

Research Activity Report

Supported by “Leading Graduate Program in Primatology and Wildlife Science”

2025.11.12	
Affiliation/Position	Wildlife Research Center/M1
Name	Alexander Hendry

1. Country/location of visit
Kyoto, Japan
2. Research project
Advanced Laboratory Skills for Field Biology
3. Date (departing from/returning to Japan)
2025.11.04 – 2025.11.07 (4 days)
4. Main host researcher and affiliation
Professor Miho Murayama, Wildlife Research Center
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.

Laboratory skills are useful for field researchers as laboratory research can help to identify the relationships between study subjects, the species of prey items consumed, and other highly relevant information to correctly interpret field observations. Although I am predominately a field-based researcher, gaining skills and experience in laboratory research techniques will help me become a more rounded researcher. This is why I decided to enroll in the Advanced Laboratory Skills for Field Biology course in November 2025.



Figure 1: Students collecting feathers at Kyoto University's main campus (Yoshida).

Day 1 – 4th of November 2025

The November 2025 Advanced Laboratory Skills focused on species identification through DNA barcoding using DNA extracted from feathers. The course commenced with the students and Professor Murayama assembling at the laboratory at the Wildlife Research Centre. We then headed to the Yoshida South campus to search for feathers. We found a few feathers at the south campus before we headed to the nearby Yoshida Shrine to look for more. Finally, we returned to the main campus where we found a few more feathers (Figure 1.). In total we collected 12 feathers at the Kyoto University campus. Participating student Eiichiro Ozasa collected two feathers at the Kyoto Imperial Palace, and we also used two stored feather samples collected from Ishigaki, Okinawa. In total we attempted to extract DNA from 16 feathers (Figure 2). Our goal was to extract DNA from the mtDNA (mitochondrial DNA) COI region. The mtDNA COI region refers to cytochrome c oxidase subunit 1 region and this region is regularly used to identify bird species as it is highly conserved between species.



Figure 2: The feathers that we had collected arranged on the laboratory bench ready for DNA extraction.

After a discussion on the importance of preventing contamination in laboratory research, we began the DNA barcoding process. The first step was the extract the DNA from the feathers. We cut the basal tip of the feathers

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off and placed them in sample tubes. We then aimed to destroy the cells within the feather tips in order to extract the DNA inside. This was done through repeated processes of incubation, centrifuging, and the addition of the enzyme proteinase K. While the samples were stored in the freezer, we prepared the gel for gel electrophoresis. Gel electrophoresis is a process used to separate DNA fragments based on their length and we used it to identify which samples we had successfully extracted DNA from. After we had made the electrophoresis gel, we then attempted to amplify the mtDNA COI gene using PCR (polymerase chain reaction). This process occurred overnight.

Day 2 – 5th of November 2025

We returned to the laboratory the following morning to continue with our DNA extraction. The PCR process was complete and we could now run the gel electrophoresis. This process involved pipetting DNA samples and dye into small wells in the electrophoresis gel. An electric current was then run through the gel which pulled the oppositely charged DNA particles through the gel. How far the DNA was pulled through the gel was dependent on its size. We found that we had successfully extracted

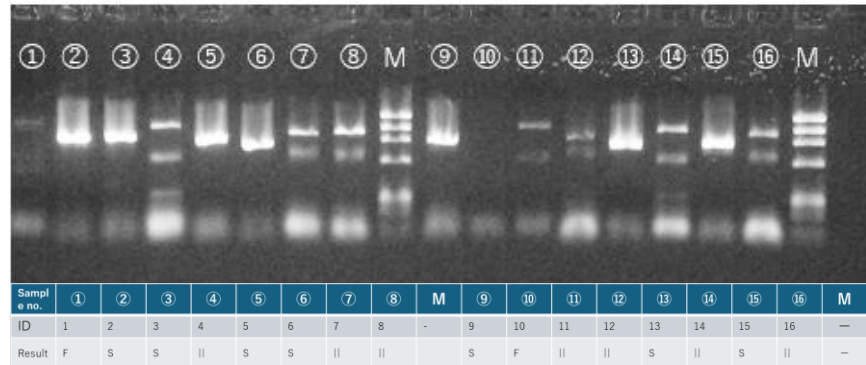


Figure 3: Gel electrophoresis results from our DNA extraction from bird feathers. In the table S standards for successful extraction, F stands for failed extraction and || standards for fragments of two different lengths of DNA extracted (partial success).

DNA from 12 of our 16 samples (Figure 3). We then purified the samples using binding buffers and washing buffers to remove primers before sequencing the DNA. Preparing the samples for sequencing was a long and detailed process. We used a DNA sequencer to identify which of the four base pairs (adenine - A, cytosine - C, guanine - G, and thymine - T) was most likely at each position on the extracted mtDNA strands. Using the FinchTV software we viewed the chromatogram base pair sequence. We then compared this sequence to sequences stored in an online library called the NCBI BLAST database. It was a very suspenseful and exciting moment to finally determine which species each of our feathers came from. Out of the successful extractions we had one oriental turtle dove (*Streptopelia orientalis* - キジバト) feather, six pigeon feathers (*Columba livia* - カワラバト), two carrion crow (*Corvus corone* - ハシボソガラス) feathers, and three sooty tern feathers (*Onychoprion fuscatus* - セグロアジサシ). Most surprisingly two of the sooty tern feathers were collected at the Kyoto University campus (the other came from Ishigaki in Okinawa). The sooty tern is a tropical species that has only been recorded in Okinawa and the Ogasawara Islands in Japan. The samples we found probably came from a vagrant sooty tern as Kyoto is a landlocked city and it is unlikely that a pelagic bird such a tern could find sufficient food in Kyoto. Alternatively, contamination with DNA from the Ishigaki samples could have occurred. Some of the feathers we collected were very small demonstrating how even small pieces of biological material can be used for DNA extraction

Also, during this day Nakamura-san showed us the gel electrophoresis results for sex identification of a brood of wild Blakiston's fish owl (*Ketupa blakistoni* - シマフクロウ) chicks from Hokkaido using blood samples. Data such as this is essential in managing the populations of endangered species and ensuring appropriate pairings. I found this fascinating, and it reminded me of the important role genetics and laboratory research can play in wildlife conservation.

Reflection

I have minimal experience in laboratory research, so every aspect of this course was new for me. I was surprised to learn how many steps the process of DNA extraction has and how long it can take. I also learnt that there are many opportunities for contamination to occur during DNA extraction and you have to be very careful and methodical in laboratory research and ensure that you are accurately labelling your samples to avoid errors. Although my interests and experience primarily lie with field research, I am grateful for the experience of doing the Advanced Laboratory Skills for Field Research Course as it has made me consider how laboratory research can complement and inform field research and conservation.

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*Murayama-sensei and the participants of this course outside the clock tower at the
Kyoto University Yoshida campus.*

6. Others

- I would like to express my gratitude to Professor Murayama for allowing me to join this course at short notice
- I would also like to thank the PWS program for supporting this course
- The tutors of this course Xorlali Azimey, Fadel Azhari, Hizuki Nakamura, and Yu Sato deserve thanks for their useful guidance and patience with students who are beginners at laboratory research