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.)

	2017. 06. 06
Affiliation/Position	Indian Institute of Science/Project Assistant
Name	Upasana Sarraju

1. Country/location of visit

Japan/Inuyama

2. Research project

Genetic Analysis of Parasites of wild mammals in Yakushima

3. Date

2017. 05. 22 – 2017. 05. 26

4. Main host researcher and affiliation

Dr.Takashi Hayakawa, Dr.Munehiro Okamoto, Primate Research Institute, 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.

The Genome Science Course continued from the results of the Field Science Course. I was part of the Parasite Research Group, consisting of four other students, led by Dr.Takashi Hayakawa and Dr. Munehiro Okamoto, Primate Research Institute, Kyoto University with tutoring by Akito Touge-san, a student of Primate Research Institute. During the Field Course, we had conducted research on ectoparasites and endoprasites of wild deer, wild field mice and ground vegetation in three different locations in Yakushima. The Genetic course aimed at genetic identification of the sampled parasites.

The endoparasites isolated from deer and mice were too few in number for successful genetic analysis Therefore, we proceeded with genetic analysis of ticks collected from wild deer and ground vegetation. We had collected and preserved 2 unidentified insects, and about 104 ticks designated as follows based on morphological identification *Haemaphysalis sp.1*, *Haemaphysalis sp.2* and *Ixodes.*

Of all the collected specimens, we selected 30 individual ticks ensuring we had sufficient representation of the different genera, species, life stage and hosts. Each of these ticks was then subjected to dissection to separate the heads from the bodies: the heads were preserved separately for morphological identification while the bodies were utilised for genetic analysis.

Fig.1 Separation of Tick Head and Body

Extraction of Genomic DNA followed standard protocols of Qiagen DNA Mini Stool Kit using the tick bodies as DNA source. However, DNA extraction methods involving NaOH digestion was attempted on six of the 30 tick samples, as described in *Ushijima et. al, J. Parasitol, 89(1),2003, pp.196-198*. The first day of lab work concluded with setup of overnight digestion for DNA extraction.

The next morning we performed the rest of the extraction protocols and setup the PCR reactions. PCR amplification was performed using a mitochondrial 16S rRNA region, a barcode sequence in ticks, as the template and with three sets of primers: tick primers for the 16S target, Lepidoptera COX1 primers (to identify the unknown insects) and mammalian *Cytb* primers (to target the mammalian blood consumed by the ticks).

Gel electrophoresis was performed to check for successful amplification based on expected product size. We found that most of the *H.sp.1* samples (11 out of 15) had amplified while most of the other species did not amplify successfully. The non-tick insect samples and the mammalian blood did not amplify. It was also noted that the positive control did not amplify indicating there was an error in the preparation of the PCR mastermix. The NaOH extraction method also proved unsuccessful.

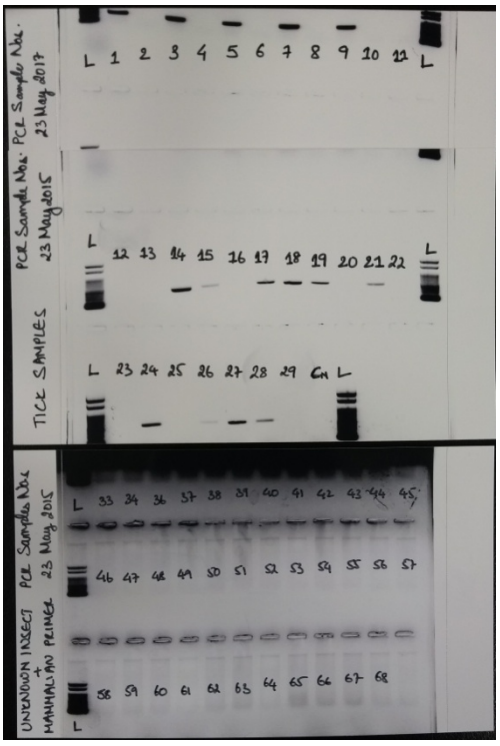

Fig 2. Gel Electrophoresis Result

To ensure better targeted amplification of *Ixodes* samples, the 16S target sequence was then amplified in fragments which were half the length of the full target sequence. PCR reactions were setup utilizing primers for the two halves of the target sequence, and a repeat reaction was set up for the full fragment as well.

Fig 3.Running a PCR on a Thermocycler

The next day, gel electrophoresis was performed to identify those samples that had successfully amplified.

We found that some *Ixodes* samples had amplified as a result of the half-fragment PCR. Purification of PCR products using Exo SAP-IT was performed on all successful amplicons. Following this, a sequencing PCR was setup using all four primers i.e. primers of both the half-fragments. The PCR products were purified using Agencourt Clean SEQ Magnetic Beads. Sequencing was performed overnight using an Applied Biosystems 3130xl Genetic Analyser.

The next day we found that sequencing had not been performed successfully indicating that there were no PCR products at the end of purification. To troubleshoot this, the successful PCR products from amplification by tick primers were purified by Isopropanol Purification. Following this, the sequencing reactions were set up again. The sequencing products were purified by Ethanol Purification.

Using the purified sequencing PCR products, sequencing of the amplicons was set up as described before.

The next day was employed in bioinformatics analysis of the sequences. Each forward and reverse sequence of samples was corrected and assembled to generate a dsDNA sequence. The sequences obtained from our samples were compared by alignment with all published sequences of ticks available in NCBI. A phyogenetic tree was constructed using Neighbour-Joining method. The phylogenetic tree revealed a perfect separation of clades containing *Hsp.1* and *H.sp.2*. The *Ixodes* samples, whose sequences were generated using only half the 16S target fragment appeared as an independent clade as well.

These interesting results suggest that during our field survey in Yakushima, we came across three new taxa of ticks. However, further study is essential to confirm our results and to genetically identify the new clades.

The results of the Genetic Course were compiled with the results of the Field Course for a poster which was to be presented later in the CETBio 6th International Seminar on Biodiversity and Evolution.

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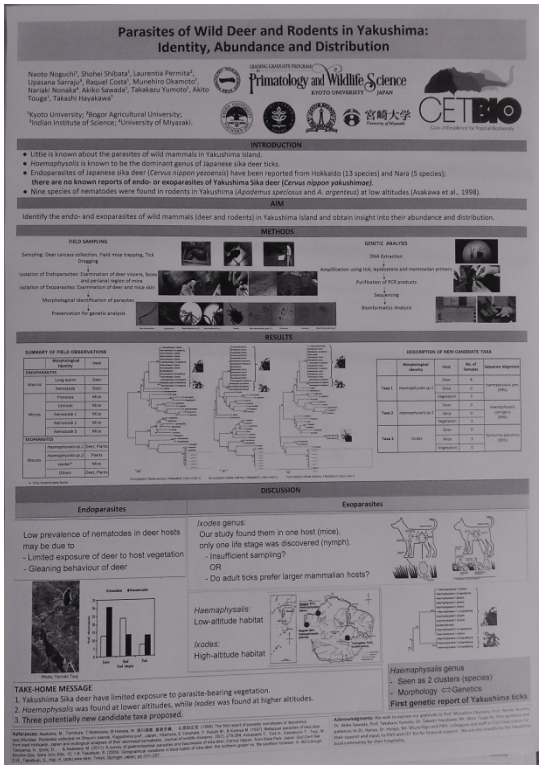

Fig 4. An image of the poster of the Parasite Research Group

6. Others

I am grateful for the support of PWS in making this visit and academic exchange possible. I am also especially grateful to my lecturers, Dr.Takashi Hayakawa, Akito Touge-san and Dr. Munehiro Okamoto, Primate Research Institute, Kyoto University. I look forward to an opportunity to visit again soon in order to take this research forward.