Genetic Structure in Long Tailed Macaque Populations in The Region of East Java: Diversity of Mithochondrial DNA in Alas Purwo and Baluran Population

Struktur Genetik Populasi Monyet Ekor Panjang di Kawasan Jawa Timur: Diversitas DNA Mitokondria pada Populasi Alas Purwo dan Baluran

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ABSTRAK

Monyet ekor panjang (*Macaca fascicularis*) telah menyebar secara luas hampir di seluruh kawasan di Indonesia, namun, kini, keberadaannya terpisah-pisah menjadi populasi lokal di dalam suatu pulau. Penelitian ini ditujukan untuk mengeksplorasi diversitas genetik populasi lokal monyet ekor panjang yang menempati Kawasan Jawa Timur. Sebagai penelitian pendahuluan, diversitas genetik populasi dikaji dengan marka molekuer *d-loop region* mtDNA menggunakan sepasang primer khusus. Sejumlah 29 sampel darah (15 dari populasi Alas Purwo dan 14 dari populasi Baluran) diekstraksi untuk mendapatkan DNA total. *d-loop region* mtDNA diamplifikasi melalui teknik PCR. Produk PCR disekuen dengan metode *dideoxynucleotide chain-termination* pada Macrogen Inc. Korea. Hasil penelitian menunjukkan bahwa sejumlah 6 haplotipe ditemukan dengan sebaran 3 jenis haplotipe di masing-masing populasi. Diversitas haplotipe berkisar 0,257 – 0,275 dan diversitas nukleotida sebesar 0,0012 – 0,0025. Diferensiasi genetik antar populasi adalah F_{ST} = 44,1%. Rekonstruksi filogeni menunjukkan bahwa seluruh haplotipe/individu membentuk dua kelompok (*haplogroup*) yang berbeda dan tidak ditemukan adanya *shared* haplotipe antar populasi. Hasil penelitian dapat disimpulkan bahwa kedua populasi (Alas Purwo dan Baluran) bersifat unik dan memiliki diversitas genetik rendah.

Kata kunci: monyet ekor panjang, diversitas genetik, *d-loop region***,** mtDNA

ABSTRACT

Long tailed macaque (*Macaca fascicularis*) has distributed throughout the regions in Indonesia, however, they, now, live separately to many local populations within an island. This research aimed to explore the genetic diversity within and between populations of long tailed macaque in the Region of East Java. The genetic diversity was examined by molecular marker of d-loop region mtDNA using a pair of specific primers. A total of 29 blood samples (15 samples from Alas Purwo population and 14 samples from Baluran population) was extracted to find out a total DNA. The d-loop region mtDNA was amplified through PCR tecnique. The PCR products were sequenced using dideoxynucleotide chain termination method at Macogen Inc. Korea. The research found 6 haplotypes, in which 3 haplotypes were found in each population. Haplotype and nucleotide diversities varied from 0.257 to 0.275 and 0.0012 to 0.0025 repectively. Genetic differentiation between populatioon was F_{ST}=44.1%. Phylogenetic reconstruction showed that all haplotypes constructed two different groups (haplogroups) and there was no shared haplotype betwen populations. It can be concluded that both populations (Alas Purwo and Baluran) are uique and have low genetic diversities.

Key words: long tailed macaque, genetic diversity, d-loop region, mtDNA

INTRODUCTION

Long tailed macaque (*Macaca fascicularis*) lives in female phylipatric social groups (Jolly, 1985). The females are being a permanent member in a social group as long as they live in thier natal groups, in contrary, the males will go out from the natal group and migrate to others (Napier & Napier, 1985; Mitchell & Erwin, 1986). The social group of

long tailed macaques belongs to multimales-multifemales group, ie, many adult males and adult females and thier offsprings in a group (Napier & Napier, 1985; Mitchell & Erwin 1986; Bennett *et al*., 1995), and it is also frequent that many subgroups were found within a group (Rowe, 1996).

A high adaptibility of the long tailed macaques (Napier & Napier, 1985) makes them spread broadly in the world (Mitchell & Erwin, 1986; Bennett *et al*., 1995). Their ability to conquer a new habitat is also supported by thier ability to change the strategy of resource utilisation for life (Brotcorne *et al*., 2014). In Indonesia, this macaques are found at almost islands in South Archipelago of Indonesia (Fooden, 1995).

Primatologist believes that the long tailed macaques in South Archipelago of Indonesia migrate from west to east, in which the Java as the origin of population. Migration of this animal from Java to Bali Island occurred many times when Java-Bali uniting in Sunda Shelf (Eudey, 1980; Fooden, 1995). To day, the long tailed macaques live in local poplation with limited habitat (Kawamoto *et al.,* 1984; Wandia, 2000; Wandia *et al.,* 2004). In such condition, it is important to examine the genetic diversity within and between populations for getting a genenically consideration in making conservation strategy for the future life.

Macaque populations in the Region of East Java play an important role in the history of their distributions in South Archipelago of Indonesia. The pulation in Alas Purwo National Park and Baluran National Park dwell the most closely to Bali Island, so these populations have a high potency to migrate to Bali Island. Exploring the geneic diversity of the populations and comparing them to those of Bali populations can enlighten the distribution history of the macaques. This preliminary research explored the genetic diversity of long tailed macaque populations at Alas Purwo National Park and Baluran National Park, in which the population genetic diversities were examined using a d-loop region of mitochondrial DNA (mtDNA) as molecular marker.

MATERIALS AND METHODS

Blood Samples

A total of 29 blood samples was collected from two populations namely 15 samples from Alas Purwo National Park and 14 samples from Baluran National Park. The blood samples were taken after the macaques were anesthetized with Ketamine (dose 10 mg/kg body weight) combined Xylazine at ratio 5:1 using blow pipe. A 5-10 ml blood was taken through femoral vein using 10 ml syringe that formerly added by 0.4 ml 10% EDTA as anticoagulant. All the blood samples have been collected in 2007 and deposed at Genetic and Tissue Culture Laboratory, Primate Research Center-Udayana Universiy.

Total DNA Extraction and D-Loop Region mtDNA Amplification

Total DNA was extracted using QIAmp DNA Blood Kits produced by Qiagen. The steps of extraction is in accordant to company protocol (Qiagen, 2007). The d-loop region mtDNA was amplified by PCR using a pair of specific sequence of primers, namely 5'-ATCACGGGTCTATCACCCTA-3' (F) dan 5'-GGCCAGGACCAAGCCTATTT-3' (R) The volume of the PCR unit was 50 µL which contain 1x PCR buffer, 3 mM MgCl2, 0.2 mM dNTP (mix), 0.2 µM each primer, and 0.625 U DNA polymerase, and 2 µL DNA template. The PCR was carried out in Applied Biosystems 2720 Thermal Cycler, repeated by 40 cycles with 58^{O}C of annealing temperature. The PCR products was electrophoresed at 50 V in 1.5% agarose gel for 30 minutes. The fragment was developed by ethidium bromide and its length was compared to 100 bp ladder standard marker. The positive PCR products were, then, sequenced with dideoxynucleotide chain termination method at the public company of Macrogen Inc. Korea.

Data Analysis

Editing and aligning the nucleotide sequences used MEGA 6 software (Tamura *et al*., 2013). Haplotype and its ditribution were

analyzed using dnaSP ver. 5.10.01 software (Rozas *et al*., 2010). Genetic diversities within population (at the level of nucleotide and haplotype) and the genetic distance (F_{ST}) were calculated with Arlequin ver 3.5.1.2 software (Excoffier *et al*., 2005). MEGA 6 software (Tamura *et al*., 2013) was also used to reconstruct a phylogenetic tree with the substitusion model of Kimura 2 parameters and the statistic mehtod of maximum likelihood.

RESULTS AND DISCUSSION

Results

Haplotype Distribution

Analysis of 29 samples of d-loop region mtDNA resulted 6 haplotypes (Table 1). In general, haplotype length was 574 nucleotides which composed of 31.22% (A), 23.76% (T), 33.76% (C), and 11.27% (G). There were 16 variable sites across haplotypes, in which haplotype hap_ap1 had 2 nucleotides deletion (Table 2). Totally, transition/tranversion mutation ratio was R=0.51.

There was no cosmopolitan haplotype. Three unique haplotypes were found in each population (Table 2). Alas Purwo population was dominated by haplotype hap_ap2 (wild type), on the other hand, Baluran population was dominated by haplotype hap_bl1 (wild type).

Table 1. Haplotypes of long tailed macaques in The Region of East Java

No	Haplotype	Nucleotide sequences*															
1	hap_ap1	T	C	A	T	G	A	G	G	-	A	-	T	C	T	A	G
2	hap_ap2	.	.	.	C	.	.	.	.	A	.	A	.	A	.	.	T
3	hap_ap3	.	A	.	C	.	.	.	.	A	.	A	.	A	.	G	T
4	hap_bl1	.	.	.	C	.	G	.	.	A	.	A	.	A	.	.	T
5	hap_bl2	.	.	T	C	A	C	A	C	A	C	A	C	A	A	.	A
6	hap_bl3	G	.	.	C	.	G	.	.	A	.	A	.	A	.	.	T

*Nucleotides provided are the variable nucleotides (16 nucleotides) that differentiate the haplotypes. The number of variable sites is not showed. Haplotypes are named arbitrarily.

Genetic Diversities Within Population

Haplotype diversities (hd) and nucleotide diversities (π) are figured on Table 3. The haplotype diversities express the probability of two haplotypes to be differ at a time of consent, and the nucleotide diversities imply the proportion of nucleotide differentiation between two sequences (Kawamot *et al*., 2013). Alas Purwo population and Baluran population had hd and π relatively low.

Table 2. Distribution of Haplotypes within Populations

No	Haplotipe	Distribution	
		Alas Purwo (n=15)	Baluran (n=14)
1	hap_ap1	0.07	
2	hap_ap2	0.86	
3	hap_ap3	0.07	
4	hap_bl1		0.86
5	hap_bl2		0.07
6	hap_bl3		0.07

Table 3. Genetic Diversities within Populations

No	Parameter	Alas Purwo	Baluran
1	Number of sample	15	14
2	Number of haplotype	3	3
3	Number of polymorphic sites	5	10
4	Haplotype diversity (hd)	0.257 (0.142)	0.275 (0.148)
5	Number of nucleotide differentiation between haplotypes	0.667 (0.541)	1.429 (0.926)
6	Nuclotide diversity (π)	0.0012 (0.0010)	0.0025 (0.0018)

Number in bracket: standard deviation

Genetic Distance and Phylogenetic Tree

The genetic distance was calculated by F_{ST} which is good for figuring out the short divergent time of two populatons (Excoffier *et al*., 2005). The genetic distance between Alas

Puwo population and Baluran population was F_{ST}=0.441 (P=0.00..; 110 permutations). The genetic distance between populations was very significant. Phylogenetic recunstruction depicted that all haplotypes composed 2 groups (haplogroups), arbitrarily called Alas Purwo clade and Baluran clade. Each haplogroup had a unique haplotype (Figure 1).

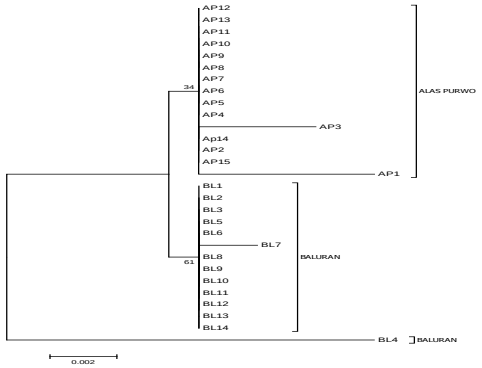

Figure 1. Phylogenetic reconstruction of Long Tailed Macaques in the Region of East Java. AP Alas Purwo, BL Baluran

Discussion

The long tailed macaque populations are very cohesive (Kawamota *et al*., 1984; Evans *et al*., 2010). Likewise, the long tailed macaque populations in the Region of East Java are very cohesive. This bonding is demonstrated by no cosmopolitan haplotype or no shared haplotype between population. Female migration is not supported by the data found, or if there is a migration between populations it would be very small number per generation. The present study showed that each population had a different wild type of haplotype. The haplotype hap_ap2 and hap_bl1 were the wild type haplotypes of Alas Purwo population and Baluran population consecutively. This finding strengthens the two populations develop an evolutionary history of live independently. The uniqueness of haplotype especially the most common haplotype can be used for a haplotype marker of population identity. Such population can be rendered in a different management unit to maintain its uniqueness of the genetic variation within population (Frankham *et al*., 2004).

Mitochondrial DNA (mtDNA) is a strictly maternally inherited molecular marker that frequently used to examine the genetic variation within population because of having a high mutation rate (Wallace *et al*., 1999; Dimauro & Davidzon, 2005; Evans *et al*., 2010). However, variation resources of mtDNA one fourth of the nuclear genome make a variant of mtDNA will reach a fixation state quicker than those of the nuclear genome (Perwitasari-Farajallah *et al*., 1999). The present study showed that Alas Purwo population and Baluran population had hd and π relatively low. These may associated to a small number of different haplotypes found in each population and also a small number of nucleotide differentation between sequences within population. Comparing to those, hd and π of 7 bonobo popuations in Congo were 0.694 – 0.980 and 0.0166 – 0.0256 consecutively (Kawamoto *et al*., 2013). The low genetic diversities within population found in present study may reflect a small or even no gene flow between populations. In this case, although mtDNA has a high mutation rate, on the other hand, it has one fourth of resource of variation and there is no female migration between popuations showed by the data, as the final result, the population will have a low genetic variation. Many other factors can attribute to that condition, these are a bottle neck effect in the past, a founder effect, a selection, and a genetic drift (Avise, 1994; Nozawa *et al*., 1996; Hartl & Clark, 1997; Wallace *et al*., 1999). A further study is needed to better understand factors that associate to a low genetic diversities in long talied macaque populatios in the Region of East Java.

A gene flow which is expressed by migration of indivudual inter populations is not only a new genetic resource within a population but also it homogenizes the genetic variation between populations (Nozawa *et al*., 1996; Hartl & Clark, 1997). The genetic distance between Alas Puwo population and Baluran population was F_{ST}=0.441, in which this genetic distance was very significant

(P=0.00..; 110 permutations). This fact indicates that there is a very small or even no gene flow between populations. Each population looks like having its own evolutionary process. This fenomenon was also found at long tailed populations in Bali Island (Wandia *et al*., 2014). The phylogenetic reconstruction also supports this event that all haplotypes were splitted to two defferent groups (haplogroups) without shared haplotype between populations (Figure 1).

CONCLUSION

Genetic Structure of long tailed macaque populaton in Alas Purwo and Baluran based on d-loop region mtDNA was unique. Haplotype diversity within population was low and genetic differentiation between populations was high.

ACKNOWLEDGEMENT

Thank to our president of Udayana University who has been funding this researh through the Contract Number: 104.16/UN14.2/PNL.01.03.00/2014, March 5, 2014.

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