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. 06, 08
Affiliation/Position	Center for Ecological Sciences, Indian Institute of Science/PhD
Name	Sanjeeta Sharma Pokharel

1. Country/location of visit

Wildlife Research Center, Kyoto

2. Research project

Genome Science Course: Molecular sexing and genotyping of Sika deers (*Cervus nippon*) using fecal samples.

3. Date (departing from/returning to Japan)

30.5.2016 to 3.6.2016 (7 days)

4. Main host researcher and affiliation

Prof. Miho Inoue-Murayama, Wildlife Research Center, 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.

Non-invasive techniques of analyzing the DNA from the fecal samples are getting wider acceptance from the wildlife biologists because of its ease of analysis and significant outcomes. To understand the mechanism behind such analysis and the way of interpretation, we formed a team of 10 students under the guidance of Prof. Miho Inoue-Murayama. The main objectives, of this course, were :

a. To identify the sex of the individual Sika deer using the DNA analysis of the fecal samples collected during Yakushima Field Science Course by the deer group
b. To identify the mitochondrial haplotype from the same samples.
c. And to calculate the successful rate of sequencing.

The results obtained from this analysis was then used to see the relationship between different haplotypes in terms of social interaction.

To attain these targets, we started our work by extracting the DNA from the fecal samples. The extraction was performed using the QIAamp Stool Kit, QIAGEN. It majorly included the process of extracting (centrifugation, absorption of unwanted particles by the absorbing tablet, degradation of cells, ethanol precipitation, filter trapping of DNA, etc.) and purifying the DNA. Hence extracted DNAs were then amplified. The PCR amplification for the sexing used KY1 and KY2 fragments while for the mitochondrial identification LD5 and H597 fragments were used. The agarose gel electrophoresis was used to see the bands under the UV Trans illuminator. PCR products were then used for sequencing. High Pure PCR product purification kit was used. The sequence was analyzed by using the sequencer Genetic analyzer. The obtained sequences were then analyzed and interpreted using MEGA7 and FinchTV softwares.

FinchTV software was used to check the correctness of the curves of reverse and forward primers. Sequences

were then sorted for both forward and reverse primers in the MEGA7 software. The complete sequence for each samples were then stored in the FASTA format for identifying the haplotypes.

Interestingly, we were able to identify the sex of the directly sighted individuals in the Yakushima island. Out of 47 samples, only two samples were misidentified. We were able to sequence 38 samples and to identify 7 different haplotypes. The association between haplotypes were also analyzed, haplotype-1 was found to be higher in number than the rest of the haplotypes.

Beside these, the DNA degradation experiment was also conducted by the deer team during the Yakushima Field Science Course, for which the same sequences and molecular sexing were used. Based on the experimental design where fecal samples were exposed to different field conditions and then they were collected for the DNA analysis. The result showed that those samples exposed in open area had faster mitochondrial DNA degradation than those samples exposed in the forested area.

Concisely, this study gave us the overall concepts of how to analyze the DNA samples from the fecal samples, to identify the sexes, haplotypes and to draw the association between them.

Personal observations and learnings:

As a major part of my research involves identifying the elephants in the crop-fields during the night in the conflict zones, this course offered me a better understanding of how to identify the sexes and if possible, also the haplotypes. I would like to enlist some of the personal learnings that I have gained from this course which shall aid me in my future work:

- How the use of SRY gene (ZFX region) could help in identifying the sex.
- Degree of DNA degradation depends on the ambient environment which it is exposed to.
- How the genetic analysis could reflect the social association between animals, in this case- the Sika deers.
- The research papers provided by Prof. Miho gave me the great insights about the genetic variations between the Sika deers and Japanese macaques:
    - Sex Determination Based on Fecal DNA Analysis of the Amelogenin Gene in Sika Deer (*Cervus nippon*) YAMAUCHI, et al, 2000.
    - Genetic Variation And Population Structure Of The Japanese Sika Deer (CERVUS NIPPON) in Hokkaido Island, based on Mitochondrial D-Loop Sequences. NAGATA et al., 1998.
    - Two Genetically Distinct Lineages of the Sika Deer, Comparison of Mitochondrial D-Loop Region Sequences *Cervus nippon*, in Japanese Islands. NAGATA et al., 1999.

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This course offers a good chance for those who are willing to learn about the DNA extraction and genetic analysis. The hands-on technique provided during the lab work gives the practical exposure requires for analyzing the DNA.

Appendix:

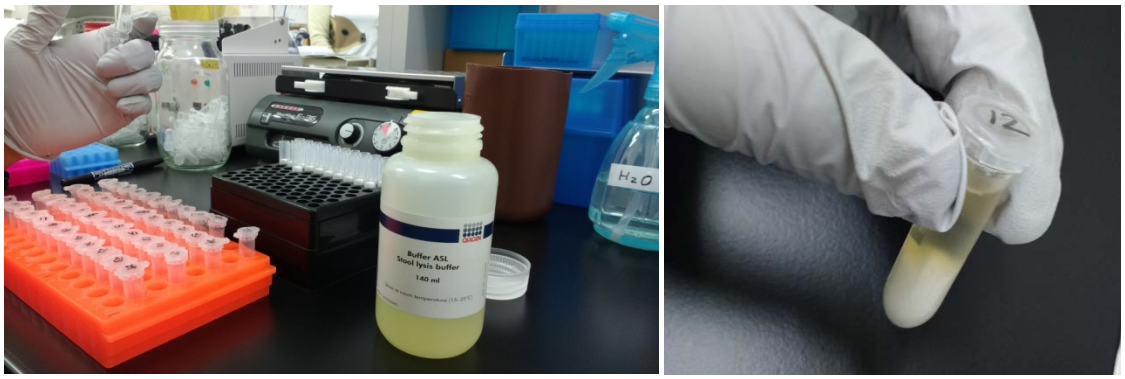

Fig 1: During DNA extraction(lysis and absorbance using the inhibitEX tablet)

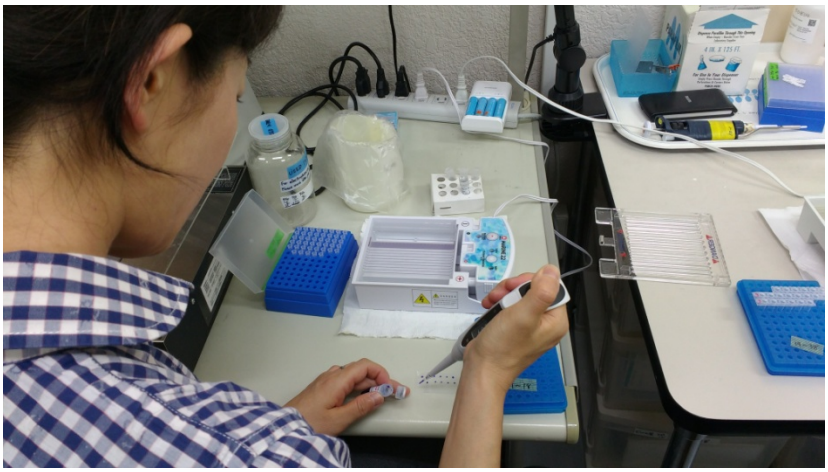

Fig 2: Prof. Miho training us on coating the Agarose gel

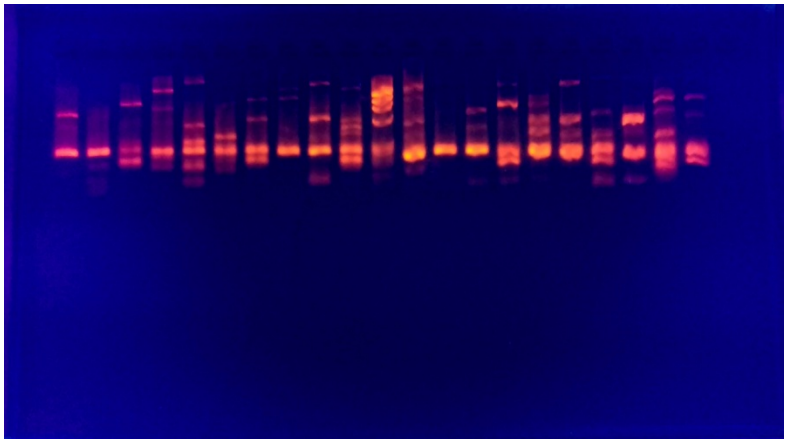

Fig 3: Bands observed under the UV Trans illuminator

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Fig 4. Preparing the plates for sequencing.

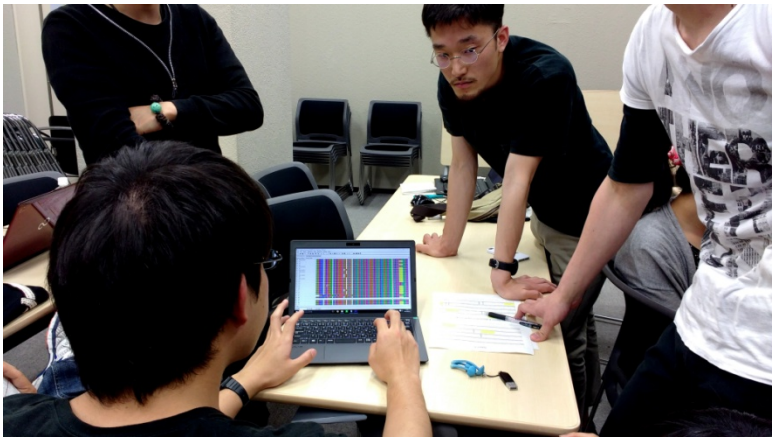

Fig 5. Classifying the haplotypes using the obtained sequences.

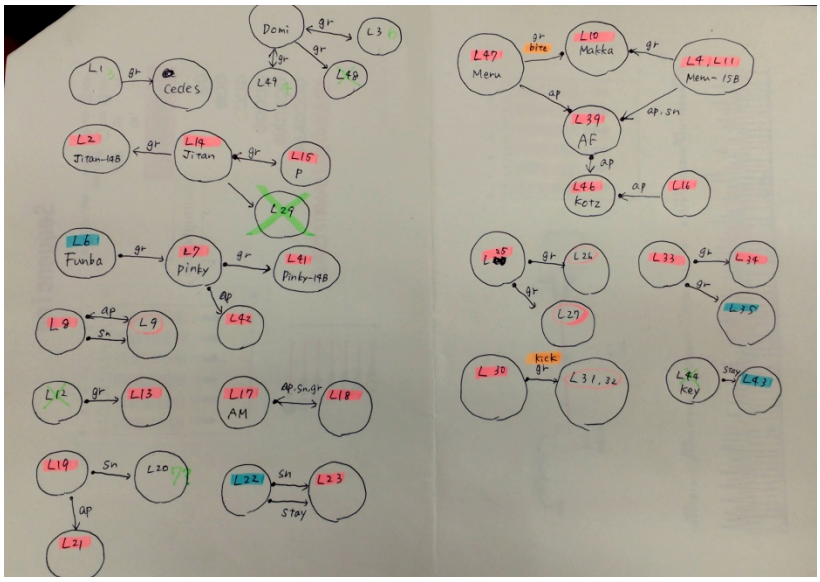

Fig 6. Genetic relation and behavioral association between individuals sketched by Mayuko Nomoto

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A team of sexing and genotyping work

6. Others

I would like to pay my deepest gratitudes to Prof. Miho Sensei, Dr. Abe Sensei, Dr. Agetsuma Sensei, Hori-san and Sato-san for training us in this technique. As always, I would like to thank all my team members for creating such a friendly-work ambiance. In person: Mao, Mayuko, Kusakabe, Rodrigo, Nurul, Akito, Utsuka, Gisele and Liu. I would also like to acknowledge Mayako Fujihara san for showing her research lab and explaining about her interesting research concept. I learnt a lot by attending the weekly lab- meeting where I got to know different researches being done under Prof. Miho. Hence, I would like to thank all of them who presented during the lab-meet and gave us the overview of their ongoing research. Last but not the least, I would like to thank WRC (Prof. Shiro Kohshima) and PWS program for giving this wonderful opportunity to me.