Research Activity Report
Supported by "JSPS Core-to-Core Program(International Core of Excellence for Tropical Biodiversity Conservation focusing on Large Animal Studies)" "Leading Graduate Program in Primatology and Wildlife Science"

(Please be sure to submit this report after the trip that supported by CETbio,PWS.)

	2018. 06, 08
Affiliation/Position	University of Oxford/Research Assistant and future DPhil Student
Name	Megan Beardmore-Herd

1. Country/location of visit

Kyoto University, Japan

2. Research project

Yakushima Genome Science Course: Plant Group

3. Date (departing from/returning to Japan)

2014. 05. 28 – 2014. 06. 01 (5 days)

4. Main host researcher and affiliation

K. Takayama (Kyoto University), W. Shinohara (Kagawa 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.

Following the Yakushima Field Science Course, I attended the Genome Science Course at Kyoto University. We aimed to analyse the gametophyte and bryophyte samples we collected in Yakushima back in the laboratory at Kyoto using DNA barcoding, in order to identify which species or genera they belonged to and compare the results with the sporophyte species identified in the same localities.

We began with DNA extraction and tissue-direct PCR. We washed each gametophyte sample in a water-filled dish and, if necessary, resized them to be 1mm x 1mm using sterile razors. The fragments were then placed in PCR micro tubes and pipette tips were used to break up the tissue. We dispensed 10µl of pre-mixed PCR reaction mixture into each PCR microtube. The microtubes were then placed into a thermal cycler and then following cycle was conducted:

PCR Parameter

1) 94°C for 5 mins
2) 94°C for 30 secs
3) 50°C for 30 secs
4) 72°C for 60 secs
5) 72°C for 7 mins
6) 10°C ∞

35 cycles

Next, we conducted a secondary PCR by repeating the same thermal cycling procedure using 0.5µl of the previous PCR product and 9.5µl of a newly mixed PCR reaction mixture. After, we conducted gel electrophoresis. First, we prepared the gel (1% agarose with Gelred) by mixing 1g of agarose with 100ml of 1X TAE buffer and heating in a microwave until dissolved. We then set the gel in a combed gel tray and, once ready, added the PCR product and a gel-loading buffer to the wells. After performing the electrophoresis, we viewed the gel on a Super LED Viewer in order to observe the DNA fragments. Next, we purified the PCR products and conducted cycle sequencing. Finally, we purified the sequencing products and applied the plates of samples to a genetic analyser.

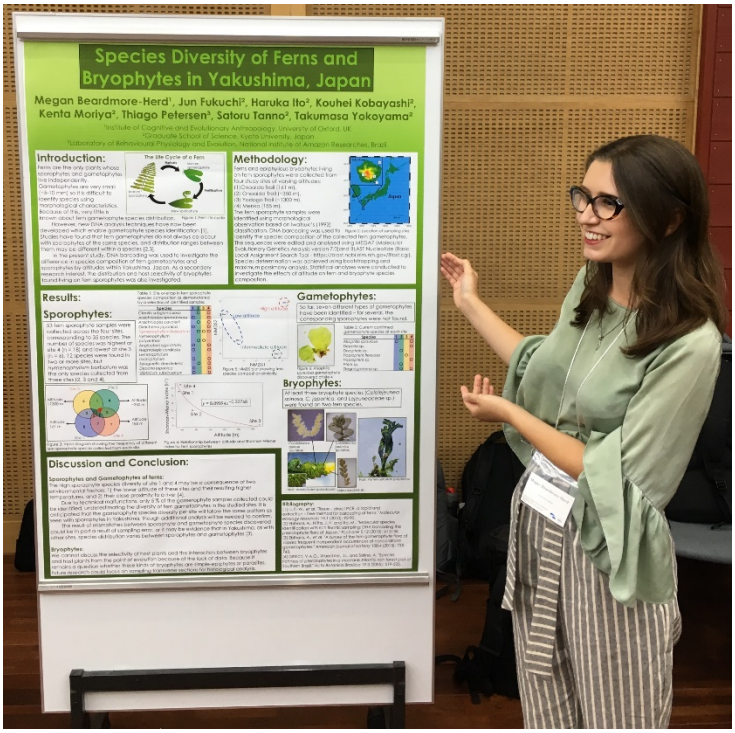
We edited and analysed the sequences using MEGA7 (Molecular Evolutionary Genetics Analysis version 7.0) and BLAST Nucleotide (Basic Local Assignment Search Tool - https://blast.ncbi.nlm.nih.gov/Blast.cgi). Species determination was achieved using bootstrapping and maximum parsimony analysis. Statistical analyses were conducted to investigate the effects of altitude on fern and moss species composition.

Due to technical malfunctions, only 5 % of the gametophyte samples collected could be identified, underestimating the diversity of fern gametophytes in the studied sites. Despite this, we managed to identify seven fern gametophyte

species. It is anticipated that the gametophyte species diversity per site will follow the same pattern as seen with sporophytes in Yakushima, though additional analysis will be needed to confirm this. Interestingly, we identified some gametophyte species for which we did not find the corresponding sporophyte species. This result could be in part due to sampling error, or it may be evidence that in Yakushima, as with other sites, species distribution varies between sporophytes and gametophytes.

Regarding the bryophytes, at least three samples were found, belonging to two species (*Cololejeunea spinosa* and one other unidentified), on two different fern species. More data could hopefully allow the assessment of host-selectivity, since our results indicate that more than one species of fern may act as a host for bryophytes.

Through this experience, I have developed my laboratory skills, which will be of great help with my future research. Additionally, we created a poster detailing our research and presented it at the 8th International Seminar on Biodiversity and Evolution, producing an output from our work and allowing me to develop my groupwork and presentation skills.



Presenting our plant group poster at the 8th International Seminar on Biodiversity and Evolution

6. Others

I would like to express my gratitude to K. Takayama, W. Shinohara, H. Kudoh, S. Fuse the laboratory assistants for their guidance and support, as well as my fellow plant team members for their hard work. Finally, I would like to thank PWS and CET-Bio for organising my attendance of the Genome Science Course and financially supporting my trip to Japan.