13–15 MYBP for dating other hominoid divergences becomes highly questionable because it will automatically produce too-recent datings of other divergences. A faster evolution in the reference (*Pongo*) will also, unless calibrated for, automatically produce too-recent datings of other divergences. The present findings are consistent with the understanding that datings based on the fragmentary paleontological record of the primates will invariably yield too-recent datings of evolutionary divergences (Martin 1993).

The difference between the Bornean control regions, ''Anna'' and ''Dennis,'' is 1.4%. These data in combination with those of five mtDNA peptide-coding genes (Table 2) suggest that the Bornean samples represent mtDNA lineages that have been separated (1/10–1/20 of the total time of separation between the Sumatran and the Bornean orangutan. The findings show that considerable evolutionary divergence has taken place within Borneo itself. The pronounced distinction between the Bornean and Sumatran mtDNA haplotypes suggests that no mtDNA exchange has taken place between the two islands via the geographical connections that existed at different times during Pleistocene and Holocene. It should be recognized, however, that, like other molecular studies of the orangutan, the geographical origin of the presently studied samples of Sumatran and Bornean orangutans is not known. A molecular population study of the Bornean orangutan will therefore necessarily require analyses of samples with known geographical origin. The present analysis underlines that any crossbreeding between Sumatran and Bornean orangutans should be avoided in order to preserve the characteristics of each species and that care should also be taken not to intermingle Bornean orangutans originating from different geographical localities.

Acknowledgments. We thank Dr. Harold M. McClure, Yerkes Regional Primate Research Center, Atlanta, for generously providing us with the samples of Sumatran orangutans and Drs. Jens Lilleør, Peter Arctander, and Bengt Widegren for samples of Bornean orangutans; also Drs. Peter Andrews, Einar Arnason, Francois Catzeflis, Marc Godinot, Dan Graur, Eric Harley, Wilfried W. de Jong, Albert Levan, and James Mallet for valuable comments on the manuscript. The work was supported by the Swedish Natural Sciences Research Council, The Crafoord Foundation, The Erik Philip-Sörensen Foundation, The Carl Tesdorpf Foundation, and by contract ERBCHR XCT 930254 from the European Commission.

References

Andrews P, Cronin JE (1982) The relationships of *Sivapithecus* and *Ramapithecus* and the evolution of the orang-utan. Nature 297:514–546

Arnason U, Gullberg A (1993) Comparison between the complete mtDNA sequences of the blue and the fin whale, two species that can hybridize in nature. J Mol Evol 37:312–322

Arnason U, Gullberg A (1996) Cytochrome *b* nucleotide sequences and the identification of five primary lineages of extant cetaceans. Mol Biol Evol 13:407–417

Arnason U, Johnsson E (1992) The complete mitochondrial DNA sequence of the harbor seal, *Phoca vitulina.* J Mol Evol 34:493–505

Arnason U, Gullberg A, Widegren B (1991a) The complete nucleotide sequence of the mitochondrial DNA of the fin whale, *Balaenoptera physalus.* J Mol Evol 33:556–568

Arnason U, Spilliaert R, Palsdottir A, Arnason A (1991b) Molecular identification of hybrids between the two largest whale species, the blue whale (*Balaenoptera musculus*) and the fin whale (*B. physalus*). Hereditas 115:183–189

Arnason U, Gullberg A, Johnsson E, Ledje C (1993) The nucleotide sequence of the mitochondrial DNA molecule of the grey seal,

Halichoerus grypus, and a comparison with mitochondrial sequences of other true seals. J Mol Evol 37:323–330

Arnason U, Xu X, Gullberg A (1996a) Comparison between the complete mitochondrial DNA sequences of human and the common chimpanzee. J Mol Evol 42:145–152

Arnason U, Gullberg A, Janke A, Xu X (1996b) Pattern and timing of evolutionary divergences among hominoids based on analyses of complete mtDNAs. J Mol Evol (in press)

de Boer LEM, Seuanez HN (1982) The chromosomes of the orangutan and their relevance to the conservation of the species. In: de Boer LEM (ed) The orang utan its biology and conservation. Dr. W Junk, The Hague, pp 135–170

Bruce EJ, Ayala FJ (1979) Phylogenetic relationships between man and the apes: electrophoretic evidence. Evolution 33:1040–1056

Caccone A, Powell JR (1989) DNA divergence among hominoids. Evolution 43:925–942

Cao Y, Adachi J, Janke A, Pääbo S, Hasegawa M (1994) Phylogenetic relationships among eutherian orders estimated from inferred sequences of mitochondrial proteins: instability of a tree based on a single gene. J Mol Evol 39:519–527

Courtenay J, Groves CP, Andrews P (1988) Inter- or intra-island variation? An assessment of the differences between Bornean and Sumatran Orang-utans. In: Schwartz (ed) Orang-utan Biology. Oxford University Press, Oxford, pp 19–29

Ferris SD, Brown WM, Davidson WS, Wilson AC (1981) Extensive polymorphism in the mitochondrial DNA of apes. Proc Natl Acad Sci USA 78:6391–6323

Groves CP (1986) Systematics of the great apes. In: Swindler DR, Erwin J (eds) Comparative primate biology I. Systematics, evolution, and anatomy. Alan R Liss, New York, pp 187–217

Groves CP, Westwood C, Shea BT (1992) Unfinished business: Mahalanobis and a clockwork orang. J Hum Evol 22:327–340

Horai S, Hayasaka K, Kondo R, Tsugane K, Takahata N (1995) Recent African origin of modern humans revealed by complete sequences of hominoid mitochondrial DNAs. Proc Natl Acad Sci USA 92: 532–536

Irwin DM, Kocher TD, Wilson AC (1991) Evolution of the cytochrome b gene of mammals. J Mol Evol 32:128–144

Janczewski DN, Goldman D, O'Brien SJ (1990) Molecular genetic divergence of orangutan *Pongo pygmaeus* subspecies based on isozyme and two dimensional gel electrophoresis. J Hered 81:375–387

Kappelman J, Kelley J, Pilbeam D, Sheikh KA, Ward S, Anwar M, Barry JC, Brown B, Hake P, Johnson NM, Raza SM, Shah SMI (1991) The earliest occurrence of *Sivapithecus* from the middle Miocene Chinji Formation of Pakistan. J Hum Evol 21:61–73

Koenigswald GHR von (1982) Distribution and evolution of the orang utan, *Pongo pygmaeus* (Hoppius). In: de Boer LEM (ed) The orang utan its biology and conservation. Dr. W. Junk, The Hague, pp 1–15

Mallet J (1995) A species definition for the modern synthesis. Trends Ecol Evol 10:294–299

Martin RD (1993) Primate origins: plugging the gaps. Nature 363:223–234

Mayr E (1940) Speciation phenomina in birds. Am Nat 74:249–278

Ruvolo M, Pan D, Zehr S, Goldberg T, Disotell TR, von Dornum M (1994) Gene trees and hominoid phylogeny. Proc Natl Acad Sci USA 91:8900–8904

Sanger F (1981) Determination of nucleotide sequences in DNA. Science 214:1205–1210

Schempp W, Toder R, Rietschel W, Gruetzner F, Mayerova A, Gauckler A (1993) Inverted and satellited Y chromosome in the orangutan (*Pongo pygmaeus*). Chromosome Res 1:69–75

Schempp W, Binkele A, Arnemann J, Glaser B, Ma K, Taylor K, Toder R, Wolfe J, Zeitler S, Chandley AC (1995) Comparative mapping of YRRM- and TSPY-related cosmids in man and hominoid apes. Chromosome Res 3:227–234

Spilliaert R, Vikingsson G, Arnason U, Palsdottir A, Sigurjonsson J, Arnason A (1991) Species hybridization between a female blue whale (*Balaenoptera musculus*) and a male fin whale (*B. physalus*): molecular and morphological documentation. J Hered 82:269–274

Stebbins GL (1950) Variation and evolution in plants. Columbia University Press, New York, pp 1–643

Xu X, Arnason U (1994) The complete mitochondrial DNA sequence of the horse, *Equus caballus:* extensive heteroplasmy of the control region. Gene 148:357–362

Xu X, Gullberg A, Arnason U (1996) The complete mitochondrial DNA (mtDNA) of the donkey and mtDNA comparisons among four closely related mammalian species-pairs. J Mol Evol 43:438–446