



# The 10th International Seminar on Biodiversity and Evolution Wildlife Metagenomics



Date:

**June 11<sup>th</sup>, 2019**

**13:00 -**

Guest Speakers:

**TOJU Hirokazu**

(Center for Ecological Research,  
Kyoto University)

**SAWADA Akiko**

(Academy of Emerging Science,  
Chubu University)

Venue:

**Science Seminar House,  
North Campus, Kyoto University**



For more info:

<http://www.wildlife-science.org/en/biodiversity-and-evolution/20190611.html>

## Program

Time	Presentation Title	Presentator	Affiliation
12:30	Registration		
13:00	Opening remarks	Shiro Koshima	WRC kyoto University
	<b>Session 1</b>		<b>chair: Takushi Kishida</b>
13:10–13:40	Talk1 Bird's-eye views of species interactions	Hirokazu Toju	Center for Ecological Research, Kyoto University
13:40–13:55	Talk2 Wildlife conservation by preserving female ovaries	Mayako Fujihara	WRC kyoto University
13:55–14:10	Talk3 Amazon river dolphins abundance at Negro River, Brazil, an area of high touristic interest	Sannie Brum	National Institute of Amazonian Research (INPA)
14:10–14:25	Talk4 Understanding past human-vegetation-climate relationship in Upper Nilgiri, India	Sarath P K	Centre for Ecological Science, Indian Institute of Science
14:25–14:40	Talk5 Microhabitat factors influence prevalence of pathogenic <i>Leptospira</i> spp. in small mammal host	Muhammad Afif Yusof	School of Biological Sciences, Universiti Sains Malaysia
14:40	coffee break		
	<b>Session 2</b>		<b>chair: Annegret Moto Naito</b>
15:00–15:15	Talk6 Molecular dietary analysis of Eurasian otter in Tangjiahe National Nature Reserve	Qiaoyun Wang	State Key Laboratory of Biocontrol, School of Life Sciences, Sun Yat-Sen University
15:15–15:30	Talk7 Characterization of Partial mtDNA Control Region of Mount Tambora Green Junglefowl, <i>Gallus varius</i> (Sumbawa Island)	Achmad Alfiyan	Department of Biology, Faculty of Mathematics and Natural Science, IPB University
15:30–15:45	Talk8 A phylogenetic analysis of colour vision in primates	Breanne Amanda Boughan	Department of Zoology, University of Oxford
15:45–16:15	Talk9 Dietary differences among three sympatric bamboo lemurs in Madagascar: who eats the most cyanogenic bamboo?	Akiko Sawada	Academy of Emerging Science, Chubu University
16:15–16:30	Talk10 From RAPDs to NGS: power and perspectives of advancing molecular tools in wildlife conservation	Giovanni Forcina	Wildlife Research Center of Kyoto University
16:30–16:45	Talk11 Population history and genomic admixture of sea snakes in the West Pacific	Takushi Kishida	WRC kyoto University
16:45	Prepare for Poster Session		
17:00–18:30	<b>Poster presentation &amp; Social Gathering</b>		
18:30	close		

The 10th International Seminar on Biodiversity and Evolution: 「Wildlife Metagenomics」  
2019.6.11

Poster

	Title	Presentator	Affiliation
Poster 1	Behavioral changes by inter individual distance between different group of Japanese macaques focusing on approach and avoidance	Maho Hanzawa	Department of Zoology, Graduate School of Science, Kyoto University
Poster 2	Social Learning Stage of Cultural Behavior Focusing on Variation of Body Orientation: Embracing Behavior of Yakushima Macaques ( <i>Macaca fuscata yakui</i> ), as an Example	Yoshiyuki Tabuse	Division of Biological Science, Graduate School of Science, Kyoto University
Poster 3	“Neutral and functional genetic variation in Japanese golden eagles: preliminary studies of microsatellites and MHC loci”	Annegret Moto Naito	WRC, Kyoto University
Poster 4	Report on the Yakushima and Genome Science Course: Species Interaction Group		
Poster 5	Report on the Yakushima Science Course : Fly Group		
Poster 6	Report on the Genome Science Course 1: Genotyping Group		
Poster 7	Report on the Genome Science Course 2: Phylogeography Group		

## Talk 1

# Bird's-eye views of species interactions

**Hirokazu Toju**<sup>1\*</sup>

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In an era of ecosystem degradation and climate change, maximizing microbial functions in natural and agricultural ecosystems has become a prerequisite for the future of humanity. However, managing species-rich communities of plant-associated microbiomes remains a major challenge. Informatics now allows us to identify members and characteristics of “core microbes” or “core microbiomes”, which may be deployed to organize otherwise uncontrollable dynamics of natural and agricultural ecosystems<sup>1,2</sup>.

By merging DNA metabarcoding (i.e., biological community profiling with high-throughput DNA sequencers) and network science, my colleagues and I have uncovered the structure of bacterial and fungal communities associated with diverse plant species across forest, grassland, and agricultural ecosystems<sup>3-5</sup>. We then found that diverse taxonomic groups of “endophytic” fungi ubiquitously interacted with plant communities, potentially playing crucial, but often overlooked, roles at the ecosystem level<sup>3,6</sup>.

Based on the patterns found in microbe-plant and microbe-microbe networks, we have begun to design optimal core microbiomes for managing agroecosystems with high resource-efficiency and stress-resistance. Moreover, we anticipate that the multidisciplinary research platform of understanding/controlling microbiomes will allow us to promote not only sustainable agriculture but also restoration of forest/grassland ecosystems.

### References:

- 1 Toju, H. *et al.* Core microbiomes for sustainable agroecosystems. *Nat. Plants* **4**, 247–257, (2018).
- 2 Toju, H. *et al.* Species-rich networks and eco-evolutionary synthesis at the metacommunity level. *Nat. Ecol. Evol.* **1**, (2017).
- 3 Toju, H., Guimarães, P. R., Jr, Olesen, J. M. & Thompson, J. N. Assembly of complex plant–fungus networks. *Nat. Commun.* **5**, 5273, (2014).
- 4 Toju, H., Guimarães, P. R., Jr, Olesen, J. M. & Thompson, J. N. Below-ground plant–fungus network topology is not congruent with above-ground plant–animal network topology. *Sci. Adv.* **1**, e1500291, (2015).
- 5 Toju, H., Tanabe, A. S. & Sato, H. Network hubs in root-associated fungal metacommunities. *Microbiome* **6**, 116, (2018).
- 6 Toju, H., Yamamoto, S., Tanabe, A. S., Hayakawa, T. & Ishii, H. S. Network modules and hubs in plant-root fungal biome. *J. R. Soc. Interface* **13**, 20151097, (2016).

### CV

Dr. Hirokazu Toju obtained his Ph.D. degree in 2007 at Kyushu University, Japan. After two-year postdoctoral research in National Institute of Advanced Industrial Science and Technology (AIST), he joined the Hakubi Center for Advanced Research, Kyoto University, as an assistant professor in 2010. Being supported by the Funding Program for Next Generation World-Leading Researchers of Cabinet Office, the Government of Japan (NEXT program), he then launched an interdisciplinary project integrating high-throughput DNA sequencing and network science. He has developed research platforms for uncovering poorly explored interactions among organisms in natural and artificial ecosystems through NEXT and JST PRESTO programs. He is now running a laboratory at Center for Ecological Research, Kyoto University.

## Talk 2

### **Wildlife conservation by preserving female ovaries.**

Mayako Fujihara, Miho Inoue-Murayama

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Within the ovary, there are thousands primordial follicles containing immature oocytes that are never ovulated or fertilized, thus becoming a wasted resource. The ability to cryopreserve the immature follicles within the ovarian tissues in association with the activation of oocyte development offers enormous opportunities to preserving female fertility in wildlife.

Using domestic dog and cat ovaries as a model for the wild carnivores, we have investigated the appropriate condition of in vitro culture and xenotransplantation which can facilitate the folliculogenesis in vitro as well as the cryopreservation technique which can preserve the primordial follicle capability. From in vitro culture study, we found that the supplementation of Retinoic acid can facilitate folliculogenesis in feline ovary. Our cryopreservation study showed that utilizing the vitrification method with DMSO+PVP as cryoprotectants were well preserved the canine ovarian tissues with normal follicle structure. Furthermore, ovarian tissues cryopreserved by this method kept holding the structurally normal follicles with some development even after 9 weeks of transplantation.

We have now started the preserving the chicken ovaries as a model of wild avian species. At first, the appropriate conditions to transfer the ovaries for cryopreservation were investigated. Among different temperature and days, the viability of ovaries stored at 4°C were maintained until 3 days of storage, whereas the ovaries stored at room temperature got damaged rapidly with increasing pH of storage medium.

By using the information obtained with model animals, we have then started freezing the ovaries of wild animals which has been died or spayed in the zoo. These ovaries could be a potential resource of female fertility regardless of sexual maturation or cycling in wild animals.

### Talk 3

## **Amazon river dolphins abundance at Negro River, Brazil, an area of high touristic interest**

Sannie Brum<sup>1</sup>, Marcele Valle<sup>2</sup>, Vera da Silva<sup>1</sup>

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The boto, *Inia geoffrensis*, was recently considered endangered and robust population information is required to guide effective management measures. In the lower Negro river, since 1998 a tourism interaction activity has been developing in which wild botos are habituated to remain close to tourists in the water, but no information on the impacts of this activity for the botos exists. There are also tucuxis, *Sotalia fluviatilis*, but its touristic use is related just to nearby groups observations. We present the first population estimates of botos and tucuxis in this area and discuss the tourism impact on boto populations. We performed boat-based surveys with double observer configuration at an area of 1666km<sup>2</sup> in 2016. Data was analyzed with R software ('mrds' package), in a mark-recapture distance sampling (MRDS) model, with trial configuration and full Independence assumption. The biological removal potential (PBR) was calculated according to Wade (1998). The abundance was estimated as 2666 botos (1499 - 4831, CV = 0.3) and 1499 tucuxis (1000 – 2332, CV = 0.21) and PBR indicated an annual withdrawal limit of 16 botos and 13 tucuxis. Hypothesising that interacting botos are withdrawn from their ecological functions, PBR can be used as a basis for monitoring the impact of this activity on botos population and this is the first proposed index. In the last five years, the estimated number of interacting animals remains at 50-60 botos and the PBR indicates a withdrawal of 80 botos from the population would not cause population decline. Monitoring and inhibiting the increase in the number of interacting animals is an easy-to-use management measure. We conclude the touristic activity with botos, if correctly managed, should not be considered a threath at a populational level. Population monitoring and close inspection of this activity are relevant management measures for boto conservation in the region.

## Talk 4

### **Understanding past human-vegetation-climate relationship in Upper Nilgiri, India**

Sarath P K<sup>1</sup>, Ramyabala P<sup>1</sup>, Devanita Ghosh<sup>2</sup>, Raman Sukumar<sup>1</sup>

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Understanding the ecological history of a region is essential in integrating conservation actions and predicting the future responses to global change. Humans have extensively altered the global environment, changing the ecosystem structure and disrupting the equilibrium between climate and vegetation. Vegetation shifts in the past of tropical montane forest-grassland ecosystem mosaic in Nilgiris is well documented using stable isotope studies and palynology. In this study we reconstructed paleofire records to understand fire-vegetation relationship and to establish role of human in altering the stability of the ecosystem mosaic. Peat samples from Sandynallah basin were used to reconstruct fire history based on charcoal records, fungal spores to understand levels of herbivory and lipid biomarkers to understand human interferences. The region features peat accumulation due to its low annual average temperature and the water logging in the valley. Radiocarbon dating of the profiles reveals a linear peat accumulation during Holocene and an abrupt jump to Last Glacial Maximum at a depth of 149 cm from the top. Macro and microcharcoal record indicated a local fire event ~3500 cal yr BP and an abundance in coprophilous fungal spores, indicating herbivory. This period is also characterized by dry arid conditions and a characteristic shift towards grassland ecosystem as inferred through various biomarker indices specifically through nC31 signatures. The late-Holocene fire horizon shows significant signatures of human and bovine fecal contamination. The fire event marks a distinct climatic period which features human migration to upper Nilgiris. Fire events recorded in our profile coincide with the C3 to C4 shift recorded in the previous studies from the region demonstrating that fire is an important disturbance event influencing and influenced by the vegetation and climate of the region. Our study also demonstrates that fire as a tool to manage landscapes played an integral role in human-environment interactions in the past.

## Talk 5

### **Microhabitat factors influence prevalence of pathogenic *Leptospira* spp. in small mammal host**

Muhammad Afif Yusof<sup>1</sup>, Siti Nabilah Ishak<sup>2</sup>, Shukor Md-Nor<sup>2</sup>, Shahrul Anuar Md-Sah<sup>1</sup>, Nurul Natasya Azhari<sup>3</sup>, Vasanthakumari Neela<sup>3</sup>, Zamberi Sekawi<sup>3</sup>, & Farah Shafawati Mohd-Taib<sup>2</sup>

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**Abstract:** Leptospirosis, a widespread zoonotic disease, is a public health problem, especially in major urban centres and is mainly reported to be associated with rats. In Malaysia, focus has been primarily given to the *Leptospira* prevalence in rodents per se, but there is lack of information on the microhabitat structure of the outbreak areas. We aimed to determine the diversity of small mammal species, microhabitat types, and their prevalence of pathogenic *Leptospira* spp. in the outbreak areas, which were categorized as urban, semi-urban and recreational forests. Sampling involved deploying 100 to 300 live traps at each study site. Kidney samples were extracted from selected individuals, for screening of pathogenic *Leptospira* spp. by PCR. Out of 537 individuals from 15 small mammal species captured, 4 species were recorded from urban, 13 from semi-urban, and 11 from recreational forest sites. From 389 individuals screened, 58 were tested positive for pathogenic *Leptospira*. Recreational forests recorded the highest prevalence with 19.4% (n=93), followed by urban, 16.6% (n=163) and semi-urban sites with 9.8% (n=133). Seven rodent species were tested positive for pathogenic *Leptospira* from all areas. *R. norvegicus* was found to harbour the highest prevalence (66.7%) in urban, *R. rattus* (53.8%) in semi-urban, whereby *M. whiteheadi* (44.4%) in recreational forest sites. Microhabitat analysis revealed that rubbish quantity contributed especially strongly to a high prevalence of *Leptospira*. This study contributes in understanding the host and microhabitat preferences of *Leptospira*, which is important in controlling the spread of this disease in human's landscapes.

**Keywords:** Rodents, Urban, Recreational forest, Leptospirosis, Prevalence, Microhabitat.



## Talk 6

### **Molecular dietary analysis of Eurasian otter in Tangjiahe National Nature Reserve**

Qiaoyun Wang, Lu Zhang, Li Yang, Pengfei Fan\*

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The Eurasian otter (*Lutra lutra*) is a top predator that mainly feed on fish in many freshwater ecosystems. Dietary analysis of otters is an important way to study food webs and to understand how preys affect the ecology of otters. Conventional dietary analysis usually acquires data through microscopic examination of food remains in feces, which requires extensive knowledge of morphology of potential preys, while providing imprecise identifications. DNA barcoding combined with Second Generation Sequencing provide an alternative method to generate precise dietary information from feces. We surveyed abundance and diversity of potential preys in Tangjiahe National Nature Reserve and collected tissue samples of each species. We designed 4 universal primers for vertebrates, and applied them on DNA extractions from tissue samples of preys and 5 otter feces to test for their application efficiency. Our Preliminary results shows otters are generalist predators. They may prefer to prey on large species. The result may similar to Optimal foraging theory, but we need analysis more samples covering the whole area and from different seasons.

Preliminary results have been obtained and used to improve primer design. Our research will provide a tool to study the diet of otters, which will help to better understand the ecology of otters in China and provide essential information for effective conservation management.

Talk 7

## **Characterization of Partial mtDNA Control Region of Mount Tambora Green Junglefowl, *Gallus varius* (Sumbawa Island)**

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*Gallus varius* is one of jungle fowl species that only existed in Indonesia, specifically in Java island and Lesser Sunda Islands. One of island in Lesser Sunda Islands that was inhabited by it is Sumbawa. There was little study about this species that inhabited in Lesser Sunda Islands, especially in Sumbawa island, even though this island is part of Wallacea region. This study aimed to characterize mtDNA control region (D-Loop) of Mount Tambora *G. varius* (Sumbawa Island) and analyzing its genetic diversity. Herein, we extracting *G. varius* DNA from plumage sample, then D-Loop region was amplified using 2 pair of primer. After that, D-Loop region was sequenced and then bioinformatic analysis was conducted. After combining the D-Loop region from 2 pair of primer, we get D-Loop region sequence with length 1227 bp, with the highest percentage of nucleotide was TTP (Thymidine Triphosphat) (33.4%) while the lowest was GTP (Guanosine Triphosphat) (13.4%). D-Loop sequence of *G. varius* from Mount Tambora showed some nucleotide variation between same species, which is 8 nucleotide substitution (5 transition and 3 transversion) and 3 insertion-deletion. Phylogenetic tree that was constructed using NJ method with Kimura 2-parameters models showed that based on partial D-Loop region, *G. varius* more closely related to *G. sonneratii* and *G. lafayetii* than to *G. gallus*.

## Talk 8

### **A phylogenetic analysis of colour vision in primates**

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Primate vision falls into one of four categories; monochromatic, dichromatic, polymorphic trichromatic, or routine trichromacy. Despite vision's key role in primate evolution, little is known about the adaptive significance of different vision categories and the ecological circumstances that select for these variations. Hypotheses to explain the evolution of primate vision focus on activity pattern or the presence of frugivory or folivory in the diet. To better understand evolutionary trends in primate colour vision, data on vision, diet, and activity pattern were collected from the literature for 299 species of primates. These data were then used to conduct Bayesian phylogenetic analyses to reconstruct ancestral states for all three variables and test for correlated evolution between them. Results suggest primates may have been polymorphic at the root of the primate lineage, allowing some females to possess trichromatic vision, and found evidence for an early reliance on fruit within the primate tree. However, there is no evidence that trichromatic vision, whether routine or through polymorphism, coevolved with frugivory. Additionally, there is no evidence for correlated evolution between activity pattern and vision. While other food items were also tested, results were difficult to interpret as the roots and directionality could not be ascertained.

Talk 9

**Dietary differences among three sympatric bamboo lemurs in Madagascar: who eats the most cyanogenic bamboo?**

Akiko Sawada<sup>1</sup>, Isabelle Clark<sup>2</sup>, Tsinjo Andriatiavina<sup>3</sup>, Onjaniaina Ramilijaona<sup>3</sup>, Brigita Tsavohitra<sup>3</sup>, Takashi Hayakawa<sup>4</sup>

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Bamboo lemurs (gentle lemurs) are small to medium-sized primates living in Madagascar. As their name clearly indicates, they are almost exclusively feed on bamboo, which is extremely rare among mammals. One of the advantages of being bamboo specialist is that they can avoid interspecific food competition over food. However, as fiber digestion is a time-dependent process, primates of the same size are generally insectivorous or frugivorous due to digestive constraints. Besides all that, some bamboo species consumed by bamboo lemurs contain a substantial amount of cyanide, which is theoretically lethal to them (Glander *et al.* 1989). Our study subjects were eastern lesser bamboo lemur (*Hapalemur griseus*), golden bamboo lemur (*H. aureus*) and greater bamboo lemur (*Prolemur simus*) inhabit Ranomafana National Park in southeastern Madagascar. The study was took place mostly in low montane forest with bamboo forest fragments where other sympatric lemur species consume non-bamboo items, such as fruits and insects. These three bamboo lemurs consumed more bamboo shoots in rainy seasons than in dry season, yet they had different preferences over bamboo species/parts. Based on feeding data and cyanide concentration we measured in different bamboo species/parts they consumed, we found *P. simus*, the largest bamboo lemur, was an exclusive bamboo specialist and ingested the largest amount of cyanide. *H. griseus*, the smallest species, were relatively generalist feeding on several non-bamboo items, while *H. aureus* were intermediate.

Talk 10

**From RAPDs to NGS: power and perspectives of advancing molecular tools in wildlife conservation**

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abstract (Font: Times New Roman, Size: 11point)

(Abstracts for Oral presenters will be about 300 words, for Poster presenter will be about 200 words.)

Evolutionary and population genetics questions related to wildlife have long been addressed with traditional DNA markers such as mitochondrial genes and microsatellites. However, the major methodological advances such as molecular indexing and next generation sequencing (NGS) coupled with an increased public awareness have recently marked the opening of a new era, with new exciting perspectives arising for biodiversity studies. This is of topical importance since estimates and tracking of genetic variability in species and populations, widely neglected as a measure of biodiversity for conservation and management over the last decades, are now mandates for governments. However, this obligation is challenging to fulfill. There is no consensus on the most appropriate way to assess genetic diversity, yet the urgent need for comparability among different studies calls for the adoption of standardized approaches. Here, I will present an overview of different studies I have taken part in over the last 11 years and the array of different and increasingly more powerful molecular tools used in the attempt to stimulate a constructive discussion about the opportunities and challenges associated with the new genomic approaches as well as the need for modern laboratories to quickly switch from the genetics to the genomics era.

## **Population history and genomic admixture of sea snakes in the West Pacific**

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Speciation in the open ocean has long been studied, but it remains largely elusive what factors promote or inhibit speciation in such an open environment. Marine amniotes, which evolved from terrestrial ancestors, provide valuable opportunities for studying speciation in the ocean because of their evident aquatic origins. Sea snakes are phylogenetically related to terrestrial elapid snakes and consist of two monophyletic groups (Hydrophiini and Laticaudini). These two groups migrated from land to water almost at the same time, but species diversities are remarkably different: there are approx. 60 species in 16 genera described for hydrophiins, whereas only eight species in a genus *Laticauda* are described for laticaudins. In this study, we focused on laticaudins to consider why they have seldom speciated. We performed whole-genome shotgun sequencing of several species of laticaudins sampled in their southmost (Vanuatu) and northmost (Ryukyu) habitats. Demographic histories of Vanuatu and Ryukyu populations suggest that populations of broadly distributed major species are geographically structured. Each species is genetically clearly distinguished, but there is a considerable amount of gene flow between two sibling species distributed sympatrically in Vanuatu. Presence of gene flow between sympatric populations of phylogenetically distant species is also implied. Broad distribution of major species combined with such genetic mixability might have prevented laticaudins from genetic isolation and speciation.

Poster 1

**Behavioral changes by inter individual distance between different group of Japanese macaques focusing on approach and avoidance**

Maho Hanzawa<sup>1</sup>, Yosuke Kurihara<sup>2</sup>

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In primates, the adjacent groups tend to avoid each other. In previous studies, the encounters were often considered to begin when the researchers noticed the other group visually. However, it remains unknown when the individuals in the groups actually notice the approach of adjacent groups and what affect their movement after the approach. We measured the temporal change of the distances between two individuals in the different groups and the direction of their movement. We found distance between the individuals became longer after the distance between them were under 410m. This indicates that they got aware of the approach of the adjacent groups when they got closer than 410m. When the nearest individuals were both females, they tended to approach after they got close. Otherwise, the individual in smaller group tended to approach, while the individual on the larger group tended to avoid. In the smaller group, the shorter the distances between the individuals in the same groups was, the higher the proportion of approach to the other group became after they got closer than 410m. These results suggest that cohesiveness within the smaller troop may affect the movement they approach to the larger group.

Poster 2

**Social Learning Stage of Cultural Behavior Focusing on Variation of Body Orientation:  
Embracing Behavior of Yakushima Macaques (*Macaca fuscata yakui*), as an Example**

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One of the cultural behaviors is embracing behavior of Japanese macaques (*Macaca fuscata*). When cultural behavior is acquired, social learning seems to play an important role. However, the development of embracing behavior is not revealed, because embracing behavior has been analyzed only for mature individuals. There is variation of embracing behavior in Yakushima, that is, there is variation of body orientation. It may be the indicator representing social learning stage. I conducted field research to collect data for Umi-A group of Yakushima macaques (*M. fuscata yakui*) from August 2018 to October 2018. Mature individuals used more variations. On the other hand, immature individuals almost always used one type of embracing behaviors, ventro-ventral embrace. In addition, when immature individuals embrace in the direction except front face, mature individuals proactively embrace in the direction of non-front face of immature individuals. In the partners of immature individuals, the same family group individuals and individuals of the same age amounted to 73%. This suggests that embracing behavior is transmitted from affiliative individuals, especially from kin individuals and that the older they are, the more variations they acquire. They may change variations of embracing behavior according to social relationships.