Polymorphism of Microsatellite Loci on Y Chromosome in Long-Tailed Macaque Populations in Bali Island, Indonesia

Polimorfisme Lokus Mikrosatelit Kromosom Y pada Populasi Monyet Ekor Panjang di Pulau Bali, Indonesia

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ABSTRAK

Monyet ekor panjang tersebar di seluruh wilayah di Pulau Bali, tetapi, kini keberadaannya telah terpisah-pisah menjadi populasi lokal yang lebih kecil. Walupun demikian, laporan yang melukiskan modus fragmentasi dan struktur genetik populasi belum banyak ditemukan. Penelitian ini bertujuan untuk mengeksplorasi polimorfisme lokus mikrosatellite kromosom Y pada populasi monyet ekor panjang di Pulau Bali dengan menggunanakan tiga primer mikrosatelit manusia, yaitu DYS390, DYS391, dan DYS393. Sejumlah 99 sampel darah dikoleksi dari monyet ekor panjang jantan yang berasal dari 8 populasi (23 dari Pulaki, 11 dari Bedugul, 13 dari Mekori, 8 dari Sangeh, 6 dari Uluwatu, 11 dari Alas Kedaton, 11 dari Ubud, dan 16 dari Bukit Gumang). DNA total diekstraksi dengan QIAamp® Blood Mini Kit. Polimorfisme lokus mikrosatelit dideteksi menggunkan teknik PCR, dan variasi alel dipisahkan melalui elektroforesis pada 7% gel poliakrilamid. Hasil penelitian menunjukkan bahwa lokus DYS390 dan DYS393 adalah monomorfik (masing-masing memiliki satu alel). Lokus DYS391 mempunyai dua alel, karena itu, lokus ini adalah polimorfik. Dari hasil penelitian dapat disarankan bahwa lokus DYS391 dapat digunakan untuk studi variasi genetik populasi monyet ekor panjang jantan di Pulau Bali.

Kata kunci: monyet ekor panjang, polimorfisme, lokus mikrosatelit, kromosom Y, Pulau Bali

ABSTRACT

The long tailed macaques inhabit throughout Bali Island, however, recently they have been fragmented into many smaller local populations. In spite of that, there are just few reports that describe the mode of fragmentation and the genetic structure of the population. This research aimed to explore the polymorphism of microsatellite loci on Y chromosome in long tailed macaque populations in Bali Island using three human microsatellite primers, namely DYS390, DYS391, and DYS393. A total of 99 blood samples were collected from male long tailed macaques originated from 8 populations (23 from Pulaki, 11 from Bedugul, 13 from Mekori, 8 from Sangeh, 6 from Uluwatu, 11 from Alas Kedaton, 11 from Ubud, and 16 from Bukit Gumang). Total DNA was extracted using QIAamp® Blood Mini Kit. The polymorphism of the microsatellite loci was detected using PCR technique, and allelic variations were separated through 7% poly acrylamide gel electrophoresis. The result of the research showed that DYS390 and

DYS393 loci were monomorphic (each had only one allele). The DYS391 locus had two alleles, therefore, this locus was polymorphic. It could be suggested that DYS391 locus could be used to study genetic variation in male long tailed macaque population in Bali Island.

Key words: long tailed macaques, polymorphism, microsatellite locus, Y chromosome, Bali Island

INTRODUCTION

Long tailed macaque (*Macaca fascicularis*) is a nonhuman primate that has distributed worldwide (Euday, 1980; Fooden, 1980; Napier and Napier, 1985). Nearly all islands of Indonesia have been dwelling by the macaques, and because of their high adaptation potency, the animals can live in various habitats (Napier-Napier, 1985; Fooden, 1995; Rowe, 1996). In the island of Bali, the macaques have dispersed almost the regions and taken place in primary and secondary forests, riverine, and coastal area (Southern, 2002).

The long tailed macaque is a female philopatry i.e. live in social group in which females remain and breed in the group of their birth. The females compose a nuclear members of the social group (Joly, 1985; Napier-Napier, 1985; Perwitasari-Farajallah *et al*., 2004). Different from the female, male macaques will not stay firmly in natal group, but migrate to other group during their growing up period. The male migration would be a genetic bridge for entering the source of genetic variation to the new population or group. This gene flow homogenizes the genetic variation inter population (Nozawa *et al*., 1996; Hartl & Clark, 1997; Li, 1997). However, the male migration some time gives a negative effect, that is, it intruduces a pathogen agent to the new population or group in which the pathogen agent can cause an illness or contagious disease (Wandia, 2003).

Many tourist destinations in Bali Island are also a habitat of the long tailed macaques like Sangeh, Ubud, Alas Kedaton, Uluwatu, Pulaki, and Bedugul. The macaques in such location give a positive impact, so that, the locations have been known as the monkey tourist destination. Many non genetic researches have been carried out in these long tailed macaques (Loudon *et al*., 2006; Lissa *et al*., 2008; Lane *et al*., 2010;, Schilaci *et al*., 2010; Brotcorne *et al*., 2011; Fuentes *et al*., 2011; Suarjana & Asmara, 2012). The research about genetic variation in long tailed macaque population in Bali has been conducted by Kawamoto *et al*. (1994) using blood protein marker. Other research i.e. characterization of microsatellite locus on somatic

chromosome has been carried out by Wandia (2003, 2004a, 2004b), and Rell *et al.* (2013). However, there was no study that exploring the genetic variation using molecular marker on Y chromosome of the long tailed macaques. This research aimed to characterize the microsatellite loci on Y chromosome of long tailed macaque populations in the Island of Bali, Indonesia. The polymorphic information of loci is very important before using them as molecular marker for assessing the genetic variation within and between populations.

MATERIALS AND METHOD

A total of ninety nine male long tailed macaque blood samples was subjected for DNA extraction. The blood samples were collected in the year 2010-2011 by research team of Primate Research Center Udayana University. The macaques were anaesthetized with the combination of Ketamin HCl (dose 10 mg/kg body weight) and Xylazin with ratio 5:1, and the drug was injected using blow pipe. These samples originated from eight local populations in Bali Island, namely Sangeh 8 samples, Uluwatu 6 samples, Alas Kedaton 11 samples, Ubud 11 samples, Bukit Gumang 16 samples, Pulaki 23 samples, Bedugul 11 samples, and Mekori 13 samples.

The total DNA was extracted using *QIAamp® DNA Blood Kits* according to company protocol (Qiagen, 2007). Three microsatellite loci on Y chromosome were examined their polymorphism using human microsatellite primer (DYS390, DYS391 and DYS393). The loci were amplified by polymerase chain reaction (PCR) tecnique, and each reaction contained 4 mM $MgCl_2$; dNTPs each 0.16 mM; a pair of primers each 0.4 mM; 0.7 U DNA Polymerase; 1.25 μl PCR *buffer* (10x), and 1 μl template DNA. Pure water was added to make the final volume of reaction 12.5 μl (Hillis *et al*., 1996). PCR for DYS390 locus was: Pre PCR: denaturation (94^o C) for 3 minutes; PCR: denaturation (94^o C) 35 seconds, annealing (50^o C) 35 seconds, and elongation (72^o C) 35 seconds; post PCR: elongation (72^o C) 5 minutes. PCR was replicated 30 cycles using Applied Biosystems 2720 Thermal Cycler. The PCR for DYS391 and DYS393 was the same as those of DYS390 with the exception of annealing temperature at 54^o C. The PCR product was separated by 7% PAGE in 1x TBE buffer and run at 125 Volt for 90 minutes. The allelic variation was developed using silver staining, and the length of allele was measured by aligning the allelic band with the bands of 100 base pairs ladder of

molecular marker. The polymorphism of loci was analyzed using Alrequin Ver 3.5 soft ware (Excoffier & Lischer, 2010**).**

RESULTS AND DISCCUSIONS

Results

Distribution of alleles and haplotypes

The results showed that two alleles were found in DYS391 locus which the length of the alleles was 282 base pairs and 292 base pairs. This locus was polymorphic, however, the two alleles were distributed oddly. The allele 292 ($DYS391^{292}$) was found in all populations, in contrary, the allele 282 ($DYS391^{282}$) was just found in population of Pulaki. Two other loci (DYS390 and DYS393) were monomorphic as long as these loci just had one allele (Table 1).

Table 1. Allele Distribution of the Microsatellite Loci on Y Chromosome of Long Tailed Macaque Populations in Bali Island

No	Loci	Allele	Relative Frequencies of allele in sampling site								Total
			PL n=23	BD n=11	MK n=13	SG n=8	AK n=11	UW n=6	UB n=11	BG n=16	Bali n=99
1	DYS390	$DYS390^{240}$	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2	DYS391	$DYS391^{292}$	0.91	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98
		$DYS391^{282}$	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
3	DYS393	$DYS393^{272}$	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Mapping of the three loci in one individual of Y chromosome will compose the haplotype. Genetic analysis showed that there were two kinds of haplotype, in which, one of them was only distributed to population of Pulaki (Table 2).

Table 2. Haplotype distribution of Y Chromosome of Long Tailed Macaque Populations in Bali Island

Haplotype	hp composition	Relative frequencies of haplotype in population								Total
		PL n=23	BD n=11	MK n=13	SG n=8	AK n=11	UW n=6	UB n=11	BG n=16	Bali n=99
1	$hp^{240:292;272}$	0.91	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98
2	$hp^{240;282;272}$	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02

Locus and Haplotype Diversities of Y Chromosome of Long Tailed Macaque Populations in Bali Island

Locus and haplotype diversities of Y chromosome of long tailed macaque populations were very low, or even zero in most populations (Table 3). It has some thing to do with the monomorphic condition of the loci. Polymorphic condition of DYS391 locus in population of Pulaki has contributed to the value of haplotype diversity (hd=0.17).

Tabel 3 Locus and Haplotype Diversities of Y Chromosome of Long Tailed Macaque Populations in Bali Island

Source	Diversity in populations								Total
	PL n=23	BD n=11	MK n=13	SG n=8	AK n=11	UW n=6	UB n=11	BG n=16	Bali n=99
DYS390 locus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
DYS391 locus	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04
DYS393 locus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Haplotype (hd)	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04

Disccusions

A Locus polymorphism level is supplied by the number of genetic variation sources in a population. The variation of mtDNA genetic marker comes from one source, mtDNA of the female (Walace *et al*., 1999; Ingman *et al*., 2000; Bowels *et al*., 2007), likewise, the variation of a genetic marker of Y chromosome just comes from Y chromosome beared by the male (Evans *et al*., 2010; Rovie-Rian *et al*., 2013). As long as a genetic marker of Y chromosome does not undergo a recombination during replication and due to the effective number of the variation source is a fourth apart of those of the somatic chromosome, this genetic marker will reach a fixation in shorter evolutionary time in a population compare to the genetic marker of somatic chromosome. The resulth of this research exibited three allales of the four alleles found were fixed or nearly fixed in all populations. Nearly fixation state of those alleles may associate with no DNA recombination during replication and low effective number of variation source as describes above. There was only one allel,

DYS391^{282}, had low frequency and just found in population of Pulaki (Tabel 1). This may reflect a new mutation of allele, therefore, the male bearing the allele has not had enough chances to distribute the allele through mating with a female within a population as well as migrating to other populations.

The low haplotype diversities are not only affected by the total number of geneticaly different male in population, but also the number of loci used and the polimorphism of the loci (Perwitasari-Farajallah *et al*., 2004). Low haplotype diversities of Y chromosome may reflects to a few genetically different male of long taild macaques in wild in Bali Island. The long tailed macaques in Bali Island were originated from Java Island and they migrated during the two islands unite to be a part of Sunda Selft (Eudey 1980; Fooden 1995). However, there is no a clear information about the frequencies of migration events and the total number of different populations had migrated. This lack of information brings to the difficulty to justify the male number of long tailed macaques as the founder population in Bali Island in the past. Kawamoto *et al.* (1984) stated that the long tailed macaques populations in Bali Island had suffered from a bottle neck effect. It was based on the blood protein data that the genetic variation of long tailed macaque populations in Bali Island was lower than those of the long tailed macaque populations in Lombok Island. The botlle neck effect may decrease severely the total number of the population members, and so do the number of the male. While this preliminary resarch used three microsatellite loci on Y chromosome and only one of them was polimorphic, the data may not express the real condition. It would be weak in differentiating individual both within and between populations. To better understand pertaining to the evolutionary history of male macaques in Bali Island, a further research using a more polimorfic microsatellite loci on Y chromosome is needed. Moreover, a broaden sampling sites (population) may be obligated including population in Java Isalnd and Lombok Island to elucidate the evolutionary history of male macaque in South Archipelago of Indonesia. However, this research has succeeded to characterize three microsatellite loci on Y chromosome at native populations of long tailed macaque in Bali Island.

CONCLUSION

Based on the result of the research, it can be concluded that each of DYS390 and DYS393 loci has one allele, so, these two loci are monomorphic in male long tailed

macaques in Bali Island. On the other hand, DYS391 locus that having two alleles is polymorphic. The locus of DYS391 could be used in the study of genetic differentiation between male long tailed macaque populations in Bali Island, but not the two others.

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