Warren, K.S., Verschoor, E.J., Langenhuijzen, S., Heriyanto,, Swan, R.A., Vigilant, L. and Heeney, J.L. (2001) *Speciation and intrasubspecific variation of Bornean orangutans, Pongo pygmaeus pygmaeus.* Molecular Biology and Evolution, 18 (4). pp. 472-480.

Speciation and Intrasubspecific Variation of Bornean Orangutans, *Pongo pygmaeus pygmaeus*

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Abstract

Mitochondrial DNA control region sequences of orangutans (*Pongo pygmaeus*) from six different populations on the island of Borneo were determined and analyzed for evidence of regional diversity and were compared separately with orangutans from the island of Sumatra. Within the Bornean population, four distinct subpopulations were identified. Furthermore, the results of this study revealed marked divergence, supportive evidence of speciation between Sumatran and Bornean orangutans. This study demonstrates that, as an entire population, Bornean orangutans have not experienced a serious genetic bottleneck, which has been suggested as the cause of low diversity in humans and east African chimpanzees. Based on these new data, it is estimated that Bornean and Sumatran orangutans diverged approximately 1.1 MYA and that the four distinct Bornean populations diverged 860,000 years ago. These findings have important implications for management, breeding, and reintroduction practices in orangutan conservation efforts.

Keywords: orangutan *Pongo pygmaeus,* mitochondrial DNA control region genetic diversity biogeography

Introduction

The endangered orangutan (*Pongo pygmaeus*) exists in the wild only on the islands of Borneo and Sumatra. Orangutans are taxonomically classified as two distinct subspecies, the Bornean (*Pongo pygmaeus pygmaeus*) and the Sumatran (*Pongo pygmaeus abelli*) (Van Bemmel 1968; Jones 1969). Current estimates suggest that the two subspecies have been geographically isolated for 10,000–15,000 years and possess only subtle morphological differentiation. Despite the presence of a pericentric inversion of chromosome 2, fertile offspring are produced by interbreeding of the different subspecies in captive conditions (Seuanez 1979). Nonetheless, there have been proposals to reclassify the subspecies as two distinct species based on past biochemical and molecular analyses (Ryder and Chemnick 1993; Ruvolo et al. 1994; Xu and Arnason 1996; Zhi et al. 1996). These studies, using isozyme variation (Bruce and Ayala 1979), mitochondrial DNA (mtDNA) restriction mapping (Ferris 1981 ; Zhi et al. 1996), isozyme and protein electrophoresis (Janczewski, Goldman, and O'Brien 1990), sequences from the COII (Ruvolo et al. 1994) and 16s rRNA (Zhi et al. 1996) genes of mtDNA, nuclear minisatellite variation (Zhi et al. 1996), and sequences of entire mtDNA molecules (Xu and Arnason 1996) all find levels of differentiation between the Bornean and Sumatran orangutans comparable to or exceeding that found between the two chimpanzee species, the common chimpanzee and the bonobo.

Today, orangutans exist in increasingly fragmented and isolated populations. While the Sumatran orangutan is primarily found in northern Sumatra, the Bornean is distributed in Central, West, and East Kalimantan, Sarawak, and Sabah. They are, however, not found in Brunei and South Kalimantan (Rijksen and Meijaard 1999). The determination of the intrasubspecific variation between isolated Bornean populations has been stated to be essential for both the management of orangutan reintroduction projects and the planning of conservation strategies to preserve the remaining wild populations (De Boer 1982; Courtenay, Groves, and Andrews 1988; Janczewski, Goldman, and O'Brien 1990; The World Conservation Union/Species Survival Commission 1993; Uchida 1996; Xu and Arnason 1996). Studies of morphological features have indicated that the extent of interpopulation differentiation within Borneo may approach that between Borneo and Sumatra (Groves, Westwood, and Shea 1992; Uchida 1998). By using discriminant analyses of orangutan skulls from different localities, Groves, Westwood, and Shea (1992) concluded that there were three distinct populations of orangutans: those in Sumatra, those in southwestern Borneo, and those in the remainder of Borneo. Avoiding the confounding effects of age-related size differences of skulls by examining tooth morphology, Uchida found significant differences between two Bornean populations from Northwest and Southwest Kalimantan that were as great in magnitude as those from the Borneo-Sumatra comparison (Uchida 1998).

Analysis of molecular variation is increasingly employed in evaluation of animal populations for purposes of taxonomic clarification, genetic variability assessment, and identification of origin of confiscated illegal pets (Morin, Moore, and Woodruff 1992; Morin et al. 1993; Zhi et al. 1996; Warren et al. 2000). Only two studies have used

samples of Bornean orangutans of known origin for the assessment of genetic variability (Zhi et al. 1996; Warren et al. 2000). The study of Zhi et al. (1996) compiled a total of 33 individuals from four areas in Borneo, as well as 6 Sumatran individuals from two locations. The analytical methods used, nuclear minisatellite loci analysis, mtDNA restriction fragment length polymorphisms, and the analysis of mitochondrial 16S rRNA sequences, revealed a separation between Bornean and Sumatran orangutans at approximately 1.5 MYA and considerable diversity within the Bornean and Sumatran subspecies. However, the methods used did not detect geographically defined genetic variation within Borneo. Recent microsatellite DNA studies provide evidence that east and west Bornean populations, while subject to genetic drift, have similar genetic backgrounds (Warren et al. 2000).

Sequence analysis of the most variable segment of the control region of the rapidly evolving mtDNA molecule has long been the method of choice for analysis of population level diversity in humans and great apes (Vigilant et al. 1991; Morin et al. 1993; Woodruff 1993; Garner and Ryder 1996; Gagneux et al. 1999). Assessment of molecular variation within Borneo requires the use of a reasonable number of samples of known origin analyzed with a highly informative genetic locus. This study presents the results of the first comprehensive analysis of mtDNA control region sequences from 41 Bornean orangutans from six locations, as well as sequences from five Sumatran orangutans.

Materials and Methods

Sample Collection

Blood and hair samples were collected from 41 Bornean orangutans and 5 Sumatran orangutans. Samples from wild and free-ranging orangutans were collected from six Bornean populations and one Sumatran population. The populations sampled were found in Southwest Kalimantan (SK), Northwest Kalimantan (NK), East Kalimantan (EK), Central Kalimantan (CK), Sabah (SAB), Sarawak (SARA), and Sumatra (SUM) (fig. 1). The samples were collected from wild (n = 22), rehabilitated (n = 22), and captive orangutans (n = 2) (table 1). Hair samples from wild orangutans were collected from nests (n = 15) or plucked from individuals during translocation procedures (n = 16). The samples were stored either in 95% alcohol or dry in envelopes placed in plastic zip-lock bags containing silica gel. The ages of nests were estimated based on classifications described by Russon and Erman (1996). Blood was collected at rehabilitation centers from individuals of known origin and with a reliable history of the location of capture or confiscation. Blood samples were diluted in 1:5 SDS storage solution (Scott-White and Densmore 1992) and stored at room temperature. Five individuals of unknown origin that were confiscated in Taiwan and Java (PA68 and OO42), or in Borneo (DO81, AN54, and 681) were included in the study to determine an estimation of their origin.

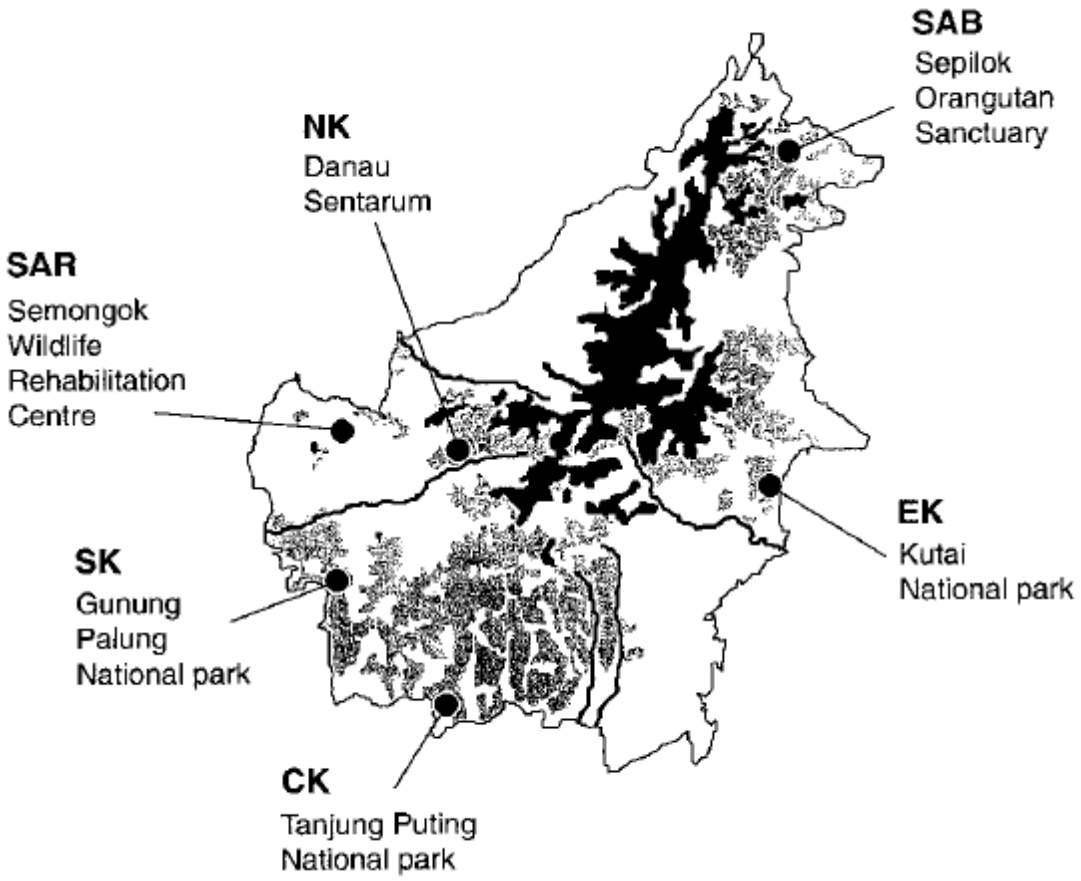

Fig. 1. - Map of sampling locations and the distribution of orangutan populations (light-gray shaded regions) on the island of Borneo. Major barrier rivers separating orangutan populations are indicated by thickened lines. Mountains higher than 500 m are darkly shaded. Sampling sites are indicated with solid dots.

Mitochondrial DNA Extraction and PCR Amplification

DNA was isolated from blood by digestion with proteinase K and 10% SDS, followed by extraction with phenol and alcohol precipitation (Sambrook, Fritsch, and Maniatis 1989). DNA was isolated from 1–5 hair follicles. It was extracted from the hair follicles according to a modification of a procedure described by Allen et al. (1998). The hair was trimmed to less than 5 mm and rinsed with ethanol and ultrapure water. Then, the hair follicle was placed in a tube containing 200 μl reaction buffer (50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris [pH 8.3], 0.45% Tween 20, 0.45% Igepal CA630, 5 μl 10 mg/ml proteinase K, and 8 μl 1 M DTT). The tubes were incubated at 56°C for 4 h and then heated at 95°C for 10 min. A nested touchdown PCR protocol was used for both stages of the nested PCR protocol. Primers were designed to amplify a fragment of the control region 278 bp in size based on conserved regions surrounding a variable region located in published sequences of control regions from orangutans, chimpanzees, and humans. The

first PCR reaction was performed in a 50-µl volume using 5 µl of DNA, 50 pmol of primers D1 (forward primer 5′-CAACATGAATATCACCC; positions 86– 102 in published sequence X97709, orangutan Dennis mtDNA control region) and D5 (reverse primer 5′- TGTGCGGGATATTGATTTCAC; positions 386–406), 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, and 2.5 U AmpliTaq Gold (PE Biosystems, the Netherlands). The touchdown amplification scheme consisted of one round of 15 min at 94°C to activate the enzyme; two rounds of 30 s at 94°C, 30 s at 65°C, and 30 s at 94°C; two rounds of 30 s at 94°C, 30 s at 61°C, and 30 s at 72°C; two rounds of 30 s at 94°C, 30 s at 59°C, and 30 s at 72°C; two rounds of 30 s at 94°C, 30 s at 57°C, and 30 s at 72°C; two rounds of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C; four rounds of 30 s at 94°C, 30 s at 53°C, and 30 s at 72°C; and 15 rounds of 30 s at 94°C, 30 s at 50°C, and 30 s at 72°C. Finally, the mixture was incubated for 5 min at 72°C. A 2-µl volume from the first PCR was then transferred to a new tube containing PCR reaction mixture, only using primers D2 (forward primer 5′-ACACAACAATCGCTTAAC; positions 104–121) and D4 (reverse primer 5′-GATGGTGAGYAAGGGATT; positions 364–381). This nested PCR mixture was amplified as described above. The PCR products were analyzed on 1.8% agarose gel. The products were isolated from agarose gel using a QIAquick gel extraction kit (Qiagen, Hilden, Germany) and cloned in the pGEM-T Easy vector (Promega Corporation, Madison, Wis.). Sequencing of the cloned insert was performed in both directions with M13 forward and reverse sequencing primers using an ABIPRISM 310 Genetic Analyzer (PE Biosystems, the Netherlands).

Analysis of DNA Sequences

Sequence analysis was conducted using MacVector, version 6.0, and AssemblyLIGN software packages (Oxford Molecular Ltd., U.K.), and aligned sequences were manually edited using the sequence alignment editor Se- Al, version 1.0a1 (Rambaut 1995). Estimates of Wright's fixation index F_{ST} (Wright 1951; Cockerham and Weir 1984) were computed using ARLEQUIN, version 1.1 (Schneider 1997). Molecular variance among groups and populations was calculated using AMOVA, which is part of the ARLEQUIN software. The interpopulation distances were calculated using the Iwave program (Harpending et al. 1993). Maximum-likelihood tree reconstruction was performed using PUZZLE, version 4.0.2 (Strimmer and von Haeseler 1999). Phylogenetic analysis of the sequences was performed with the PHYLIP package, version 3.572 (Felsenstein 1995). Trees were visualized using TREEVIEW, version 1.5.3, software (Page 1998). Most recent common ancestor (MRCA) estimations were performed using FLUCTUATE (Kuhner 1998).

Nucleotide Sequences

The nucleotide sequences of the orangutan mitochondrial DNA control regions described in this article have been deposited in the EMBL/GenBank data libraries (accession numbers AJ391095–AJ391103 and AJ391105–AJ391141.

Table 1
Origins of Orangutan Samples and Sequences Used in this Study

Code	Subspecies[a]	Status[b]	Origin/Reference[c]	Sample[d]
OU TNK41	B	W	Kutai National Park, East Kalimantan	Hair, D, nest @ 2 months
OU TNK39	B	W	Kutai National Park, East Kalimantan	Hair, D, nest 1 day
OU TNK38	B	W	Kutai National Park, East Kalimantan	Hair, D, nest @ 2 months
OU TNK37	B	W	Kutai National Park, East Kalimantan	Hair, D, nest @ 2 months
OU TNK36	B	W	Kutai National Park, East Kalimantan	Hair, D, nest @ 2 weeks
OU LI64	B	R	Wanariset, confiscated in East Kalimantan	Blood
OU SO69	B	R	Wanariset, confiscated in East Kalimantan	Blood
OU KPC	B	W	Sangatta, East Kalimantan	Hair, D, plucked
OU MU1	B	R	Wanariset, confiscated in East Kalimantan	Hair, D + A, plucked
OU KA1	B	W	Sangatta, East Kalimantan	Hair, D + A, plucked
OU PU1	B	R	Wanariset, confiscated in East Kalimantan	Hair, D, plucked
OU SB71	B	W	Sandakan, Sabah	Hair, D, plucked and stored dry for 1 year
OU SB60	B	W	Kinabatangan, Sabah	Hair, D, plucked and stored dry for 1 year
OU SB70	B	W	Lahad Datu, Sabah	Hair, D, plucked and stored dry for 1 year
OU SB57	B	W	Sukau, Kinabatangan, Sabah	Hair, D, plucked and stored dry for 1 year
OU SB372	B	R	Sepilok, Sabah	Blood
OU TP14	B	R	Tanjung Puting, Central Kalimantan	Hair, A, plucked
OU DO71	B	R	Wanariset, confiscated in Central Kalimantan	Blood
OU TP15	B	R	Tanjung Puting, Central Kalimantan	Hair, A, plucked
OU TP6	B	W	Tanjung Harapan, Tanjung Puting, Central Kalimantan	Hair, D, nest @ few days old
OU TE4	B	R	Wanariset, confiscated in Central Kalimantan	Blood
OU GPJA	B	W	Gunung Palung, Southwest Kalimantan	Hair, D, nest @ few days old
OU GPMA	B	W	Gunung Palung, Southwest Kalimantan	Hair, D, nest @ 12 days
OU GP31	B	W	Gunung Palung, Southwest Kalimantan	Hair, D, stored dry for 2.5 years
OU GPUN	B	W	Gunung Palung, Southwest Kalimantan	Hair, D, nest 2–3 weeks
OU TP24	B	W	Tanjung Puting, Southwest Kalimantan	Hair, D, nest 1–2 weeks
OU DSRA	B	W	Radai, Danau Sentarum, Northwest Kalimantan	Hair, A, nest @ 3 months
OU DSME1	B	W	Meliau, Danau Sentarum, Northwest Kalimantan	Hair, A, nest @ 2 months
OU DSLE1	B	W	Leboyan, Danau Sentarum, Northwest Kalimantan	Hair, A, nest @ 2 months
OU DS29	B	W	Leboyan, Danau Sentarum, Northwest Kalimantan	Hair, A, nest @ 1 week
OU DSME2	B	W	Meliau, Danau Sentarum, Northwest Kalimantan	Hair, A, nest @ 1 week
OU SEUA	B	R	Semongok, Sarawak	Hair, A, plucked
OU SEBU	B	R	Semongok, Sarawak	Hair, D, taken from a stuffed animal
OU SEOA	B	R	Semongok, Sarawak	Hair, A, plucked
OU SE8	B	R	Semongok, Sarawak	Blood
OU SEAH	B	R	Semongok, Sarawak	Hair, A, plucked
OU SUHS	S	C	Known Sumatran origin, Perth Zoo	Hair A, collected from night den @ 1 day
OU SUUT	S	C	Known Sumatran origin, Perth Zoo	Hair A, collected from night den @ 1 day
OU SU46	S	R	Bohorok, North Sumatra	Hair, D, plucked
OU SU45	S	R	Bohorok, North Sumatra	Hair, D, plucked
OU SU76	S	R	Bohorok, North Sumatra	Hair, D, plucked
OU PA68	B	R	Wanariset, confiscated in Taiwan, origin unknown	Blood
OU OO42	B	R	Confiscated in Semarang, Java, origin unknown	Blood
OU 681	B	R	Sepilok, Sabah, confiscated, origin unknown	Blood
OU DO81	B	R	Wanariset, confiscated, origin unknown	Blood
OU AN54	B	R	Wanariset, confiscated, origin unknown	Blood
OU Dennis	B	P	Xu and Arnason (1996), origin unknown	EMBL accession no. X97709
OU Anna	B	P	Xu and Arnason (1996), origin unknown	EMBL accession no. X98472
OU SUMA	S	P	Xu and Arnason (1996), Sumatran origin	EMBL accession no. X97708

[a] B = Bornean; S = Sumatran.

[b] W = wild; R = rehabilitation center; P = published sequence; C = captive in zoo.

[c] Kutai National Park GPS - 00º31´53.82"N, 117º27´52.67"E; Gunung Palung, southwest Kalimantan GPS - 01º13´03.82"S, 110º06´26.14"E; Leboyan, Danau Sentarum, Northwest Kalimantan GPS - 00º48´34.28"N, 112º13´37.55"E; Meliau, Danau Sentarum, Northwest Kalimantan GPS - 00º56´10.39"N, 112º20´42.03"E

[d] D = dry; A = alcohol; @ = approximate age

Results

The 46 individual orangutans analyzed produced 41 unique sequences or mtDNA types. The four pairs of individuals with identical sequences were from Sumatra (OU SU76 and OU SU46) and the Bornean regions of Sarawak (OU SEOA and OU SE8), East Kalimantan (OU MU1 and OU KPC), unknown regions of Borneo (OU AN54 and OU 681), and a final pair in which one individual was from Southwest Kalimantan (OU GP31) and the other was from Central Kalimantan (OU DO71). For some analyses, published sequence information from two Bornean individuals of unknown geographic origin (Anna and Dennis) and from one Sumatran individual (SUMA) were used (Xu and Arnason 1996). The total data set of the 41 new sequences plus these 3 published sequences has an average length of 245 bp and contains 63 polymorphic sites. The polymorphisms consist of 51 transitions and 24 transversions. Single- base insertion/deletion events occurred at two positions, and one individual (DO71) possessed a 79-bp deletion. Length mutations were not used in diversity calculations or phylogenetic reconstruction.

Population pairwise F_{ST} values and percentages of sequence divergence were calculated for the groups of individuals from the six Bornean regions and those with a Sumatran origin (table 2). Results indicate that the six regions sampled in Borneo represent four significantly differentiated (P = 0.05) populations, with the individuals collected from the geographically adjacent regions of Sarawak and Northwest Kalimantan and those collected from Southwest and Central Kalimantan, respectively, not being genetically distinct. At the more stringent 1% significance level, the individuals from Sabah were also undifferentiated from the other two northerly populations from Northwest Kalimantan and Sarawak.

Table 2

Percentages of Sequence Divergence and F_{ST} Values

	BORNEO						
	Central Kalimantan (CK)	Southwest Kalimantan (SK)	Northwest Kalimantan (NK)	Sarawak (SARA)	East Kalimantan (EK)	Sabah (SAB)	SUMATRA (SU)
CK	2.2	2.1	6.5	5.4	5.3	5.0	19.2
SK	**−0.12**	2.6	6.3	5.3	5.2	5.1	17.5
NK	**0.54****	**0.53****	3.4	2.6	4.7	5.9	16.5
SARA	**0.63****	**0.63****	**0.07**	1.5	3.5	4.6	16.7
EK	**0.56****	**0.59****	**0.48****	**0.49****	1.9	4.1	17.2
SAB	**0.49****	**0.50****	**0.50***	**0.56***	**0.48****	2.6	16.9
SU	**0.71****	**0.68****	**0.66****	**0.70****	**0.76****	**0.68****	7.8

NOTE. – Percentages of sequence divergence are indicated on and above the diagonal F_{ST} and value are indicated below the diagonal in boldface type.

* Significant at the 0.05 level

** Significant at the 0.01 level

The percentages of sequence divergence between the Sumatran group and the six Bornean populations ranged from 16.5% to 19.2% (table 2). Within Borneo, the pairwise population sequence divergences ranged from 2.1% (Central vs. Southwest Kalimantan) to 6.5% (Central vs. Northwest Kalimantan).

AMOVA analysis was used to further substantiate the apportionment of the genetic diversity within Borneo (table 3). The first analysis classified the Bornean sequences into six populations (SARA, NK, SAB, EK, SK, and CK) and considered these six populations one group for comparison with the single population of the Sumatran group. The analysis indicated that most of the variation (70.8%) distinguished the Bornean and Sumatran groups. Since the six Sumatran individuals did not constitute a geographically defined population and an understanding of variation within Borneo was of interest, the analysis was performed with the same six Bornean populations and using the three Bornean groups that were defined by the population pairwise F_{ST} analysis (CK/SK, EK, and SARA/SAB/NK). This analysis showed that most of the diversity (46.4%) was found within populations but that a considerable proportion of the diversity distinguished populations within groups (26%), as well as the groups themselves (27.6%).

A neighbor-joining (NJ) tree depicting evolutionary relationships between the mtDNA types from individual orangutans is shown in figure 2 . Distances were calculated using the Kimura two-parameter method. The tree was rooted by using chimpanzee control region sequences as the outgroup. Irrespective of whether published chimpanzee, human, or gorilla control region sequences were used as the outgroup, the root was consistently placed at the node between Sumatran and Bornean orangutans. Both Bornean and Sumatran orangutans form monophyletic clusters, and closer examination of the Bornean clade confirmed the results of the population analysis. The NJ tree also revealed four major subclades, each containing individuals from the same geographic area. Importantly, individuals from Northwest Kalimantan and Sarawak and from Southwest and Central Kalimantan were found not to be distinctly separate from each other. One exception was found: TNK38, an individual from East Kalimantan, consistently fell into the cluster formed by individuals from Northwest Kalimantan and Sarawak.

Table 3

Hierarchical Analysis of Molecular Variance Between Groups and Populations of Orangutans

Geographical Arrangement	Variance	% of Total	*P*
Borneo vs. Sumatra			
Among groups	0.708	70.8	<0.0001
Among populations/within groups	0.414	14.9	<0.0001
Within populations	0.801	14.3	<0.0001
Within Borneo			
Among groups	0.276	27.6	0.0450
Among populations/within groups	0.358	26.0	<0.0001
Within populations	0.536	46.4	<0.0001

NOTE.—Groups for the within-Borneo test are defined as Northern (SARA, NK, and SAB), Eastern (EK), and Southern (SK and CK). See table 2 for definitions of abbreviations.

Seven additional Bornean orangutans of uncertain origin were also included in the NJ tree analysis. Two individuals fell into the clade of types from Central or Southwest Kalimantan (PA68 and OO42), and another was within the Sabah clade (Anna). The four other individuals (DO81, Dennis, 681, and AN54), the latter two having identical sequences, fell within the Borneo clade but outside of the four defined subclades. This suggested a Bornean origin distinct from the populations sampled in this study.

Maximum-likelihood (ML) tree reconstruction using the quartet-puzzling method was also applied to the data (Strimmer and von Haeseler 1997). The transition/transversion ratio was estimated from the data using a Tamura- Nei model of substitution (Tamura and Nei 1993), and the gamma-distribution parameter alpha, which describes the extent of rate heterogeneity among sites, was estimated assuming gamma-distributed rates (table 4). Comparison of these parameters for the control region segment in bonoboos, chimpanzees, and humans revealed similar levels of rate heterogeneity but a two- to threefold reduced transition-transversion ratio in orangutans. ML analysis produced trees consistent in their branching patterns with the ones obtained from the NJ analyses (data not shown). ML trees were constructed with and without the assumption of a molecular clock. Results indicated that all data sets analyzed (all orangutans plus the chimpanzee outgroup, all orangutans, and only Bornean orangutans) failed the clock test at the 5% significance level. This result was likely an effect of analyzing a large number of lineages that were differentiated by a relatively small number of mutations, and the ML approach may not be the best way to test for constancy of evolutionary rates in large mtDNA data sets (A. von Haeseler, personal communication). The program FLUCTUATE (Kuhner 1998) was then used to estimate the time to the MRCA of the mtDNA segment studied. The Bornean orangutan female effective population size (N_e) was estimated to be 22,000, and the age of the MRCA was estimated to be 860,000 years ago. The combined data set of Bornean and Sumatran orangutans led to an estimate of 28,000 for the female N_e and an MRCA of 1.1 MYA. These estimates were derived using the assumptions of a mutation rate for the sequence of 0.33 changes per site per million years, a mutation rate based on human data (Ward et al. 1991), a generation time of 20 years, a constant population size, and the ML estimator of τ. The estimates have a large variance. To put the values in perspective, the same assumptions were used to derive estimates for the ages of the MRCAs of west African chimpanzees (61 individuals; MRCA 420,000 years ago) and bonobos (31 individuals; MRCA 340,000 years ago) (Gagneux et al. 1999). These values suggest that the genetic depth of Bornean orangutans is twice as deep as that of western chimpanzees, and 2.5 times as deep as that of bonobos.

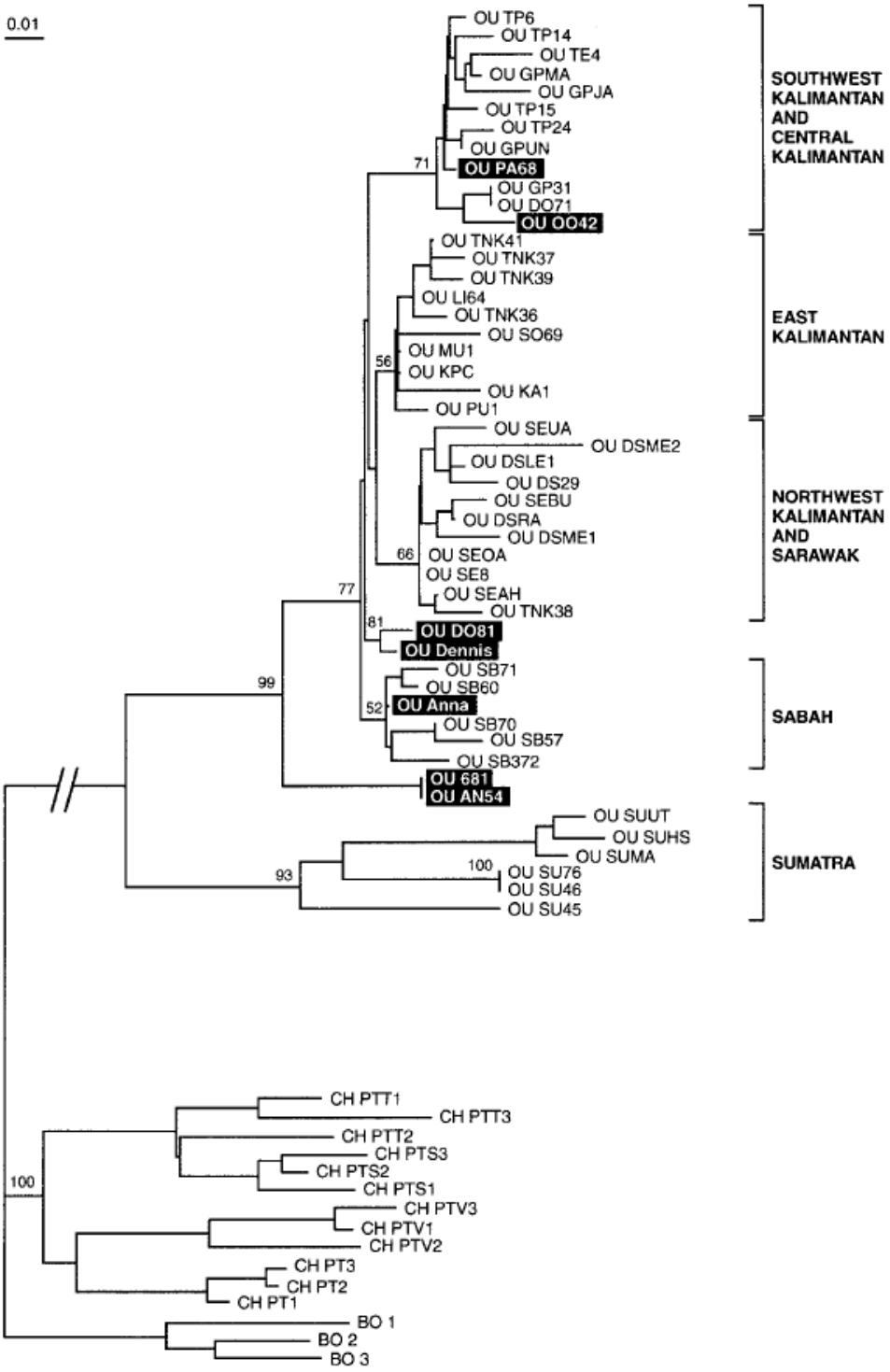

Fig. 2.—Phylogenetic tree derived from mtDNA control region sequences comparing Bornean orangutans with orangutans from Sumatran and with bonobos and the different subspecies of common chimpanzees. The PHYLIP program SEQBOOT was used to bootstrap data in which 1,000 data sets were analyzed. DNADIST and NEIGHBOUR (neighbor joining) were used to create dendrograms. CONSENSE was used to create consensus trees, which were visualized using TREEVIEW. BO = bonobo; PTT = Pan troglodytes troglodytes; PTS = Pan troglodytes schweinfurtii; PTV = Pan troglodytes verus; PT = Pan troglodytes vellerosus (Gonder et al. 1997). Orangutans of unknown origin are indicated in shaded boxes

Table 4

Estimated Parameters for the Control Region mtDNA Segment from Different Data Sets

Parameter[a]	All Orangutans	Bornean Orangutans	Bonobos[b]	Western Chimpanzees[b]	Humans[c]
n	44	39	29	61	124
α	0.62	0.53	0.55	0.52	0.42
κ	4.9	5.6	16.3	9.8	16.1

[a] *n* = number of sequences in the data set; α = rate heterogeneity parameter; κ = transition-transversion parameter
[b] Sequences from Gagneux et al. (1999)
[c] Sequences from Vigilant et al. (1991)

Discussion

From these new data, it is evident that the Bornean orangutan population has at least four subpopulations defined by mitochondrial DNA variation: (1) Southwest and Central Kalimantan (SK/CK), (2) Northwest Kalimantan and Sarawak (NK/SARA), (3) Sabah (SAB), and (4) East Kalimantan (EK). The results from our phylogenetic analyses differ from those of others (Zhi et al. 1996) in that our analysis provides strong evidence of geographic clustering. In this respect, our results strongly support Groves, Westwood, and Shea (1992) and demonstrate that there has been an underestimate of the complexity of geographic variation in orangutans. The geographic structuring of mtDNA control region variation seen in orangutans is in marked contrast to the pattern seen in chimpanzees, bonoboos, and humans (Vigilant et al. 1991 ; Gagneux et al. 1999). The results of pairwise sequence differences and AMOVA calculations indicate that there is much diversity within orangutans. The proportion of variation is quite different from that seen in humans and east African chimpanzees (Goldberg and Ruvolo 1997), for which approximately 80%–85% of the diversity is contained within all populations, and only 15%–20% of genetic diversity is specific to particular isolated populations. This suggests that there is relatively little to moderate diversity among human or chimpanzee populations. However, in orangutans, the distribution within all populations is 50.9% and the distribution among populations is 49.1%. Therefore, unlike humans and chimpanzees, there seems to be much more genetic diversity between different groups. This would indicate that Bornean orangutans have not undergone a severe genetic bottleneck, one of the proposed theories behind low genetic diversity in humans and chimpanzees (Goldberg and Ruvolo 1997).

Our phylogenetic analyses confirm the findings of wide genetic variation between Bornean and Sumatran orangutans. Despite the identification of distinct geographic clusters of Bornean orangutans, no particular cluster was clearly more related to the Sumatran apes than any other Bornean population. These data contrast with findings by Groves, Westwood, and Shea (1992) that suggested that the southwest population (SK) was more related to the Sumatran population than any other orangutan population in Borneo. However, it should be noted that only the skulls from Southwest Kalimantan,

Northwest Kalimantan, Sarawak, and Sabah were studied, and individuals from Central and East Kalimantan were not included.

Our observations may have implications for the interpretation of the proposed migration routes of the Bornean orangutan ancient ancestors from Sumatra to Borneo across the Sunda landmass (fig. 3) (Rijksen and Meijaard 1999). Natural geographic barriers may have forced the isolated colonization of at least four different regions of Borneo. This notion is now supported by the more recent genetic diversification of orangutans observed in the four different regions of Borneo (table 3). Estimations of the MRCA suggested that Sumatran and Bornean populations diverged approximately 1.1 MYA. Furthermore, our data suggest that at least four distinct Bornean subpopulations diverged 860,000 years ago. This is in agreement with previous estimates of divergence dates for Bornean and Sumatran orangutans, which ranged from 0.6 to 3.4 Myr (Bruce and Ayala 1979; Janczewski, Goldman, and O'Brien 1990 ; Ruvolo et al. 1994; Zhi et al. 1996). Thus, the Bornean and Sumatran orangutans were reproductively isolated long before the islands were geographically isolated by rising seas in the Late Pleistocene. This isolation could have been due to geographical barriers such as ancient river systems or due to behavioral barriers (Zhi et al. 1996). Indeed, differences in social interactions have been recorded in field studies of Sumatran and Bornean orangutans (Galdikas 1978; Rijksen 1978; Peters 1995).

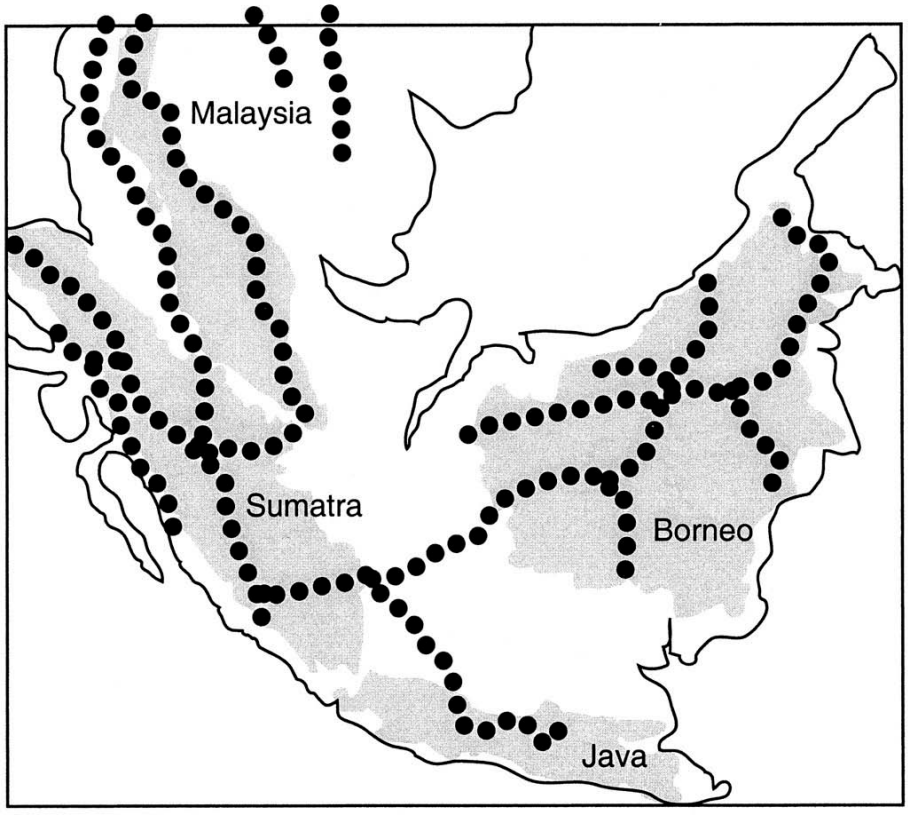

Fig. 3.—Map indicating the proposed dispersal routes of ancient orangutan populations through Sumatra to Borneo across the Sunda landmass, and migration into different regions throughout Borneo

Migration of prehistoric orangutans can provide some information as to the distribution of orangutan populations within Borneo. Orangutans migrated across the Sundaland from southern China as far east as what is now known as Java and Northeast Borneo. They entered Borneo from the southeast corner of Sumatra, and once in Borneo their dispersal was determined by geographical barriers (fig. 3). It is thought that dispersal occurred along the foothills of central mountain ranges that acted to geographically isolate populations. According to Rijksen and Meijaard (1999), many of these populations were ecological culs-de-sac, and it is likely that these populations have been isolated from each other since the population dispersed initially. Figure 3 illustrates how the main four isolated populations identified in this study may have developed historically.

The results of this study are consistent with behavioral research that indicates that female orangutans have smaller ranges than males and generally stay in specific geographic locations (Rijksen and Meijaard 1999). In fact, geographic boundaries formed by rivers and mountains prevent isolated orangutan populations from traversing such terrain. Recent microsatellite studies revealed no differentiation between East and West Kalimantan (Warren et al. 2000). However, it is important to note that microsatellite analyses provide data reflecting a more historical time point than the mtDNA, which is less conservative and has a rapid mutation rate. Current data suggest that historically, the orangutan populations within Borneo were large enough within specific regions to enable gene flow and prevent a genetic bottleneck. However, the mtDNA does indicate that the four distinct subpopulations within Borneo represent reproductively isolated populations that show significant genetic diversity.

The phylogenetic analyses enabled the determination and confirmed the probable place of origin for most of the confiscated individuals that were used in this study. There was also evidence that there may be even greater genetic variation within Borneo, indicated by the few individuals that fell outside of the main subpopulation clusters. There was only one case in which an orangutan was not located in the expected geographical cluster. Hair samples were collected from the nest of a wild individual, TNK 38, in Kutai National Park. However, on phylogenetic analysis, this individual was consistently located in the cluster from Northwest Kalimantan/Sarawak (fig. 2). Occasionally, ex-captive orangutans were released into Kutai National Park unofficially prior to the establishment of new regulations in 1995 governing orangutan reintroduction practice. Thus, the probable explanation is that this animal represents an ex-captive orangutan originating from the Northwest Kalimantan/Sarawak population that was subsequently released into Kutai National Park.

In conclusion, this study provides evidence for (1) marked divergence and speciation of Sumatran and Bornean orangutans and (2) at least four distinct subpopulations of Bornean orangutans. Based on mtDNA analysis, these populations are estimated to be equally genetically distinct from each other. It can be debated whether there is sufficient divergence for the four Bornean populations to be classified as subspecies. However, they are clearly genetically isolated populations that are in the process of divergence. In this regard, these populations should be treated as “subpopulations,” and their genetic

diversity should be maintained. Furthermore, given the most recent catastrophic destruction of orangutan habitat by fires and logging, more detailed studies will be required to further define other distinct populations as well as to generate predictive data about the future survival of smaller orangutan populations isolated in increasingly fragmented habitats. These findings provide evidence to strengthen arguments for further conservation and management efforts to secure greater areas of orangutan habitat. The orangutan subpopulations should be protected in each geographic region to ensure their genetic diversity and survival.

Acknowledgements

We thank the staff of the Wanariset Orangutan Reintroduction Project for their assistance and support, particularly Adi Susilo, Adriansyah, and Willie Smits. We also thank Dr. Cheryl Knott and staff at Gunung Palung, West Kalimantan; staff of ODA and A. Erman at Danau Sentarum, West Kalimantan; Dr. Edwin Bosi and staff at Sepilok orangutan sanctuary; and the staff at the Semongok Wildlife Rehabilitation Center in Malaysia. We are thankful for the financial assistance provided by BHP Minerals Ltd., Melbourne, the Chicago Zoological Society, and the Merck Company Foundation on behalf of Merial, without which this research could not have been conducted. We are extremely grateful to Henk van Westbroek for his expertise with the figures and Jeannette Schouw for compiling this manuscript. Herman Rijksen provided engaging discussions about the biology of orangutans and current survival efforts. We are also grateful to our colleagues Henk Niphuis, Nel Otting, Natasja de Groot, and Ronald Bontrop for their assistance and support. K.S.W. and E.J.V. both contributed equally to this work.

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