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.)

	2015. 11. 1
Affiliation/Position	Institute for Frontier Medical Science, Kyoto University / M1
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1. Country/location of visit

Kyoto, Kyoto Prefecture, Japan

2. Research project

Species composition and phenology in fern sporophyte and gametophyte

3. Date (departing from/returning to Japan)

2015. 10. 26 – 2015. 10. 30 (5 days)

4. Main host researcher and affiliation

Dr. Fuse of Kyoto University and Dr. Sato of Center for Ecological Research

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 genome science course, we analyzed samples collected in Yakushima field science course using techniques of molecular biology. I belonged to the Plant team. We identified the species of ferns collected in Yakushima by analyzing DNA sequence, and studied their distribution in Yakushima Island. In previous field science course, we could morphologically identify the species of sporophytes. However, we could not morphologically identify the species of gametophytes because they are tiny and indistinguishable. Therefore, we tried to identify the species of gametophytes by analyzing DNA sequence.

Methods are as follows:
1st day: We five selected 8 gametophytes and 8 sporophytes per person, and total 80 samples were analyzed. We crashed sporophytes and extracted DNA. We did not crashed gametophytes. We amplified rbcL (ribulose bisphosphate carboxylase/oxygenase large subunit) gene using PCR technique. rbcL gene is commonly used for species identification.
2nd day: We checked amplification of rbcL gene and performed cycle sequence (dideoxy sequencing), and then analyzed samples by ABI3130 sequencer.
3rd day: We performed PCR of gametophytes because we could not get enough rbcL gene of gametophytes.
4th day: We identified the species of gametophytes and sporophytes by using BLAST
5th day: We drew phylogenetic tree of samples we collected, and prepared for presentation.

Result
We failed in sequencing of gametophytes DNA. We got only few data and therefore we could not compare about locations and seasons. Also sequence data is too short to identify the species by BLAST.
But we identified the genus name of gametophytes referred to phylogenetic tree (we cannot identify specific epithet). Based on these results, we discovered 3 genera of gametophytes which live in different locations from locations its sporophytes live in. Moreover, some gametophytes were collected only in Spring or Autumn.

What I regret is failing in sequencing gametophytes DNA. We got only few data, which made discussion difficult. We were going to compare our data in Autumn with previous data in Spring 2014, but we could not because data is not enough. Therefore, I suggest 2 improvements about methods: 1. Gametophytes should be crashed and sequencing will be succeeded. 2. We should have selected samples from fewer locations. In this course, we selected 8 samples from all 6 locations and almost failed in sequencing, therefore we could not compare about locations and seasons. By selecting samples of fewer locations, we can get more samples and information about one location, which will make discussion easy.

We are going to present these results with outcome of previous field course on November 5th.

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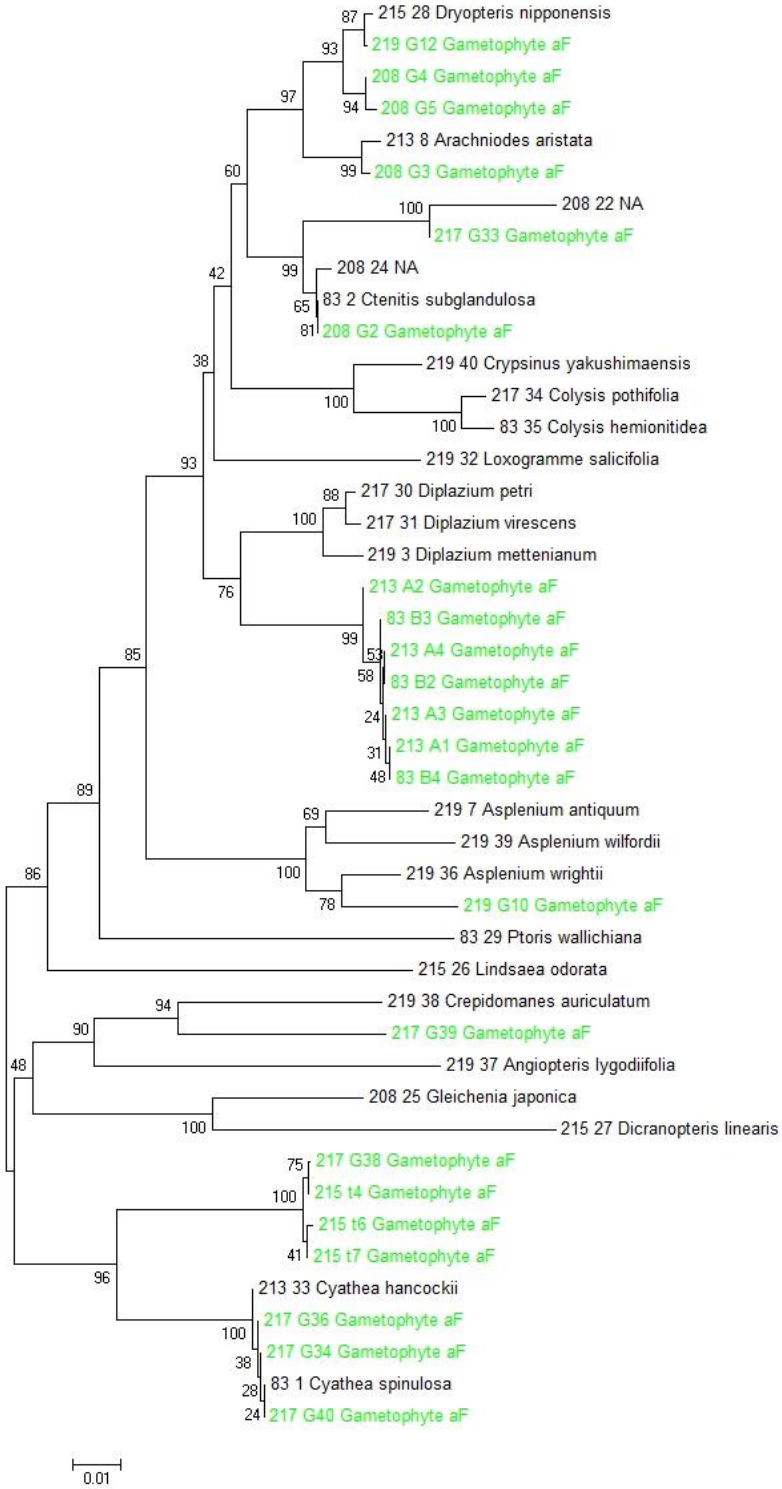

Phylogenetic tree of samples we collected

6. Others

I thank Dr. Fuse, Dr. Sato, Dr. Shinohara and members of plant team.