学術変革領域研究 (A)
共進化表現型創発：延長された表現型の分子機構解明
Co-evolutionary Emergence of Extended Phenotypes

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ICE2024 Kyoto Symposium
“Extended phenotypes emerging across insects, plants and microbes” 開催報告

表紙　シンポジウム講演者の集合写真（2024年8月26日京都国際会館）

目次

ICE2024 Kyoto Symposium “Extended phenotypes emerging across insects, plants and microbes” 開催にあたって

佐藤 拓哉（京都大学）

8 月 25 日～30 日に京都で開催された ICE2024 において、学術変革領域研究（A）「共進化表現型創発：延長された表現型の分子機構解明」では、標記のシンポジウムを開催しました。当領域の計画研究代表者 9 名が一堂に会するとともに、アメリカ、イギリス、オランダ、中国、ドイツ、フランスから関連分野を先導する一線の研究者が口頭発表をおこないました。まず領域代表者の勝間進から、当領域研究のコンセプトと絡めてシンポジウムの目的が説明されました。その後、行動操作、生殖操作、形態操作、発生操作、共生表現型変容、ウイルスー昆虫ー植物の共生機構といった多岐にわたる計 16 題の講演がおこなわれました。

本シンポジウムは、当学術変革領域研究の発足以来、「延長された表現型」を主題とする 2 回目の国際シンポジウムです。第1回は、2024 年 6 月に米国バージニア州に所在するハワードヒューズ医学研究所のジャネリアリサーチキャンパスで開催された「Mechanisms of Inter-organismal Extended Phenotypes」で、深津武馬ほか多くの計画研究代表者が参加しました。この国際シンポジウムでは、「延長された表現型」の分子機構解明について、国際的機運の大きな高まりを感じるとともに、すでに学術変革領域を発足して研究を進展させている我々の先進性や優位性を確認することができました。一方、第2回となる今回のシンポジウムでは、我々の国際的なプレゼンスを高めることに加えて、国内の研究者に当該研究分野の面白さや将来性をより広く知ってもらい、領域研究のすそ野を広げることも企図しました。5 時間にわたるシンポジウムの間じゅう、コーヒーブレイク後のわずかな時間を除いて満席となり、立ち見・座り見の聴衆も多くみられました。質疑応答も活発に行われ、講演者と聴衆の双方にとって、有意義なシンポジウムになったのではないかと思います。国際昆虫学会には、昆虫を対象とした分子生物学から自然史研究まで、様々な研究者が参加しています。今回のシンポジウムが、多様な昆虫研究者の目に留まり、今後、「生物個体を超えた階層に拡張した生物学」を日本発で創り上げていくことの一助になればうれしく思います。

シンポジウム終了後は、講演者一同で鴨川沿いの「川床」へと場を移し、ICE2024 の最初の基調講演者である Nancy Moran 博士（米国テキサス大学オースチン校；専門は昆虫と細菌の共生進化）も迎えて、京都の夏を関係者一同で楽しみ、国際的な交流を深める夜となりました。

【Symposium Timetable】

Section 10. “Insect-Microbe Interactions”

Symposium 10-2. “Extended phenotypes emerging across insects, plants and microbes”

Mon. Aug 26, 2024 1:30 PM - 6:15 PM Room 510

Chair: Takuya Sato (Kyoto University), Susumu Katsuma (University of Tokyo), Takema Fukatsu (National Institute of Advanced Industrial Science and Technology), Carolyn Elya (Harvard University), Charissa de Bekker (Utrecht University)

1:30 PM - 1:45 PM
[10-2-01] Molecular mechanisms underlying baculovirus-induced host behavior manipulation
*Susumu Katsuma[1], Ryuhei Kokusho[1] (1. The University of Tokyo, Japan)

1:45 PM - 2:00 PM
[10-2-02] Neural mechanisms of fruit fly behavioral manipulation by the killer fungus *Entomophthora muscae*
*Carolyn Elya[1], Danylo Lavrentovich[1], Emily Lee[1], Cassandra Pasadyn[1], Jasper Duval[1], Maya Basak[1], Valerie Saykina[1], Benjamin de Bivort[1] (1. Harvard University, USA)

2:00 PM - 2:15 PM
[10-2-03] Hijacked! Investigating the molecular strategies used by a zombie-making fungus to manipulate carpenter ant behavior
*Charissa de Bekker[1] (1. Utrecht University, Netherlands)

2:15 PM - 2:30 PM
[10-2-04] Adaptive and maladaptive consequences of enhanced polarotaxis for the extended phenotype of nematomorph parasites in human-dominated environments
*Takuya Sato[1], Yuna Sawada[1], Midori Sakura[2] (1. Kyoto University, Japan, 2. Kobe University, Japan)

2:30 PM - 2:45 PM
[10-2-05] Behavioural and proteomic analysis of the water-entry behaviour in crickets (*Acheta domesticus*) infected with the nematomorph *Paragordius varius*.
*Louise Coates[1], Mara Carey-Wood[1], Dominika Lastik[1], Chris Vennard[1], Jean-François Doherty[2], Eddy Dowle[3], Vicky Hunt[1] (1. University of Bath, UK, 2. University of British Columbia, Canada, 3. University of Otago, New Zealand)

2:45 PM - 3:00 PM
[10-2-06] Brain manipulation of mammalian host by intracellular parasite, *Toxoplasma gondii*
*Yoshifumi Nishikawa[1] (1. Obihiro University of Agriculture and Veterinary Medicine, Japan)

3:00 PM - 3:15 PM

[10-2-07] Why do some vertically transmitted viruses kill male hosts?
*Daisuke Kageyama[1], Keisuke Nagamine[1], Toshiyuki Harumoto[2], Masayoshi Watada[3], Yoshinori Shintani[4] (1. NARO, Japan, 2. Kyoto University, Japan, 3. Ehime University, Japan, 4. Minami Kyushu University, Japan)

3:15 PM - 3:30 PM
[10-2-08] Commonality and difference in male-killing mechanisms caused by insect symbionts
*Toshiyuki Harumoto[1], Masayoshi Watada[2], Daisuke Kageyama[3] (1. Kyoto University, Japan, 2. Ehime University, Japan, 3. National Agriculture and Food Research Organization, Japan)

Coffee Break（3:30 PM - 4:15 PM）

4:15 PM - 4:30 PM
[10-2-09] Evolutionary and genomic insights into male-killing and non-male killing *Spiroplasma* endosymbionts associated with the pea aphid
Hiroshi Arai[2], Fabrice Legeai[1], Romuald Cloteau[1], Frédérique Mahéo[1], Ruyichi Koga[3], Daisuke Kageyama[2], Akiko Sugio[1], *Jean-Christophe Simon[1] (1. INRAE, France, 2. NARO, Japan, 3. AIST, Japan)

4:30 PM - 4:45 PM
[10-2-10] Identification of the gall-inducing peptide from a gall-inducing aphid, *Schlechtendalia chinensis*
*Tomoko Hirano[1], Masa H Sato[1] (1. Kyoto Prefectural University, Japan)

4:45 PM - 5:00 PM
[10-2-11] Manipulation of Plant Morphology by Gall-Forming Social Aphids
*Mayako Kutsukake[1] (1. National Institute of Advanced Industrial Science and Technology (AIST), Japan)

5:00 PM - 5:15 PM
[10-2-12] Venom proteins of the endoparasitoid wasp *Asobara japonica* induce epithelial cell death in the host *Drosophila* species and ensure parasitism success
Takumi Kamiyama[1], Yuko Shimada-Niwa[1], Naoki Tani[2], Akira Takasu[3], Hitoha Mori[1], Toshiya Senda[3], Akira Nakamura[2], *Ryusuke Niwa[1] (1. University of Tsukuba, Japan, 2. Kumamoto University, Japan, 3. High Energy Accelerator Research Organization, Japan)

5:15 PM - 5:30 PM
[10-2-13] Exploitation of behavioral fever as a defense strategy against parasitoids
Yifeng Sheng[1,2], Zhi Dong[1,2], *Jianhua Huang[1,2] (1. Institute of Insect Sciences, Ministry of Agriculture Key Lab of Molecular Biology of Crop Pathogens and Insect Pests, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, China, 2. Key Laboratory of Biology of Crop Pathogens and Insects of Zhejiang Province, Zhejiang University, Hangzhou, China)

5:30 PM - 5:45 PM
[10-2-14] Symbiont-induced modification of host's adaptive phenotypes
*Takema Fukatsu[1] (1. National Institute of Advanced Industrial Science and Technology (AIST), Japan)

5:45 PM - 6:00 PM
[10-2-15] Paleocene origin of a streamlined digestive symbiosis in leaf beetles
*Marleny Garcia Lozano[1], Christine Henzler[1], Miguel Ángel González Porras[1], Inès Pons[1], Aileen Berasategui[1], Christa Lanz[1], Heike Budde[1], Kohei Oguchi[2], Yu Matsuura[3], Yannick Pauchet[4], Shana Goffredi[5], Takema Fukatsu[6], Donald Windsor[7], Hassan Salem[1,7] (1. Max Planck Institute for Biology, Germany, 2. University of Tokyo, Japan, 3. University of the Ryukyus, Japan, 4. Max Planck Institute for Chemical Ecology, Germany, 5. Occidental College, USA, 6. National Institute for Advanced Industrial Science and Technology, Japan, 7. Smithsonian Tropical Research Institute, Panama)

6:00 PM - 6:15 PM
[10-2-16] Identification of plant virus proteins responsible for the manipulation of host phenotype and vector behavior
*Quentin Chesnais[1], Maxime Verdier[1], Véronique Brault[1], Raymonde Baltenweck[1], Philippe Hugueney[1], Martin Drucker[1] (1. INRAE - University of Strasbourg, France)

聴衆で満員のシンポジウム会場　立ち見、座り見の姿もみえる

[10-2-01] Molecular mechanisms underlying baculovirus-induced host behavior manipulation

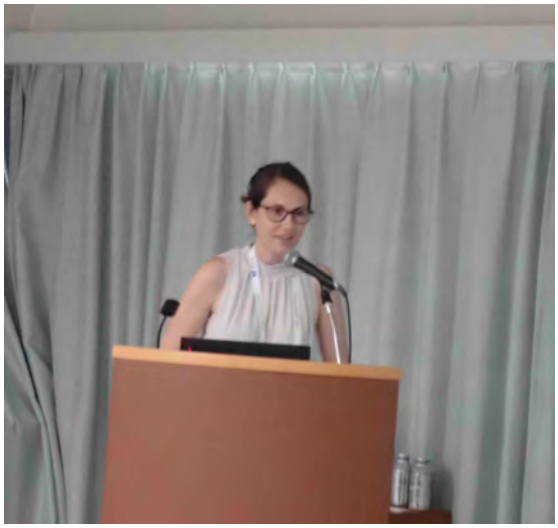
*Susumu Katsuma[1], Ryuhei Kokusho[1] (1. The University of Tokyo (Japan))

Pathogens sometimes alter the behavior of their hosts so that progeny transmission is maximized. One of the earliest documented examples of such behavior modification is *Wipfelkrankheit*, a baculovirus-induced disease that causes caterpillars to migrate to the upper foliage of food plants where they die. We and other groups have identified two baculovirus genes, *ptp* (*protein tyrosin phosphatase*) and *egt* (*ecdysteroid UDP-glucosyltransferase*), as the key factors for baculovirus-induced abnormal behavior during the late stage of infection (Hoover et al., 2011, *Science*; Kamita et al., 2005, *PNAS*; Katsuma et al., 2012, *PLoS Pathog.*). Interestingly, both of them are likely captured from ancestral lepidopteran insects by horizontal gene transfer, suggesting that the modern baculovirus uses captured host genes for manipulation of host behavior. On the other hand, host genes involved in baculoviral behavior manipulation remain largely unknown. We recently identified a host gene *T3up1* that is induced in the larval brain of silkworms during baculovirus-induced behavior modification. Functional analysis strongly suggested that activation of *T3up1*-mediated signaling triggers baculovirus-induced enhanced behavior. In this talk, we will discuss the functions of host and viral genes involved in baculovirus-induced host behavior manipulation.

[10-2-02] Neural mechanisms of fruit fly behavioral manipulation by the killer fungus *Entomophthora muscae*

*Carolyn Elya[1], Danylo Lavrentovich[1], Emily Lee[1], Cassandra Pasadyn[1], Jasper Duval[1], Maya Basak[1], Valerie Saykina[1], Benjamin de Bivort[1] (1. Harvard University (United States of America))

Many parasites are known to manipulate the behavior of their animal hosts, but how this occurs remains poorly understood. Recently, I discovered a strain of the mind-controlling pathogen *Entomophthora muscae* in wild fruit flies and developed robust methods to culture the fungus in the model organism *Drosophila melanogaster*. Before sunset on their final day of life, infected flies perform the "zombie" behaviors characteristic of *E. muscae* infection: they climb to a high location (a behavior known as "summiting"), extend their proboscises, and raise their wings in a pose that facilitates spore dispersal. Using a combination of approaches that range from behavioral neuroscience to molecular biology, we have established a neuro-mechanistic model of summiting behavior in zombie flies. Future work will identify the fungal inputs to the fly summiting circuit as well as revealing the genetic and circuit underpinnings of additional zombie behaviors driven by *E. muscae*.



[10-2-03] Hijacked! Investigating the molecular strategies used by a zombie-making fungus to manipulate carpenter ant behavior

*Charissa de Bekker[1] (1. Utrecht University (Netherlands))

The evolutionary arms race between parasites and their hosts can culminate into complex extended phenotypes that benefit disease progression and transmission. The fungus-adaptive changes in behavior as seen in *Ophiocordyceps*-infected carpenter ants are a prime example. These “zombie ants” demonstrate a suite of behaviors that are thought to circumvent the social immune responses of the colony. Subsequently, the hijacked ant climbs and attaches itself at an elevated position that benefits fungal spore development and dispersal. These fungus-induced behaviors are not unique to this particular infection as parallel behaviors have also been observed in invertebrate infections by other parasite taxa. This suggests that these behavioral hijacking strategies and their underlying mechanisms have convergently evolved. Unraveling how parasites can corrupt insect behavioral pathways and physiology to alter behavior can teach us how normal insect behavior is regulated as well. However, the precise mechanisms that are involved are currently largely unknown. To begin to unravel these mechanisms, we have conducted extensive fieldwork and developed the *Ophiocordyceps*-ant interaction into an integrative model system that allows us to study parasitic behavioral manipulation in greater detail in the lab. By combining fungal culturing and lab infections with behavioral assays and multiple omics approaches, we propose several comprehensive mechanistic hypotheses about the fungal proteins and ant receptors involved in this phenomenon. These hypotheses include specific fungal “manipulation” effectors of interest and their potential binding to ant proteins involved in light perception, biogenic amine binding and daily rhythms. To test these hypotheses we are currently, for the first time in this model, beginning to integrate functional genetics assays to determine the function of presumed fungal “manipulation” effectors, the host behaviors they elicit, and the host pathways that underly those phenotypes. Our results will provide detailed insights into fungus-animal interactions in general while giving some of the first insights into parasitic hijacking of animal behavior in particular.

[10-2-04] Adaptive and maladaptive consequences of enhanced polarotaxis for the extended phenotype of nematomorph parasites in human-dominated environments

*Takuya Sato[1], Yuna Sawada[1], Midori Sakura[2] (1. Kyoto University (Japan), 2. Kobe University (Japan))

A wide range of parasites manipulate the behaviours of their hosts in order to complete their life cycle, in a well-known example of the “extended phenotype”. Alteration of phototaxis is thought to be involved in host manipulation in many cases. However, very little is known about what features of the light (intensity, spectrum, polarization) alter behaviour. Nematomorph parasites induce their terrestrial insect hosts, such as mantids and crickets, to enter water, where the parasites must emerge to reproduce. This is one of the most fascinating examples of host manipulation by parasites, but the key mechanism triggering the host’s entry into water has long been wrapped in mystery. A previous report argued that parasite-induced positive phototaxis can increase the encounter rate between hosts and bodies of water. However, luminous environments are ubiquitous in nature, including forest openings, bright sandy habitats or grassland reflecting sunlight or moonlight. Therefore, a simple alteration of the response to light intensity may not be sufficient for hosts to effectively find and enter bodies of water. The reflection of sunlight off of water bodies is horizontally polarized, a feature that is used by many arthropods to either seek out or avoid water habitats in luminous environments. In this talk, we will first explain that an induced positive polarotaxis can explain water entry by a mantid host infected with a nematomorph parasite. Then, we will show a series of our laboratory and field experiments testing whether the enhanced polarotaxis can cause adaptive and maladaptive consequences for the extended phenotype (i.e., water-entry behavior of host mantids) of the nematomorphs in human-dominated environments. We will finally introduce potential molecular mechanisms of the water-entry behavior of mantids infected by the nematomorphs.

[10-2-05] Behavioural and proteomic analysis of the water-entry behaviour in crickets (*Acheta domesticus*) infected with the nematomorph *Paragordius varius*

*Louise Coates[1], Mara Carey-Wood[1], Dominika Lastik[1], Chris Vennard[1], Jean-François Doherty[2], Eddy Dowle[3], Vicky Hunt[1] (1. University of Bath (UK), 2. University of British Columbia (Canada), 3. University of Otago (New Zealand))

Nematomorpha are a group of parasitic worms which infect arthropods as their definitive host. To complete their lifecycle, these worms manipulate the host behaviour, resulting in water entry by the host. The mechanism influencing this behavioural manipulation is unknown, though light attraction, water attraction and erratic behaviour are thought to play a role. Here we investigated the attraction to water hypothesis using crickets (*Acheta domesticus*) exposed to the nematomorph *Paragordius varius*. A 'Y' maze choice test with a water trough at the end of one arm and an empty trough at the end of the other was used to assess the water attraction behaviour of crickets. As expected, crickets infected with a nematomorph parasite were more likely to enter water than uninfected control crickets. Post-infected crickets, allowed to expel their worms prior to testing, did not enter or interact with the water and instead favoured the dry trough. This could indicate the reversibility of the water seeking behaviour once the parasite has been expelled. To understand if the water seeking behaviour is driven by similar mechanisms to dehydration, uninfected dehydrated crickets were tested. Dehydrated crickets were slightly more attracted to water than uninfected control crickets, but not to the extent of infected crickets suggesting that other mechanisms are involved. The dehydrated group did not fully enter the water trough, as the infected group did, and instead submerged their heads under the water for a prolonged period of time, indicating a level of consciousness of the danger of the water, which was not observed in the infected group. Because it is not clear if the time of day affects the water seeking behaviour, all experiments were carried out in both day and night conditions. To investigate the molecular basis of behavioural manipulation, quantitative mass spectrometry was carried out for haemolymph from crickets from all test conditions. We have identified changes in protein levels in the host and identified parasite-derived proteins that are candidates for host behaviour manipulators.

[10-2-06] Brain manipulation of mammalian host by intracellular parasite, *Toxoplasma gondii*

*Yoshifumi Nishikawa[1] (1. Obihiro University of Agriculture and Veterinary Medicine (Japan))

Parasitic organisms are basically living organisms that live inside or on the surface of their host's body and cannot survive without the presence of a host. Many parasitic organisms have distinctive life cycles, and one of their unique parasitic strategies is the phenomenon of host manipulation. Our research focuses on *Toxoplasma gondii* as a host-manipulating parasite. *Toxoplasma* is a commensal intracellular parasitic protozoan that uses felines as its definitive host and most of warm-blooded animals including humans as intermediate hosts. It is estimated that about one-third of the world's population is infected with *T. gondii*, making it the most successfully spread pathogen. When *T. gondii* infects an intermediate host, it migrates into the brain and muscles of that host, eventually forming dormant cysts and establishing chronic infection. Dormant parasites are not pathogenic in hosts with normal immune systems, but can sustain infection for the lifetime of the host. In other words, *T. gondii* is thought to be a life-long parasite in the brain and muscles. Because chronic *Toxoplasma* infections rarely show clinical signs, the effects of infection on the central nervous system have been neglected. However, studies in recent decades have reported that chronic *Toxoplasma* infection increases the risk of developing human psychiatric disorders and alters rodent behavior. However, mechanism of brain manipulation by *T. gondii* is largely unknown. Therefore, we established a mouse model of toxoplasmosis and analyzed behavioral changes in hosts during the acute, chronic, and reactivation phases of infection. In mice during the acute and reactivation phases of infection, the appearance of core symptoms of "depression" dependent on activation of the kynurenine pathway was observed. On the other hand, in chronically infected mice, abnormal neurotransmitter production and neuronal dysfunction in the brain were inferred, and impairment of the ability to fix fear memories was confirmed. These results suggest that *T. gondii* infection disrupts the central nervous system. Future detailed studies will elucidate *Toxoplasma*'s parasitic strategy and reveal the true significance of host manipulation by this parasite.

[10-2-07] Why do some vertically transmitted viruses kill male hosts?

*Daisuke Kageyama[1], Keisuke Nagamine[1], Toshiyuki Harumoto[2], Masayoshi Watada[3], Yoshinori Shintani[4] (1. NARO (Japan), 2. Kyoto University (Japan), 3. Ehime University (Japan), 4. Minami Kyushu University (Japan))

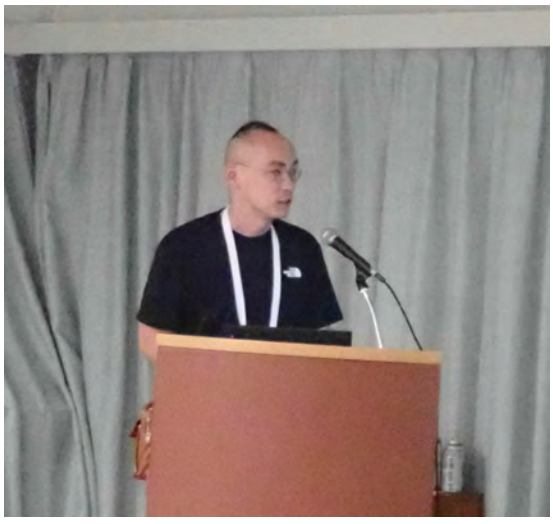
Recent metagenomic studies have revealed a diverse array of viruses inhabiting insects, but much remains unknown about their phenotypic characteristics. While considerable attention has been paid to insect pathogens or those transmitted by insects that are pathogenic to plants or animals, viruses reside in insects and are transmitted vertically from parent to offspring have received less attention. These viruses coexist intimately with their specific hosts, potentially leading to various phenotypic outcomes. Our focus lies on viruses that induce specific male host mortality, known as male-killing viruses, owing to their intriguing traits. Male-killing viruses have been identified in three distinct hosts: partiti-like viruses in the tea tortrix moth *Homona magnanima* (OGV), a partiti-like virus in the fly *Drosophila biauraria* (DbMKPV1), and a tombus-like virus in the tobacco cutworm *Spodoptera litura* (SlMKV). Notably, all these viruses belong to the category of segmented RNA viruses. Since the male-killing gene identified from DbMKPV1 has no homology to any of the genes found in OGV and SlMKV, the male-killing ability is thought to have been acquired independently by each virus. When DbMKPV1 is microinjected into uninfected *D. biauraria*, the segment encoding the male-killing gene can be lost in subsequent generations, resulting in a stable fly line infected with a non-male-killing virus. Our presentation will address whether male killing is truly an adaptive trait for DbMKPV1 and to explore the potential phenotypes associated with other genes possessed by DbMKPV1.

[10-2-08] Commonality and difference in male-killing mechanisms caused by insect symbionts

*Toshiyuki Harumoto[1], Masayoshi Watada[2], Daisuke Kageyama[3] (1. Kyoto University (Japan), 2. Ehime University (Japan), 3. National Agriculture and Food Research Organization (Japan))

About half of all insects on earth are estimated to have some symbiotic microorganisms within their bodies. Such symbiosis underpins the survival and prosperity of insects, for instance, by provisioning essential nutrients as well as providing resistance against their enemies. Meanwhile, some symbionts selfishly manipulate host reproduction to spread their infection within the host population. We have been studying the symbiotic microorganisms of *Drosophila* species, whose infection selectively kill male progeny during development. This phenotype, known as male killing, has been well known to be widespread in insect-bacterial symbiosis; however, recent studies have started to unveil the existence of male-killing viruses in some insect taxa.

In this presentation, we first would like to summarize our current mechanistic understanding of male killing caused by *Spiroplasma*, a bacterial symbiont of *D. melanogaster*, especially focusing on the action mechanism of a male-killing toxin Spaid. Next, we will focus on a novel male-killing factor recently discovered from a viral symbiont of *D. biauraria* and will introduce our efforts on elucidating how the viral protein selectively reduces the survival of male offspring. Together, we highlight the commonality and difference of the underlying molecular mechanisms of male killing, which are evolved in distinct microbial symbionts associated with closely related host insect species.



[10-2-09] Evolutionary and genomic insights into male-killing and non-male killing *Spiroplasma* endosymbionts associated with the pea aphid

Hiroshi Arai[2], Fabrice Legeai[1], Romuald Cloteau[1], Frédérique Mahéo[1], Ruyichi Koga[3], Daisuke Kageyama[2], Akiko Sugio[1], *Jean-Christophe Simon[1] (1. INRAE (France), 2. NARO (Japan), 3. AIST (Japan))

Insects are frequently associated with microorganisms whose effects on the host range from parasitism to mutualism. Understanding the evolutionary trajectories of these associations and the mechanisms underlying the phenotypes induced by microorganisms in their hosts is of considerable importance to the field of host-microorganism interactions. Aphids are hosts to several symbiotic bacteria. First, they harbour a primary endosymbiont, *Buchnera aphidicola*, which is essential for aphid survival by providing essential amino-acids missing in the diet. Second, they can be infected by an array of facultative symbionts that are not essential to aphids but can induce phenotypes that are advantageous to their hosts. The pea aphid, *Acyrthosiphon pisum*, can host various facultative symbionts from different bacterial lineages, notably *Spiroplasma ixodetis* from the mollicutes. The prevalence of *Spiroplasma* in pea aphid populations is highly variable, ranging from zero to 60%, with no clear explanation of the factors responsible for these fluctuations. Previous work has shown that, despite the fact that aphids reproduce mainly by parthenogenesis, certain strains of *Spiroplasma* can induce male-killing in their hosts. Furthermore, it has been shown that *Spiroplasma* can also protect *A. pisum* from infection by entomopathogenic fungi, as well as from attacks by parasitoid Hymenoptera. In this presentation, we will first report the genome characteristics of a *Spiroplasma* strain that induces male-killing in the pea aphid, which we have compared with those of other strains of *S. ixodetis*. We will also present the genomic diversity and phylogenetic relationships of *Spiroplasma* strains associated with pea aphid populations but also with other insects. Finally, we will provide the first insights into the mechanisms that induce male-killing in pea aphids, based on genomic and histological analyses.

[10-2-10] Identification of the gall-inducing peptide from a gall-inducing aphid, *Schlechtendalia chinensis*

*Tomoko Hirano[1], Masa H Sato[1] (1. Kyoto Prefectural University (Japan))

Some insects manipulate plants to produce an 'insect gall', where they spend a safe larval period before becoming adults.The insect gall is not a simple cluster of cells, but a highly ordered organ adapted to insects, consisting of a nutrient-rich tissue in an inner layer, vascular bundles to transport water and nutrients, and hard tissue to protect against external enemies in an outer layer.Gall formation has been a mysterious phenomenon for many centuries and its molecular mechanism is still largely unknown. It has been well known that "the mechanism of gall formation cannot be generalized" because different insects, such as aphids, moths, flies, bees and weevils, form different insect galls on specific host plants. On the other hand, the gall-inducing insects and host plants have not been modelled. First, we found the common features of 'insect gall' by comparing gene expression analyses of different types of gall and concluded that insect gall formation is caused by the partial expression of floral organ genes and fruit genes. Second, we developed a novel bioassay, Ab-GALFA (Arabidopsis-based gall formation assay), to analyze the mechanism of gall formation using model plants, based on the discovery that the model plant Arabidopsis responds to gall-inducing insect extracts by forming a gall-like structure. Third, we used a novel screening approach to identify the effectors of gall formation.Using the effectors, we were finally able to construct an artificial gall without any insects.In this talk, we will present progress in identifying effectors and plant responses during gall formation.

[10-2-11] Manipulation of plant morphology by gall-forming social aphids

*Mayako Kutsukake[1] (1. National Institute of Advanced Industrial Science and Technology (AIST) (Japan))

Galls, also known as plant galls, are uniquely shaped structures formed on plants by insects and other organisms. They are formed when insects physically or chemically stimulate specific parts of plants, inducing hypertrophy or hyperplasia of cells in young plant tissues and the development of conspicuous plant morphologies not typically observed. These galls provide inducer insects with an isolated and exclusive habitat, a constant and high-quality food supply, a physical barrier against predators and parasites, and mitigation of environmental stresses such as desiccation and temperature fluctuations. Interestingly, gall morphology is characteristic and diverse depending on insect species rather than plant species. However, it remains stable and reproducible within a species, suggesting that gall formation is precisely controlled by genetic factors in inducer insects. Therefore, the morphological traits of the galls are often regarded as the “extended phenotypes”of the inducer insects.
Here I will talk about several intriguing cases from our findings of sophisticated insect-plant interactions related to gall-forming social aphids. In the social aphid *Nipponaphis monzeni,* when the gall is damaged, altruistic individuals known as soldiers promptly repair the hole on the gall wall by discharging and plastering their body fluid. Subsequently, the soldiers continuously stimulate the wound site around the breach, leading to the proliferation and regeneration of plant tissue, ultimately restoring the tissue integrity of the inner gall wall. This phenomenon represents one of the sophisticated plant-insect interactions in a social context (Kutsukake *et al.* 2009, 2019). Recently, to gain insight into the molecular mechanisms of gall formation and functions induced by aphids, we are focusing on *Ceratovacuna nekoashi*, whose banana-shaped galls exhibit an intriguing phenomenon known as “late flowers” originating from failed galls. While much remains to be explored, I would like to present some results obtained from field experiments and transcriptome analyses conducted so far.

References:
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[10-2-12] Venom proteins of the endoparasitoid wasp *Asobara japonica* induce epithelial cell death in the host *Drosophila* species and ensure parasitism success

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Parasitoid wasps are one of the most diverse groups of animals. Their ecological and taxonomic characteristics have been actively studied for a long time. Among them, a group of endoparasitoid wasps inject various venoms into their hosts to control their development and physiology to achieve successful parasitism. However, the molecular mechanisms of venom-induced host manipulation at the molecular and cellular level remain largely unexplored.To address this issue, we use *Asobara japonica*, a Braconidae endoparasitoid wasp, and the host species *Drosophila* as a model of endoparasitoid wasp-host interaction. *A. japonica* parasitizes a wide range of *Drosophila* species. The adult wasp lays a single egg inside the larval body of the host *Drosophila*. The mode of parasitism of *A. japonica* is “koinobiont,” as the wasp larva preys on its host body only after the host becomes a pupa. Eventually, the adult wasp emerges from the host fly pupal case.We found that *A. japonica* infection rapidly induced imaginal disc degeneration (IDD), which was mediated by apoptosis, autophagy, and inhibition of cell proliferation, while other tissues appeared intact. Furthermore, IDD was caused by the venom injected into the host by the wasp. To elucidate the molecular mechanism of the venom-mediated IDD, we performed whole genome sequencing of *A. japonica* [Kamiyama et al. *DNA Res.* 2022]. Transcriptomics, proteomics, and comparative genomics approaches allowed us to narrow down 63 candidate genes that are highly and exclusively expressed in the *A. japonica* venom gland. After screening these candidates using double-stranded RNA injection-based gene knockdown, we identified two candidate genes, both of which encode novel secretory proteins. RNAi of either of these two genes almost completely abrogated the venom-induced apoptosis, autophagy, and inhibition of cell proliferation in the host imaginal discs, significantly suppressing IDD. Notably, the RNAi wasps exhibited a lower parasitism success rate than the control wasps. Our study highlights a novel hijack strategy of the endoparasitoid wasp, which contributes to successful parasitism by preventing host metamorphosis.

[10-2-13] Exploitation of behavioral fever as a defense strategy against parasitoids

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Behavioural fever that defined as an acute change in thermal preference plays an important role in the survival of ectotherm animals post infection by pathogens. However, the underlying mechanisms remains unclear. Here, we found that *Drosophila* larvae displayed a behavioural fever and they preferred a much higher temperature zone post infection by *Leptopilina* wasps. This shift in temperature preference caused the mortality of parasitoids and increased the survival of parasitized hosts. Further experiments revealed that the behavioural fever was triggered by the increased expression of *Heat shock protein 70* (*Hsp70*) genes in parasitized hosts. Specifically, the absence of Hsp70 could cause the disappearance of behavioral fever response in the parasitized host larvae; while overexpression of Hsp70 alone would induce a preference for a higher temperature in normal host larvae. Furthermore, we observed that the behavioral fever induced the upregulation of antimicrobial peptides (AMPs) in parasitized hosts, leading to the dysfunction of gut microbiota of parasitoid wasps and the subsequent mortality. Together, our findings provide valuable insights into the mechanisms of behavioural fever for the ectotherm animals.

[10-2-14] Symbiont-induced modification of host’s adaptive phenotypes

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There are interesting examples where host insect phenotypes, such as external appearance and behavior, are markedly and adaptively altered by interactions with mutualistic symbiotic bacteria present in the host insect's body. Using the obligatory mutualistic relationship between stinkbugs and their gut symbiotic bacteria as model system, we aim at elucidating the molecular basis of host color change caused by symbiont infection and host behavioral change associated with vertical symbiont transmission to hatching larvae, thereby gaining insight into the coevolutionary trajectories from parasitism to mutualism by comparing them with the host manipulation mechanisms in parasitic relationships.

[10-2-15] Paleocene origin of a streamlined digestive symbiosis in leaf beetles

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Timing the acquisition of a beneficial microbe relative to the evolutionary history of its host can shed light on the adaptive impact of a symbiotic partnership. Here, we investigated the onset and molecular evolution of an obligate symbiosis between Cassidinae leaf beetles and *Candidatus* Stammera capleta, a γ-proteobacterium. Residing extracellularly within foregut symbiotic organs, *Stammera* upgrades the digestive physiology of its host by supplementing plant cell wall-degrading enzymes. We observe that *Stammera* is a shared symbiont across tortoise and hispine beetles that collectively comprise the Cassidinae subfamily, despite differences in their folivorous habits. In contrast to its transcriptional profile during vertical transmission, *Stammera* elevates the expression of genes encoding digestive enzymes while in the foregut symbiotic organs, matching the nutritional requirements of its host. Despite the widespread distribution of *Stammera* across Cassidinae beetles, symbiont acquisition during the Paleocene (~62 Mya) did not coincide with the origin of the subfamily. Early-diverging lineages lack the symbiont and the specialized organs that house it. Reconstructing the ancestral state of host-beneficial factors revealed that *Stammera* encoded three digestive enzymes at the onset of symbiosis, including polygalacturonase – a pectinase that is universally shared. While non-symbiotic cassidines encode polygalacturonase endogenously, their repertoire of plant cell wall-degrading enzymes is more limited compared to symbiotic beetles supplemented with digestive enzymes from *Stammera*. Highlighting the potential impact of a symbiotic condition and an upgraded metabolic potential, *Stammera*-harboring beetles exploit a greater variety of plants and are more speciose compared to non-symbiotic members of the Cassidinae.

[10-2-16] Identification of plant virus proteins responsible for the manipulation of host phenotype and vector behavior

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Host-vector manipulation by plant viruses is an evolutionary concept describing the effects of viruses on host plant phenotype and vector behavior, in ways that favor their transmission. To date, the effects of viral infections on host-plant/insect vector interactions have been well characterized, but the underlying molecular mechanisms are largely unknown. We are studying cauliflower mosaic virus (CaMV, *Caulimoviridae*) and turnip yellows virus (TuYV, *Solemoviridae*), two viruses transmitted by the green peach aphid (*Myzus persicae*) with different modes of transmission (semi-persistent non-circulative and persistent circulative, respectively), and therefore subjected to very different selection pressures. We have characterized the manipulative effects of these viruses and identified two CaMV proteins and one TuYV protein responsible for altering different sub-phases of the aphid probing or feeding behavior on infected plants. These virus-specific behavioral alterations (e.g., increased number of intracellular penetrations on CaMV-infected plants, longer phloem sap ingestion on TuYV-infected plants, etc.) appear well adapted to the specific transmission mode of each virus and could have important repercussions on virus acquisition by the vector. Transcriptomic and metabolomic analyses of the infected plants revealed a high number of deregulated genes and changes in metabolic pathways (such as those involved in the biosynthesis of ethylene, glucosinolate, and jasmonic acid) that could be responsible for the aphid behavioral alterations observed. Additional analyses on viruliferous aphids revealed metabolic changes (e.g., modification of the aphid lipid contents), which correlated with modifications of the aphid's longevity and dispersion abilities that could favor virus propagation. To get closer to a comprehensive understanding of this tripartite molecular dialogue, functional validation work is currently underway. Finding the genes and metabolic pathways that viruses manipulate in their host plants and insect vectors might help design strategies to stop virus spread by selecting, for example, plants that are less susceptible to virus manipulation.

シンポジウム講演者の集合写真

ICE 基調講演者の Nancy Moran 博士らを囲んでの国際交流

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