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Genetic Characterization of Long-tailed Macaques (*Macaca fascicularis*) on Tabuan Island, Indonesia

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ABSTRACT. Protein and mitochondrial DNA variations (D-loop region PCR-RFLP) were analyzed for 7 serum and 40 clot samples collected from long-tailed macaques (*Macaca fascicularis*) living on Tabuan Island, Indonesia. Protein polymorphisms were examined electrophoretically for 5 and 12 kinds of protein in serum and erythrocytes, respectively. Each of the protein loci tested showed a monomorphic pattern. Polymorphisms were detected in the analysis of the D-loop-containing region of mtDNA (PCR-RFLP) using 32 restriction endonucleases. Two haplotypes, differing 1.03% in sequence divergence were observed, and both were previously undetected in other local populations. Based on genetic features and differences in pelage color as outlined in FOODEN's (1995) morphological analysis, the present results suggest that long-tailed macaques on Tabuan Island are a unique population. From the genetic analyses performed here, Tabuan monkeys are considered to be the same species group as those populations of Sumatra and Java (FOODEN, 1995).

Key Words: Protein variation; mtDNA PCR-RFLP; *Macaca fascicularis*; Tabuan Island.

INTRODUCTION

Long-tailed macaques (*Macaca fascicularis*) are widely distributed throughout tropical Southeast Asia including the southern part of Burma, the eastern part of Bangladesh, the Indochinese Peninsula, the Malay Peninsula, Sumatra, Java, Borneo, the Lesser Sunda Islands, the Philippine Islands, the southernmost Nicobar Islands, and many small adjacent islands. On the basis of morphological traits such as dorsal pelage color, this species in the Indonesian Archipelago can be classified into two groups: one inhabiting the mainland (Java, Sumatra, and Kalimantan) and the other on fringing islands which are characterized by a darker color than the mainland monkeys (FOODEN, 1995).

Tabuan Island is an offshore island in Lampung Province, Sumatra, Indonesia (Fig. 1). The island, consisting of mountainous terrain with primary and secondary tropical rain forest, is about 7,500 ha in size. KYES et al. (1996) reported the natural distribution of long-tailed macaques on Tabuan Island. A short census conducted by AMIR et al. (1991) estimated a population size of 11,000 – 14,000 monkeys on Tabuan.

Tabuan monkeys have a darker pelage color than those in West Java and Southern Sumatra (KYES et al., 1996), which is consistent with the second group of FOODEN's (1995) classification mentioned above.

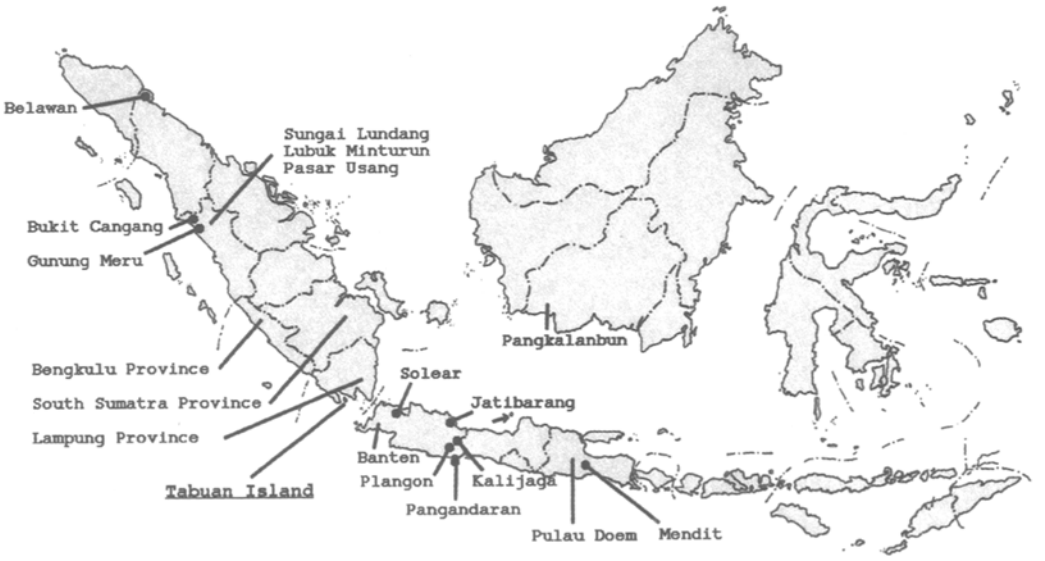

Fig. 1. Tabuan Island and other populations examined in this study. Black circles denote localities of social groups from which samples were collected. Arrow indicates the geographical position of Karimunjawa and Kemujan Islands.

To date, no information is available on genetic or evolutionary features of Tabuan macaques. In this brief report, we focus on the characterization of protein and mitochondrial DNA variation (PCR-RFLP) of long-tailed macaques on Tabuan Island and compare it to those of other Indonesian *fascicularis*. We undertook this study to gain insight into whether phylogenetic analyses from protein and mtDNA in *M. fascicularis* concur with FOODEN's morphological analysis (1995).

MATERIALS AND METHODS

SAMPLES

We used a total of 7 serum and 40 clot samples from long-tailed macaques inhabiting Tabuan Island in 1996 and 1997. All samples examined for protein and DNA analyses are presented in Tables 1 and 2. Table 1 consists of the data obtained in the present study (Tabuan Island) and reanalyzed data from previous studies (KAWAMOTO, 1982; KAWAMOTO et al., 1984) as reference populations. Furthermore all data presented in Table 2 were obtained in the present study.

PROTEIN ELECTROPHORESIS

Protein polymorphism was examined electrophoretically for 5 of serum proteins (*Alb, TBPA, Tf, LAP*, and *Pi*) and 12 of erythrocyte proteins (*PHI, 6PGD, ADA, CA-I, G6PD, MDH, LDH, TO, LAP, Pi, Cell-Es*, and *IDH*). Electrophoretic examination was done using starch gel electrophoresis (SGE) and polyacrylamide gel electrophoresis (PAGE). Running, staining conditions and typing of alleles followed KAWAMOTO (1982) and KAWAMOTO et al. (1982).

Table 1. Origin, number of samples examined, comparison of allele frequencies, and genetic variability of Indonesian long-tailed macaques.

Sampling locality	Sample size	Year of sampling	*Alb*				*TBPA*		*PHI*			
			A	*B*	*C*	*D*	*F*	*S*	*1*	*5*	*13*	*14*
Tabuan Island	7*	1996	1.0	–	–	–	1.0	–	1.0	–	–	–
	40**	1996										
Reference population												
West Java												
+Jatibarang	26	1980	1.0	–	–	–	1.0	–	1.0	–	–	–
+Pangandaran	41	1980	1.0	–	–	–	0.915	0.085	1.0	–	–	–
Banten	10	1979	1.0	–	–	–	0.995	0.005	1.0	–	–	–
East Java												
+Mendit	20	1980	1.0	–	–	–	1.0	–	1.0	–	–	–
Pulau Doem	24	1980	1.0	–	–	–	0.979	0.021	1.0	–	–	–
South Sumatra												
Lampung Province	70	1979	0.986	–	0.014	–	0.920	0.080	1.0	–	–	–
Palembang (South Sumatra Province)	13	1979	0.962	–	0.038	–	0.962	0.038	1.0	–	–	–
Bengkulu Province	21	1979	1.0	–	–	–	0.881	0.119	1.0	–	–	–
West Sumatra												
+Gunung Meru	66	1980	1.0	–	–	–	0.995	0.006	–	–	–	–
+Bukitcangang	9	1980	1.0	–	–	–	1.0	–	1.0	–	–	–
Sungai Lundang	26	1980	0.981	0.019	–	–	0.981	0.019	0.904	0.096	–	–
North Sumatra												
+Belawan	43	1981	0.488	0.465	–	0.047	0.872	0.128	1.0	–	–	–
Total	416											

Table 1. (continued)

Sampling locality	*Tf*#												
	B^1	B^2	C^1	C^2	C^3	D^1	D^2	E^1	E^2	F^1	G^1	G^2	H^1
Tabuan Island	–	–	–	–	–	–	–	–	–	–	–	–	1.0
Reference population													
West Java													
+Jatibarang	–	–	–	–	0.692	0.308	–	–	–	–	–	–	–
+Pangandaran	–	–	–	0.037	0.183	0.597	–	–	–	–	–	0.183	–
Banten	–	0.044	–	0.061	0.055	0.571	0.017	–	0.028	0.022	0.011	0.159	0.033
East Java													
+Mendit	–	–	–	0.075	–	0.775	–	–	–	–	–	0.150	–
Pulau Doem	–	–	–	0.521	–	0.333	–	–	–	0.042	–	0.104	–
South Sumatra													
Lampung Province	0.050	0.007	0.007	0.186	0.007	0.543	–	0.007	0.022	–	0.014	0.036	0.121
Palembang (South Sumatra Province)	0.039	–	–	0.039	0.039	0.615	–	–	–	–	0.077	–	0.191
Bengkulu Province	–	–	–	0.238	–	0.691	–	–	–	–	–	–	0.071
West Sumatra													
+Gunung Meru	–	–	–	0.235	0.061	0.614	–	–	–	–	–	–	0.090
+Bukitcangang	–	–	–	0.389	0.056	0.333	–	–	–	–	–	–	0.222
Sungai Lundang	–	–	–	0.423	0.019	0.404	–	–	0.019	–	–	0.019	0.116
North Sumatra													
+Belawan	–	–	–	–	–	0.919	–	–	–	–	–	–	0.081

Table 1. (continued)

Sampling locality	*6PGD*		*ADA*	*CA-1*			*G6PD*	*MDH*	*LDH*			*TO*	*LAP*
	A	*C*	*A*	*a*	*b*	*c*	*B+*	*1*	*A*		*B*		
									1	*3*	*1*	*1*	*a*
Tabuan Island	1.0	–	1.0	1.0	–	–	1.0	1.0	1.0	–	1.0	1.0	1.0
Reference population													
West Java													
+Jatibarang	1.0	–	1.0	1.0	–	–	1.0	1.0	1.0	–	1.0	1.0	1.0
+Pangandaran	1.0	–	1.0	1.0	–	–	1.0	1.0	1.0	–	1.0	1.0	1.0
Banten	1.0	–	1.0	1.0	–	–	1.0	1.0	1.0	–	1.0	1.0	1.0
East Java													
+Mendit	1.0	–	1.0	1.0	–	–	1.0	1.0	1.0	–	1.0	1.0	1.0
Pulau Doem	1.0	–	1.0	1.0	–	–	1.0	1.0	1.0	–	1.0	1.0	1.0
South Sumatra													
Lampung Province	0.940	0.060	1.0	0.950	0.007	0.043	1.0	1.0	1.0	–	1.0	1.0	1.0
Palembang (South Sumatra Province)	1.0	–	1.0	0.885	–	0.115	1.0	1.0	1.0	–	1.0	1.0	1.0
Bengkulu Province	1.0	–	1.0	1.0	–	–	1.0	1.0	1.0	–	1.0	1.0	1.0
West Sumatra													
+Gunung Meru	1.0	–	1.0	1.0	–	–	1.0	1.0	1.0	–	1.0	1.0	1.0
+Bukitcangang	1.0	–	1.0	1.0	–	–	1.0	1.0	0.944	0.056	1.0	1.0	1.0
Sungai Lundang	0.957	0.043	1.0	–	–	–	1.0	1.0	1.0	–	1.0	1.0	1.0
North Sumatra													
+Belawan	0.558	0.442	1.0	–	–	–	1.0	1.0	0.872	0.128	1.0	1.0	1.0

Table 1. (continued)

Sampling locality	*Pi*			*Cell-Es*		*IDH*			
	A	*B*	*C*	*1*	*3*	*1*	*2*	*3*	*4*
Tabuan Island	–	1.0	–	1.0	–	1.0	–	–	–
Reference population									
West Java									
+Jatibarang	–	1.0	–	1.0	–	1.0	–	–	–
+Pangandaran	–	0.915	0.085	1.0	–	0.244	0.756	–	–
Banten	0.006	0.827	0.167	1.0	–	0.766	0.228	–	–
East Java									
+Mendit	–	0.325	0.675	1.0	–	0.325	0.675	–	–
Pulau Doem	–	0.729	0.271	1.0	–	0.667	0.333	–	–
South Sumatra									
Lampung Province	0.029	0.750	0.221	1.0	–	0.900	0.079	0.007	0.014
Palembang (South Sumatra Province)	–	0.846	0.154	1.0	–	0.885	0.077	0.038	–
Bengkulu Province	–	0.904	0.095	1.0	–	0.976	0.024	–	–
West Sumatra									
+Gunung Meru	–	0.700	0.300	1.0	–	0.992	0.008	–	–
+Bukitcangang	–	0.611	0.389	1.0	–	1.0	–	–	–
Sungai Lundang	–	0.957	0.043	1.0	–	0.978	0.022	–	–
North Sumatra									
+Belawan	–	1.0	–	0.977	0.023	0.640	0.337	–	0.023

Table 1. (continued)

Sampling locality	*AK* 1	*Es-D* 1	Proportion of polymorphic loci $P_{poly} \pm$ S.E.	Mean of heterozygosity $\bar{H} \pm$ S.E.
Tabuan Island	1.0	1.0	0	0
Reference population				
West Java				
+Jatibarang	1.0	1.0	0.056 ± 0.053	0.024 ± 0.024
+Pangandaran	1.0	1.0	0.222 ± 0.098	0.041 ± 0.028
Banten	1.0	1.0	0.278 ± 0.106	0.073 ± 0.042
East Java				
+Mendit	1.0	1.0	0.167 ± 0.088	0.069 ± 0.038
Pulau Doem	1.0	1.0	0.222 ± 0.098	0.083 ± 0.044
South Sumatra				
Lampung Province	1.0	1.0	0.389 ± 0.115	0.089 ± 0.041
Palembang (South Sumatra Province)	1.0	1.0	0.333 ± 0.111	0.078 ± 0.036
Bengkulu Province	1.0	1.0	0.222 ± 0.098	0.049 ± 0.028
West Sumatra				
+Gunung Meru	1.0	1.0	0.278 ± 0.106	0.061 ± 0.037
+Bukitcangang	1.0	1.0	0.389 ± 0.115	0.070 ± 0.045
Sungai Lundang	1.0	1.0	0.389 ± 0.115	0.061 ± 0.036
North Sumatra				
+Belawan	1.0	1.0	0.389 ± 0.115	0.120 ± 0.045

Name of alleles followed KAWAMOTO (1982) and KAWAMOTO et al. (1982). +: Sample collected from social group; *serum; **clot blood; #: PAGE (Polyacrylamide Gel Electrophoresis) method; others: SGE (Starch Gel Electrophoresis).

For further analysis of protein data, we reanalyzed the data of KAWAMOTO (1982) and KAWAMOTO et al. (1984) for 17 proteins as we screened for the Tabuan population.

DNA EXTRACTION AND AMPLIFICATION

Genomic DNA was extracted from serum and clot samples using QIAamp Blood Kits (Qiagen) and the Easy-DNA™ Kit (Invitrogen). In order to amplify a 1.8-kb mtDNA fragment containing the D-loop region, we used the primers and PCR conditions outlined by LAWLER et al. (1995). PCR amplification was successful for all serum samples but for only half of the clot samples.

PCR-RFLP

A two-stage process was employed in mtDNA typing. Primary typing was performed using the following five restriction endonucleases: *Hae*III, *Msp*I, *Sau*3AI, *Mbo*II, and *Hinf*I. These restriction endonucleases were specifically chosen to detect prominent differences among individuals. In the second step, we applied 27 restriction endonucleases (*Taq*I, *Apa*I, *Ase*I, *Bcl*I, *BstE*II, *Cla*I, *Dra*I, *Eco*O109I, *Hinc*II, *Hind*III, *Kpn*I, *Sac*II, *Sca*I, *Stu*I, *Sac*I, *Eco*RI, *Pst*I, *Pvu*II, *Bgl*II, *Csp*45I, *Hpa*I, *Xho*I, *Bam*HI, *Nae*I, *Nru*I, *Xba*I, and *Apa*L1) which recognized four, six or multiple base sequences for quantification of sequence divergence among haplotypes. We selected one representative sample from each haplotype of each population. Digestion conditions were as specified by the supplier. The digested DNA fragments were separated and visual-

Table 2. Origin and number of samples examined for DNA analysis and comparison of genetic variability of Indonesia long-tailed macaques.

Sampling locality	Sample size	Year of sampling	Haplotype (first step of analysis)	Individuals	Haplotype (second step of analysis)	*n*	*k*	*p*	Variance (*p*)
Tabuan Island	27	1996	6	3	6	2	3	0.00726	0.00002
			7	24	7				
Reference population									
Java									
West Java									
+Jatibarang	14	1994	1	14	1	1	–		
+Plangon	16	1994	2	16	2	1	–		
+Kalijaga	7	1994	3	7	3	1	–		
+Solear	5	1994	4	5	4	1	–		
+Pangandaran	46	1995	5	46	5	1	–		
Banten area	10	1979	2	3	8	5	6	0.01504	0.00002
			9	2	9				
			10	3	10				
			11	1	11				
			12	1	12				
East Java									
+Mendit	20	1980	13	8	13	2	4	0.00979	0.00002
			14	12	14				
Total						12	16	0.04552	0.00004
Sumatra									
South Sumatra									
Lampung Province	5	1979	3	2	3	3	8	0.02054	0.00003
			16	2	16				
			17	1	17				
Palembang (South Sumatra Province)	2	1979	2	1	8	2	5	0.01238	0.00003
			19	1	19				
Bengkulu Province	4	1979	20	1	20	3	4	0.00979	0.00002
			21	2	21				
			22	1	22				
West Sumatra									
+Gunung Meru	10	1980	24	10	24	1	–		
+Bukitcangang	5	1980	25	5	25	1	–		
Sungai Lundang	5	1980	23	5	23	1	–		
Lubuk Minturun	4	1981	23	4	23	1	–		
Pasar Usang	2	1980	23	2	23	1	–		
North Sumatra									
+Belawan	5	1981	26	5	26	1	–		
Total						12	20	0.06015	0.00005
Borneo									
Central Kalimantan Pangkalanbun	9	1994	3	1	3	3	2	0.00479	0.00001
			27	6	27				
			28	2	28				
Total	196		25		26				

n: The number of haplotypes; *k*: the number of polymorphic sites; *p*: estimation of the proportion of polymorphic sites (HUDSON, 1982).

ized in 4 – 8% polyacrylamide gel with silver staining. The size of restriction fragments was estimated using a molecular size marker (BIORAD).

The sequence divergence between haplotypes was estimated by the equations given by NEI (equations 5.3 and 5.50, 1987). The proportion of polymorphic nucleotide sites (nucleotide diversity) was estimated by the method of HUDSON (1982). The relationships among populations based on sequence divergence were evaluated using UPGMA option in the NEIGHBOR program of PHYLIP version 3.57 (FELSENSTEIN, 1993).

RESULTS

PROTEIN POLYMORPHISM

By comparison to human isozyme patterns (HARRIS & HOPKINSON, 1978), 17 proteins were postulated to be encoded by 18 loci. All of the loci tested were monomorphic in the Tabuan population. We compared this result with 12 social groups from regional populations (369 samples) in Sumatra and Java (Table 1). Table 1 gives allele types, estimates of allele frequencies, proportion of polymorphic loci (P_{poly}), and mean heterozygosity ($\bar{H}$) for Tabuan and other populations.

Figure 2 represents a dendrogram constructed from NEI's (1972) standard genetic distance using UPGMA. In this analysis, reanalyzed data from KAWAMOTO (1982) and KAWAMOTO et al. (1984) of Sumatra and Java were compared with our results (Table 1). The Tabuan was found to be distantly related to other populations in Sumatra and Java.

MITOCHONDRIAL DNA VARIATION

We compared Tabuan (27 samples) with 17 social groups or regional populations (169 samples) in Sumatra, Java, and Borneo.

Typing of mitochondrial DNA was accomplished using 5 restriction endonucleases that each recognized 4 or 5 base sequences, yielding 25 haplotypes. Using 27 restriction endonucleases that recognized 4, 6 or multiple base sequences, we identified 26 haplotypes (one more haplotype was found). These haplotypes were assigned numerical letters and their distribution is given in Table 2. Sixteen restriction endonucleases (*Bcl*I, *BstE*II, *Cla*I, *Hinc*II, *Kpn*I, *Stu*I, *Eco*RI, *Pst*I, *Pvu*II, *Csp*45I, *Hpa*I, *Xho*I, *Bam*HI, *Nae*I, *Nru*I, and *Xba*I) revealed no cleavage sites in the RFLP analysis. Using the combination of the other 16 enzymes, a total of 25 polymorphic sites were identified, 3 of which were variable in the Tabuan population. We found two mtDNA haplotypes in 27 Tabuan long-tailed macaques (frequencies of types 6 and 7 are 0.111 and 0.889 respectively). These haplotypes displayed a sequence divergence of 1.03% and have not been observed previously in the West Java (PERWITASARI-FARAJALLAH et al., 1999) or Sumatran populations (Table 2). Pairwise sequence divergence estimates between Tabuan haplotypes and other haplotypes varied from 0.0172 to 0.0449 with a mean of 0.0309. This is within the range of other Indonesian *fascicularis* (ranging from 0.0039 to 0.0449).

The proportion of polymorphic sites of mtDNA in the Tabuan population was 7.26×10^{-3}, 6 to 8 times smaller than that observed in the study populations of Java or Sumatra islands (Table 2). Assuming a normal distribution, the proportion of polymorphic sites of the Tabuan population differs significantly from the Java and Sumatra populations (t=4.94 and 6.32, p<0.001). However, the Tabuan population is not significantly different from the Bornean population (t=0.54, 0.5<p<0.6), possibly due to the small number of samples that were collected in a limited area in Pangkalanbun, Central Kalimantan.

Figure 3 presents the genetic relationship between study populations constructed from sequence divergence using the UPGMA method. In this clustering, Tabuan types formed an outgroup with a haplotype in Pangandaran (West Java).

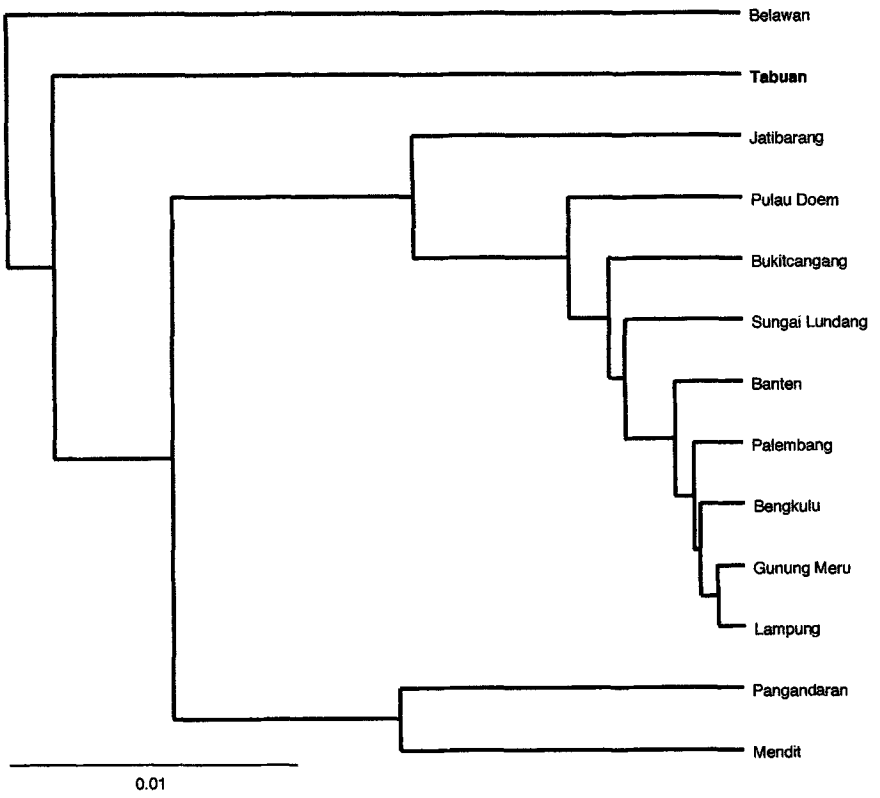

Fig. 2. Genetic relationship of island populations estimated from protein data using UPGMA clustering of NEI's standard genetic distance (NEI, 1972).

DISCUSSION

PROTEIN DIVERSITY

Due to limitations imposed by sample availability, we examined mainly those polymorphic protein loci known from previous studies. Among the loci examined, transferrin is known to be highly polymorphic (KAWAMOTO, 1982, 1996; NOZAWA et al., 1982; KAWAMOTO et al., 1984; KAWAMOTO & SURYOBROTO, 1985). However, Tabuan was fixed to allele H^1, the third most common allele in South Sumatra.

The results of the clustering analysis indicated that the Tabuan population was greatly differentiated from other populations, including its mainland counterparts in Sumatra such as the populations of Lampung, Bengkulu, and the South Sumatra provinces (Fig. 2). Geographically, Tabuan is a part of the Sunda Shelf. It is located 30 km off the south coast of Sumatra and it was connected to mainland Sumatra during glacial periods in the Pleistocene and most recently about 10,000 years ago (HANEBUTH et al., 2000; FAIRBANKS, 1989). All of the protein alleles observed on Tabuan have been previously detected in Sumatran populations (KAWAMOTO et al., 1984). Thus, the Tabuan and Sumatran populations differed quantitatively only in allele frequencies. The scarcity of private mutant and the low variability in protein loci are remarkable features of the Tabuan population which resulted in a specific topology when analyzed in a dendrogam with other Indonesian *fascicularis* populations (Fig. 2).

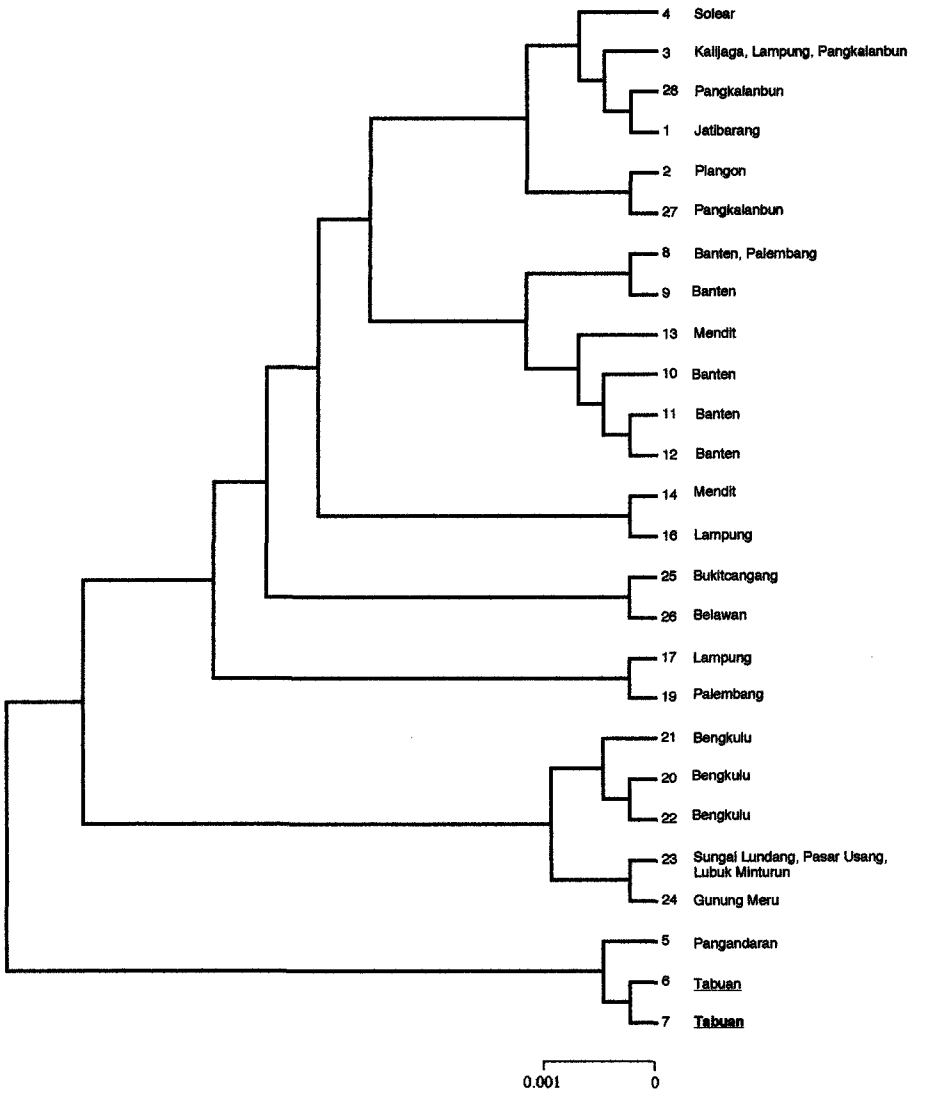

Fig. 3. Relationship of mtDNA haplotypes estimated by UPGMA with sequence divergence measures (NEI, 1987). The haplotype numbers are the same as in Table 2.

MITOCHONDRIAL DNA DIVERSITY

Analysis of social groups in Sumatra, Java, and Borneo revealed a distinct lack of variability in haplotype as compared to regional populations (Table 2). This is consistent with previous studies of other non-human primates (HOELZER et al., 1994; ROSENBLUM et al., 1997; GOLDBERG, 1998). This lack of mtDNA haplotype variation may result from its specific mode of heritance, i.e. maternal inheritance without recombination (HUTCHINSON et al., 1974; GILES et al., 1980), and sociological features such as female philopatry and lineage sorting in group division (MELNICK & HOELZER, 1992, 1996). Bearing this in mind, despite the unknown origin of the social group(s) of Tabuan samples, two haplotypes found there could be assumed to belong to different social groups on the island.

DIFFERENCES IN PROTEIN AND MITOCHONDRIAL DNA DIVERSITY

The present study noted that the degree of protein diversity was in sharp contrast with that of mtDNA. Protein loci show monomorphism in addition to sharing alleles with Sumatran populations. Conversely, mitochondrial DNA diversity was observed in the population, including two specific mtDNA haplotypes that were unique to Tabuan. This discrepancy could be associated with differences in protein and mtDNA features as well as the history of the population's geographic isolation. Protein loci generally display Mendelian inheritance, however mtDNA displays maternal transmission. By reason of these differences, the effective population size of mtDNA was smaller than that of protein, leading to a higher degree of genetic drift (BIRKY et al., 1989), as well as a high fixation rate as a selectively neutral marker (BROWN et al., 1979). As a result, variability of mitochondrial DNA is expected to be lower than that of protein loci. However, the results were a contrast in the Tabuan population. In light of this evidence, we consider population history to be a key factor for explaining this inconsistency. During the most recent glacial period, ca. 10,000 year BP (HEANEY, 1978), Tabuan should have been connected to mainland Sumatra. At this time, if a population existed on Tabuan, male migration may have contributed to the homogenization of protein throughout a wide geographic range. However it may have had less impact on the distribution of mtDNA due to the restricted movement pattern of female macaques (female philopatry) (MELNICK & HOELZER, 1992, 1996). Lack of protein variability at present in Tabuan could be attributed to genetic drift after separation of the island from mainland Sumatra. Theoretically, and in accordance with founder size, genetic diversity of an isolated population should be expected to decrease and it may take a long time to recover depending on population growth (NEI et al., 1975). Not only protein diversity was affected by geographic isolation, but mtDNA diversity as well was similarly influenced. Two possible scenarios could account for the observed mtDNA polymorphisms. One is that new mutants occurred after the isolation due to the high mutation rate of mtDNA, and the other is an old type remained in the population. In the former case, within the relatively short isolation time (less than 10,000 years), it is unlikely that two haplotypes having at least three restriction site differences and other undetectable intermediate haplotypes would have been generated. Therefore, we consider that the observed mtDNA polymorphisms are a result of the persistence of an old type. At present, we have insufficient data to determine whether or not natural selection operates on the processes maintaining mtDNA polymorphisms.

PHYLOGENETIC INTERPRETATION

As indicated by the clustering analysis of protein and mtDNA, it is hard to state conclusively the origin of the Tabuan population. There are, however, three conceivable interpretations of their ancestry.

1. Old ancestry: Tabuan was found to lack protein variability. Also, the estimated range of pairwise sequence divergence of mtDNA haplotypes between Tabuan and mainland populations was comparable to the range for *fascicularis* populations on Java and Sumatra. However, two unique mtDNA haplotypes existed on Tabuan and given the fact that this island has been isolated from mainland Sumatra for less than 10,000 years (HEANEY, 1978), it seems improbable that they would have arisen by novel mutations. Therefore, an old ancestry seems to be a logical explanation for the origin of Tabuan population.

2. Human introduction: Tabuan monkeys differ morphologically from mainland populations in

that they have a darker pelage color (KYES et al., 1996). Furthermore, the population shows substantial differentiation in the allelic composition of the transferrin locus from the mainland populations of Sumatra and Java. Thus, the possibility of human recent introduction is not strongly supported.

3. Natural distribution in relation to geological development of the Sunda Shelf during the Pleistocene: This possibility finds support from a recent taxonomic study conducted by FOODEN (1995). Despite the fact that the Tabuan monkey is distinguished by its dark pelage color (KYES et al., 1996), the result from mitochondrial DNA analysis revealed the genetic distance between Tabuan and mainland populations to fall within the distance range of *fascicularis* populations on the Sunda Shelf (genetic distance value varied from 0.0172 to 0.0449 with mean of 0.0309). Therefore, the present results support FOODEN's (1995) contention that shallow-water fringing-island populations such as Tabuan *fascicularis* are placed in the same taxon with mainland *fascicularis*. Furthermore, FOODEN noted that, in general, monkeys of shallow-water fringing islands have a slightly darker, more erythristic pelage and are smaller than those of mainland populations. In order to determine whether Tabuan long-tailed macaques can be categorized as old as those on Karimunjawa and Kemujan islands (see FOODEN, 1995), further evidence from morphological and genetic study are required.

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