Research Activity Report
Supported by "Leading Graduate Program in Primatology and Wildlife Science"
(Please be sure to submit this report after the trip that supported by PWS.)

	2016. Jun, 14
Affiliation/Position	Primate Research Institute/M1
Name	Mao Asami

1. Country/location of visit

Japan, Kyoto

2. Research project

Genome training

3. Date (departing from/returning to Japan)

2016. May. 30 – 2016. Jun.7 (9days)

4. Main host researcher and affiliation

Dr. Miho Murayama, Professor at Wildlife Research Center of Kyoto University

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.

In this course, I have belonged Deer team that aim was to understand the way to analyze the DNA which extracted from fecal samples. We conducted sex identification and mitochondrial haplotype identification of Japanese deer (*Cervus Nippon yakushimae*) to reveal following three points.

- The sex and mitochondrial haplotype of observed deer from non-invasive samples
- Relationship between social interaction and mitochondrial haplotype
- Rate of successful sequencing mitochondrial DNA from samples that left outside

Those results were combined with that of Yakushima field course, and given a presentation on the 5th International Seminar on Biodiversity and Evolution.

< Schedule of the course >
May 30th : Move to Kyoto, DNA extraction, and PCR amplification
May 31st to June 3rd :Sexing and Mitochondrial haplotype identification
June 6th :Preparation for presentation
June 7th Poster presentation

1) Sex identification

Three types of sample were investigated.

a) Fresh 47 fecal samples (collected just after defecation in Yakushima field course,)
b) 20 samples that left outside for few days (1day to 8 days)
c) 5 samples reserved in a freezer for one year.

We did DNA extraction of fecal samples and PCR amplification for agarose gel electrophoresis. The first electrophoresis did not show any bands, so we did the same procedure again. The second one was works and all samples could be identified by using Amelogenin gene which is located on the sex chromosome.

These results showed that even feces left outside could identify the sex at least until 8 days and sex of observed deer correctly.

Photo1: Learning PCR method

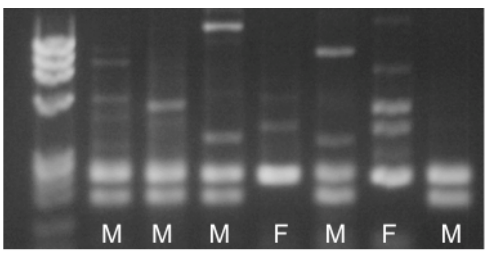

Photo2: The result of electrophoresis (M) male (F) female

2) Mitochondrial haplotype identification
We investigate sample (a) and (b) using amplified mitochondrial control region (D-loop) by 3130xl sequencer Genetic analyzer. Same as sexing, first sequencing was failed. Therefore, we analyzed second sequencing results using MEGA7. 7 haplotype were detected from 38 samples that succeed sequencing. There were same samples that could not read their sequences even sex identification was succeeded.
The result showed that most of the social interaction happened between the same haplotype. Additionally, all social interactions between different haplotype were male-female interactions.

From this course, I've learned basic techniques of sexing and sequence analyses. For a future prospect, I am interested in what Yoshimi-san taught me that there is the method that can get DNA sample from old bones or fossil. To investigate the evolution of animals this great experience will help my own research.

Photo3: Members of deer team

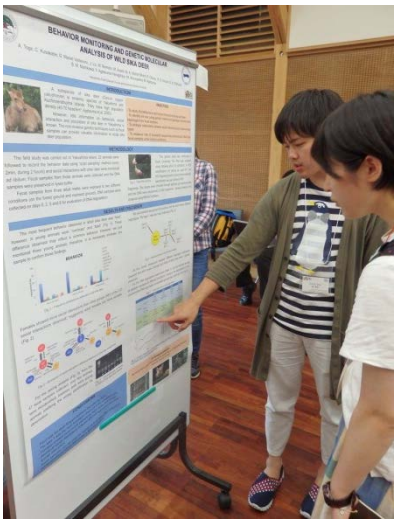

Photo4: Poster presentation

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6. Others

I would like to express my deep gratitude for Dr.Murayama and Yoshimi-san to teach how to do the experiments. And also, I thank Sato-san to help us a lot. My sincere gratitude to PWS program and members of deer group for giving this opportunity include interesting research.