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Variation in Blood Proteins and Mitochondrial DNA Within and Between Local Populations of Longtail Macaques, *Macaca fascicularis* on the Island of Java, Indonesia

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ABSTRACT. We examined 31 blood protein loci, and restriction fragment length profiles of a PCR product of mitochondrial DNA containing the D-loop region using five kinds of restriction endonucleases (*Hae*III, *Hinf*I, *Mbo*II, *Msp*I, and *Sau*3AI) in order to quantify the level of genetic variation of longtail macaques, *Macaca fascicularis*. Samples were collected from nine social groups in five localities of West Java, Indonesia. The average heterozygosity per individual ($\overline{H}$) was 0.060 and 15.7% of the loci were polymorphic (P_{poly}) over all populations in the protein analysis. There was no mtDNA haplotype variation within either social groups or local populations. To the contrary, great diversity was observed among local populations. Both nuclear diversity (measured by NEI's standard genetic distance) and mitochondrial diversity (measured by sequence divergence) showed a significantly positive correlation with geographic distance. There was no significant correlation between these two genetic markers, however. The genetic structure of the population was evaluated in terms of local inbreeding and temporal changes in allele frequency. Differences between nuclear and mitochondrial data are discussed in relation to gender specific migration and lineage sorting.

Key Words: Longtail macaques; Blood proteins; mtDNA; Genetic variation; Java Island.

INTRODUCTION

The longtail macaque (*M. fascicularis*) is distributed in the eastern part of Bangladesh, southern Burma, the Indochinese peninsula, the Malay peninsula, Sumatra, Borneo, Java, the Lesser Sunda Islands, the Philippine Islands, and the southernmost Nicobar islands (FOODEN, 1995). Studies on morphology (FOODEN, 1995, 1997), ecology and social behavior (SOUTHWICK & CADIGAN, 1972; WHEATLEY, 1980; KOYAMA et al., 1981; SUSSMAN & TATTERSALL, 1981, 1986; WHITTEN & WHITTEN, 1982; VAN SCHAIK & VAN NOORDWIJK, 1985; SUGARDJITO et al., 1989) reveal that the species possess a high degree of intraspecific differentiation. This tendency is also found in blood protein studies (KAWAMOTO et al., 1981, 1984, 1988; KAWAMOTO, 1982; TANAKA et al., 1989, 1991; KONDO et al., 1993; SCHEFFRAHN et al., 1996), and other molecular studies (HARIHARA et al., 1986, 1988, 1991; TAKENAKA et al., 1989, 1991; CROVELLA et al., 1994; LAWLER et al., 1995).

Population genetic studies of local populations in the Indonesian longtail macaque using blood proteins revealed relatively low genetic variability within small island populations and extremely great diversity among them (KAWAMOTO et al., 1981, 1984; KAWAMOTO & SURYOBROTO, 1985). This special feature of local genetic differentiation is discussed in relation to geographic

isolation as well as the possibility of intergradation during the glacial period (KAWAMOTO et al., 1984). Interestingly, a tendency for clinal variation has also been found in the distribution of the allele frequencies of blood proteins. Nevertheless, the main factors responsible for the extremely great diversity among island populations and relatively low genetic variability within small islands remain unclear and should be analyzed in greater detail.

The most abundant genetic information for use in the assessment of genetic variation of non-human primates comes from blood protein variation detectable by gel electrophoresis. Recently a number of techniques have been applied to DNA analysis such as sequencing, restriction fragment length polymorphisms (RFLP) and microsatellite analyses (HAYASAKA et al., 1991; LAWLER et al., 1995; ROSENBLUM et al., 1997; MORIN et al., 1997). They are highly suitable approaches for the study of population genetics.

Despite the fact that both nuclear and mitochondrial genes can be utilized as genetic markers for the quantification of variation in populations, the degree of concordance between results obtained from protein and mtDNA data for the same sample has rarely been tested.

The primary aims of this study are to examine polymorphism and to elucidate intra- and inter-populational genetic variation in longtail macaques using blood protein and mitochondrial DNA D-loop PCR-RFLP analyses for the same samples. The assessment of genetic variability within and between social groups and local populations presents basic information for this species and provides further insight for the discussion of population structure and population history.

MATERIALS AND METHODS

SAMPLING

Blood samples were collected from five localities in West Java (Fig. 1). The populations surveyed and number of samples examined are given in Table 1. These samples were taken from members of social groups. The study populations lived in remnant forests or in areas close to human habitation, such as secondary forests, tourist areas, and sacred places (SURYOBROTO et al., unpubl. data).

SAMPLE PREPARATION

Blood samples were separated into erythrocytes, plasma and buffy coat by centrifugation at 3,000 rpm, for 15 min. Erythrocytes were then washed three times with saline (0.86%). Plasma

Table 1. Populations and number of samples examined in this study.

Sampling locality	Social group	Abbreviation	Sample size	Year of sampling
Jatibarang	Jatibarang	JT	14	1994
Plangon	East Plangon	EP	5	1994
	West Plangon	WP	11	1994
Kalijaga	Kalijaga	KAL	7	1994
Solear	Solear	SOL	5	1994
Pangandaran	Cirengganis	PCG	9	1995
	Goa Parat	PGP	9	1995
	Goa Jepang	PGJ	9	1995
	Cikamal	PCK	19	1995
Total			88	

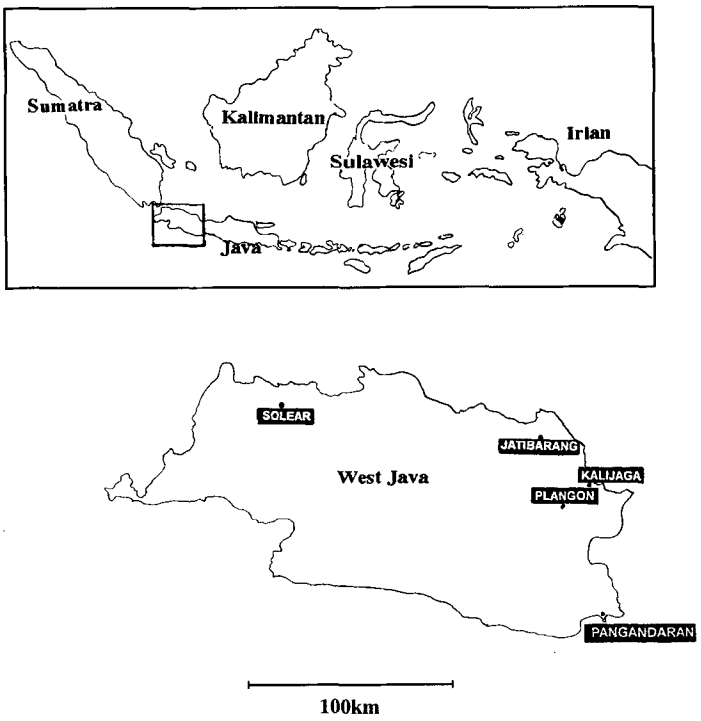

Fig. 1. Map of sampling localities in the present study.

and erythrocytes were used for protein analysis and the buffy coat for DNA analysis. They were stored at −20°C until examination. Whole genomic DNA were extracted from the buffy coat by the method of KAN et al. (1977) with slight modifications.

DETECTION OF PROTEIN VARIATION

Thirty-one genetic loci were examined by starch gel electrophoresis (SGE), polyacrylamide gel electrophoresis (PAGE), or iso-electric focusing (IEF). The loci examined are listed in Table 2. Electrophoresis and staining were carried out as described by NOZAWA et al. (1982) and KAWAMOTO et al. (1984).

AMPLIFICATION OF MITOCHONDRIAL DNA D-LOOP REGION

Mitochondrial DNA containing the D-loop region was amplified by polymerase chain reaction (PCR) using the primers described by LAWLER et al. (1995). PCR amplification was performed in a 100 μl volume by Perkin Elmer Thermalcycler (PJ 2000), containing 1μg of total DNA, 1.6 μM dNTP, 0.2 μM of each primer, 2.5 units Taq DNA polymerase (TAKARA), 10 μl 10 x reaction buffer (100 mM Tris-HCl, pH 8.3; 500 mM KCl; 15 mM $MgCl_2$) and overlaid with mineral oil. Denaturing, annealing, and extension conditions were 95°C for 45 sec, 58°C for 40 sec, and 70°C for 2 min, respectively. The total number of cycles was 30 and followed by a final extension for 5 min at 70°C.

Table 2. Blood protein and protein loci used for electrophoretic examination.

Blood protein	Locus	Source
Acid phosphatase	*Acp*	Erythrocyte
Adenosine deaminase	*ADA*	Erythrocyte
Adenylate kinase	*AK*	Erythrocyte
Carbonic anhydrase	*CA-I*	Erythrocyte
	CA-II	Erythrocyte
Catalase	*Cat*	Erythrocyte
Esterase	*Cell-Es*	Erythrocyte
Esterase D	*Es-D*	Erythrocyte
Glucose-6-phosphate dehydrogenase	*G6PD*	Erythrocyte
Hemoglobin α	*Hbα-I*	Erythrocyte
	Hbα-II	Erythrocyte
Hemoglobin β	*Hbβ*	Erythrocyte
Isocitrate dehydrogenase	*IDH*	Erythrocyte
Lactate dehydrogenase	*LDH-A*	Erythrocyte
	LDH-B	Erythrocyte
Malate dehydrogenase	*MDH*	Erythrocyte
NADH-diaphorase	*Dia*	Erythrocyte
Phosphohexose isomerase	*PHI*	Erythrocyte
6-phosphogluconate dehydrogenase	*PGD*	Erythrocyte
Phosphoglucomutase I	*PGM-I*	Erythrocyte
Phosphoglucomutase II	*PGM-II*	Erythrocyte
Albumin	*Alb*	Plasma
Alkaline phosphatase	*Alp*	Plasma
Ceruloplasmin	*Cp*	Plasma
Cholinesterase	*Ch Es*	Plasma
Haptoglobin	*Hp*	Plasma
Leucine aminopeptidase	*LAP*	Plasma
Protease inhibitor	*Pi*	Plasma
Slow-α_2-macroglobulin	α_2	Plasma
Thyroxin-binding prealbumin	*TBPA*	Plasma
Transferrin	*Tf*	Plasma

TYPING OF PCR PRODUCT

Restriction fragment length polymorphism (RFLP) analysis was performed using five kinds of restriction endonucleases (*Hae*III, *Hinf*I, *Mbo*II, *Msp*I, and *Sau*3AI). The digested fragments were resolved in 4 and 8% polyacrylamide gel electrophoresis (PAGE). The size of the fragments were determined by using the DNA size standard of 50-2,000 bp ladder (Amplisize-BIORAD) and visualized by silver staining (TEGELSTRÖM, 1986).

PROTEIN DATA ANALYSIS

Allele frequencies were calculated by the simple gene counting method, except for the hemoglobin α-II (*Hb-αII*) locus. The hemoglobin α subunit was postulated to be encoded by a duplicated α-globin gene (SMITH & FERRELL, 1980; TAKENAKA et al., 1989, 1991). Assuming dominant and recessive alleles, the frequencies of two presumed alleles at a duplicated locus (*Hb-αII* locus), denoted as *0* (null) and *2*, were estimated by the square-root method.

The amount of variation was measured by the proportion of polymorphic loci (P_{poly}) and average heterozygosity per individual ($\overline{H}$). A locus was considered polymorphic when the frequency of the most common allele was equal to or less than 0.99.

Intra and interpopulation diversity was calculated by NEI's statistics: H_T, H_S, and G_{ST} (NEI, 1973, 1987). In proportion to the hierarchical population structure, we took into account that total population is divided into a number of local populations. And also, each local population is

Table 3. Allele frequencies estimated for the polymorphic protein loci in the study populations of West Java longtail macaques.

		Social group								
Locus	Allele	JT (14)	EP (5)	WP (11)	KAL (7)	SOL (5)	PCG (9)	PGP (9)	PGJ (9)	PCK (19)
Tf	B^1	–	–	0.091	–	–	–	–	–	–
	B^2	–	–	–	0.071	–	–	–	–	–
	C^2	–	0.100	0.045	–	–	–	–	–	–
	C^3	0.429	–	0.045	–	0.300	0.333	0.111	0.278	0.105
	D^1	0.571	0.900	0.591	0.786	0.500	0.444	0.444	0.556	0.579
	E^2	–	–	0.045	–	0.100	–	–	–	–
	F^1	–	–	0.045	–	–	–	–	–	–
	G^1	–	–	0.136	0.071	–	–	–	–	–
	G^2	–	–	–	0.071	0.100	0.222	0.444	0.167	0.316
CA-II	*a*	0.542	0.100	0.318	0.071	–	–	–	–	–
	b	0.458	0.900	0.682	0.929	1.000	1.000	1.000	1.000	1.000
PGM-I	*1*	1.000	0.900	0.864	1.000	0.800	1.000	1.000	1.000	1.000
	3	–	0.100	0.136	–	0.200	–	–	–	–
IDH	*1*	1.000	0.900	0.591	0.786	0.700	0.170	0.220	0.390	0.370
	2	–	0.100	0.409	0.214	0.300	0.830	0.780	0.610	0.630
Hbα-II	*0*	1.000	0.900	0.909	1.000	0.600	0.670	0.500	0.670	0.610
	2	–	0.100	0.091	–	0.400	0.330	0.500	0.330	0.390
Pi	*B*	–	1.000	0.773	1.000	1.000	0.610	0.830	0.780	0.740
	C	1.000	–	0.227	–	–	0.390	0.170	0.220	0.260
TBPA	*F*	1.000	1.000	1.000	1.000	1.000	1.000	0.890	0.830	1.000
	S	–	–	–	–	–	–	0.110	0.170	–
PGM-II	*1*	1.000	1.000	0.955	1.000	0.800	0.720	0.830	0.610	0.710
	x	–	–	0.046	–	0.200	–	–	–	–
	5	–	–	–	–	–	0.280	0.170	0.390	0.290

divided into a number of social groups. Therefore, we used H_S, H_L, G_{SL}, H_T, and G_{LT} instead of H_T, H_S, and G_{ST} for intra and interpopulation diversity (NEI, 1973). Genetic distances between populations were calculated by NEI's standard genetic distance (NEI, 1972). Local inbreeding in West Java populations was analyzed using Wright's fixation index derived by NEI and CHESSER (1983).

PCR-RFLP ANALYSIS

According to the differences in electrophoretic patterns, restriction profiles were classified with alphabetic letters for each endonuclease. Then, from comparison of combinations of enzymatic restriction patterns, mtDNA haplotypes were defined. The gain and loss of restriction sites were evaluated by measuring DNA fragment size with size markers.

The sequence difference between two haplotypes was estimated in terms of distance p (the estimated number of substitutions per site) according to NEI (1987). Using the iteration procedure (NEI, 1987; equations 5.3 and 5.50), the estimate of sequence divergence was obtained.

To assess the proportion of intra and interpopulational variation in the mtDNA D-loop region, the proportion of the total mtDNA diversity attributable to intra and interpopulation differences was measured using the G_{ST} statistic of NEI (1973) modified by TAKAHATA and PALUMBI (1985).

Table 4. Proportion of polymorphic loci (P_{poly}) and average heterozygosity ($\overline{H}$) for each social group.

Social group	L	P_{poly}±S.E	$\overline{H}$±S.E
Jatibarang	2	0.065±0.04	0.032±0.022
East Plangon	5	0.161±0.067	0.029±0.012
West Plangon	7	0.226±0.076	0.077±0.030
Kalijaga	3	0.097±0.054	0.027±0.016
Solear	5	0.161±0.067	0.070±0.031
P-Cirengganis	5	0.161±0.067	0.072±0.031
P-Goa Parat	6	0.194±0.072	0.071±0.029
P-Goa Jepang	6	0.194±0.072	0.084±0.032
P-Cikamal	5	0.161±0.067	0.074±0.031
(Mean)		(0.157)	(0.060)

L: The number of polymorphic loci.

RESULTS

PROTEIN VARIATION

In comparison with human isozyme patterns (HARRIS & HOPKINSON, 1978) the 28 blood proteins examined (Table 2) were presumed to be encoded by 31 loci. Among the 31 loci, 8 were found to be polymorphic; these are *Tf*, *CA*-II, *PGM*-I, *IDH*, *Hbα*-II, *Pi*, *TBPA*, and *PGM*-II (Table 3). All except alleles *x* and *5* of *PGM*-II locus were observed previously in the Java populations (KAWAMOTO et al., 1984). In general, the study populations commonly shared allele types.

VARIABILITY WITHIN LOCAL POPULATIONS

The average heterozygosity ($\overline{H}$) fell within the range of 0.027−0.084 with a mean of 0.060 and the range of observed values of polymorphic loci (P_{poly}) between 0.065 and 0.226 (Table 4) with a mean of 0.157. These values were comparable to those obtained in previous studies of longtail macaques in Java (KAWAMOTO et al., 1981, 1984).

TEST FOR RANDOM MATING

Occurrence of local inbreeding in each social group, neighboring groups in the same locality and local populations was tested using Wright's fixation index F and χ^2. No case deviated significantly from $F=0$ (data not shown), thus all of the study groups or populations were regarded as being in Hardy-Weinberg equilibrium.

VARIABILITY BETWEEN LOCAL POPULATIONS

Gene Diversity (G_{LT})

Table 5 summarizes the estimates of gene diversity and population differentiation in West Java longtail macaques. The total gene diversity (H_T=0.079) can be apportioned into intra (H_S=0.060) and interpopulational ($D_{LT}=H_T-H_L$=0.019) gene diversity. G_{LT} ($=D_{LT}/H_T$) was 0.241; indicating that about 24% of the total gene diversity was attributed to the differences between populations.

Table 5. Gene diversity and population differentiation in West Java longtail macaques.

Total population	Local population	Social group	H_S	H_L	H_L-H_S	G_{SL}			
	Plangon	East Plangon West Plangon	0.055	0.057	0.002	0.035			
	Pangandaran	Cirengganis Goa Parat Goa Jepang Cikamal	0.091	0.095	0.004	0.042			
						0.039 (mean)			
							H_T	H_T-H_L	G_{LT}
West Java	Jatibarang Plangon Kalijaga Solear Pangandaran			0.060			0.079	0.019	0.241

H_S: Gene diversity within social group in the local population; H_L: gene diversity within local population; G_{SL}: H_L-H_S/H_L: relative gene diversity between social groups in the local population; H_T: gene diversity within total population; G_{LT}: H_T-H_L/H_T: relative gene diversity between local populations.

NEI's *Standard Genetic Distance (D)*

The absolute value of genetic diversity between populations was estimated by NEI's standard genetic distance (NEI, 1987) as given in Table 6. We treated data with regards to the social group. Genetic differentiation between neighboring social groups was generally less than that between non-neighboring groups. Genetic differentiation between neighboring groups and between local populations in West Java fell within the range of longtail macaques (KAWAMOTO et al., 1984) (Table 7). However the maximum *D* value of 0.009 in longtail macaque populations between neighboring groups was less than that of Japanese macaques, *Macaca fuscata* (NOZAWA et al., 1982) and toque macaques, *Macaca sinica* (SHOTAKE & SANTIAPILLAI, 1982). Furthermore, the maximum *D* value of 0.060 in longtail macaques between local populations was higher than that of macaque species and grivet monkeys, *Cercopithecus aethiops aethiops* (SHIMADA & SHOTAKE, 1997).

We investigated the relationship between genetic distance and geographic distance. For this we used the straight-line distance (km) between sites for measuring geographic distance (Table 6).

Table 6. NEI's standard genetic distance (below diagonal) and geographic distance (km; above diagonal) between social groups of West Java longtail macaques.

Social group	JT	EP	WP	KAL	SOL	PCG	PGP	PGJ	PCK
JT		50	50	45	140	150	150	150	150
EP	0.047		0	20	155	105	105	105	105
WP	0.032	0.009		20	155	105	105	105	105
KAL	0.013	0.002	0.007		160	115	115	115	115
SOL	0.057	0.011	0.012	0.012		200	200	200	200
PCG	0.055	0.035	0.019	0.029	0.020		0	0	0
PGP	0.072	0.032	0.021	0.027	0.015	0.005		0	0
PGJ	0.056	0.023	0.016	0.019	0.012	0.004	0.006		0
PCK	0.055	0.022	0.014	0.018	0.012	0.003	0.003	0.002	

For abbreviation of group name see Table 1.

Table 7. Comparison of genetic differentiation of non-human primates as estimated by NEI's standard genetic distance.

	M. fascicularis West Java (present study)	*M. fascicularis* (KAWAMOTO et al., 1984)					*M. fuscata* (NOZAWA et al., 1982)	*M. sinica* (SHOTAKE & SANTIAPILLAI, 1982)	*Cercopithecus aethiops aethiops* (SHIMADA & SHOTAKE, 1997)
		Sumatra	Java	Bali	Lombok	Sumbawa			
Between social groups in the same locality	0.002–0.009	0.003	0.002–0.008	0.001	0.017	0.003–0.006	0.003–0.010	0.001–0.011	
Between local populations in the same islands	0.005–0.060	0.005–0.037	0.010–0.062	0.001–0.010	0.003–0.007	0.001–0.011	0.002–0.052	0.009–0.045	0.002–0.023

The correlation between genetic distance and geographic distance was significantly positive ($r=+0.43$; $0.001<p<0.01$) among social groups. Eliminating Jatibarang, which was relatively distant from other populations in protein analysis, a stronger positive correlation was observed ($r=+0.52$; $0.001<p<0.01$).

Temporal Change of Allele Frequency in Jatibarang and Pangandaran Populations

Allele frequency data obtained in different periods of sampling were available for the Jatibarang and Pangandaran populations (KAWAMOTO et al., 1981; present study). Using those data, it was possible to test whether the temporal changes in allele frequencies follow stochastic change or not.

The results of the comparison for the two sampling periods (1980 and 1994–1995) revealed that there had been no significant change in allele frequencies in the Jatibarang population (Table 8). However, in the case of Pangandaran, the *Pi* locus showed a significant change in allele frequencies.

Table 8. Changes in allele frequencies between two periods of sampling (1980–1994) in Jatibarang and Pangandaran populations.

Local population	Locus	Allele	q (1980)	q' (1994)	$\Delta q=(q-q')/2$	$\sigma_{\delta q}=\sqrt{q(1-q)/2N}$	$C=\Delta q/\sigma_{\delta q}$	p
Jatibarang	*Tf*	D^1	0.6923	0.5714	−0.0605	0.0769	−0.7860	$0.4<p<0.5$
	CA-II	*a*	0.5192	0.5833	+0.0321	0.0833	+0.7695	$0.7<p$
Pangandaran	*Pi*	*B*	0.9186	0.7391	−0.0898	0.0212	−4.2335	$p<0.001$
	TBPA	*F*	0.9091	0.9457	+0.0183	0.0233	+0.7854	$0.4<p<0.5$
	IDH	2	0.7273	0.6304	−0.0485	0.0346	−1.4003	$0.1<p<0.2$

The fluctuation of frequencies of an allele was tested as follows: $E\{\delta q\}=\{(q'-q)-\Delta q\}/\sqrt{q(1-q)/2N}$; $E\{\delta q\}$: expectation of change of allele frequency caused by random fluctuation; N: effective population size (1/3×census data) (NOZAWA, 1972); In the case of a neutral marker, Δq (directional change due to natural selection) is zero, then $E\{\delta q\}=(q'-q)/\sqrt{q(1-q)/2N}=C$; C is expected to follow approximately a normal distribution with mean=0 and variance=1; effective population sizes are assumed to be 18 and 83 for the Jatibarang and Pangandaran populations, respectively; Δq: estimation of allele frequency change per generation (14 years were approximately considered to be two generations of monkey).

Table 9. Mitochondrial DNA haplotypes found in the populations of *Macaca fascicularis* in West Java.

Population	*n*	Haplotype	Composite types				
			*Hae*III	*Hinf*I	*Mbo*II	*Msp*I	*Sau*3AI
Jatibarang	14	1	A	A	A	A	D
Plangon	16	2	A	B	A	A	A
Kalijaga	7	3	A	A	A	A	A
Solear	5	4	A	A	A	B	C
Pangandaran	46	5	A	A	A	A^+	B

n: Number of sample individuals.

Mitochondrial DNA Variation

Restriction Site Polymorphisms

The approximate size of amplified segments of mitochondrial DNA containing the D-loop region was 1.8 kb. Three of five restriction endonucleases (*Hinf*I, *Msp*I, and *Sau*3AI) showed polymorphisms in their restriction pattern. A total of five different haplotypes (composite types) were found in the present study (Table 9). Geographic distribution of the mtDNA haplotype was restricted in each sampling locality and individual variation was not observed within local populations. The total number of recognition sites and the number of sites shared which were counted from five restriction endonucleases are presented in Table 10. Due to the technical hindrance in DNA fragment's calibration by PAGE (polyacrylamide gel electrophoresis), we determined the minimum number of restriction site differences (see Appendix).

Gene Diversity and Sequence Divergence

The estimate of total gene diversity of mtDNA variation (H_T) was 0.198. As there was no individual difference within local populations, intrapopulational gene diversity (H_S) was zero. This resulted in $G_{ST}=1$, indicating that all of the diversity was attributable to the component between local populations.

The estimates of sequence divergence between the PCR products of mtDNA containing D-loop region are summarized in Table 10. The overall mean of the sequence divergence was 0.0308.

From the quantification of sequence divergence between mtDNA haplotypes, the relationship between mtDNA diversity and geographic distance was evaluated. The analysis suggested a positive correlation between sequence divergence and geographic distance ($r=+0.61$; $0.05<p<0.1$). When the Jatibarang population was omitted, a stronger positive correlation was observed ($r=+0.81$; $0.001<p<0.01$).

Table 10. The number of recognition sites shared (above diagonal), the total number of recognition sites (diagonal), and estimates of sequence divergence between PCR products of mtDNA containing D-loop region among populations of West Java longtail macaques (below diagonal).

	Jatibarang	Plangon	Kalijaga	Solear	Pangandaran
Jatibarang	17	13	14	15	15
Plangon	0.0354	13	13	12	13
Kalijaga	0.0252	0.0093	14	13	14
Solear	0.0310	0.0555	0.0436	17	14
Pangandaran	0.0236	0.0270	0.0170	0.0408	16

Table 11. Comparison of two indices of genetic variation, average proportion of polymorphic loci (P_{poly}) and average heterozygosity ($\bar{H}$) in non-human primates.

Species	Population	No. of social group	No. of loci	P_{poly}	$\bar{H}$	Reference
M. fascicularis	West Java	9	31	0.16	0.060	Present study
M. fascicularis	Java	6	33	0.11	0.042	KAWAMOTO et al., 1984
M. fascicularis	Sumatra	4	33	0.19	0.057	KAWAMOTO et al., 1984
M. fascicularis	Bali	8	33	0.11	0.025	KAWAMOTO et al., 1984
M. fascicularis	Lombok	4	33	0.11	0.032	KAWAMOTO et al., 1982
M. fascicularis	Sumbawa	7	33	0.11	0.044	KAWAMOTO et al., 1982
M. fascicularis	Thailand	12	31	0.26	0.090	KAWAMOTO et al., 1989
M. sinica	Sri Lanka	8	32	0.23	0.078	SHOTAKE & SANTIAPILLAI, 1982
M. fuscata	Japan	33	32	0.09	0.013	NOZAWA et al., 1982
M. mulatta	Pakistan	5	35	0.14	0.053	MELNICK et al., 1984
Cercopithecus aethiops aethiops	Ethiopia	11	33	0.11	0.030	SHIMADA & SHOTAKE, 1997

DISCUSSION

CHARACTERISTICS OF GENETIC VARIATION

Levels of Variability Within a Population

A comparison of two indices of genetic variation, proportion of polymorphic loci (P_{poly}) and average heterozygosity ($\bar{H}$) for social groups of non-human primates is given in Table 11. Here, the average heterozygosity ($\bar{H}$) was in the range of 1.3−9.0%. In general, West Java populations followed the tendency of island populations to have lower heterozygosity compared to continental populations as discussed by FOODEN and LANYON (1989). Reduced genetic variability in island populations is primarily due to genetic drift and the founder effect. These two factors may have a greater impact on the gene pool of island populations than on continental populations.

Genetic Differentiation Between Local Populations

We defined three hierarchical categories of study population to discuss genetic differentiation of longtail macaques in West Java, namely social groups, local populations, and the total population. Mean proportion of gene diversity between social groups in the same locality was 0.039 (Table 5), and the proportion of gene diversity between local populations was 0.241. With reference to the partition of populations, the proportion of gene diversity for the West Java populations could be summarized as follows; 24.1% of the total gene diversity existed between local populations and 75.9% (=1−0.241) within local populations. Of the total gene diversity within local populations (75.9%), 3.0% (=0.759×0.039) is contributed from the variation between social groups in the same local population and the remaining 72.9% (=75.9%−3.0%) from social groups. The observed low differentiation between social groups in the same local population may result from frequent gene flow by adult males transferring among neighboring social groups. The mean of the effective number of migrants per social group per generation (N_em) according to the estimation from G_{ST} (equation 8.15; SMITH, 1989) is 6.1. The field observations of longtail macaques reported by KOYAMA et al. (1981) and WHEATLEY (1980) have shown that adult males migrate from their natal social group into other social groups. In contrast, the genetic differentiation between local populations coupled with positive correlation between

protein genetic distance and geographic distance ($r=+0.43$; $0.001<p<0.01$) may be caused by limitation of gene flow between local populations (mean $N_em=0.8$).

Population Structure

The present study revealed a panmictic condition within local populations and differentiation between them according to their geographic distribution. These features could be explained by the hierarchical population structure model presented by Nozawa et al. (1982) for Japanese macaques. This model assumes that the species population consists of a number of local populations each of which is composed of several social groups. The adjacent social groups in the same locality are considered to be associated genetically by frequent adult male transfer (Kawanaka, 1973). On the contrary, male dispersion between local populations is considered to be restricted, causing local genetic differentiation. The genetic features found in this study support the idea that the population structure of longtail macaques in Java is principally similar to that of Japanese macaques.

At present, the information about geographic distribution of longtail macaques in West Java is unavailable. As a result, we could not clarify the enviromental factors which come into play in fragmentation of monkey populations. Notwithstanding, in general, population subdivision is considered to be the most conceivable situation which is attributable to a certain habitat fragmentation (Wilson & Wilson, 1975; Supriatna et al., 1996).

Previous discussions have dealt with the genetic structure of a population with respect to the problem of spatial structure. The problem of time is another important aspect. However, little is known about this due to a lack of long-term studies. The results of changes in allele frequencies in two sampling periods (1980–1994) in the Jatibarang and Pangandaran populations revealed that although one case was exceptional (*Pi* in Pangandaran) the changes in allele frequencies were generally within the range of random fluctuation or stochastic change. This means that protein polymorphism in a local population of longtail macaques is stable with respect to time and space.

As reported previously by Kawamoto et al. (1981), the Jatibarang population was characterized by having a low level of genetic variability. This situation may result from isolation from other populations. The changes in allele frequency in the social group of Jatibarang or Pangandaran over 15 years (about two generations) were within the range of random fluctuation and suggest genetic equilibrium in the study group. Although the possibility of some forces of natural selection could not be ruled out, such conditions may result from panmixia in the local population during the 15 year period. In Pangandaran populations the result seems to support admixture in the gene pool of a local population due to frequent gene flow by adult male transfer between social groups within the local population. On the other hand, in the Jatibarang population, restricted gene flow by male transfer from other local populations and small population size may result in genetic equilibrium and low genetic variability.

Differences Between Nuclear and Mitochondrial Data

Contrasting Features in Variations Within and Between Local Populations

Five distinct mtDNA haplotypes were found in the local populations of longtail macaques by PCR-RFLP analysis. Each local population examined has a unique haplotype. A similar situation

has also been found in Japanese macaques (HAYASAKA et al., 1991), rhesus macaques (ZHANG & SHI, 1993), and toque macaques in Polonnaruwa (HOELZER et al., 1994) and also in a PCR-RFLP analysis of pigtail macaques, *Macaca nemestrina* (ROSENBLUM et al., 1997).

The relative magnitude of interlocal differentiation for nuclear DNA (G_{LT}=0.241) was much smaller than that for mtDNA (G_{ST}=1). Extremely great differentiation between local populations in mtDNA was primarily in accordance with expectations from the molecular evolutionary rate of being five to ten times larger than nuclear genes (BROWN et al., 1979). But the scarcity of individual mtDNA variation in local populations could not be explained only by this rate difference. Contrast in mtDNA and nuclear DNA differentiation has been pointed out in a previous study on rhesus macaques (MELNICK & HOELZER, 1992). Two factors were pointed out to give rise to this condition. One is the difference in effective population size. As mtDNA is transmitted maternally without recombination (HUTCHINSON et al., 1974; GILES et al., 1980; OHNO, 1997), its effective population size is one fourth that in nuclear DNA. This finally leads to a higher degree of genetic drift (BIRKY et al., 1989), as well as high fixation rate as a selectively neutral marker (BROWN et al., 1979). Consequently, reduction of variability becomes larger in mitochondrial DNA than in nuclear genes.

The other factor is the nature of female philopatry in the social organization of macaque species. With respect to population structure, the features of female philopatry and group division (lineage sorting) have a prominent role in causing this contrast (HAYES & HARRISON, 1992). In macaques, male migrants can contribute to the shuffling of the nuclear gene pool but not of the mitochondrial gene pool. This gender specific migration pattern in macaque species contrasts to that in humans (MELNICK & HOELZER, 1992).

Correlation of Local Genetic Differentiation Between Nuclear and Mitochondrial Genes

Since we examined either uniparentally (mtDNA) or biparentally (nuclear) inherited markers from the same individuals, the obtained data allows us to see the relation of their geographic differentiation patterns. Despite significantly positive correlations between geographic distance and both protein genetic distance and mitochondrial sequence divergence, the correlation between the two genetic distance measures was not obvious ($r=+0.23$; $0.1<p<0.2$; number of combination=36). This inconsistency may result from the mechanisms that rule out the geographic nature of genetic differentiation. In nuclear markers such as proteins, restricted gene flow among subpopulations or isolation by distance give rise to the positive correlation. However, gene flow by males has no contribution to correlation of mtDNA differentiation. This seems to be the case for longtail macaques. Females typically remain at their natal group and historical splitting of maternal lineages by group division seems to be a major cause of spatial differentiation of the genetic marker in this species. Whether this explanation is reasonable or not can be tested by adding more population samples in future studies.

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Appendix. Restriction morph and fragment size estimates (in base pairs) of all fragment patterns observed in mitochondrial DNA containing the D-loop region.

RE	Morph	Fragment size (bp)								Total
*Hae*III	A	675	575	420	120	60				1850
*Hin*fI	A	1080	480	290						1850
	B	1080	770							1850
*Mbo*II	A	1350	500							1850
*Msp*I	A^+	1210	560	45	45					1860*
	A	1210	550	45	45					1850
	B	1210	470	90	45	45				1860*
*Sau*3AI	A	680	510	375	255	30				1850
	B	510	375	350	255	250	80	30		1850
	C	510	375	330	320	255	30	30		1850
	D	510	330	320	255	220	155	30	30	1850

*We detected electrophoretically 10 bp difference in total fragment size. For calibration, we assumed morph A and A^+ shared the same restriction sites. Calibration unit: for large fragment: 10 bp (1000 bp<), for small fragment: 5 bp (<1000 bp).

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