Research Activity Report
Supported by "Leading Graduate Program in Primatology and Wildlife Science"

	2017. 06, 07
Affiliation/Position	Universiti Sains Malaysia, MSc. Candidate.
Name	Christopher Chai Thiam WONG

1. Country/location of visit

Kyoto University, Kyoto, Japan.

2. Research project

Monkey Team no.2

The Diversity and Phylogeography of Mitochondrial DNA in Japanese Macaque (*Macaca fuscata yakui*) of Yakushima Island.

3. Date (departing from/returning to Japan)

22nd – 26th May 2017, Kyoto University, Kyoto, Japan.

4. Main host researcher and affiliation

Dr. Takushi Kishida (Program-Specific Assistant Professor, Wildlife Research Centre of Kyoto University)

Kei Matsushima (lecturer)

5. Progress and results of your research/activity (You can attach extra pages if needed)

Please insert one or more pictures (to be publicly released). Below each picture, please provide a brief description.

Laboratory:
Room no. 035 (BF1), Graduate School of Science BLD#1, Kyoto University.

Returning from Yakushima, Genome Course divided the original (8 people) Monkey and Deer Group into two smaller groups. I was assigned to Group 2 to carry out experiment in investigating 'Diversity and Phylogeography of Mitochondrial DNA if Japanese Macaque (*Macaca fuscata yakui*) of Yakushima Island.

Pre Genome Course.
Prior to the start of Genome Course in Kyoto University, Dr. Kishida sent the team scientific journals as references for the experiments. Protocols of the lab work were also sent as guides. I find the materials very useful as they prepared me for the things to expect during the laboratory work.

22nd May 2017 (Day 01)
The lab work started with introduction by Dr. Kishida and his assistant for the week Mr. Kei (a PhD candidate). The Monkey Group no.2 also introduced ourselves where we provided background of individuals' studies.
Before the lab work began, Dr. Kishida briefed on the lab safety procedures – the dos and don'ts in the lab. We were strictly warned about using gloves and mask at all time to prevent contamination of our samples. He then proceeded to demonstrate on proper use of a pipette. The task for the day was 'DNA extraction and purification from fecal samples'. There were almost 30 steps in the process. All 25 samples were done processing at around 1600hrs. Samples were then undergone the nanodrop test to check the concentration of the DNA in the processed samples.

23rd May 2017 (Day02)
In day 02, we received 25 more samples from Group 01. In those samples, DNA extraction and purification have been completed, hence the samples were processed along with samples from Group 02 without additional steps. The aim on the second day was to complete i) PCR amplification and ii) 2.Removal of primers and dNTPs. Quantity of certain mix was modified for easier preparation. The day ended with the samples being inserted for thermal cycles for approximately 2 hours.

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24th May 2017 (Day03)
On day03, we were guided to prepare the samples for i) sequencing and ii) alcohol precipitation. During this process, a cancer causing solution – HiDi – was required. All students were again reminded about the importance of wearing gloves and mask when performing lab work. All process was completed around 1700 hrs. Samples were then bought to another lab where they were placed in a DNA sequencing machine to be sequenced. A lab assistant kindly offered his help to run those samples in the sequencer.

25th May2017 (Day04).
Results from the sequencer came in. The Group cleaned up the lab before proceeded to analysing the results. We moved to Higashi Ichijokan (another Kyoto University out-of-campus facility) for discussion. Using Mega7, the results were read and interpreted.

26th May 2017 (Day05).
The process of interpretations of the results continues on day 03. All results were then compiled and merged by Dr. Kishida. Together with Dr. Kishida, and Kei, the team discussed the results. The remaining time of the day was spent to prepare for the poster presentation for 6th International Seminar on Biodiversity and Evolution .

Results:
1. Several haplotype detected in this experiment were also detected in Hayaishi and Kawamoto (2006). One of the haplotype could be the first observation of expansion or/and migration in Yakushima Island. The same haplotype is also being recorded at much higher elevation from the previous study.
2. The team also found that success rate of samples is highly influenced by the condition of the feces collected. Fresher samples yield better results than older feces samples.
(For methods and full results, refer to Yanagi et. al., 2017 – Poster attached).

Reference:
Hayaishi, S. and Kawamoto, Y. (2006) Low genetic diversity and biased distribution of mitochondrial DNA haplotypes in the Japanese macaque (Macaca fuscata yakui) on Yakushima Island. *Primates* **47**, 158-164

Photos

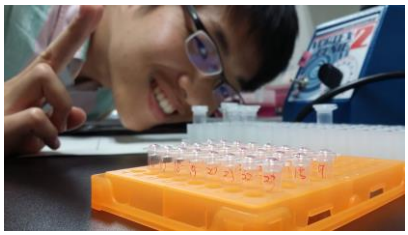

Fig 1: Feces samples in tubes.

Fig 2: Samples in incubator.

Fig 3: Dr. Kishida gave explanation on PCR machine.

Fig 1: Gel electrophoresis

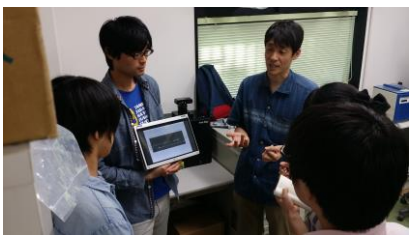

Fig 1: Results from gel electrophoresis.

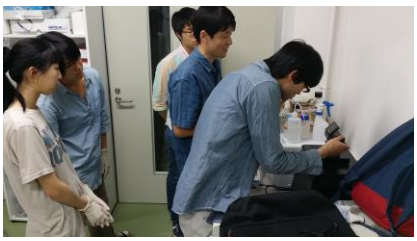

Fig 1: Taking photos of the gel.

Fig 7: Laboratory.

Fig 8: Used pipette tips.

6. Others

Being unfamiliar with lab work, I highly appreciate Dr. Kishida's and Kei Matsushima's patience in explaining the process in the experiment. Never to be forgotten my team mates Taku Ohtsubo for guiding me on the lab work, Moe Yanagi and Mikaze Kawada for preparing the poster results. Special thanks also to Kyoto University for allowing the use of its facilities.

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The Diversity and Phylogeography of Mitochondrial DNA in Japanese Monkeys (*Macaca fuscata yakui*)

Moe YANAGI[1], Taku OTSUBO[2], Mikaze KAWADA[3] and Christopher Chai Thiam WONG[4,5].

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Introduction

Yakushima, a World Natural Heritage site, is home to an endemic primate – the Japanese macaque (*Macaca fuscata yakui*). Various and great amount of research has been carried out on the species including morphological, physiological, ecological, and social features since mid-1900 (Yamagiwa, 2010). Over the past 30 years, researchers have been studying more on genetic information to supplement and strengthening natural history information of the Japanese macaques (e.g. Inoue et al. 1990; Hayakawa and Takenaka 1999).

Method

Genetic analysis

- Extracted mtDNA from fecal samples using QIAamp DNA Stool Mini Kit(QIAGEN).
- To amplify 624 nucleotides, we used primers[forward, 5'-ATCACGG GTCTATCACCCTA-3' and reverse, 5'-GGCCAGGACCAAGCCTATTT-3'] originally designed by Hayasaka et al (1991).
- The amplified DNA was directly sequenced on ABI3130.
- We also used two additional internal primers[forward, 5'-TAT GCTGACTCCCACCACAT-3' and reverse, 5'-GTTTGGATGAAGGTC GGAGA-3'] originally designed by Kawamoto et al (2007) for sequencing.
- Each base sequence was verified using MEGA7.

Success rate analysis

- Success rates for genomic analysis were compared for
  i) samples collected on sunny and light raining day
  ii) old and new feces samples.
- Success samples are defined as samples which based sequence Y1* is obtained and can be read
  (*following sequence ID by Hayaishi and Kawamoto, 2006)
- Fisher's exact test were performed for significance

Results

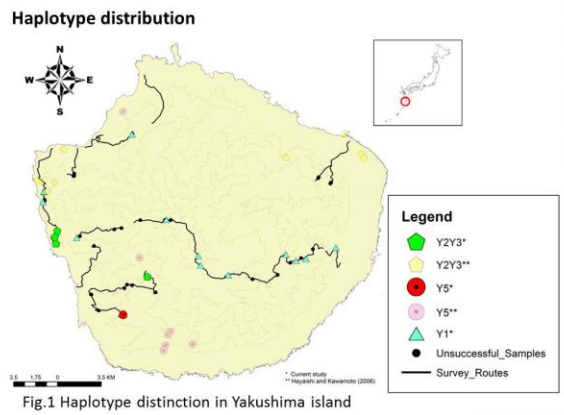

Fig.1 Haplotype distinction in Yakushima island

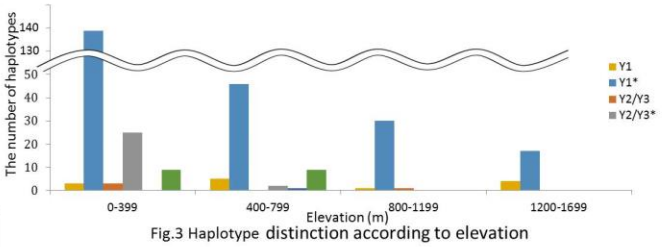

Fig.3 Haplotype distinction according to elevation

Three types of haplotype were observed, namely Y1, Y2/Y3, and Y5 (the same as, hence following the naming by Hayaishi and Kawamoto, 2006). Y1 haplotype was widely distributed in Yakushima Island. The widespread is also observed by this study that we detected 11 fecal samples were haplotype Y1 (Fig 1). Although they at being detected at a very low frequency, there is a possibility of an expansion or/and migration of haplotype Y2/Y3. Four fecal samples containing haplotype Y2/Y3 were found outside of the region where they were previously reported by Hayaishi and Kawamoto (2006). One of the Y2/Y3 haplotype is also found at elevation 1087m a.s.l, much higher than previous recorded. In previous study, the highest elevation range that haplotype Y2/Y3 was found was from 400-799m a.s.l. Haplotype Y5 is also found in one sample, and in proximity to Y5 detection region of Hayaishi and Kawamoto (2006).

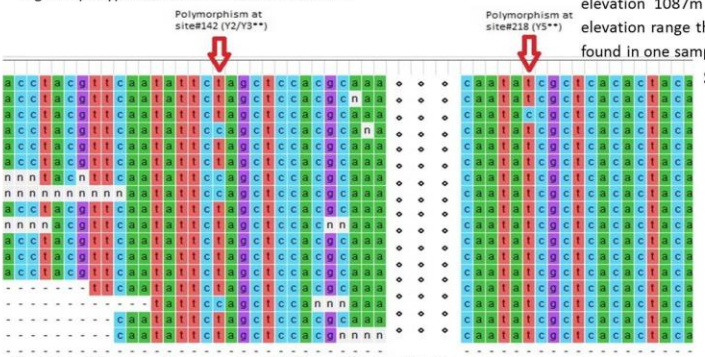

Fig.2 The difference of haplotypes at Y2/Y3 and Y5 found in the Japanese macaques.

Success rate analysis

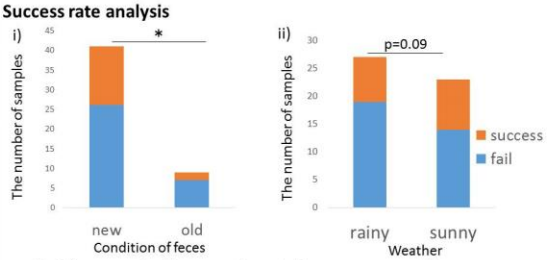

Fig.4 Success rates for genomic analysis were compared for
i) samples collected on sunny and light raining day
ii) old and new feces samples.

Discussion

Mutation

- ✓ Y2/Y3 were found outside of the region where they were previously reported (Hayaishi and Kawamoto, 2006).
- ✓ One of the Y2/Y3 haplotype is also found at elevation 1087m a.s.l
- ➢ In previous study, the highest elevation range where haplotype Y2/Y3 was found was from 400-799m a.s.l.
- → **first record of expansion of haplotype Y2/Y3 Japanese Macaque to higher elevation?**
- ✓ Y1 was distributed widely, whereas the other haplotypes were observed only in restricted areas
- ✓ Y5 was found in same area that previously reported (Hayashi and Kawamoto, 2006).
- → **some haplotypes group has still remained since 15 years ago, but genetic diversity is still low**

Success rate

- ✓ New feces were significantly successful for reading mtDNA sequences
- ✓ We had sunny days and a rainy day for collecting fecal samples, but it was not significantly related with success rate.
- → **The freshness of feces is important for success on PCR**
  Rainfall doesn't affect on PCR success, within 2 days? (Brinkman, 2010)

Acknowledgement
We appreciate Primatology and Wildlife Science Leading Graduate Program and CET-Bio.

References:

1. Yamagiwa J. (2010) Research history of Japanese macaques in Japan. In: (Nakagawa N, Nakamichi M, and Sugiura H, eds) The Japanese macaques. Springer, Tokyo. pp. 3-25.
2. Kawamoto Y, Shotake S, Nozawa K, Kawamoto S, Tomari K, Kawai S, Shirai K, Morimitsu Y,Takagi N, Akaza H, Fujii H, Hagihara K, Aizawa K, Akachi S, Oi S, and Hayaishi S. (2007) Primates 48, 27-40.
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