MITOCHONDRIAL DNA (MTDNA) VARIATION OF SULAWESI BLACK MACAQUE (*Macaca nigra*) LIVED IN TWO NATIONAL PARKS (TANGKOKO AND DUA SAUDARA) OF NORTH SULAWESI

Hapry F.N. Lapian
Faculty of Animal Husbandry, Sam Ratulangi University, Manado. 0431-863186
hlapian@yahoo.it

ABSTRACT

Hypervariable II (HVII) regions of mitochondrial DNA (mtDNA) D-loop regions of Macaca nigra lived in Tangkoko and Dua Saudara National Parks have been analysed. An approximately 600 base pairs (bp) of HVII regions was sequenced to characterize genetic variation among 20 samples of Macaca nigra lived in Tangkoko and Dua Saudara National Parks. Using an outgroup of Macaca fascicularis of Kalimantan, a neighbor-joining and maximum parsimony trees both identified that all DNA samples of Macaca nigra were arranged in similar cluster. The low nucleotide diversity among samples of Macaca nigra in Tangkoko and Dua Saudara suggests either there is a direct mix among species or that they may have similar ancestor among them.

***Key words**: Macaca nigra, mtDNA, D-loop Region, Genetic Diversity, Tangkoko-Dua Saudara*

INTRODUCTION

Sulawesi black macaque (*Macaca nigra*) is the most widely distributed nonhuman primates in North Sulawesi. This species is only found in the northern part of Sulawesi. *M. nigra* is also the only macaque that has no tail. Because of their physical performances, *M. nigra* might be the better nonhuman primate model for medical research in various domains such as infectious diseases, tissue engineering, bone marrow graft, gene therapy, immune response against pathogens or new vaccines, and infectious diseases and particularly Simian Immunodeficiency Virus (SIV)-induced AIDS or emergent pathogens. In all these models, the genetic background of the animals could be of great importance.

Population of *M. nigra* tends to decrease because of local hunting (Lee 1997; O'Brien & Kinnaird *et al.,* 1999; Rosenbaum *et al*.,1998; Sugardjito *et al*., 1989). Because of their existence, conservation efforts to maintain population of this macaque have to be formulated. Genetic conservation in this term could provide the accurate and important data for species management. The importance of genetic data of *M. nigra* is not supported by molecular researches. Very few analysis have included information DNA sequence data of *M. nigra*. As part of a study on the complete genetic relationships of Macaque, the Sulawesi Black Macaque play an importance role, because of the high number of endemic species and the low amount of currently available genetic data.

In the efforts to provide valuable data for investigating intraspecific variation, population structure, phylogeography, and demography in macaque species, maternally inherited mitochondrial (*mt*)DNA has been widely used (Harihara *et al.*, 1988). In the present study, the molecular genetic relationships between Sulawesi Black Macaque populations lived in Tangkoko National Park, based on mtDNA sequence data have been investigated. Herein, approximately 600 bp in *hypervariable* segment 2 (HVII) of the D-loop of a Sulawesi Black Macaque populations have been sequenced. Focus on the D-loop region of DNA is considered because mutation rate in this region is higher than another region of mtDNA (Ishida *et al.*, 1994). Parker *et al.* (1988) mention that because of the higher mutation rate, study on the D-loop would be important to investigate divergent population. The D-loop has also provided advantageous evidence for confirmation population distribution pattern and genetic variation among population (Avise *et al.*, 1984; Loftus *et al.*, 1994; Giuffra *et al.*, 2000; Luikart *et al.*, 2001; Ponomarev *et al.*, 2003; Loehr *et al.*, 2006; Jia *et al.*, 2007).

MATERIALS AND METHODS

Sample Collection

This study included 20 species *M. nigra* lived in Tangkoko and Dua Saudara National Park. In order to safely keep species, sample was collected from faeces. This technique is known as *non-invasive method.*

DNA Analysis

Isolation of the genomic DNA was performed using protocols of **QIAmp® DNA Stool Handbook** of QIAGEN. Amplification conditions were conducted under following

profiles: 95° C for 5 minutes; 95° C for 60 s; 55° C for 60 s; and 72° C for 60 s; followed by final step at 72° C for 10 minutes. s using HotStarTaq Master Mix Kit (Qiagen). Stock solution was formulated as follows: buffer 10 X sebanyak 3 µl; DnTP 10mM 0.6 µl; MgCl2 25 mM 4 µl; Primer Saru 4F (5'-ATCACGGGTCTATCACCCTA-3') (Blancher *dkk*., 2008) 10 pmol 1 µl; Primer Saru 5R (5'-GGCCAGGACCAAGCCTATTT-3') (Blancher *dkk*., 2008) 10 pmol 1 µl; BSA 20mg/ml 0.5 µl; Taq 5 µ/µl 0.2 µl; H20 16.7 µl and DNA template 5 µl. Product was then purified using **QIAquick Gel Extraction Kit** (Qiagen) according to manufacturer's recommended protocol. The sequencing reaction product was then sequenced on an automated DNA sequencer (ABI PRISM 310, Applied Biosystems).

Phylogenetic Analysis

The trees were produced by *Neighbor-Joining* (NJ) and maximum parsimony (MP) using the program of MEGA4 (Tamura *dkk.,* 2007). NJ was constructed by *p-distance* model with 1000 bootstraps. The MP tree was produced following *Close-Neighbor-Interchange* (CNI) algorithm with 3 *search level* and using random stepwise additions with 100 replications. 1000 bootstrap replications have been performed.

RESULTS

Figure 1 and 2 showed phylogenetic tree obtained by Neighbor-Joining method. Numbers in node is bootstrap values and phylogenetic tree obtained by Maximum Parsimony method. Numbers in node is bootstrap values.

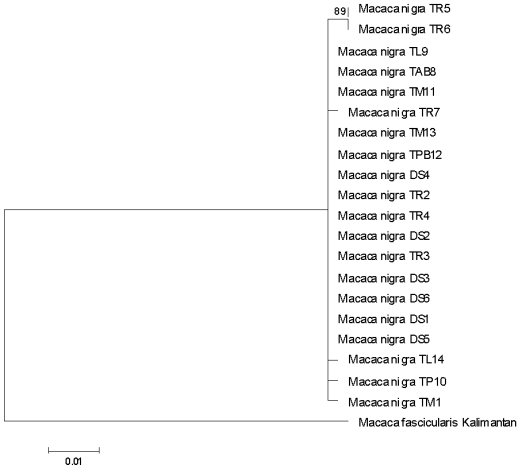

Figure 1. Phylogenetic tree obtained by Neighbor-Joining method. Numbers in node is bootstrap values.

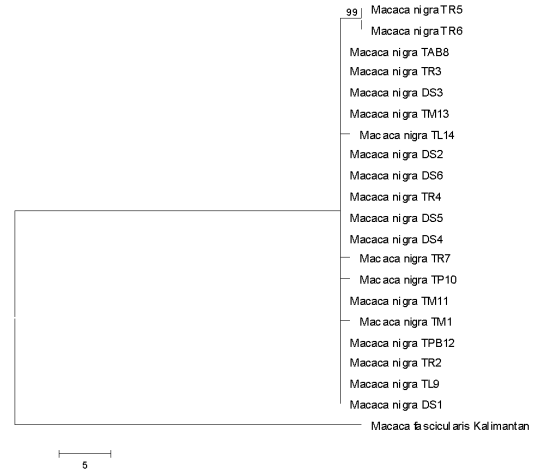

Figure 2. Phylogenetic tree obtained by Maximum Parsimony method. Numbers in node is bootstrap values.

DISCUSSION

Twenty samples of DNA are sucesfully sequenced from 27 feaces of *M. nigra*. With all *M. nigra* samples, a single band by PCR amplification have been clearly obtained. The amplified fragments were analyzed for their sequences. Phylogenetic analyses of these sequences produced trees consistent with the known macaque phylogeny. The determined length of mitochondrial *HVII* D-loop in the different *M. nigra* samples ranged from 531 to 595 nucleotides. The phylogenetic tree, rooted by *Macaca fascicularis*, obtained by NJ and MP methods (Fig. 1 and 2 respectively) shows that the 20 individual of *M. nigra* considered are not clearly separated into different group. The distance matrix shows that there are very small genetic distances (0.01) among the *M. nigra* species lived in both Tangkoko and Dua Saudara National Park.

Based on the phylogenetic trees mentioned above, it could be confirmed that *M. nigra* from different area of these two National Parks are a mixed origin among others. This suggests the current presence in mtDNA lineages of Tangkoko's and Dua Saudara's *M. nigra* is coming directly from similar ancestor. This has also been confirmed by the topography of these national parks. There is no significant barrier that clearly blocks migration among the macaques that live in two different area.

The low genetic and nucleotide diversities between internal population of Tangkoko suggest that there is also a mixed population among group. This is also found in the internal population of Dua Saudara. A small species of some group might colonize the other groups.

The results also suggest that the D-loop region of mitochondrial in species of *M. nigra* are very highly conserved sequences reflecting the very close genetic relationships among the species of *M. nigra* lived in Tangkoko and Dua Saudara. The D-loop region of mitochondrial DNA relationships discussed here provide valuable insight into *M. nigra* migration in or out of Tangkoko and Dua Saudara National Park. However, increased sampling is essential to bring the history of this species into greater focus. More intensive investigation are required in order to answer the phylogeography pattern of this animals. The present study has already revealed the posibility of mixing of population among different social group of macaques.

From the sequences of D-loop region of mitochondrial, it could also be confirmed that the *M. nigra* species have lower evolution rate. In addition, the low D-loop region of mitochondrial variation suggested that the *M. nigra* species may be at risk of extinction. Loss of specific, genetic, and ecological diversities is a very serious problem in Indonesia. Forests destruction either by human made or natural influences negatively affects wild animals such as the *M. nigra*. It reduces habitat for wild animals and cause population fragmentations. This could isolate population of animals and could rapidly lose genetic heterogeneity and become vulnerable to environmental change and risk extinction. One or more factor that leading to genetic loss is the ability of interspecific intercross caused by close evolutionary relationship. The genetic of species might be lost. In order to resolve the genetic relationships among the *M. nigra* in different area of National Parks (Tangkoko anf Dua Saudara) properly, it is important to not only study a larger sample, but to consider comparative behavioral studies as well.

So, based on the mtDNA D-loop sequences, it could be concluded that *M. nigra* population in Tangkoko and Dua Saudara was arranged in similar cluster. The low nucleotide diversity *Macaca nigra* in Tangkoko and Dua Saudara suggests either there is a direct mix among species or that they may have similar ancestor among them.

REFERENCES

Avise JC, Neigel JC, Arnold J, 1984. Demographic influences on mitochondrial DNA lineazge survivorship in animal population. *J. Mol. Evol*., 20, 99–105.

Blancher A, M Bonhomme, B Crouau-Roy, K Terao, T Kitano, and N Saitou, 2008. Mitochondrial DNA Sequence Phylogeny of 4 Populations of the Widely Distributed Cynomolgus Macaque (Macaca fascicularis fascicularis). *J. of Heredity* 99(3):254–264.

Giuffra E, Kijas, JMH, Amarger V, Carlborg O, Jeon JT, Andersson L, 2000. The Origin of the domestic pig: independent domestication and subsequent introgression. *Genetics* 154:1785–1791. (http://animaldiversity.ummz.umich.edu/site/accounts/information/Bubalus_bubalis.html).

Harihara S, Saitou N, Hirai M, Aoto N, Terao K, Cho F, Honjo S, Omoto K, 1988. Differentiation of mitochondrial DNA types in *Macaca fascicularis. Primates*. 29:117–127.

Ishida N, Hasegawa T, Takeda K, Sakagami M, Onishi A, Inumaru S, Kamtsu M, Mukoyama H, 1994. Polymorphic sequence in the D-loop region of the equine mitochondrial DNA. *Anim. Genet* 25: 215–221.

Jia S, Chen H, Zhang G, Wang Z, Lei C, Yao R, Han X, 2007. Genetic variation of mitochondrial D-loop region and evolution analysis in some Chinese cattle breeds. *J. Genet. Genomics* 34: 510–518.

Kinnaird MF, O'Brien TG, Suryadi S. 1999. The Importance of Figs to Sulawesi's Imperiled Wildlife. *Tropical Biodiversity* 6(1&2): 5–18.

Lee RJ, 1997. The Impact of Hunting and Habitat Disturbance on The Population Dinamic and Behavioral Ecology of The Crested Black Macaque (*Macaca nigra*) [disertation]. Oregon: The University of Oregon.

Loehr J, Worley K, Grapputo A, Carey J, Veitch A, Coltman DW, 2006. Evidence for cryptic glacial refugia from North American mountain sheep mitochondrial DNA. *J. Evol. Biol*. 19: 419–430.

Loftus RT, MacHugh DE, Bradley DG, Sharp PM, Cunningham P, 1994. Evidence for two independent domestications of cattle. *Proc. Natl. Acad. Sci. USA*. 91: 2757–2761.

Luikart G, Giellly L, Excoffier L, Vigne JD, Bouuvet, J, Taberlet P, 2001. Multiple maternal origins and weak phylogeographic structure in domestic goats. *Proc. Natl. Acad. Sci. USA*. 98: 5927–5932.

O'Brien TG, Kinnaird MF, 1997. Behavior, Diet, and Movement Patterns of the Sulawesi Crested Black Macaque (*Macaca nigra*). *International Journal of Primatology* 18(3):321–51.

Parker GP, Snow AA, Schub MD, Booton GC, Fuerst P (1998) What molecules can tell us about populations: choosing and using a molecular marker. *Ecology* 79:361–382

Ponomarev AG, TD Tatarinova, VV Bubyakina, FO Smagulova, IV Morozov, TA Kashentseva, and NG Solomonov, 2004. Genetic Diversity in Siberian Crane (Grus Leucogeranus) Based on Mitochondrial DNA D-Loop Polymorphism. *Doklady Biological Sciences* 397: 321–323.

Rosenbaum B, O'Brien TG, Kinnaird M, Supriatna J, 1998. Population Densities of Sulawesi Crested Black Macaques (*Macaca nigra*) on Bacan and Sulawesi, Indonesia: Effects of Habitat Disturbance and Hunting. *American Journal of Primatology* 44:89–106.

Sugardjito J, Southwick CH, Supriatna J, Kohlhaas A, Erwin J, Froelich J, Lerche N, 1989. Population Survey of Macaques in Northern Sulawesi. *American Journal of Primatology* 18:258–301.

Tamura K, Dudley J, Nei M, and Kumar S, 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24:1596–1599.