

# Genetic basis of species differentiation in Sulawesi macaques, Indonesia

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## Background

Biodiversity is one fundamental feature of our planet. One of the greatest challenges faced by evolutionary biologists is to explain the enormous diversity. In Sulawesi Island, seven *Macaca* species are rapidly diverged into exceptionally variable in body shape and coat color from common ancestor. These seven species are allopatrically distributed in the island, with three species on the northern peninsula, one species in the central part of the island and the rest three species in the southern peninsula, respectively.

Many molecular markers have been applied to phylogenetic analyses of Sulawesi macaques. Recent a RADseq study showed that the seven species formed a monophyletic clade and are sister groups to *M. nemestrina*. The phylogenetic tree in coincidence to the geography distribution of species. In this study, I aim to explore: 1. What are the important traits that have diversified, and what are the genes and gene combinations underlying the traits (Chapter 2), 2. How do the gene function change and how do they shape phenotypic differentiation (Chapter 3). Presumably, highly differentiated loci between two contact species are expected to have been the basis for investigating differentiation in the genome.

## Methods

Saliva samples were collected from captive monkeys in Sulawesi macaques. In chapter 2, I compared the exome-wide sequence between neighboring species, to explore genetic variation that contribution to species differentiation. In total, five species (*M. nigra*, *M. nigrescens*, *M. hecki*, *M. tonkeana* and *M. maurus*) have been studied into four pairwise species comparisons with ten individuals in each species. Fixation index was adopted to screen out differentiated sites which most possibility responsible for species differentiation. Specifically, genes involving in pigmentation system were investigated in deeper for their possible functional enrichment. To clarify the genetic and evolutionary basis for coat color variation in Sulawesi macaques, in chapter 3, I investigated the selection signal in *MC1R* coding region and confirmed its functional consequence of species-specific variants in *vitro*.

## Result

In chapter 2, I found that hundred thousand SNPs were completely differentiated between species and significantly enriched in pigmentary system. Several differentiated SNPs located in key pigmentation genes were predicted to cause functional consequence of the proteins. Specifically, a key pigmentation related gene, melanocortin-1 receptor (*MC1R*) was found to have differentiated SNPs in all four pairwise species comparisons. These results suggested genes related to pigmentation

largely diverge between species and contribute to species differentiation. In chapter 3, I evidenced the MC1R have fixed variants and resulted in different functional characteristics in the six specie. Six species-specific variants corresponded with variation in agonist-induced and basal activity. Inconsistent with the dark coat color, four substitutions independently caused decreases in the basal activity of MC1R in four species, respectively.

## **Discussion**

This study represents the first exome-wide differentiation of Sulawesi macaques. I identified a lot of SNPs with fixed differences, which may contribute to local adaptation and prevent expansion of hybridization between contact species. Above the low genetic variation background, an exome-wide  $F_{ST}$  profile clearly shows genetic regions of high differentiation. These regions are highly possible under divergent selection. These differentiated SNPs will provide useful genetic markers for the future conservation management for Sulawesi macaques.

Coat color is considered as the product of natural and sexual selection, since coat color plays a role in conspecific signaling, thermoregulation, camouflage and possibly protection from pathogens. The result suggested that the pigmentation system was significantly differentiated between contact species. Moreover, I confirmed the fixed species-specific MC1R alleles displayed functional differentiation *in vitro*. Whether the color variability among Sulawesi macaques is due to random genetic drift or is a result of natural selection remains to be determined. It could be caused by random genetic drift if the color variability occurred before the geographical isolation disappeared between species. Further analysis on the divergent time of pigmentation related genes will be needed to clarify. Fujita suggested that Sulawesi macaques discriminated conspecific from closely related species. It suggested that the seven Sulawesi macaques are all different groups both in terms of their behavioral differentiation and from the monkeys' point of view. The differentiation on coat color might work as an external clue to prevent overall intergradation of Sulawesi macaques. Further investigation on possible functional impacts of differentiated SNPs will be needed to illustrate the possible mechanism of the pigmentation development process.

## **Conclusion**

An understanding of the evolutionary processes sharpening species differentiation is fundamental for understanding biodiversity. By studying on Sulawesi macaques, I tried to describe genetic factors shaping phenotypic variation at the population level. In the present study, I generated plenty of pigmentation related SNPs, producing a comprehensive resource that can be used in a variety of ways to promote further discoveries. Outcomes from this research can potentially improve our ability to trace environmental factors promoting ecological adaptation in mammals. It may also serve as a model for studying phylogeographic relationships of endemic fauna in Sulawesi or other island populations.