# PWS symposium 2023

## Thursday, Nov 16

JST

| Time | Event |
|------|-------|
| 13:30 | **Keynote Talk** <br> Dr. Andersson <br> (13:30-14:30) |
| 14:00 | |
| 14:30 | **Registration** |
| 15:00 | **Opening remarks** |
| | **Oral session 1** <br> (15:10-15:50) |
| 15:30 | |
| | Break (20 min) |
| 16:00 | |
| | **Poster session** <br> Lightning Talk |
| 16:30 | 2 min/person <br> (16:10-16:50) |
| 17:00 | Core time <br> with snacks and drinks <br> (16:50-18:00) |
| 17:30 | |
| 18:00 | |

## Friday, Nov 17

JST

| Time | Event |
|------|-------|
| 10:00 | **Oral session 2** <br> (10:00-12:00) |
| 10:30 | |
| | Break (10 min) |
| 11:00 | |
| 11:30 | |
| 12:00 | Lunch break <br> (~13:30) |
| 13:30 | **Keynote Talk** <br> Dr. Otani <br> (13:30-14:30) |
| 14:00 | |
| 14:30 | Break (10min) |
| | **Oral session 3** <br> (14:40-16:40) |
| 15:00 | |
| 15:30 | Break (10 min) |
| 16:00 | |
| 16:40 | **Closing remarks** |

## Oral session 1 (Nov 16)

| Time | min | Speaker | Affiliation | Title |
|---|---|---|---|---|
| 15:10~ | 20 | Nahoko TOKUYAMA | Wildlife Research Center, Kyoto University | The Experience Of Encountering Bonobos And Its Impact On Conservation Awareness Among Secondary Students Living Inside And Outside A Reserved Area |
| 15:30~ | 15 | Hiroto YOSHIMURA | Wildlife Research Center, Kyoto University | Fecal DNA metabarcoding analysis of wild snow leopard (*Panthera uncia*) and sympatric mammals focusing on dietary plant species. |
| 15:45~ | 15 | Fadel A. AZHARI | Wildlife Research Center, Kyoto University | Genetic Diversity and Population Structure of *Cybister rugosus* and My Research Plan in WRC |

## Oral session 2 (Nov 17)

| Time | min | Speaker | Affiliation | Title |
|---|---|---|---|---|
| 10:00~ | 20 | Yoko MITANI | Wildlife Research Center, Kyoto University | Future for sea otters in Hokkaido |
| 10:20~ | 15 | Shun HONGO | Center for African Area Studies, Kyoto University | Predicting wildmeat biomass from simple indicators applicable to harvest-based monitoring in a Cameroon rainforest |
| 10:35~ | 15 | Himani NAUTIYAL | Howard University | Anti-predator strategies of adult male Central Himalayan Langurs (*Semnopithecus schistaceus*) in response to domestic dogs in a human-dominated landscape |
| Break | 10 | | | |
| 11:00~ | 15 | James BROOKS | Institute for Advanced Study, Kyoto University | Oxytocin modulates group organization in free-moving horses |
| 11:15~ | 15 | China KOBAYASHI | Wildlife Research Center, Kyoto University | Revealing the effect of oxytocin on social relationships in semi-free-ranging horses under food competition with behavioral tracking using deep learning |
| 11:30~ | 15 | Kenneth KEUK | Wildlife Research Center, Kyoto University | Raccoon dog invasion in Yakushima: is raccoon dog distribution associated with risk of environmentally transmitted parasites? |
| 11:45~ | 15 | David FASBENDER | Wildlife Research Center, Kyoto University | Assessing the distribution and Ecology of the Bonobos of the Lomami National Park with a Combination of Indirect Methods |

## Oral session 3 (Nov 17)

| Time | min | Speaker | Affiliation | Title |
|---|---|---|---|---|
| 14:40~ | 20 | Susumu TOMIYA | CICASP/EHUB, Kyoto University | Going Down with Primates: Paleontological Insights into Coordinated Dynamics of Mammalian Diversity under Forest Loss |
| 15:00~ | 15 | Jie GAO | Wildlife Research Center, Kyoto University | Body part categorization in chimpanzees |
| 15:15~ | 15 | Rena NUMABE | EHUB, Kyoto University | Correlation between the functional polymorphisms in TAS2Rs and human bitter taste perception for caffeine |
| Break | 10 | | | |
| 15:40~ | 15 | Ena ONISHI | Wildlife Research Center, Kyoto University | Urination Behavior in Captive Chimpanzees: Synchrony, Contagion, and Social Patterns |
| 15:55~ | 15 | Christen LIN | Wildlife Research Center, Kyoto University | Measuring Emotional States through Judgment Bias via a Go/no-go Touch Screen Task in Chimpanzees: |
| 16:10~ | 15 | Yige PIAO | Wildlife Research Center, Kyoto University | Investigating the importance of observed causal effects during the social learning of tool set use in chimpanzees: preliminary report |
| 16:25~ | 15 | Raquel COSTA | Japan Monkey Center | People and Gorillas, from Uganda to Japan: the impacts of interactions between tourists and gorillas on their behavior and species conservation |

# The Experience Of Encountering Bonobos And Its Impact On Conservation Awareness Among Secondary Students Living Inside And Outside A Reserved Area

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Experiences with nature and interactions with animals, especially during childhood, are known to enhance environmental conservation awareness. Traditionally, the conservation of endangered species in tropical regions has been dominated by a "fortress conservation" approach. However, this approach restricts the natural use of the area by local communities, leading to a loss of knowledge and interest in animals. In recent years, there has been a growing interest in "community-based conservation," which emphasizes the participation of local residents and aims for coexistence between wildlife and humans. However, the impact of continued interaction with nature and animals through this approach on the conservation awareness of local communities has not been thoroughly examined.

The Luo Scientific Reserve in the Democratic Republic of Congo was established in 1991 as a conservation area focusing on the coexistence of the endangered bonobos and the local population. This study examined the impact of knowledge about bonobos and experiences of encountering bonobos on conservation awareness, particularly focusing on the younger generation. A survey using anonymous questionnaires, consisting of 34 questions related to 1) knowledge about bonobos, 2) experiences of encountering bonobos, and 3) conservation awareness of bonobos, was conducted among students from three secondary schools in Wamba village and two secondary schools in the nearby village of S.

The percentage of students who had encountered bonobos was 83% in Wamba village and 67% in S village. Among them, 51% in Wamba village and 15% in S village had encountered bonobos more than ten times. Seventy-eight percent of students in Wamba village felt "happy," while 51% in S village felt the same. Conversely, 20% of students in Wamba village felt "fearful," while 48% in S village felt the same. In response to the question of whether they would eat bonobos if given the opportunity, the percentage of students answering "yes" was lower among students who had encountered bonobos (5.7%) compared to those who hadn't (24%) in Wamba village. In S village, students who had more frequent encounters with bonobos were less likely to answer "yes" to the same question. Additionally, in Wamba village, students with more frequent encounters with bonobos were more likely to answer "I like bonobos/like them a lot."

These results suggest that the frequency of encounters with bonobos and the emotions experienced during these encounters influence conservation awareness, although the patterns differ between the reserve and its neighboring areas.

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# Fecal DNA metabarcoding analysis of wild snow leopard (*Panthera uncia*) and sympatric mammals focusing on dietary plant species.

Hiroto Yoshimura[1*], Takashi Hayakawa[2,3], Dale M Kikuchi[4], Kubanychbek Jumabay-Uulu[5], Huiyuan Qi[1], Taro Sugimoto[6], Koustubh Sharma[5,7] and Kodzue Kinoshita[8]

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Felids, commonly known as cats, are the obligatory carnivores. However, they also consume plants both in the wild and in captivity, raising questions about their interactions with plants. This is particularly true for the snow leopard (*Panthera uncia*), a big cat native to central Asia's high mountains, which has been observed to consume plants more frequently than other members of its genus, Panthera.

Our study aimed to identify the prey and plants that snow leopards and six other mammals living in the same area consume. We used DNA metabarcoding methods on 126 scat samples collected from the Sarychat-Ertash Nature Reserve in Kyrgyzstan. After whole scat DNA was extracted, five DNA barcode regions (*12SV5* for prey vertebrates and *ITS2*, *rbcL*, *trnL*, *ITS1* for dietary plants) were amplified by PCR and then massively sequenced by an Illumina MiSeq. We also determined the sex of the snow leopards based on their DNA. Probabilistic co-occurrence analysis of vertebrates and plants was conducted to check the effect of gut contents of prey vertebrates.

Our findings show that the main prey for carnivores in this region were Siberian ibex (*Capra sibirica*), Argali sheep (*Ovis ammon*), and marmots (*Marmota baibacina*). Interestingly, Argali sheep appeared to be consumed only by male snow leopards, although the sex difference was not statistically significant ($p=0.601$, 999 permutation test). In terms of plants, the snow leopards mainly consumed species from the Poaceae, Tamaricaceae (specifically the genus *Myricaria*), and Asteraceae families. We discovered that certain plants and prey tended to appear together in the scats, suggesting a secondary consumption. However, we found that Myricaria was often present in scat samples that lacked any DNA of animal prey, indicating that snow leopards might have eaten this plant particularly when their digestive tract was empty. There was also a significant difference in plant composition in scat between male and female snow leopards ($p<0.05$, 999 permutation test), and possibly between sampling seasons ($p=0.09$, 999 permutation test).

Our research revealed a comprehensive repertoire of plants in scat of snow leopard and sympatric mammals in the study area. This will contribute to hypothesis formulation and determination of future research directions to understand the adaptive significance of the plant-eating behavior of felids.

# Genetic Diversity and Population Structure of *Cybister rugosus* and My Research Plan in WRC

Fadel Abdurrahman Azhari[1,2*], Etsuko Moritsuka[3], Junko Kusumi[2,3], Kunio Araya[2,3], Miho Inoue-Murayama[1], Yu Sato[1]

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The aquatic beetle *Cybister rugosus* has a distribution range extending from Southeast Asia to the Ryukyu Archipelago. This species was recently listed as endangered on the Red List by the Japanese Ministry of Environment and is also designated as a specified class II nationally rare species of wild fauna and flora. However, genetic information, which is essential for endangered species to design effective conservation strategies, is limited in *C. rugosus*. In this study, the genetic diversity and population structure of *C. rugosus* from Cambodia and the islands of the Ryukyu Archipelago were inferred by using the COI gene and ten microsatellite markers. The results showed significant genetic differentiation between the Japanese and Cambodian populations and pronounced clustering in the South and Central Ryukyu populations. Based on these findings, it can be concluded that the Japanese and Cambodian populations represent different evolutionary significant units (ESU).

Different to undergraduate study, instead of insects, in my Master's Course, I aim to study a keystone species with a different methodology to expand my knowledge of conservation genetics. Thus, for my research plan, I aim to perform whole genome analyses of Japanese and European White-tailed eagles, *Haliaeetus albicilla*, which is considered as an Endangered Species by the Ministry of Environment. I also plan to conduct a population viability analysis (PVA) of the Japanese captive population. The research aims to infer the population structure of White-tailed eagles, estimate their genetic diversity level, and inform the best breeding strategy of the captive population. Thus, information obtained from this research will be useful for developing effective conservation strategies, including the integration of in-situ and ex-situ approaches.

# Future for sea otters in Hokkaido

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Sea otters (*Enhydra lutris*) are members of the Mustelidae family and have a relatively recent evolutionary history (~5 Mya) compared to other marine mammals. Historically, they were distributed continuously along the North Pacific Rim from Hokkaido, Japan to the Baja California, Mexico. In Japan (including the Kuril Islands, which were Japanese territory at that time), they were hunted to near extinction due to the maritime fur trade in the 19th and early 20th centuries, before receiving protection in 1911. Along the east coast of Hokkaido, there have been periodic sea otter sightings after a long absence, and now they seem to be recolonizing coastal ecosystems around this area. Sea otters forage for a variety of benthic invertebrates, such as sea urchins, crabs, and shells. They consume ca. 25% of their body mass per day. Therefore, one concern about their return are possible conflicts with fisheries, especially the sea urchin harvest. On the other hand, the sea otter is globally known as an iconic species of healthy coastal ecosystems and has a potential to be an important resource for tourism in Japan as same as cases in US and Canada. Since the distribution of sea otters in Japan continues to expand, it is necessary to find a way for humans to coexist with sea otters and continue to share coastal ecosystem services. Therefore, we are approaching the scientific aspects of sea otter ecology by conducting visual observations and diving surveys to clarify the relationship between sea otter habitat use, feeding ecology, and the environment. We conducted interviews and questionnaires with stakeholders to clarify human dimensions on sea otter conservation and management. In this presentation, we will present the results obtained so far and discuss future measures.

# Predicting wildmeat biomass from simple indicators applicable to harvest-based monitoring in a Cameroon rainforest

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Tropical forests face the wildmeat crisis and require locally-based monitoring for sustainable wildlife management. The monitoring needs simple indicators that comply with local knowledge and accurately predict wildmeat biomass—the total biomass of animals targeted for hunting. This study explores indicators predicting wildmeat biomass in a rainforest of southeast Cameroon. Employing camera traps and the Random Encounter and Staying Time (REST) model, we estimated the spatial density variations of five mammals (Peters's duikers, bay duikers, blue duikers, brush-tailed porcupines and pouched rats) at three sites. We then estimated the wildmeat biomass from the estimated density and body mass. We also estimated the spatial variations in six candidate indicators from camera-trap capture rates. Finally, we assessed the relationships between wildmeat biomass and each indicator. The results showed that the R/B ratio (red-to-blue duiker ratio) and the D/R ratio (duiker-to-rodent ratio) exhibited positive linear-like correlations to wildmeat biomass at all sites. The correlation lines were moderately similar across sites in the R/B ratio but largely different in the D/R ratio, suggesting that the latter is unsuitable for sharing information between neighbouring communities. Given a large sample size, the two indicators based on captured animal composition may effectively predict the total biomass of the main target species for local hunting. Because local hunters are aware of depletion-related changes in the species composition of caught animals, these indices may be effectively incorporated into harvest-based wildlife monitoring.

**Anti-predator strategies of adult male Central Himalayan Langurs (*Semnopithecus schistaceus*) in response to domestic dogs in a human-dominated landscape**

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Anti-predation strategies are act largely to diminish the cost of being captured by a predator. One strategy is to emit alarm calls in the presence of predators and some primate species are known to confront predators directly, by what is known as 'mobbing' or 'counterattacking. Several hypotheses regarding the benefits to males performing such costly behaviors have been tested in a variety of different taxa, but only a handful of studies have looked at this aspect in primates. We investigated four hypotheses using dog-langur predation encounters in a group of 78 Central Himalayan Langur (CHL) individuals living in a high-altitude landscape of northern India. In total, 1917 hours (mean 213 ±23.89 SE hours per male) of focal observations (15 minutes per session) of 9 adult males were recorded from May 2017 to November 2018. A total of 1,971 (mean 219±37.32 SE per male) adult male langur responses were observed during 312 dog-langur encounters. Study pursued Multinomial Logistic Regression analysis and an information theory approach to examine multiple hypotheses simultaneously and. The results showed that the likelihood of CHL adult males performing a high-cost counterattack and alarm call over low-cost flee and/or freeze is better explained by an integrated model (AIC=2493, weight=1) including the variables residency duration (*parental care* hypothesis; $z=5.675$; $p<0.01$), genetic relatedness (*kin selection* hypothesis; $z=-1.794$; $p<0.01$), grooming rate (*reciprocal altruism* hypothesis; $z=3.780$; $p<0.01$), rank, and mating rate (*costly signaling* hypothesis; $z= -4.099$; $p<0.01$). Natural selection and kin selection have both contributed to the evolution of CHL anti-predator tactics.

# Oxytocin modulates group organization in free-moving horses

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The oxytocinergic system has been suggested to make up an important part of the endocrine basis of group cohesion. However, controlled studies of animals in open-group settings have not been performed. We here investigated the impact of exogenous intranasal oxytocin, compared to saline placebo control, on the group-level social organization of horses. After treatment, we recorded positional data at 1Hz frequency of all members of 5 groups of horses (N = 58) in open field settings, and conducted social network analysis to measure oxytocin's effect on metrics of group cohesion. Across measures, we find oxytocin decreased social differentiation between group members, resulting in more evenly distributed but more dispersed groups. These results reinforce and extend oxytocin's importance to collective behaviour and to the evolution of group-based sociality across taxa.

# Revealing the effect of oxytocin on social relationships in semi-free-ranging horses under food competition with behavioral tracking using deep learning

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Oxytocin, a hormone which is conserved across mammals, has been suggested to contribute to the formation of bonds within groups, while possibly promoting a hostile attitude towards outgroup. But there have been few studies investigating these effects specifically outside a narrow range of species. The purpose of this study was to reveal the effects of exogenous oxytocin on social relationships in a group of horses, a highly social species which forms multilevel society in natural conditions. We intranasally administrated oxytocin to two groups of semi free-ranging captive horses (n=11 and 12, respectively), and observed their social interaction around scattered hay, facilitating the observation of competitive and cooperative relationships. We developed two main hypotheses: (1) the general bonding hypothesis, in which oxytocin generally reduces competition and increases cooperative behaviors. (2) the selective bonding hypothesis, in which oxytocin enhances existing differences, making close associates more friendly and aggressive relationships more aggressive. These hypotheses will be tested by behavioral analyses focusing on social approach, co-feeding, and agonistic interactions between all individuals. Using multiple animal tracking software based on deep learning, behavioral data was collected by pose estimation of three body points (head, neck, and tailhead) of each horse from drone movies.

# Raccoon dog invasion in Yakushima: is raccoon dog distribution associated with risk of environmentally transmitted parasites?

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Originating from the Far East, the raccoon dog (*Nyctereutes procyonoides*) has become one of Europe's most successful invasive carnivores, posing threats to conservation and public health by facilitating the spread of pathogens, including zoonotic ones. Although native to Japan, invasions have occurred on two locations within the archipelago since the 1980s: Yakushima and the Oki islands. Invasions putting at risk Yakushima's UNESCO-protected ecosystem, previously devoid of meso-predator.

Raccoon dogs have been identified as important hosts for numerous parasites within and outside their native range. In contrast, the last and only description of raccoon dog parasites in Yakushima dates to 2006 from 14 individuals, and little is known about raccoon dogs' actual distribution on the island. To investigate how raccoon dogs shape disease risk for other sympatric hosts in Yakushima's western coastal forest (3.6 km²), we estimated their abundance and its spatial variation, using latrine survey and camera trapping in 2022. Additionally, we collected ~300 fecal samples for parasite identification and quantification (microscopy and PCR), focusing on gastrointestinal helminths. Preliminary analyses suggest densities of 30-40 latrines/km² and high abundance of *Toxocara tanuki* (up to 7000 eggs/g) in sampled raccoon dogs feces. Results of complete analyses will be presented, along with a test of the prediction that raccoon dog local density and parasite abundance and richness will be positively correlated, supporting an increased risk for other potential hosts. These include an endemic and endangered subspecies of Japanese weasel, as well as feral cats and dogs that can further transmit these parasites to domestic animals and humans. Yakushima provides a unique opportunity to study invasion dynamics and explore the correlation between invasive species ecology and disease risk. This research contributes essential knowledge to conserve this ecosystem and highlights the broader implications of invasive species on the environment and public health.

# Assessing the distribution and Ecology of the Bonobos of the Lomami National Park with a Combination of Indirect Methods

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In 2007, surveys in what is now the Lomami National Park uncovered a surprisingly large and expansive bonobo population extending the bonobo geographic range. Subsequently, comparative studies using fecal samples and museum skeletons have shown this bonobo population to be genetically and morphologically distinct. Nevertheless, little is known about the behavior and ecology of these unhabituated and understudied bonobos. Since 2014, the Lomami Conservation Project of the Frankfurt Zoological Society (formerly the TL2 Project) has been using a combination of indirect methods to piece together a better understanding of their distribution and ecology. Patrols across the park recording bonobo indicators reveal a patchy bonobo distribution with, contrary to predictions, the highest concentrations at the southern forest savanna ecotone. Marked nest counts were used to calculate bonobo densities confirming high concentrations of bonobos at the ecotone, even in unprotected areas outside of the park. Camera trap studies confirmed similarities between the social organization of these ecotone bonobos and other bonobo populations as well as variation in the presence of other frugivores and predators across the park. Finally, implementation of passive acoustic monitoring will hopefully enable monitoring of the ranging patterns of a targeted bonobo community relative to food and poaching. While these methods each provide insight into a different aspect of bonobo socioecology, they also require immense investments in time and resources. Therefore, integrating methods has proven more efficient, building off of patterns from large-scale lower-intensity patrols to spatially target intensive camera trap studies and refine study questions. Hopefully, with new methods and techniques we can continue to improve our understanding of the distribution, ecology and status of this important bonobo population.

# Going Down with Primates: Paleontological Insights into Coordinated Dynamics of Mammalian Diversity under Forest Loss

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The global climatic trajectory of the Cenozoic Era (66 million years ago [Ma] to Present) is characterized by gradual declines in temperature and precipitation. In mid-latitude regions, this broad-scale climatic transition brought about replacement of tropical rainforests with more open subtropical to temperate woodlands and grasslands. It is well known that these biomic shifts have profoundly influenced the course of macroevolution for mammals, but our mechanistic understanding of regional faunal transitions has not yet advanced sufficiently to generate predictive models of mammalian diversity dynamics under forest loss. With the latter goal in mind, my international collaborators and I have been investigating patterns of vertebrate faunal transitions in the middle Eocene (ca. 48–37 Ma) of North America, which experienced a complete loss of once-diverse primates.

To date, we have analyzed diversity dynamics of carnivorous mammals against declining primate diversity in two particularly fossil-rich regions. In the Washakie Basin of the central Rocky Mountains region, analyses of ~5,200 vertebrate fossil occurrence data using Bayesian hierarchical occupancy–detection modeling revealed a substantial loss (with an estimated median of 40%) of carnivore species around 46 Ma, concomitant with geologically sudden disappearance of previously-abundant primates.

In southwestern North America (encompassing present-day southern California and Trans-Pecos, Texas)—a likely holdout for a forest-adapted North American fauna that saw a delayed and more protracted decline of primates compared to the rest of the continent—mammalian carnivore diversity as a whole does not appear to have decreased as dramatically as in the central Rocky Mountains region. However, it was marked by gradual decrease in proportional diversity of hypo- and mesocarnivores from ca. 42 to 38 Ma. This trend culminated in the appearance of earliest hypercarnivorous carnivoraforms (crown-group carnivorans and their close relatives) in North America.

These observations illustrate independent responses of carnivore assemblages that, while exhibiting some contextual dependencies, are both consistent with expectations for forest-to-woodland transitions in which opening-up of forest canopies reduces the dimensions of spatial and food resources. Our findings augment eco-geographic patterns of carnivoran diversity observed today, providing a deep temporal perspective that is not confounded by anthropogenic disturbances.

# Body part categorization in chimpanzees

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Humans are able to categorize body parts, which reflects our knowledge about bodies and could potentially help with higher-level activities involving bodies. In this study, we tested whether humans' closest living relatives, chimpanzees, can categorize body parts as humans do. Six chimpanzees were trained to perform a body-part matching-to-sample task using sets of pictures of the same individuals. The body parts were heads, torso, arms, and legs. Five of the chimpanzees passed the training and received test sessions. There were three kinds of trials mixed in the test sessions: (1) used pictures of same-individual pairs; (2) new same-individual pairs; and (3) new different-individual pairs. All individuals had performances that were better than the chance level in all the conditions and for all the body parts. For their first encounters with the new different-individual pairs, their performances were also better than the chance level. The analysis for their accuracies in the novel different-individual pairs suggested significant differences among all four body parts (from high to low): when the samples were heads, torso, arms, and then legs. An analysis in response time in these pairs also showed a significant difference: arms and legs had longer time than heads and torso. A multidimensional scaling analysis of their choices suggested that they were mostly confused with arm-leg pairs. Overall, the results showed that chimpanzees are able to match and categorize body parts, even across different chimpanzee bodies. Their sensitivities to each body part vary, and they seem to be more sensitive to heads and torso compared to arms and legs. This indicates that knowledge about body parts may play an important role in their lives. We hope this study could inspire future studies on the evolution of body perception in primates.

# Correlation between the functional polymorphisms in TAS2Rs and human bitter taste perception for caffeine

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**Introduction**: Bitter is an innate sense to avoid the toxic substances and bitterness is recognized by TAS2Rs (taste 2 receptors). 25 TAS2Rs have been known as the bitter taste receptors in human, which can totally recognize thousands of bitter compounds. Some TAS2Rs have genetic polymorphism, which are suggested to be responsible for interindividual differences of bitter taste sense. The relation of TAS2R functions and human taste sense has been still under discussion. For example, there are some papers discussing the relationship between coffee bitterness and Single Nucleotide Polymorphism (SNP) in TAS2Rs. Caffeine is a well-known bitter compound in coffee; therefore, we focus on the influence of the SNPs in TAS2Rs to people's bitter perception to coffee and caffeine. SNPs near the loci of TAS2R43 and TAS2R46 are reported to be related to the caffeine bitterness and liking of coffee. In this study, we examined the relationship of functional differences in protein haplotypes of TAS2R43 and TAS2R46 with the threshold of human bitter taste sense.

**Materials and Methods:** TAS2R43 has 2 major haplotypes due to 2 SNPs. Combination of W at position 35 and H at position 212 is suggested as the ancestral type during the molecular evolution which mainly distribute in Africa. Another combination of S and R is suggested as the derived type, which mainly distribute in East Asia. TAS2R46 has also 2 haplotypes, in which the ancestral type of L at position 228 and the derived type of M. Because these polymorphisms cause the functional differences in these TAS2Rs to some toxic ligands, we examined the response of these TAS2Rs to caffeine and related compounds using the expressed protein in cultured cells (HEK293T cell). We carried out human tasting test with volunteer subjects, to compare their threshold of bitterness perception with their genotyping data of the TAS2Rs.

**Results**: We found the difference in the function to caffeine between the haplotypes of expressed TAS2R43 and TAS2R46 protein. In both receptors, ancestral types showed higher response and lower threshold compared to the derived type. Also, there was a tendency that people having the ancestral type can detect caffeine bitterness at lower concentration than those having the derived type in TAS2R43 and TAS2R46.

**Discussion**: The SNPs of TAS2R43 and TAS2R46 can affect their protein response to caffeine which causes the difference in people's bitter perception.  Interestingly, other bitter compounds in coffee (mozanbioside and cafestol) were reported to stimulate only TAS2R43 and -46. Therefore, the taste of coffee may be different among the people's TAS2R43/-46 genotypes. Considering the distribution of the haplotypes, these SNPs in the TAS2R43/-46 may be corelated to human spread from Africa. One of the candidates for the differences in the SNPs abundance ratio is difference in vegetation of toxic plants in each area. Another possibility is related to the foods and drinks contain caffeine. The SNPs in TAS2R43/-46 may contribute to the change in the tase of foods/drink which is changed in the human evolution.

# Urination Behavior in Captive Chimpanzees: Synchrony, Contagion, and Social Patterns

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Behavioral contagion, a fundamental element of group dynamics, has recently piqued scientific interest due to its relevance in understanding the evolution of group cohesion among social animals, including humans. In our study, we investigated the urination behavior of captive chimpanzees, aiming to explore the existence of contagious urination and elucidate the associated social patterns. We conducted direct observation of four groups of five captive chimpanzees each (N=20) at Kyoto University Kumamoto Sanctuary for 421 cumulative hours. Our data collection included scan data of interindividual proximity, social behavior of all individuals at two-minute intervals, as well as all occurrences of urination and the immediate proximity of individuals during urination events. We then analyzed chimpanzee urination behavior in three key aspects: synchrony, contagion, and the underlying social factors. First, to assess synchronization, we compared our observational data to computer-simulated sessions. Findings indicated a significant level of synchronization in the observed urination events, surpassing that observed in simulations. Second, we employed generalized linear mixed models (GLMM) to investigate the role of contagion. We found that proximity to a urinating individual has a significant effect on the likelihood of urination ($p < 0.01$), providing compelling evidence for contagion in the urination behavior of chimpanzees. Third, we explored the social characteristics of initiators and followers, considering social dominance and social centrality to discern social patterns of contagion. We find a significant effect of dominance status, with lower-ranking individuals more likely to follow the urination of higher-ranking individuals ($p < 0.01$). Our findings therefore suggest that urination in captive chimpanzees does exhibit clear signs of behavioral contagion, and the observed connection between social rank and urination contagion may provide insights into chimpanzee leadership dynamics.

# Measuring Emotional States through Judgment Bias via a Go/no-go Touch Screen Task in Chimpanzees:

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Emotional contagion can be challenging to directly investigate in non-human animals because it is oftentimes difficult to determine their emotional states with complete certainty. As such, the judgment bias paradigm can be used to infer the emotional state of animals. This design utilizes the tendency of individuals experiencing negative emotions to judge ambiguous situations pessimistically, and those feeling positive emotions to judge ambiguous situations optimistically. In this judgment bias task animals are firstly trained to associate different stimuli with positive and negative outcomes respectively, and once training is complete their response rate and speed towards novel, neutral stimuli can be recorded to infer the valence of their emotional state. With the goal of eventually investigating emotional contagion through video presentation in chimpanzees, we trained 7 chimpanzees on a touch screen judgment bias go/no-go task. During training chimpanzees would be presented with a touch screen panel, upon which buttons of light or dark color would be presented periodically. After pressing buttons of one of the colors the chimpanzees would be rewarded with food, while pressing the opposite color would not result in anything. The individuals that completed this training phase proceeded onto the next phase, in which snake images were utilized as emotional stimuli to confirm if a change in emotions would result in a speed or frequency shift for button-pressing. We found that there was a difference in response latency between individuals, suggesting that the effectiveness of this method may be individual-specific. It is possible that this judgment bias task can be used to successfully determine emotional valence for some chimpanzee individuals, thus potentially establishing an experimental basis for investigating emotional contagion with this paradigm using video stimuli of conspecifics expressing emotions.

# Investigating the importance of observed causal effects during the social learning of tool set use in chimpanzees: preliminary report

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Chimpanzees are one of the most proficient tool users in the wild, and previous studies have emphasized social learning in the propagation of their tool-using behaviors. However, the demonstrated behaviors in previous studies are mostly simple tool-use consisting of single-step manual manipulation, where physical understanding and social facilitation are difficult to distinguish. Wild chimpanzees can also master more complex tool-using behaviors, like tool set use, involving two or more tools in multiple steps and sometimes operates opaquely, viz the goal of each step is not obvious or not directly linked to immediate reward. Studies have shown that such kind of complex tool use could hardly be individually innovated and should be a product of social learning. With these features, tool set use could be a feasible way to discern the effects of physical understanding and social facilitation during the learning process. The current study therefore aims to investigate how visual information may interact with social learning in tool set use. We developed the "flowing peanut task", which requires subjects to use water and sticks sequentially to obtain the peanut. We presented it to 13 captive chimpanzees and confirmed that no individuals solved the task either individually or socially in the absence of a demonstrator. The next experiments will offer first human, then conspecific, models, in both transparent and opaque conditions to the subjects. Under the controlled presentation of different information, results of the learning process will indicate the role of both a model and the observed causal effects in their understanding of and ability to execute more complex tool-use.

# People and Gorillas, from Uganda to Japan: the impacts of interactions between tourists and gorillas on their behavior and species conservation

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Tourism can have a significant impact on the conservation of species, especially when it involves emblematic species that serve as flagship and umbrella for their habitat. A prominent example of this phenomenon is Gorilla Tourism, which is often advocated to be a primary driver for the growth in the population of mountain gorillas (*Gorilla beringei beringei*) through funding the protection of their habitat. However, research indicates that tourists can exert negative influences on primate species, leading to reasonable concerns that gorillas may also experience similar adverse effects. We assessed stress-coping mechanisms (prosocial behaviors of social buffering), behavioral indicators of stress (self-scratching), direct interactions with humans (agonistic, neutral and avoidance behaviors), and changes in social cohesion patterns (time spent with and number of individuals in close association), in the presence and absence of tourist groups visiting one group of mountain gorillas living in Bwindi Impenetrable National Park, Uganda. Generalized linear mixed models and Social network analysis were used to analyze differences in gorilla behavior as a function of (a) presence vs. absence of tourists and (b) proximity to tourists (<3 m vs >3 m). Contrary to guidelines, tourists spent 60% of their viewing time within 3 m of the gorillas. During tourist visits, gorillas increased time spent in prosocial behavior and rates of self-scratching and human-directed behavior, increasing also social cohesion. When tourists approached gorillas within 3 m, prosocial behavior, human-directed behavior, and social cohesion increased, with adult males presenting a pick of self-scratching rates. We conclude that tourists are influencing gorilla behavior and we recommend following and enforcing the IUCN guidelines by keeping a minimum 7 m distance when viewing gorillas. In a subsequent study conducted in captivity, we discovered that gorillas are the preferred species among zoo visitors. This finding holds significant importance as those visitors who express a preference for gorillas represent potential future Gorilla tourists. Consequently, zoos take on a central role in educating and raising awareness regarding close interactions with wildlife and responsible tourism practices.

Poster session (Nov 16)

Ligthing talk: 2 min/person in the poster session

| | Presenter | Affiliation | Title |
|---|---|---|---|
| P-1 | Alisa TOBE | Wildlife Research Center, Kyoto University | Analysis of Genes Related to Toxic Resistance in Crested Serpent Eagle Preying on Poisonous Invasive Alien Species |
| P-2 | Kazuya TODA | The Graduate University for Advanced Studies, Sokendai | Equal Mating Opportunities Among Male Bonobos? |
| P-3 | Takuto SUGIMOTO | Wildlife Research Center, Kyoto University | Horses with sustained attention evaluate humans based on their problem-solving abilities |
| P-4 | Momoka SUZUKI | Wildlife Research Center, Kyoto University | Mitochondrial DNA haplotype analysis of killer whales (*Orcinus orca*) around Hokkaido suggests a refugium for killer whales off Japan during the Last Glacial Maximum |
| P-5 | Xiaochan YAN | EHUB, Kyoto University | Gene expression pattern of hair follicles to unravel Melanism Color Evolution |
| P-6 | Yume OKAMOTO | Wildlife Research Center, Kyoto University | *Androgen Receptor* Gene Microsatellite Polymorphisms and Vocal Communication in Domestic Cats (*Felis catus*) |
| P-7 | Negin ESLAMIBIDGOLI | Wildlife Research Center, Kyoto University | Investigating the Latrine Behavior of Invasive Raccoon Dogs (*Nyctereutes procyonoides*) on Yakushima Island |
| P-8 | Sanjana P. KADAM | EHUB, Kyoto University | Exploring Attentional Bias in Chimpanzees |
| P-9 | Emi TOKUSHIGE | Kyoto University | An Approach to Comprehensive Analysis of Trichuris Parasites in Wild Primates Using the Global Mammal Parasite Database |
| P-10 | Marie SEKI | Wildlife Research Center, Kyoto University | Tsushima Leopard cats may scavenge on carcasses in Tsushima Island, Japan |
| P-11 | Mohamed M. SAIDI | Wildlife Research Center, Kyoto University | Genetic diversity of critically endangered captive black rhinoceros in Japan: Implication for conservation |
| P-12 | Li HEPING | Wildlife Research Center, Kyoto University | Feeding habits of migratory pinnipeds in the adjacent waters of northern Japan |
| P-13 | Ayumi OGAWA | Wildlife Research Center, Kyoto University | How do horses achieve joint action?: a case of mutual grooming |
| P-14 | Chinatsu NISHIMOTO | Wildlife Research Center, Kyoto University | Improvement of oocyte in vitro maturation techniques using domestic cats' ovaries to establish assisted reproductive technology in wild cats |
| P-15 | Kana ARAI | Wildlife Research Center, Kyoto University | Epigenetic Age Estimation of Asian Elephants (*Elephas maximus*) |
| P-16 | Shimei SHIRASAWA | Wildlife Research Center, Kyoto University | Research plan to measure emotional states in free-ranging Japanese macaques using an infrared thermal camera. |
| P-17 | Lin CHAOYU | Wildlife Research Center, Kyoto University | Primate origin of risk propensity and its effect on social status: field cognitive experiments with free-ranging Japanese monkey groups |
| P-18 | Abdullah LANGGENG | Wildlife Research Center, Kyoto University | Of Hot Spring and Bacteria: Linking hot spring bathing behaviour and gut microbiome in Japanese macaques |
| P-19 | Elio BORGHEZAN | Wildlife Research Center, Kyoto University | Females of the sailfin tetra prefer red-lightened environments |
| P-20 | Shintaro ISHIZUKA | Toho University | Sex - Specific Effects of Juvenile Offspring on their Mothers' Social Relationships with Other Females in Japanese Macaques (*Macaca fuscata*) on Shodoshima Island |
| P-21 | Chika ZEMMOTO | Wildlife Research Center, Kyoto University | Study plan on domestic yak's (*Bos grunniens*) population genomics |

# Analysis of Genes Related to Toxic Resistance in Crested Serpent Eagle Preying on Poisonous Invasive Alien Species

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The crested serpent eagle (*Spilornis cheela perplexus*) is an endemic top predator of Ishigakijima and Iriomotejima Islands and contributing to the maintenance of the balance in the island ecosystems with its wide variety of food animals. However, the eagle is classified as endangered (class IA) by the Japanese Ministry of the Environment, with approximately 100 individuals on each island. Moreover, the introduction and establishment of various non-native species is a significant problem on Ishigakijima Island. One such invasive alien species is the poisonous cane toad (*Rhinella marina*), known to be lethal to some of its predators, raising concerns about the negative effects to the native species of Ishigakijima Island. Surprisingly, despite frequent predation on this toad by crested serpent eagles, there have been no reported cases of poisoning. In previous studies, it was found that some animals which live with this toad in its original habitat have resistance of the toad's toxins. Crested serpent eagle also seems to have resistance to the toxin, even without any previous studies. The aim of this study is to confirm the genetic basis of the crested serpent eagle's toxic resistance. Additionally, we will also assess the population sustainability of the eagle based on the analysis of genetic diversity.

We utilized available annotated reference genome of crested serpent eagle for our analysis. Based on previous studies which identified mutations in functional genes (ATP1A) contributing to resistance to cane toad toxins, we explored three paralogs of ATP1A regions within the reference genome and conducted an analysis of finding mutations related to resistance. We conducted additional WGS on seven crested serpent eagle individuals (three from Ishigakijima Island and four from Iriomotejima Island) and compared the mutations in ATP1A gene. Additionally, we tried genomic analysis of the two island populations for comparing the genome-wide genetic diversity using PCA and heterozygosity.

The results of our study indicate the presence of specific mutations associated with resistance to cane toad toxins in one paralog of ATP1A within the crested serpent eagle's reference genome. In this presentation, we will also present the findings of mutation comparisons between individuals and the analysis of genetic diversity within two island populations.

# Equal Mating Opportunities Among Male Bonobos?

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In several primates living with multi-male/multi-female groups, female sexual swelling plays a role of the signal to advertise the timing of ovulation. Bonobos (*Pan paniscus*) are unique in that females exhibit sexual swelling even outside the timing of ovulation and are capable to frequently mate with males. Such a long-term sexual receptivity of female bonobos has been hypothesized to mitigate mating competition between males through the decrease in the ratio of the number of males to the number of estrus females at a given time ("estrus sex ratio" hypothesis: Furuichi 2011). However, Ryu et al. (2022) indicate that male bonobos expend more their mating effort toward estrous females around the timing of ovulation than pseudo-estrus females. Thus, male bonobos seem to detect female reproductive state with some manner ("males know" hypothesis). Actually, in two populations of wild bonobos, the reproductive skew was much biased towards high-ranking males. Nonetheless, if so, what is the function of long-term sexual receptivity in female bonobos? As the first step to explore an ultimate factor of the non-reproductive mating behavior in bonobos, I examined a correlation between mating frequency of male bonobos and their dominance ranks, using observational data collected with all-day focal animal sampling from wild bonobos at Wamba, Luo Scientific Reserve, in Democratic Republic of the Congo. Male bonobos were observed to engage in mating interactions at least once for 33 out of 41 days (80%). Also, their mating frequencies were not correlated with rank, on the contrary to results of three previous studies at the same field site. Mating opportunities seem to be considerably equal for all males of the group, in regardless of their reproductive success. It could be possible that non-reproductive mating interactions have proximate mechanisms in reducing the escalation of male aggression. The next study plans to investigate physiological responses of male bonobos after mating interactions by measuring urinary hormone levels.

# Horses with sustained attention evaluate humans based on their problem-solving abilities

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The ability to recognize and evaluate others is considered to be an important ability in selecting cooperative partners. There have been some studies on this ability in both humans and nonhuman animals, however, little empirical research has been conducted to examine how horses perceive humans. Here, we investigated whether horses can evaluate the problem-solving ability of humans in an unsolvable task. We showed horses a pair of two experimenters, one who can open the lid of a container (problem solver) and the other who cannot open the lid of the container but unintentionally open it (problem nonsolver), trying to open the lid of the container either with or without food in it. Gaze duration toward each experimenter was recorded and used as a proxy measurement of the attention of the horses. Furthermore, we investigated the horses' visual attention levels toward human experimenters to evaluate the relationship between their motivation and their performance of the task. Horses that sustained high attention levels watched the problem solver longer than the problem nonsolver in the food condition. They also watched the problem solver for longer in the food condition compared to the no food condition. These results suggest that horses can recognize the problem-solving ability of humans. This study also indicate that motivation for the task should be taken into account when examining the cognitive abilities of animals.

# Mitochondrial DNA haplotype analysis of killer whales (*Orcinus orca*) around Hokkaido suggests a refugium for killer whales off Japan during the Last Glacial Maximum

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Glacial periods can have impacts on range of habitats and population size of animal species. Killer whale (*Orcinus orca*), which is globally distributed, has a widespread population bottleneck during the Last Glacial Maximum (LGM). Then, some ice-free sea areas were used as refugia for them, so the populations retained in such regions often preserve stable and rich genetic diversity. In the eastern North Pacific, there are two haplotypes among the fish-eating killer whales, Southern Resident (SR) and Northern Resident (NR), though each habitat is adjacent. SR haplotype is also frequently found in the western North Pacific, indicating the ancestors of SR survived during the LGM there. Recently, Filatova *et al*. (2023) found a new haplotype among the killer whales sampled in Nemuro Strait, by control region in mitochondrial DNA haplotype and acoustic analyses. The new haplotype suggests a high genetic diversity level, indicating that off Japan could be used by killer whales as a refugium during the LGM. About killer whales off Japan, it became clear that there are fish-eating group and marine mammals-eating group, by analyzing D-loop and cytochrome *b* regions in mitochondrial DNA (Mitani *et al*. 2021). However, there are few studies about their genetic diversity using samples around Hokkaido.

In this study, we made a neighbor-joining tree and a haplotype network using control regions or those cropped from mitochondrial genomes of fish-eating groups to assess genetic diversity. We used sequences of mitochondrial genomes of 4 samples around Hokkaido (2 from off Rausu and 2 from off Kushiro). A comparison of our samples with NR (haplotype 35 in Morin *et al*. (2010)), SR (haplotype 32 in Morin *et al*. (2010)), and the new one found by Filatova *et al*. (2023), suggests our samples may share another new haplotype if there are no gaps in the beginning 6 bases of the mitochondrial DNA control region. If it is a novel one, there can be multiple haplotypes off Hokkaido. Furthermore, in focusing on our samples, we also found that 1 from Rausu and 2 from Kushiro share whole mitogenome, and in the control region, all our samples share the sequence. It indicates there is intercourse among killer whales off Kushiro and Rausu. These results suggest the ice-free region in the western North Pacific including off Japan was an important refugium for them during the LGM.

# Gene expression pattern of hair follicles to unravel Melanism Color Evolution

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Animal use coloration to conceal themselves from predators, to regulate body temperatures, to advertise their toxicity, or to attract mates. Animal coloration, including coloration and patterning, has often been taken as evidence of adaptation driven by selection. Such adaptations respectively occurred across the animal kingdom, offer a rich example for integrating our understanding of 'evolution' at macro to micro scale, i.e., ecology, genetics, and molecular biology. Most mammals exhibit darker dorsal and lighter in ventral and/or limbs pelage, probably for both concealment and thermoregulation. Studies have repeatedly found the same locus (MC1R and ASIP) or genomic regions to be responsible for melanism adaptation in domesticated mammalian species. However, due to the technical challenges of comprehensively analyzing wild animal population, there are few examples that clarified coloration adaptation in non-domesticated mammals. By addressing these aspects, the study will focus on coloration and patterning evolution of Sulawesi macaques, which rapidly evolved from common ancestor and diverged into melanism coloration and specific color patterns in each species. First of all, we collected hair roots from black(dark) hair and white(bright) hair of *M. ochreata* individual and determined total RNA expression responsible to different color phenotype. We found black hair samples have more similar expression pattern which are clustered in PCA analysis. While white hair samples showed large variation. Further, we found key genes evolving in melanogenesis have no expression difference between black and white hair, rather genes for melanosome transportation and transferring have significantly high expression in black hair samples. So far, our result suggested the sufficient method of RNA sampling from hair roots and showed the possible internal causality of body color pattern. Further analysis and comparison between different coloration phenotype among Sulawesi macaques will provide a deeper understanding of how coloration in primates evolves and adapts to different environments.

# *Androgen Receptor* Gene Microsatellite Polymorphisms and Vocal Communication in Domestic Cats (*Felis catus*)

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Domestic cats (*Felis catus*) are popular companion animal and play an important role in the society through human-cat relationship. They show various behaviors while communicating with humans and other cats, such as vocalizations and aggressions. It is reported that in many species, behaviors are associated with genetic backgrounds in addition to environments. Recent genetic investigations have examined that *androgen receptor* gene (*AR*) microsatellite polymorphism in exon 1 region located on the X chromosome is in relation to various traits in many animal species, for example, violent criminal behavior in men (Rajender et al., 2008) and aggression in male Japanese Akita Inu (Konno et al., 2011). A previous study on domestic cats also revealed the CAG repeat polymorphisms in *AR*, but their association with behavior is still not known (Kato et al., 2007).

The objective of this study is to investigate association between *AR* microsatellite polymorphism and behavior in domestic cats. An online questionnaire survey was conducted for behavior assessment using Feline Behavioral Assessment and Research Questionnaire (Fe-BARQ). We also extracted DNA from cats' buccal cells and analyzed CAG repeat number in *AR*. A total of 155 male mixed breed was used for the assessment. All individuals were spayed. 7 alleles ranging from repeat numbers 15 to 21 were found. We divided alleles into 2 groups using medians as cut-off, then categorized alleles with 18 repeats or fewer as short type (S)  and those with 19 repeats or more as long type (L). The result of Wilcoxon rank sum test showed that individuals having S/- genotype showed higher "Directed calls / vocalizations ($p = 0.020$)" and "Purring ($p = 0.005$)" scores than L/- genotype.

Domestic cats' vocal communication is suggested to be used to attract people (Mertens & Turner, 1988), and purring is used in the context of promoting contact, for example, to solicit food from their human (McComb et al., 2009). Therefore, a short CAG repeat polymorphism may positively relate to affiliative behavior in male cats. This finding is contrary to previous studies which have suggested that shorter CAG repeat relates to aggression-related behavior in humans and dogs (Rajender et al., 2008; Konno et al., 2011). A possible explanation for this might be an interaction with another polymorphic region. The previous research on men had reported that *AR* exon 1 contains 2 polymorphic regions (GGN repeat and CAG repeat) and suggested that short CAG is associated with extraversion, only in the case of individuals carrying long GGN (Westberg et al., 2009). Therefore, it might be possible to consider that social behaviors might reflect a combination of polymorphisms in *AR*. A further study on other polymorphisms is therefore suggested. The research on genetic backgrounds of domestic cats' behavior would contribute to enhancing future human-cat relationships.

# Investigating the Latrine Behavior of Invasive Raccoon Dogs (*Nyctereutes procyonoides*) on Yakushima Island

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In recent decades, invasive alien species (IAS) have become one of the major causes of biodiversity loss and species extinction. Invasive species compete over various resources with native inhabitants, posing a threat to the biodiversity in their new environment. Also, they could potentially introduce new pathogens and promote disease transmission within the native community, and consequently harm the health of other species, including humans, as well as cause economic losses. The Japanese raccoon dog ("tanuki"; *Nyctereutes procyonoides*) is an opportunistic generalist species that is native to mainland Japan but invasive on Yakushima Island. One of the fundamental behaviors of tanukis is their communal defecation and urination sites, called latrines. However, this behavior has not been extensively studied and its function is not yet known in any detail. Following research on other latrine-forming species, our hypothesis is that latrines serve a role in communication between individuals for various purposes, for instance, between males and females during the mating season. To discover more about the function of tanuki latrines, in this study, we mainly ask three questions: (1) are there sex biases in latrine use? (2) do kin preferentially use the same set of latrines? and, (3) can environmental factors such as change of season influence patterns of latrine use? We will use a combination of ecological and molecular analyses to conduct this study. The core methodology for this project relies on latrine surveys and sampling. **Fresh feces are being collected from latrines in Yakushima's western coastal forest and beaches around Nagata village**. With those samples, we will conduct **genetic analyses** for sex identification, for example using sex-determining genes such as SRY, and individual identification using microsatellite markers from the literature on tanuki and other canids. Through this research, we aim to provide a deeper understanding of the tanuki invasion on Yakushima by focusing on a fundamental behavior. We also plan on downstream analyses such as creating social networks to study social behavior and infectious disease transmission. Finally, our data will allow for comparative studies on genetic diversity in tanuki on Yakushima and mainland Japan, and potentially comparisons of latrine behavior between invasive and native tanuki throughout Japan.

# Exploring Attentional Bias in Chimpanzees

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Attentional bias is a cognitive tendency to focus on specific information while disregarding the rest. In animals, the exact time taken to process specific information which can affect these biases is still being discussed. Our study utilizes attentional blink to shed light on what information affects attention and processing time for chimpanzees (*Pan troglodytes*). To investigate this, we conducted a rapid serial visual presentation task on chimpanzees, exposing them to a stream of images. While the chimpanzees are still undergoing training, initial findings may suggest a trend towards images in specific positions within the image stream. This type of bias can be adaptive in various situations, particularly when facing threats, as it enables individuals to focus on the most dangerous elements in their environment and respond effectively.

# An Approach to Comprehensive Analysis of Trichuris Parasites in Wild Primates Using the Global Mammal Parasite Database

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Researchers have been accumulating knowledge on soil-transmitted helminths (STHs) that infect various primates through investigations over time. However, few study have taken the perspective of carefully-selected individual parasite species or genera and comprehensively analyzed their infection patterns from this sea of information. We attempted such an analysis of whipworms (*Trichuris* spp.), which are widely distributed throughout the world from the data of the papers on wild primates.

The Global Mammal Parasite Database (GMPD: https://parasites.nunn-lab.org/ ) is a database focused on records of host-parasite associations from the published literature.

We downloaded as csv files all records that met the criteria of involving whipworms (genus *Trichuris*) from the GMPD's Query Primate Data. First, we summarized the records in terms of the number of studies, the primate species involved, the region of study, and so on. Then, we statistically analyzed the occurrence of whipworms in primate hosts according to various host conditions, including geographic and socioecological characteristics, and tested for sampling biases and knowledge gaps.

A total of 374 primate populations from 142 papers were included in our data set, coming from 79 primate species were included as hosts. Chimpanzees had the largest number of individual reports, followed by Japanese macaques. Most records involved catarrhine primates, with only four reports about platyrrhines. Most papers used morphological descriptions to identify *Trichuris* through egg shape and did not use DNA analysis. Therefore, although some papers were identified to species, which is insufficient to distinguish between species of whipworm. Statistical analyses are ongoing.

We also identify challenges with research using databases like the GMPD. For example, the number of papers may be large for primates that are easy to observe or approach in their habitats or are of high interest, resulting in a strong publication bias affecting the data. What is needed to solve these problems and conduct highly accurate, systematic, and bird's-eye view analyses will also be discussed.

# Tsushima Leopard cats may scavenge on carcasses in Tsushima Island, Japan

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Scavenging is the consumption of animal carcasses. Whether vertebrates are able to scavenge depends on the accessibility and availability of carcasses, which can be affected by human hunters.

On Tsushima Island, more than 8,000 wild boar and deer are hunted each year. Despite the appropriate deer population size in Tsushima of just 3,500 individuals, there are more than 40,000 individuals and they cause serious damage to crops and the forest floor.

The Tsushima leopard cat (*Prionailurus bengalensis euptilurus*) is endemic to Tsushima Island and is a subspecies of the mainland Leopard cat. There are approximately 100 individuals that inhabit the island, and their diet is still not yet well understood. We hypothesize that the Tsushima leopard cat may utilize (i.e. consume) deer and wild boar carcasses, and that this utilization rate may be affected by carcass availability which is affected by hunting.

Two methods will be conducted to test this hypothesis. First, we will conduct species identification of the hair collected from the feces of Tsushima leopard cats by observing its surface and thickness. Also, using DNA from the fecal samples we will conduct species identification. We will set primers for the Cytochrome b regions of each species (deer, wild boar, leopard cat) and select a specific region, and the frequency of occurrence (FO) will be calculated. Next, we will create a model in which we analyze the correlation between the rate of FO as an explanatory variable and other data as objective variables, including the number of hunted deer and wild boar, the number of individuals captured by camera traps, and the season in which the fecal sample was collected. If the consumption of deer and wild boar is confirmed, this study will be the first to verify the possibility that the Tsushima leopard cat scavenges on such carcasses. This study will also verify utilization rate of such carcasses in relation to hunting.

# Genetic diversity of critically endangered captive black rhinoceros in Japan: Implication for conservation

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Black rhinoceros (*Diceros birconis*) is under great threat of extinction in the wild condition due to illegal hunting for their horns and habitat loss (Lacy, 2019). Their risk of extinction is exacerbated by less chance of migration, loss of genetic diversity and management in fragmented sub-populations (Frankham, 2016). Maintaining captive population is important as the insurance of wild population (McGowan et al, 2017). Success of captive breeding program depends on maintaining genetically health population that is capable to reproduce, withstand and evolve in response to environmental changes, diseases, pests and parasites (Gaines et al, 2010).

Therefore, this study intends to evaluate and provide information of genetic diversity with 21 captive black rhinoceros, those are almost all individuals kept in Japan, so as to inform future conservation and management interventions.

DNA samples were extracted from blood or feces provided from 10 zoos in Japan (Asa, Kanazawa, Yagiyama, Tennoji, Ueno, Yokohama Zoorasia, Tobe, Kamine, Higashiyama and Kumamoto). We analyzed haplotypes of mtDNA control region sequence (477bp), and compared with the previous report of wild populations in Africa (Moodley et al, 2017) for revealing genetic diversity in Japan.

Four haplotypes of maternal lineage were identified with overall haplotype diversity of $h = 0.65$. This suggests a slight loss of genetic diversity in Japan, comparing with reported haplotype diversity ($h = 0.88$) of east African haplogroup (Moodley et al, 2017).

Three Japanese haplotypes showed the same sequence as Eastern African haplogroup, and remaining one haplotype was as Southern African haplogroup. Therefore, classified into sub species *Diceros bicornis michaeli* and *Diceros bicornis nyasae* distributed across Kenya, Tanzania and Zimbabwe respectively. The result suggests that Japanese population possibly maintains a sub species (*Diceros bicornis nyasae*) which has not been recognized in recent years in Africa. However, further analysis based on the whole genomic data is required.

We recommend that, current level of diversity be maintained and improved by allowing additional variation by re-introduction of genetically diverse individuals into Japanese gene pool, although further analysis is required with nuclear genome such as microsatellites. Therefore, the analysis of 11 microsatellite markers is currently ongoing with the same sample set. Our genetic data has the potential for contributing to develop effective conservation managements.

# Genetic diversity of critically endangered captive black rhinoceros in Japan: Implication for conservation

Mohamed M. Saidi[1,2], Yu Sato[1], Sherif Ramadan[3] & Miho Inoue-Murayama[1*]

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# How do horses achieve joint action?: a case of mutual grooming

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Many social animals interact jointly, but only humans and some other primate species are considered to experience a specific sense of obligation toward their co-participants, a joint commitment. Joint commitment is not only a mental state but also a process that reveals itself in the coordination efforts deployed during entry and exit phases of joint action. Here we investigate whether horses engage in behavioral coordination during entry phases of their allogrooming. Allogrooming in horses is primarily in the form of mutual grooming, i.e. two individuals groom each other simultaneously, which should requires more active cooperative engagement of the participants compared to unidirectional grooming. Therefore, we predicted that horses demonstrate some communicative signals during the entry phase of mutual grooming. We also hypothesized that if such communicative signals have a function of soliciting joint commitment, they should be affected by the social dimensions between partners in similar ways as in humans, e.g. social distance and social rank. In this study,  we analyzed videos of feral horses living in Serra d'Arga, Portugal, and identified a total of 124 grooming events. Of these, 24 events were accompanied by headbobbing, a conspicuous vertical head shaking, before mutual grooming. Social factors that may influence the occurrence of headbobbing will be investigated.

# Improvement of oocyte in vitro maturation techniques using domestic cats' ovaries to establish assisted reproductive technology in wild cats

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Assisted reproductive technologies (ART), including artificial insemination, *in vitro* fertilization, *in vitro* maturation, and gamete cryopreservation, have been developed for the treatment of human infertility medicine and can be useful tools for maintaining genetic diversity in endangered species and overcoming the problem of infertility in those species in captivity. ART using oocytes have not been studied in comparison to sperm, due to the difficulty of obtaining them including felids. The ability to utilize oocytes for *in vitro* fertilization in association with *in vitro* maturation offers enormous potential to preserve female fertility including infertile individuals and even after death. However, the optimal culture condition for *in vitro* oocytes maturation remains to be developed in the feline family, which has many endangered species.

Using domestic cats as a model of wild feline species, we aimed to develop the optimal culture condition for *in vitro* maturation of oocytes for future application for *in vitro* fertilization. Firstly, several analyses were performed to confirm the oocytes viability. Then, we focused on sirtuin genes which maintain and activate cellular functions and examined the influence of its activator, nicotinamide mononucleotide (NMN), on oocyte maturation. Cat oocytes were cultured with three different concentrations (10, 100, 1000 μM) of NMN for 28 h, and their effects on oocyte maturation were evaluated by tubulin staining.

Currently, 225 oocytes have been recovered from six individuals. In the viability analysis, neutral red staining was effective, and we confirmed that a large number of oocytes were viable when the ovaries were received one day after the sterilization. In the maturation evaluation, tubulin staining successfully differentiated the five of six maturation stages including metaphase-II (MII) oocytes, which were considered to be fully matured. By using these analysis and increasing the sample size, we plan to further investigate the effects of NMN on oocytes maturation in domestic cats to apply for wild feline species.

# Epigenetic Age Estimation of Asian Elephants (*Elephas maximus*)

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Asian elephants (Elephas maximus) are classified as endangered by the International Union for Conservation of Nature (IUCN) due to anthropogenic effects. This has led to a decline in population with only approximately 50,000 individuals left. Knowing an animal's age is an important ecological tool in wildlife conservation. This is such as providing information on demographic trends and population viability in the wild and allowing appropriate care in captivity. However, current methods to estimate age are not always accurate and difficult to observe in the wild for many species. This is also applicable for Asian elephants where age is estimated through observing their physical features, mostly from culled individuals and not living. Visual estimation in long-lived species like the elephants is also prone to gross overestimation and/or underestimation. As they mature slowly, the visual determination of 'maturity is subjective and difficult. Thus, another reliable method to estimate age is required. DNA methylation is an epigenetic modification consisting in the addition of a methyl group in cytosine-guanine loci. Changes in DNA methylation at specific CpG sites have been used to build predictive models to estimate animal age. Here, we have generated a reduced representation bisulphite sequencing (RRBS) data set from captive Asian elephants ($n = 66$) across Japanese zoos, aged from 0.25–65 years. RRBS is suggested to have a higher resolution than the most used microarray-based method, which typically only probe a subset of CpG sites in the genome. Here, we will report the results of our RRBS analysis to reveal the methylation levels and the correlation between CpG sites and chronological age in captive Asian elephants. This study highlights the potential further use of age-associated DNA methylation dynamics which could inform conservation and management practices, as well as linking ecological and evolutionary understanding of biological ageing for future studies.

# Research plan to measure emotional states in free-ranging Japanese macaques using an infrared thermal camera.

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While understanding animal emotions has long been a challenging topic in zoology, it has not been fully studied due to its inherent complexities. Traditionally, such studies were especially difficult because they required measurements to be physically attached to the animals, and research was primarily conducted in laboratories. In contrast, contact-free methods such as infrared thermal cameras (IRT) offer the potential to uncover deeper emotional states in wild animals during social interactions. Recent studies using IRT imaging have revealed the physiological states of animals when exposed to ecologically relevant situations. For example, grooming, a form of affiliative behavior, is observed across various primate species. This behavior occurs regardless of an individual's rank within a group. However, the actual feelings of comfort or discomfort experienced by the individual being groomed remain uncertain. It's known that lower-ranking individuals often exhibit an increased heart rate in the presence of a higher-ranking member, a response akin to nervousness in humans. Prior research indicates that nose surface temperature decreases during states of competition, excitement, and tension.

In this study, we will be using an IRT to measure variations in the nasal surface temperature of free-range Japanese monkeys (Macaca fuscata) on Koshima island, Japan, during their grooming interactions, especially between kin and non-kin individuals. This will provide insights into the emotional valence associated with grooming.

# Primate origin of risk propensity and its effect on social status: field cognitive experiments with free-ranging Japanese monkey groups

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Primates encounter complex survival situations and social settings that necessitate taking risks when the time is right, thus enhancing their survival odds. Unraveling the evolution of risk-averse cognition is indeed a task with intricate details and factors at play. Studies shows that behaviors in primate species involve both risks and benefits such as consuming unfamiliar food and engaging in innovative activities, which could be harmful but bring new benefits to them. Japanese macaques are an ideal species to study the origins and implications of risk-taking behavior. Among the social behaviors of Japanese macaques, some outstanding behaviors seem to imply the specificity of their cognitive level and risk propensity preference, such as they also consuming high risky foods which does not belong to their recipes. This behavior highlights a possible correlation between risk-taking choices and behavioral patterns in Japanese macaques. Our research purview will encompass these factors, appraising their potential impact on the inclination towards risk. This research distinguishes itself as the first to employ field cognitive experiments with free-ranging Japanese macaques, aiming to decode their intricate understanding of risk-taking. Precisely, our aim is to explore the connection between risk preference and individuals' properties such as age, sex and social status in free-ranging Japanese macaques. This study will provide a more nuanced understanding of risk-taking behavior, potentially revealing variations in survival strategies among different demographic and social groups within the macaque population.

# Of Hot Spring and Bacteria: Linking hot spring bathing behaviour and gut microbiome in Japanese macaques

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Hot spring bathing behaviour (HSBB) is a form of balneotherapy, generally beneficial for health and stress relief. The practice is primarily performed by humans and has not been reported in other animals, except in a population of Japanese macaques (*Macaca fuscata*) at the Jigokudani Snow Monkey Park (JMP) in Nagano, Japan, particularly during cold seasons. There have been several cases showing that sharing hot water for bathing facilitates the transmission of certain (micro)organism. For example, it has recently been reported that the similarity of faecal microbial communities between family members who shared bathtub water was significantly closer than between those who did not. The Japanese macaques at JMP provide an ideal (and perhaps the only) model to study the effects of HSBB on the gut microbiome of non-human primates. Our research aimed to investigate whether bathing would affect gut microbial communities, either directly through differential bacterial exposure or indirectly through hormonal responses. However, we did not make specific predictions about the exact way in which the gut microbiome might be affected. The study was carried out over two winter seasons between 2019 and 2021. We collected fresh faecal samples from sixteen adult female macaques (9 bathers and 7 non-bathers) that varied in age, reproductive cycle and rank. The samples were processed and subjected to 16S rRNA Illumina sequencing. Our results showed no significant differences in the alpha and beta diversity of the gut microbiome between bathers and non-bathers. However, four microbial genera (*Methanobrevibacter, Granulicatela, Fusobacterium* and *Acinetobacter*, some of which are opportunistic pathogens when the host immune system is compromised) were found to be enriched in non-bathers. Future studies should consider incorporating additional variables, such as hormonal and immunological aspects, in order to assess the impact of bathing.

Key words: *Macaca fuscata*, gut microbiome, hot spring, wildlife tourism, animal behaviour

# Females of the sailfin tetra prefer red-lightened environments

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Colours and their preference have profound impacts on individuals' fitness of visually-oriented species. Colours are important to find food resources and mates of better quality and modulate several physiological and behavioural features. Such processes may be more important to sexually dimorphic species, especially dichromatic ones since visual signals play an important role in intra- and interspecific communication. Amazon small streams vary widely regarding the amount of dissolved organic carbon resulting in black (red-biased) and clear (mostly transparent) water types. The sailfin tetra *Crenuchus spilurus* is a small-sized and sexually dimorphic and dichromatic species composed of two main genetic lineages that live in black and clear water types. Recently, we have shown that the female sexual preference for male ornaments colouration differs between individuals inhabiting black and clear water types. Females from black waters do not choose males primarily based on colouration under strongly red-biased lighting, but females from clear waters do, which suggests that the importance of red colours for sexual behaviour differs between lineages of the sailfin tetra. Here, we evaluated the sailfin tetra female colour preference through the choice between a red and a full spectrum enlightened environments. Females from black waters and clear waters preferred red-lightened environments. Such results suggest that the females are attracted to the red colouration outside the context of mating, irrespective of the prevailing colouration of their native environment. Such preference for red colours may be important for several processes in the sailfin tetra like feeding, predator avoidance and mating opportunities.

# Sex-Specific Effects of Juvenile Offspring on their Mothers' Social Relationships with Other Females in Japanese Macaques (*Macaca fuscata*) on Shodoshima Island

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Females of female-philopatric species form lifelong affiliative relationships with one another. Given that adult daughters can provide benefits to mothers in the future, mothers may invest more in juvenile daughters than juvenile sons. A potential implication of this sex difference in investment is that mothers of juvenile daughters have reduced capacity to form affiliative relationships with other adult females. We tested whether the strength of female–female affiliative relationships varies depending on the sex of their juvenile offspring in free-ranging Japanese macaques (*Macaca fuscata*). We analyzed affiliative interactions (spatial proximity and social grooming) of 14 adult females to assess the strength of affiliative relationships between them over a 3-month period. We found that affiliative relationships between females with juvenile daughters were weaker than those between females without juvenile daughters. In contrast, the presence of juvenile sons did not significantly influence the strength of affiliative relationships between females. Mothers associated more with juvenile daughters than with juvenile sons, suggesting that mothers invested more in associating with juvenile daughters than with juvenile sons. Our data confirmed that females form strong affiliative relationships with kin, as is typical for this species. The results imply that females invest more effort in offspring with whom they can maintain affiliative relationships after maturing, which may reduce their level of affiliative relationships with non-kin. Further studies over longer time periods are required to determine whether the reduction in non-kin affiliative relationships is temporary or permanent.

# Study plan on domestic yak's (*Bos grunniens*) population genomics

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The yak (*Bos grunniens)* is a bovine animal widely distributed in the highlands of Eurasia. In recent years, genetic studies on yaks have been conducted, and the whole genome information of wild and captive individuals has been read and registered. Remarkable results have been achieved not only in population genetic analysis but also in the elucidation of structural genes that may contribute to adaptation to upland environments. These results suggest that yak domestication began approximately 7,300 years ago in Qinghai, China. In addition, based on mitochondrial genomes, populations in the Pamir Mountains, which border Central Asia and China, are thought to be genetically distant from populations in Qinghai, suggesting migration of yak populations. These studies have been conducted primarily in Xinjiang Uygur Autonomous Region and Qinghai Province. On the other hand, actual yaks are widely distributed not only in Tibet but also in Eurasia, and it is difficult to fully understand the actual situation of widely distributed populations only with current data. In this study, we will perform whole-genome resequencing of two populations in Kyrgyzstan, which borders China across the Tien Shan Mountains in Central Asia. We aim to estimate the past effective population size (Ne) variation using the already registered wild yak (*Bos mutus*) and domesticated yaks in China and the two individuals in this study, and to estimate the time of divergence from the Tibetan Plateau population.