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. Oct, 31
Affiliation/Position	Kyoto University Primate Research Institute/M1
Name	Nelson Broche

1. Country/location of visit

Inuyama, Aichi (Kyoto University PRI)

2. Research project

Genome Science Course (genomic techniques and cortisol & testosterone hormonal assay training)

3. Date (departing from/returning to Japan)

2016. Oct. 24 – 2016. Oct. 28 (5 days)

4. Main host researcher and affiliation

Dr. Kodzue Kinoshita, Dr. Takashi Hayakawa (both researchers affiliated with Kyoto University PRI)

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.

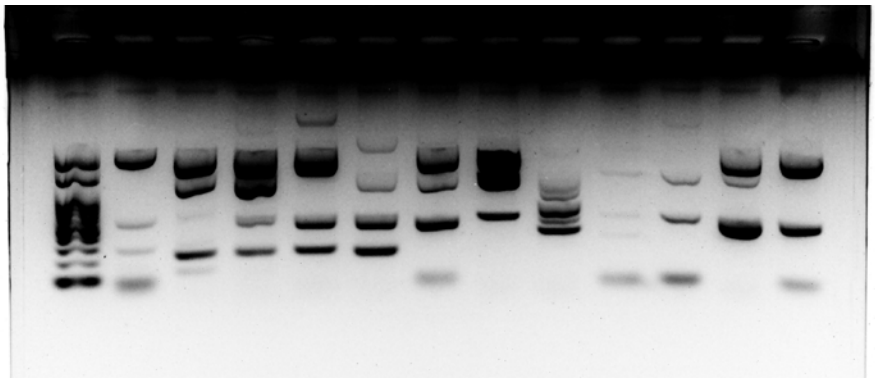
This report is a direct continuation of the Yakushima field course report. For the Genome Science Course, our goal was to use genome techniques for our Yakushima macaque (*Macaca fuscata yakui*) fecal samples in order to more confidently determine the sex of collected samples. Not all fecal samples we collected were directly observed in the field during defecation, and some individuals were difficult to distinguish sex due to young age. The next step of our lab work consisted of assaying the immunosuppressive hormones cortisol and testosterone. Then we looked for any sex difference relationships between immunosuppressive hormones and our sample prevalence of nematode parasitism.

For the genome portion, we extracted DNA from 72 out of 91 total fecal samples. We were not entirely confident using observations of determining sexes for non-adult (i.e. below reproductive age) Yakushima macaques, therefore these samples took precedence for sex determination genome sequencing. DNA fecal samples were kept in 1 ml lysis buffer until DNA extraction. We then used proteinase and RNase in order to break down proteins and RNA, leaving mostly intact DNA in the samples. Next we binded working DNA to tube columns, followed by washing the columns, which allows for more purification of the DNA sample. Through elution we removed DNA from the columns. We then amplified genes SRY (only located on Y chromosome) and ZFXY (associated with both sexes) by polymerase chain reaction (PCR) and identified sex by electrophoresis. We found that the electrophoresis results were difficult to read, from which Dr. Hayakawa commented that further amplification by PCR could make the electrophoresis results clearer to read. We had more confidence in our observational data for sex determination than our DNA samples. In the end used observational data sex determination in our statistical analysis.

For hormone assaying, feces samples were freeze dried, then placed in a "crusher" with 3 ml of 80% methanol, which prepared the fecal samples for extraction. Extraction involved vortexing for 30 minutes, centrifugation, and finally removing 0.5 ml of supernatant, which became our fecal extraction sample. Testosterone and cortisol concentrations were quantitatively measured by a double antibody enzyme immunoassay. The 1st antibody was added to the plate coated in the 2nd antibody. Then we added HRP binding antigen and fecal extraction sample to each well, incubated the plates overnight in the dark at 4°C. Substrate buffers were then added to the wells, followed by sulfuric acid in order to stop the reaction. A standard curve was used to measure hormone concentration. We found no significant relationship between our sample prevalence of nematode parasitism and the immunosuppressive hormones testosterone and cortisol. However, as expected, we did find that males had significantly higher levels of testosterone than females and juveniles.

Through the Genome Science Course I have learned that using genomic techniques in order to determine sex identification is an intricate process that requires careful precision. I am now more able to clearly see how genomic techniques can be a useful tool to help answer research questions. As for hormone assaying, I plan to further study the endocrine response to stress within primates and personally found it very practical for me to become familiar with hormone assaying. The techniques learned in this course will be necessary for later work with my own research

studying stress in Japanese macaques (*Macaca fuscata*) and other macaque species.



Amplifying by PCR once more would have likely made our electrophoresis results clearer to read.

6. Others

Thank you to Dr. Kodzue Kinoshita and Dr. Takashi Hayakawa for patiently and kindly answering hormone and genome questions, allowing us to use the lab space, and for leading this course. Also, thank you to Dr. Takushi Kishida and Ms. Yu Sato who assisted us in the lab. I am grateful to PWS for funding this course. And finally thanks to my fellow lab mates who I very much enjoyed working together with during this course.