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.)

	2018.06.10
Affiliation/Position	Laboratory of Ethology, Department of Zoology, Kyoto University / M1
Name	Kouhei Kobayashi

1. Country/location of visit

Japan, Kyoto

2. Research project

Advanced training and Advanced laboratory skills in field biology in 2018

3. Date (departing from/returning to Japan)

2018.05.28 – 2018.06.01 (5days)

4. Main host researcher and affiliation

Koji Takayama (Faculty of Science, Kyoto Univ.)

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.

During the genome course, we conducted DNA barcoding using fern gametophytes and bryophytes on the fern leaves collected in Yakushima.
The species composition of fern is sometimes different between sporophytes and gametophytes even in the same place. Also, some gametophytes can reproduce by themselves without the stage of sporophytes, so they sometimes live in the place where same sporophytes species doesn't exist at all. In the genome research using fern gametophytes, the aim was to examine the difference of species composition between sporophytes identified in Yakushima and gametophytes identified by using BLAST. Regards to bryophytes, we had two aims in genome research; identifying species lives in Yakushima and revealing how many species were on one fern pinnule.

Day 1 (5/28)
We washed and cut each sample (gametophytes and bryophytes) and put them into each PCR micro tubes. We dispensed PCR mix into each tube and conducted Tissue-direct PCR (first).
(*We used different primers to gametophytes and bryophytes.)

Day 2 (5/29)
First, we conducted Tissue-direct PCR (second). Then, we conducted electrophoresis experiment to check the PCR fragments. After that, we purified PCR products which certainly amplified using ExoSAP-IT.

Day 3 (5/30)
(Regards to PCR products which didn't amplify, Mr. Takayama and his students conducted PCR again, and checked by electrophoresis experiment.)
First, we purified additional PCR products. Then, we conducted Cycle sequencing using purified PCR products.
（Primer of gametophytes were rbcL-af and rbcL-888R. Primer of gametophytes were rbcL-HrL1and rbcL-1301RL.）
In the afternoon, we purified Cycle sequencing products and finally, we applied plates to a next generation sequencer.
(* 83 gametophytes samples and 7 bryophytes samples. Prepared forward and reverse.)

Day 4 (5/31)
Because a next generation sequencer failed, we made the poster for international seminar in the morning.
In the afternoon, we received the lecture about how to identify species from results of sequence by MEGA7 and BLAST.

Day 5 (6/1)
We identified species from a few sequence data. (*10 samples- 1 bryophytes and 9 gametophytes.) Also, we discuss the results of field research in Yakushima again and made the poster.

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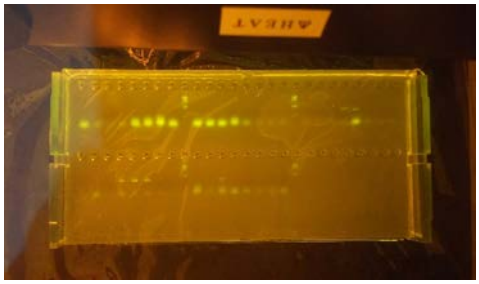
(6/4)
We finished the poster.

(6/5)
We showed our poster (mainly about field research and a few data of genome research) at International seminar held in Kyoto Univ.

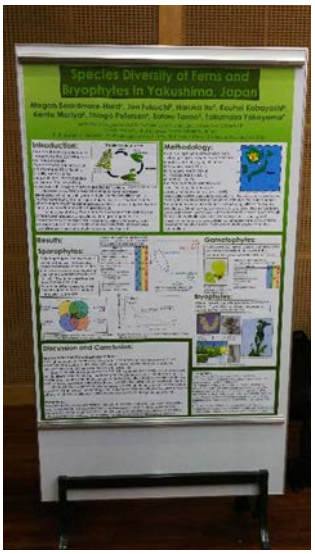
Unfortunately, we don't get enough genome data now because of the defect of sequencer. However, Mr. Takayama will fix it and conduct again. So, after that, our team will identify fern gametophytes and bryophytes on the fern leaves, and discuss the result.

Working table in experimental room



Day 2 result of electrophoresis experiment

A next generation sequencer



Poster in International Seminar

6. Others

Although we couldn't get enough DNA barcoding results during this course, I was able to experience technique of DNA analysis which I have never done in my usual study. In our discussion, I had a little difficulty of communicating in English with a few members, but thanks to other member's assistance, it was very meaningful opportunity for me. Again, throughout field and genome research, I really appreciate professors and staffs who held those courses.