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. 03
Affiliation/Position	Wildlife Research Center/D1
Name	Miho Saito

1. Country/location of visit

Inuyama, Japan

2. Research project

Genome course (deer team)

3. Date (departing from/returning to Japan)

2015.10.26 - 2015.10.30 (5 days)

4. Main host researcher and affiliation

Dr. Hayakawa, Dr. Kishida and Dr. Kinoshita

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.

We used fecal samples from Yakushima field course for the experiments and did DNA analysis to identify if this fecal was from female or male and also did hormone analysis for the same purpose.

1. DNA analysis

<Methods>

DNA extraction, purification->PCR->Electrophoresis

<Results and improvements>

- We collected 54 samples and put each samples into small plastic bags. However, we noticed that there were 2 samples which was named as "sample No.20". We didn't write down additional information like a sampling time/ person who collected that sample. Therefore, we needed to remove these two samples from analysis because we couldn't identify which is the correct sample as "20". Based on this experience, if I have a chance to do this kinds of field work, I would write more information on sample bad.

- We forgot to run electrophoresis with control. Control is important to know the level of noise, therefore we should not make a same mistake from next time.

- Two electrophoresis were conducted for the same time duration. However, the marker of one of them didn't dissociate clearly. Therefore, it was difficult to know the bp of samples. We may mistook to calculate the composition of the gel. We need to pay more attention when creating gel.

- Some of samples were difficult to detect sex because purification was not good enough. Maybe the reason is our way of swabbing feces was not appropriate. To improve a methods, it is suggested to establish more efficient way of collecting DNA from ungulate's dry feces or try to use different buffer.

- As a result, most of the samples which could assume sex from electrophoresis showed a same result from direct observation. Therefore, DNA analysis is an efficient method to detect a sex of individuals. Giraffe is much easier to detect the sex, however, sometimes juvenile/calf is difficult to detect it. In that case, I would like to collect feces of that individuals and perform DNA analysis. I am interested in studying blood relationship among females, so this course was a good chance for me to learn the basic method of DNA analysis.

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.)

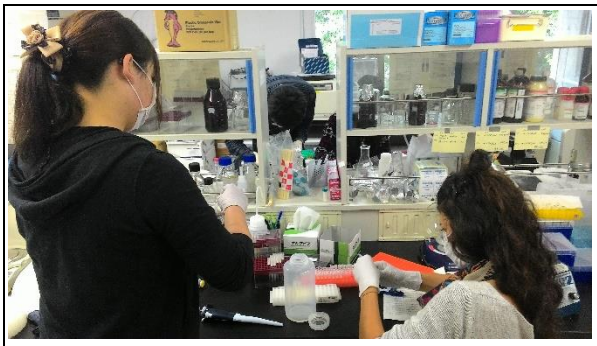

Figure 1. Purification of DNA from feces

Figure 2. Set samples into gel for electrophoresis

2. Hormonal analysis

<Methods>

1. Dilution of sample with ethanol. Performed vortex. Collect supernatant liquid and mix it with EIA buffer
2. Prepared standard from S1 to S10
3. Prepared HRP antigen and antibody
4. injected the products from step 1, 2 and 3
5. Performed incubation after added substrate buffer
6. After added N-H_2SO_4, read the plates with a microplate reader

<Results and improvements>

- It was my first time to do hormonal analysis, so it was good experience to know new methods and new instruments.

- There were many technics which I could not learn by just reading text, for example, I need to absorb sample with pipet-man at vertical angle to absorb correct amount. Therefore, it was my good opportunity to learn such kinds of experimental technics from experts.

- Our team was responsible for progesterone. As a result, we could state that the value of progesterone in feces of female in breeding season were higher than that of non-breeding season. Additionally, that hormonal level in feces were higher in female than in males. Therefore, hormonal analysis of progesterone is an efficient way to assume breeding season of female and also to assume the sex of each individual.

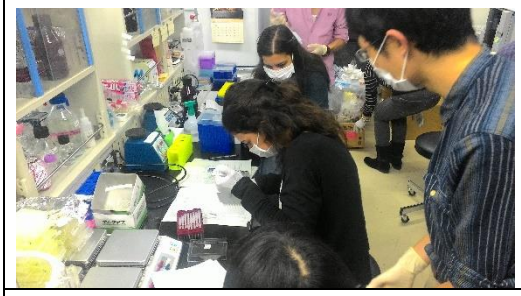

Figure 3. Adding substrate buffer before incubation

6. Others

I would like to express my sincere gratitude to the PWS program for supporting this course. Many thanks to Dr. Hayakawa, Dr. Kishida and Dr. Kinoshita for guiding this course.