15th International Symposium *on* Primatology *and* Wildlife Science

March 1–2, 2021

Primate Research Institute founded 1967 Kyoto University

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Cover Photo: Bonobos at Luo Scientific Reserve, Democratic Republic of the Congo (Credit: Nahoko Tokuyama)

The 15th International Symposium on Primatology and Wildlife Science

PROGRAM

Note: Pre-recorded talks and live Q&As will take place on **Zoom**, and poster sessions on **LINC Biz**.

Day 1 (Monday, March 1)

Time	Abstract	(min)	Title	Speaker	Affiliation
10:00–10:05		(5)	Opening Remarks	Gen'ichi Idani	Wildlife Research Center, Kyoto University
Wild Animals and Conservation					Chair: Nahoko Tokuyama
10:05–10:25	O1	(20)	Examining the Possible Mechanisms and Adaptive Significance of Single and Multi-male Groups in Horse Societies	Pandora Pinto	Wildlife Research Center, Kyoto University
10:25–10:40	O2	(15)	Pair Chemistry in Golden Eagles: Relationship between MHC Genes and Breeding Success	Annegret Moto Naito	Wildlife Research Center, Kyoto University
10:40–10:55	O3	(15)	Roosting Dynamics Predicted by Nepotism in a Cooperatively Breeding Bird	Fumiaki Nomano	Department of Evolutionary Studies of Biosystems, Sokendai
10:55–11:35	K1	(40)	The Dawn of Animal Linguistics: Exploring Referentiality and Compositionality in Bird Calls	Toshitaka Suzuki	The Hakubi Center for Advanced Research, Kyoto University
11:35–13:00		(85)	<Lunch Break>		
Cognition and Welfare in Primates I					Chair: Yuko Hattori
13:00–13:15	O4	(15)	Chimpanzee (*Pan troglodytes*) Reinforcement Learning via Eligibility Traces	Yutaro Sato	Wildlife Research Center, Kyoto University
13:15–13:30	O5	(15)	Oxytocin Promotes Species-relevant Outgroup Attention in Bonobos and Chimpanzee	James Brooks	Wildlife Research Center, Kyoto University
13:30–13:45	O6	(15)	The Effect of 'Brightness Contrast' in Luminance Discrimination Tasks for Chimpanzees and Killer Whales	Ayumu Santa	Primate Research Institute, Kyoto University
13:45–14:00	O7	(15)	Material Discrimination Based on Concepts in Chimpanzees	Mikuho Yokoyama	Primate Research Institute, Kyoto University
14:00–14:15	O8	(15)	Socially Contingent Video Can Facilitate Referential Information Utilization in Chimpanzees: A Progress Report	Shenwen Xu	Primate Research Institute, Kyoto University
14:15–14:30	O9	(15)	Feeding Decisions Predict Parasite Infection in Sanctuary-housed Bonobos	Cécile Sarabian	Primate Research Institute, Kyoto University
14:30–15:00		(30)	<Coffee Break>		

Cognition and Welfare in Primates II					Chair: Andrew MacIntosh
15:00–15:15	O10	(15)	Evaluation of a Novel Activity Monitor for Captive Japanese Macaques (*Macaca fuscata*)	Vanessa Gris	Primate Research Institute, Kyoto University
15:15–15:35	O11	(20)	Exploring Non-invasive Methods to Better Understand the Stress Response of Japanese Macaques (*Macaca fuscata*)	Nelson Broche	Primate Research Institute, Kyoto University
15:35–15:50	O12	(15)	Play Escalation in Japanese Macaques: It is a Matter of Relative Rank and Relative Age	Sakumi Iki	Department of Evolutionary Studies of Biosystems, Sokendai
15:50–16:30	K2	(40)	Why Conservation Needs Anthropology	Joanna Setchell	Department of Anthropology, Durham University
16:30–17:00		(30)	<Coffee Break>		
Poster Session I					
17:00–18:30		(90)	Posters on LINC Biz [see p. iv for titles]		
Conserv'session Discussions: *Close-proximity Pictures with Wildlife*					
18:30–19:30			[See the attached flyer for details]		

Day 2 (Tuesday, March 2)

Time	Abstract	(min)	Title	Speaker	Affiliation
Primate Bodies & Minds					Chair: Lira Yu
10:00–10:15	O13	(15)	Examining Long Bone Phenotypic Plasticity: A Case Study of Skeletal Differences between Northern and Southern Japanese Macaques	Rose Leach	Department of Anthropology, Kent State University
10:15–10:30	O14	(15)	Female Fertility Rates between Subspecies of Captive Rhesus Macaques	Emilee Hart	Department of Anthropology, Kent State University
10:30–10:45	O15	(15)	Species-specific Loss of Function of Melanocortin-1 Receptor Gene (MC1R) in Seven Endemic Species in Sulawesi Island	Xiaochan Yan	Primate Research Institute, Kyoto University
10:45–11:00	O16	(15)	Staring "Death" in the Face: Chimpanzees' Attention towards Conspecific Skulls and the Implications of a Face-module Guiding this Behavior	André Gonçalves	Primate Research Institute, Kyoto University
11:00–11:40	K3	(40)	Mirrors, Yawns, and Death: Connections?	James Anderson	Department of Psychology, Graduate School of Letters, Kyoto University
Poster Session II & Lunch Break					
11:40–13:00		(80)	Posters on LINC Biz (see p. v for titles) and Lunch		

Wildlife Science and Conservation					Chair: Gen'ichi Idani
13:00–13:40	K4	(40)	From Himalaya to Ocean, from Glacier to Tropical Rain Forest: Studies by a Strange Biologist	Shiro Kohshima	Wildlife Research Center, Kyoto University
13:40–14:00	O17	(20)	Re-evaluate Ethno-Ornithological Recognitions of Kazakh Eagle Falconry for Conservation of Golden Eagle, Western Mongolia	Takuya Soma	Hakubi Center & WRC, Kyoto University
14:00–14:30	O18	(30)	The Planet and Humans at a Crossroad and SDGs	Masahiko Horie	Ministry of Foreign Affairs of Japan
14:30–15:00		(30)	<Coffee Break>		
Japan–Australia Wildlife Science Collaboration					Co-Chairs: Frank Grützner, Takashi Hayakawa
15:00–15:05		(5)	Introduction	Takashi Hayakawa	Faculty of Environmental Earth Science, Hokkaido University
15:05–15:45	K5	(40)	Genomes & Threatened Species Conservation	Carolyn Hogg	School of Life and Environmental Sciences, University of Sydney
15:45–16:00	O19	(15)	The First Reference Genome for the *Antechinus* Genus Provides a Resource for Investigating the Genetic Basis of Semelparity and Age-related Neuropathologies	Parice Brandies	School of Life and Environmental Sciences, University of Sydney
16:00–16:05		(5)	Introduction to Monotreme Biology	Frank Grützner	School of Biological Sciences, University of Adelaide
16:05–16:20	O20	(15)	Characterising the Gut Microbiomes in Wild and Captive Short-beaked Echidnas Reveals Diet-Associated Changes	Tahlia Perry	School of Biological Sciences, University of Adelaide
16:20–16:25		(5)	Introduction to Japan–Australia Collaboration	Takashi Hayakawa	Faculty of Environmental Earth Science, Hokkaido University
16:25–16:40	O21	(15)	Stress Monitoring in Captive Koalas (*Phascolarctos cinereus*) Using Urinary Cortisol Concentration	Kodzue Kinoshita	Wildlife Research Center, Kyoto University
16:40–16:55	O22	(15)	What is the Favorite Leaf for Koalas: Koalas' Diet Preference and Welfare Studies in Japan	Tadatoshi Ogura	School of Veterinary Medicine, Kitasato University
16:55–17:00		(5)	Closing Remarks	Hiroo Imai	Primate Research Institute, Kyoto University

Poster Session I

Monday, March 1, 17:00–18:30 (core presentation time 17:00–18:00)

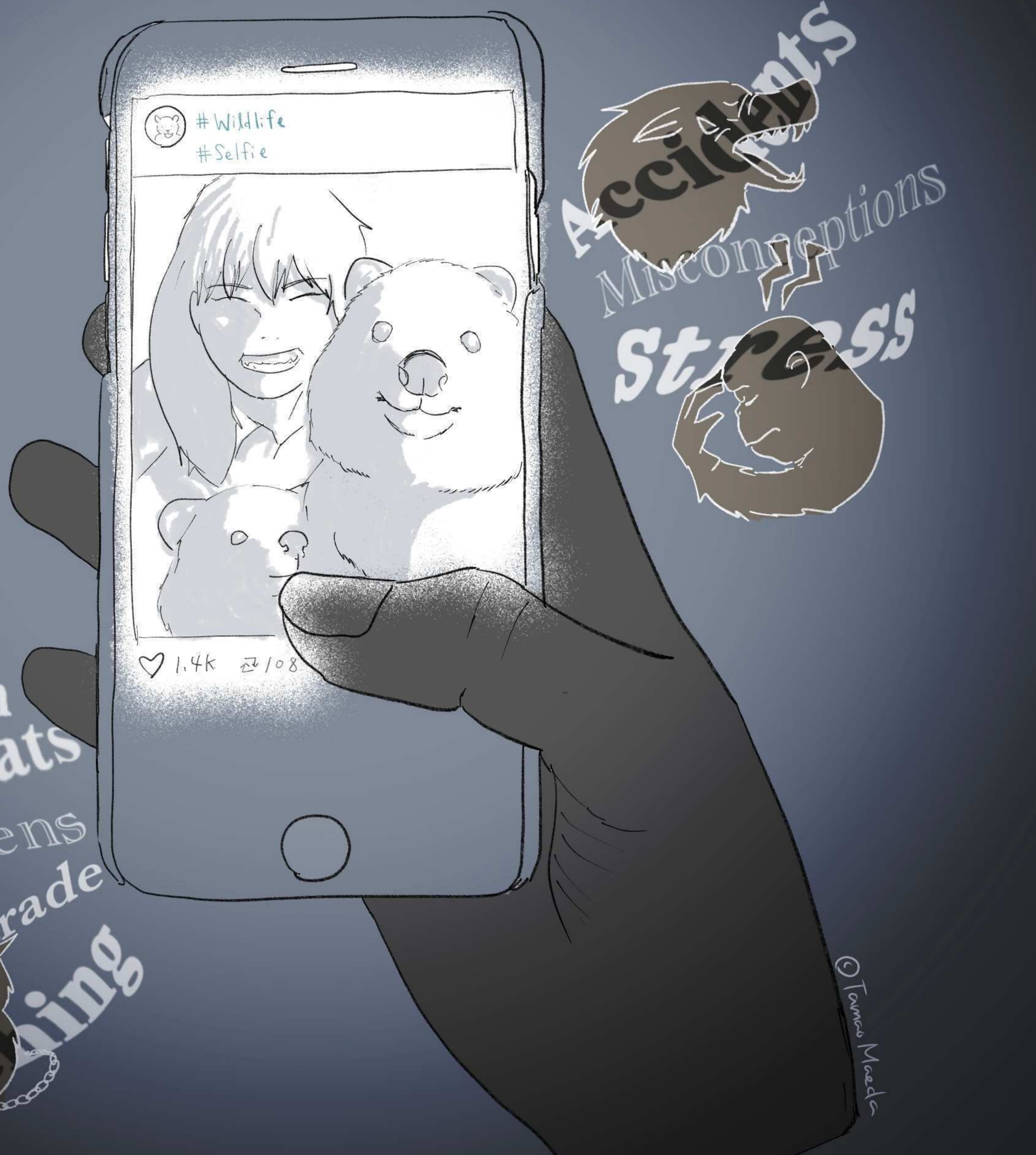
P1	Abdullah Langgeng	Diversity of Gastrointestinal Helminths in Japanese Macaques of the Jigokudani Snow Monkey Park
P2	Aki Sugita	Selective Foraging of an Arboreal Folivore, the Japanese Giant Flying Squirrel (*Petaurista leucogenys*)
P3	Akihiro Itahara	"Corvids Tracking Studio": The Motion Capture System as a Novel Tool to Study the Gaze of Corvids
P4	Akiho Muramatsu	Longitudinal Study of Chimpanzees' Working Memory
P5	Boyun Lee	High- & Middle-ranked Mothers Aggressively Handle Little Higher-ranked 2-3-month-old Infants in Yakushima Japanese macaques (*Macaca fuscata yakui*)
P6	Chika Zemmoto	Preliminary Characterization of Personality and its Genetic Factors in Two Dog Breeds
P7	Chisato Tanaka	Physiological and Behavioral Studies for Reintroduction in Tsushima Leopard Cat (*Prionailurus bengalensis euptilurus*)
P8	Cody Ruiz	Macaque Y-chromosome Introgression: Bioinformatic Analysis of Y-Chromosome Proteins in *Macaca mulatta*, *M. fascicularis*, and their Indochinese Hybrids
P9	David Fasbender	Socioecological Implications of the Unusual Distribution of Bonobos in the TL2 Landscape
P10	Ena Onishi	Behavioral Synchronization: Timing of Urination among Captive Chimpanzees
P11	Gema Palacino-Gonzalez	The Development of Mother-Calf Interactions in Wild Harbour Porpoises (*Phocoena phocoena*) Studied with Unmanned Aerial Vehicles (UAVs)
P12	Hana Goto	Gene Expression Analysis in a *Hylobates lar* Brain: Toward the Elucidation of the Molecular Mechanism of Song
P13	Hiroto Yoshimura	Does Over-marking Effective on Mate Search? A Mathematical Simulation
P14	Kana Arai	What Influences Eggshell Gas Exchange and is Personality based on Physical Characteristics in Different Avian Species? Research Plan and My Goals at PWS
P15	Kenneth Keuk	Enter SimuNet: a Social Network Simulation Framework, with a Zest of Empiricism
P16	Koki Yoshimura	What is "Tameness" in Captive Red Foxes?: with Reference to Self-domestication Hypothesis
P17	Kotaro Kondo	Genetic Origin of Koalas Captive in Japan: toward the Investigation of Innate Diet Preference
P18	Linda Shearwin-Whyatt	Monotremes Shed Light on Mammalian Evolution

Poster Session II

Tuesday, March 2, 11:40–13:00 (core presentation time 11:45–12:45)

P19	Madoka Hattori	Comparison of Facial Morphology between Wildcats and Domestic Cats
P20	Makiko Take	Food Transfer in Golden-faced Saki: Developmental Change in Juveniles' Begging Behavior
P21	Qi Luan Lim	Phylogenetic Relationship of the Malayan Tapir in Japan
P22	Rena Numabe	The Change of Bitter Sensitivity to PTC in Each of Individuals and the Relationship between TAS2R38 Polymorphism and Food Preference
P23	Shohei Shibata	What Did He See on the Surface of the Puddle? A Male Chimpanzee Produced an Alarm Call at a Water Puddle
P24	Sofia Vilela	Impact of Game Hunting on Physiological Stress Levels of Wild Red Deer
P25	Sok Hwan Lee	The Evolution of Social Status: Investigation on Prestigious Behavior in Chimpanzees and Bonobos
P26	Sota Inoue	Influence of Age of Breeding Mare is Not Negative on the Racing Performance of their Offspring as Commonly Assumed
P27	Takumasa Yokoyama	Variable Functions of Genito-Genital Rubbing among Female Bonobos (*Pan paniscus*)
P28	Takumi Ezawa	Genetic Analysis of Captive Echidnas in Japanese Zoos using Noninvasive Spine Samples
P29	Takuto Sugimoto	Research on the Social Behavior of Horses
P30	Tamao Maeda	How is Spatial Structure of Multilevel-society Decided?
P31	Tomoe Torii	Spatial Relationships in Finless Porpoises by Aerial Videos
P32	Toshiki Minami	New Trends of Infant Handling by Japanese Macaques (*Macaca fuscata*) in a Provisioned Group
P33	Yeongju Lee	Acoustic Communication by Non-vocal Sound in the Free-ranging Horse Group
P34	Yige Piao	The Operant Conditioning of Short-tailed Pit Vipers (*Gloydius brevicaudus*) Based on Different Infrared Shapes
P35	Yuko Kuriyama	Socioecology of Iriomote Cat (*Prionailurus bengalensis iriomotensis*)

CLOSE-PROXIMITY PICTURES WITH WILDLIFE



DR. JOANNA SETCHELL × CONSERV'SESSION

2021.03.01 18:30(JST) @SPATIAL CHAT

K1

The Dawn of Animal Linguistics: Exploring Referentiality and Compositionality in Bird Calls

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Establishing the theory of language evolution is an ongoing challenge in science. One profitable approach in this regard is to seek the origins of linguistic capabilities by comparing language with the vocal communication systems of closely related relatives (i.e., the great apes). However, several key capabilities of language appear to be absent in non-human primates, which limits the range of studies, such as direct phylogenetic comparison of linguistic capabilities. A further informative approach lies in identifying convergent features in phylogenetically distant animals and conducting comparative studies. This approach is particularly useful with respect to establishing general rules for language evolution. In this article, I review recent findings on linguistic capabilities in a passerine bird species, the Japanese tit (Parus minor). Field experiments have revealed that Japanese tits produce unique alarm calls when encountering predatory snakes, which serve to enhance the visual attention of call receivers with respect to snake-like objects. Moreover, tits often combine discrete types of meaningful calls into fixed-ordered sequences according to an ordering rule, conveying a compositional message to receivers. These findings indicate that two core capabilities of language, namely, referentiality and compositionality, have independently evolved in the avian lineage. I describe how these linguistic capabilities can be examined under field conditions and discuss how such research may contribute to exploring the origins and evolution of language.

K2

Why Conservation Needs Anthropology

Joanna M Setchell[1*], Emilie Fairet[2], Kathryn Shutt-Phillips[3], Siân Waters[1] and Sandra Bell[1]
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Biodiversity conservation is one of the grand challenges facing society. Many people interested in biodiversity conservation have a background in wildlife biology. However, the diverse social, cultural, political, and historical factors that influence the lives of people and wildlife can be investigated fully only by incorporating social science methods, ideally within an interdisciplinary framework. Cultural hierarchies of knowledge and the hegemony of the natural sciences create a barrier to interdisciplinary understandings. I will review three different projects that confront this difficulty, integrating biological and ethnographic methods to study conservation problems. The first project involved wildlife foraging on crops around a newly established national park in Gabon. Biological methods revealed the extent of crop loss, the species responsible, and an effect of field isolation, while ethnography revealed institutional and social vulnerability to foraging wildlife. The second project concerned great ape tourism in the Central African Republic. Biological methods revealed that gorilla tourism poses risks to gorillas, while ethnography revealed why people seek to get (too) close to gorillas. The third project focused on humans and macaques sharing a landscape in Morocco. Incorporating shepherds in the coproduction of ecological knowledge about primates built trust and altered attitudes to the primates. These three case studies demonstrate how the integration of biological and social methods can help us to understand the sustainability of human–wildlife interactions, and thus promote coexistence. In each case, an integrated biosocial approach incorporating ethnographic data produced results that would not otherwise have come to light. Research that transcends conventional academic boundaries requires the openness and flexibility to move beyond one's comfort zone to understand and acknowledge the legitimacy of 'other' kinds of knowledge. It is challenging but crucial if we are to address conservation problems effectively.

K3

Mirrors, Yawns, and Death: Connections?

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Comparative research over the last 50 years has established that, among primates, reliable and compelling evidence for visual self-recognition is limited to humans and the great apes. Prosimians and monkeys show no signs- certainly no more than highly questionable signs- of visual self-recognition. The weight of these data suggests the existence of a qualitative difference in self-awareness between, on the one hand prosimians and monkeys, and on the other hand, great apes including humans. Research over the past 15 years has also revealed the existence of a similar, though more nuanced, gap in susceptibility to contagious yawning, which some authors attribute to species differences in empathic abilities. And finally, research in the past 10 years has started to address in a more scientific way how other primate species react emotionally and cognitively to death, approaching topics such as grief, and understanding what death means. In this presentation, I briefly review some of my contributions to these three fields of research, and suggest how three phenomena: self-recognition, contagious yawning, and death awareness, may be psychologically connected.

K4

From Himalaya to Ocean, from Glacier to Tropical Rain Forest: Studies by a Strange Biologist

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I would like to talk about my strange research career as a biologist because this will be my last presentation in the PWS sympodium. I started my research career from the study on insects living in the snow and ice. Although biological activity on glaciers has been believed to be extremely limited, we found various biotic communities specialized to the glacier environment in various part of the world, such as Himalaya, Patagonia, Alaska and Greenland. Some of these glaciers hosted various cold-tolerant insects, annelids and copepods living in the glacier by feeding on algae and bacteria growing in the snow and ice. Thus, the glaciers are simple and relatively closed ecosystems sustained by the primary production in the snow and ice. Since the microorganisms growing on the glacier surface are stored in the glacial strata every year, we showed that they could be new environmental signals for the past environment studies. We also showed that blooms of algae and bacteria on the glacier darken the surface and accelerate the glacier melting. Although I continue to study on glacier ecosystems, I have also studied ecology and behavior of various organisms with many strange students who studied dolphins (resting behavior, habitat use, etc.), seals (vocal communication), primates including humans (eye morphology), orangutans, civet (feeding ecology), porcupines, tapirs (social structure), and elephants (vocal communication). For these studies, we have conducted field studies in various part of the world such as Alaska, Amazon, Borneo, India and Africa. I will introduce some of these studies and our project in Amazon to develop “Field Museum” that can contribute not only to study and conservation of wildlife but also to environmental education and sustainable development of the local communities.

K5

Genomes & Threatened Species Conservation

Carolyn Hogg[1*]
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Globally we are immersed in a biodiversity crisis with over 1 million species now under threat of extinction. Of the 13,500 animal species listed as threatened by the IUCN, less than 1% have genomic resources. To combat this, global genome initiatives have been established to create genomic resources for all eukaryotic life on earth. Yet a reference genome does not conserve a species. Having the reference genome allows us to develop a suite of tools to understand both genome-wide and functional diversity within and between species. Conservation practitioners can then use these genetic resources to inform their decision-making. I will present case studies from Australia on how having genomic data has helped us make conservation decisions for some of our most iconic species. From translocating Tasmanian devils, that are threatened by an infectious cancer, to managing the metapopulation of bilby behind fences and islands, to developing new genomic tools for koala populations. By working closely with our conservation partners, we are able to integrate the latest genomic tools into management practice in real-time. Using the latest genome technologies we are able to make a significant contribution to understanding both the biology of our iconic Australian species as well as to their long-term conservation.

O1

Examining the Possible Mechanisms and Adaptive Significance of Single and Multi-male Groups in Horse Societies

Pandora Pinto[1*], Renata Mendonça[1], Monamie Ringhofer[2], Kodzue Kinoshita[1] and Satoshi Hirata[1]

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In some feral horse populations, adult females (mares) are either associated with a single male or multiple males (stallions). However, little is known on why such groups with more than one male exist, considering that stallions fight to monopolize females. The proposed study aims to address this question by reconsidering previously proposed hypotheses and by assessing intraspecific variations in stallions' traits. Research was conducted on feral Garrano horses in Serra d'Arga, Northern Portugal.

(1) Body size is often an important determinant of male fighting ability and/or dominance rank and, consequently, reproductive success. Stallions may, therefore, vary in "quality" which could be a differentiating factor between single-stallion and multi-stallion males. We examined the relationship between body size, sex, group type and number of females. Using a non-invasive laser distance meter, we determined individual body length and height by overlapping photos of the horses and a measuring tape. Results showed no significant differences in body length and height for all parameters assessed, suggesting that size is not a determinant factor for feral horse society.

(2) Testosterone is often linked to aggressive and dominant behavior in males, which provides a mechanism for reproductive competition. In view of that, we examined the relationship between fecal testosterone levels, group type (single-stallion, multi-stallion or bachelor group), number of females in the group and dominance rank, during the breeding season. Preliminary results showed that males in multi-stallion groups had higher testosterone concentrations than single and bachelor males. Subordinate males averaged higher than their dominant counterparts in multi-stallion groups and males in single-stallion groups, while the number of females in the group didn't seem to have much effect.

(3) Mate parasitism, by-product mutualism, reciprocal altruism and the consort hypothesis have been used in the past to explain the multi-stallion group structure. In this preliminary study, we test the advantages and functional characteristics predicted by these hypotheses. We tested for differences in female group size and stability, disparities in group defense effort by males, activity budget of stallions and females, and stallion → mare aggression levels. We found no evidence that the males in multi-stallion groups benefited from their relationship in terms of group size and stability, and activity budgets. Subordinate males in multi-stallion groups contributed to group defense, while their dominant counterparts were the least involved in female protection. Finally, mares from single and multi-stallion groups did not exhibit different grazing and resting experiences, nor were they significantly subjected to male harassment.

Pair Chemistry in Golden Eagles: Relationship between MHC Genes and Breeding Success

Annegret M. Naito*[1], Yu Sato[1,2], Miho Inoue-Murayama[1]
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Genes of the major histocompatibility complex (MHC) are said to influence immunocompetence and mate choice in many vertebrate species, and are of great interest to conservation geneticists due to their impacts on individual and population fitness. In this study, we investigated the MHC gene region in the Japanese golden eagle (*Aquila chrysaetos japonica*), a large apex predator listed as endangered by the Ministry of Environment. This subspecies' reproductive success has been continuously declining in the wild, and captive breeding success remains limited to certain family lineages. We therefore aimed to shed light to any potential genetic underpinnings of reproductive success by investigating the MHC genes of the captive Japanese golden eagle population. Using amplicon sequencing, 18 individuals (10 pairs) were analyzed at the highly polymorphic MHC class II *DRB* exon 2, which encodes for the peptide binding region (PBR) of the antigen presenting MHC class II protein. For each breeding pair, the number of *DRB* alleles, proportion of shared alleles, the predicted mean amino acid distances at the PBR and non-PBR, and relatedness (data from a previous study using 19 microsatellite loci) were calculated. The number of eggs laid, proportion of fertilized and hatched eggs, proportion of stopped broods, and number of successful adoptions were extracted from the past 10 years' reproduction data provided by zoos across Japan. A generalized linear mixed model approach was used to analyze the relationship between the aforementioned MHC gene variables and reproductive data. Results showed a strong positive correlation between the proportion of eggs hatched and amino acid distance at the PBR ($p < 0.0001$, $R^2 = 0.865$), and weak negative correlations between the proportion of stopped broods and amino acid distance at the PBR ($p < 0.05$, $R^2 = 0.249$) and non-PBR ($p < 0.05$, $R^2 = 0.220$). These findings imply that reproductive success increases if individuals of a pair are more dissimilar at MHC loci. An MHC-based disassortative mating strategy has been observed in many vertebrate species and is thought to have fitness benefits – choosing a mate with dissimilar MHC genotypes will increase the chances of one's offspring possessing more MHC alleles, increasing the offspring's immunocompetence against various types of pathogens. Although the sample size of our study is limited, the results suggest the possibility of MHC-based disassortative mating in the Japanese golden eagle. Specific mechanisms underlying our findings are yet to be investigated, but it is possible that MHC dissimilarity in breeding pairs leads to lowered pre-hatching death rates in chicks, thereby increasing hatching success. These results are valuable for informing future captive breeding plans, as well as understanding trends in reproductive success in the wild. We hope to apply this information towards in-situ and ex-situ conservation of the Japanese golden eagle.

O3

Roosting Dynamics Predicted by Nepotism in a Cooperatively Breeding Bird

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Family living is a stepping stone to higher sociality such as cooperative breeding. Nepotism has been suggested to promote family living but evidence is scarce. Nepotistic interactions may occur during any group activities that enhance fitness of group members but entail a potential of unequal sharing of such benefits. I studied huddle roosting in the chestnut-crowned babbler, *Pomatostomus ruficeps*, a cooperatively breeding bird endemic to the semi-arid zone in south-eastern Australia. Group members sleep in a roost nest and save night-time energy loss through huddling inside. However, birds near the entrance hole may not gain from the huddling as much as the rest of the group. Roosting behaviour of identified individuals was monitored, using a PIT (passive integrated transponder)-tag detection system. Late-entering individuals were more likely to exit earlier in the morning, and had a higher chance of coming close to the entrance hole during the night. A closer look at tag records during roost entrance revealed tag detection patterns in line with competition over earlier entrance. Subordinate females unrelated to the dominant pair was later to enter than all other social classes. These roosting dynamics are likely to reflect the nepotistic interaction during which immigrants are disadvantaged in securing a favourable roosting position in the nest. Because most dispersers join existing social groups in this species, such selective exclusion of immigrants from the central huddle can be a cost of dispersal promoting family group formation via delayed dispersal.

O4

Chimpanzee (*Pan troglodytes*) Reinforcement Learning via Eligibility Traces

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Associating an action with its outcome lies at the core of animal learning. It becomes difficult when the outcome is delayed in time and other decision-making events occur between the causal action and its outcome, raising so-called credit-assignment problem. In the framework of reinforcement learning, one solution for this is by means of eligibility trace. It keeps past actions eligible for value update for a while according to the elapsed time since its occurrence. Learning mechanisms like this may play a determinant role in regulating timescale of animal learning abilities. Experiments with humans have shed light on roles played by eligibility traces in learning by using behavioral tasks that are specifically designed for that aim. This approach is useful to better know the properties of eligibility trace like the speed of its decay while suppressing contributions of other learning mechanisms. However, this line of research is lacking in nonhuman animal literature. Here, following a previous human study, I devised a delayed-feedback task tailored to nonhuman animals and examined whether chimpanzees (*Pan troglodytes*) could solve this task using a touchscreen experiment. In this task, participants needed to learn the value of three choice options. A food reward was delivered depending on participant choices, but the choices did not affect which options are presented in following trials, that is, state transitions were independent of actions. Of particular interest was whether participants could appropriately learn the value of an option associated with a delayed reward. The results show that two of five chimpanzee participants could learn it appropriately, indicating that at least some chimpanzees can learn by utilizing their action history via mechanisms like eligibility traces, as humans can. Further, three human participants underwent basically the same task, and all of them could learn much faster than those chimpanzees. This highlights a potential inter-species difference between chimpanzees and humans, although it is difficult to conclude it and, in particular, which aspect of learning process differs between the species, due to the small sample size.

Oxytocin Promotes Species-relevant Outgroup Attention in Bonobos and Chimpanzee

James Brooks[1*], Fumihiro Kano[1], and Shinya Yamamoto[1,2]
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[2] *Institute for Advanced Study, Kyoto University, Kyoto, Japan*
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The hormone neuropeptide oxytocin (OT) has attracted considerable research attention over the last decade for its role in human ingroup-outgroup psychology. However, comparative research on the evolutionary basis of OT's role in intergroup relations is lacking. Bonobos and chimpanzees are humans' two closest relatives and display remarkable differences in intergroup relations, with lethal intergroup aggression led by males in chimpanzees and largely tolerant intergroup associations led by females in bonobos. Previous research has found that bonobos preferentially attend to ingroup females and chimpanzees to ingroup males, that OT is released in advance of chimpanzee border patrols, and that exogenous OT affects the two species' social attention differently. We thus compared the effect of OT on group-based social attention. We found that OT selectively shifted attention toward the outgroup in the sex primarily involved in intergroup encounters, eliminating the bias found in previous studies. This demonstrates that despite their divergent patterns of intergroup relations OT promotes outgroup attention in both bonobos and chimpanzees in species-relevant ways. We thus suggest the OT system may have a conserved role in hominid intergroup relations but supports the evolution of divergent species-relevant instantiations.

O6

The Effect of 'Brightness Contrast' in Luminance Discrimination Tasks for Chimpanzees and Killer Whales

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Cognitive abilities are products of evolution and are shaped by both phylogenetic and environmental factors. In order to examine how environmental factors have affected on the evolution of cognitive abilities, comparative studies on mammals living in underwater environment, which is far different from terrestrial environment, are essential.

Cetaceans (dolphins and whales) have been highly adapted to the underwater environment and have developed their cognitive abilities according to the characteristics of the environment. While many researches have been conducted on auditory abilities of cetaceans, it is not well understood how they use their visual abilities. Then, it is suggested that contrast of luminance may play an important role in object recognition of cetaceans with low visual acuity and no color vision. In order to examine how cetaceans perceive contrast, I decided to examine whether one of the visual effects called 'brightness contrast' occurred or not. When the effect of 'brightness contrast' is occurred, contrast of luminance between object and background is enhanced and helps observers to separate objects visually from background.

In this study, I examined this effect by conducting similar luminance discrimination tasks for 6 chimpanzees in Primate Research Institute of Kyoto University and 2 killer whales in Port of Nagoya Public Aquarium. Research aim is to develop a new research method which enables us to conduct visual tasks for killer whales and to examine the effect of "brightness contrast" in these two species. Through luminance discrimination tasks, I succeeded to develop a new experimental method for killer whales and the results of experiments showed that the effect of 'brightness contrast' was observed in both chimpanzees and 1 killer whale. Therefore, it was suggested that contrast of luminance play an important role not only in terrestrial mammals but also in visual objects recognition of the underwater mammals. In the future, I will examine the magnitude of the effect for two species and if possible, conduct comparison between them.

O7

Material Discrimination Based on Concepts in Chimpanzees

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Animals including humans live surrounded by a lot of objects made of various materials. Without touching them, we can effortlessly perceive what materials they made of and tell what kind of features they have. This ability is known as material discrimination and it helps us to judge the quality of the objects and make a behavioral decision. A lot of studies found that humans are extremely good at visual discrimination of materials and the reason is that they have the concepts of each material. They also show the concepts have shaped by integrating multiple level information sensed by multiple modalities through the previous experiences and this process is facilitated by labeling the information by language. Chimpanzees, one of the most evolutionally closest species to us, are also surrounded by a lot of materials and discriminate them in their daily life like us although they do not share any language system with us. Therefore, we conducted two experiments to chimpanzees to examine how they discriminate materials and whether their discrimination is based on concepts of materials. In the first experiment, we conducted an identical matching-to-sample task by using images of 6 materials to reveal whether they have visual sensitivities toward differences between material categories. The results showed not only did they have the sensitivities, but they spontaneously grouped stimuli by material categories. Moreover, it is also known that humans integrate multiple levels of visual information from low-level one such as luminance or contrast to high-level one such as glossiness or roughness to shape concepts of materials. To examine how chimpanzees use the information for their discrimination, we conducted three kinds of image analysis to stimuli used in the experiment and compared them with the performances of chimpanzees. The results revealed that they did not solely depend on low-level information but possibly integrate multiple levels of image information. In the second experiment, we added auditory stimuli to the first experiment as interferences and their performances of visual discrimination were degraded by them. This indicates they perceived materials not only from vision but also auditory sensation. Together, these findings show that chimpanzees integrate multiple level of information both within one modality and between multiple modalities into concepts of materials without labeling by language. This suggests that material discrimination of humans based on concepts of materials emerged before language evolution.

Socially Contingent Video Can Facilitate Referential Information Utilization in Chimpanzees: A Progress Report

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Humans are able to recognize out-of-sight events and objects referred by language. For such referential information comprehension, social contingency has been implicated as an important catalyst in early language development. However, it remains unclear whether the role of social contingency in referential ability is shared in non-linguistic animals. To address this, we tested whether chimpanzees would utilize referential information more from contingent videos compare to noncontingent videos. We employed a tool-choice task using two rooms. In the first room, chimpanzees engaged in a social interaction with the experimenter and watched a food-hiding event via video. They, then, received a choice task to pick up one of two tools, which is necessary to get food in the next room. After that, chimpanzees moved to the next room to obtain food using the tool. Only if they have had the right tool in hand, they can reach and extract the food. Results showed that only one out of five chimpanzees performed better than expected by chance without contingent cues, whereas more individuals performed at greater rates with contingent cues. The data so far suggested that like humans, videos without social contingent cues may not be sufficient for most of the chimpanzees to comprehend referential information; in contrast, contingent cues can facilitate it.

O9

Feeding Decisions Predict Parasite Infection in Sanctuary-housed Bonobos

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Intense selection pressure from parasites on free-living animals has resulted in behavioral adaptations that help potential hosts avoid sources of infection. In primates, such 'behavioral immunity' is expressed in different contexts and may vary according to the ecology of the host, the nature of the infectious agent, and the individual itself. In this study, we investigated how the sensitivity of sanctuary-housed bonobos toward surrounding contaminated food associates with levels of parasite infection. To do this, we assessed bonobos' responses to soil- and fecally-contaminated food in behavioral experiments, and then compared the results with an estimate of protozoan infection across individuals. We found that aversion toward contaminated food correlated negatively with *Balantioides coli* infection, a potentially pathogenic protozoan transmitted through the fecal-oral route. This relationship was mainly observed in experiments involving intermediate and low levels of contamination. In the case of high contamination, most subjects were aversive to the presented food item and this may have mitigated any relationship between feeding decisions and infection. We controlled for factors such as age category and sex in our statistical models of infection to avoid potential confounds. The behavioral immunity observed may be a consequence of the direct effects of parasites (infection), reflecting the first scale of a landscape of disgust: individual responses. Indirect effects of parasites, such as lowered food consumption and reduced social interactions, are also discussed in light of individual fitness and primate evolution.

Evaluation of a Novel Activity Monitor for Captive Japanese Macaques (*Macaca fuscata*)

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Accelerometers are non-invasive activity monitors that provide real-time data on the frequency, duration and intensity of physical activities. While it does not describe the exact behavior, an increase or decrease in the amount of activity suggests modification of the routine, leading to the investigation of the cause.

So far, validity and reliability of a novel veterinary monitor (Plus Cycle®, JARMeC, Kanagawa, Japan) have been reported in cats. Given that captive macaques are in constant observation regarding their health status and stress levels, non-invasive monitoring is valuable tool for the improvement of welfare in captivity. This study aimed to evaluate the relationship between activity monitor physical activity count and directly observed behavior. Three healthy female macaques (8.4 kg – 10.3 kg; 9 years old) housed in pairs in indoor cages were evaluated. The accelerometer was accommodated in a double-layer hand-made nylon collar and put in the macaque's neck. The accelerometer epoch was set at 1 minute and data was expressed as counts per minute. The behavior was assessed by videorecording each macaque using an infrared night vision camera (CAM-3, UENO-JP, Japan) from 17:00 to 10:00 for 8 days. Video were analyzed to define four levels of activity: rest (0; no movement of the trunk), light (1; short movements of trunk and limbs), moderate (2; movement of trunk and limbs, eventual walk, body shake or scratch), vigorous (3; prolonged period of walk, jump, scratch, body shake). In a second video analysis, accelerometer and videos were synchronized and three hundred minutes were randomly selected to be classified from 1 to 3 according to the activity level observed.

Linear regression showed a Pearson's correlation coefficient (R^2) of 0.8316, indicating a high correlation between measured activity count and activity observed. Video observation showed that the macaques were restless and frequently touched the adapted collar on the first day. Along the week the signs of discomfort decreased gradually, indicating that a period of acclimatation is necessary. Cage mates did not try to touch or bite the collar from one another. Preliminary results suggest that Plus Cycle® can be used to quantify spontaneous activity and activity intensity in captive macaques.

This is an ongoing study and data is being collected to determine the activity profile, accuracy for jumping measurement, optimal sampling interval and suitability of the device for the species in this type of housing condition.

Exploring Non-invasive Methods to Better Understand the Stress Response of Japanese Macaques (*Macaca fuscata*)

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What is stress? Although we all have an intuitive understanding, the concept of stress can be difficult to precisely define. From a biological perspective, stress is often described as any environmental, social, and/or physiological demand placed on an organism that unbalances its natural regulatory state. A primary physiological system for studying stress is the hypothalamic-pituitary-adrenal (HPA) axis, which when activated releases cortisol, a hormone that adaptively responds to stress in order to return the body back to homeostatic balance. However, due to the biological costs of this response, frequent or chronic release of stress-related hormones can become maladaptive and result in immune dysregulation, impaired cognitive abilities, and as often reported in captive non-human primates an increase in self-injurious behavior. Therefore, studying the stress response of non-human primates may provide us with strategies to more effectively manage the health of populations under human care. Japanese macaques are closely interconnected with human activities such as being kept in captivity; whether in zoos and research facilities, or even provisioned at monkey parks, where they often naturally inhabit areas near local human populations. This species is ideal for developing and applying non-invasive tools to better monitor their stress in a wider range of environmental contexts. This talk will cover several of my projects that have aimed to monitor stress throughout both short- and long-term periods in Japanese macaques using non-invasive methodologies to analyze stress hormones found in saliva or feces. In the first study, a method for cooperative saliva collection was developed in captive individuals in order to explore the temporal dynamics of salivary alpha-amylase (sAA) enzyme in relation to acute, or short-term stress. In human studies, sAA levels are shown to be positively correlated with increases of the hormone norepinephrine, allowing sAA to act as a biomarker for sympathetic nervous system (SNS) activity. The SNS is associated with the *fight-or-flight* response and is a separate but parallel stress response system to the hypothalamic-pituitary-adrenal (HPA) axis. Similar to human studies, we found sAA responds quickly to mild stress in Japanese macaques. Next, I will briefly report on an ongoing project where cooperative saliva collection was expanded to the semi wild population of Japanese macaques on the island of Koshima, Miyazaki. Focal individuals were continuously followed to record behavioral data and saliva samples were collected after behaviors such as grooming, conspecific aggression, and foraging. This project aims to determine both cortisol and sAA concentrations in saliva in order to better understand acute stress in a wild setting. Finally, I will report on an ongoing study that tracked fecal cortisol concentrations and behavior over 6 weeks in a captive population to better understand the relationship between environmental factors and stress within a captive setting.

Play Escalation in Japanese Macaques: It is a Matter of Relative Rank and Relative Age

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The question of how animal communication fails has received much less attention than the question of how animals successfully communicate. Using data from a group of Japanese macaques (*Macaca fuscata*), this study focused on escalation from play fighting into serious fighting as an incident of communication breakdown. We defined an individual who expressed negative responses (e.g. screaming) when play escalated as the "victim", and the other as the "aggressor". We found that when play escalated, individuals with lower ranks or younger individuals were more likely to be victims compared to their play partners. These results implied that play escalation was likely to be caused by the disproportionate use of force by relatively higher-ranked or older players. We also found that victims' post-escalation behaviors varied according to the dominance relationships with the aggressors. That is, victims with higher rankings than aggressors were more likely to counterattack their counterparts immediately after escalation, while victims with lower rankings were more likely to evade their counterparts. Overall, our results suggest that dominance relationships between individuals become manifest upon escalation.

Examining Long Bone Phenotypic Plasticity: A Case Study of Skeletal Differences between Northern and Southern Japanese Macaques

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Neanderthals exhibit a robust somatotype, thick cortical diaphyseal bone, shorter limb, and larger thoraxes, relative to modern humans. While phenotypic differences could be a result of a distinct genome, they may also reflect somatic plasticity in response to colder climates. Geographical rules developed by Bergmann and Allen state that mammals in warmer temperatures have a reduced thorax and longer limbs to aid in heat dissipation, whereas the robust thorax and shorter limbs in cold climates serve to conserve heat. Japanese macaques have a longer period of developmental exposure to their immediate environment and reside in areas of Japan ranging from the colder regions of northern Honshu to the hot climates in southern regions like Yakushima Island, providing an opportunity to examine these theories. Climate may affect Japanese macaque skeletal ontogeny, implying some level of plasticity and/or cold climate adaptation. Therefore, we expect northern Japanese macaques will exhibit significant differences in osteological architecture from southern Japanese macaques. This study sampled adult macaques (*M. fuscata fuscata* and *M. fuscata yakui*, n=85) from the Kyoto University Primate Research Institute Skeletal Collection. Sex-balanced data for each macaque were collected using osteometrics. Preliminary plots show minimal variation in limb length between groups and no clear cline with increasing temperature. The results suggest that the cline does not exist, in part, due to the limited range in climate used in the study.

Female Fertility Rates between Subspecies of Captive Rhesus Macaques

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Other than humans, Rhesus macaques (*Macaca mulatta*) are the most widely dispersed primate species covering a geographic range from as far west as Afghanistan to the eastern Chinese coast. Previous studies have compared female fertility rates among macaque species and has shown that the sinica group was more prolific than the fascicularis group, but these studies did not separate the *M. mulatta* species into their subspecies which represent the extremes of their geographic range. The current study focuses on two of the subspecies of captive *M. mulatta*: Indian-derived and Chinese-derived housed within the long-established breeding colony at the Tulane National Primate Research Center (TNPRC) in southeastern Louisiana to determine whether the average number of female infants produced by a female (GRR) is consistent with previous findings. This study will also compare the m(x) fertility curves between this subspecies and other macaque species in previous literature.

The records of 840 Indian-derived and 217 Chinese-derived females spanning a 30-year period were analyzed to establish whether they had given birth while belonging to a designated age group. Results show that the GRR is 3.801 for Indian-derived and 2.246 for Chinese-derived. The results of this study are compared to the female fertility rates found in the wild *M. mulatta* population in Florida and the captive population at CPRC.

O15

Species-specific Loss of Function of Melanocortin-1 Receptor Gene (*MC1R*) in Seven Endemic Species in Sulawesi Island

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The melanocortin-1 receptor gene (*MC1R*) encodes a G-protein coupled receptor and plays a key role in regulating synthesis of the dark eumelanin and the red/yellow pheomelanin in mammals. Mutations in MC1R have been shown to cause changes from light to dark in coat color. Several gain-of-function in MC1R result in increased production of eumelanin in coat, whereas loss-of-function mutations result in increased pheomelanin production, including red hair in human. Sulawesi macaques are seven endemic species in Sulawesi island. Distinguishing from the other macaques, Sulawesi macaques commonly have dark coat color, with variation on color brightness and color pattern. To understand whether MC1R is related to the coat variation in Sulawesi macaques, we investigated nucleotide sequences of MC1R gene from total 54 individuals. We identified 26 single-nucleotide polymorphisms (SNPs), of which 10 SNPs resulted in species-specific nonsynonymous variants. All the 10 nonsynonymous substitutions responsible for six species-specific haplotypes, while *M. ocheata* and *M. brunescens* shared the amino acid haplotype. We predicted that 5 amino acid substitutions to the consensus sequence do affect MC1R function. To clarify the effect of the substitutions, we tested the functional property of agonist α-MSH binding activity of fixed variants in each species. We found that species-specific MC1R variants exhibited partial loss-of-function in either agonist sensitivity or basal activity. Specifically, MC1R of *M. maura* exhibited extremely low basal activity and low sensitivity to agonist, highly corresponding to its light coat color. The changes in MC1R function are not simply associated with coat color in the other six species. Our results suggested that fixation of MC1R occurred among Sulawesi macaques, and MC1R variants evolved into different functional characteristic among species. The apparent of loss-of-function MC1R variants in Sulawesi macaques suggested that MC1R may have other important functions which are unique to each species.

Staring "Death" in the Face: Chimpanzees' Attention towards Conspecific Skulls and the Implications of a Face-module Guiding this Behavior

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Chimpanzees exhibit a variety of behaviors surrounding their dead ranging from several physical interactions to stationing around for extended periods and even revisiting the corpse. Much less is known how they behave around or even if they show any interest in conspecific skeletons. In an important contribution to the field of comparative thanatology, McComb and colleagues (2006), noticing wild elephants' natural propensity to interact with conspecific skeletons, devised an experiment whereupon they measured the elephant's responses towards conspecific skulls and ivory, using other controls such as buffalo and rhinoceros' skulls and similar sized chunks of wood. Their conclusions supported the notion that African bush elephants are significantly more attentive towards conspecific stimuli than not, therefore suggesting some conspecific recognition phenomenon taking place, due to the limitations of their study, McComb and colleagues could not control for factors such as color, luminosity and size. By means of a looking-time paradigm in chimpanzees, we tested their visual attention towards conspecific and non-conspecific skulls (cat, chimp, dog, rat), shown simultaneously in four corners of a screen in distinct positions (frontal, diagonal and lateral). Additionally, both faces and skull shaped stones of these animals were used following the same methods. Overall, chimpanzees where attracted to conspecific related stimuli. The results show that 1) chimpanzees are significantly attracted towards conspecific skulls , particularly forward faced and to a lesser extent diagonally; 2) they are significantly attracted to conspecific faces in forward and diagonal angles; 3) they show a higher looking trend towards conspecific skull-shaped stones facing forward but non-significantly and; 4) similarly to elephants, within forward facing skull stimuli (diagonal/frontal), chimpanzees show significant interest in teeth which are a visible cue for live conspecific faces. We suggest chimpanzee skulls still retain relevant, albeit impoverished, face-like features that arguably activate a domain specific face-module in the chimpanzee's brain, guiding their attention to them. Although, unlike wild chimpanzees, but perhaps notably, these captive subjects have never interacted with conspecific skeletons, this study proposes that, apart from learned, similar interest exhibited by wild chimpanzees towards conspecific skulls can be explained by the same recognition mechanism.

Re-evaluate Ethno-Ornithological Recognitions of Kazakh Eagle Falconry for Conservation of Golden Eagle, Western Mongolia

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There are deep history of human and raptors interactions between Kazakh eagle hunters and Golden Eagle in the Altai Mountains. Eagle masters only tame female one, and they are release to wild again after 4~5 year (after sexual maturity). Their art and knowledge for taming eagles and, hunting strategy, are irreplaceable unique folklore, so called traditional ecological knowledge (T.E.K.), fostered centuries-lasting culture amongst the Kazakh nomadic herding community. That knowledge always includes connotations for sustainable interactions with Golden Eagles. I will discuss about how we should integrate unique taming art and knowledge accumulated by Kazakh eagle hunters into conservation of Golden Eagles. The presentation shows concrete ethnography of Kazakh eagle masters living in Sagsai village in Bayan-Ulgii Province, Mongolia. The main focus of this presentation is to discuss that taming procedures of Golden Eagle may be, in some point, benefit to develop a total survivability of tamed eagles. Once tamed eagle can obtain non-humanphobic nature, at the same time, accepts wide range of dietary palatability due to adaptation to unusual foods (such as dead meat, fish, dog, cattle, etc.). This presentation shows a potential case study implementing the actor-integrated wildlife conservation, in reference to ethno-ornithological recognitions inherited by deep cultural contacts between Kazakh eagle hunters and Golden Eagles.

The Planet and Humans at a Crossroad and SDGs

Ambassador Masahiko Horie
Special Assistant to the Foreign Minister of Japan
Special Advisor to the President of Meiji University

The Planet and Humans are at a crossroads. The world population is over 7 billion today and will be reaching 10 billion in the near future. We need to alleviate poverty of over ten billion people in the world by supporting their economic development.

However, many developing countries' endeavors for their nation building, in many cases, lead to further deterioration of environment and huge biodiversity loss.

At this lecture, we will review this conflict between Development and Environment including problems of global warming and consider what we should do to save this Planet, Humans and Biodiversity. All the peoples of the world need to uphold the Sustainable Development Goals (SDGs) which was adopted at United Nations in 2015.

The First Reference Genome for the *Antechinus* Genus Provides a Resource for Investigating the Genetic Basis of Semelparity and Age-related Neuropathologies

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Antechinus are a genus of mouse-like marsupials that are distributed throughout Australia and New Guinea and possess a number of unique characteristics that warrant further investigation. For example, Antechinus exhibit a rare reproductive strategy known as semelparity whereby increased levels of corticosteroids during the annual breeding season results in extreme resource shifts from immunity to reproduction, resulting in a complete die-off of all males in the weeks following mating. Additionally, antechinus have been found to naturally develop age-related neuropathologies similar to that of humans, suggesting that they may be a suitable model for Alzheimer's disease. A reference genome is key to exploring how the unique traits of the antechinus may be encoded at the genetic level. We provide the first annotated antechinus reference genome for the brown antechinus (*Antechinus stuartii*). The reference genome is 3.3Gb in size with a scaffold N50 of 73Mb and 93.3% complete mammalian BUSCOs. Using bioinformatic methods we assign scaffolds to chromosomes and identify 0.78Mb of Y-chromosome scaffolds representing the largest amount of Y-chromosome sequence data available for any marsupial species. Comparative genomics revealed interesting expansions in the NMRK2 gene and the protocadherin gamma family, which have previously been associated with aging and age-related dementias respectively. Transcriptome data from a variety of tissues displayed expression of common Alzheimer's related genes in the antechinus brain and highlight the potential of utilising the antechinus as a disease model. The first antechinus reference genome will promote future research that could have large implications for both humans and other wildlife.

Characterising the Gut Microbiomes in Wild and Captive Short-beaked Echidnas Reveals Diet-Associated Changes

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The short-beaked echidna (*Tachyglossus aculeatus*) is an iconic Australian animal as one of only three egg-laying mammals alongside the long-beaked echidna and the platypus. The short-beaked echidna is the most widespread native mammal in Australia and commonly held in zoos, however, much of their biology is still unknown as they are cryptic animals making them difficult to study in the wild. To combat this, we developed an Australia-wide citizen science project, EchidnaCSI, to gather population data and scat material to perform molecular analysis on. Through this innovative approach and in combination with collaborations with Taronga and Perth Zoos, we investigated for the first time the gut microbiomes of wild and captive-held echidnas. This research revealed a high level of variability in echidna gut microbiomes between individuals and locations, and a major divergence between echidnas held in zoos in comparison to their wild conspecifics. Echidna gut microbiomes are mostly dominated by taxa belonging to the phyla Firmicutes, Proteobacteria, Bacteroidota, Actinobacteriota, and Fusobacteriota. Although echidnas are often mistakenly categorised as myrmecophagous mammals (diet consisting mostly of ants and termites), their gut microbiome consists of many putative plant-fermenting bacteria, suggesting plant matter may play a significant role in their diet. This first analysis of echidna gut microbiome highlights extensive microbial diversity in wild echidnas and changes in microbiome composition in managed populations as well as changes as a result of different diets. This is a first step towards using microbiome analysis to better understand gastrointestinal biology and improve management in these iconic animals.

Stress Monitoring in Captive Koalas (*Phascolarctos cinereus*) Using Urinary Cortisol Concentration

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In many species, environmental changes and physiological changes are considered to be factors that cause stress in individuals. The health status, including stress in captive animals, is often monitored by observing changes in behavior such as facial expression and appetite, and the state of excrement. However, koalas sleep for an average of 18 to 20 hours a day making it is difficult to interpret their expressions and to note small behavioral changes. Therefore, behavioral observations alone cannot determine their health condition, including stress. We evaluated whether measuring cortisol concentration can capture their stress status using urine. Fresh urine samples were collected from six adult koalas at the Kanazawa Zoological Gardens for two years from October 2015 to November 2017 (n = 865) and preserved at -20 °C. After centrifuging the urine thawed at room temperature, the cortisol concentration was measured by enzyme immunoassay, and the value corrected by the creatinine level was used. In addition, their behaviors were also recorded, and their relationship with the cortisol values was evaluated. Our result showed that the urinary cortisol concentration was significantly high during the periods related to estrus, pairing with a male, delivery, and illness. In addition, the day after the construction of their exhibition, the cortisol concentration was high. This suggests that the urinary cortisol concentration clearly reflected the physiological stress of koalas.

What is the Favorite Leaf for Koalas: Koalas' Diet Preference and Welfare Studies in Japan

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Environmental education and scientific research on wild animals are included in the missions of many modern zoos. Nevertheless, most Japanese zoos have limitations in terms of manpower and budget costs to conduct education programs and research activities independently. Similarly, educational facilities, such as universities, have difficulties in keeping their own collections of wild animals for academic investigation and classroom education. Therefore, our laboratory, at Kitasato University, has worked together with zoos on educational and research activities focusing on animal behavior and environmental enrichment. The project did not involve only researchers, but also students and zoo staff members too, producing the durable educational and research benefits.

As one of our research activities, we have studied feeding preference of koalas (*Phascolarctos cinereus*). The koala is an arboreal folivore that specialises on the leaves of Eucalyptus spp. Although koalas have a strong preference for this diet, the cues used to discriminate between preferred and unpreferred leaves are still unclear. A series of our studies have investigated what the factors affecting their preference are. For example, the smelling behavior of koalas was studied using a food selection experiment to determine if volatile compounds were related to koala food selection. The behavioral observation on 10 captive koalas housed in Higashiyama Zoo and Kanazawa Zoo, Japan, revealed the subjects ate the leaves more after sniffing than without it, suggesting that volatile compounds may be an important cue when choosing preferred leaves. Next, four koalas in Higashiyama Zoo were fed a choice of six Eucalyptus leaf species simultaneously. The duration of the koalas' feeding behavior and the Eucalyptus species on which they fed were recorded. The volatile compounds contained in the leaves were investigated using gas chromatography and gas chromatography-mass spectrometry. The subsequent multiple regression analysis showed that the amount of certain volatile compounds such as β-linalool, α-thujenal, geranial and so on, the majority of which were classified as a group of secondary metabolites, terpenes, was related to the foraging duration on leaves, but was expressed differently for each subject. These results from the behavioral and odorant analyses, namely koalas sniff leaves before eating or rejecting them and the feeding duration depends on the amount of specific volatile compounds, suggested that koalas smell the specific volatile compounds when determining their individually different preference from the available choices. Our researches on koalas' feeding preferences will enable captive management caring their diet requirement and welfare.

P1

Diversity of Gastrointestinal Helminths in Japanese Macaques of the Jigokudani Snow Monkey Park

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The Japanese macaque is the most northerly-living non-human primate, distributed from warm-temperate to cool-temperate forests across Japan. Like all primates, Japanese macaques host several kinds of parasites, including helminths, lice, protozoa, etc. Generally, geographical and climatic factors play important roles in the diversity of gastrointestinal (GI) parasites infecting a host. Two decades ago, a coprological survey of gastrointestinal helminths infecting Japanese macaques across their range showed a strong latitudinal gradient in parasite species richness (Gotoh, 2000), mimicking the general pattern of increasing biodiversity approaching the equator. Our study aimed to replicate this earlier parasitological survey at one of the sites: the Jigokudani Snow Monkey Park in Nagano, where monkey hot spring bathing behavior occurs. Fecal samples were collected opportunistically from sixteen adult female macaques of different social ranks during two study periods: winter 2019 – 2020 and summer 2020. Coproscopic examination of 146 fecal samples collected during both seasons uncovered four species of GI nematodes, belonging to the genera *Trichuris*, *Streptopharagus*, *Oesophagostomum*, found in all sixteen subjects, and *Strongyloides*, found in three subjects. Sample-to-sample prevalence and infection intensity were variable across the study. Our results demonstrate significantly higher parasite diversity than was found in the previous study, when only *Trichuris* was detected in the troop. Whether our results simply reflect increased sampling effort, methodological improvements, random variation or recent changes in parasite distribution remains unclear, but further investigation of other populations of Japanese macaques are now needed to confirm patterns of parasite biodiversity across Japan.

Keyword: Japanese macaque, gastrointestinal helminths, infectious disease

P2

Selective Foraging of an Arboreal Folivore, the Japanese Giant Flying Squirrel (*Petaurista leucogenys*)

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It is widely known that plants contain phytochemicals which act as digestion inhibitor and toxin, and also that fibers composing plants reduce digestion efficiency. To cope with these difficulties, folivores show morphological and behavioral adaptation such as having large and long guts and showing selectivity in their food.

Many arboreal folivores select foraging parts and species based on nutritional content. Japanese giant flying squirrels are an arboreal folivore that distribute widely in Japan. Similar to other arboreal folivores, this species has elongated guts and forage wide range of trees species and tree parts. Still, the criteria of food selectivity remain unclear. For further understanding of giant flying squirrels' foraging behavior, their selectivity in food items use needs to be examined.

Fieldworks were conducted at Tama science garden, Hachioji, Tokyo, Japan. From July 2017 to June 2019, we searched for food remnants with unique bite marks of giant flying squirrels under the canopy of all the trees taller than 8m heights within the study site (n=2305). During the two years of survey, we obtained 477 food marks form 33 tree species. It was found that they used various types of trees including arboreal, deciduous, broad leaf and conifer trees. They also used various tree parts as their food. Statistical analyses will be performed on the selectivity in their food use.

P4

Longitudinal Study of Chimpanzees' Working Memory

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With regards to chimpanzees' working memory, previous studies have revealed that child chimpanzees have a greater ability than adult chimpanzees and adult humans. These studies found similarities and differences between chimpanzees' and humans' memorizing capacities and strategies. Furthermore, their results suggested that chimpanzees' working memory might change throughout their life. There are, however, no longitudinal studies of working memory in a single chimpanzee. Even in human studies, while there are reports and studies about developmental and aging effects on working memory ability, most studies are based on the comparison of a young group and an elder group.

This study aims to chart the change in chimpanzees' working memory over their life course. Chimpanzees in Kyoto University Primate Research Institute have participated in memory studies for more than a decade. I collected and analyzed ten years of data covering the life phase of chimpanzees from child/sub-adult to adult and from adult to elderly. I will report a progress of my analysis.

P5

High- & Middle-ranked Mothers Aggressively Handle Little Higher-ranked 2–3-month-old Infants in Yakushima Japanese Macaques (*Macaca fuscata yakui*)

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In Yakushima Japanese macaques, females rarely handle other females' infants in aggressive ways except their behaviors (e.g., foraging, resting) are interrupted by the infants. We, however, found that high- & middle-ranked females aggressively handle others' infants, although they are not interrupted by the infants. This aggressive infant handling, which is defined as a handling followed by negative responses (e.g., crying, trying to escape) from the infant handled and by continuous handling despite the negative responses, has certain characteristics: (a) the handler handles the infant whose mother is little higher-ranked than her; (b) no lowest-ranked female does this kind of handling; (c) the handler and the infant are not close relatives; (d) the handler has her own infant in many cases; (e) the infant handled is 2–3 month old—more immature or more mature infants not aggressively handled in this way.

P6

Preliminary Characterization of Personality and its Genetic Factors in Two Dog Breeds

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Dogs have lived with humans since ancient times and even today they enrich our lives as working dogs and as companions. Humans have created various breeds of dogs by selecting not only for their size, hair and shape but also for their behavioral characteristics. Their intraspecific differences in personality are well known. Understanding individual differences is important in pet-keeping, so it would be useful to know their personalities in advance. Therefore, survey of genetic polymorphisms and their effect on differences in personality among dog breeds is important.

In this study, we aimed to investigate the genetic contribution to dog personality. We examined the relationship of personality and genotypes in two dog breeds, Miniature Dachshunds and Toy Poodles, which were selected because of their popularity and accessibility to large sample sizes compared to other breeds. We conducted a questionnaire survey of 169 Miniature Dachshunds and 257 Toy Poodles enrolled in Anicom pet insurance. The questionnaires consisted of 39 items and were collected along with swab samples. We performed exploratory factor analyses on the questionnaire data from each breed. The results suggested that the breeds had different numbers of personality factors, indicating that each breed should be treated separately in future personality studies. We checked the candidate genes in these breeds and found weak associations between androgen receptor short repeat variants and high “aggressiveness” in both breeds. Then we performed genome-wide association studies on all personality factors in each breed and found novel SNPs which may be related to “dog-directed sociability” in Miniature Dachshunds and “carelessness” and “aggressiveness” in Toy Poodles.

P8

Macaque Y-chromosome Introgression: Bioinformatic Analysis of Y-Chromosome Proteins in *Macaca mulatta*, *M. fascicularis*, and their Indochinese Hybrids

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Species in the genus *Macaca* typically live in multimale-multifemale social groups with male macaques exhibiting some of the largest testis: body weight ratios among primates. Males are believed to experience intense levels of sperm competition. Several spermatogenesis genes are located on the Y-chromosome and, interestingly, occasional hybridization between two species has led to the introgression of the rhesus macaque (*Macaca mulatta*) Y-chromosome deep into the range of the long-tailed macaque (*M. fascicularis*). These observations have led to the prediction that the successful introgression of the rhesus Y-haplotype is due to functional differences in spermatogenesis genes compared to those of the native long-tailed Y-haplotype. We examine here four Y-chromosomal loci—*RBMY*, *XKRY*, and two nearly identical copies of *CDY*—and their corresponding protein sequences. The genes were surveyed in representative animals from north of, south of, and within the rhesus x long-tailed introgression zone. Our results show a series of non-synonymous amino acid substitutions present between the two Y-haplotypes. Protein structure modeling via I-TASSER revealed different folding patterns between the two species' Y-proteins, and functional predictions via TreeSAAP further reveal physicochemical differences resulting from non-synonymous substitutions. These differences inform our understanding of the evolution of primate Y-proteins involved in spermatogenesis and primate speciation.

Behavioral Synchronization: Timing of Urination among Captive Chimpanzees

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Behavioral synchronization, including phenomena such as contagious yawning and facial mimicry, has gained increased scientific attention. It is a fundamental ability required for social animals to maintain group cohesion and operate in collective behavior. This study examined the possibility of synchronized urination in captive chimpanzees (*Pan troglodytes*). To our knowledge, no previous study has examined such a phenomenon. We aimed to quantitatively demonstrate the existence of synchronized urination of captive chimpanzees. We then investigated the effects of social closeness on such synchronization to investigate possible mechanisms of the phenomenon. We observed 4 groups of captive chimpanzees (N=20) for cumulative 421 hours at Kumamoto Sanctuary. We recorded all urinations with a resolution of 1 second as well as grooming interactions and physical proximity at 2-minute intervals. The observed results were then compared to a simulation (100 sessions) of random urination timings. We found synchronizations within 68 seconds occurred at a higher rate than predicted by the simulation. We did not find a clear relationship with social bond strength so far, which needs further investigation with larger sample size.

The Development of Mother-Calf Interactions in Wild Harbour Porpoises (*Phocoena phocoena*) Studied with Unmanned Aerial Vehicles (UAVs)

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Studying cetacean behaviour in the wild can be difficult, due to the aquatic lifestyle and wide distribution of these animals. One of the less known species is the harbour porpoise (*Phocoena phocoena*). Significant gaps still remain in our understanding of the behaviour and development of the species. Such information is of great importance for protecting porpoises, especially calves during the most vulnerable period of their life cycle. Unmanned aerial vehicles or drones represent an innovative and effective way to observe cetaceans in their natural environment. The aim of this study was to assess the affiliative relationship of mother-calf pairs by measuring the length and relationship (in terms of distances) between the mother and her calf in two different periods of the neonate´s life cycle. Data analysis was performed from drone-based video recordings with the help of Drone Video Measure software, a new computer program developed at the University of Southern Denmark (SDU Dronecenter. Mærsk Mc-Kinney Møller Instituttet). Results show that, during the first months after birth, calves stayed significantly closer to their mother and synchronized their breathing with her more often than during the final part of the lactation period. This shows that the bond between the mother and her calf is strongest during the first months after birth and gradually decrease during lactation. This has important implications for our understanding of harbour porpoise behaviour and how to design relevant and adequate conservation measures for the species.

Keywords: *mother-calf pair; harbour porpoise, unmanned aerial vehicle, drone, social behaviour, affiliation, photogrammetry*.

Gene Expression Analysis in a *Hylobates lar* Brain: Toward the Elucidation of the Molecular Mechanism of Song

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Gibbons (Hylobatidae, Primates) are a group belonging to Hominoidea. They morphologically, karyologically are classified into four distinct genera (Hoolock, Hylobates, Nomascus, and Symphalangus). They are extremely arboreal and inhabit in subtropical to tropical rainforest of Southeast Asia. They mainly use forest canopy and form specialized locomotion using their long upper limbs, which is called brachiation. Their species-specific, sex-specific vocalization, called the song, is also unique among primates. Some gibbon species perform duet between heterosexual pairs. It was reported that hybrid gibbons indicated a significant genetic component of the song (Brockelman and Schilling, 1984). Many studies of the gibbon song have been conducted based on the acoustic analysis, but few on the molecular analysis. To investigate the genetic basis of the song, we comprehensively analyzed mRNA from several brain regions of a deceased Hylobates lar using a next-generation sequencer (RNAseq). We identified gene expressions potentially involved in vocalization, such as FOXP2. We will discuss the difference of gene expression patterns among the regions in terms of neural development and regulation of the gibbon song.

Does Over-marking Effective on Mate Search? A Mathematical Simulation

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Scent marking is a common behavior among animals. For instance, felids leave scent-marks with urine, feces or glandular secretions. When mammals encounter a scent-mark of another individual, they often place their own scent-mark on top of or very close to it. This phenomenon is known as “over-marking”. Over-marking occurs among individuals in various relationships such as breeding pairs, same-sex competitors, social group members and sometimes different species. In this study, we focused on scent communication among different sexes. Although function of over-marking within different sexes is still uncertain, mate-attraction has been suggested to be one of the possible functions of this phenomenon. Especially for solitary animals which have broad activity ranges, scent marks can be an important tool to acquire information about location and reproductive status of potential mates. Several studies reported that marking frequency increased during the mating season among various species, supporting the role of scent marking in mating. However, few studies investigated the effect of over-marking on mate search.

The present study hypothesized that over-marking is effective to efficiently encounter mates. We compared the efficiency of mate search of individual animals performing over-marking with that of individual animals not performing over-marking in silico under a variety of conditions. The preliminary results indicated that over-marking might be effective to reduce time and traveled distance to encounter another individual. Although in silico analyses do not completely reflect actual movements of animals, the present study provides insights into adaptive significance of over-marking in the context of mate search.

Enter SimuNet:
a Social Network Simulation Framework, with a Zest of Empiricism

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The SARS-CoV-2 pandemic teaches us two things: (1) we need to improve our understanding of disease transmission, especially in wildlife, and (2) pandemics heavily hamper a researcher's ability to travel to the field to study wildlife. Should you find yourself stuck in the middle of these two, stick along/fear not! In the last few decades, disease ecology and epidemiology gained great insights from empirically-inspired simulations. This approach – coined *in silico* research – allows to test a broad range of hypotheses under our current best way of modelling biological phenomenon like disease transmission. Over the last few decades, animal social network analysis arose as a powerful tool to describe transmission patterns within interconnected individuals. But because graph theory – the science of networks – was historically first transposed to more abstract fields such as computer network or the world wide web, there is currently no network simulation framework that can accommodate the empiricism of animal social research. For this purpose, I developed SimuNet, an R package, to flexibly represent the different steps in which empirical and practical considerations can happen in animal social network analysis. SimuNet can be used to generate a broad range of weighted networks taking real-life animal social observations. And because all the smallest intermediate steps are simulated and can easily be tweaked, social systems now also have a framework in which to test empirical hypotheses *in silico*. How does your choice of behavioural sampling affect your results? What happens when you tend to miss the peripheral individuals in a group? What happens to the network when a central individual disappears? How can this change disease transmission in the group? Now you can address these questions as easily as installing a package.

What is "Tameness" in Captive Red Foxes?: with Reference to Self-domestication Hypothesis

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In this study, I re-evaluated the self-domestication hypothesis by observation of foxes' (*Vulpes vulpes*) reactions to humans. Domesticated animal species are known to have some physical and behavioral traits in common. Such "domestication syndrome" includes feminized head, depigmentation, reduced aggression, enhanced social cognition, etc. Because the similar syndrome is also found in non-domesticated primates such as bonobos (*Pan paniscus*) and humans (*Homo sapiens*), these species are sometimes said to be "self-domesticated." The "self-domestication" process of these primates is often inferred from that of dogs, as the latter is also said to be self-domesticated in its earliest phase. On the other hand, dogs' domestication process has been modeled with a domestication experiment (artificial selection of "tame" animals) of red foxes. Thus, bypassed by the domestication of dogs, primate "self-domestication" is often discussed as a process of selection of "tameness." However, there are two major concerns with this inference. The first is that the fox domestication experiment was conducted in a confined environment, whereas the self-domestication of dogs should have occurred in an open environment. The second is that the experiment was conducted by artificial selection, whereas "self-domestication" should have occurred without it. In this study, I observed foxes in the North Fox Farm, in Hokkaido, Japan, a tourist facility where foxes are kept in a wide enclosure and have free access to other foxes and human visitors. The Farm do not control the mating of foxes thus they are not artificially selected. Focal animals were thirteen 0–1-year-old individuals. I recorded and typified foxes' reactions to humans (tourists and staff). The principal component analysis showed that fearful responses (escape) and friendly responses (contact and approach) were not opposite reactions. Rather, the opposite of the friendly responses appeared to be ignoring. Aggressive responses were not observed in this study. Foxes ignored humans when the distance between them was not likely to be close, but escaped when the distance was likely to be close. These results suggest that the behaviors of unfriendly foxes may change from "ignoring" to "fearful responses" to "aggressive responses" as the environment becomes more confined so that foxes have less options to avoid approaches by humans. If this is also applicable to dogs, the current picture of self-domestication process of dogs may require a reconsideration. Although the selection to reduce aggression is now believed to be the key, this idea may be falsified. Because the environment in which dogs would have self-domesticated should have been an open environment, and it is likely that unfriendly canine ancestors would have easily escaped from humans rather than to show aggressive responses. As suggested by this study, the friendly and the aggressive/escape responses may not have been opposite reactions in the self-domestication of dogs. Therefore, we will still need to re-examine which behavior was really selected for in the self-domestication of dogs, before we easily apply the current inference to the self-domestication process of primates.

Genetic Origin of Koalas Captive in Japan: toward the Investigation of Innate Diet Preference

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Koalas (*Phascolarctos cinereus*) are arboreal marsupials that inhabit eucalypt forests of eastern Australia. They feed almost exclusively on leaves of *Eucalyptus* spp. There are more than 600 species in the genus *Eucalyptus*; nevertheless, a koala selectively chooses and feeds on limited species of *Eucalyptus*, and also chooses certain individual trees within a species. Thus, koalas are known as the fussy eaters. It has not been well known how koalas choose their diet.

The sense of taste is important to assess whether the food is acceptable or not. The taste of foods is recognized with taste receptors expressed on the taste buds of the tongue. Taste receptor genes evolutionarily change their copy numbers and/or nucleotide sequence that causes the functional changes contributing to the adaptation to new novel environments and diet. Koalas are distributed from north to south in eastern Australia. This geographical variation provides difference of the species availability of eucalyptus trees to koalas. Therefore, there might be genetic variants of taste receptors specific to regions to adapt their habitat environments and diets.

Aiming future investigation of the innate diet preference of koalas, we first investigated the genetic origin of 11 koalas in Higashiyama zoo, Nagoya, where their diet preferences were surveyed by behavioral studies. Phylogenetic analysis with mitochondrial DNA control region has shown that koalas can be divided into four linages corresponding geographically: two northern lineages, a central lineage and a southern lineage. The nucleotide sequence of their mitochondrial DNA control region showed that there are all the 4 lineages. Thus, we can investigate the difference in preference between the four lineages. Based on this result, we will investigate genetic variations of taste receptors and discuss the relationship with diet preferences and geographical origin. This will also lead to sustainable husbandry of koalas based on their innate diet preference.

Monotremes Shed Light on Mammalian Evolution

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The platypus and echidna (monotremes) are the only egg-laying mammals and are an extraordinary amalgam of mammalian and reptilian features. They represent the most ancient, extant mammalian lineage, having diverged from all other (therian) mammals approximately 187MYA[1]. As such they provide invaluable insight into mammalian evolution. With the generation of the first draft echidna genome and greatly improved platypus genome assemblies[1], we have begun to identify genetic alterations that are unique to the monotreme lineage compared to therian mammals and identify genetic differences that underlie the unique biology of the platypus or the echidna. We have shown that loss of genes involved in gastric function is common to both the platypus and echidna and that monotremes lack a key gene in the haemoglobin breakdown pathway, haptoglobin. The haptoglobin gene was previously thought to be common to all mammals but our finding suggests that monotremes have evolved a unique mammalian haemoglobin breakdown system. Together these findings advance our understanding of the molecular basis of monotreme biology and mammalian evolution.

Comparison of Facial Morphology between Wildcats and Domestic Cats

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Domesticated animals commonly display low cortisol secretion, reduced aggression and juvenilized head known as the domestication syndrome. Previous studies have shown that domestic cats have low cortisol levels than wildcats; however, their behavior and morphology have not been investigated in a comparative way. In this study we compared facial morphology of four categories in cats: African wildcats *(Felis lybica),* feral mongrels, owned domestic mongrels, and owned domestic purebred cats *(Felis catus).* We measured their facial depth (nose length adjusted by distance between eyes) and eye angle. We also collected feces from owned domestic mongrels and owned domestic purebred cats to measure cortisol levels, and investigated behavioral characteristics by questionnaires to the owners. The results showed that face depth of domestic cats was shorter than that of African wildcats and the distance of owned domestic purebred cats was significantly shorter than that of owned domestic mongrels. In terms of the angle, owned domestic purebred cats was found to be lower than owned domestic mongrels. There were no significant differences in fecal cortisol levels, sociability, and aggression. These results suggest that domestication causes the facial morphology of domestic cats to be more juvenilized than African wildcats but further studies are needed to investigate its effects on their endocrine and behavior.

Food Transfer in Golden-faced Saki: Developmental Change in Juveniles' Begging Behavior

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The dietary shift from mother's milk to solid foods is the common challenge for all mammals. Some neotropical primates are known to fill the gap by the frequent food transfer from caregivers to infants. The importance of food transfer should be high especially for primates in the subfamily Pitheciinae which is recognized as "seed eater", because of their extractive foraging style requiring manual dexterity, robust teeth, and powerful jaws. However, little is known about their food transfer or the developmental process of the feeding behavior. We observed two free-ranging groups of Golden-faced Saki (*Pithecia chrysocephala*) in Manaus, Brazil. Through 908 hours of observation, we recorded 398 food transfer events. All events were initiated by juveniles' begging and no proactive food offerings was observed. Among them, 52% of the begging were by 4 individuals of 0-year-old, and 48% were by 7 individuals of 1-year-old. The begging frequency was not different between 0-year-old and 1-year-old, but the success rate of the begging was higher in 1-year-old. The diversity of begged fruits was less in 1-year-old. They begged more frequently for fruits that require longer handling time. These results suggested that older juveniles were begging more selectively to optimize their food-intake efficiency.

P21

Phylogenetic Relationship of the Malayan Tapir in Japan

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The Malayan tapir (or Asian tapir; *Tapirus indicus Desmarest*) is the only Asian member of the Tapiridae family in the order Perissodactyla. The species is native to Peninsular Malaysia, southern Thailand and Myanmar, and Sumatra of Indonesia. It is listed in CITES Appendix I and IUCN Red List ("endangered") with a population estimation of not more than 2,500 individuals worldwide. Japan as one of the countries with a captive breeding programme of the Malayan tapir has for decades maintained a captive tapir population in the zoos and a breeding center, but genetic analysis has been scarce. Mitochondrial marker such as the control region, or D-loop, is a maternally-inherited neutral marker useful for the inference of intra- and interspecies phylogenetic and phylogeographic relationships. The captive Malayan tapir population in Japan has not been assessed with this marker, albeit an attempt by a previous study was made to investigate the phylogenetic and phylogeographic relationships of the Japanese captive population using the mitochondrial cytochrome *B* gene on captive samples originated from Malaysia, Thailand and Sumatra of Indonesia. To further understand the genetic diversity of the maternal lineages of the Malayan tapir in Japan captivity, in this study, we used a partial sequence of the mitochondrial control region (507 bp) to compare the genetic diversity of the Japanese captive population ($n = 20$) with the captive and/or wild populations in Peninsular Malaysia ($n = 44$) and Thailand ($n = 37$). We found a total of 32 variable sites within the 101 samples, and among which 27 sites were parsimony-informative. Based on the parsimony-informative sites, we detected three novel haplotypes on top of 19 haplotypes found in Malaysian and Thailand samples. Haplotype network indicated two major clades where the Japanese samples could be found, representing seven out of 22 haplotypes detected in total samples. Potential loss of mitochondrial genetic diversity is high given that only three haplotypes were possessed by four females in the Japanese sample. We hope to analyze more Japanese samples in the future to get a better picture of the haplotypic distribution in Japan captive populations.

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P22

The Change of Bitter Sensitivity to PTC in Each of Individuals and the Relationship between TAS2R38 Polymorphism and Food Preference

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Introduction. Human has the innate ability to perceive thousands of compounds as bitter, and 25 taste 2 receptors (TAS2Rs) have been known as the bitter taste receptors. TAS2R38 is the bitter receptor having polymorphisms and it is said that this gene is one of the responsible genes for interindividual difference of bitter perception. It is identified that one of the agonists of TAS2R38 is PTC and the polymorphisms should influence the preference to Brassicaceae vegetables. Besides, some studies reported genotypes of TAS2R38 may have relations to smoking and alcohol intake. In many studies, the taste tests for dividing participants' bitter sensitivity into 3 groups (supertasters, medium tasters, and non-tasters) was done only once. In this study, I focus on the changes of taste sense in each of the individuals and the relationship between TAS2R38 genotypes and people's bitter sensitivity or food preference.

Methods. The participants had the taste tests using PTC test papers performed once a week, for 2 months. I collected DNA samples from oral swab samples to identify their genotypes of TAS2Rs by DNA sequence. And the data of the participants' age, sex, the change of their physical condition, and preference of vegetables and drinks were collected by the questionnaire.

Results. The TAS2R38 genotypes of most of the participants were PAV/PAV, PAV/AVI and AVI/AVI. The changes of the bitter sensitivity to PTC were observed in all participants, however, there exist the significant difference between PAV/PAV (tasters) group and AVI/AVI (non-tasters) group continuously. The PAV/PAV carriers tend to perceive PTC bitterness stronger than the AVI/AVI carriers, consistent with the previous studies. There was no correlation of their physical condition with the change of the results of the taste tests. Also, there was a little correlation between the bitter sensitivity to some of Brassicaceae vegetables including glucosinolate and TAS2R38 genotypes. Because the structure of glucosinolate is similar to that of PTC, preference for Brassicaceae vegetables could be connected to the sense of bitterness. On the other hand, there was no association between TAS2R38 genotypes or their bitter sense for other foods and the bitter sense.

Discussion. The change of the bitter sensitivity to PTC suggests that the classification of taste sensitivity by only once taste test may be uncertain result. In order to avoid picking outliers, it is reasonable that we repeat taste test several times and use the median for analysis. No significant correlation between TAS2R38 genotype and people's bitter sensitivity or food preference would be because that the questionnaire was performed without actual food tasting, and the bitterness and taste depended on personal image. Based on this data, I am going to continue to perform the taste test and questionnaire with more participants. Because similar polymorphisms of TAS2R38 was observed in other primate species, such as macaques and chimpanzees, it will help to know the better way to feed them or their health conditions.

What Did He See on the Surface of the Puddle? A Male Chimpanzee Produced an Alarm Call at a Water Puddle

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Chimpanzees (*Pan troglodytes*) are known to have a fear of water, a condition known as hydrophobia. However, the extent of the fear depends on populations; adolescent chimpanzees in Mahale put their fingers into the water of a stream as play and both matured and juvenile chimpanzees of a group in Senegal even soak in water puddles. The factors that cause those differences in hydrophobia are still unclear.

Here we share a series of videos of a male chimpanzee of the M group producing alarm call at a water puddle and responses from other males recorded in Kalinzu Forest Reserve, Uganda. In the middle of traveling on a large path, one male suddenly produced an alarm call at a water puddle next to him and jumped back. The call panicked the party for about fifteen seconds. After calming down, three chimpanzees carefully inspected the puddle and two of them displayed at the puddle. One hypothesis is that the male felt afear response upon seeing an “unfamiliar” face and/or figure of a conspecific.

We also observed inspection behaviors similar to this report at another place with a small stream both in rainy season and dry season. In dry season, there is little water at that place and they inspected where the water runs in rainy seasons. Those places are not covered by trees and the surface of water reflects images. This report provides the hypothesis that the image reflections of water affect behaviors of chimpanzees and shape the differences in hydrophobia.

P24

Impact of Game Hunting on Physiological Stress Levels of Wild Red Deer

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Hunting is a very common activity all over the planet. However, its practices may induce intense stress responses in wildlife, which can have important consequences for prey populations and, consequently, for hunting management. The wild population of red deer (*Cervus elaphus*) is one of the most harvested during the seasonal hunting in the Lousã Mountain, Portugal.

To evaluate the impact of hunting activity on the red deer population, different biological samples were collected from hunted individuals during two hunting seasons. Short and long-term stress levels were examined by assessing cortisol and its metabolites in blood, feces and hair. The immunological and physical conditions of the animals were also evaluated. As expected, results showed an increment of cortisol levels measured in the hair during the months of harvesting. Surprisingly, we found a tendency for plasma cortisol levels to decrease during the hunting season, which could be interpreted as habituation, or as a result of the duration of the hunt itself. Regarding fecal cortisol metabolites, and contrary to our predictions, the concentrations in feces did not show any clear patterns across months.

Taken together, our results suggest an influence of hunting activities on the physiological stress levels in red deer. We highlight the potentially important role of hair to assess physiological stress, although more studies are needed to fully understand its suitability as an indicator of long-term stress. The framework of the present study emphasizes the importance of simultaneously using multi-temporal procedures to assess short and long-term effects in studies focused on physiological stress reactions.

P26

Influence of Age of Breeding Mare is Not Negative on the Racing Performance of their Offspring as Commonly Assumed

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In general, it is considered that the ageing of breeding mares would negatively affect the racing performance of their offspring. Although this general idea has never statistically examined so far, certainly, the older breeding mares may be a risk, considering the issue of chromosomal abnormalities. However, there is room for doubt as to whether the effects of pregnancy at old age will continue to affect horses that have grown to the point where they can participate in racing. Therefore, the mechanism by which pregnancy at old age reduces the race performance of the offspring is unknown. We constructed two hypotheses. 1) pregnancy at old age affects the development of their offspring; 2) low quality stallions are likely to be chosen as a partner of an older breeding mare. To address which hypothesis is plausible, we examined the effect of the age of the breeding mare on the racing performance of their offspring with controlling the effect of stallions' quality, age, and ID, sex of the offspring, trainer, and the location of training center. We sampled information of racing horses which were registered to Japan Racing Association from the website of Japan Bloodhorse Breeders' Association. We confirmed the racing performance of horses which was born from older mares was lower than those which born from younger mares. However, results of Generalized linear mixed model (GLMM) with hurdle model showed that the effect of age of breeding mare was not significant. On the other hand, the quality of stallions showed the significant effects. Furthermore, age of breeding mare was negatively correlated to the quality of stallions, although the variance inflation factor was low. Therefore, we concluded that the effect of the age of the breeding mare was negligible or only limited and rather the quality of the stallion had important influence of the racing performance of the offspring. This conclusion seems to be likely, considering the production system of racing horses. Low quality stallions, in other words, cheap stallions would be likely to be chosen as a partner of older mares which potentially have a risk on giving birth. The present study showed that the general idea that the horses which was born from the older mare would show low performance may not be understood correctly.

Variable Functions of Genito-Genital Rubbing among Female Bonobos (*Pan paniscus*)

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Primates, including humans, engage in various sexual behaviors that are not for reproductive success directly. Homosexual behaviors include such sexual behaviors which do not directly result in reproductive success. The adaptive functions of homosexual behaviors in humans are still poorly understood, despite many previous studies that have referred to the function of homosexual behaviors in non-human primates in order to understand the evolutionary origin of human homosexual behaviors.

Female bonobos (*Pan paniscus*), who are the closest living relatives of humans, engage in same-sex socio-sexual behaviors frequently, called genito-genital rubbing (GG rubbing). GG rubbing appears to be multifunctional (*e.g.*, reconciliation, mate attraction, tension regulation, expression of social status, social bonding), which helps us receive valuable insight for understanding the evolutional functions of homosexual behaviors.

In the current study, we examine the function of GG rubbing among female bonobos at Wamba, Luo Scientific Reserve, Democratic Republic of the Congo. Our results showed that the social rank of females was related symmetrically in the initiation of GG rubbing, which previous studies reported different results. The more females had proximity and engaged in grooming behaviors, the more they engaged in GG rubbing, supporting the social bonding hypothesis. In Wamba, we could not confirm the clear linear dominance hierarchy among females, and the female-female dominance ranks were stable. These results indicate that the function of GG rubbing may be variable depending on female-female relationships among multiple bonobo populations.

Genetic Analysis of Captive Echidnas in Japanese Zoos using Noninvasive Spine Samples

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Short-beaked echidnas are documented as having five subspecies but little is known about genetic basis of their phylogeny. A previous study showed that the D-loop sequence region of mitochondrial DNA of the short-beaked echidna did not clearly reflect the formal subspecies classification and divided echidna populations into three clusters (two groups in Australia and one group in New Guinea) (Summerell et al. 2019). The study concluded that further research is required to clarify the regional distribution of echidna subspecies.

There are captive short-beaked echidnas in Japan. They imported to Japan several dozen years ago due to their longevity, but their geographical origins are unknown. In this study, we analyzed their genetics using noninvasive spine samples. After washed and rinsed, we cut 1-cm from the root end of the spine, crushed it using the bead beater homogenizer, and then extracted DNA using the QIAamp Investigator Kit. After PCR amplification of the D-loop region of mtDNA, sequenced it and created a phylogenetic tree.

As the first trial, we analyzed four noninvasive spine samples of two echidnas in Higashiyama Zoo. We successfully amplified their mtDNA sequences and their mtDNA haplotype is identical to Queensland & New South Wales haplotypes. We will analyze their auto- and sex chromosomes, add individuals in other zoos, and investigate recodes at the time when they were imported so as to discuss phylogeography of captive echidnas.

How is Spatial Structure of Multi-level Society Decided?

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Some mammalian species of different taxonomic groups, including humans, have developed social structures with nested levels of organization. In this multilevel society, the smallest core groups are called 'unit', and multiple units aggregate to form higher-level group. A multilevel society is one of the most complex social systems in animals, but their function and evolutional process are still poorly understood. Investigation on factors that affect social relationships and spatial structure of a group may provide the implication on the mechanism and the function on the society. The current study aimed to investigate how unit-level social relationships are decided in a multilevel society of feral horses by comparing the spatial structure in 2018 and 2019. During 2018, the feral horse population experienced a significant decrease because of the human capturing, and the unit composition largely changed. The analysis suggested that the absence of the bachelor, males which could not hold any female to form a harem unit, weaken the cohesion among harems. This implies the bachelor threat hypothesis, that multilevel society benefits harems to have more effective protection from bachelors' harassments. In addition, the social network analysis revealed that the inter-harem spatial proximity is correlated to the inter-male relationships, suggesting harem-male have an important role to maintain the inter-unit cohesion. Relatedness had no significant correlation to the inter-male and inter-harem relationships. In other species forming multilevel society, genetic relationships are considered as the most important factor to decide relationships among units, but our study indicates that non-genetic aspects could be also important.

P31

Spatial Relationships in Finless Porpoises by Aerial Videos

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Finless porpoises (*Neophocaena asiaeorientalis sunameri*) are distributed throughout the shallow (usually <50 m deep) coastal waters of Japan. Their group size has been reported to be 1.97 individuals in previous research, yet aggregations of more than 100 individuals have been observed. A recent study revealed that a bird's-eye observation technique using a drone may generate new considerations on finless porpoise behavior and sociality, as finless porpoises have no dorsal fins, which reduces their visibility for surface observations. We planned a drone study for estimating more accurate group size through direct observations. At the Misumi West Port, Ariake Sound, Japan, settled finless porpoises are well recognized among local people. We conducted an observational study for collecting day-long behavioral data at Misumi West Port by drones. We collected data for 5131 minutes from November 2019 to November 2020. We defined groups by using interindividual distances, and mean group size was 1.47 individuals.

New Trends of Infant Handling by Japanese Macaques (*Macaca fuscata*) in a Provisioned Group

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Infant handling is a behavior by nonhuman primates in which individuals other than the mother (hereafter, 'handlers') handle infants (e.g., grooming, holding, carrying, and aggressive interactions). A lot of studies from a variety of species have investigated which individuals handle infants more frequently. In Japanese macaques (*Macaca fuscata*), which is the subject animals in this study, previous works showed that (1) females handle infants more than males, (2) juveniles handle infants more than adults, and (3) handlers handle kin infants more than non-kin infants. In a provisioned group, however, we found some different trends from these reported tendencies. The current presentation reports the novel trends of infant handling by Japanese macaques and makes implications for the mechanism and function of this behavior. The subject animals were Japanese macaques in Arashiyama Monkey Park Iwatayama, Kyoto, Japan. Monkeys in this park have been provisioned since this park was open in 1954. All 13 infants who were born in 2020 were selected as focal subjects. Behavioral data recording was conducted from August to December 2020. By focal animal sampling, contact time between infants and handlers during infant handling and handlers' name were recorded. The total focal time for each infant was more than 500 minutes (average: 542.2 minutes, SD = 25.5). There were the similar results with previous studies; (1) females handled infants more than males and (2) juveniles handled infants more than adults. However, some unreported trends were also confirmed; (3) juvenile handlers handled non-kin infants more than kin infants (19.2% towards kin infants), and (4) infant handling by adult females were regularly observed. Focusing on infant handling by adult females, we found (5) adults who did not have their own offspring under 3 years old handled infants more than adults who had their own offspring, (6) adults who had kin infants handled infants more than adults who did not have kin infants, and (7) adults who had kin infants did not necessarily handled their kin infants (only 19.4% of all handling by those adults were directed to kin infants). These results provide some useful implications for the mechanism and function of infant handling especially by adult female Japanese macaques.

Acoustic Communication by Non-vocal Sound in the Free-ranging Horse Group

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Horses communicate in diverse ways using visual, chemical, tactile, and auditory signals. As a highly social species, they are known to recognize other individuals as well as the social category and convey their emotional states with various vocalizations and non-vocal sounds. Studies on acoustic communication of horses have mainly focused on the vocalizations—involving the use of larynx—such as whinny, a call seeking social contacts with herd mates who are out of view. However, non-vocal sounds could also play important roles in their communication, given that horses have very sensitive hearing, and that non-vocal sounds can be subtler signals making them less detected by predators than vocalizations. I plan to examine whether and how the non-vocal sound types are used in the communication of horses living in social groups throughout the year. This would further the understanding of the details of horse social life and contribute to improving the management and welfare of horses.