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, 04
Affiliation/Position	Universiti Sains Malaysia
Name	Evan Quah Seng Huat

1. Country/location of visit

Inuyama, Japan

2. Research project

Preliminary assessment of the phylogenetic relationships of fig (*Ficus*) and fig wasps species found on Yakushima Island, Japan

3. Date (departing from/returning to Japan)

2016. 05. 30 – 2016. 06. 03 (5 days)

4. Main host researcher and affiliation

1. Professor Takashi Hayakawa (Primate Research Institute, Kyoto University)
2. Professor 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.

When we got into Inuyama after our field trip the week before at Yakushima, we started the genome portion of the course at the Primate Research Institute (PRI). There were a number of objectives of this part of the course. The first was to extract, purify and sequence the DNA from the fig and insect samples. This was followed by accurate identification of the different species to the lowest taxonomic level by cross referencing the barcode nucleotide sequences against sequences uploaded on GenBank. Subsequently phylogenetic trees of the figs and wasps collected was constructed in an attempt to clarify to the systematics of the species found on Yakushima.

In the few short days we spent at PRI, our team achieved a lot as we extracted, amplified, purified and sequenced DNA from eight fig samples and 48 insect samples and analyzed all the sequence data. For the fig samples we sequenced data from gene atpB-rbcL found in the chloroplast while for the insects we sequenced the 28S nuclear gene, both of which are non-coding genes. The preliminary phylogenetic trees have revealed some very interesting results like *Ficus pumila* and *F. sarmentosa* are a complex of closely related species in need of taxonomic reappraisal, and the difference in the number of wasp species found in monoecious and dioecious species of figs. Monoecious species like *F. microcarpa* and *F. superba* are occupied by many species of wasps whereas dioecious species like *F. pumila* and *F. erecta* have specific species pollinator wasp, such *Wiebesia pumilae* in *F. pumila* and *Blastophaga nippocia* in *F. erecta*.

Although I have a little bit of experience with laboratory procedures used in genetic analyses, this course gave me the opportunity to learn many new techniques and protocols I have not been exposed to before. It was the first time I used Zirconia beads to extract DNA from samples and it was also the first time I learned how to prepare PCR samples for sequencing using the Dye terminator or Sanger method. Previously back at my institution we do not have a sequencer and samples are sent out to companies that run the sequencing for us. I especially enjoyed learning how to purify sequencing reaction products using magnetic beads that was also totally new for me. Another new method I was exposed to was the using of GelGreen Mix dye with agarose gels to stain the DNA for electrophoresis that is a safer alternative to the usage of Ethidium Bromide (EtBr) for staining.

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Through this portion of the course, I have been reminded how important it is to be very careful and meticulous when conducting very sensitive laboratory experiments like DNA extraction and PCR which are very prone to contamination. Despite the precautionary measure we took during our experiments like carefully recording every insect specimen that we had selected for analyses with a digital photograph prior to DNA extraction and sequencing, some of our samples had to be discarded due to some technical mix-up and contamination. I am also glad to have been exposed to alternative molecular methods and protocols with will be of great use to me when I return to my home country to conduct research such as the use of GelGreen mix to dye the amplified PCR products that is much safer to use than EtBr.

Once again I am very grateful for the guidance of the lecturers involved in the course and the efforts of all my team mates who worked hard to make the project a success. Many of the participants did not have any previous experience with molecular analyses but together we overcame the many challenges we faced. Our findings and results are currently being refined to be presented as a poster for the 5th International Seminar on Biodiversity and Evolution: New Methodology for Wildlife Science in Kyoto on the 7th of June 2016.

Fig wasp (*Wiebesia* sp.) collected from the syconia of *Ficus microcarpa* photographed prior to DNA extraction.

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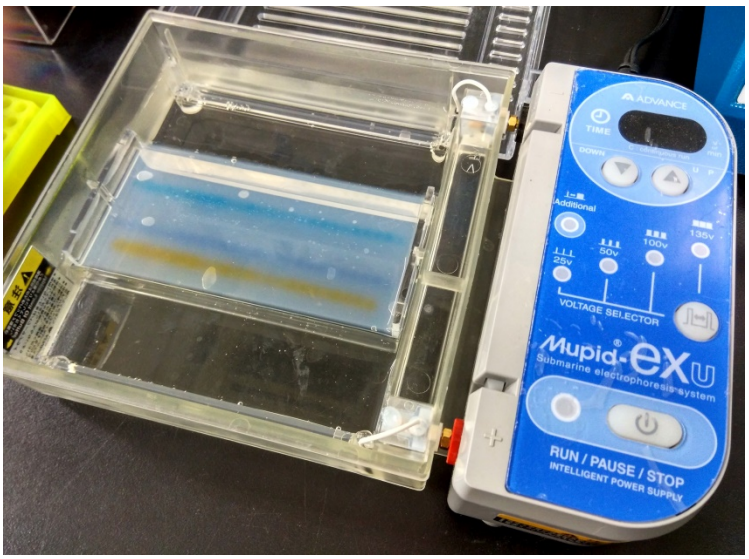
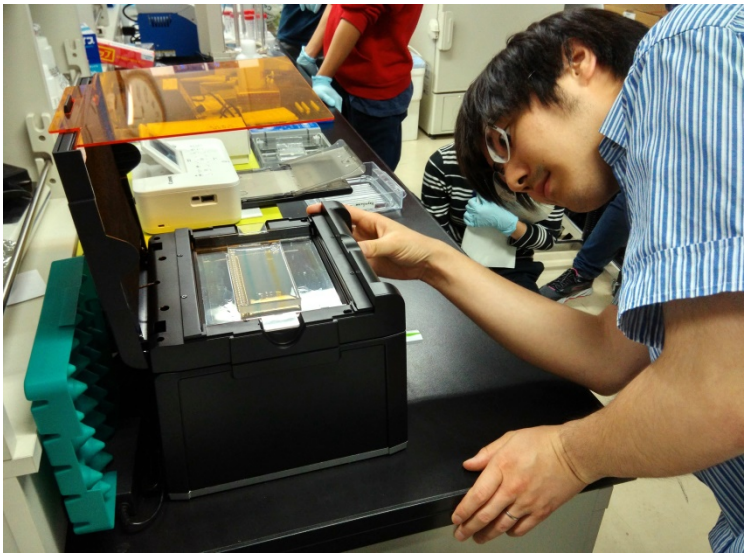
Preparing samples for PCR with teammate Anna Kawakita



Gel electrophoresis of PCR products



Dr. Hayakawa demonstrating how to visualise electrophoresis gels using FAS-LED BOX

Dr. Hayakawa demonstrating the usage of the sequencer and loading samples to be sequenced.

6. Others

Apart from the time we spent at the laboratory extracting, sequencing and analyzing the sequences of samples collected at Yakushima, we had the opportunity to visit some other facilities at the Primate Research Institute (PRI) and the Japan Monkey Centre (JMC). Both these institutions are renowned for the work on the cognitive and behavioral aspects of primates. I was impressed by the large collection of species at the JMC from all around the world which serves as a great education platform for the public to get to learn more about primates in addition to this facility contributing to research together with PRI. In addition, I enjoyed the displays at the visitor's center of the JMC where the specimens were beautifully preserved and prepared. Since a young age I have been very fascinated with taxidermy. There are very few trained taxidermist in the world today and it is a dying art form which few young generation scientist have a passion in picking up and I think that it is a real shame. Taxidermy is a discipline which allows researchers to combine the field of science with art which can result in masterpieces. By bringing into the laboratory knowledge about an animals biology or behavior, one can prepare mounted specimens

from deceased animals that accurately reflect what it would be doing in nature such as the poses it might adopt in natural settings. Thus taxidermy can give these creatures a second lease of life when they are properly immortalized for display.

Visiting the Chimpanzee research facilities at the Primate Research Institute.

Visiting the collection at the visitor's center of the Japan Monkey Centre.

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Meeting a Ring-tailed Lemur at the Japan Monkey Centre.