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Genetic Divergence of Orangutan Subspecies (*Pongo pygmaeus*)

Yun-wu Zhang,[1,2] Oliver A. Ryder,[1] Ya-ping Zhang[2]

[1] Center for Reproduction of Endangered Species, Zoological Society of San Diego, San Diego, CA 92112, USA
[2] Laboratory of Cellular and Molecular Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650223, China

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Abstract. Microsatellites and mitochondrial DNA sequences were studied for the two subspecies of orangutans (*Pongo pygmaeus*), which are located in Borneo (*P. p. pygmaeus*) and Sumatra (*P. p. abelii*), respectively. Both subspecies possess marked genetic diversity. Genetic subdivision was identified within the Sumatran orangutans. The genetic differentiation between the two subspecies is highly significant for ND5 region but not significant for 16s rRNA or microsatellite data by exact tests, although F_{ST} estimates are highly significant for these markers. Divergence time between the two subspecies is approximately 2.3 ± 0.5 million years ago (MYA) estimated from our data, much earlier than the isolation of their geological distribution. Neither subspecies underwent a recent bottleneck, though the Sumatran subspecies might have experienced expansion approximately 82,000 years ago. The estimated effective population sizes for both subspecies are on the order of 10^4. Our results contribute additional information that may be interpreted in the context of orangutan conservation efforts.

Key words: Orangutan — Genetic diversity — Genetic differentiation — mtDNA-Microsatellites.

Introduction

The orangutan, *Pongo pygmaeus,* is the only extant non-African great ape species. The current wild populations of orangutan exist only in the islands of Borneo and Sumatra, although the fossils of orangutans have been found in sites widespread throughout Southeast Asia (Smith and Pilbeam 1980), documenting a previously wider distribution. In the wild, the species has become severely threatened because of poaching and habitat destruction. It is estimated that populations in the wild may have declined by as much as 30–50% between 1983 and 1993 (Sodaro 1997).

Traditionally, the two island populations are regarded as two subspecies: *Pongo pygmaeus pygmaeus* (Linnaeus, 1760) on Borneo and *P. p. abelii* (Lesson, 1827) on Sumatra, based primarily on their distinctive morphological and behavioral characteristics (Groves 1971; Rijksen 1978; Nowak 1991; Demitros 1997). The two subspecies differ cytogenetically by a pericentric inversion of chromosome 2, and this difference has been used as an index of subspecies for captivity management (Seuanez et al. 1979; Ryder and Chemnick 1993). However, molecular genetic data from protein electrophoresis (Bruce and Ayala 1979), DNA hybridization (Caccone and Powell 1989), isozyme, two-dimensional electrophoresis (Janczewski et al. 1990), mtDNA-RFLP, mtDNA sequences, and minisatellites (Ferris et al. 1981; Ryder and Chemnick 1993; Xu and Arnason 1996a; Zhi et al. 1996; Muir et al. 2000) have demonstrated that the differences between the two subspecies are almost the same as or even higher than those between recognized species, e.g., chimpanzee (*Pan troglodytes*) vs. bonobo (*Pan paniscus*), horse (*Equus caballus*) vs. donkey (*Equus asinus*), etc. Some authors suggest the two orangutan subspecies should be elevated to species status (Xu and

Correspondence to: O. A. Ryder; E-mail: oryder@ucsd.edu

Arnason 1996a), although this analysis has been challenged (Muir et al. 1998).

Knowledge of genetic structure and taxonomic distinctions of orangutan subspecies is important not only for systematic issues but also for population studies relevant to species conservation, sample origin identification, and reintroduction efforts (O'Brien 1994; Zhi et al. 1996). In this study, we assess the genetic differentiation within and between the two subspecies, using two genetic measures of variation: mitochondrial DNA sequences and nuclear DNA microsatellite loci.

Materials and Methods

Samples

DNA was extracted from blood, tissues, and/or cell lines kept in the Center for Reproduction of Endangered Species, San Diego Zoo. For the Bornean orangutans, there were 13 male and 8 female samples; for the Sumatran orangutans, there were 8 male and 15 female samples. All individuals were born in the wild and regarded as unrelated, except one female Sumatran orangutan that was born in captivity and assumed to be unrelated to the other individuals. However, the exact original capture sites of those wild-born animals were not known. The karyotypes of all individuals, except the one born in captivity, were investigated and were consistent with their subspecies classification (Ryder and Chemnick 1993).

Microsatellite Screening

Twenty tri- or tetrameric microsatellite loci were screened here using primers developed for humans: D1S550, D2S1326, D2S1363, D2S425, D3S2459, D4S1627, D4S2408, D5S1505, D6S501, D7S1869, D10Q0002, D13S321, D13S765, D14S581, D16S540, D17S1289, D17S1303, D19S190, D20S476 and D22S684. One of each pair of primers was fluorescent dye-labeled. PCR was performed as follows: 94°C 40s, 56°C 40s, 72°C 30s, for 35 cycles, with 94°C 12 min at the beginning and 72°C, 10 min at the end. Sizing was scored using Model 373 automated sequencer with GENESCAN 672 software (PE Applied Biosystems).

ND5 Sequencing

An approximately 550 bp fragment of ND5 near the 5′-region was amplified using primers orang-ND5f: 5′-TAA CCG CCC TCA CCT TAA CTT CCC-3′, and orang-ND5r: 5′-GGT CAG GAT GAA GCC AAT GTC G-3′. PCR conditions were the same as those for microsatellite screening. Both strands were sequenced using ABI 373 sequencer (PE Applied Biosystems). Sequence alignment was performed using Sequencher 3.1 (Gene Codes Corporation, Inc.).

Data Analysis

In addition to ND5 data, orangutan DNA sequences from other mitochondrial regions have been previously reported (Ruvolo et al. 1994; Xu and Arnason 1996a; Zhi et al. 1996; Muir et al. 2000). Here we also reanalyzed 16s rRNA data from Zhi et al. (1996) (see Table 1). However, a preliminary test showed that 16s rRNA did not evolve at a constant rate in the two orangutan subspecies, so we did not focus our analysis on this region but rather on ND5. Other authors have contributed microsatellite studies of chimpanzees and bonobos (Morin 1992; Morin et al. 1994; Reinartz et al. 2000). Some of their data and/or results were utilized here for comparison (see Table 1).

Unless otherwise identified, data analyses were performed using the Arlequin software package (Schneider et al. 1997). Genetic diversities at the nucleotide level (π) for ND5 and 16s rRNA were estimated. Different substitution models are available in Arlequin and they gave similar results for our data set, so here we only listed results from the Kimura-2-parameter model (Kimura 1980). Differentiation between the two subspecies was evaluated by two methods: the pairwise F_{ST} (R_{ST}) values, which can be used as short-term genetic distances between populations (Slatkin 1995; Schneider et al. 1997) and the exact test, which explores all potential states of the contingency table to estimate the probability of observing a table equal or less than the observed sample configuration under the null hypothesis of panmixia (Raymond and Rousset 1995; Goudet et al. 1996; Schneider et al. 1997). To test for the presence of significant association between pairs of microsatellite loci, a likelihood ratio test was performed (Schneider et al. 1997), with >16,000 permutations which guaranteed less than 1% difference with the exact probability in 99% of the cases (Guo and Thompson 1992). Estimates of the gene exchange index, *Nm*, between the two subspecies, were calculated by using equations (15a and 15b) of Slatkin (1995).

Mismatch distribution is usually multimodal in samples drawn from populations at demographic equilibrium, but unimodal in recently expanded populations (Rogers and Harpending 1992; Schneider et al. 1997). The raggedness index of the observed distribution defined by Harpending (1994) takes larger values for multimodal distributions than for unimodal and smoother distributions (Schneider et al. 1997). Here mismatch distribution was only performed for ND5 data, and population expansion time was estimated by using Rogers and Harpending's method (1992).

The relative rate test was performed by PHYLTEST program (Kumar 1996) to evaluate if the ND5 region evolves constantly in the two orangutan taxa, using one *Gorilla gorilla gorilla* sequence (Xu and Arnason 1996b, GenBank accession number X93347) as the outgroup. Since a preliminary test showed a constant evolutionary rate between chimpanzees and bonobos from our unpublished data, using 2.5 ± 0.5 MYA as divergence time for the two *Pan* species (Morin et al. 1994), we estimated that the substitution rate of this ND5 region was about 1.1×10^{-8} substitution/site/year. This substitution rate was used here to estimate divergence time between the two orangutan subspecies.

Parsimony analysis for ND5 gene was performed with PAUP3.1

Table 1. Genetic diversity within different ape groups

	π value (mtDNA)[a]		Microsatellite[b]	
	(ND5) (SD)	(16s rRNA) (SD)	mean A (SE)	mean Ho (SE)
Bornean	0.35% (0.24%)	1.38% (0.80%)	4.3 (0.62)	0.47 (0.08)
Sumatran	1.07% (0.60%)	0.71% (0.51%)	4.2 (0.43)	0.52 (0.06)
Chimpanzee	0.77% (0.49%)	0.00% (0.00%)	9.9 (1.16)	0.73 (0.07)
Bonobo	0.37% (0.25%)	0.28% (0.23%)	4.9 (0.51)	0.48 (0.06)

[a] For mtDNA data analysis, the Kimura-2-parameter model (Kimura 1980) was used for molecular distance estimate. ND5 results for chimpanzees and bonobos were from our unpublished data. Partial 16s rRNA sequences (387bp) of these apes were from Zhi et al. (1996, GenBank accession number U63489-63505), in addition to another three sequences of chimpanzees: NC_001643, D38113 (Hixon and Brown 1986), and X93335 (Arnason et al. 1996); and seven sequences of bonobos: AB050147-Ab050151 (Saitou et al. 2000), NC_001644, and D38116 (Hixon and Brown 1986). Sequence alignments were according to Zhi et al. (1996)

[b] Microsatellite results of chimpanzees and bonobos were from Reinartz et al. (2000); here we used data set representing a Pan-African sample for chimpanzees, which were derived from Morin (1992) and Morin et al. (1994). Mean A and mean Ho are mean allele numbers and mean observed heterozygosity over loci, respectively

SD: standard deviation; SE: standard error

	1 1 1 1 1 1 2 2 2 2 2 2 2 2 2 3 3 3 3 3 3 3 3 3 3 4 4 4 4 4 4 5 5
	7 8 1 1 3 6 7 8 0 1 2 4 8 8 8 9 9 0 5 5 5 5 6 6 7 7 8 9 0 3 4 5 7 9 9 0 2
	2 7 4 7 5 2 4 7 1 6 2 0 2 3 6 1 7 6 1 2 4 9 6 9 8 9 1 4 5 5 4 9 7 2 8 4 5
bor5(6)	C G G T T T G A T A G T G T G C T G C T C A C C T A C A C G A A T C A C C
bor1(2)	. . . . . A . . . . .
bor9(5)	. . . . C . . . . .
bor2(5)	. . . . C . . . . . T . . A . . . . .
bor13(3)	. . . . . C . . . . .
sum5(2)	T A . C . C A . C G A C A C A T C A T C . T . T C G . G . A . G C T . T T
sum4(7)	T A T C . . A G C G A C A C A T C A T C . T T T C . . G T . G G C . G T T
sum1(1)	T A T C . . A G C . A C A C A T C A T C . T T T C . . G T . G G C . G T T
sum8(1)	T A . C . . A G C G A C A . A T C A . C T T . T . G . G . . . G C . G T T
sum15(9)	T A . C . . A G C G A C A . A T C A . C . T . T . G . G . . . G C . G T T
sum2(3)	T A . C . . A . C G A C A . A T C A . C . T . T . G . G . . . G C . G T T

Fig. 1. Sequence variation between orangutan subspecies; "bor" and "sum" denote the Bornean and the Sumatran orangutans, respectively. Numbers above denote location of the nucleotide in the ND5 gene (calculated from the initiation codon). Numbers in brackets denote the number of individuals sharing the same haplotype.

(Swofford 1993), using the same gorilla sequence as the outgroup. All characters were assigned as unordered and given equal weight or alternatively different weights to transitions and transversions (see Results). Bootstrap analysis consisted of 1,000 heuristic replications. Distance methods Neighbor-Joining (NJ) and UPGMA by MEGA1.02 (Kumar et al. 1993) were also used for ND5 gene tree construction.

A bottleneck test was performed using BOTTLENECK 1.2.01 (Cornuet and Luikart 1996). Both the infinite allele model (IAM) and the stepwise mutation model (SMM) were tested. This program also calculated observed heterozygosity (Ho) and expected heterozygosity (Heq) at each locus.

We estimated effective population size, *Ne*, based on expected heterozygosity (Heq) at each of the microsatellite loci in each subspecies. The following formulae were used:

$$\text{SMM: } Ne = \{[1/(1-\text{H})]^2 - 1\}/8\mu$$
$$\text{IAM: } Ne = \text{H}/4\mu(1-\text{H})$$

where H is Heq and μ represents mutation rate (Nei 1987; Lehmann et al. 1998). For microstellite data, both SMM and IAM were used to calculate *Ne* with $\mu = 10^{-4}$ (Edwards et al. 1992; Weber and Wong 1993). For ND5 data, two methods were used to estimate *Ne* with $\mu = 1.1 \times 10^{-8}$: (1) the IAM model; (2) Ruvolo's (1997) method. First we estimated the mean time to coalescence for mitochondrial haplotypes that differ most (Ruvolo et al. 1993), then we calculated *Ne* by using the formulae of Ruvolo (1997), with 20 years as generation time.

Results

Analysis of ND5 Sequences

A 536bp fragment of the ND5 gene near the 5′-region was PCR amplified and sequenced. For data analysis, we used a 483bp region that encodes 161 amino acids of the ND5 protein (from amino acid 16 to 176). All sequences have been submitted to GenBank and their accession numbers are AF255448–AF255458. There were 5 and 6 haplotypes found in the Bornean and Sumatran orangutans, respectively (Fig. 1). Differences between the two subspecies were obvious: among 37 variant sites, 18 distinguished the Sumatran orangutans from the Bornean orangutans. Most variations occurred at the third codon position (30 sites) and only six variations occurred at the first codon position (sites 187, 283, 286, 352, 379, and 394) and one variation occurred at the second codon position (site 359). The ratio of transitions to transversions is about 25.

The result of the relative rate test showed that this ND5 region evolved constantly between the two orangutan taxa. When using 1.1×10^{-8} as the substitution rate, it is estimated that the two orangutans separated at about 2.3 ± 0.5 MYA. This result is slightly higher than those estimated from other molecular data (0.73–1.7 MYA) (Janczewski et al. 1990; Zhi et al. 1996).

Distinct Bornean and Sumatran clades for ND5 gene trees were obtained regardless of which method was used or whether characters were weighted equally or unequally (transition: transversion as 1:25) (Fig. 2). The trees from distance methods had a tendency to separate the Sumatran orangutans into two groups (sum1 and sum4 and sum2, sum8, and sum15.

Genetic Diversity Within Orangutan Subspecies

There were appreciable amounts of ND5 nucleotide diversity estimated in both subspecies (Table 1). These results are similar to those from mtDNA-RFLPs, which estimated π at 0.33% (Bornean) and 1.75% (Sumatran), (Zhi et al. 1996). The Sumatran values are particularly high and surpass those of the common chimpanzees and the bonobos from our unpublished data.

A 387bp fragment of the 16s rRNA gene was also analyzed here (see Table 1). There was marked diversity within the two orangutan subspecies, but with a much higher diversity within the Borneans than within the Sumatrans. There was no variation within the chimpanzee sequences. The bonobos showed a relatively low diversity.

Of twenty microsatellite loci screened here, nineteen are polymorphic in one or both subspecies (Fig. 3). One locus, D16S540, is monomorphic in both subspecies and

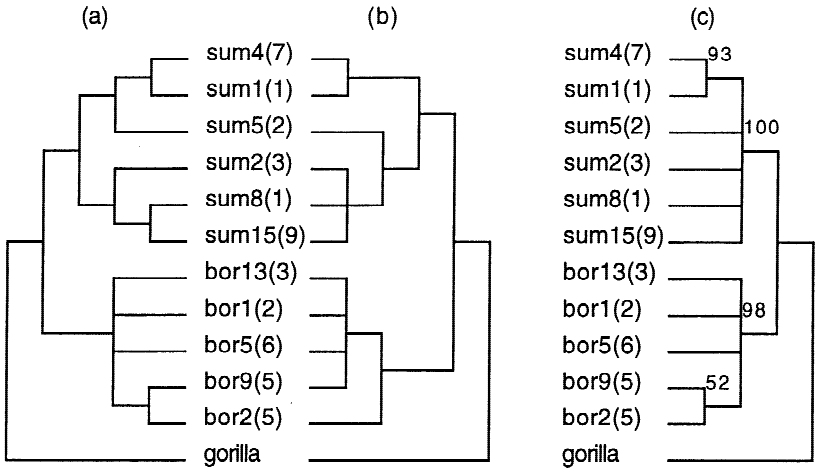

Fig. 2. Gene trees constructed using ND5 sequences. Numbers in brackets denote the number of individuals sharing the same haplotype. **(a)** and **(b)** NJ and UPGMA trees by MEGA, respectively. **(c)** Parsimonious tree by PAUP; numbers on the branch are the confidence by 1,000 bootstrap replications.

was not used for further analysis. In addition to D16S540, the Bornean subspecies has five more monomorphic loci, whereas the Sumatran subspecies has only one additional monomorphic locus. At locus D3S2459, we found no shared alleles between the two orangutans. The mean allele numbers (A) of the two orangutan subspecies are almost the same over the examined loci. Genetic diversity estimated as the mean observed heterozygosity (Ho) over the microsatellite loci showed a higher diversity within the Sumatran than within the Bornean orangutans (Table 1). To assess differences in mean A and mean Ho between the two orangutans, we used one-way analysis of variance (ANOVA; Sokal and Rohlf 1995). Our results showed no significant difference between the two orangutan taxa for either allelic diversity (ANOVA: $F = 0.017$, $P > 0.05$) or heterozygosity diversity (ANOVA: $F = 0.237$, $P > 0.05$), whereas the results of Reinartz et al. (2000) for chimpanzee/bonobo comparison showed significant difference at both (ANOVA for allelic diversity: $F = 13.259$, $P = 0.003$; for heterozygosity diversity: $F = 10.273$, $P = 0.003$).

Genetic Differentiation Between Subspecies

Differentiation between the two subspecies was assessed by F_{ST} (for both mtDNA and microsatellites) and R_{ST} (for microsatellites only). All these F_{ST} and R_{ST} values were highly significant (Table 2). Genetic divergence represented by F_{ST} values between the Bornean and the Sumatran orangutans were nearly the same as those between chimpanzees and bonobos. However, the exact test of population differentiation resulted in a highly significant difference only for ND5 data. Neither 16s rRNA data nor microsatellite data were found to be significantly different by the exact test.

Gametic Linkage Disequilibrium

All pairwise comparisons of different microsatellite loci were calculated. For each subspecies, there were 171 independent comparisons, so we would expect 1–2 of them to be significant at the 0.01 level by chance alone. Our results showed that there were only 2 and 3 values were statistically significant at the 0.01 level within the Bornean and the Sumatran subspecies, respectively (Table 3). These loci are likely stochastically significant, without showing much biological meaning, e.g., rather than loci located on the same chromosome and demonstrating linkage because of their physical location.

Effective Population Size

The effective population size *Ne* provides a measure of the effect of genetic drift on a population. *Ne* depends on demographic factors such as population density, movement pattern, and the mating system. Knowledge of this parameter is fundamental in understanding population structure (Lehmann et al. 1998). From microsatellite data, *Nes* estimated by using the SMM at each locus were always larger than those from the IAM (Table 4). The mean *Ne* from the IAM was in thousands; the mean *Ne* from the SMM was an order of magnitude larger. Both methods for ND5 data estimated *Nes* in 10^4 or higher, larger than those from microsatellite data. *Nes* estimated from the IAM model were nearly 4 times those from Ruvolo's (1997) method.

Bottleneck Test

The program BOTTLENECK 1.2.01 (Cornuet and Luikart 1996) was used to test if the microsatellite loci showed a departure from the mutation-drift equilibrium. Both the IAM and the SMM were tested. For the Bornean orangutans, under the SMM, a deviation from mutation-drift equilibrium was not supported by either the Sign test ($P > 0.20$) or Wilcoxon test ($P > 0.39$, two tailed). The results of the standardized difference test are not reported here because at least 20 polymorphic loci are required for this test. Under the IAM, the Wilcoxon

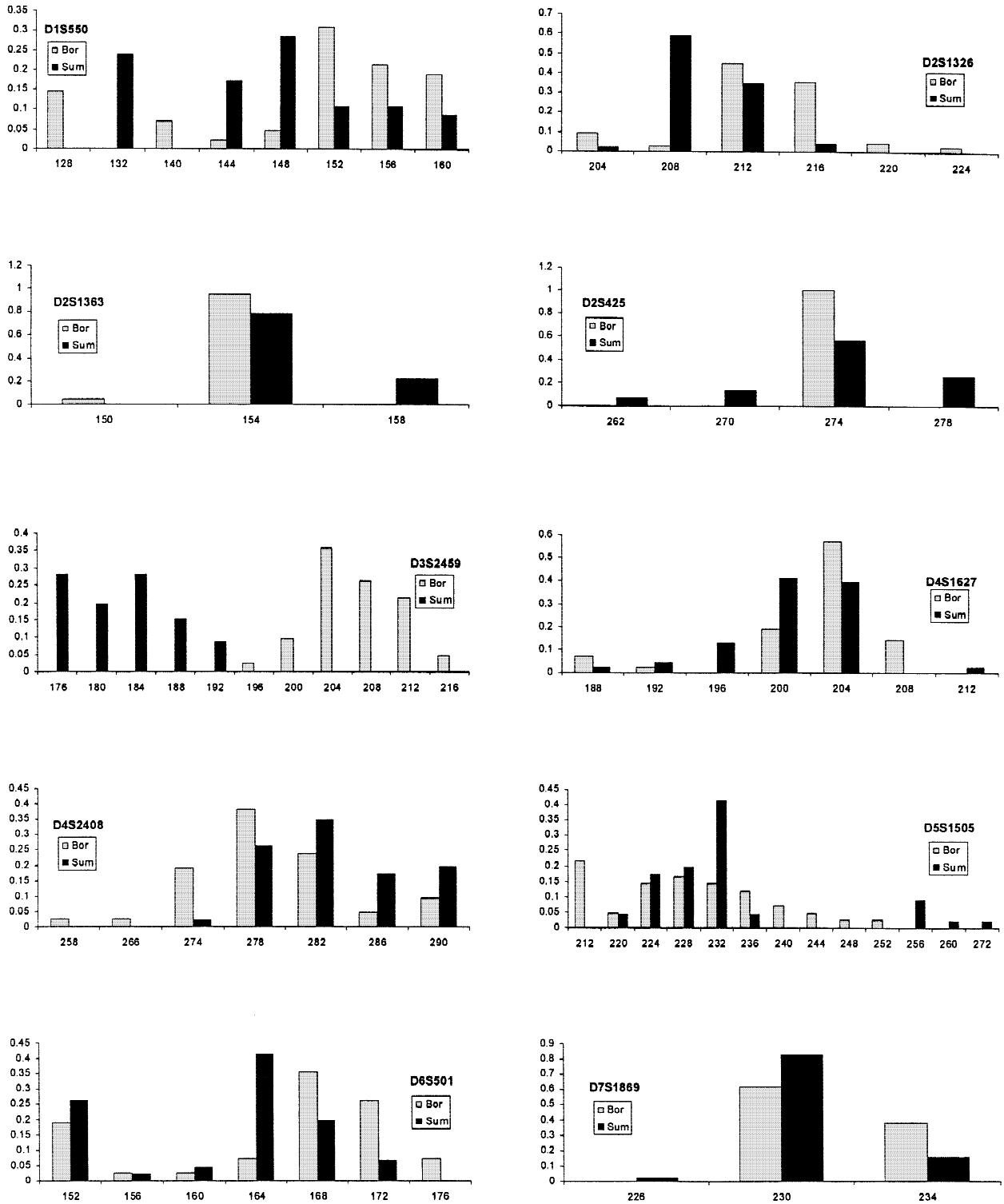

Fig. 3. Allele distribution of microsatellite loci in the two orangutan subspecies. The monomorphic locus D16S540 is not shown. Note that there are no alleles shared by the two subspecies at locus D3S2459.

test supported a deviation ($P < 0.004$) although the Sign test did not ($P > 0.06$). For the Sumatran orangutans, under the SMM, neither test supported a deviation (Sign test, $P > 0.20$; Wilcoxon test, $P > 0.32$), although under the IAM both tests supported a deviation (Sign test, $P < 0.02$; Wilcoxon test, $P < 0.04$). Since microsatellites with 3- to 5-bp repeats are thought to evolve predominantly under the single-step SMM (Shriver et al. 1993; Cornuet and Luikart 1996), we consider the SMM results more suitable for our data. Thus, our results suggested that

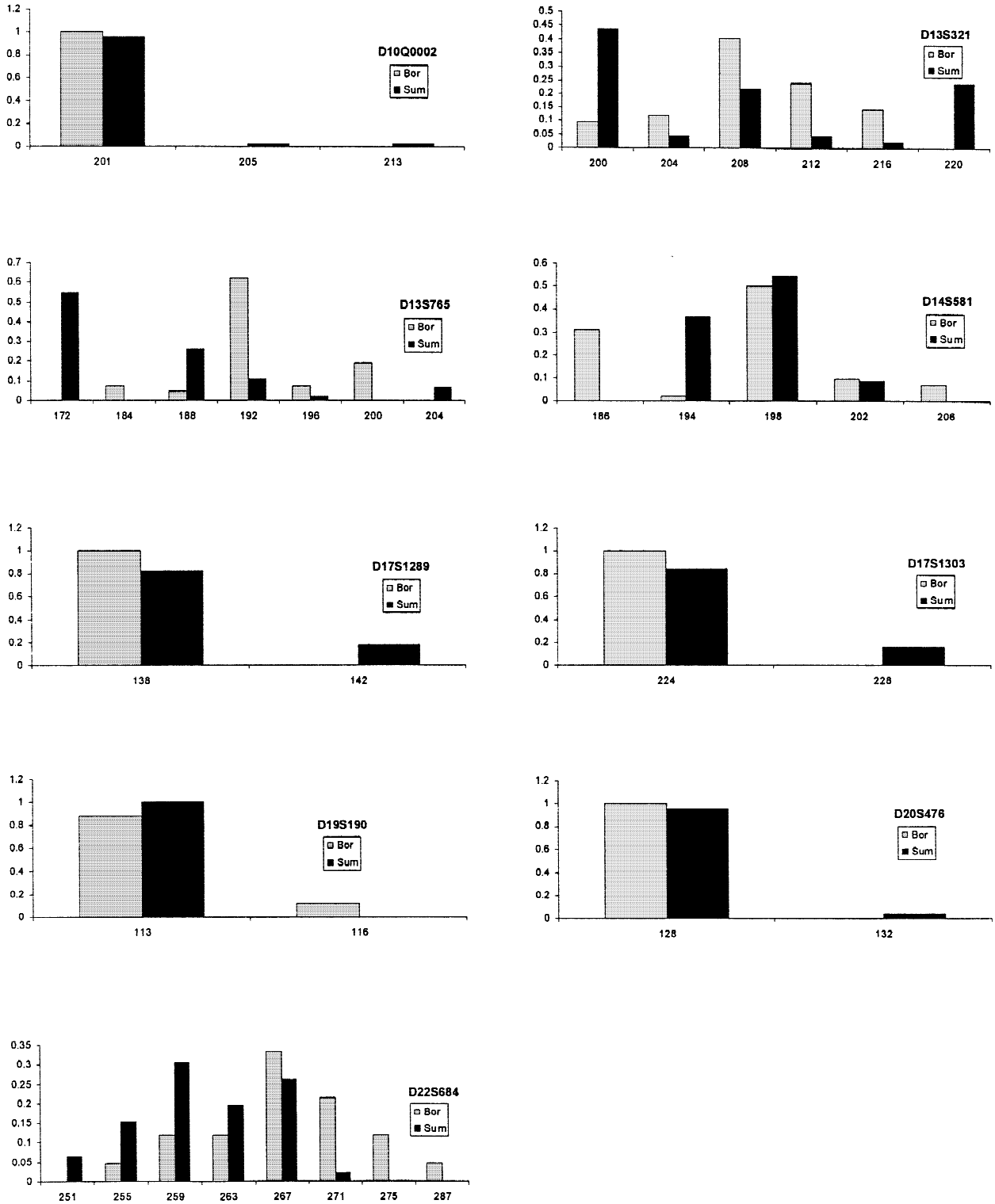

Fig. 3. Continued.

both orangutan subspecies may be in mutation-drift equilibrium at the microsatellite loci examined. Finally, the analysis of allele frequency distribution revealed an L-shape for both subspecies, which is expected for a population that did not experience a recent bottleneck (Luikart et al. 1998).

Expansion Time Dating

The mismatch distribution test from ND5 data cannot be performed for the Bornean orangutans because the parameters of expansion cannot be estimated by the Arlequin software based on the observed mismatch distribu-

Table 2. Genetic differentiation between different ape groups[a]

	Bornean/Sumatran	Chimpanzee/Bonobo
F_{ST} (*Nm*)		
ND5	0.910*** (0.025)	0.780*** (0.071)
16s rRNA	0.734*** (0.091)	0.885*** (0.032)
Microsatellites	0.142*** (1.511)	–
R_{ST} (*Nm*)		
Microsatellites	0.444*** (0.157)	–
Exact test		
ND5	***	***
16s rRNA	Not significant	Not significant
Microsatellites	Not significant	–

[a] Data sources are the same as described in Table 1
For F_{ST} and R_{ST} estimate, 1,000 permutations were performed to test significance
For exact test, the default values were used, i.e., 10,000 steps in Markov chain, 1,000 steps in dememorization, and the required precision on probability is 0.01
Nm: gene exchange index
*** $P < 0.001$
– Comparison not available

tion. Results for the Sumatran orangutans gave the raggedness index as 0.157 and the tau value as 1.744. Because orangutan females usually breed for the first time at 15 years, whereas males may not begin to breed until 18–20 years (Markham 1994), we assume 20 years as the generation time. Using Roger and Harpending's (1992) method and 1.1×10^{-8} as substitution rate, we calculated the expansion time for the Sumatran orangutans to be approximately 82,000 years ago.

Discussion

Taxonomic Status of the Two Orangutans

The taxonomic classification of orangutans is relevant not only to systematic issues but also to species conservation (O'Brien 1994; Zhi et al. 1996). The Bornean and the Sumatran orangutans are traditionally regarded as different subspecies (Nowak 1991). Hybrids have been produced in captivity, and preliminary data suggest that hybrid fertility is not especially reduced, at least compared to that of purebred Borneans (Markham 1985; Courtenay et al. 1988). However, for most species, the most important criterion for recognizing species, that of reproductive isolation, is hard to apply in practice, and the degree of differentiation becomes the main consideration in determining the level of taxonomic relatedness (Mayr 1969; Courtenay et al. 1988). Recently, studies of chromosomes, proteins, DNA hybridization, mtDNA-RFLPs, mtDNA sequences, minisatellites, etc., have demonstrated that the genetic differences between the two orangutans are almost the same as or even higher than those of other putative species like chimpanzee/bonobo, horse/donkey, etc. (Bruce and Ayala 1979; Ferris et al. 1981; Caccone and Powell 1989; Janczewski et al 1990; Ryder and Chemnick 1993; Xu and Arnason 1996a; Zhi et al. 1996; Muir et al. 2000). Based on these differences, some researchers have suggested that the two orangutans should be elevated to be separate species (Xu and Arnason 1996a). Recently, on the "Taxonomy for the New Millennium" workshop in Orlando, the Asian subpanel also recommended that the Sumatran and Bornean orangutans be elevated to full species due to the accumulating evidence of the great genetic differences between them (Dr. Caro-Beth Stewart, personal communication). But Muir et al. (1998) doubted this elevation and questioned the connection of conservation issues and academic systematics arguments. Courtenay et al. (1988) compared differences between the two orangutans from morphological, behavioral, and genetic data reported from other authors, finding that although the genetic difference is obvious between the two orangutans, the total differences are less impressive.

Our results from Arlequin analysis showed that F_{ST} and R_{ST} between the Bornean and the Sumatran orangutans are highly significant, with a low gene exchange index *Nm* (Table 2). However, while the exact test of population differentiation showed a highly significant difference for ND5 data, neither 16s rRNA data nor microsatellite data supported significant population differentiation by this test. Analyses of allelic and heterozygosity diversity of the two orangutan taxa showed no significant differences between them. While using the same analyses, Reinartz et al. (2000) found differences between chimpanzees and bonobos to be significant for both allelic and heterozygosity diversity. Even though other microsatellite loci than ours were studied, their results still provided comparable information.

In general, the two orangutan taxa are highly differentiated at the genetic level. However, the effectiveness of elevating the two orangutans as separate species is still controversial. Although genetic differentiation between the two orangutans is comparable to those between well-recognized species, there are no objective criteria clarifying how significant the difference will be to diagnose species status. Furthermore, genetic divergence estimated from different genetic markers may be different. e.g., the exact test of population differentiation gave contrary results for ND5 and 16s rRNA/microsatellite data. Melnick et al.'s (1993) studies on macaque monkeys showed that mtDNA diversities within species surpass those between species, even though their conclusions were based on RFLP data and may not adequately sample diversity. Recently, Segesser et al.'s (1999) microsatellite work on Barbary macaques also demonstrated that differentiation between isolated subpopulations could be significant. We suggest caution before using only genetic differentiation as index for taxonomic classification in the absence of more objective criteria and consensus data sets (Ryder 1986). If we separate the

Table 3. Linkage disequilibrium between pairs of loci in the Bornean (above diagonal) and the Sumatran orangutans (below diagonal)[a]

Loci	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
D1S550	*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
D2S1326	−	*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
D2S1363	−	−	*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
D2S425	−	−	−	*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
D3S2459	−	−	−	−	*	−	−	−	−	−	−	−	−	−	−	−	−	−	−
D4S1627	−	−	−	−	−	*	−	−	−	−	−	−	+	−	−	−	−	−	−
D4S2408	−	−	−	−	−	−	*	−	−	+	−	−	−	−	−	−	−	−	−
D5S1505	−	−	−	−	−	−	−	*	−	−	−	−	−	−	−	−	−	−	−
D6S501	−	+	−	−	−	−	−	−	*	−	−	−	−	−	−	−	−	−	−
D7S1869	−	−	−	−	−	−	−	−	−	*	−	−	−	−	−	−	−	−	−
D10Q0002	−	−	−	−	−	−	−	−	−	−	*	−	−	−	−	−	−	−	−
D13S321	−	−	−	+	−	−	−	−	−	−	−	*	−	−	−	−	−	−	−
D13S765	−	−	−	−	−	−	−	−	−	−	−	−	*	−	−	−	−	−	−
D14S581	−	−	−	−	−	−	−	−	−	−	−	−	−	*	−	−	−	−	−
D17S1289	−	−	−	−	−	−	−	−	−	−	−	−	−	−	*	−	−	−	−
D17S1303	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	*	−	−	−
D19S190	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	*	−	−
D20S476	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	*	−
D22S684	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	*

[a] * represents diagonal
+ Significant at 0.01 level
− Not significant at 0.01 level

Table 4. Effective population size *Ne* estimated from microsatellite loci and ND5 sequences

	Bornean				Sumatran			
Locus	A	Ho	Ne_{IAM}	Ne_{SMM}	A	Ho	Ne_{IAM}	Ne_{SMM}
Microsatellites[a]								
D1S550	7	0.82	5,778	28,787	6	0.81	4,464	1,7973
D2S1326	6	0.67	4,602	18,750	4	0.54	2,431	7,183
D2S1363	2	0.09	768	1,089	2	0.35	701	1,045
D2S425	1	0.00	–	–	4	0.62	2,317	7,183
D3S2459	6	0.76	4,663	19,575	5	0.79	3,369	1,2276
D4S1627	5	0.62	3,612	12,921	6	0.69	4,425	1,8590
D4S2408	7	0.77	6,003	28,206	5	0.76	3,369	1,2100
D5S1505	10	0.88	10,521	69,415	8	0.77	7,079	3,7330
D6S501	7	0.78	5,806	28,206	6	0.73	4,542	1,9074
D7S1869	2	0.49	709	1,083	3	0.30	1,487	3,463
D10Q0002	1	0.00	–	–	3	0.09	1,494	3,463
D13S321	5	0.75	3,524	12,365	6	0.72	4,542	18,279
D13S765	5	0.58	3,553	12,826	5	0.63	3,328	12,187
D14S581	5	0.66	3,495	12,187	3	0.57	1,449	3,481
D17S1289	1	0.00	–	–	2	0.29	734	1,027
D17S1303	1	0.00	–	–	2	0.26	697	1,070
D19S190	2	0.22	697	1,089	1	0.00	–	–
D20S476	1	0.00	–	–	2	0.09	705	1,058
D22S684	7	0.82	5,889	28,206	6	0.78	4,503	18,750
Mean	4.3	0.47	4,259	19,621	4.2	0.52	2,869	10,863
(SE)	(0.62)	(0.08)	(675)	(4,526)	(0.43)	(0.06)	(418)	(2,206)
mtDNA[b]								
ND5(1)			79,800				245,800	
ND5(2)			23,500				56,900	

[a] For microsatellite data, mutation rate is 10^{-4}. A, allele number, Ho, observed heterozygosity, Ne_{IAM} and Ne_{SMM}, estimated effective population size based on the IAM and the SMM, respectively
[b] For ND5 data, mutation rate is 1.1×10^{-4}. ND5(1): the IAM model method; ND5(2): Ruvolo's (1997) method

two orangutans as different species, then should we reconsider the status of some other great apes? For the genetic differentiation between western gorillas vs. eastern gorillas and western chimpanzees vs. eastern and central chimpanzees are also apparent (Ruvolo et al. 1994; Garner and Ryder 1996; Morin et al. 1994; our unpublished data).

Genetic Diversity and Orangutan Conservation

The distribution of the Bornean orangutans is discontinuous and some of these gaps may be due to local extinction caused by overhunting (Rijken 1978; MacKinnon and Ramono 1993). The Bornean orangutans are fragmented into four independent populations, whereas the Sumatran orangutans are confined to the very northern end of the island Sumatra even though they once had a wider distribution (Janczewski et al. 1990; Kaplan and Rogers 1994). It is estimated that orangutan populations in the wild may have declined by as much as 30%–50% between 1983 and 1993 (Sodaro 1997). Habitat loss through conversion for timber, plantations, and agriculture, as well as hunting and capture for the pet trade have all contributed to this decline. Road construction leads to fragmentation and isolation of formerly continuous populations (Sodaro 1997).

Zhi et al. (1996) compared orangutans from different Bornean populations, and concluded that there was little genetic differentiation among them. There was no apparent genetic subdivision within our Bornean samples. Assuming they accurately represent the diversity of Bornean populations, the conclusion that fragmentation of the Bornean orangutans took place very recently is upheld. Previously, there may have been substantial gene flow within the whole Bornean populations. If a thorough survey from both Bornean orangutans in the wild and captivity also concludes the same, then these findings can shed light on some conservation problems: if possible, confiscated Bornean orangutans should be returned to original populations; if not possible, there seems no significant genetic harm to using confiscated animals to build new populations. This might be helpful to artificially extend their distribution, and also help to resolve the suspicions about disease infection from confiscated animals to wild populations (Karesh et al. 1997).

Gametic Linkage Disequibrilium

Linkage disequilibrium measures the departure of the observed association of alleles of different loci from expected values derived on the basis of random association. Natural populations are usually under linkage equilibrium unless (1) the loci are tightly linked, often due to inversions; (2) the gene pool is subdivided; (3) selection maintains disequilibrium; or (4) there is strong genetic drift (Lehmann et al. 1998). Our results suggest that gametic phase disequilibrium was not widespread in both subspecies, or the two subspecies are likely under linkage equilibrium.

Interestingly, there are no shared alleles between the two orangutans at locus D3S2459, which locates on chromosome 3 in humans. Because chromosome 3 in humans is homologous to chromosome 2 in orangutans, on which the inversion occurred between the two subspecies (Seuanez et al. 1979), non-overlapping alleles at this locus may be associated with the pericentric chromosome inversion.

Effective Population Size

Because the SMM represents a more conservative model than the IAM (Cornuet and Luikart 1996), within microsatellites, estimates of *Ne* from the SMM should be larger than those from the IAM. Our results were consistent with this hypothesis (Table 4). Since the SMM model is more suitable for our data, *Ne* estimates from the SMM should be more reasonable. However, because the actual average mutation rate of microsatellites may be lower than 10^{-4} estimate (Edwards et al. 1992; Weber and Wong 1993; Lehmann et al. 1998), *Nes* estimated from the SMM might be still underestimated.

Although our estimate of the ND5 mutation rate ($\mu = 1.1 \times 10^{-8}$) is nearly the same as the average mutation rate ($\mu = 10^{-8}$) of the whole mitochondrial DNA (Nei 1987; Lehmann et al. 1998), the actual mutation rate for ND5 region is probably higher due to multiple substitution. Thus, *Nes* from the IAM model might be largely overestimated. On the other hand, although *Nes* from Ruvolo's (1997) method might also be overestimated due to an underestimated mutation rate, since the estimated coalescence time only reflects the most different haplotypes that we observe from limited samples and thus is a conservative estimate, the *Ne* estimates were correspondingly compromised. We consider *Nes* estimated from Ruvolo's (1997) method may reflect a more reasonable estimate.

In general, the effective population sizes *Nes* for both orangutan subspecies approximate 10^4. These results are nearly the same as *Nes* estimated in humans, chimpanzees, and gorillas (Nei and Graur 1984; Ruvolo 1996; 1997). It is estimated that the number of extant orangutans is about 5,000–6,000 in Sumatra and 37,000–40,000 in Borneo (Rijksen 1978; Nowak 1991; MacKinnon and Ramono 1993), of which only an estimated 9,000 or so are protected in the Indonesian reserve system (MacKinnon and Ramono 1993). By comparing the extant numbers and estimated *Nes*, the prospects for preservation of genetic diversity within both orangutan taxa are not optimistic. This is especially serious for the Sumatran orangutans, which possess high genetic diversity (the present study; Zhi et al. 1996) but seem to be subdivided, while their extant number is low compared with the *Ne* estimate.

Divergence of the Two Orangutans

Our estimate of divergence time between the two orangutan subspecies is about 2.3 ± 0.5 MYA based on ND5 sequence data. Janczewski et al. (1990) estimated it as 0.73–1.13 MYA, and Zhi et al. (1996) estimated it as 1.5–1.7 MYA. However, the two islands Borneo and Sumatra were connected historically several times by land bridges due to glacial effects and most recently these land bridges persisted from about 60,000 years ago until as recently as 10,000 years ago, or half of the last years (Muir et al. 2000). These results suggest that the two extant orangutans are representatives of populations that were genetically isolated from one another long before the physical separation of the two islands.

Rijken (1978) found two types of Sumatran orangutans based on their appearances: the dark-haired, long-fingered type and the light-haired, short-fingered type. However, many intermediates seem to exist between these two extreme forms, and both types co-exist in the same population. Karesh et al. (1997) reported two matriarchal lines that exist sympatrically in Sumatra dating back to 0.6 MYA. Recently, based on the identification of highly diverged mitochondrial haplotypes within the Sumatran orangutans and other paleogeographic evidence, Muir et al. (2000) proposed a hypothesis that the modern Sumatran orangutans are polyphyletic and the ancestor of the Bornean orangutans might also have contributed to the modern Sumatran orangutan gene pool. However, our results do not serve as support for this hypothesis, especially the point of ancestral Bornean contribution. First, we did not find that Bornean-alike haplotypes existed within the Sumatran samples at either the ND5 or 16s rRNA region. Secondly, genetic diversity of 16s rRNA within the Borneans was much higher than those within the Sumatrans. Thirdly, although the ND5 data have a tendency to cluster the Sumatrans into two groups (sum1 and sum4) and (sum2, sum8, and sum15) (Fig. 2), their divergence time can be dated back to only 0.88 ± 0.28 MYA, later than the divergence time (2.3 ± 0.5 MYA) between the whole Sumatrans and the Borneans. *P* distances (uncorrected) between the two Sumatran groups (1.66%–2.10%) are also less than those between the whole Sumatrans and the Borneans (4.56%–6.83%).

Historical Events

Historical events such as a bottleneck and/or expansion within a population can distort its genetic diversity and thus might be a step to speciation. Consequently, evaluation of historical events that might have affected genetic structure of extant orangutan populations is an interesting area for investigation. We performed a bottleneck test; however, there is no strong evidence to support that either subspecies underwent a recent bottleneck. On the contrary, there is evidence to suggest that the Sumatran subspecies might have experienced expansion after the two orangutan subspecies diverged.

Simple Test About Social Organization

Based on the hypothesis that kin selection has influenced the evolution of social structure, Morin et al. (1994) studied degrees of relatedness among chimpanzee males, who are philopatric, and among chimpanzee females, who usually disperse at adolescence. Their results showed that the mean number of alleles per locus shared between chimpanzee males was significantly higher than that between females, indicating that chimpanzee males are more related to one another than are females. Using their method, we also compared microsatellite data between orangutan males and females in each subspecies. Our results showed no significant difference between them in either subspecies. Our results could derive from the randomness of sampling, but on the other hand, the results might also be partly due to social structure differences between chimpanzees and orangutans. The social structure of orangutan may be less organized than for other great apes; they tend to move solitarily or in small "natal" groups, i.e., mother with offspring (Rijksen 1978; Kaplan and Rogers 1994).

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