

Microsatellite DNA Polymorphisms for Colony Management of Long-tailed Macaques (*Macaca fascicularis*) Population on the Tinjil Island

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ABSTRACT

Polymorphic genetic markers are the basic requirement for studies on population and conservation genetics of non-human primates. In this paper, we screened microsatellites for their polymorphism and gene typing of DNA samples from blood of wild long-tailed macaques (*Macaca fascicularis*) from Tinjil Island population. Among the three primer sets tested, two are polymorphic. They were D1S548 and D3S1768. Average observed heterozygosity (H) within populations ranged between 0.264-0.555. D1S1768 locus was highly polymorphic and 24 alleles were detected among two loci. Estimation of genetic variability for the Tinjil population (H) was 0.485. The results obtained provide further insight into the long-term viability of the population and help in creating genetic management of both captive and natural habitat breeding colonies of primates.

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Key words: microsatellite, variations, social groups, long-tailed macaques, *Macaca fascicularis*.

INTRODUCTION

Microsatellite sequences are valuable genetic markers due to their dense distribution in the genome, great variation, co-dominant inheritance and easy genotyping. In recent years, they have been extensively used in parentage testing, linkage analyses, population genetics and other genetic studies (Goldstein and Pollock, 1997). They are very useful to analyze the degree and pattern of genetic variability within and between populations. Their variation is mainly explained by factors such as genetic drift, gene flow and mutation, since they are generally considered non-selective markers (Pérez-Lezaun et al., 1997). Although they show some limitations in the analyses of phylogenetically distant organisms, due to their irregular mutation processes involving range constraints and asymmetries (Nauta and Weissing, 1996), they have proven very useful in intra-species population studies.

At present, many PCR based primers designed for one species are applicable to closely related species.

For non-human primates in particular, cross species amplification has benefited greatly by the human genome project that cloned far more microsatellite in human than in any other living organisms (Morin et al., 1997; Nurnberg et al., 1998). One can assign sizes to tetranucleotide alleles more reliably than dinucleotide alleles using a variety of instruments. To accomplish maximal genetic information at the lowest possible costs, the loci included on the test should also exhibit high estimates of gene diversity. These markers also enable one to make unique genetic characteristics.

Tinjil Island is located off the south coast of west Java (Banten Province), Indonesia. The island, approximately 600 ha in size, was established as a natural habitat breeding facility (NHBF) for simian retrovirus (SRV)-free long-tailed macaques (Kyes, 1993). Between 1988 and 1994, 520 adult macaques (from sites in West Java and South Sumatra; 58 ♂ and 462 ♀) were released onto the island to establish a free-ranging breeding population (Kyes et al., 1998). Over the past few years, an additional 83 macaques (3 ♂ and 80 ♀) have been released to introduce new genetic stock. In 2007 survey data indicated a population of approximately 2000 individuals on the island. The Primate Research Center at Bogor Agricultural University has maintained a long-term study of the socio-ecology and serology of the Tinjil macaques.

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The present study aimed to generate important baseline information on microsatellite DNAs for genetic management of both captive and natural habitat breeding colonies of primates. We hope to contribute to the development of a standardized genetic management strategy for breeding colonies using well-characterized microsatellite markers.

MATERIALS AND METHODS

During 2005 a total 55 blood samples were collected from long-tailed macaques in seven different social groups on Tinjil Island, Indonesia namely Buntung (n=6), Jambul (n=13), Jebag (n=4), Mata Beruk (n=6), Ranca (n=7), Sipit (n=13), and Topeng (n=6). Genomic DNA was extracted from buffy coat using the modified method of Kan et al. (1977).

Amplification of microsatellite

The 55 macaque samples were screened for each of the three tetranucleotide repeats. All these loci are located on different chromosomes. The loci were selected based on the results of a preliminary study on long-tailed and pig-tailed macaques (Perwitasari-Farajallah et al. 2004, Perwitasari-Farajallah 2007) and research on rhesus macaques, *M. mulatta* (Smith et al., 2000). PCR amplification of three human microsatellite loci was carried out in 12.5 µL volumes with a reaction mix containing 25 mM MgCl₂, 0.83 U Taq polymerase and its buffer (PROMEGA), 2.5 mM dNTP, and 25pM of each forward and reverse primers (Table 1).

Amplifications were performed in a GeneAmp PCR System 9600 thermocycler (Applied Biosystems) using the following cycling parameters: 30 cycles of 40 s denaturation at 94°C, 40-60s primer annealing at 48-57°C, and 5 min final extension at 72°C.

All PCR products were separated on a 5% polyacrylamide gel and silver-stained following the technique described by Tegelström (1986). Allele sizes were determined using DNA size standard of 20 bp ladder (BioRad).

Data analyses

Allele frequencies were calculated by direct count. The amount of variation was measured by average heterozygosity (H_o : observed heterozygosity).

Estimates of gene diversity were obtained according to Nei (1987) equation as follows:

$$\bar{H} = 1 - \frac{\sum_{i=1}^m X_i^2}{m}$$

X_i is the frequency of the i -th allele, m is the number of alleles and average proportion of heterozygosity (\bar{H}) is the average over all loci.

Relative proportion of gene diversity between social groups (D_{ST}), within social groups (H_{ST}), and total gene diversity (H_T) were calculated using Hartl and Clark (1997) equations. Genetic distance (D) was estimated by applying the equation of Nei (1987).

RESULTS AND DISCUSSION

We were able to reliably amplify DNA from all 55 samples. Of three loci screened, two loci revealed polymorphisms. They were D1S548 and D3S1768. D3S1768 locus was highly polymorphic and 24 alleles were detected. D1S548 and D3S1768 were found to be polymorphic in rhesus macaques, *M. mulatta* (Kanthaswamy et al., 2006). Additionally the results obtained by Chu et al. (1999) in Taiwanese macaques, *M. cyclopis*, Nurnberg et al. (1998) in rhesus macaques and Goossens et al. (2000) in chimpanzees, *Pan troglodytes troglodytes* demonstrated that D1S548 locus was polymorphic. The present study indicated that D2S1777 was monomorphic as observed in our previous study (Perwitasari-Farajallah et al., 2004). In contrast Perwitasari-Farajallah (2007) and Ely et al. (1998) demonstrated that this locus was polymorphic in pigtailed macaques (*M. nemestrina*) and chimpanzees (*P. troglodytes troglodytes*) respectively. The present results indicate that these two loci are useful for assessing genetic variability in longtailed macaques.

Overall average heterozygosity within social groups ranged between 0.26 for Topeng to 0.56 for Sipit (Table 2). An estimate of genetic variability (\bar{H}) for the Tinjil population was 0.485.

In comparison with previous studies in long-tailed and pig-tailed macaques using D1S548, D2S1777, D3S1768, and D5S820 loci with average heterozygosities (\bar{H}) 0.5796 and 0.6141, respectively

Table 1. Description of chromosomal location, GenBank Accessions Numbers, and sequences of each primer

Locus	<i>M. mulatta</i> chromosome	GenBank accession number	Repeat motif	Primer sequences (5'→3')	Size range (bp) in human
D1S548	1	GDB: 228890	tetra	F: GAACTCATTGGCAAAGGAA R: GCCTCTTTGTTGCAGTGATT	212
D2S1777	-	GDB: 693873	tetra	F: TCCCCAAGTAAAGCATTGAG R: GTATGTAGGTAGGGAGGCAGG	242
D3S1768	2	GDB: 228929	tetra	F: GGTGCTGCCAAGATTAGA R: CACTGTGATTGCTGTTGGA	197

Note: F: forward; R: reverse

(Perwitasari-Farajallah et al., 2004; Perwitasari-Farajallah, 2007), the present study revealed slightly low genetic variability. The data however suggest high genetic diversity if the Topeng social group is excluded from the analyses. In fact, Topeng social group could be classified as an outlier, at present however we have no detail information including behaviour observation to explain the low genetic diversity of Topeng social group. Additionally protein analyses using three protein loci Transferrin (*Tf*), Thyroxine binding pre-albumin (*TBPA*) and Albumin (*Alb*) performed in the same group revealed an average heterozygosity (\bar{H}) of 0.23 (Perwitasari-Farajallah, unpublished data). Although only two loci showed polymorphisms (*Tf* and *TBPA*), it has been showed other in macaque population genetics studies that *Tf* is the most polymorphic locus (Kondo et al., 1993; Kawamoto et al., 1984; Perwitasari-Farajallah et al., 1999; Perwitasari-Farajallah et al., 2001; Kawamoto et al., 2008), and initially it can be applicable for assessment of allelic diversity.

Table 2. Estimates of genetic variability within groups.

Social group	n	$\bar{H} \pm SE$
1. Buntung	6	0.54 ± 0.27
2. Jambul	13	0.55 ± 0.28
3. Jebag	4	0.50 ± 0.25
4. Mata Beruk	6	0.53 ± 0.26
5. Ranca	7	0.46 ± 0.23
6. Sipit	13	0.56 ± 0.28
7. Topeng	6	0.26 ± 0.13

The total gene diversity ($H_T = 0.583$) of the seven social groups can be distributed into intra- ($H_S = 0.485$) and inter- ($D_{ST} = H_T - H_S = 0.098$) social groups gene diversity. G_{ST} ($= D_{ST}/H_T$) was 0.167, it represented that 17% of the total gene diversity was attributed to differences between social groups. This result suggested that low differentiation between social groups may result from frequent gene flow by adult male migration among neighboring social groups (Koyama et al., 1981; de Ruiter and Geffen, 1998; Perwitasari-Farajallah et al., 1999). Males leave their natal group typically before sexual maturity and may change social groups many times during their lifetime (de Ruiter and Geffen, 1998). Changing social groups is associated with high variation in male reproductive success (de Ruiter et al., 1992; Keane et al., 1997) and even mortality (Dittus, 1975). This behavior serves as a guard against inbreeding and homogenizes the distribution of genetic variation in the nuclear genome within a deme (Melnick and Hoelzer, 1992, 1996). Upon the obtained results further studies were essential due to lack of samples on the present study.

The value of the absolute amount of genetic diversity between social groups was calculated by Nei's standard genetic distance (Nei, 1987) as given in Table 3.

Table 3. Nei's genetic distance (D) and genetic similarity (I) between social groups.

	1	2	3	4	5	6	7
1		0.187	0.258	0.196	0.151	0.104	0.167
2	0.829		0.219	0.226	0.183	0.085	0.485
3	0.773	0.803		0.291	0.259	0.211	0.558
4	0.822	0.798	0.748		0.287	0.107	0.332
5	0.860	0.832	0.771	0.750		0.172	0.345
6	0.901	0.919	0.810	0.899	0.841		0.397
7	0.846	0.616	0.572	0.718	0.708	0.672	

Note: above diagonal: Nei's genetic distance (D); below diagonal: genetic similarity; 1-7: social groups as indicated in Table 3.

The largest distance was found between Jebag and Topeng (average of $D = 0.558$), while Jambul and Sipit revealed the smallest genetic distance (average $D = 0.085$). Genetic differentiation between adjacent groups estimated by Nei's standard genetic distance in general was less than those between non-adjacent groups (Perwitasari-Farajallah et al., 1999).

CONCLUSIONS

Microsatellite loci which were cloned from the human genome can provide informative genetic markers for studying population structure and genetic differentiation of long-tailed macaques, *Macaca fascicularis*. D1S548 and D3S1768 were polymorphic loci, and can be applied for estimating allelic diversity.

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