

*The 16th International Symposium  
on Primateology and Wildlife Science*



*September 29-30, 2021*



# The 16th International Symposium on Primatology and Wildlife Science

Schedule at a Glance (updated Sep. 22, 2021)

Wednesday, September 29			Thursday, September 30						
JST	Morning	JST	Afternoon	JST	Morning	JST	Afternoon		
10:00	Opening Remarks	13:10	G-1 Hanya	Defaunation: loss of seed dispersal services due to extinction of megafauna	10:00	O-7 Yan PWS Finishing Talk	Molecular & Cellular Insights		
	O-1 Zemmoto	13:30	G-2 Corlett		10:30	O-8 [Withdrawn]		13:30	O-10 Brooks
10:30	O-2 Itahara		G-3 Nakabayashi		10:30	O-9 Hayashi		13:30	O-11 Fasbender
	O-3 Gris PWS Finishing Talk	14:00	G-4 Bernard			Coffee/Tea Break	14:00	O-12 Xu	
11:00	Coffee/Tea Break		G-5 Horie		11:00	Poster Session II Core Time: P-15 –P-28	14:00	O-13 Sato PWS Finishing Talk	
	O-4 He	14:30	General Discussion			Present during entire session: P-15 Onishi; P-16 Boyun Lee; P-17 Maeda; P-18 Minami; P-19 Yokoyama; P-20 Yeongju Lee; P-21 Langgeng; P-22 Pratap Kadam; P-23 Vilela; P-24 Ruiz; P-25 Jones; P-26 Chaney; P-27 Santa; P-28 Nakamura	14:30	O-14 Shibata PWS Finishing Talk	
11:30	O-5 Keuk	15:00	Coffee/Tea Break		11:30	Lunch Break		Coffee/Tea Break	
	O-6 Sukumar	15:30	Poster Session I Core Time: P-1 –P-14		12:00	Lunch Break	15:00	G-6 Sarania	
12:00	Lunch Break	16:00	Present during entire session: P-1 Naito-Liederbach; P-2 Sugimoto; P-3 Hattori; P-4 Hirata; P-5 Numabe; P-6 Palacino Gonzalez; P-7 Sok Hwan Lee; P-8 Arai; P-9 Piao; P-10 Tanaka; P-11 Kuriyama; P-12 Lim; P-13 Yoshimura; P-14 Toyoda		12:30	Lunch Break	15:30	O-15 Ryu	
12:30	Lunch Break	16:30	Coffee/Tea Break		13:10	Lunch Break	15:50	Closing Remarks	

# The 16th 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 Wednesday, September 29th, 2021

Time (JST)	min.	Abstract	Title	Speaker	Affiliation
10:00-10:10	(10)		Opening Remarks	Gen'ichi Idani	Wildlife Research Center, Kyoto University
<b>Individual Perspectives</b>			<b>Chair: Susumu Tomiya</b>		
10:10 - 10:25	(15)	O-1	Some novel SNPs may affect dogs' personality in two breeds	Chika Zemmoto	Wildlife Research Center, Kyoto University
10:25 - 10:40	(15)	O-2	"Corvid Tracking Studio" The motion capture system as a novel tool to study the gaze of corvids	Akihiro Itahara	Wildlife Research Center, Kyoto University
10:40 - 11:00	(20)	O-3	Acute pain in Japanese macaques: development of assessment tools	Vanessa N. Gris	Primate Research Institute, Kyoto University
11:00 - 11:15	(15)	[ Coffee/Tea Break ]			
<b>Forest Animal Ecology</b>			<b>Chair: Nahoko Tokuyama</b>		
11:15 - 11:30	(15)	O-4	Reducing food particle size may not be important for eating fruits: examining determinants of fecal particle size in Japanese macaques	Tianmeng He	Primate Research Institute, Kyoto University
11:30 - 11:45	(15)	O-5	Bugs in animal social networks: from the field to simulations	Kenneth Keuk	Primate Research Institute, Kyoto University
11:45 - 12:10	(25)	O-6	Fitness consequences of crop raiding by elephants	Raman Sukumar	Centre for Ecological Sciences, Indian Institute of Science
12:10 - 13:10	(60)	[ Lunch Break ]			

Time (JST)	min.	Abstract	Title	Speaker	Affiliation
<b>[Special Session] Defaunation: loss of seed dispersal service due to extinction of megafauna</b>					
<b>Chair: Ikki Matsuda</b>					
13:10 - 13:40	(30)	G-1	Defaunation in Japan: seed dispersal by Japanese macaques in Yakushima and their extinction in a neighboring island	Goro Hanya	Primate Research Institute, Kyoto University
13:40 - 14:10	(30)	G-2	Defaunation and the loss of seed dispersal services in tropical Asian forests	Richard T. Corlett	Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences
14:10 - 14:20	(10)	[ Coffee/Tea Break ]			
14:20 - 14:50	(30)	G-3	Seed dispersal of small seeded-plants, <i>Ficus</i> on Borneo	Miyabi Nakabayashi	Graduate School of Advanced Science and Engineering, Hiroshima University
14:50 - 15:20	(30)	G-4	Do riparian reserves support native terrestrial small mammal fauna in oil palm-dominated landscapes?	Henry Bernard	Institute for Tropical Biology & Conservation, Universiti Malaysia Sabah
15:20 - 15:40	(20)		General Discussion		
15:40 - 15:55	(15)	[ Coffee/Tea Break ]			
<b>Sustainable Developmental Goals</b>					
<b>Chair: Gen'ichi Idani</b>					
15:55 - 16:35	(40)	G-5	The Planet and Humans at a crossroad and SDGs	Masahiko Horie	Special Assistant to the Foreign Minister of Japan
16:35 - 16:50	(15)	[ Coffee/Tea Break ]			
16:50 - 18:00	(70)	Poster Session I (on LINC Biz) Core Time: P-1 ~ P-14			

**DAY 2** Thursday, September 30th, 2021

Time (JST)	min.	Abstract	Title	Speaker	Affiliation
<b>Molecular &amp; Cellular Insights</b>			<b>Chair: Takashi Hayakawa</b>		
10:00 - 10:20	(20)	O-7	Genetic basis of the speciation in Sulawesi macaques, Indonesia	Xiaochan Yan	Primate Research Institute, Kyoto University
10:20 - 10:35	(15)	O-8	[Withdrawn]		
10:35 - 10:50	(15)	O-9	A zoo-born white wallaby: disruption of the melanin synthesis pathway due to an insertion mutation	Sakura Hayashi	Department of Biological Science, Kyoto University
10:50 - 11:00	(10)	[ Coffee/Tea Break ]			
11:00 - 12:10	(70)	Poster Session II (on LINC Biz) Core Time: P-15 ~ P-28			
12:10 - 13:10	(60)	[ Lunch Break ]			
<b>Behavior, Learning, and Ecology of <i>Pan</i></b>			<b>Chair: Yuko Hattori</b>		
13:10 - 13:25	(15)	O-10	Oxytocin promotes social grooming in bonobos: evidence for a biobehavioural feedback loop?	James Brooks	Wildlife Research Center, Kyoto University
13:25 - 13:40	(15)	O-11	Foraging together: food characteristics associated with large party size in bonobos	David Fasbender	Primate Research Institute, Kyoto University
13:40 - 13:55	(15)	O-12	Chimpanzees can learn to utilize referential information from video to choose a tool for future use	Shenwen Xu	Primate Research Institute, Kyoto University
13:55 - 14:15	(20)	O-13	Understanding of others in chimpanzees ( <i>Pan troglodytes</i> ): cognitive and affective underpinnings	Yutaro Sato	Wildlife Research Center, Kyoto University
14:15 - 14:35	(20)	O-14	How do males associate with other males in their group? comparative studies of male aggression and association pattern of the genus <i>Pan</i>	Shohei Shibata	Primate Research Institute, Kyoto University
14:35 - 14:50	(15)	[ Coffee/Tea Break ]			
<b>Working with Communities</b>			<b>Chair: Michael A. Huffman</b>		
14:50 - 15:30	(40)	G-6	Shifting our gaze – towards a just, inclusive approach to research in the field	Bidyut Sarania	Centre for Ecological Sciences, Indian Institute of Science
15:30 - 15:45	(15)	O-15	Citibats: citizen science-based bat monitoring project in Korea	Heungjin Ryu	Department of Life Sciences, Ulsan National Institute of Science and Technology
15:45 - 15:50	(5)		Closing Remarks	Takakazu Yumoto	Primate Research Institute, Kyoto University

## Poster Session I

Coretime: Wednesday, September 29th, 2021, 16:50 - 18:00

Abstract	Title	Speaker	Affiliation
P-1	Diversity of MHC genes of endangered Japanese raptors: a preliminary comparison between golden eagles and mountain hawk-eagles	Annegret M. Naito-Liederbach	Wildlife Research Center, Kyoto University
P-2	How horses evaluate third-party interactions between humans	Takuto Sugimoto	Wildlife Research Center, Kyoto University
P-3	Comparison of facial morphology in African wildcats, feral mongrels, and owned domestic cats	Madoka Hattori	Wildlife Research Center, Kyoto University
P-4	Estimation of food habits of Japanese macaques based on tooth microwear: detection of variations among regional populations	Kazuha Hirata	Primate Research Institute, Kyoto University
P-5	Individual difference in the sensitivity to bitter compounds in coffee based on TAS2R gene polymorphism	Rena Numabe	Primate Research Institute, Kyoto University
P-6	Comparative studies on the social behaviour of magpies and domestic horses	Gema Palacino-Gonzalez	Department of Zoology, University of Extremadura
P-7	Effect of prestigious behavior in social status of chimpanzees ( <i>Pan troglodytes</i> ) and bonobos ( <i>Pan paniscus</i> )	Sok Hwan Lee	Wildlife Research Center, Kyoto University
P-8	Preliminary analysis on the epigenetic age estimation of captive Asian elephants ( <i>Elephas maximus</i> )	Kana Arai	Wildlife Research Center, Kyoto University
P-9	Dipping or sucking? Choice of tube-using techniques in chimpanzees and bonobos before and after video demonstration	Yige Piao	Wildlife Research Center, Kyoto University
P-10	Relationship between stress hormone concentration and behavioral changes in captive Tsushima leopard cats ( <i>Prionailurus bengalensis euptilurus</i> )	Chisato Tanaka	Wildlife Research Center, Kyoto University
P-11	Reproductive physiology of Iriomote cat ( <i>Prionailurus bengalensis iriomotensis</i> )	Yuko Kuriyama	Wildlife Research Center, Kyoto University
P-12	Whole-genome resequencing of endangered Malayan tapir for novel discovery of genome-wide single nucleotide polymorphism loci	Qi Luan Lim	Wildlife Research Center, Kyoto University
P-13	Plants for carnivores: Metabarcoding analysis of wild snow leopards ( <i>Panthera uncia</i> ) focusing on dietary plant species	Hiroto Yoshimura	Wildlife Research Center, Kyoto University
P-14	Ontogenetic differences in mandibular morphology and their adaptive significance in Japanese macaques and long-tailed macaques	Naoto Toyoda	Primate Research Institute, Kyoto University

## Poster Session II

Coretime: Thursday, September 30th, 2021, 11:00 - 12:10

Abstract	Title	Speaker	Affiliation
P-15	Urination behavior of captive chimpanzees: preliminary study of social factors	Ena Onishi	Wildlife Research Center, Kyoto University
P-16	Selective infant handling in Yakushima Japanese macaque ( <i>Macaca fuscata yakui</i> )	Boyun Lee	Primate Research Institute, Kyoto University
P-17	Power-law relationships between a group size and a social interaction number in animal societies	Tamao Maeda	Wildlife Research Center, Kyoto University
P-18	Infant Japanese macaques have the most infant-like faces at around 3 weeks of age during the first 10 weeks	Toshiki Minami	Primate Research Institute, Kyoto University
P-19	The effects of genito-genital rubbing on affiliative relationships among female bonobos ( <i>Pan paniscus</i> )	Takumasa Yokoyama	Primate Research Institute, Kyoto University
P-20	The influence of social bonds of mothers on the offspring's sociality in feral horse groups	Yeong-ju Lee	Wildlife Research Center, Kyoto University
P-21	Hot spring bathing behavior and helminth infection in Japanese macaques at Jigokudani	Abdullah Langgeng	Primate Research Institute, Kyoto University
P-22	Comparison of foraging patterns between smokers and non-smokers	Sanjana Pratap Kadam	Primate Research Institute, Kyoto University
P-23	Empathy in elephants: integrative approach of behavioral ecology and cognitive science	Sofia Vilela	Centre for Functional Ecology, University of Coimbra
P-24	Copy number variation among multicopy Y-chromosome gene families of rhesus and long-tailed macaques	Cody A. Ruiz	Department of Anthropology, Kent State University
P-25	Japanese and rhesus macaques differ in amygdala cannabinoid 1 receptor-immunoreactive axon density	Danielle N. Jones	Department of Anthropology, Kent State University
P-26	Captive male marmosets (Genus <i>Callithrix</i> ) are prone to dental abnormalities, regardless of hybrid status	Morgan E. Chaney	Department of Anthropology, Kent State University
P-27	The effect of 'brightness contrast' in luminance discrimination tasks for killer whales	Ayumu Santa	Primate Research Institute, Kyoto University
P-28	Morphological and histoanatomical studies of the larynx in lemurs	Kanta Nakamura	Primate Research Institute, Kyoto University

## **Defaunation in Japan: seed dispersal by Japanese macaques in Yakushima and their extinction in a neighboring island**

Goro Hanya<sup>1</sup>\*

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Unlike many tropical countries, increase, rather than decrease, of large animals is now regarded to be a conservation issue in Japan. However, many species of Japanese wildlife suffered from a high hunting pressure and habitat loss until a few decades ago. Local and even species extinction occurred in many regions in the Japanese archipelago, and some populations have not recovered since then. Yakushima is an island in southern Japan where the long-term study of Japanese macaques (*Macaca fuscata*) has been conducted since the 1970s. Seed dispersal is one of the topics which is intensively studied for this population and most of the aspects of pre-dispersal and dispersal phases have been clarified. Japanese macaques have gone extinct in Tanegashima, a neighboring island of Yakushima, until the late 1960s. The two islands are comparable in size and vegetation, but are quite contrasting in the terrain, human population and their land use. There is one preliminary study suggesting that the extinction of Japanese macaques in Tanegashima has resulted in a considerable decrease of seed dispersal services there, compared with Yakushima where a large number of Japanese macaques are still surviving. In this talk, I introduce previous studies of seed dispersal of Japanese macaques in Yakushima, discuss how their extinction can potentially affect forest regeneration and introduce our new ongoing project trying to reveal the cause and consequences of defaunation in Tanegashima.



## Defaunation and the Loss of Seed Dispersal Services in Tropical Asian Forests

Richard T. Corlett<sup>1\*</sup>

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The rapid loss of most large vertebrates from botanically intact forests in tropical Asia, and their failure to re-colonize regenerating forests on abandoned land, risks an impoverishment of the future forest flora as many plant species lose their major dispersal agents. The major dispersal agents of seeds in large (>2 cm diameter) fruits—large birds, large bats, elephants, rhinoceroses, primates, and some carnivores—have different degrees of access to the canopy, different sensory capabilities, different oral and digestive processing, and thus different impacts on seed fates, so their ecological roles are largely complementary and non-substitutable. To a subsistence or recreational hunter, however, they are all meat, and the progressive downsizing of the fauna does not necessarily lead to reduced hunting pressure. Seeds in the largest fruits (>4 cm diameter) are dispersed efficiently only by elephants and probably rhinoceroses, but these megafruits make up < 1% of the regional flora and it is the larger number in the 2–4 cm range that are of greater concern. This size range includes single- or few-seeded bird fruits, swallowed whole by hornbills, fruit pigeons, and a few other large bird species, similar-sized fruits taken largely by flying foxes, primate fruits with a peelable protective layer, and other fruits taken by a wider variety of animals. If we do not reverse defaunation, or compensate by enrichment planting, future forests will be dominated by wind-dispersed and small-fruited (<2 cm) taxa, with lower diversity and carbon, and a lower capacity to support large vertebrates if they are later reintroduced.

## Seed dispersal of small seeded-plants, *Ficus* on Borneo

Miyabi Nakabayashi<sup>1\*</sup>, Abdul Hamid Ahmad<sup>2</sup> and Eyen Khoo<sup>3</sup>

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*Ficus* is famous for its obligate pollination mutualism with fig wasps. To maintain population of pollination wasps, each *Ficus* individual produces ripe fig fruits asynchronously and aseasonally, and therefore, each *Ficus* population exhibits continual fruiting throughout the year. Because of their year-round fruiting patterns, *Ficus* are considered keystone food resources for animals in tropical rainforests, especially when the availability of preferred fruits is low. There are some animals depend their diet largely on fig fruits. Binturongs and helmeted hornbills are examples of these animals.

About 80% of binturongs' diet consists of fig fruits, and over 70% of the *Ficus* species are hemi-epiphytic figs on Borneo. Available data on helmeted hornbills' diet on Borneo indicates they are specialists of fig fruits. In this talk, we introduce how these medium-sized animals contribute to seed dispersal of figs, especially hemi-epiphytes by comparing the contribution of Müller's gibbons. Our study indicates that binturongs are the most effective seed dispersers among the three animal species in terms of both quantity and quality at *Ficus* individual level, and helmeted hornbills are also important seed dispersers at *Ficus* population level. Unfortunately, these two important seed dispersers for hemi-epiphyti figs, especially bearing large-sized figs, are listed as endangered animals in the IUCN Red List. Considering that fig fruits serve as keystone food resources for various Bornean animals, loss of these important disperser would negatively affect other animals and in consequence, ecosystem. More efforts are urgently needed to implement practical conservation policies for these important seed dispersers.

At last, we will show a tentative result of the comparison of densities of *Ficus* species in healthy and empty forests on Borneo. Because of the covid-19 pandemic, we stopped this study now but the result shows that number of *Ficus* species and fig size in an empty forest are smaller than those in a healthy forest. This indicates that even small-seeded plants may also affected by loss of large seed dispersers, and collapse of seed dispersal system might have happened. We need prompt actions to prevent whole ecosystem collapse.

## Do riparian reserves support native terrestrial small mammal fauna in oil palm-dominated landscapes?

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Defaunation, generally defined as the loss of wildlife due to human causes such as deforestation for agricultural purposes, has affected much of Southeast Asia. Retaining riparian reserves, i.e. narrow strips of natural forests flanking rivers, within disturbed or converted habitat, is considered a useful way to mitigate wildlife loss in farmland. However, little is documented for non-volant small mammals in the tropics. We used standardised grid-based sampling to compare small mammal species richness, abundance and community composition in 14 riparian reserves in old growth forest, logged forest and oil palm plantations, with that found alongside oil palm rivers with no riparian vegetation. We also investigated whether the small mammal communities were associated with riparian habitat covariates to gain an insight into possible optimum characteristics of riparian reserves for the small mammal conservation. We found that on average riparian reserves in logged forest and oil palm plantations had significantly more specialist species but fewer generalist species than areas in oil palm without reserves. All sites had comparable capture rates, but the abundance of specialist species was higher in logged forest riparian and riparian in oil palm plantation compared to oil palm without riparian. The opposite trend was observed for generalist species. Two invasive rats, *Rattus rattus* and *R. exulans*, dominated oil palm plantation sites, but were near absent from riparian reserves, indicating that reserves do not harbor pest rats. Higher overall small mammal species richness was generally positively associated with closed canopy forest. While higher small mammal abundance was generally associated with taller canopies and slightly lower vegetation biomass. The abundance of generalist and invasive species was negatively associated with canopy closure and understory vegetation cover. The proportion of forest alongside rivers did not explain the variations in the overall species richness and abundance, but it was significantly positively associated with variations in the richness of forest species. In conclusion, retaining forest-like remnants along rivers in oil palm plantations is a useful way to enhance small mammal diversity, including forest specialists, and does not appear to promote pest rats in the plantation.

## The Planet and Humans at a crossroad and SDGs

Ambassador Masahiko Horie<sup>1,2\*</sup>

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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.

## **Shifting our gaze – towards a just, inclusive approach to research in the field**

Bidyut Sarania<sup>\*</sup>, Krishnapriya Tamma<sup>1</sup>, Samira Agnihotri<sup>1</sup>, Subhashini Krishnan<sup>1</sup> and Sutirtha Lahiri<sup>1</sup>

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Over the last few years, there has been a growing awareness of the need for decolonizing ecological research and conservation programmes. Dominant ideas in conservation and ecology include those that view nature and humans as separate entities, which value the exploration of “pristine” wild areas and the discovery of “new species”, and the need to create awareness of conservation issues among indigenous and local communities. However, these ideas contain implicit value judgments that many of us will have encountered, expressed with phrases like “my knowledge is better than yours”. Researchers are also usually completely ignorant of the history of a landscape and its people. Since ecological research is frequently conducted with help from individuals belonging to ‘native communities’, it is important that we acknowledge how our ignorance can influence our own field research and practice. It is often impossible to conduct field research without the guidance of these individuals, whom we commonly known as field assistants. We strongly feel that there needs to be a paradigm shift – from this framework, and away from extractive practices, towards a culture of practice that is just, inclusive and collaborative.

## Some novel SNPs may affect dogs' personality in two breeds

Chika Zemmoto<sup>1\*</sup>, Minori Arahori<sup>1,2</sup>, Yuki Matsumoto<sup>2</sup> and Miho Inoue-Murayama<sup>1</sup>

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Behavioral characteristic is important for dog keepers. These characteristics are affected by both genetic and environmental factors. Genetic polymorphisms associated with personality traits have been reported in several animal species, including dogs. In this study, we aimed to investigate genetic differences which are related to personality in dogs (*Canis lupus familiaris*) using SNPs (Single Nucleotide Polymorphism). We targeted two popular breeds, Miniature Dachshunds and Toy Poodles. To assess personality, we conducted questionnaires on 154 Miniature Dachshunds and 222 Toy Poodles enrolled in Anicom insurance, Inc. The questionnaires consisted of 39 items and were collected along with DNA samples. We performed exploratory factor analyses on the questionnaire data from each breed and found 6 personality factors (human-directed sociability, aggressiveness, dog-directed sociability, lively, nervousness, carelessness) in Toy Poodles and 8 personality factors (human-directed sociability, dominance, tolerance, lively, nervousness, aggressiveness, dog-directed sociability, carelessness) in Miniature Dachshunds. Then, we performed genome-wide association studies (GWAS) on all personality factors in each breed and found novel SNPs associated with these factors ( $p < 10^{-5}$ ). These results provide information about molecular underpinnings of traits which can threaten the safety of captive individuals and their keepers, such as aggressiveness, dominance and sociability.

## **“Corvid Tracking Studio” The motion capture system as a novel tool to study the gaze of corvids**

Akihiro Itahara<sup>1\*</sup> and Fumihiko Kano<sup>2,3</sup>

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Gaze behavior has been frequently used to examine cognition of nonhuman animals in comparative psychology. However, those studies exclusively tested primates but not bird species, although many bird species are known for equivalent cognitive abilities. This is due to the lack of technology and the difficulty in tracking the direction of attention in freely-moving birds. This study thus developed a novel tool set, which we called “Corvid Tracking Studio (CTS)”, to track head movement of crows using infrared motion-capture system. We also developed a custom-made application that reconstructs 3-dimensional positions of each bird’s eyes and beak tip from a few still images taken by a standard camera. Experiments tested accuracies of the head-centered coordinate system reconstructed from the eyes and beak positions of birds and confirmed that estimated errors in its axes were all well within a degree. We thus show that a motion-tracking system has a good potential to examine the attention of freely-moving birds with several customizations. In future, we aim to first quantify their use of visual field when attending to a visual target, and then examine how they use their gaze in socio-cognitive experiments by analyzing key gaze behaviors such as eye contact/avoidance, gaze-following, and anticipatory-looking.

## Acute pain in Japanese macaques: Development of assessment tools

Vanessa N. Gris<sup>1\*</sup>

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Changes in facial expression work as non-verbal signals of pain and provide cues for assessing emotional states in mammals. While biomedical research has a continuing demand for primate models, it is necessary to ensure that laboratory animals are efficiently evaluated and provided pain relief. However, a validated scale for assessing pain in macaques has yet to be developed, further aggravating the difficulties inherent to the discrimination of the presence and intensity of pain in the species. In the past decade, we observed a growing interest in the development of tools to detect facial expressions of pain. Geometric morphometrics (GMM) stands among these new methods and has been successfully applied to the analysis of facial features in humans and domesticated species. This talk will cover several of my projects that have aimed to identify signals of pain in captive Japanese macaques. In the first study, we applied GMM in order to analyze facial shape in female Japanese macaques who experienced surgery. The facial images were collected from video footage of the macaques in two separate conditions: at least one day before and 1 day after surgery. Forty-four facial landmarks were selected based on the underlying facial musculature and their corresponding facial action units and then annotated on each image. Shape information was extracted from the landmark coordinates with a generalized Procrustes fit and summarized with principal component analysis. Results showed that pain seems to be associated with tightening of the muzzle, as has been observed in other species, and apparent raising of the hairline, likely due to piloerection. We hope to exploit our innate observational bias to focus on the facial region to improve the ability to recognize pain in non-human animals. Next, I will report on the use of Artificial Neural Networks to automate the detection of pain in Japanese macaques based on their facial expression using the same sample set from the previous study. Finally, it is worth mentioning that behavioral changes are an important component of pain evaluation. I will briefly report on an ongoing analysis regarding behaviours associated with stress and pain. The results from these experiments may provide us with a better understanding of the behavioral and facial signals of pain in captive macaques, which will ultimately lead us to efficiently performing health evaluations and improving their overall care.



## **Reducing food particle size may not be important for eating fruits: examining determinants of fecal particle size in Japanese macaques**

Tianmeng He<sup>1\*</sup>, Wanyi Lee<sup>1</sup> and Goro Hanya<sup>1</sup>

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Fecal particle size has been widely used in studies on folivores to understand their feeding and digestion. In folivores, diet composition, dietary toughness, and age-related factors such as tooth wear are important proximate determinants of fecal particle size. However, primate fecal particle size studies find different proximate determinants in this taxon, especially in frugivorous and omnivorous species. Thus, it requires further study on interpreting fecal particle size results in these taxa. This study aims to find out the influence of age-sex class and diet on fecal particle size in omnivorous Japanese macaques in Yakushima. We documented the diet composition, dietary toughness, and fecal particle size of Japanese macaques in the lowland area of Yakushima. We also conducted *in vitro* digestibility assay on food samples with different particle sizes to estimate the influence of particle size on digestion. Fecal particle size showed limited variation among different months and no difference among age-sex classes. Dietary toughness did not influence fecal particle size. The consumption of fruits showed marginally significant negative effects on fecal particle size. Particle size had a less obvious influence on the *in vitro* digestibility of fruits than it of leaves and seeds. These results indicate that diet and age-sex-related factors are not important determinants for fecal particle size in our study subject. Fecal particle size and digestion efficiency may not be negatively correlated in frugivores and omnivores primate as studies in folivores suggested. Factors other than what we examined in this study, such as the physical structure of food and chewing behavior, should not be neglected when interpreting fecal particle size in primate studies.

## Bugs in animal social networks: from the field to simulations

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Group living has its advantages, but it also comes at various costs. Our times of pandemic highlight that infectious disease transmission is one of them; think how lockdowns were decided to limit transmission and protect public health. Animals form groups of varied size, composition, or structure in the wild. Studying animal social systems can provide insights in Ecology, Evolution, Cognitive studies, infectious disease-related fields like Epidemiology or Disease Ecology, and further inform us on the pros and cons of group living.

However, a group is *more* than the sum of its parts that compose it – the individuals. Even studying all the pairs of individuals within a group might not tell the full story: a group is a *complex system*. This becomes obvious when a social group is modelled as a social network: in such mathematical object, individuals are represented as the nodes of the network, connected via their relationships or associations, represented as the network edges. Social networks inform us on more aspects of the group: how central or peripheral individuals are within it, how sub-divided or how connected the group is, etc.

Animal Social Network Analysis proved to be a powerful and flexible tool to study social systems. To showcase this, I demonstrate how animal social networks and individual network centrality correlates to infection, in the case of the Japanese macaques of Kōshima and their gastro-intestinal helminths. However, many aspects of Social Network Analysis (SNA) still deserve more study and the refinement of dedicated tools to help improve analyzing social systems. One such aspect is network uncertainty, or put differently: how confident can one be that what has been measured in a network is variable or not around the measure? I present how Bayesian inference and computer simulations – in the form of the R package *SimuNet* I developed – can help study network uncertainty, and why this is important to study animal social systems.

## Fitness consequences of crop raiding by elephants

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Crop raiding by free-ranging elephants is increasingly common in Asia and Africa. The causes of elephant-human conflicts are related to a complex suite of factors, including proximate factors such as habitat loss, degradation and fragmentation, adverse climatic events such as droughts, elephant population increase at local scales, increasing greening afforestation and agriculture outside forests, or ultimate factors related to optimal foraging choices by elephants, behavioural ecology, and cultural transmission of behaviour.

Elephants take considerable risks in venturing out of their natural habitats to raid agricultural crops as they may be injured in several ways or even die in the process. We hypothesize therefore that elephants derive considerable benefits from a crop raiding strategy and that the benefits obtained potentially outweigh the costs incurred during their crop raiding forays outside their relatively safe natural habitats. While benefit-cost ratios and their ultimate fitness consequences are difficult to measure without long-term studies of populations, we can measure some components (such as body condition, stress levels, musth in male elephants, and reproductive rates) of fitness benefits in elephants.

The most obvious benefits that elephants derive from crop raiding is superior nutrition from feeding on cultivated plants: several studies have clearly shown that elephants obtain higher quantity of forage per unit time spent in crop fields and nutrients such as protein and certain minerals. Male elephants translate the superior nutrition derived from crops into faster growth in body size and expression of musth duration and intensity, potentially enhancing their reproductive success, as seen in studies both in Africa and in Asia. Bulls are form larger groups with complex hierarchies for crop raiding and learning. Female elephants in family groups which raid crops in human-dominated landscapes also maintain better body condition than those which are confined to protected forests. Further, the former also exhibit lower levels of physiological stress as seen from glucocorticoids measured in faeces, perhaps because of superior nutrition (especially dietary protein) obtained from cultivated plants.

Anecdotal evidence also suggests that elephants ranging predominantly in agricultural landscapes have higher reproductive rates. Although, the overall benefit-cost ratios in terms of fitness have not been quantified as yet, the rapid range expansion of elephant populations in many regions strongly suggests that the fitness consequences of a crop raiding strategy may be beneficial to elephants under the present regime of wildlife conservation in many countries. This has implications for the mitigation of elephant-human conflicts and the management of elephant populations.

## Genetic basis of the speciation in Sulawesi macaques, Indonesia

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When populations face with different environments, they underwent different directional selection pressures on the traits. This process often leads to reproductive isolation and ecological speciation. Although numerous cases indicate the importance of ecological speciation in nature, very little is known about the genetics of the process. Sulawesi macaques, seven endemic species in Sulawesi Island, Indonesia are optimal for studying the mechanism of genes driving speciation. They have rapidly speciated from the common ancestor in a relative short period. They widely distributed from southern to northern Sulawesi and are morphologically diversified. Exceptionally, they exhibited distinct dark coat color but with difference in color pattern between the species. To clarify the genetic basis of speciation in Sulawesi macaques, we sequenced the exome (all exons) of five species and screen out the high diverged SNPs and genes. In total, we collected 10 saliva samples of each species in Sulawesi Island. After determined the exome sequence using human exome library, we computed the sites  $F_{st}$  value of each two neighboring species and filter the top 5%  $F_{st}$  SNPs as high diverged sites. We found that about 310 genes were highly diverged in all the five species. These genes might be responsible to the speciation and evolve in directional selection on the traits. Further we investigated the pigmentation-related genes and confirmed the functional divergence of the genes in vitro. We found about 20 pigmentation-related genes were involved in speciation. We determined the cAMP induced ability of the melanocortin-1 receptor (MC1R), which regulates the synthesis and density of melanin production. The MC1R exhibited fixed amino acid sequence in each species and showed specific functional characteristics in each species. These results demonstrate the genetic basis of the speciation in Sulawesi macaques and the possibility of coat color divergence mechanism.

## A zoo-born white wallaby: disruption of the melanin synthesis pathway due to an insertion mutation

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A baby girl with white fur and red eyes was born to a mother wallaby of the regular body color at a zoo in Japan. My analysis of the daughter's genome revealed that her albino phenotype was caused by an insertion sequence in the *tyrosinase* gene, which is essential for melanin synthesis. Furthermore, the insertion sequence was found to be an endogenous retrovirus which was named *walb*. Albino animals generally have disadvantages in survival in wild circumstances because of higher sensitivity to UV rays, weakened eyesight, and bright body color easily found by predator. Therefore, mutant genes leading to the albino phenotype tend to be eliminated from the gene pool of the host organism. This tendency suggests that *walb* was transposed into the *tyrosinase* gene recently and still retains transposition activity. The *walb* element is a unique example to respond to the question: why virtually all endogenous retrovirus copies are inactivated in mammals while there are many active copies in insects and plants.

In addition, I found that *walb* occurs in the genomes of multiple Australian marsupials, including wallabies, kangaroos, and koala. This wide distribution suggests that *walb* was introduced into the genome in their common ancestor. Although *walb* has long been residing in the genome, its transposition activity has been maintained in a limited host lineage. In general, endogenous retrovirus and its host genome are in the relationship of evolutionary arms race, that is, competing with each other. The *walb* element may have given some positive effect on the wallaby genome and hence may have been maintained there stably.

## Oxytocin promotes social grooming in bonobos: evidence for a biobehavioural feedback loop?

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Oxytocin has attracted research attention for its role in mammalian social bonding. The biobehavioural feedback loop hypothesis suggests that the oxytocin system has evolved to support the formation and maintenance of social bonds through a positive feedback loop, where oxytocin promotes social behaviours which then release oxytocin themselves. Oxytocin is known to be released following social grooming in chimpanzees and female-female sexual behaviour in bonobos, though it is not known if these social behaviours are involved in such a loop. We therefore tested the effect of exogenous oxytocin on social behaviour in captive female bonobos. Bonobos engaged in social grooming more frequently in the oxytocin condition compared to placebo control, supporting the notion that oxytocin has evolved to form a feedback loop in *Pan* sociality. They displayed sexual behaviour only once during the observation period (oxytocin condition) probably due to low social tension, precluding formal analysis. Additionally, there was a trend for lower rates of abnormal behaviour (regurgitate and reingest) in the oxytocin condition, possibly related to its known anxiolytic effects. These results demonstrate that exogenous oxytocin affects naturalistic great ape social behaviour and provide support for the biobehavioural feedback loop hypothesis of oxytocin in *Pan* social evolution.

## **Foraging together: food characteristics associated with large party size in bonobos**

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Food availability is considered one of the primary constraints on group size in animal societies. Paradoxically, the societies of our hominin ancestors grew larger and more complex in increasingly food-scarce, seasonal environments. To identify social strategies or environmental factors that enable social cohesiveness when food is scarce, we can turn to the closest living models of our ancestors, bonobos and chimpanzees. This research focuses on the cohesiveness of bonobo parties when foraging in different habitats and seasons. Specifically, it considers whether the large party size of foraging bonobos is better explained by overall high food availability or by characteristics of favorite bonobo foods. Data on bonobo party size and composition and ripe fruit availability previously collected at two field sites: ‘Luzaka’ and ‘Wamba’ in DR Congo. At both sites food data was collected with monthly transect surveys of ripe fruit. Party size and composition were estimated with camera traps at Luzaka and direct observation at Wamba. Weak but significant positive relationships were found between party size and total fruit abundance at both sites. Preliminary analysis suggests abundance of several favorite foods were also significant predictors of party size. At Luzaka abundance of two favorite foods were stronger predictors than total food abundance. Ecological characteristics of favorite foods such as dispersion/clustering, patch size and ephemerality were calculated as these qualities are associated with social cohesiveness in other primates including humans. Future research will use a much bigger dataset of bonobo feeding and movement patterns at Wamba to look at whether bonobos appear to shape their ranging patterns to the distribution of these favorite foods.

## **Chimpanzees can learn to utilize referential information from video to choose a tool for future use**

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Humans make extensive use of referential information to prepare for future encounter with the corresponding referent. However, we know relatively little about the comparable referential processing in nonhumans. In a series of four test conditions, we examined if chimpanzees would use video information to choose a tool for future use. Specifically, we used two rooms throughout the test. In the first room, we presented a video showing an experimenter preparing either peanuts or honey in another room where chimpanzees would visit later. Chimpanzees were then asked to choose one of two tools, a stick or a straw. The stick was functional only for peanuts and the straw was for honey. We increased social contingent cues stepwise throughout the conditions, by adding interaction between the experimenter and the subjects prior to the video presentation. We found most chimpanzees performed better than expected by chance in the fourth condition where the experimenter had a direct interaction and then an extra interaction through video with the subjects before preparing the food. The results suggest that chimpanzees could learn to utilize referential information in video. To rule out the possibility that chimpanzees simply learned the discriminative cues in the videos, future study need to confirm their understanding of video with novel situations.



## Understanding of others in chimpanzees (*Pan troglodytes*): cognitive and affective underpinnings

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For social animals, cognitive abilities to understand other beings are important to guide their behaviors appropriately for social and ecological demands. I examined chimpanzees' abilities related to understanding of others using eye tracking experiments. First, I examined chimpanzees' reactions to others' injury. I also measured psychophysiological responses (i.e., nasal skin temperature) via infrared thermography. Second, I examined their understanding of biomechanical rules underlying others' movements. I also attempted to use pupillometry as a proxy for arousal, although the data was difficult to interpret due to some confounding effects (i.e., visual properties of stimuli). Those studies involved visual information processing in chimpanzees. Yet, for chimpanzees, auditory information can also be a rich source of information about other beings. Third, I examined cross-modal processing between visual and auditory information. Specifically, I examined chimpanzees' associations between conspecific vocal signals (i.e., alarm and food-associated calls) and images of relevant objects (i.e., snakes and fruits, respectively). Those studies shed light on unexplored aspects of understanding of others in chimpanzees and provide methodological implications for future great ape cognition research.

## How do males associate with other males in their group? Comparative studies of male aggression and association pattern of the genus *Pan*

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Chimpanzees (*Pan troglodytes*) and bonobos (*Pan paniscus*) are known for their large difference in male aggression. While male chimpanzees frequently show severe aggressive behaviors in various contexts, aggression among male bonobos is much less intense. Although prolonged receptivity in infertile periods in female bonobos is regarded to reduce male competition over mating, a previous study suggested that higher-ranking males sire more offspring than lower-ranking ones. It is still unclear how and to which extent it reduces aggression among male bonobos. These two species are also different from each other in terms of their grouping patterns. Although both species have fission-fusion societies as a common basis, the stabilities of their temporary parties are largely different. Chimpanzees form parties which vary in size and members while bonobos form large and stable parties which include most party members. It is possible that those differences in their aggression and grouping patterns are related. In this talk, I will share reports focusing on intragroup aggression and the grouping patterns of males within these two species. In the first study, I conducted focal animal observations of adult male chimpanzees in the M group at Kalinzu Forest Reserve, Republic of Uganda for approximately 6 months in 2018. I found that the number of males in a given party were significantly larger in the presence of females showing maximum sexual swelling (MS) than in the absence of those females. I also found that low-ranking males spent more time alone than other males in the absence of females with MS present in the group. In total, 177 cases of aggressive interactions were observed. Regardless of the absence/presence of females with MS, the frequency of male aggressive behaviors per number of males observed in a party increased with the number of males per respective party. These results suggest that male chimpanzees of different ranks utilize varying behavioral strategies during party attendance, likely with the aim of reducing the probability of being attacked by other party males. Second, from 2019, I conducted field observations on 11 male bonobos of the E1 group at Wamba, Luo Scientific Reserve, Democratic Republic of the Congo. The number of bonobo males in a given party were stable and they rarely ranged alone. I observed 86 cases of aggressive interactions among males and more than half of those interactions were expressed toward two particular individuals. Those two individuals are siblings and often showed provocative behaviors toward two higher-ranking individuals from another family line. These observations suggest that biological kinship plays a large role concerning male aggression among bonobos. Lastly, I will report on ongoing analyses comparing male association patterns and discuss the differences in male association patterns between the two species.

## Citibats: citizen science-based bat monitoring project in Korea

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Citizen science – public engagement in science – is a fast-growing field in science for the past few decades. Citizen science is also an efficient way to facilitate public education and to change public attitudes towards the target species. In 2020, there were increasing concerns on bats conservation due to the COVID-19 outbreak. For example, bats had become even a persecution target in some countries. To save bats from such devastating conditions and debunk misinformation about bats, it was urgent for me to establish a public education program in Korea. With Kids Science Donga and Korea Safety Health Environment Foundation, I was able to launch a citizen science program ‘Citibats’. The project aimed for monitoring bats in Korea and deepening public understanding of bats. Despite the pandemic situation, over 50 citizen scientists participated in the project. Children were particularly motivated and engaged well in the project. In 2020, we were able to conduct 8 waterway surveys and found 32 bat biodiversity hotspots in Korea. We also identified 14 to 16 bat species across the country. As there are 21 to 24 bat species in South Korea, we were able to find about 70% of bat fauna in Korea. The project is being continued this year as a part of the Earth Loving Explorers project – the biggest citizen science platform in Korea in which over 3000 people participated every year. Despite the even worse COVID-19 situation in 2021, more and more citizen scientists are reporting bats and the data quality is getting better. This experience made me realized that a citizen science project will facilitate public awareness of bats and contribute to bat conservation. A citizen science-based program, therefore, will be a good option for researchers who are looking for an efficient way to facilitate public awareness and conservation of the study species.

## Diversity of MHC genes of endangered Japanese raptors: a preliminary comparison between Golden eagles and Mountain hawk-eagles

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Genes of the major histocompatibility complex (MHC) play a crucial role in the immune system of vertebrates. In theory, the higher the diversity of MHC genes an individual has, the more pathogens it can defend against. Understanding MHC diversity in endangered species is especially important because such information can be used to predict how resilient populations are to pathogens and whether conservation strategies such as genetic rescue are necessary. Here, we performed a preliminary comparison of the diversities of MHC *DRB* exon 2 genes of two species of endangered raptors, the Golden eagle (*Aquila chrysaetos japonica*) and the Mountain hawk-eagle (*Nisaetus nipalensis orientalis*). Both species are important apex predators of forest/mountain ecosystems in Japan, and are listed as endangered IB by the Japanese Ministry of the Environment – the population sizes of the Golden eagle and Mountain hawk-eagle are ca. 500 and 2000, respectively. Next generation amplicon sequencing technology (Illumina MiSeq) and bioinformatic techniques (online server tool AmpliSAT) were used to genotype MHC *DRB* exon 2 sequences. We found that the number of alleles (*Na*), mean and maximum number of alleles per individuals were higher in wild Golden eagles ( $n = 21$ ,  $Na = 12$ , mean = 6.14, max = 8) than in wild Mountain hawk-eagles ( $n = 11$ ,  $Na = 8$ , mean = 4.91, max = 6). This difference could be due to the difference in sample size, but may also be influenced by (1) sampling locations, (2) ecological characteristics, and (3) evolutionary histories: (1) In this study, Golden eagles were sampled from both northern and western Japan, whereas Mountain hawk-eagles were mostly sampled from western Japan. (2) It is known that juvenile Japanese Golden eagles can disperse long distances from their nests (longest record is 300 km) resulting in gene flow throughout the country, while Mountain hawk-eagles do not disperse as far and have been observed to remain in their natal territories for several years. (3) Golden eagles have a circumglobal, distribution, so they may have had more opportunities to evolve immunity towards various pathogens compared to the Mountain hawk-eagle, which is found only in Asia. Moreover, it is known that Japanese Golden eagles have experienced gene flow from two continents (North America and continental Asia) during the last glacial period, which may have contributed to a diverse pool of alleles in the population. We plan to deepen the analysis to better understand these differences as well as their functional consequences and hope to apply the results to conservation.

## How horses evaluate third-party interactions between humans

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Humans evaluate others by observing their interactions with third parties that are not directly related to the interests of the observer. However, such third-party evaluation in non-human animals has not been well-studied. Here, we asked whether horses socially evaluate humans based on third-party interactions. We used the same helping paradigm as that in monkeys (Anderson, Kuroshima, Takimoto, & Fujita, 2013): horses watched one of two actors (attempter) try to open a transparent container to get an object inside, and then the attempter asked the other actor (helper/non-helper) for help. In the Helper condition, the actor (helper) responded to the request and helped, whereas in the Non-helper condition the actor (non-helper) refused to the request by turning away. After the interaction, the two actors offered food to the horse, and the horse was allowed to choose which actor to take food from. Horses showed no preference or avoidance in either condition, but older horses tended to avoid the non-helper in the Non-helper condition. Therefore, in contrast to monkeys and dogs, horses may not have a robust third-party evaluation ability. However, the results also suggest that horses' social experience with humans may improve this ability in horses.

## Comparison of facial morphology in African wildcats, feral mongrels, and owned domestic cats

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For domestic animals, facial morphology is important in their interactions with humans. Previous studies have shown that infantile faces cause caretaking behavior in adults. In this study we focused on the facial morphology of cats to clarify changes in facial morphology due to their relationships with humans. First we compared the facial morphology of four categories of cats: African wildcats (*Felis lybica*), feral mongrels, owned domestic mongrels and owned domestic purebred cats (*Felis catus*). The results showed that the nose length of domestic mongrels and domestic purebred was shorter than that of African wildcats and feral mongrels. The eye angle of owned domestic purebred cats was significantly looser than that of the other categories. Two types of changes in facial morphology can be considered: transgenerational changes (domestication syndrome) and changes at the level of individual development. We therefore conducted a questionnaire with owners of domestic mongrels and domestic purebred cats to investigate developmental changes. The results showed that there was no relationship between the number of ownership years, sociability, aggression and facial morphology. These results suggest that there are changes in cat facial morphology due to human influence and that these changes may not be "developmental changes" but "genetic changes" (domestication or de-domestication).

## Estimation of food habits of Japanese macaques based on tooth microwear: detection of variations among regional populations

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Animal teeth play an important role in food comminution through mastication. Contact with food particles results in scars on their enamel surfaces. The shape and depth such so called microwear features are strongly correlated to the physical properties of the ingested food, so it is possible to estimate the diet from the microwear. Among the microwear analyses, dental microwear texture analysis (DMTA) is the most popular at present. DMTA utilizes a 3D enamel surface data by a confocal microscope and quantifies using standardized surface roughness parameters. This method allows objective, repeatable 3D measurements without observer-bias. Since this method is relatively new, it is necessary to verify whether each parameter is suitable to capture differences in dietary habits. For this purpose, it is useful to compare microwear texture of different local populations within the same species, where tooth shape and mastication process are identical. Japanese macaques (*Macaca fuscata*) are fit for this verification because they vary in diet among regional populations. For example, populations in high latitude region feed a lot of leaves while populations in low latitude region consume many fruits. In this study, we aimed to detect variations of food habits among regional populations of Japanese macaques and test how well DMTA is suitable for estimating the food habits.

We selected about 20 skeletal specimens for each sample of wild Japanese macaques collected in 6 regional populations (Shimokita, Kinkazan, Hakusan, Boso, Koshima, and Yakushima). The 3D coordinate data of the occlusal tooth surface were obtained using a confocal laser microscope (VK-9700). The data were analyzed using surface texture analysis software (MountainsMap 9) and two kinds of surface roughness parameters (ISO25178 and SSFA) were used for comparison between regional populations.

In ISO parameters, we found that these regional populations divided into two groups: the Shimokita–Hakusan–Boso population (Group A), with relatively low "height" and "volume" parameters, and the Kinkazan–Yakushima–Koshima populations (Group B), characterized by the opposite tendency of Group A. In Group A, Shimokita and Boso populations consume leaves at high rate while Group B consumes them at lower rate (Tsuji, 2015). Though dietary data in Hakusan haven't be compiled, the diet in Hakusan likely resembles to that of Shimokita because both of them are belong to the heavy snow area in Japan. Proportion of leaves in diet is likely significantly correlated with the microwear texture. Thus, ISO parameters seem to detect variations of food habits among regional populations of Japanese macaques, at least in terms of differences in leaf consumption rate. In terms of SSFA, each parameter was correlated with the rate of fruit consumption (Calandra et al., 2012). However, the results in this study didn't clearly show such tendency. Whether the SSFA detect dietary variation in Japanese macaques should be verified more carefully.

## Individual difference in the sensitivity to bitter compounds in coffee based on TAS2R gene polymorphism

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The bitter receptor family is consisted by 26 TAS2R genes in human, and each of them can recognize from a few to hundreds of bitter compounds. There are many Single Nucleotide Polymorphism (SNP) in TAS2Rs compared to other taste receptors. It can be one of the factors of inter-individual difference of people's sensitivity to bitterness or their threshold of recognizing it. In this study, we focus on the bitterness of coffee, which is one of the most consumed beverages worldwide despite its bitterness. We targeted TAS2R43 and TAS2R46, which recognize some bitter compounds contained in coffee like caffeine. First, we examined the difference of the responsiveness to the bitter compounds in coffee among the haplotypes by using the proteins expressed in cultured cell. As the results, there were differences in the responsiveness among their haplotypes in both of TAS2R43 and -46. Second, we performed the tasting test to know people's threshold of recognizing the bitter compound included in coffee. Comparison of the threshold data and genotypes of TAS2R43 and -46 would provide a clue for the inter-individual difference of the bitter taste sense to coffee. In this presentation, I want to discuss about the effect to the difference of food preference and culture, because the ratio of TAS2R43 and -46 haplotypes is different among the world wide populations.



## Effect of prestigious behavior in social status of chimpanzees (*Pan troglodytes*) and bonobos (*Pan paniscus*)

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In human society, dominance and prestige are both considered viable strategies for attaining social rank. By sharing novel knowledge with others, humans achieve social rank through prestige. In contrast, many previous studies have considered only dominance as a means of achieving social status in nonhuman animals, assuming that forceful and powerful individuals tend to become high-ranked and thus monopolize vital resources. However, recent studies on a few primate and bird species have shown similar results to human prestige, discovering an increase in social centrality of performers after sharing their novel skills with group members. These results raise the question of whether prestige exists in and could affect the social status of nonhuman animals. To acquire prestige, humans display desirable abilities to followers that contradict dominant characteristics, indicating that each is decoupled from the other. Yet, most studies on nonhuman animals have focused only on dominance, and as such there is still a lack of investigations on nonhuman animal prestige. Here we propose a plan to study the role of prestigious behavior in attaining social status in chimpanzees and bonobos in Kumamoto Sanctuary. We will seed novel knowledge and skill by training a few individuals to operate a novel apparatus, and then allow other group members to observe and learn the skill from them. Then, we will compare the change in the individuals' dominance ranks and social centrality in grooming and proximity networks before and after the introduction of the skill. Based on the results from previous studies, we predict to see an increase in centrality, but no change in dominance rank. From this study, we hope to reveal how prestige has emerged and diverged from dominance in humans.

Keywords: Social status, prestige, dominance, centrality in social network, *Pan troglodytes*, *Pan paniscus*

## Preliminary analysis on the epigenetic age estimation of captive Asian elephants (*Elephas maximus*)

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Asian elephants (*Elephas maximus*) are classified as endangered by the International Union for Conservation of Nature (IUCN) due to habitat destruction and poaching in the wild. This has led to a continuous decline in population size which has caused the number of individuals to decrease to less than 50,000. Knowing an animal's age provides essential ecological and conservational information such as population demographics in the wild and appropriate care in captivity. However, current methods are not always accurate and difficult to observe in the wild for many species. This is also applicable for Asian elephants where age estimation is usually done through observing their height, size of tusks and foot dimensions, mostly from culled individuals and not living. Thus, a better method to estimate their age such as from faecal DNA is needed.

It is suggested that DNA methylation-derived epigenetic clocks are the most promising method to estimate age. Epigenetic clocks based on DNA methylation have been shown to accurately estimate age on humans, mice, primates, cetaceans, dogs, bats, etc.

This study aims to find age-related markers, related to epigenetic changes in Asian elephants that will allow us to estimate age accurately, ultimately to apply for wild individuals. In this preliminary study, we have extracted DNA from blood samples of known-aged captive individuals provided by the collaboration of zoos. So far, we extracted DNA from a total of 35 blood samples of 11 individuals (3 males and 8 females). Age-range was 0.5 – 65 years old. Then we referred to candidate marker genes using information on age-associated DNA methylation in humans and mouse genes, to design age-associated primers for Asian elephants. To do this, we relied on different programs such as BLAST and have re-designed the genes, ELOVL2 and RALYL. Through finding the right RT-PCR conditions, amplification success was seen in both genes suggesting that these genes could be potential age-related primers for Asian elephants. We are now looking to design more potential age-related primers and increase the number of samples (blood and faecal samples). DNA methylation profiles will then be made to see the correlation between the CpG methylation frequency of specific genes and chronological age using methylation-sensitive high-resolution melting (MS-HRM).

Successfully identified epigenetic clocks could be used for future uses in conservation efforts where accurate estimates of age are needed to predict demographic trends.

We thank Oji Zoo and Kyoto City Zoo for providing samples.

## **Dipping or sucking? Choice of tube-using techniques in chimpanzees and bonobos before and after video demonstration**

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The presence of culture in great apes has been confirmed and studied for several decades, and researches have shown that it is the social learning processes that underpin much of the primitive culture in these apes. Compared with the sister species, chimpanzees (*Pan troglodytes*), culture phenomena in bonobos (*Pan paniscus*) has been relatively less investigated, which is partly due to their scarce tool-use behaviors in feeding contexts. To achieve a comprehensive understanding of the evolution of human culture, it is essential to promote tool-use researches in bonobos and conduct comparative studies on the two closely related yet unique Pan species. In the present study, we plan to compare the spontaneous choice of tube-using techniques in chimpanzees and bonobos before and after the social learning process, and investigate their visual attention during the learning process. The apes will be offered a translucent tube, which can be used as either a stick to dip the juice or a straw to more efficiently suck the juice. The initial method of their tube-using techniques will first be recorded. Then, the apes will be provided a video demonstration of the sucking method, and their visual attention will be analyzed during this learning process. Later, the apes will be given a second chance to use the tube, and their tube-using techniques will be compared with the initial method. We already found some individual variations of tool use techniques. The impending studies and future results may further demonstrate how the apes allocate their visual attention to different video demonstrations and whether they will show some shift or persistency in the tube-using techniques.

Keywords: Bonobo, Chimpanzee, Eye tracking, Social learning, Tool use

## **Relationship between stress hormone concentration and behavioral changes in captive Tsushima leopard cats (*Prionailurus bengalensis euptilurus*)**

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The Tsushima leopard cat is one of only two known wild cat species in Japan, and is an endangered species. Because of its rarity, various efforts are being made to conserve them, and it is the only mammal in Japan for which a technique of reinforcement has been developed. Reinforcement is a conservation activity to release captive animals into existing populations of conspecifics in their natural habitat and then recover their population. However, the success rate reported in other species, especially mammals is totally low, and the technical improvement is required. One of the important keys is stress. Transfer between facilities and excessive contact with humans is likely to be stressful for the animals. Previous research has confirmed that prolonged elevation of glucocorticoids (stress hormones) can have a negative impact on reproductive and immune functions. It has also been reported that high levels of glucocorticoids affect cognitive function in animals, and if this condition persists for a long time, it may damage neural tissues involved in learning and memory. However, not all stress has a negative impact on animals and maintaining a certain degree of stress is also thought to be important for reinforcement. In addition, it is difficult to identify what is causing stress when the concentration of stress hormones is high by simply tracking the fluctuations of stress hormone levels. Therefore, in order to clarify what events are stressors, I have monitored their behavior change in addition to analyzing the stress hormone concentration.

Stress hormones will be monitored continuously by collecting feces from individuals kept at the wild acclimatization station of the Tsushima leopard cat and measuring the cortisol concentration in the feces using the enzyme-immunoassay method. I have also measured stress hormone concentrations of them before and after the transfer to other facility (including zoos) to investigate how stressful the transfer and environmental changes are for the individuals. Behavioral observations have been conducted using video data. Since some facilities don't allow for visual confirmation of the animal's location, I plan to attach GPS loggers to the individuals. In addition, by attaching an activity meter, the exercise status of the individuals will be evaluated. These observations will be continued and compared with changes in stress hormone concentrations in an effort to identify stressors.

Key word: reinforcement, leopard cat, stress hormone, behavioral observation

## Reproductive physiology of Iriomote cat (*Prionailurus bengalensis iriomotensis*)

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The Iriomote cat (*Prionailurus bengalensis iriomotensis*) has been listed as a Critically Endangered on the IUCN Red List, and their population is only about 100 animals. Although their diet, habitat, and behavioral areas have been revealed, the physiological underpinnings of the reproductive cycle have not been undertaken. In order to increase the population, stable breeding is necessary, and not only behavior, but also the understanding of their reproductive physiology is required to promote their breeding.

With the cooperation of Dr. Masako Izawa, who has studied on Iriomote cat for long time, I have a plan in the physiological study. Firstly, I'll confirm whether hormones in feces and urine samples of Iriomote cat which have already been collected at the study site over the past 20 years had been preserved. And then, I will collect new samples at the study site and conduct on-site environmental survey. Using these old and new samples, I'll compare their physiological state between environmental differences and time scales. The hormones to be analyzed are estrogen, the sex hormone, and cortisol, the stress hormone. By analyzing these, I'll clarify seasonal physiological conditions about breeding, land suitable for breeding, differences in stress values by location and season.

Key words: *Prionailurus bengalensis iriomotensis*, endangered species, reproductive physiology, hormone analysis

## Whole-genome resequencing of endangered Malayan tapir for novel discovery of genome-wide single nucleotide polymorphism loci

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The Malayan tapir (*Tapirus indicus* Desmarest) is the only Asian member of the Tapiridae family in the order Perissodactyla. This species is native to Peninsular Malaysia, southern Thailand and Myanmar, and the Sumatran Island 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. Population genetic research that are orientated for conservation and population management goals has been few but accumulating since 2008. With the advancement of sequencing technologies, reduced costs, and international consortiums such as 1000 Genomes Project and DNA Zoo, genomic data becomes more affordable as well as accessible for development of new genome-wide markers (e.g., single nucleotide polymorphism or SNP) and for sophisticated genomic analysis. Genome-wide SNP markers have been increasingly developed and used for genomic analysis in many species, including the threatened species, but not in the Malayan tapir. Here, we used genetic profiles (microsatellite and mitochondrial DNA control region) of a group of Malayan tapirs (n = 31) in Japanese zoos to select three samples from three genetic clusters (putatively unrelated and each from an origin of Malaysia, Thailand and Sumatra) for paired-end whole-genome resequencing on a Novaseq 6000. The raw reads were treated with the following main pipeline using a combination of bioinformatics tools: trimming and filtering based on base/read quality and removing of unpaired reads with *fastp*, mapping of the filtered paired reads to the public Hi-C genome assembly of the Malayan tapir (available from the DNA Zoo) with *bwa-mem2*, and removing of duplicated reads from the alignments with *samtools*; before SNP variant calling with *freebayes*. The called variants were filtered using *bcftools* for high-quality biallelic SNPs based on Phred-scaled quality scores and depth of coverage. Variant sites that were monomorphic or carried missing data were removed from the SNP dataset. The mean total reads before pipeline was 398.65 million (M) reads (min: 345.19 M, max: 479.48 M) and after mapping 348.98 M (min: 306.57 M, max: 412.12 M). The mean depth of coverage in the mapped alignments was  $20.01 \times$  (min:  $17.59 \times$ , max:  $23.64 \times$ ). Initial variant calling for all three samples discovered a total of 5,686,003 SNPs, of which 226,443 high-quality biallelic SNPs were retained for future analysis. The SNP dataset will be useful for preliminary analysis of genetic distance of the three individuals representing three different regions, for performing a Pairwise Sequential Markovian Coalescent analysis for demographic history, and for future design of a panel of SNP markers for population genetic/genomic analysis to inform conservation management. We hope to increase the number of samples for whole-genome resequencing in the future to increase the accuracy of SNP variant calling.

## Plants for carnivores: Metabarcoding analysis of wild snow leopards (*Panthera uncia*) focusing on dietary plant species

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Although felids (colloquially called cats) are the obligate carnivores, they sometimes eat plants both in the wild as well as in captivity. However, little is known about why exclusive carnivores eat plants. A big cat, snow leopard (*Panthera uncia*) inhabits high mountains in Central Asia. Interestingly, their scats contain plant materials more frequently than other *Panthera* species. Identification of plant species leads to figure out the interaction between carnivores and plants in alpine ecosystems and understand the mechanism and adaptation of plant eating in carnivores.

In this study, we aimed to identify plant species in wild snow leopard scats using a DNA metabarcoding method. 37 scat samples of wild snow leopards in Kyrgyz Republic were investigated. After whole scat DNA was extracted, five DNA barcode regions (*12SV5* for prey vertebrates and *ITS2*, *rbcL*, *trnL*, *ITS1* for dietary plants) were amplified by PCR and then massively sequenced by a Illumina MiSeq. The collected site, altitude, and date of each scat and sex of each host were tested as potential factors which affect taxonomic composition of detected plants. The spatial structures were constructed using Moran's eigenvector maps (MEMs).

As a result, the family Asteraceae and the genus *Myricaria* species were dominant plant taxa in snow leopard scats, that is, frequencies of occurrences were 27/37 (73%) and 21/37 (57%), respectively. There was no clear correlation between prey and plant species in the identical scat (Yule's Q <0.9). The effect of collected month on plant taxonomic composition was prominent (a permutation ANOVA, P =0.011) but the constrained proportion was not high (8.1%).

*Myricaria* spp. were generally not preferred by herbivores. They contain anti-inflammatory compounds and are known as a traditional medicine in Tibet. Asteraceae species also contains a variety of plant secondary metabolites and traditionally used for medication by many ethnic people. There were lacks of the correlation between detected vertebrates and plants, indicating that snow leopard voluntary eat plants because detected plants are not derived from gut contents of prey vertebrates, and perhaps, the detected plants may have self-medication roles. Analyzing prey animal scats and/or gut contents as well will clarify whether detected plants from snow leopard scats were derived from prey vertebrates. Vegetation survey of study sites is also important to know their preference to plant species.

## **Ontogenetic differences in mandibular morphology and their adaptive significance in Japanese macaques and long-tailed macaques**

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The mandibular morphology is determined not only by dietary habits, but also by sexual selection and allometry (size-shape relationships) in primates. The interspecific facial shape differences in African papionini (including baboons, geladas, mandrills and drills, and mangabeys), which are phylogenetically closely related to macaques, are well known to be formed by truncation or extension of a common ontogenetic allometric trajectory, a phenomenon called ontogenetic scaling. In this study, we compared the ontogenetic patterns of the mandibular morphology in two species of macaques with different body sizes, Japanese macaques (*Macaca fuscata fuscata*, hereafter Mff) and long-tailed macaques (*Macaca fascicularis*, hereafter Mfa), using geometric morphometrics with landmark coordinates, to show the ontogenetic phases when the interspecific differences occur and discuss about their adaptive significances in terms of life history.

The two species of macaques show a major common ontogenetic allometry, whereas the shape-size regression lines are parallel to each other with no significant differences in the developed shape; that is, no ontogenetic scaling occur. This size-shape relationship probably spared the large-sized Mffs from increased stress at the mandibular symphysis (i.e., mechanical restrictions) and simultaneously allowed them to increase their body size adaptively to climatic fluctuation in the Pleistocene (i.e., Bergmann's effects). Rather, the interspecific differences in mandibular shape are amplified by an extension of a minor allometry which occurs only before weaning in Mffs. Such an allometric effect makes the mandible tall and short in Mffs. These shape differences reflect the mechanical differences in mastication required after molar eruptions, e.g., the beginning of adult dietary habits: Mffs take tough food items, including barks in winter. Both species show the same shape development reflecting sexual dimorphism, i.e., canine enlargement and the modifications of its associated features in males, but the interspecific differences developed in the early phase, i.e., before weaning, are maintained in later stages.

Thus, while the shape differences in adults are made during a whole growth period in African papionins, the differences were developed before the time of weaning in these macaques. Our comparison of the ontogenetic trajectories successfully provided evidence strongly supporting for the view that the interspecific differences in mandibular shape were made for solving the mechanical requirements for dietary habits in a given species of macaques which have survived in the fluctuated climate of Pleistocene Asia.



## Urination behavior of captive chimpanzees: preliminary study of social factors

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Urination is a basic physiological phenomenon in most animals including humans. However, while urine itself is widely used in physiological studies such as measuring hormones, there is a lack of research on urination itself in behavioral science. Even in chimpanzees, one of our evolutionary closest relatives, there has been no detailed study on urination behavior. The purpose of this study is to gather basic behavioral information of urination such as its frequency and the time of day when urination is likely to occur. We also preliminarily investigated social factors on urination behavior. We observed four 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 inter-individual proximity at 2-minute intervals. The results showed that the average interval of urinations was 3595 sec (SD=3078), but the frequency varied greatly among individuals. We did not find any clear trend across individuals about the time of day when urination was most frequent. Our preliminary analysis found no clear effects of social bonding on consecutive urination between individuals. Further investigation is needed for further understanding of urination behavior.

## **Selective infant handling in Yakushima Japanese macaque (*Macaca fuscata yakui*)**

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Female Yakushima Japanese macaques (*Macaca fuscata yakui*) commonly handle other females' infants since those infants are only a week old. Every female watches for a chance to handle others' infants several times a day, but not every infant is their target. It is clearly observed that females (attempt to) handle specific infants rather than other infants staying around them. This selective infant handling would be shaped by individual or social factors: (1) it is a result of seeking for a high success rate of infant handling, (2) it is a form of infant care and (3) it enhances established social bonds of the handlers, (4) it establishes social bonds with high ranking females. We studied patterns of infant handling in a bunch of cases that a handler stays nearby (within 3m) several infants in a large macaque group at the western lowland forest of Yakushima island. Considering individual information (e.g., age, rank) of handlers and infants, relatedness between them and behavioral states or events observed in each case, we try to reveal the meaning of the inequality in infant handling.

## Power-law relationships between a group size and a social interaction number in animal societies

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Power-law (or scaling) relationships between variables  $X$  and  $Y$  is  $Y \sim X^\beta$ . It is well known that such a power law could be found in various natural phenomena, for example, magnitude and frequency of earthquakes, and allometric laws in animal body size. Recently, one study suggested the scaling relationships between a number of social interactions and group size across species (Rocha et al., *Sci. Rep.* 2021:11:12584). The total edge numbers “ $E$ ” in three types of social networks, (cooperation, proximity and human friendships), scaled super-linearly with group size “ $N$ ”, i.e.,  $E \sim N^\beta$ , where exponent  $\beta$  was 2.08, 1.54 and 1.15 respectively. This study argued that this super-linear exponent was derived from the hierarchical structure of social networks. The result is important in that it may reveal common principles in the group structures across animal species and type of social interactions. Since the previous study conducted meta-analysis, inter-specific variations in social structures and environmental differences were not addressed, although they could largely affect the social and spatial networks. To fully verify those effects, it is necessary to see whether animals flexibly change the network density according to the changes in a group size, either experimentally or by long-term observation of a same population. Moreover, the original model assumed that a sub-group size increases according to a power of three (e.g., 5 (1<sup>st</sup> level), 15 (2<sup>nd</sup>), 45 (3<sup>rd</sup>), ...). However, in reality, animal sub-group size may have more variations. In this study, we investigated a natural horse population to verify the power law relationships, and further optimized the hierarchical model using simulation. Horses form multilevel society, where several small groups called “units” gather to form a larger group, “herd”. During 2018 – 2019, a herd size ranged from 15 to 123 individuals. With those variations we found that the spatial proximity network scaled super-linearly with herd size, where the exponent was 1.62, pretty consistent with the previous study. Our simulations also suggested that  $E \sim N^{1.6}$  relationship could be found in various sub-group structures.

## **Infant Japanese macaques have the most infant-like faces at around 3 weeks of age during the first 10 weeks**

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Human infants have characteristic faces compared with adults. Infants have a wider face, a longer forehead, larger eyes, and a smaller nose and mouth than adults. Some studies show that these features get more and more salient for some months after birth. Many researchers may agree that nonhuman primates also have infantile faces. However, less studies investigate whether nonhuman primate infants really have characteristic faces, and which facial features indicate infancy in each species. Moreover, no study has examined how infantile faces develop during infancy in nonhuman primates. This study investigated infantile facial features in Japanese macaque (*Macaca fuscata*) and the developmental process of infantile faces during the first 10 weeks after birth. The subject was a provisioned group of Japanese macaques in Arashiyama Monkey Park Iwatayama, Kyoto, Japan. TM took photos of the frontal face of 70 monkeys more than 5 years old in the group. In addition, TM took facial photos of eight infants which were born in 2021 every week from their 0 to 10 weeks old. All facial photos were analyzed and the relative size of each facial features to the head or face were calculated. Additionally, infants' behaviors were recorded by ad-libitum sampling every week. Comparisons of each facial features between infants and adults revealed that a wider face, a longer forehead, larger eyes, and a smaller nose were facial features characteristic of infants. Unlike humans, the size of the mouth was not an infantile facial feature in Japanese macaques. This implies that facial features that indicate infancy may differ between primate species. Next, these infantile facial features were used to calculate the infantile face score (IFS) in all facial photos of infants. Based on this score, the development of infantile faces during infancy was examined, and it was found that, on average, IFS increased from the birth to 3 weeks of age, and then gradually decreased until 10 weeks of age. Finally, the relationship between IFS and infant's behaviors was examined. As a result, 3 weeks of age, when the IFS got the highest, coincided with the time when all infants could walk on their own and some infants began to explore away from their mother. In humans, infantile faces attract attention and motivate adults to take care of infants. In addition, human infants are rated as the cutest at 9-11 months of age, when their mobility begins to develop. Our findings may suggest that, as with humans, infant Japanese macaques can attract strong attention of other individuals by having most infantile faces at the time when they have still poor mobility and they are considered to be the most at risk. More detailed investigations of the relationship between infantile facial features and behaviors of infants and caregivers should make fruitful suggestions for mechanisms of caretaking behaviors and for surviving strategies of infants in nonhuman primates.

## The effects of genito-genital rubbing on affiliative relationships among female bonobos (*Pan paniscus*)

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Primates, including humans, often engage in homosexual behaviors among both male and female sexes. Although many previous studies have referred to homosexual behaviors in non-human primates to better understand the evolutionary processes of homosexual behaviors, the explicable functions of homosexual behaviors in humans have yet to be fully elucidated. Bonobos (*Pan paniscus*), who are the closest living relatives of humans, frequently engage in genito-genital rubbing (GG rubbing) among female members, categorized as homosexual behaviors. GG rubbing appears to be multifunctional (*e.g.* reconciliation, tension regulation, expression of social status, social bonding, mate attraction), which helps us receive valuable insight for understanding the explicable functions of homosexual behaviors in humans. Considering previous studies in bonobos, the multiple functions of GG rubbing may vary in their magnitude among allopatric bonobo populations. In the current study, I examined the functions of GG rubbing among female bonobos at Wamba, Luo Scientific Reserve, Democratic Republic of the Congo, and particularly focused on the hypothesis of social bonding. I found that the more dyads had close physical proximity, the more they engaged in GG rubbing, but social grooming did not affect the proportion of GG rubbing. Ages were close in the dyads, which suggested that their dominance ranks also were an important factor on the proportion of GG rubbing. My results suggest that the dyads whose ages and dominance ranks were close kept close physical proximity and engaged in GG rubbing frequently to maintain their social relationships. Comparing the functions of homosexual behaviors between primates, including humans, and within the same species in different populations is helpful to shed light on hypotheses concerning evolutionary explicable functions of homosexual behaviors.

## **The influence of social bonds of mothers on the offspring's sociality in feral horse groups**

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Female horses are known to form and maintain social bonds with other females in a polygynous group ("harem"). The mothers living in this social environment affect their offspring's various social traits, as a social role model as well as their first social partner. It has been reported that foals tend to show affiliative behaviors toward the human who contacted friendly with the mother horses, and that foals preferentially associate with another foal whose mother is the most preferred partner of their mothers. The maternal effect on the way foals build social relationships, however, was little studied in the feral horse group living in natural social settings. I planned to examine whether and how the mother's social style, such as the number of social partners and the strength of the social bonds, influences the offspring's sociality and the dynamics of social relationships. In free-roaming Misaki horse harems with foals and yearlings, the affiliative/agonistic social behaviors and proximity between horses have been recorded using a video camera and a drone from summer through early autumn. As time went by, foals tended to be less close to their mothers and interact with other group members, including their siblings or other foals if they exist, more often than before. Given that most Misaki horses maintain the harem groups until early winter, more observations would be needed to examine the development of social relationships of the horse offspring and the maternal effect on it.

## Hot spring bathing behavior and helminth infection in Japanese macaques at Jigokudani

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The population of Japanese macaques at Jigokudani Snow Monkey Park, Nagano, display hot spring bathing behavior (HSBB) during the cold season. HSBB is known to benefit thermoregulation and reduce stress, but in several human cases it has also been shown to accommodate transmission of heat-resistant waterborne parasites. To our knowledge, there are no studies about the impacts of HSBB on parasitism in nonhuman primates. Thus, we tested for a relationship between HSBB and gastrointestinal helminth parasite infection risk in Japanese macaques. HSBB might increase the risk of infection if hot springs and their surroundings are a source of infection. On the other hand, HSBB might decrease the risk of infection if it reduces the immunocompromising effects of stress. The study was conducted over two winter seasons between 2019 and 2021. A total of 148 fecal samples were collected opportunistically from sixteen adult female Japanese macaques (9 bathers and 7 non-bathers) varying in age, reproductive cycle, and rank. Parasite prevalence (proportion infected) and abundance (egg per gram feces: EPG) were determined using microscopy. We detected three species of geohelminth (all Phylum Nematoda): *Trichuris trichiura*, *Oesophagostomum aculeatum*, and *Strongyloides fuelleborni*. Two species, *O. aculeatum* and *S. fuelleborni*, were excluded from analysis due to their low frequency in the host population. Generalized linear mixed models show that there is a statistical trend of *T. trichiura* being more prevalent in bathers than non-bathers ( $p=0.061$ ), but we found no noticeable difference in its abundance. We also found that the reproductive cycle was associated with *T. trichiura*, where its prevalence was lower in cycling than lactating females. While HSBB may have certain benefits, we should not discount the potential costs, so greater effort is now needed to further test the link between HSBB and other potential negative health outcomes.

Keywords: *Macaca fuscata*, parasite transmission, Monkey Parks, Wildlife Tourism, Animal Behavior

## Comparison of foraging patterns between smokers and non-smokers

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The dual information processing system is a model that indicates how our brain can roughly be divided into two distinct types of information processing systems: one for heuristic processes, system 1, and the analytical system, system 2. The heuristic process, immediate and automatic response system, is associated with affective function, but causes biased judgment known as cognitive bias. This preliminary study aims to understand the mechanism of cognitive biases and its relation to addiction, in particular those with and without smoking habits through foraging patterns.

Healthy control subject (past smokers: n = 5, non-smokers: n = 10) and those with smoking habits (n = 5) were recruited. Recruited participants answered standardized questionnaires to measure stress, worry and depression, and Foraging Tasks were conducted to measure exploration tendencies with regards to risk factors. Those with smoking habit showed increased exploration tendencies, choosing to explore for new and unknown reward and non-smokers showed the increased exploitation tendencies, exhausting the resources before moving onto the next one. This could indicate higher risky behavior in smokers.

Our study suggests that smokers may show trends towards more biased behaviors in the absence of nicotine which could lead to riskier decision making. Understanding individual differences in smoking habit can help us to elucidate the mechanisms of addiction leading to novel strategies for smoking cessation.



## Empathy in elephants: integrative approach of behavioral ecology and cognitive science

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Empathy plays a vital role in the rich social life of humans. The ability to relate to the other and properly respond to social interactions can be crucial in species with extensive parental care, cooperative activity and complex societies. However, there is no consensus on definitions, mechanisms and evolution of empathy. This is largely due to the lack of comparative studies with non-human animals. Therefore, it is particularly relevant to investigate empathy in other social species and assess to what extent related phenomena can occur. Elephants, having sophisticated cognitive abilities and complex social organization, are a pertinent study target to contribute to comparative work in animal empathy. The goal of this project is to investigate the empathy-related phenomena in Asian elephants. By joining methods and theories of behavioral ecology and cognitive research, through behavioral observations, data will be collected on Asian elephants on captive settings and in free-ranging conditions in South India. It is aimed, specifically, to i) investigate the occurrence and the socio-ecological context of empathy-related phenomena (e.g. consolation) and to ii) examine the differential hemispheric processing of emotions (negative vs positive) reflected in distinct lateral tendencies in the behavior, as a tool to assess emotions in non-human animals. Overall, the present project intends to contribute to the understanding of the mechanisms and functions associated with empathy phenomena and, ultimately, to increment the comparative work on animal empathy.

## Copy number variation among multicopy Y-chromosome gene families of rhesus and long-tailed macaques

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The rhesus macaque (*Macaca mulatta*) Y-chromosome is prevalent in Indochinese populations of the parapatric long-tailed macaque (*M. fascicularis*) due to introgression via occasional hybridization. Mammalian Y-chromosomes harbor many male-specific singleton and multicopy gene families directly related to spermatogenesis and aspects of male fertility. Copy number variation (CNV) analyses have shown that greater copy numbers of a gene are typically correlated with increased protein expression. We hypothesize that macaques with the rhesus Y-haplotype, including Indochinese long-tailed populations north of the Khlong Marui Fault, will have greater copy numbers of spermatogenesis-related Y-genes than those with the “native” long-tailed Y-haplotype (i.e. macaques from peninsular Malaysia and the Sunda Shelf islands). Custom CNV target assays were designed for each of the four multicopy macaque Y-gene families – *HSFY*, *TSPY*, *DAZ*, *CDY* – in addition to a custom reference assay designed from the macaque *RnaseP* gene. Target and reference assays were subsequently run on a QuantStudio3 with genomic DNA from rhesus and long-tailed macaques of known provenience. Raw assay data were then transformed in CopyCaller v2.1. Preliminary results of each assay are discussed in the context of rhesus Y-introgression and primate spermatogenesis.

## Japanese and rhesus macaques differ in amygdala cannabinoid 1 receptor-immunoreactive axon density

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Endogenous and exogenous cannabinoids signal through the cannabinoid 1 receptor (CB1R) to modulate various aspects of social behavior, including aggression and anxiety. In rodents and primates, CB1R expression in the basolateral amygdala is dense and cannabinoid signaling in this region has been reported to influence social behavior. Little is known about how endocannabinoid signaling in the amygdala contributes to primate social diversity. The behaviorally diverse and species-rich cercopithecoid monkey genus *Macaca* is an ideal model for addressing this topic. Japanese (*Macaca fuscata*) and rhesus macaques (*M. mulatta*) display similar social styles in some respects; however, there is evidence to suggest they differ in their stress response, amygdala structure, and monoaminergic signaling. To further assess the molecular basis of social style in Japanese and rhesus macaques, we used immunohistochemistry and stereological methods to compare CB1R-immunoreactive (CB1R-ir) axon density in the basolateral amygdala, which is comprised of the lateral, basal, and accessory basal nuclei. Our study sample included 6 Japanese and 5 rhesus macaques. Repeated-measures ANOVAs were used to evaluate species differences, with amygdala region as the within-subjects measure and species as the between-subjects factor. This revealed significant main effects for species and area ( $p$  values  $< 0.05$ ) with no interaction. Post hoc tests revealed higher CB1R-ir axon density in the basal and accessory basal nucleus of rhesus macaques compared to Japanese macaques. Our results suggest that CB1R-mediated signaling in the lateral nucleus of the amygdala is comparable between the two species, while the differences we observed in the basal and accessory basal nuclei may contribute to the nuanced behavioral differences observed between them.

## **Captive male marmosets (Genus *Callithrix*) are prone to dental abnormalities, regardless of hybrid status**

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Hybridization is evolutionarily important because of its potential to transmit genetic information between otherwise distinct evolutionary lineages, and several species of *Callithrix* are known to hybridize in the wild and captivity. In previous work, hybrid primates have exhibited dental abnormalities at significantly higher frequencies than non-hybrids. Therefore, we expected the same for the hybrid marmosets in this large sample (N = 223). Using captive and wild marmosets sampled in northeastern and southeastern Brazil, we examined all teeth for breakage, missingness, dislocation, or other abnormalities. We then used logistic regression to build a generalized linear model describing the main predictors of dental irregularities. Surprisingly, hybrid status was not significantly predictive of dental problems; rather, the best fitting model contained a significant interaction between sex and captivity as the strongest predictor for dental irregularities ( $p = 0.007$ ). In this model, captive male marmosets, regardless of hybrid status, were 3.96 times more likely than free-ranging males to have dental abnormalities (95% CI: 1.26-12.46). Conversely, while free-ranging females were 1.9 times as likely as captive females to exhibit this response, this comparison was non-significant ( $\chi^2 = 1.29$ ,  $p = 0.26$ ). The lack of association between dental irregularities and hybrid status is notable in light of the preexisting work documenting a positive link between these two phenomena in other species.

## The effect of ‘brightness contrast’ in luminance discrimination tasks for 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, it is essential to conduct comparative studies on mammals living in underwater environment, which is far different from terrestrial environment. 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.

Through previous researches on cetaceans, it is suggested that contrast of luminance may play an important role in their visual object recognition of cetaceans with low visual acuity and no color vision. In order to examine how cetaceans perceive contrast, the effect of ‘brightness contrast’ was examined through the luminance discrimination tasks for 2 killer whales in Port of Nagoya Public Aquarium. Research aim was to develop a new research method which enables us to conduct visual tasks for killer whales and to examine whether the effect of “brightness contrast” was observed or not.

Through luminance discrimination tasks, we succeeded to develop a new experimental method controlled by a computer and monitors. The results of experiments showed that the effect of ‘brightness contrast’ was observed in these killer whales. Furthermore, one killer whale showed the same tendency as humans that the larger the inducer area, the stronger the effect of brightness contrast was observed. In conclusion, it was suggested that the effect of ‘brightness contrast’ was observed not only in terrestrial mammals but also in marine mammals. When the effect is occurred, contrast of luminance between object and background is enhanced and helps observers to separate objects visually from background. Therefore, it was suggested that contrast of luminance could be essential for visual object recognition not only of terrestrial mammals but also of underwater mammals.

## Morphological and histoanatomical studies of the larynx in lemurs

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Madagascar lemurs are well known to have a rich vocal repertoire. The phonation of the larynx generates the vocal source and determine the major acoustics of the voice, e.g., the length, loudness, intensity, and chaotic phenomena. In humans, the vibrations of the vocal folds play a central role in phonation. On the other hand, unique morphological variations in the laryngeal structures are expected to facilitate vocal repertoires in lemurs.

Diffusible iodine-based contrast-enhanced computed tomography (diceCT) was used to examine embalmed larynges from 5 genera of lemurs and 3 genera of lorises and galagos for comparison with catarrhines. The specimens were soaked in 5% solution of I<sub>2</sub>KI, to visualize the muscles and soft tissues on the CT scans. The histological structures of an embalmed larynx of a ring-tailed lemur were also examined.

Lemurs had several derived morphological and histoanatomical features. They had a transverse recess formed in the laryngeal vestibule to make another set of upward folds similar to the vocal folds. The thyroarytenoid muscle are also inserted into the submucosa of the upward folds. These features were not observed in lorises and galagos used here that show solely a vocal fold as in catarrhines. The upward folds have histological features comparable to the vocal fold. An areolar connective tissue occupied the lamina propria of the mucosa of the ventricular folds and stratified squamous epithelium covered them.

The contraction of the vocal muscle takes part in moving the bilateral vocal folds toward the glottal midline. This action probably moves the upward folds simultaneously in lemurs. The histoanatomical structures found in the upward folds are distinctive to the vocal folds and they facilitate vibrations of the vocal folds during phonation. Thus, the present findings are consistent with the hypothesis that lemurs also use the vibrations of the “second” vocal folds solely or together with the true vocal folds during phonation. While the acoustic effects of the sole or simultaneous vibrations are not yet examined, they are expected to take part in a variety of their vocalizations.