

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.)

2015.11.01	
Affiliation/Position	PRI/D1
Name	Duncan Wilson

1. Country/location of visit
Yakushima (Kagoshima Prefecture) and Kyoto University North Campus, Japan
2. Research project
Species composition and phenology in fern gametophytes (Yakushima and Genome Science courses)
3. Date (departing from/returning to Japan)
2015. 10.18 – 2015.10.30 (12 days)
4. Main host researcher and affiliation
Yakushima: Prof. Hiroshi Kudoh, Prof. Wataru Shinohara, Genome: Prof. Hirotohi Sato, Prof. Shizuka Fuse
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.
<p>I participated in the Fall Yakushima Field Science and Genome Science courses as a member of the ‘Plant Team’. The aim of the project was to investigate species composition and phenology in fern gametophytes in Yakushima. Ferns typically observed in forests are in their sporophyte stages. Compared with sporophytes, fern gametophytes are very small and to date no sufficient morphological characteristics useful for species identification have been found. Recently, however, developments in molecular analysis are helping with gametophyte species identification. We collected gametophytes from several places and attempted to compare their species composition to that of samples collected in spring the previous year. For the gametophyte samples collected from Yakushima, we identified the species or the genus to which they belong using molecular analysis. We extracted DNA from the samples to determine rbcL gene sequences from them. We then compared these sequences with registered DNA sequences in a gene bank.</p> <p>Yakushima - Fern sporophyte and gametophyte collection: Day 2 (Oct, 19): Sample collection at three locations (53m, 136m, and 467m altitude) at Miyanoura River basin. Day 3 (Oct, 20): Sample collection at two locations (27m, 58m altitude) at Onna River basin. Day 4 (Oct, 21): Sample collection at one location (193m altitude) at Hanaage River basin. Day 5 (Oct, 22): Sporophyte preliminary identification and gametophyte preparation, presentation preparation. Day 6 (Oct, 23): Presentation.</p> <p>Kyoto University - Fern sporophyte and gametophyte identification: Day 9 (Oct, 26): DNA extraction (sporophytes) and Polymerase Chain Reaction (PCR) (gametophytes). Day 10 (Oct, 27): PCR (sporophytes), electrophoresis, purification, cycle sequencing, denaturing and genetic analysis. Day 11 (Oct, 28): PCR repeated (rbcL gene amplification failed the previous day). Day 12 (Oct, 29): Editing of raw sequence data. Day 13 (Oct, 30): Editing of raw sequence data, analysis, and presentation preparation. Nov, 5: Presentation on results of both courses.</p> <p>We found species composition of fern sporophytes varied with location, but not season (only the Hanaage River basin was sampled in spring, so sporophyte distribution was unknown). We did not have enough gametophyte samples to determine whether gametophyte species composition varied with location or season. Our findings are discussed in detail in an academic report submitted separately to this report.</p> <p>During these courses I learnt many different laboratory techniques and used specialised equipment, potentially useful for my future career. I also learnt how to work together in a fieldwork team through collecting samples, analysing data, preparing presentations and writing academic reports with other members. In addition, it was a great opportunity to see many indigenous species on the island, including Yakushima macaques, Sika deer, ancient Japanese Cedar trees, and of course, ferns! I would like to express my gratitude to the course organisers for their invaluable assistance.</p>

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6. Others



Fern collection guidance (Photo: Hiroshi Kudoh)



Fern sample preparation



Giant fern (Photo: Hiroshi Kudoh)



Ferns (Photo: Hiroshi Kudoh)



Gametophyte collection (Photo: Hiroshi Kudoh)



‘Kigensugi’ Cedar tree (est. 3000 years old!)



Yakushima macaques (Photo: Hiroshi Kudoh)



Sika deer (Photo: Hiroshi Kudoh)

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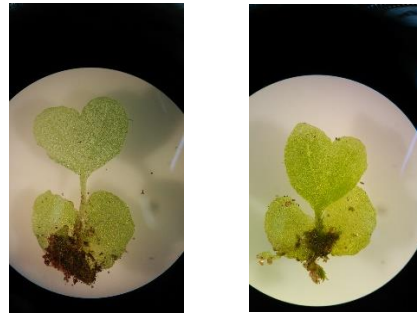
Miyanoura River basin



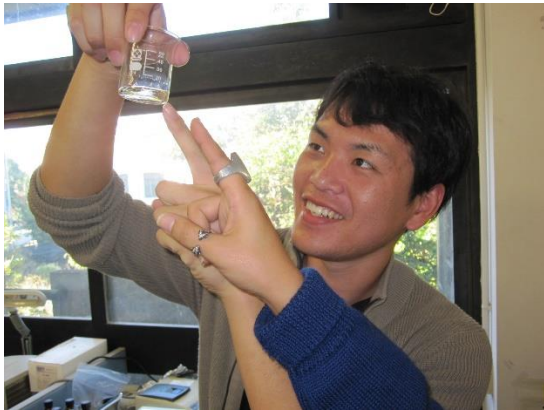
The Plant Team (Photo: Wataru Shinohara)



Sporophyte identification (Photo: Hiroshi Kudoh)



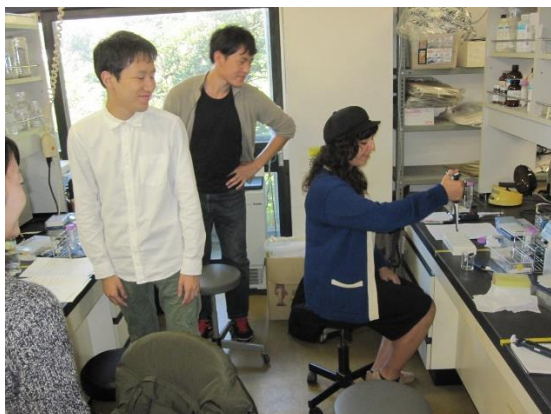
Gametophytes (lower) with attached sporophytes



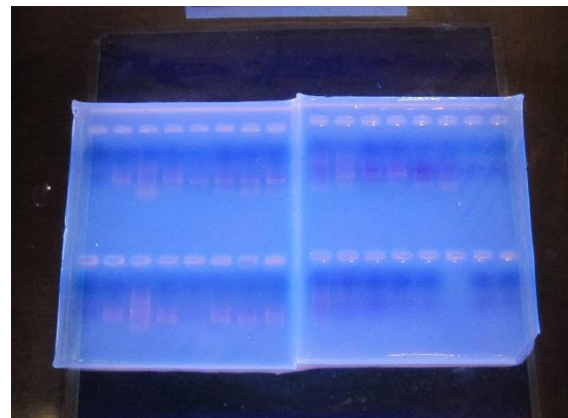
Preparing gametophyte samples for DNA analysis



A ‘TissueLyser’ grinding up our sporophyte samples



PCR (Polymerase Chain Reaction) to amplify DNA



Electrophoresis to separate DNA fragments