Field Science Course and Genome Science Course in 2015

1.1 Outline

"Field Science Course" aims to train students to do fieldwork on the UNESCO World Natural Heritage Site on Yakushima Island, Japan. Young scientists from abroad and graduate students in Kyoto University attend the course together, using English as an official language.

"Genome Science Course" aims to train students in the molecular biology, from rudimentary DNA sequencing techniques to the analyses of high-throughput next-generation sequencer data. In this course, various samples collected in the preceding "Field Science Course" will be analyzed. Through the two courses, students will experience the whole process of scientific research, sampling in the field, analyzing in the laboratory, conducting data analysis and presentation of the results. Three 'beginners' courses and an 'advanced' course will be provided.

No previous experience is required to take these courses and we welcome both students who engage in fieldwork and those who engage in laboratory work. We also welcome students who have few chances to communicate in English. Please communicate with foreign students of the same generation.

In the Field Science Course, students will learn the fundamental methods to study the ecology and behavior of various wild animals. We stay in a small village, having local food. We hope you enjoy the nature and culture of Yakushima Island.






1.2 Application

A graduate student of Biological Science, Faculty of Science, Graduate School of Kyoto University (from April 2015) can apply for the courses. Please note that we accept a limited number of participants due to safety for fieldwork and limited capacity of accommodation and transportation.

We will hold the course twice this year, in spring and fall. The contents of spring and fall courses are different, and students may take only one of them. If an applicant was unaccepted for the spring course, he/she may apply again for the fall course.

In both seasons, the Field Science Course will be held in Yakushima Island. We hold the Genome Science Course at Yoshida Campus of Kyoto University in Kyoto City in spring, and at the Primate Research Institute in Inuyama City in fall.

Students may apply to either the Field Science Course or Genome Science Course, though we encourage to take both of the courses.

1.3 Fee

Typically, no fee is required. During the Field Science Course in Yakushima, please pay by yourself the cost of optional activities such as entrance fee of sightseeing sites, public bath and snacks and meals other than those provided in our field station.

2. Schedule

Field and Genome Science Courses in spring

April 16 Deadline for application by students of Kyoto University (both for the Field Science Course and Genome Science Course)

May 18, 4:00 p.m. Guidance for the Field Science Course and Genome Science Course, at WRC in Yoshida Campus. We will have a welcome party for participants from abroad.

<u>Field Science Course (Yakushima Island, Kagoshima Prefecture)</u>

May 23 Field Science Course starts (Move to Yakushima on this date)
May 24-27 Fieldwork in Yakushima
May 28 Data analysis, presentation in the afternoon
May 29 Leave Yakushima

<u>Genome Science Course at Kyoto University (Yoshida Campus, Kyoto City)</u>

June 1-5 Genome experiments, at several laboratories in Yoshida Campus of Kyoto Univ.
June 8 Preparation for presentation
June 9 Presentation of the results of the courses at international symposium (held at Yoshida Campus)

Field and Genome Science Courses in fall

Beginning of September	Deadline for application (both for the Field Science Course and Genome Science Course)
Middle of October	Guidance for the Field Science Course and Genome Science Course, at PRI, Inuyama.

Field Science Course (Yakushima Island, Kagoshima Prefecture)

October 18	Field Science Course starts (Move to Yakushima on this date)
October 19-22	Fieldwork in Yakushima
October 23	Data analysis, presentation in the afternoon
October 24	Leave Yakushima

Genome Science Course at Kyoto University (Primate Research Institute, Inuyama City)

October 26-30	Genome experiments, at several laboratories in Inuyama (animal) and Yoshida (plant) Campus of Kyoto Univ.
November 5	Presentation of the result of the courses at Primate Research Institute

3. Field Science Course in Spring (May 23-29)

3.1 Participants

Nine students who learn biology from India, Malaysia, Brazil and Tanzania.
About 12 graduate students of Biological Science, Kyoto University
About 10 teaching staff, including professors, post-doc and graduate students who study in Yakushima and/or subject species

3.2 Groups

We form four groups: monkeys, insects, mushrooms and deer. Each group will engage in different tasks. Choose your preference of the groups, from first to fourth. Please note that we cannot ensure your first preference, due to limited capacity of each group.

A) Monkey group

Title

Ecological role of monkeys in yamamomo seed dispersal: Are they true dispersers?
ヤマモモ種子散布過程におけるサルの役割－散布者か捕食者か？

Lectures

Akiko SAWADA (Wildlife Research Center, Kyoto Univ.) 澤田晶子(京都大・野生動物)
Takafumi SUZUMURA (Wildlife Research Center, Kyoto Univ.) 鈴村崇文(京都大･野生動物)

Akiko TAKAHASHI (Wildlife Research Center, Kyoto Univ.)　高橋明子(京都大･野生動物)
Yosuke KURIHARA (Primate Research Institute, Kyoto Univ.)　栗原洋介(京都大・霊長研)

Abstract

We will observe wild Yakushima monkeys *(Macaca fuscata yakui*), one of the endemic species on Yakushima Island. Monkeys in the western coastal area are well-habituated to human due to long-term studies since 1975. While Yakushima monkeys are thought as a seed disperser of drupes, it has also been reported that they sometimes crack the seeds.

Through behavioral observation and fecal analysis (e.g., counting cracked/intact seeds in feces), we will discuss the ecological role of Yakushima monkeys in seed dispersal. Particularly, we will focus on fruits of yamamomo (*Myrica rubra*), the main food item for the monkeys during the course. We will evaluate whether the monkeys are seed dispersers or seed predators for yamamomo trees.

B) Insect group

Lectures

Munehiro OKAMOTO (Primate Research Institute, Kyoto Univ.) 岡本宗裕 (京都大・霊長研)
Kiyokazu AGATA (Dept. of Biophysics, Kyoto Univ.)　阿形清和（京都大・理・生物物理）

Abstract

Insect ecology is very diverse. Some insects may fly, walk on the ground, gather on flowers, gather near light, etc. In the insect group, we will learn how to collect such various types of insects and learn how to identify those insects to their family or genus classification.

During the practical fieldwork of 2013 and 2014, we collected the feces of Yakushima macaques that ate insects. We collected the feces in hopes of being able to identify the insects' DNA contained inside the feces. However, there were very few DNA data found in the feces, which resulted in insufficient analysis. Therefore, the goal of this year's Genome Science Course will be to instead collect the insects that are likely to be eaten by the Yakushima macaques, and conduct a mitochondrial DNA sequencing in order to create a DNA barcode database of those insects. We will mainly collect insects at lowland broad-leaved evergreen forests.

C) Mushroom group

Title

Spatial aggregation and segregation of mushroom forming fungi in Yakushima
（屋久島のキノコ類で共通して見られる分布パターンとすみわけについて）

Lectures

Hirotoshi SATO (Center for Ecological Research, Kyoto Univ.) 佐藤博俊（京都大・生態研）
Takaya IWASAKI (Center for Ecological Research, Kyoto Univ.) 岩崎貴也（京都大・生態研）

Yoriko SUGIYAMA (Faculty of Science, Kyoto Univ.) 杉山賢子（京都大・理学部）

Abstract

We aim to reveal whether mushroom-forming fungal species in Yakushima show spatially aggregated or segregated patterns. To do this, we collect mushroom-forming fungi along line transects at low-altitude zone and high-altitude zone forests in Yakushima, record each sampling location, and compare spatial distribution of these fungi. We welcome anyone who is interested in the natural history of mushroom-forming fungi with or without preliminary knowledge of fungi.

D) Deer Group

Title

Individual identification from fecal DNA of sika deer and its application to ecological study

Lectures

Hideki SUGIURA (Wildlife Research Center, Kyoto Univ.) 杉浦秀樹(京都大・野生動物)

We will invite one or two expert(s) of ecological study of sika deer

Abstract

We will collect DNA samples from feces of Yakushima deer, a subspecies of sika deer (*Cervus nippon yakushimae*). Using these samples, we will try to identify the individual of the host in the genome course. We will collect DNA from fresh feces of identified individual to examine whether we can identify individuals from DNA. We also collect DNA from feces whose hosts are unknown, to estimate the density of deer. Note that fecal sampling of deer may demand endurance and we may walk in hilly mountain areas for long distances.

3.2 Reports on the past program

Global COE website (in English)

http://gcoe.biol.sci.kyoto-u.ac.jp/gcoe/eng/report/2011/01/report_of_fieldworkgenome_trai.php

http://www.wildlife-science.org/en/reports.html

フィールド＆ゲノム科学実習　CCTBio HP (in Japanese)

http://www.wrc.kyoto-u.ac.jp/core-to-core/training_old.html

http://www.wildlife-science.org/ja/reports.html

4. Genome Science Course in spring (June 1-9)

Following the Field Science Course will be the Genome Science Course. If you are planning to take both the Field Science and Genome Science Courses, we will be using samples collected from the Field Science Course in Yakushima Island for the Genome Science Course. Therefore, we recommend that you take a Genome Science Course accordingly to the Field Science course you took (if you take the Field Science Course). In addition, if you do not have experience with genome experiments, we recommend Genome Science Courses A) - C). These combinations enable you to analyze the samples collected by yourselves. If you are interested in NGS and data analysis for NGS, take course D), regardless of the group you took in the Field Science Course (if you did take any Field Science Courses).

Genome Science Course	Field Science Course
A) Individual identification of fecal DNA	A) Monkey group and D) Deer group
B) Insect DNA barcoding	B) Insect group
C) Mushroom DNA barcoding	C) Mushroom group
D) Whole genome analyses	(Any group)

A) Individual identification of fecal DNA

Lectures

Eiji INOUE (Dept. of Zoology, Kyoto Univ.) 井上英治 (京都大・理・動物)
Akiko SAWADA (Wildlife Research Center, Kyoto Univ.) 澤田晶子 (京都大・野生動物)
Takushi KISHIDA (Wildlife Research Center, Kyoto Univ.) 岸田拓士(京都大・野生動物)
Kiyokazu AGATA (Dept. of Biophysics, Kyoto Univ.) 阿形清和 (京都大・理・生物物理)

Abstract

We will focus on DNA individual identification method based on genotyping. DNA of monkeys and deer will be extracted from the feces collected during the Field Science Course. After DNA extraction, we will identify the sex using genetic marker and will identify individuals using several microsatellite loci.

B) Insect DNA barcoding

Munehiro OKAMOTO (Primate Research Institute, Kyoto Univ.) 岡本宗裕(京都大・霊長研)
Takushi KISHIDA (Wildlife Research Center, Kyoto Univ.) 岸田拓士(京都大･野生動物)
Kiyokazu AGATA (Dept. of Biophysics, Kyoto Univ.) 阿形清和（京都大・理・生物物理）

The Insect group will collect and identify insect species in Yakushima. Using these collected insects, we will sequence the mitochondrial DNA in order to provide a DNA barcode catalogue of insects in Yakushima. Based on the DNA barcode catalogue thus obtained, we will analyze the food repertories of Yakushima monkeys using the monkey feces-derived

sequence data which were obtained by the members of 2014 Genome Training Course using MiSeq next generation sequencer.

C) Mushroom DNA barcoding

Lectures

Hirotoshi SATOH (Center for Ecological Research, Kyoto Univ.) 佐藤博俊（京都大・生態研）
Shizuka FUSE (Department of Botany, Kyoto Univ.) 布施静香（京都大・理・植物）

Abstract

The Mushroom DNA group will collect and identify mushroom species in Yakushima. Using these collected mushrooms, we will sequence the ITS region of the genomic DNA in order to provide a DNA barcode catalogue of mushrooms in Yakushima Island.

D) Whole genome analyses of the Yakushima monkey

Lectures

Takushi KISHIDA (Wildlife Research Center, Kyoto Univ.) 岸田拓士 (京都大･野生動物)
Takashi HAYAKAWA (Primate Research Institute, Kyoto Univ.) 早川卓志 (京都大・霊長研)
Kiyokazu AGATA (Dept. of Biophysics, Kyoto Univ.) 阿形清和（京都大・理・生物物理）

Abstract

Lecturers will provide a whole genome shotgun (WGS) sequence of a Yakushima monkey, *Macaca fuscata yakui* determined by HiSeq2000 high-throughput sequencer. Using this data, we will try to analyze the functional gene sequences (*e.g.*, sensory receptor gene repertoire), genetic diversity in the genome-wide scale, genetic differences with other macaque species, and historical changes of the effective population sizes of Yakushima monkeys in order to reveal the ecology and evolution of Yakushima monkeys. This group will not do "wet" laboratory experiments, but will focus on "dry" bioinformatics using personal- and super-computers.

International Seminar

Date: June 9 (Tue), 2015
Place: Science Seminar House, Yoshida Campus of Kyoto University.

We will have talks from visiting foreign students and host researchers. We will also present the results of Field Science Course and Genome Science Course in the poster session.

5. Field Science Course in fall (October 18-24)

5.1 Participants

About 20 graduate students of Biological Science, Kyoto University
About 6 teaching staff, including professors, post-docs and graduate students who study in Yakushima and/or the subject species

5.2 Groups

We form into two groups, deer and plant groups, and each group engages in different tasks. Choose your first and second preferences for group assignment. Please note that we cannot ensure your first preference due to capacity limitations.

A) Deer group

Title

Deer feces as a source of added information to enhance population census data
シカの糞から個体群調査のための情報を得る

Lectures

Takakazu YUMOTO (Primate Research Institute, Kyoto Univ.) 湯本貴和(京都大･霊長研)
Goro HANYA (Primate Research Institute, Kyoto Univ.) 半谷吾郎(京都大･霊長研)
Kodzue KINOSHITA (Primate Research Institute, Kyoto Univ.) 木下こづえ(京都大･霊長研)
Akiko SAWADA (Wildlife Research Center, Kyoto Univ.) 澤田晶子(京都大・野生動物)

Abstract

Fecal pellet counting is a conventional census technique used in wild ungulate populations to estimate their abundance. The value of the data would be much improved if we could obtain more information from the feces that were counted in the census. Participants will collect fresh feces while following habituated deer individuals in the forest, and examine the whether variation in fecal pellet size is correlated with the animal's biological characteristics observable in the field, such as body size, age, sex, and reproductive status. The fecal samples will be used for molecular analyses of DNA and hormones to determine sex, individual, and reproductive status of the deer in the following Genome Science Course. The correlations between observable characteristics in the field and molecular profiles determined in the laboratory may contribute to interpreting results achieved during the population census.

B) Plant group

Title

Species composition and phenology in fern gametophyte
シダ植物の配偶体における種構成とフェノロジー

Lectures

Wataru SHINOHARA (Kagawa Univ.) 篠原渉(香川大学)
Hiroshi KUDOH (Center for Ecological Research, Kyoto Univ.) 工藤洋(京都大・生態研)

Abstract

Ferns that we typically observe in forests are in their sporophyte (胞子体) stages. Compared with sporophytes, fern gametophytes (配偶体) are very small, about 1 cm at most in size, and to date no sufficient morphological characteristics useful for species identification have been identified, as is the case for other tiny characterless kinds of organisms. Recently, however, developments in molecular analysis are shedding light on species identification for these small organisms. This year, the plant team focuses on studying phenology of fern gametophytes in Yakushima. We plan to collect gametophytes from several places and compare their species compositions to that of samples collected in the other seasons during the previous year.

6. Genome Science Course in fall (October 26-30)

Following the Field Science Course, we will have the Genome Science Course, which uses samples collected during the Field Science Course on Yakushima Island. We also recommend taking the following course that corresponds to that which you took during the Field Science course. These combinations enable you to analyze the samples you yourself have collected. Prior knowledge in molecular biology is not necessary for the Genome Science Course in fall. This course is open either at the Primate Research Institute, Kyoto University in Inuyama City (deer group) or at the Graduate School of Science, Kyoto University in Yoshida Campus (plant group).

Genome Science Course	Field Science Course
A) Deer group	A) Deer group (at Primate Research Institute)
B) Plant group	B) Plant group (at Yoshida Campus, Kyoto Univ.)

A) Deer group

Title

DNA and hormonal analyses of deer feces for the determination of sex and reproductive status

Lectures

Hiroo IMAI (Primate Research Institute, Kyoto Univ.) 今井啓雄(京都大・霊長研)
Kodzue KINOSHITA (Primate Research Institute, Kyoto Univ.) 木下こづえ(京都大・霊長研)

Takushi KISHIDA (Wildlife Research Center, Kyoto Univ.) 岸田拓士(京都大・野生動物研究センター)
Takashi HAYAKAWA (Primate Research Institute, Kyoto Univ.) 早川卓志(京都大・霊長研)

Abstract

By applying two molecular methods (DNA and hormonal analyses), participants will determine the sex and reproductive status of Yakushima deer from feces collected during the Field Science Course. First, deer genomic DNA will be extracted from the feces. The participants will determine the deer sex by amplification of genes located on the sex chromosomes. Second, participants will also analyze the sex steroid hormones (estrogen, progesterone, and testosterone) from the same deer feces to determine sex. In addition, by measuring the concentration of each hormone, the participants will estimate the reproductive status of deer. Because fall is the breeding season for deer, if female and male adults are in estrus and in rut, respectively, female (estrogen/progesterone) and male (testosterone) hormones will show high concentrations. Based on the results from these two molecular analyses, we will try to develop practical methods for estimating reproductive status of wild deer.

B) Plant group

Title

Species composition and phenology in fern gametophyte

Lectures

Shizuka FUSE (Faculty of Science, Kyoto Univ.)　布施静香(京都大・理学研究科)
Wataru SHINOHARA (Kagawa Univ.)　篠原渉(香川大学)
Hiroshi KUDOH (Center for Ecological Research, Kyoto Univ.)　工藤洋(京都大・生態研)

Abstract

For the gametophyte samples collected from Yakushima, we will try to identify the species or the genera to which they belong using molecular analysis. We will extract DNA from the samples and determine rbcL gene sequences from them. Subsequently, we will compare these sequences with registered DNA sequences in the gene bank.

7. Information on fieldwork and life in Yakushima Island

7.1. Fieldwork

Research in lowland forest

The main study site is located in the western lowland forest in Yakushima. In the forest, the canopy is closed and the forest floor is dark. Undergrowth is sparse and not bushy. There are no trails in the forest, but we can walk through most parts of the forest easily. We sometimes walk on steep slope and cross streams. Please wear long trousers and shoes to protect your legs and feet in case you lose your balance.

During the month of May, you may get bug bites (mostly mosquitoes). A long-sleeved shirt is good for protection from insect bites. Wear a long-sleeved shirt, if you have sensitive skin. We also walk on the road to search for animals and their feces. Sunlight is strong on the road so you will need a hat.

In the forest, visibility is poor and you need to confirm your location using a map and compass.

Weather

Air temperature is about 18-26 °C in May and 19-25 °C in October in lowland. Note that air temperature is much lower in high-altitude mountainous areas.

Yakushima Island is famous for rainy climate. Early June is the beginning of rainy season and it is likely to rain in late May.

What to wear on the field

・Long-sleeved shirt, long trousers, hat or cap

In general, it is better to wear a long-sleeved shirt and long trousers to cover your skin on the field. They will protect you from insect bites, scratches and strong sunlight. Sunlight is very strong in May. Long trousers should be soft and loose enough to walk on steep slopes. Avoid tight jeans, because they become heavy and hard when they are wet.

・Shoes

Avoid slippery shoes or those that do not cover your foot, such as sandals.

Mountaineering shoes are the best, if you have. We recommend them, if you do not have enough experience in walking in hilly forests.

Jogging shoes or sneakers are good in lowland forest, though they are not water proof. They should be tough enough to walk on the rocky slope.

If you have old mountaineering shoes, jogging shoes or sneakers (more than 3 years), check their soles. Old bond may become weak and the soles can fall apart. Almost every year, one or two participants have had their soles fall apart.

Long boots are also good, if you are experienced in walking in the mountain with long

boots.

・Gloves

You may wear gloves if you like. Thin gloves are good for manipulation, such as taking notes.

7.2. Accommodation and meals

In Yakushima, we stay in the field station (PWS House Yakushima) of Kyoto University in Nagata Village. All meals are provided during our stay in the field station. For dinner, you can enjoy local foods cooked by local people. Students and staffs will not cook except for the BBQ on the last night. We wash dishes and clothes and clean the rooms by ourselves.

Do it yourself in the field station

We have no housekeeper in the station. You will be responsible for maintaining the station and doing daily chores such as cleaning, washing clothes, packing lunch, washing dishes, taking out the garbage, etc. Please do these things actively and cooperatively.

Meals

Meals are cooked by residents in Yakushima. Please help them cook when necessary. If you have food allergy or food(s) to avoid (e.g., meat for vegetarian), please let us know.

We pack lunch by ourselves. Bring a lunchbox of your preference and utensils necessary (spoon, fork, chopsticks, etc.).

Room and bedding

In the Field Station, there is a dining hall, kitchen, 3 shower rooms and 3 restrooms. You will share one of 5 bedrooms with other students (4 people/room). Each bedroom has two bunk beds with mattresses. Please bring your own sleeping bag and/or warm clothes, as the temperature may drop to 15 degrees at night. Expensive, high-quality sleeping bag is not necessary as we stay inside the house at night.

Bath

There are three shower rooms. We prepare shampoo and soap, which you can use freely for bathing. If you prefer your own shampoo or soap, please bring them. Have your own towel for bathing.

As it takes a long time for everyone to take a shower, some of us may go to public bath. You can try Japanese public bath, if you like. When you go to a public bath, have soap and a towel with you.

Washing clothes

Two washing machines are available. We will provide the laundry detergent. Please wash your clothes together with those of other station members, to save time. We prepare mesh bags

for washing, for which you can put your clothes in. Do not start washing after 10 p.m., to avoid making noise and inconveniencing others.

Others

You may bring sandals, which may be useful for walking around the station.

Shopping

In Nagata Village, you can buy snacks, drinks and daily necessities at a small shop. There are no supermarkets or convenience stores in the village. There is a supermarket in Miyanoura (20 km from the field station), the largest town in Yakushima Island, but you will not have time to go shopping there. You will have some time for shopping, on the last day.

7.2. List of personal equipment

Equipment for field research (common to all groups)

In addition to below, some other equipments will be necessary depending on your activity. Special equipment in each group will be announced later.

□ backpack (20-30 litters is enough)
□ notebook (pocket size is good)
□ pen / pencil
□ marker (with which you can write on plastic sample bags and plastic tubes)
□ canteen (you may use a PET bottle)
□ lunch box and spoon, fork, chopstick (bring a plastic container to pack your lunch to Yakushima)
□ flashlight (and batteries)
□ whistle (we can lend some whistles. Please let us know if you do not have it)
□ sampling bag, tube, etc. (they are given to participants at Yakushima)
□ maps (they are given to participants at Yakushima or Kyoto)
□ poison remover (they are given to participants at Yakushima)
□ backpack cover, or plastic bag inside the backpack (optional, when it is rainy)

□ long trousers (and a belt, must)
□ socks (must)
□ rainwear / umbrella (when it is rainy)

□ insect repellent (optional)
□ antipruritic (optional)
□ sunscreen (optional)
□ snack, candy, etc. (optional)

□ adhesive tape (optional, each lecturer has a first-aid kit)
□ towel (optional)
□ tissue (optional)

□ vest (optional)
□ waist pouch (optional)
□ spats optional)
□ gloves (optional)

□ camera (optional)

Other equipments for stay at the field station

□ sleeping bag (must)
□ underwear
□ clothes (which you can wear in the house)
□ sandals
□ towel
□ soap (for public bath)
□ toilet kit
□ laptop (optional; a personal laptop computer is useful for data analysis and presentation of the results)