Field Science Course and Genome Science Course in 2016

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. Students can choose a topic which is suitable for their interest and prior experience in molecular biological experiments. 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.

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, Graduate School of Science, Kyoto University (as of April 2016) 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 either at Yoshida Campus of Kyoto University in Kyoto City or at the Primate Research Institute in Inuyama City (depends on the group).

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

Application form is available at the following site
http://www.wildlife-science.org/ja/curriculum/

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 15 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.

Field Science Course (Yakushima Island, Kagoshima Prefecture)

May 21 Field Science Course starts (Move to Yakushima on this date)

May 22-25 Fieldwork in Yakushima

May 26 Data analysis, presentation in the afternoon

May 27 Leave Yakushima

Genome Science Course at Kyoto University (Yoshida Campus, Kyoto City or PRI, Inuyama)

May 30 – June 3 Molecular experiments and analyses, at several laboratories.

Whole-genome and deer genotyping groups will be held in Kyoto, and the fig/insect group will be held in Inuyama.

June 6 Preparation for presentation (both for Field and Genome Science Courses)

June 7 Presentation of the results of the courses at international symposium (held at Yoshida Campus)

Field and Genome Science Courses in fall

September 9	Deadline for application (both for the Field Science Course and Genome Science Course)
October 7	Guidance for the Field Science Course and Genome Science Course

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 and Yoshida Campus of Kyoto Univ.

3. Field Science Course in Spring (May 21-27)

3.1 Participants

Seven students who learn biology from India, Malaysia, and Brazil.

About 13 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 three groups: monkeys, fig/insects, and deer. Each group will engage in different tasks. Choose your preference of the groups, from first to third. Please note that we cannot ensure your first preference, due to limited capacity of each group.

A) Monkey group

Title

Effect of gut microbe of Japanese macaques on *in vitro* digestibility
ニホンザル腸内細菌の *in vitro* 消化率に与える影響

Lecturers

Goro HANYA (Primate Research Institute, Kyoto Univ.)　半谷吾郎(京都大･霊長研)
Kazunari USHIDA (Graduate School of Life and Environmental Sciences, Kyoto Prefecture Univ.)　牛田一成(京都府立大・生命環境)
Akiko SAWADA (Primate Research Institute, Kyoto Univ.)　澤田晶子(京都大・霊長研)

Abstract

We will and examine the effect of gut microbe on digestive ability of the wild Yakushima macaques *(Macaca fuscata yakui*). Yakushima macaques are one of the endemic species on Yakushima Island and live in various habitats in Yakushima along the elevational gradient from the coast to above 1900 m a.s.l. Macaques in the lowland much fruits and seeds, but those in the highland depend largely on leaves. Gut microbe may be one of the characteristics that macaques can flexibly adapt to the different food conditions.

We will collect fresh feces of wild Yakushima macaques both in the highland and lowland and bring them to the field station. We will conduct *in vitro* digestibility assay using the fecal samples as inoculum and various carbohydrates or ground leaves as substrate. We examine the hypothesis that the gut microbe in the highland helps to digest high-fiber diet in the highland.

B) Fig/insect group

Title

Fig-fig wasp relationships in Yakushima Island

Lecturers

Takakazu YUMOTO (Primate Research Institute, Kyoto Univ.) 湯本貴和 (京都大・霊長研)
Munehiro OKAMOTO (Primate Research Institute, Kyoto Univ.) 岡本宗裕 (京都大・霊長研)

Abstract

Seven species of figs (*Ficus* spp.) occur naturally in Yakushima Island. It is well known that each species of *Ficus* has a specific pollinating fig wasp as its partner. However, besides pollinating fig wasps, there are parasite wasps that never pollinate flowers but predate ovules or larvae of pollinating wasps. We will collect syconia of figs as many species as possible in Yakushima Island. We will dissect syconia (inflorescence of figs regarded as multiple and accessory fruit/果嚢) to observe inside by microscopes, and make specimens. Then, the specimens will be carried over to Genome Course to try identifying species of pollinating wasps and/or parasitic wasps.

C) Deer Group

Title

Social interaction and genetic relation among wild sika deer.

Lecturers

Naoki AGETSUMA (Field Science Center for Northern Biosphere, Hokkaido Univ.) 揚妻直樹
(北海道大・北方生物圏フィールド科学センター)
Yoshimi AGETSUMA-YANAGIHARA 揚妻芳美
Mari NISHIKAWA (Graduate School of Science, Kyoto Univ.) 西川真理(京都大・理)

Abstract

By now, littele information is available on social behaviors of wild Japanese sika deer (*Cervus nippon)*. The wester coastal forest of Yakushima is one of the best study sites for behavioral observation of wild sika deer. We will observe individually identified deer by focal animal sampling method, and record social interaction between focal deer and others. We will analyze types of interactions (social grooming, aggression, contact call, and proximity), frequencies of the interactions and attribution of participant deer (age-class and sex). We expect different patterns of social interactions between adult males and females.

During behavioral observation, we will collect DNA samples from feces of focal deer and socially interacting deer for “Genome Science Course”. We will analyze genetic relations among the observed deer. We could examine relation between social behaviors and genetic types between deer individuals.

Note that behavioral observation and fecal sampling of deer may demand endurance and we may walk in hilly mountain areas for long distances every day.

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

CCTBio HP (in Japanese)
http://www.wrc.kyoto-u.ac.jp/core-to-core/training_old.html

Leading Graduate Program of Primatology and Wildlife Sciences (in English)
http://www.wildlife-science.org/ja/reports.html
(2014 fall: http://www.wildlife-science.org/pdf/reports/YakushimaReport_FINAL.pdf)
(2015 fall: http://www.wildlife-science.org/pdf/reports/Report-2015-10-18-plantteam.pdf, http://www.wildlife-science.org/pdf/reports/Report-2015-10-18-deerteam.pdf)

4. Genome Science Course in spring (May 30-June 3)

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 the samples collected during the Field Science Course in Yakushima Island for the Genome Science Course. Therefore, we recommend that you join in a group in Genome Science Course that corresponds to the one in the Field Science course you took. In addition, if you do not have past experience with genome experiments, we recommend Genome Science Courses A) or B). These combinations enable you to analyze the samples collected by yourselves. If you are interested in NGS and data analysis for NGS, take course C), regardless of the group you took in the Field Science Course (if you did take any Field Science Courses).

Recommended combinations

Genome Science Course	Field Science Course
A) Sexing and genotyping of fecal DNA	C) Deer group
B) Plant and insect DNA barcoding	B) Fig/Insect group
C) Whole genome analyses	(Any group)

※ Members of A) Monkey group are recommended to participate in any group in the Genome Science Course

A) Sexing and mitochondria genotyping of fecal DNA

Lecturer

Miho MURAYAMA (Wildlife Research Center, Kyoto Univ.) 村山美穂（京都大・野生動物）

Abstract

DNA of 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 genotype mitochondrial haplotype. Then we will compare data with the observed distribution.

B) Plant and insect DNA

Lecturer

Takashi HAYAKAWA (Japan Monkey Centre / Primate Research Institute) 早川卓志（日本モンキーセンター/京都大・霊長研）

Abstract

There are seven species of figs in Yakushima, and it is known that each species of figs are pollinated by species-specific agaonid wasps. In this course, we will extract DNA from figs and wasps collected in Yakushima and infer phylogenetic trees. Microsatellite analyses for individual identification of fig individuals will also be tried.

C) Whole genome analyses of the Yakushima macaque

Lecturer

Takushi KISHIDA (Wildlife Research Center, Kyoto Univ.) 岸田拓士 (京都大･野生動物)

Abstract

The lecturer will provide a whole genome shotgun (WGS) sequence of a Yakushima macaque, *Macaca fuscata yakui* determined by HiSeq2000 high-throughput sequencer. Using this data, we will try to analyze the genetic diversity in the genome-wide scale, genetic differences with other macaque species, and historical changes of the effective population sizes of Yakushima macaques in order to reveal the ecology and evolution of Yakushima macaques. This group will do little or no "wet" laboratory experiments, but will focus on "dry" bioinformatics using personal- and super- computers.

International Seminar

Date: June 7 (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 15-21)

5.1 Participants

About 12 graduate students of Biological Science, Kyoto University
About 5 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, monkey 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) Monkey group

Title

Testing the trade-off between parasite resistance and the immunosuppressive hormones cortisol and testosterone

Lecturers

Andrew JJ MACINTOSH (Primate Research Institute, Kyoto Univ.)
Hideki SUGIURA (Wildlife Research Center, Kyoto Univ.)　杉浦秀樹（京都大・野生研）
Takakazu YUMOTO (Primate Research Institute, Kyoto Univ.) 湯本貴和 (京都大・霊長研)

Abstract

Parasites are ubiquitous in nature and are known to affect the health and fitness of both wild and domestic animals worldwide. Recently, research into primate parasitism has increased, partly because roughly half of all species are now threatened with extinction, and partly because primates are an important source of zoonotic infection in humans, especially in areas where humans continue to encroach into primate habitat. Yet, we still know very little about the dynamics of infection for the majority of infectious organisms and the majority of wild primate species.

This course will attempt to test for a trade-off between resistance to gastrointestinal parasites and the production of immunosuppressive hormones: cortisol, a critical component of the stress response, and testosterone, a critical component of male reproduction. The monkey group will locate and collect fecal samples from Yakushima macaques along the Western coastal road. Samples will then be processed and analyzed at the field laboratory, during which all parasites (helminths and protozoa) will be identified and quantified to estimate infection size in each sampled macaque. Students will thus assess the prevalence and abundance of each parasite infecting Yakushima macaques, and determine the diversity (richness) of macaque parasites in the area. These parasitological data will be used for comparison with the endocrinological data produced during the subsequent laboratory course at the Primate

Research Institute.

B) Plant group

Title

Species composition in fern gametophyte
シダ植物の配偶体における種構成

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 usually observe in forests are in their sporophyte stages. Compared with sporophyte, fern gametophyte is very small and about 1 cm at most in size and has no sufficient morphological character using for species identification so far, as well as other tiny characterless kind of organisms. However, recently, developing molecular analysis shed right on the species identification for these small organisms. This year, plant team focuses on studying phenology of fern gametophytes in Yakushima. We plan to collect gametophytes from several places and compare the species constitution across the collection sites.

6. Genome Science Course in fall (October 24-28)

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) Monkey group	A) Monkey group (at Primate Research Institute)
B) Plant group	B) Plant group (at Yoshida Campus, Kyoto Univ.)

A) Monkey group

Title

Testing the trade-off between parasite resistance and the immunosuppressive hormones cortisol and testosterone

Lectures

Kodzue KINOSHITA (Primate Research Institute, Kyoto Univ.) 木下こづえ(京都大･霊長研)
Takashi HAYAKAWA (Primate Research Institute, Kyoto Univ.) 早川卓志(京都大･霊長研)
Andrew MACINTOSH (Wildlife Research Center, Kyoto Univ.)
Munehiro OKAMOTO (Primate Research Institute, Kyoto Univ.) 岡本宗裕（京都大・霊長研）

Abstract

Participants will apply two molecular methods (DNA and hormonal analyses) to analyze macaque feces collected during the Field Science Course to determine the sex, reproductive status and stress levels of each host macaque. First, macaque genomic DNA will be extracted from feces to determine host sex by amplifying genes located on the sex chromosomes. Second, participants will also analyze cortisol from the same set of monkey feces, and testosterone from the samples identified as coming from males. Reproductive status and stress levels will be estimated by measuring the concentration of each hormone in the feces.. Because fall is the breeding season for Yakushima macaques, male testosterone is expected to show high concentrations, and since stress may also be high, we expect relatively high concentrations of cortisol as well. We will test the hypothesis that these hormones may affect immune function by comparing hormonal data with parasitological data across samples, and then discuss the possible trade-off between resistance to gastrointestinal parasites and the production of immunosuppressive hormones, which are essential for survival and reproduction. Finally, if possible, a quantitative PCR will be performed on extracted feces to measure copy numbers of parasite DNA as an index of parasite intensity for comparison with microscopic and hormonal data.

B) Plant group

Title

Species composition in fern gametophyte

Lecturers

Hiroshi Azuma (Faculty of Science, Kyoto Univ.)　東 浩司(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 equipment 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)