Memorial Symposium for
the 26th International Prize for Biology

Biology of Symbiosis
-Celebrating Dr. Nancy A. Moran-

December 7th and 8th, 2010
Tsukuba International Congress Center
Tsukuba, Japan
Tsukuba International Congress Center (EPOCHAL Tsukuba) Floor Guide

Symposium and Special Lecture: Convention Hall 300 (3F)
Coffee Break and Poster Session: Conference Room 102 (1F)

http://www.epochal.or.jp/eng/floor_guide/index.html
Tsukuba International Congress Center (EPOCHAL Tsukuba) Access Guide

Seats on the bus from Tsukuba to Narita must be reserved.

Access

*Diagram Showing Location of Bus Stops of Narita Airport [Terminal 1] [Terminal 2]

http://www.epochal.or.jp/eng/access/index.html
Area Map

http://www.epochal.or.jp/eng/access/map_kouiki.html
Map & Parking

Go up the escalator at Exit A3. Turn right at a ticket office, then go up the front steps. Go straight approximately for 800m.

http://www.epochal.or.jp/eng/access/map_shuhen.html
Program
<Day 1: Tuesday, December 7th, 2010>

9:30-10:30 Registration & Poster Setup

10:30-10:40 Makoto Asashima (National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan; Chair of the IPB Selection Committee)
“Opening address”

Session 1: Insect-Microbe Symbiosis (1)
Chair: Hiroaki Noda (National Institute of Agrobiological Sciences (NIAS), Tsukuba, Japan)

10:40-11:15 Serap Aksoy (Yale University, USA)
“Microbial influences on host physiology: Lessons learned from tsetse fly”

11:15-11:40 Tsutomu Tsuchida (RIKEN, Wako, Japan)
“Symbiotic bacterium modifies aphid body color”

11:40-12:15 John H. Werren (Rochester University, USA)
“The evolutionary consequences of Wolbachia symbionts”

12:15-12:40 Daisuke Kageyama (National Institute of Agrobiological Sciences (NIAS), Tsukuba, Japan)
“Endosymbiont-induced sex reversal in insects”

12:40-14:00 Lunch & Poster Session

Session 2: Insect-Microbe Symbiosis (2)
Chair: Takema Fukatsu (National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan)

14:00-14:35 Scott L. O’Neill (University of Queensland, Australia)
“Wolbachia symbionts and pathogen interference in insect hosts: implications for basic and applied biology”

14:35-15:00 Ryuichi Koga (National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan)
“Comparison of the vertical transmission processes between the obligate endocellular symbiont of aphid and the obligate gut symbiont of plataspid stinkbug”
15:00-15:35 **Howard Ochman** (Yale University, USA)
“Determinants of bacterial genome size and complexity”

15:35-16:00 **Shuji Shigenobu** (National Institute for Basic Biology (NIBB), Okazaki, Japan)
“Genomic revelations of a mutualism: the pea aphid and its obligate bacterial symbiont”

16:00-16:30 Coffee Break & Poster Session

**Session 3: Special Lecture by Dr. Nancy A. Moran**

16:30-16:50 **Takema Fukatsu** (National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan)
“The recipient of the 26th International Prize for Biology, Dr. Nancy A. Moran”

16:50-18:00 **Nancy A. Moran** (Yale University, USA)
“Genome evolution in heritable symbionts, and why it matters for their hosts”

18:00-18:30 General Discussion

18:30 **Isao Inoue** (University of Tsukuba, Tsukuba, Japan)
“Concluding remark of the day 1 program”

19:00- Reception Party
<Day 2: Wednesday, December 8th, 2010>

9:00-9:30 Registration

Session 4: Animal/Protist-Microbe Symbiosis (1)
Chair: Ken-ichiro Ishida (University of Tsukuba, Tsukuba, Japan)

9:30-9:55 Yoshitomo Kikuchi (National Institute of Advanced Industrial Science and Technology (AIST), Hokkaido, Japan)
“Amazing diversity of bacterial symbiosis in leeches”

9:55-10:30 Margaret McFall-Ngai (University of Wisconsin, USA)
“The chronic colonization of epithelia by Gram-negative bacteria: Insights from the experimental squid-Vibrio model”

10: 30-10:55 Yuichi Hongoh (Tokyo Institute of Technology, Tokyo, Japan)
“Termite, protists, and bacteria: a multilayered symbiosis for thriving on lignocellulose”

10:55-11:25 Coffee Break & Poster Session

Session 5: Animal/Protist-Microbe Symbiosis (2)
Chair: Masahiro Fujishima (Yamaguchi University, Yamaguchi, Japan)

11:25-12:00 Nicole Dubilier (Max Planck Institute, Germany)
“Chemosymbiosis: animal-microbe associations fueled by dark energy”

12:00-12:25 Yuuki Kodama (Kochi University, Kochi, Japan)
“Infection process of symbiotic Chlorella sp. to the alga-free Paramecium bursaria”

12:25-13:40 Lunch & Poster Session

13:40-14:15 John M. Archibald (Dalhousie University, Canada)
“Endosymbiosis and genome mosaicism in microbial eukaryotes”

14:15-14:40 Haruyo Yamaguchi (University of Tsukuba, Tsukuba, Japan)
“Is secondary endosymbiosis ongoing in Hatena arenicola?”

Session 6: Plant-Microbe Symbiosis (1)
Chair: Hiroshi Kouchi (National Institute of Agrobiological Sciences (NIAS), Tsukuba, Japan)
14:40-15:15 Eva Kondorosi (Institut des Sciences du Végétal, CNRS, France) “Disharmony in Rhizobium-legume symbiosis: the secret of plant dominance”

15:15-15:40 Haruko Imaizumi-Anraku (National Institute of Agrobiological Sciences (NIAS), Tsukuba, Japan) “Importance of Ca\(^{2+}\) signal sensing by CCaMK: a key regulator for accommodation of two different symbionts”

15:40-16:10 Coffee Break & Poster Session

16:10-16:35 Satoko Yoshida (RIKEN Plant Science Center, Yokohama, Japan) “Life as a parasite: host recognition by the parasitic plant Striga hermonthica”

**Session 7: Plant-Microbe Symbiosis (2)**
Chair: Makoto Hayashi (National Institute of Agrobiological Sciences (NIAS), Tsukuba, Japan)

16:35-17:10 Guillaume Bécard (CNRS/Université Paul Sabatier, France) "Has the ancestral arbuscular mycorrhizal symbiosis paved the way for other plant biotic interactions?"

17:10-17:45 Paola Bonfante (Department of Plant Biology, University of Torino, Italy) “Plants and arbuscular mycorrhizal fungi: born to be friends”

17:45-18:10 Yoshihiro Kobae (Nagoya University, Nagoya, Japan) “P and N transporters in mycorrhizal roots”

18:10-18:35 Seishi Ikeda (National Agricultural Research Center for Hokkaido Region (NARCH), Hokkaido, Japan) “Plant genetics meets microbial ecology: A new strategy for unraveling plant-microbes interactions”

18:35-19:00 General Discussion

19:00 Isao Inoue (Tsukuba University, Tsukuba, Japan) “Closing remark”
Abstract of Special Lecture
Symbiotic associations between insects and bacteria have evolved many times and are driven by a variety of effects of symbionts on hosts, including protection from enemies, manipulation of reproductive systems, and nutritional provisioning. Insect-bacterial symbioses based on nutrition are often obligate with strict maternal inheritance persisting over many millions of years; a primary example of an obligate nutritional symbiosis is that between aphids and \textit{Buchnera}. Symbionts with this unusual life style exist in relatively small, asexual populations. Genomic studies of nutritional symbionts show remarkable complementarity of gene repertoires of host and symbiont and of co-resident symbionts in the same host, as illustrated most dramatically in the sap-feeding leafhoppers, cicadas, and spittlebugs. These symbiont genomes also show extreme levels of genome reduction and include the four smallest known cellular genomes, derived from four different bacterial divisions. Despite their small size, these bacteria retain the central components of cellular information processing machinery, a feature that distinguishes them from organelles. Among capabilities lost by obligate nutritional symbionts are DNA repair functions, and most gene regulatory machinery. Symbionts seem to be reliant on regulatory mechanisms that are encoded in the host genome; these remain to be explored. Genome reduction in the symbionts sometimes erodes the pathways underlying the original contributions of symbionts to host nutrition. But hosts cannot easily escape their addiction to long-term symbionts, even if their nutritional needs are met without them, because host coadaptation results in developmental processes dependent on the presence of the symbiont cells.

Thus, obligate heritable symbiosis can be seen as a double-edged sword. On the one hand, it enables novel capabilities especially in animals, which are depauperate in biosynthetic capacity, and underlies expansion in ecological niches of hosts. Symbiont-host coadaptation makes their association more efficient. But, on the other hand, some degenerative processes are ongoing and irreversible, since lost genes are not recovered in these systems.
世代を経て伝えられる共生微生物のゲノム進化 —宿主生物との関わりの謎を解く—

ナシキー・アン・モラーン
イェール大学 生態・進化研究部門 教授

昆虫類と微生物の間の共生関係は、天敵への防御、生殖の操作、栄養の供給など、共生者が宿主に与えるさまざまな効果を背景として、何度も繰り返し進化してきました。昆虫と細菌の間の栄養的な関係は、しばしば生存に必須となり、厳密な母性遺伝によって何百万年、何億年にもわたり安定に維持されてきました。そもそももっともよく知られた例がアブラムシと細胞内共生細菌フナネラの間の栄養共生関係でしょう。このような風変わりな生活様式をもつ共生微生物は、比較的きわめて小型な無性生殖集団として生存しています。このような栄養共生微生物のゲノム研究から、宿主と共生微生物、さらには同じ宿主体内に共存する異なる共生微生物の間に、驚くべき遺伝子レパートリーの相補性が明らかになってきました。そのような仮想的な実例は、ヒメジョバイ、セミ、アワフキムシなどにみられます。これらの共生微生物のゲノムは著しくサイズが縮小しており、既知のもっとも小さな4種の細菌ゲノムを含みますが、それらは4つの異なる細菌群から独立に進化したもののです。これらの微生物のゲノムは極端に縮小しているものの、細胞情報処理の中核的な分子機構は維持されている点で、ミトコンドリアや葉緑体のような細胞内小器官とは一線を画しています。必須栄養共生微生物において失われた能力として、DNA修復機構や、ほとんどの遺伝子発現調節機構などがあります。こういった共生微生物は、宿主のゲノムにコードされた調節機構に依存しているようですが、その解明は今後の課題です。共生微生物におけるゲノム縮小は、時にもっとも宿主への栄養供給に必要だったはずの代謝経路の崩壊にまで及びることがあります。しかし宿主は長大な年月にわたる共生微生物への依存のくびきから容易に逃れることはできません。たとえ共生微生物が栄養要求性が満たされる状況となっても、共適応の帰結として宿主の発生過程が共生微生物の存在に依存してしまうからです。

したがって、世代を経て伝えられる必須な共生微生物は諸刃の剣であるといえます。一方で、共生微生物の獲得は宿主にとって新規な能力をもたらし、新たな生態的環境の開拓を可能にします。宿主と共生微生物の共適応により、その関係の効率はより高まるでしょう。しかし他方では、ゲノムや代謝経路の崩壊は着実にしかも不可逆的に進み、共生系から遺伝子や機能は失われていく一方なのです。
Abstract of Invited Lectures
Microbial influences on host physiology:
Lessons learned from tsetse fly
Serap Aksoy
Yale School of Public Health, Yale University, USA

Beneficial microbial symbionts serve important functions within their hosts, including dietary supplementation and maintenance of immune system homeostasis. Insects provide excellent systems to study the dynamic interplay between eukaryotic taxa and microbial fauna. We use the tsetse fly, *Glossina morsitans*, and its obligate mutualist, *Wigglesworthia glossinidia*, to investigate the co-evolutionary adaptations that influence host physiological processes. The obligate symbiont *Wigglesworthia* is maternally transmitted to tsetse’s intrauterine larvae, and we can produce flies that lack this symbiont by interfering with this process. Such offspring give rise to adults that exhibit a largely normal phenotype, with the exception being that they are reproductively sterile. Our results indicate that when reared under normal environmental conditions adults that lack *Wigglesworthia* have a highly compromised immune system. Our results demonstrate that *Wigglesworthia* in particular is required during the development of immature progeny in order for tsetse’s immune system to function properly during adulthood. This phenomenon provides evidence of yet another important physiological adaptation that further anchors the obligate symbiosis between tsetse and *Wigglesworthia*. 
Symbiotic bacterium modifies aphid body color

Tsutomu Tsuchida
Molecular Entomology Laboratory, RIKEN Advanced Science Institute,
Wako 351-0198, Japan

The world is full of colors, and many animals have color vision, recognizing their environment, habitat, food, enemies, rivals and mates by visual cues. Body color is thus an ecologically important trait, often involved in species recognition, sexual selection, mimicry, aposematism and crypsis.

In the pea aphid *Acyrthosiphon pisum*, red and green color morphs are found in the same populations. The color variation is also involved in the predation and parasitism pressure in natural aphid populations. An unexpected recent discovery by Moran and Jarvik showed that a carotenoid synthesis gene transferred from a fungus to the aphid genome is responsible for the red or green coloration of the aphids. Here we present another unexpected layer underlying aphid color polymorphism: a previously unrecognized endosymbiont that modifies insect body color in natural populations.

While screening pea aphid strains from natural populations collected in France, we found several strains of green aphids producing red nymphs. As the nymphs grew, their body color changed from reddish to greenish, and the adults became green. A survey of endosymbiotic microbiota in these aphid strains identified a novel aphid endosymbiont of the genus *Rickettsiella*. Diagnostic PCR surveys detected 7.9% (28/353) *Rickettsiella* infection in Western Europe populations of the pea aphid. Antibiotics treatments, artificial infection experiments and quantitative PCR analysis revealed that the *Rickettsiella* infection is responsible for green body color in at least some green pea aphids in natural populations.

Aphid body color mainly consists of yellow-red carotenoid and blue-green polycyclic quinones, and the *Rickettsiella* infection increased amounts of blue-green pigments while affecting yellow-red carotenoid pigments less. Considering that all these pigments increased in a similar pattern and no new pigments appeared upon the *Rickettsiella* infection, *Rickettsiella* probably does not synthesize the green pigments for itself but somehow interferes with the aphid metabolism to activate the green pigment production.

The effect of the endosymbiont on body color is expected to influence prey-predator interactions. Our finding would shed new light on some aspects in ecology and environmental adaptation of aphids and other living organisms.
The evolutionary consequences of *Wolbachia* symbionts

John (Jack) H. Werren

Department of Biology, University of Rochester, Rochester, NY 14627 USA

werr@mail.rochester.edu

*Wolbachia* are among the most abundant intracellular parasites on the planet, infecting upwards of 65% of terrestrial arthropods and many filarial nematodes. These bacteria are intimately associated with host cells and affect cell and reproductive biology of their hosts in diverse ways. Whether *Wolbachia* accelerate adaptive evolution and speciation in their hosts remains an open and controversial topic. Here I present data relating to the diversity of *Wolbachia*, their co-evolutionary interactions with hosts, and their role in accelerating host adaptive evolution. Lateral gene transfers from *Wolbachia* to invertebrate genomes are common and widespread. An important question is whether these transfers can lead to acquisition of novel gene functions. Findings relating to this question will be discussed.
Endosymbiont-induced sex reversal in insects
Daisuke Kageyama
National Institute of Agrobiological Sciences, Japan

Wolbachia pipientis, known as an intracellular symbiont of invertebrates, manipulates the reproductive system of its hosts in a variety of ways. By exerting these phenotypes (called reproductive manipulations), Wolbachia can rapidly spread and/or stably be maintained in host populations. Although potential impact of Wolbachia on host populations is attracting applied entomologists, almost nothing is currently known about the underlying mechanism of Wolbachia-induced manipulations. In some isopods, butterflies and leafhoppers, Wolbachia feminizes genetic males.

In the butterfly Eurema mandarina, genetically male individuals with a particular infection status develop to adults that display complete female phenotype. Furthermore, antibiotic treatment on larvae results in intersexual phenotype. Does Wolbachia affect sex-determining genes of Eurema? How and when does Wolbachia exert its effect? Does Wolbachia have a feminizing effect on other systems such as cell culture? In this symposium, I will talk about the fascinating biology of feminizing endosymbionts and recent findings on the mechanism underlying Wolbachia-induced feminization.
Wolbachia symbionts and pathogen interference in insect hosts: implications for basic and applied biology

Scott L. O’Neill

The School of Biological Sciences, University of Queensland, Australia

Recent studies have shown that Wolbachia symbionts of insects protect their hosts from a range of pathogens including RNA viruses, bacterial pathogens, nematodes and other eukaryotic parasites. These observed interactions have the potential to change our view of Wolbachia from acting exclusively as a reproductive parasite to a more complicated interaction with the host that includes reproductive parasitism and mutualism. This in turn may better help us understand Wolbachia invasion biology and provide a more comprehensive framework than provided solely by Bartonian wave dynamics. Pathogen interference may also present a major applied opportunity to use Wolbachia infections as a mechanism to prevent insect-transmitted disease of humans.
Comparison of the vertical transmission processes between the obligate endocellular symbiont of aphid and the obligate gut symbiont of plataspid stinkbug

Ryuichi Koga

National Institute of Advanced Industrial Science and Technology (AIST), Japan

Insects that feed on plant sap, such as aphids, coccids, planthoppers, cicadas and others, harbor a symbiotic bacterium specific to each host species. These symbionts are transmitted almost exclusively from mother to daughter for generations, that is to say, transmitted vertically. They provide their host insects with nutrients, such as essential amino acids and vitamins, and in turn, the hosts feed and shelter the symbionts in their body. Hence, the insects and bacteria are interdependent and involved in an obligately mutualistic relationship.

In this presentation, I will talk about two obligate symbionts of sap-feeding hemipteran insects. One is *Buchnera aphidicola* found in the most of extant aphid species, and the other is *Ishikawaella capsulata* harbored by the plataspid stinkbug *Megacopta punctatissima*. Both of the obligate symbionts play an equivalent nutritional role essential for survival and reproduction of their hosts. However, these symbionts occupy different organs in their host body. *Buchnera* is an endocellular symbiont that is housed within cells specialized for the symbiosis, called bacteriocytes. On the other hand, *Ishikawaella* is a gut symbiont that exclusively populates a characteristic midgut part bearing many crypts. Accordingly, it is also different how the insect hosts securely and vertically transmit their obligate symbionts, whose loss leads to death of the hosts. I will describe the details of the vertical transmission processes of these obligate symbionts, from histology to molecular levels, and discuss how the insect and bacteria suit their behavior, morphology, development, physiology and even genome to their symbiotic lifestyle.
Determinants of bacterial genome size and complexity

Howard Ochman
Department of Ecology & Evolutionary Biology, Yale University, USA

Bacteria are the most ancient, abundant, and genetically diverse organisms on Earth. The current repertoire of fully sequenced bacterial genomes has genome sizes ranging from 0.16 to over 13 megabases, with obligate pathogens and symbionts showing particularly small genomes sizes. By analyzing the genome size and contents of numerous bacterial lineages, we evaluate the roles of mutation, selection and genetic drift as causes of evolutionary change in genomes and in the functional capabilities of bacterial lineages. We find that variation in level of genetic drift coupled with the inherent bias toward deletions in bacterial genomes are the key forces that govern the evolution of genome size and complexity in bacteria.
Genomic revelations of a mutualism: the pea aphid and its obligate bacterial symbiont

Shuji Shigenobu
National Institute for Basic Biology, Okazaki 444-8585, Japan

Aphid species bear intracellular symbiotic bacteria in the cytoplasm of bacteriocytes, specialized cells for harboring the bacteria. The mutualism is so obligate that neither can reproduce independently. The newly released 464 Mb draft genome sequence of the pea aphid, *Acyrthosiphon pisum* [1], in consort with that of bacterial symbiont *Buchnera aphidicola* [2], illustrates the remarkable interdependency between the two organisms. Genetic capacities of the pea aphid and the symbiont for amino acid biosynthesis are complementary. The genome analysis revealed that the pea aphid has undergone characteristic gene losses and duplications. The IMB antibacterial immune pathway is missing several critical genes, which might account for the evolutionary success of aphids to obtain beneficial symbionts. Lineage-specific gene duplications have occurred in genes of broad range of functional categories, which includes signaling pathways [3], miRNA machinery, chromatin modification and mitosis. The importance of these duplications for the symbiosis remains to be determined. We found several instances of lateral gene transfer from bacteria to the pea aphid genome. Some of them are highly expressed in bacteriocytes.

Aphid research is entering post-genome era. We analyzed the transcriptome of the aphid bacteriocytes using RNA-seq technology featuring a next-generation DNA sequencer [unpublished]. We found thousands of genes over-represented in the symbiotic organ in comparison with the whole body. Many genes for amino acid metabolisms are found to be over-represented as expected: the plant sap-eating insect depends on the bacterial symbionts to supply essential amino acids. In addition, many kinds of novel secretion proteins that are found only in aphid species are extremely enriched in the bacteriocytes. We also found that bacteriocytes express *Distal-less (Dll)*, a homeodomain-containing transcription factor throughout the life cycle [4]. The future study should focus on dissecting the genetic network of these components, which should allow us to understand the genetic basis on which symbiosis generates evolutionary novelty.

References:
Amazing diversity of bacterial symbiosis in leeches

Yoshitomo Kikuchi

National Institute of Advanced Industrial Science and Technology (AIST), Hokkaido, Japan

Most animals that feed exclusively on vertebrate blood, such as tsetse flies and lice, harbor endosymbiotic bacteria within their body. These symbionts provide their hosts with vitamin B compounds that are scarcely contained in blood. Among bloodsuckers, leech species exhibit an amazing diversity of bacterial endosymbiosis. The following three distinct bacterial associations have been found: (i) the mycetocyte, which is a large symbiont-containing cell associated with the esophagus; (ii) the bladders, in which a stratified bacterial-community occurs; and (iii) the digestive tract, where two bacterial species predominantly habitate the gut microbiota. Recent advancement in biological molecular techniques, such as molecular phylogeny, fluorescence in situ hybridization (FISH), and microbial genetics, enables us to analyze the leech microbiota. Here, I first introduce the various mycetocyte and bladder symbionts in leech species, wherein a broad range of bacteria, mainly the Proteobacteria and Bacteroidetes, have been characterized as leech endosymbionts. Thereafter, the molecular basis of the host-symbiont interactions in the leech gut symbiosis, specifically that of Aeromonas veronii and Hirudo verbana, will be highlighted. The leech gut symbiont, A. veronii, is culturable and genetically manipulable. These biological features enabled the synthesis of a transposon mutant library. Screening the transposon mutants of A. veronii for colonization defects within the leech host revealed that the type III secretion system (T3SS), previously characterized as a virulence factor in many disease agents, was pivotal for symbiont colonization in the leech gut. Comparative studies based on the T3SS reveal the differences and similarities occurring between symbiosis and pathogenesis.


The chronic colonization of epithelia by Gram-negative bacteria: Insights from the experimental squid-Vibrio model

Margaret McFall-Ngai
University of Wisconsin-Madison, USA

The application of new genomic methods over the last decade has revealed that the most prevalent symbiotic relationships are the essential, coevolved partnerships that animals have with beneficial bacteria, or mutualistic symbionts. The majority of such associations share a set of common characteristics: they are established anew each host generation, remain stable throughout the host's lifetime, and occur as interactions at the apical surfaces of epithelial cells along the mucosal surfaces. These new findings afforded by advances in biotechnology beg the questions: (i) how do animals initially recognize their appropriate partners, and (ii) how do they maintain them in balance? Biologists are taking a variety of approaches to address these questions. Because in humans and other vertebrates these alliances involve hundreds to thousands of microbial species, several invertebrate associations, which typically have much simpler symbiont communities, are being exploited as natural experimental models. In much the same way as the fruit fly and worm have been used to establish basic principles of developmental biology, these invertebrate symbioses provide a window into the conserved mechanisms underlying interspecies co-existence and communication. This presentation will describe work in one such model, the association between the Hawaiian bobtail squid, Euprymna scolopes, and its bioluminescent bacterial partner, Vibrio fischeri. Both partners are independently culturable under laboratory conditions and, because the principal product translocated to the host by the symbiont is luminescence, the fitness of the host under non-symbiotic conditions is not compromised. Genetics have been developed in the bacterial symbiont, and several full genome sequences of various V. fischeri strains are now available. A unigene set of 14,000 unique clusters, derived from ESTs of the host light organ, has been generated. These resources have been used to characterize the transcriptomic responses of the host and symbiont to the stages of the symbiosis. Specifically, we have used this binary system to study cellular, biochemical and molecular mechanisms underlying harvesting of the symbiont from the environment, the processes of recognition and specificity, reciprocal partner development in response to symbiosis, and the dynamic stability of the mature association. One unifying theme is the prevalence of features typically associated heretofore with microbial pathogenesis of animal tissues. Because biologists are now recognizing that beneficial symbiosis are more prevalent than pathogenesis in animals, mutualism is likely to be the principal driving selection pressure on the characters of animal-bacterial interaction.
Termites, protists, and bacteria: a multi-layered symbiosis for thriving on lignocellulose

Yuichi Hongoh
Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Tokyo 152-8550, Japan

Termites are key stone animals in the global carbon cycle. They vigorously decompose dead plant matters and contribute 2% of the total CO2 emissions from land. Their ability to feed solely on recalcitrant, nitrogen-poor lignocellulose is mostly attributable to the activities of the gut microbes, which comprise protists, bacteria, and archaea. Extensive physiological and biochemical analyses of the entire gut have revealed that the gut microbes are responsible for lignocellulose degradation, nitrogen fixation, and reductive acetogenesis or methanogenesis from H2 plus CO2. However, the difficulties in cultivation of most microbial species in the gut have hampered the clarification of the detailed community structure and the functions of the individual community members.

Our research group has adopted culture-independent approaches and has been revealing the phylogenetic and spatial community structure of the gut microbes (1). We have also been attempting to unveil the functions of and interrelationships among the individual microbial species in the gut by acquiring their complete genome sequences using isothermal whole genome amplification (WGA) technique. We targeted unculturable bacterial species, named Rs-D17 and CfPt1-2, which are intracellular symbionts of the unculturable cellulolytic protists Trichonympha agilis and Pseudotrichonympha grassii, respectively, present in termite guts. There had been no information on the physiology of these bacteria. Several hundred cells of each of the intracellular symbionts were collected and subjected to WGA. From the amplified samples, we successfully reconstructed single circular chromosomes of Rs-D17 (2) and CfPt1-2 (3) without ambiguity.

In my talk, I summarize the recent achievements of our research group and outline this highly evolved, multi-layered symbiotic system.

References:

Email: yhongo@bio.titech.ac.jp
HP: http://www.hongoh.bio.titech.ac.jp/
Chemosymbiosis:
animal-microbe associations fueled by dark energy

Nicole Dubilier
Symbiosis Group, Max Planck Institute for Marine Microbiology,
Celsiusstr. 1, D-28359 Bremen, Germany.
ndubilie@mpi-bremen.de

The discovery of deep-sea hydrothermal vents in 1977 was one of the most profound findings of the 20th century, revolutionizing our perception of energy sources fueling primary productivity on earth. These ecosystems are based on chemosynthesis, i.e., the fixation of carbon dioxide into organic compounds, as in photosynthesis, but using inorganic compounds such as sulfide or methane as energy sources instead of sunlight. Most of the biomass at hydrothermal vents is generated through symbiotic associations between chemosynthetic bacteria and marine invertebrates in which the bacteria “feed” their hosts via carbon fixation, analogous to the chloroplasts of algae and plants.

Remarkably, it took the discovery of these symbioses in the deep-sea for scientists to realize that chemosynthetic symbioses occur worldwide in a wide range of habitats including colds seeps, whale and wood falls, shallow-water coastal sediments, and continental margins. The evolutionary success of these symbioses is visible in the diversity of animal groups that have established associations with chemosynthetic bacteria with at least seven invertebrate phyla currently known to host these symbionts. The phylogenetic diversity of the bacterial symbionts is equally high, and phylogenetic analyses show that these associations have evolved multiple times in convergent evolution. In my presentation, I will describe what is currently known about the interactions between chemosynthetic symbionts and their hosts, focusing on the molecular, metagenomic, and metaproteomic methods used in my research group to study these associations.
Infection process of symbiotic *Chlorella* sp. to the alga-free *Paramecium bursaria*

Yuuki Kodama
Research and Education Faculty, Natural Sciences Cluster, Sciences Unit, Kochi University, Kochi, Japan

*Paramecium bursaria* cells harbor several hundred symbiotic algae in their cytoplasm. Each symbiotic alga is enclosed in a perialgal vacuole (PV) derived from the host digestive vacuole DV, which protects the alga from lysosomal fusion. Although the association of *P. bursaria* with *Chlorella* sp. has long been regarded as a mutualistic symbiosis, alga-free paramecia and symbiotic algae are capable of growing independently and paramecia can be reinfected experimentally by mixing them. This phenomenon provides an excellent model for studying cell-to-cell interaction and the evolution of eukaryotic cells through secondary endosymbiosis between different protists. However, the detailed algal infection process remained unknown for a long time. Using pulse labeling of the alga-free paramecia with the isolated symbiotic algae and chase method, the timing of acidification and lysosomal fusion of the DVs and of algal escape from the DVs were clarified. Furthermore, we found four necessary cytological events for establishing endosymbiosis. (1) At about 3 min after mixing, some algae show resistance to the host lysosomal enzymes in the DVs, even if the digested ones are present. (2) At about 30 min after mixing, the alga starts to escape from the DVs as the result of the budding of the DV membrane into the cytoplasm. (3) Within 15 min after the escape, the DV membrane enclosing a single green alga differentiates to the PV membrane, which provides protection from lysosomal fusion. (4) The alga localizes at the host cell cortex by affinity of the PV to unknown structures of the host. At about 24 h after mixing, the alga multiplies by cell division and establishes endosymbiosis. Infection experiments with infection-capable and infection-incapable algae indicate that the infectivity of algae is based on their ability to localize beneath the host surface after escaping from the DVs. This algal infection process differs from known infection processes of other symbiotic or parasitic organisms to their hosts.
Endosymbiosis and genome mosaicism in microbial eukaryotes

John M. Archibald

Canadian Institute for Advanced Research, Program in Integrated Microbial Biodiversity, Department of Biochemistry & Molecular Biology, Dalhousie University, Halifax, Nova Scotia, Canada

The process of endosymbiosis has had a profound impact on the evolution and diversification of the eukaryotic cell. Plastids, the photosynthetic organelles of modern-day plants and algae, evolved from free-living cyanobacteria that were taken up by eukaryotic hosts and transformed into a permanent sub-cellular compartment. Unlike the endosymbiotic origin of mitochondria, which probably occurred in the common ancestor of extant eukaryotes, the endosymbiosis that gave rise to plastids took place after the deepest divergences in eukaryotic evolution had already occurred. This pivotal event paved the way for the evolution of a diverse array of algal lineages and for the spread of plastids between unrelated eukaryotic lineages by ‘secondary’ (i.e., eukaryote-eukaryote) endosymbiosis. In this presentation I will (i) provide an overview of the genetic, molecular and cell biological aspects of this complex process, (ii) discuss the genome biology of two secondary plastid-bearing algae of pivotal evolutionary significance, the cryptophyte *Guillardia theta* and the chlorarachniophyte *Bigelowiella natans*, and (iii) present evidence for extensive genome mosaicism in these and other algae.
Is secondary endosymbiosis ongoing in *Hatena arenicola*?

Haruyo Yamaguchi

Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan

Eukaryotes, comprising eight supergroups, are a major component of life on present-day earth. The evolution and diversification of eukaryotes were realized by an increase in primary production. Oxygenic photosynthesis, originally evolved in cyanobacteria, was transferred to eukaryotes by endosymbiosis. The Plantae is a supergroup that is believed to have acquired a cyanobacterium and integrated it as a plastid. However, plastids are also distributed in five additional supergroups. The photosynthetic groups of these supergroups are believed to have acquired plastids via engulfment of eukaryotic algae. These evolutionary events, known as secondary endosymbioses, were a major driving force of the diversification of eukaryotes. However, our understanding of the evolutionary processes of secondary endosymbiosis is fragmented. It is therefore important to study organisms that are in the early stages of plastid acquisition.

*Hatena arenicola* is a flagellate belonging to the phylum Kathabrepharida and possessing green endosymbionts originating from *Nephroselmis* spp. (Chlorophyta). Ultrastructural studies show that the symbiont plastid is enlarged, whereas other organelles either remain or are completely lost. In addition, the eyespot of the symbiont is always positioned at the cell apex of the host cell. These facts indicate that there are certain functional associations between *H. arenicola* and *Nephroselmis*, although *H. arenicola* accepts several closely related species of *Nephroselmis* as symbionts.

The most interesting feature of *H. arenicola* is cell division. The symbiont is inherited by only one daughter cell, resulting in one colorless cell and one symbiont-harboring green cell. Without exception, the symbiont is inherited by the right-hand daughter cell when viewed from the dorsal side of the cell, suggesting that *H. arenicola* controls the inheritance of the symbiont. In the colorless cell, a complex feeding apparatus appears at the cell apex, the location occupied by the eyespot in symbiont-harboring cells. The colorless cell then engulfs free-living *Nephroselmis* from the outside and returns to the symbiont-harboring cell.

To establish permanent symbiosis, both the limitation of the symbiont to a single species and the synchronized cell division between *H. arenicola* and the symbiont should evolve. This organism is a good example of an early stage of secondary endosymbiosis, and it would be an ideal system to study the “pioneer symbiotic genes” required in the early stage of endosymbiosis.
Disharmony in *Rhizobium*-legume symbiosis:
the secret of plant dominance
Eva Kondorosi

BAYGEN Institute, 6726 Szeged, Hungary, Institut des Sciences du Végétal
CNRS UPR 2355, 91198 Gif sur Yvette, France

Symbiosis between legume plants and *Rhizobium* soil bacteria results in the formation of nitrogen fixing root nodules. This relationship is mutually beneficial as bacteria support plant growth by reduction of the atmospheric nitrogen to ammonia inside the nodule cells while the plant supplies the bacteria with photosynthetic products and the energy for nitrogen fixation. Nodule development is induced by bacterial lipochitooligosaccaride signal molecules, the Nod factors. Nodule maturation and functioning require, however, further and still uncovered signaling events between the partners. In addition, symbiotic cell functioning in *Medicago truncatula* and closely related species (belonging to the IRL Clade), necessitates a remarkable, plant controlled differentiation process of the bacteria resulting in living, non-dividing nitrogen fixing bacteria called bacteroids that are polyploid, largely elongated and branched with definitive loss of cell division capacity. Thus, in these interactions, the benefit of symbiosis is imbalanced as the plant exploits the nitrogen fixation ability of the bacteroids and inhibits their return to the free living state.

We have identified a wide spectrum of nodules specific peptides in *M. truncatula* resembling antimicrobial peptides of the innate immunity that govern this irreversible terminal differentiation of bacteroids. The majority of these peptides (>400) belong to the NCR family. The *NCR* genes are only expressed in the symbiotic cells but at different stages of bacteroid development. The NCR peptides are targeted to the bacteroids via the secretory pathway of the nodule cells, and are essential for bacteroid differentiation. Direct action of NCRs on bacteria, inducing features of terminal bacteroid differentiation, has been demonstrated both *in planta* and *in vitro*. Our present studies are focused on the identification of plant peptides present in the bacteroids, their bacterial targets and mode of action and how their concerted actions control bacterial cell number and cell fate in symbiosis.
Legumes have mutualistic symbiotic relationships with rhizobial bacteria and arbuscular mycorrhizal (AM) fungi. Two distinct symbioses share a single signaling pathway designated as “common symbiosis pathway (CSP)”. In response to signal molecules derived from these symbiotic-microbes, a periodic change of cytosolic Ca$^{2+}$ concentration, Ca$^{2+}$ spiking, is induced. Because almost all loss-of-function mutants of genes belonging to CSP lose the ability to induce Ca$^{2+}$ spiking, it is generally accepted that the induction of Ca$^{2+}$ spiking through CSP is essential for the symbiotic interactions.

CCaMK (Calcium Calmodulin-dependent protein kinase) has been isolated as a member of CSP and is positioned downstream of Ca$^{2+}$ spiking. Due to its unique structural features that enable CCaMK to sense both CaM and Ca$^{2+}$, CCaMK is supposed to be a strong candidate for the decoder of Ca$^{2+}$ signals induced by microbial signal molecules. However, it remains unknown how CCaMK is activated differently by these two different symbionts. In this presentation, I will focus on the recent advances on the differential requirement of Ca$^{2+}$/CaM for activation of CCaMK in accommodation of rhizobia and AM fungi and propose a scheme for CCaMK activation in the symbiotic signal transduction.

References
Life as a parasite:
host recognition by the parasitic plant *Striga hermonthica*

Satoko Yoshida
RIKEN Plant Science Center, Yokohama, Japan
satokoy@psc.riken.jp

Approximately 1% of angiosperm species are adapted to parasitic life style. Parasitic plants infest their host plants and rob them of water and nutrients. Therefore weedy parasitic species cause serious damages on agriculture. Especially *Striga hermonthica* (common name witchweed), an obligate root parasite, severely affects the crop yields in sub-Saharan Africa, as it parasitizes major crops such as sorghum, maize and upland rice. The estimated yield losses are up to 7 billion US dollars per year.

Life cycle of *S. hermonthica* depends on host-derived signals. *Striga* seeds remain dormant until they are exposed to host-exudated germination stimulants called strigolactones. Recently, strigolactones were found to have hormonal function to inhibit shoot branching (Umehara et al. 2008, *Nature*, 455, 195-200). *Striga* exploits a host developmental signal to recognize the presence of the host. A germinated *Striga* seedling forms a specialized penetration organ called haustorium in response to host-derived induction factors. The root tips of the parasite develop radial swelling and haustorial hairs that function as attachment anchors and penetration pegs. After successful attachment and penetration of a host root, the parasite establishes vascular connections.

*S. hermonthica* parasitizes monocotyledonous Poaceae species but not dicotyledonous species. However, the germination inducible strigolactone and haustorium inducing factors are generally found in both monocots and dicots. We investigated the *S. hermonthica* interaction with various host and non-host species. *S. hermonthica* is able to penetrate even in non-host Arabidopsis and cowpea, but their growth stops after vasculature connection. Interestingly, using other parasitic species *Phtheirospermum japonicum* as a potential host, *S. hermonthica* could not penetrate *P. japonicum* roots. Parasitic plants may possess a system to avoid parasitize each other. Thus compatibility between *S. hermonthica* and its host is likely to be defined by multiple factors (Yoshida and Shirasu, 2009, *New Phytol*. 183, 180-189).

We performed the large scale EST analysis to set up molecular analysis tools for *S. hermonthica* research. Through this analysis, we found an example of horizontal gene transfer of host nuclear-encoded gene to *S. hermonthica* (Yoshida et al. 2010, *Science*, 328, 1128). Intimate relationship between host and parasite results in transfer of a genetic component during evolution.
Has the ancestral arbuscular mycorrhizal symbiosis paved the way for other plant biotic interactions?

Guillaume BECARD
SCSV, UMR 5546 CNRS/Université Paul Sabatier, 31326,
CASTANET-TOLOSAN, France

A molecular communication takes place very early between the partners of the arbuscular mycorrhizal (AM) symbiosis. Before physical contact each partner secretes diffusible molecules that can trigger in the opposite partner metabolic responses, new gene expression and developmental modifications. Trace molecules such as strigolactones present in root exudates serve as plant host recognition signals for AM fungi. They induce spore germination and/or hyphal branching at extremely low concentrations by rapidly stimulating the fungus mitochondrial activity. Interestingly strigolactones are also germination stimulant of the obligate parasitic weeds *Orobanche* and *Striga* and they have recently been proposed as a new class of plant hormones controlling bud outgrowth.

Symmetrically, based on genetic, molecular, biochemical and physiological evidence AM fungi were also expected to produce important early signals perceived by their plant host. The so called Myc factors were expected to trigger the plant mycorrhization programme via the common SYM pathway. These Myc factors have recently been identified. They are simple lipochitooligosaccharides (LCOs) that can stimulate at extremely low concentrations mycorrhization of non legumes. They can also stimulate lateral root formation in the model legume *Medicago truncatula*. This activity on root development is dependent on DMI1, DMI2, DMI3 and NSP2, four proteins involved in the nodulation signalling pathway triggered by Nod factors.

The fact that strigolactones and LCOs, involved in a very ancient plant biotic interaction, are also important signals in “modern” interactions with parasitic weeds and with rhizobia, raises the question of their possible occurrence in other plant biotic interactions. It also encourages to pursuing the search for other signals involved in later stages of the AM symbiosis.
Arbuscular mycorrhizas (AMs) are symbiotic associations, which are commonly described as the result of co-evolution events between Glomeromycota fungi (Schussler et al., 2001) and plants where both partners benefit from the reciprocal nutrient exchange (Bonfante and Genre, 2010). Data from fossil records, recent characterizations of AM fungi in basal plant taxa, and live cell imaging of angiosperm colonization processes indicate the ancient origin of AM interaction and provide evidence of conserved cellular traits. Among them, the presence of a symbiotic interface compartment which allows fungal development inside the cell lumen and maintains host cell integrity is considered a landmark of AM establishment.

This presentation will focus on mechanisms which are associated with the perception of the AM fungus and its accommodation inside the lumen of the host plant cell, leading to the assembly of the perifungal membrane and symbiotic interface. Our findings, based on an in vivo confocal microscopy approach, demonstrate that root cells perceive AM fungal signals and trigger calcium-mediated signaling in their nucleoplasm, both before and upon direct contact with the fungus (Chabaud et al., 2010). Such calcium signals position within the so-called SYM pathway, the signal transduction pathway that controls AM establishment. Nuclear calcium spiking is a pre-requisite to the cellular reorganization that initiates after the adhesion of the fungal hyphopodium to the root and leads to the assembly of the so-called prepenetration apparatus (PPA) inside one or more contacted epidermal cells. Lastly, the PPA seems to be instrumental for the assembly of the interface construction (Genre et al., 2008).

These features have been identified in legumes and in non-legume plants, but so far not in naturally collected plants or in lower taxa. Answers to these questions may help to solve ongoing debate on plant-fungal co-evolution and on the functional role of AMF in natural systems.

References
P and N transporters in mycorrhizal roots
Yoshihiro Kobae
Graduate School of Bioagricultural Sciences, Nagoya University, Japan

The majority of land plants acquire soil nutrients, such as phosphorus and nitrogen, not only from the root surface but also through arbuscular mycorrhizal (AM) fungi. In the latter case, periarbuscular membranes, which envelope the highly branched hyphae within cortical cells, have been thought to be the major site of nutrient exchange. We are investigating mechanisms of nutrient absorption through AM fungi, using rice and soybean, the most important cereal and leguminous crops in the world. We prepared transgenic rice plants that express a fusion of rice AM-inducible Pi transporter (PT) OsPT11-GFP, and grew them with AM fungi. The fluorescence of the fusion PT was specifically observed around arbuscule branch domain. We also invented a simple device for in vivo observation of fluorescent proteins in living AM roots. In cortical cells, arbuscules were cell-autonomously developed and seemed to be functional for only 2.5 days. Suddenly, the arbuscular branches shrank. At this stage, however, the periarbuscular membranes appeared intact. Then, the fluorescence of the transporter disappeared within only 2.5 h. In addition, we found 14 PT genes on the soybean genome. We extracted total RNA from AM fungi-infected soybean roots and non-infected roots, and then performed RT-PCR experiments using specific primer pairs for each PT gene. As a result, we identified three AM-inducible PT genes of soybean. Their phylogenetic relationship and expression patterns will be presented. Next, in the rice genome databases, we found 12 ammonium transporter (AMT) genes. Among them, only one AMT gene (OsAMT3.1) was specifically induced in AM roots to a high level. We also found 16 AMT genes in the soybean genome, five of which were AM-inducible. Among them, promoter-reporter analysis indicated that the strongest one, GmAMT4.1, showed specific expression in arbuscule-containing cortical cells. Moreover, limited localization of fusion proteins of OsAMT3.1-GFP and GmAMT4.1-GFP on the branch domain of periarbuscular membranes of rice and soybean, respectively, where active ammonium transfer must occur, was demonstrated for the first time.
Day 2, 18:10~18:45

Plant genetics meets microbial ecology:
A new strategy for unraveling plant-microbes interactions
Seishi Ikeda
National Agricultural Research Center for Hokkaido Region (NARCH), Hokkaido, Japan

The phytosphere is an attractive habitat for microorganisms due to an abundance of nutrients and relative environmental stability. Conceptually, this unique environment consists of three main habitats for plant-associated microbes: the phyllosphere (leaf), the endosphere (stem) and the rhizosphere (root). Each of these habitats provides a considerably diverse physical, chemical and biological environment, and as consequence can support a wide range of microbial groups. Diverse microorganisms that occupy these habitats assist in the uptake of nutrients from soils and can exert considerable influence upon the overall health of host plants. These microorganisms range from neutral microorganisms with no obvious effects upon the host plants to pathogens and mutualistic symbionts with deleterious and beneficial effects, respectively. However, questions about driving forces shaping the community structures of these plant-associated microbes are still largely unknown as the phytosphere contains a phylogenetically as well as functionally broad spectrum of microorganisms with different degree of interaction with their host plants.

During their evolution, leguminous plants developed two systems to attain mutual symbioses with rhizobia and mycorrhizae. The genetic requirements for rhizobial and mycorrhizal interactions in plants overlap in a common symbiosis pathway (CSP) leading to successful symbioses. Legumes are also known to negatively control the degree of nodulation and mycorrhization of roots, respectively. This autoregulatory mechanism occurs through a long-distance signaling between shoots and roots. Plants deficient in the autoregulation of nodulation fail to control the nodulation and mycorrhization and develop hypernodulated roots. Because of these great successes of plant genetics in revealing the systems to attain symbioses with rhizobia as well as mycorrhizae, a part of above questions now can be addressed using legume mutants relevant to genes for symbioses. In addition, recent technical advances in microbial ecology also now enable the tracing and assessment of plant-associated microorganisms using rapid and simple molecular methods with free of any culture-dependent bias.

By taking advantage of the situation described above, the global diversity of symbiotic bacteria in field-grown soybean was investigated in relation to different nodulation genotypes and nitrogen fertilization levels. The results revealed that a subpopulation of Proteobacteria in aerial parts of soybean was likely to be regulated through both the autoregulation system for plant–rhizobium symbiosis and the nitrogen sensing system in a similar manner. Phylogenetic analyses of the bacterial communities revealed that legumes accommodate a taxonomically characteristic microbial community including novel bacterial species. More importantly, the results with hypernodulated soybean, which is genetically impaired for the autoregulation of nodulation, imply that the presence of novel plant signaling pathway for regulating symbiotic bacterial community. Moreover, multivariate analyses of the community structures clearly indicated that a nitrogen fertilization level could be a dominant force in shaping the community structures of plant-associated bacteria for aboveground plant tissues, over and above nodulation phenotype and the tissue specificity of bacterial colonization, suggesting the importance of the plant nutrient status in plant–microbe interactions.

These results clearly indicate that the interdisciplinary works between plant genetics and microbial ecology will provide a new strategy to unravel complex plant-microbes interactions and will promise to lead a whole new level of understanding for symbioses in phytosphere for viewpoints of both basic and applied sciences.
Poster Presentations
P01 Evolutionary maintenance of selfish homing endonuclease genes in the absence of horizontal transfer
* Koji Yahara (1,2), Masaki Fukuyo (3), Akira Sasaki (4,5,6), Ichizo Kobayashi (3)

P02 Genetic basis of flower color evolution in daylily (*Hemerocallis fulva*) and nightlily (*H. citrina*)
* Kozue Nitta (1), Yumi Sakaguchi (1), Misako Mishima (1), Yoshihiro Ozeki (2), Akiko A. Yasumoto (3), Tetsukazu Yahara (1)
(1) Kyushu Univ., (2) Tokyo Univ. Agr. Tech., (3) Kyoto Univ./Univ. Zurich

P03 Coevolution and the origin of species: speciation gene acts for anti-predator adaptation by pleiotropy for left-right reversal in snails
* Masaki Hoso (1), Yuichi Kameda (2), Shu-Ping Wu (3)

P04 Mothers never miss the moment: vertical transmission mechanism of the gut symbiont in a subsocial insect
* Takahiro Hosokawa (1), Mantaro Hironaka (2), Hiromi Mukai (3), Takema Fukatsu (1)
(1) AIST, (2) Hamamatsu Univ. Sch. Med., (3) Saga Univ.

P05 Community-wide effects of belowground rhizobia on aboveground arthropods
* Noboru Katayama (1), Zhi Qi Zhang (1), Takayuki Ohgushi (1)
(1) Kyoto Univ.

P06 *Wolbachia* population density is regulated in *Drosophila* germline through its transport from germline to soma
* Kazuya Hashiyama (1), Chiyo Noda (1), Satoru Kobayashi (1)

P07 The role and function of the *Wolbachia* effector protein
* Manabu Ote (1), Morio Ueyama (2), Daisuke Yamamoto (1)
(1) Tohoku Univ. (2) Soka Univ.

P08 Female mating strategy during precopulatory mate guarding in spider mites
* Keiko Oku (1)
(1) NARC

P09 Intracellular symbiosis of double strands RNA in ant species, *Camponotus yamaokai*
* Toshiyuki Satoh (1), Saori Miyazaki (2), Hiromitsu Moriyama (1), Toshiyuki Fukuhara (1)

P10
Group-hibernating gynes of the paper wasp *Parapolybia indica* (Hymenoptera, Vespidae, Polistinae) and their endoparasitic nematode (Tylenchomorpha, Sphaerularioidea, Sphaerularia)
*Fuki Saito (1), Iki Kawamura (1), Jun-ichi Kojima (1)
(1) Ibaraki Univ.

P11
Metabolomic and enzymatic analysis of cellulose digestion in termites
*Gaku Tokuda (1), Kumiko Kihara (2), Yuuri Tsuboi (2), Shigeharu Moriya (2), Jun Kikuchi (2)
(1) Univ. Ryukyus, (2) RIKEN

P12
Altruistic colony defense by post-reproductive females in a social aphid
*Keigo Uematsu (1), Mayako Kutsukake (2), Takema Fukatsu (2), Masakazu Shimada (1), Harunobu Shibao (1)
(1) Univ. Tokyo, (2) AIST

P13
Gall repair and regeneration by soldier aphids in the social aphid, *Nipponaphis monzeni*
*Mayako Kutsukake (1), Harunobu Shibao (2), Keigo Uematsu (2), and Takema Fukatsu (1)
(1) AIST, (2) Univ. Tokyo

P14
Male killing caused by a *Spiroplasma* symbiont in the small brown planthopper *Laodelphax striatellus*
Sachiyo Sanada-Morimura (1), Masaya Matsumura (1), Hiroaki Noda (2)
(1) KONARC, (2) NIAS

P15
Genes specifically expressed in the bacteriome of the leafhopper *Nephotettix cincticeps*
*Makoto Tomizawa (1, 2), Hiroaki Noda (1, 2)
(1) NIAS, (2) Univ. Tokyo

P16
Glycosome-like organelles in diplonemids
*Takeshi Nara (1), Takashi Makiuchi (1, 2), Takeshi Annoura (3), Muneaki Hashimoto (1), Tetsuo Hashimoto (3)
(1) Juntendo Univ., (2) NIID, Japan, (3) Univ. Tsukuba

P17
Trans-splicing in the intron-poor eukaryotic parasite *Giardia intestinalis*
*Ryoma Kamikawa (1), Yuji Inagaki (1), Masaharu Tokoro (2), Andrew J. Roger (3), Tetsuo Hashimoto (1)
(1) Univ. Tsukuba, (2) Kanazawa Univ., (3) Dalhousie Univ.

P18
EST-based screening for genes involved in host–symbiont crosstalk in the bean bug *Riptortus pedestris*
*Ryo Futahashi (1), Kohjiro Tanaka (1), Masahiko Tanahashi (1), Yoshitomo Kikuchi (1), Takema Fukatsu (1)
(1) AIST

P19
QTL mapping of a larval-performance trait in the leaf mining moth *Acrocercops transecta*
*Issei Ohshima (1), Mitsuyasu Hasebe (1,2)

P20
Wolbachia infection is associated with sex determination and mating systems in ambrosia beetles (Curculionidae: Scolytinae)
*Yuuki Kawasaki (1), Hisashi Kajimura (1)
(1) Nagoya Univ.

P21
A preliminary report of Wolbachia infection in moths from two climatic regions of Japan
Zenobia Lewis (1, 2), *Takahisa Miyatake (1)
(1) Okayama Univ., (2) Univ. Liverpool

P22
Host cell immune responses against infection of Wolbachia, Cardinium and Rickettsia
*Yuki Nakamura (1), Kenji Watanabe (1), Hiroaki Noda (1)
(1) NIAS

P23
Molecular evidence for the residual nuclear genome of the green algal endosymbiont in the dinoflagellate Lepidodinium chlorophorum
*Takuya Matsumoto (1), Ryoma Kamikawa (1), Tetsuo Hashimoto (1), Yuji Inagaki (1)
(1) Univ. Tsukuba

P24
Multiple endobionts of both bacteria and kleptoplast in Virgulinella fragilis (foraminifera): distribution, cytological and genetic characteristics
*Masashi Tsuchiya (1), Takashi Toyofuku (1), Katsuyuki Uematsu (2), Volker Brüchert (3), John Collen (4), Hiroyuki Yamamoto (1), Hiroshi Kitazato (1)
(1) JAMSTEC, (2) Marine Works Japan, Ltd., (3) Stockholm Univ., (4) Victoria Univ. Wellington

P25
Evolution of situation depending mutualism
*Shin Fukui (1), Atsushi Yamauchi (1)
(1) CER Kyoto Univ.

P26
Aphids acquired symbiotic genes via lateral gene transfer: evidence at the protein level
**P27**

**Novel bacteriome and endosymbiont evolved in the seed bugs of the genus *Nysius***

*Yu Matsuura (1, 2), Ryuichi Koga (1), Xian-Ying Meng (1), Yoichi Kamagata (1), Takema Fukatsu (1)*

(1) AIST, (2) Univ. Tsukuba

**P28**

**Partial cytoplasmic incompatibility in *C. analis***

*Yukihiko Toquenga (1), Natsuko Kondo (2)*

(1) Univ. Tsukuba, (2) NIES

**P29**

**Phylogeny, systematics and evolutionary history of the turtle and whale barnacles (Cirripedia: Balanomorpha: Coronuloidea)**

*Ryota Hayashi (1)*

(1) Chiba Univ.

**P30**

**Quantitative analysis of lytic cycle of WO bacteriophage in *Wolbachia*, an endosymbiont of arthropods**

*Seiichi Furukawa (1), Kohjiro Tanaka (2), Takema Fukatsu (2), Tetsuhiko Sasaki (3)*

(1) Univ. Tsukuba, (2) AIST, (3) Tamagawa Univ.

**P31**

**Early and late male-killing phenomenon found in field populations of oriental tea tortrix, *Homona magnanima* (Lepidoptera, Tortricidae)**

*Ayako Hirao (1), Yusuke Tsugeno (1), Sayaka Morimoto, Kazuko Nakanishi, Mayu Hoshino (2), Madoka Nakai (1), Yasuhisa Kunimi (1)*

(1) Tokyo Univ. Agr. Tech., (2) SDS Biotech

**P32**

**Cellulose decomposition proteins search in the termite symbiotic system**

*Kumiko Kihara (1), Takashi Nobori (2), Yuuri Tsuboi (1), Jun Kikuchi (1), Shigeharu Moriya (1)*

(1) RIKEN, (2) Yokohama City Univ.

**P33**

**Development of environmental metabolomics for planktonic microbial communities**

*Craig Everroad (1), Yuri Tsuboi (1), Seiji Yoshida (2), Jun Kikuchi (1), Shigeharu Moriya (1)*

(1) RIKEN, (2) Yokohama City Univ.

**P34**

**Cytoplasmic incompatibility of *Homona magnanima* by its symbiont *Wolbachia***

*Akizuki N (1), Nakai M (1), Hirao A (1), Kunimi Y (1)*

(1) Tokyo Univ. Agr. Tech.
P35
Cyanobacterial symbiosis and photosynthetic performance in coral reef ascidians
*Akio Murakami (1), Hiroko Uchida (1), Fumiya Hamada (1), Seiji Akimoto (1), Euichi Hirose (2)
(1) Kobe Univ., (2) Univ. Ryukyus

P36
Relic plastid and absence of its own DNA in the oyster parasite Perkinsus marinus
*Motomichi Matsuzaki (1), Haruko Kuroiwa (2), Tsuneyoshi Kuroiwa (2), Hisayoshi Nozaki (1), Kiyoshi Kita (1)
(1) Univ. Tokyo, (2) Rikkyo Univ.

P37
Transition of symbiotic protist community structure after hybridization of two termite species, Reticulitermes speratus and R. kanmonensis
Akane Wakui (1), *Osamu Kitade (1)
(1) Ibaraki Univ.

P38
Emerging symbioses between platypodid ambrosia beetles and three fungal lineages
*Rikiya Endoh (1, 2), Seitaro Hashimoto (1), Yuko Takeuchi (1), Kazuyoshi Futai (1)
(1) Kyoto Univ., (2) RIKEN

P39
Reductive bacterial genome evolution in insect gut environment
*Naruo Nikoh (1), Takahiro Hosokawa (2), Kenshiro Oshima (3), Masahira Hattori (3), Takema Fukatsu (1)
(1) Open Univ. Japan, (2) AIST, (3) Univ. Tokyo

P40
Unique genome evolution of Symbiobacterium thermophilum
Hiromi Nishida (1), *Choong-Soo Yun (1)
(1) Univ. Tokyo

P41
Application of (feed-termite-intestinal microflora) system for biogas production
*Hideki Aoyagi (1), Tsuyoshi Yoshimura (2)
(1) Univ. Tsukuba, (2) Kyoto Univ.

P42
History of grass-endophyte coevolution elucidated by chloroplast DNA markers and endophyte infection status; cases seen in Lolium grasses and Epichloë (Neotyphodium) fungal endophytes
*Koya Sugawara (1), Hiroyuki Tobina (2,3), Akira Arakawa (1), Masayuki Yamashita (2), Hitoshi Sawada (2), Ikuko Okabe (1), Takao Tsukiboshi (1), Christopher L. Schardl (4)
(1) NILGS, (2) Shizuoka Univ., (3)NARC Tohoku, (4) Univ. Kentucky

P43
Genome analysis on the horizontally transmitted bacterial endosymbiont of deep-sea
vent gastropod in the Central Indian Ridge
*Shigeru Shimamura (1), Satoshi Nakagawa (2), Yoshihiro Takaki (1), Tomoo Sawabe (2), Ken Takai (1)
(1) JAMSTEC, (2) Hokkaido Univ.

P44
Resistance to rice leaf bug, *Trigonotylus caelestialium* (Heteroptera: Miridae), was enhanced in important forage/turf grass, *Lolium* species, by its fungal endo-symbionts, *Neotyphodium* endophytes
*Takuya Shiba (1), Koya Sugawara (1), Akira Arakawa (1)
(1) NILGS

P45
Effects of elevated CO₂ and soil nutrients and water conditions on photosynthetic and growth responses of *Alnus hirsute*
*Hiroyuki Tobita (1), Akira Uemura (2), Mitsutoshi Kitao (1), Satoshi Kitaoka (2), Yutaka Maruyama (1), Hajime Utsugi (1)
(1) FFPRI Ibaraki, (2) FFPRI Sapporo

P46
KLV mediates the long-distance negative control of nodulation in *Lotus japonicus* and interacts with HAR1
*Hikota Miyazawa (1,2), Erika Oka-Kira (2), Naoto Sato (2), Hirokazu Takahashi (2), Guo-Jiang Wu (2), Shusei Sato (3), Masaki Hayashi (4), Shigeyuki Betsuyaku (2), Mikio Nakazono (2), Satoshi Tabata (3), Kyuya Harada (4), Shinichiro Sawa (2), Hiroo Fukuda (2), Masayoshi Kawaguchi (1,5)
(1) NIBB, (2) Univ. Tokyo, (3) KDRI, (4) NIAS, (5) CREST

P47
“DEEP IMPACT” on biodiversity: The invasion of land snail predator (flatworm), *Platydemus manokwari* on Chichijima Island in the Ogasawara (Bonin) Islands, Japan
*Takashi Ohbayashi (1, 2, 4), Koji Takeuchi (1, 4), Isamu Okochi (3), Hiroki Sato (3), Tsuyoshi Ono (1, 4), Satoshi Chiba (5) and Naoki Kachi (2)
(1) Tokyo Metropolitan Agr. Forest. Res. Center, (2)
Tokyo Metropolitan Univ., (3) FF PRI, (4) Ogasawara Subtropical Branch, Tokyo Metropolitan Agr. Exp. Station, (5) Tohoku Univ.

P48
Phospholipid biosynthesis in the gut of *Spodoptera litura* larvae and effects of tannic acid ingestion
*Takako Aboshi (1), Naoki Mori (1)
Univ. Kyoto (1)

P49
Molecular phylogeny of the *Sacoglossa*, with a discussion of gain and loss of kleptoplasty in the evolution of the group
*Taro Maeda (1), Tadashi Kajita (2), Tadashi Maruyama (1,3), Yoshiaki Hirano (2)
(1) Tokyo Univ. Marine Sci. Tech. (2) Univ. Chiba, (3) JAMSTEC
P50
Wolbachia infections in world populations of bean beetles (Coleoptera: Chrysomelidae: Bruchinae)
*Natsuko I. Kondo (1), Midori Tuda (2), Yukihiro Toquenaga (3), Yen-Chiu Lan (4), Sawai Buranapanichpan (5), Shwu-Bin Horng (6), Masakazu Shimada (7), Takema Fukatsu (8)
(1) NIES, (2) Kyushu Univ., (3) Univ. Tsukuba, (4) Leader Univ., (5) Chiang Mai Univ., (6) National Taiwan Univ., (7) Univ. Tokyo (8) AIST

P51
Complehensive understanding of eco-system by omics science
*Shigeharu Moriya (1)
(1) RIKEN

P52
The identification and characterization of the antigens related to the deep-sea clam Calyptogena endosymbiosis by the monoclonal antibodies with the gill tissue specificity
*Yoshimitsu Nakamura (1), Yuki Hongo (1, 2), Kazue Oishi (1), Akihiro Tame (3), Masatoshi Nakazawa (4), Masahiro Fujishima (5), Katsunori Fujikura (1), Tadashi Maruyama (1) and Takao Yoshida (1)

P53
Breeding of insect resistant Italian ryegrass (Lolium multiflorum) cultivar for forage using plant endo-symbiont; the application of fungal endophyte, Neotyphodium occultans, and infected grass in Japan
*Akira Arakawa (1), Koya Sugawara (1), Takuya Shiba (1), Kazuhiro Uchiyama (1), Kazuhiko Mizuno (1)
(1) NIRGS

P54
Cordyceps specimens in Japan
*H. Sato (1), S. Ban (2), H. Masuya (3) and T. Hosoya (4)
(1) FFPRI-Kyushu, (2) NITE, (3) FFPRI, (4) TNS

P55
The host legume gene, SEN1, is crucial for symbiotic nitrogen fixation
*Tsuneo Hakoyama (1,2), Kaori Niimi (2), Takeshi Yamamoto (2), Sawa Isobe (2), Shusei Sato (3), Yasukazu Nakamura (3), Satoshi Tabata (3), Hirotaka Kumagai (1), Yosuke Umehara (1), Katja Krause (4,5), Thomas R. Petersen (4), Niels Sandal (4), Jens Stougaard (4), Michael K. Udvardi (5), Masanori Tamaoki (6), Masayoshi Kawaguchi (7), Hiroshi Kouchi (1) and Norio Suganuma (2)

P56
Use of phylogenetic-based multi-species comparison to identify the key determinants in plant-pollinator interactions
*Yudai Okuyama (1), Tomoko Okamoto (2), Ryutaro Goto (3), Makoto Kato (3)
Analysis of the protein import machinery in the *Entamoeba* mitochondrial remnant
*Takashi Makiuchi (1), Fumika Mi-Ichi (1), Kumiko Nakada-Tsukui (1), Tomoyoshi Nozaki (1)
(1) NIID

P58 Isolation of symbiont-like bacteria from *Osedax* spp.
*Masayuki Miyazaki (1) Masaru Kawato (1), Yuichi Umezu (2), Florence Pradillon (1), Yoshihiro Fujiwara (1,2)
(1) JAMSTEC, (2) Hiroshima Univ.

P59 A newly discovered female-specific mycangium and the associated microbial communities in stag beetles
*Masahiko Tanahshi (1), Tadatsugu Hosoya (2), Kohei Kubota (3), Takema Fukatsu (1)
(1) AIST, (2) Univ. Kyushu, (3) Univ. Tokyo

P60 Phylogenetic implication of evolution in the endosymbiosis between the foraminifer and red algae
*Akiko Yokoyama (1)
(1) Univ. Tsukuba

P61 The effect of plant hormone cytokinins on *Toxoplasma gondii*
Syed Bilal Ahmad Andrabi (1,2), Michiru Tahara (1), Hiroka Aonuma (1), Tomoko Toyama (3), Kazuyuki Tanabe (3), Tomoyoshi Nozaki (1), *Kisa Nagamune (1)
(1) NIID, (2) Keio Univ., (3) Osaka Univ.

P62 Isolation of diazotrophic endophytes from sweet potato
*Junko Terakado-Tonooka (1,2), Yoshinari Ohwaki (1), Shinsuke Fujihara (1)
(1) NARC, (2) JSPS Research Fellow

P63 Population genomic analysis of the chemoautotrophic endosymbiont in *Calyptogena* clams
*Yoshihiro Takaki (1), Takao Yoshida (1), Shigeru Shimamura (1), Masatoshi Tsukahara (2,3), Maiko Nezuo (2,3), Morimi Teruya (3,4), Shimoji Makiko (2), and Tadashi Maruyama (1)
(1) JAMSTEC; (2) TTC; (3) OCGP; (4) OITC

P64 Symbiotic associations of the geological past: how can we approach?
*Yuichiro Kashiyama (1), Robert G. Jenkins (2), Yasuhiro Iba (3), Yoshito Chikaraishi (4), and Naohiko Ohkouchi (4)
(1) Univ. Tsukuba, (2) Yokohama Nat. Univ., (3) NMNS, (4) JAMSTEC

P65 The characterization of a lectin from a hard coral *Ctenactis echinata*
*Mitsuru Jimbo (1), Chie Tanaka (1), Koji Muramoto (2), Hiroaki Tateno (3), Jun
Inoculation of *Carex kobomugi* seedlings with endophytic bacteria could enhance the plant growth in sand coast soil

*H. Matsuoka (1), M. Akiyama (2), K. Kobayashi (1), K. Yamaji (1)
(1) Univ. Tsukuba, (2) Geosci. Res. Lab.

Diversity and evolution of symbioses at whale falls

*Yoshihiro Fujiwara (1, 2), Masaru Kawato (1), Masayuki Miyazaki (1), Florence Pradillon (1), Tadashi Maruyama (1, 2)
(1) JAMSTEC, (2) Hiroshima Univ.

Association between insect-transmissibility and plasmids of phytopathogenic phytoplasma

*Yoshiko Ishii (1), Shigeyuki Kakizawa (1), Kenro Oshima (1), Shigetou Namba (1)
(1) Univ. Tokyo

Reconstruction of primary metabolism and efficient cellulose utilization caused by independent lateral gene transfers in symbiotic protists in the gut of the termite *Coptotermes formosanus*

*Jun-ichi Inoue (1), Moriya Ohkuma (1)
(1) RIKEN BRC-JCM

New test formation in a euglyphid testate amoeba *Paulinella chromatophora*

*Mami Nomura (1), T. Nakayama (1), K. Ishida (1)
(1) Univ. Tsukuba

The effect of spatial structure on the evolutionary dynamics of mutualism: a dual lattice model with continuous Prisoner's Dilemma game

*Hideo Ezoe (1)
(1) Osaka Pref. Univ.

Genetic and phenotypic diversity of rhizobia isolated from coastal legumes in Japan and Canada

*Seishiro Aoki (1), Motomi Ito (1)
(1) Tokyo Univ.

Role of plant volatile in host-finding behavior of parasitoid fly, *Exorista sorbillans*

*Aya Tanaka (1), Yooichi Kainoh (1), Jun Tabata (2), Hajime Sugie (2), Ryoko T. Ichiki (3), Satoshi Nakamura (3)
P74
Common molecular and biological properties among endogenous dsRNAs in plants (Endornaviruses)
*Ryo Okada (1), Eri Kiyota (1), Chee Keat Yong (1), Hiromitsu Moriyama (1), Toshiyuki Fukuhara (1), Sead Sabanadzovic (2), Rodrigo A. Valverde (3)

P75
Endogenous dsRNA (Endornavirus) in Malabar spinach (*Basella alba*)
*Eri Kiyota (1), Ryo Okada (1), Hiromitsu Moriyama (1), Toshiyuki Fukuhara (1)
(1) Tokyo Univ. Agr. Tech.

P76
Detection of identical sequences in *Wolbachia* strains
*Takuto Sumi (1), Masako Katsuki (1), Takahisa Miyatake (1), Kazuki Miura (2, 3)
(1) Okayama Univ., (2) WeNARC, (3) Hiroshima Univ.
Sponsors
Japan Society for the Promotion of Science (JSPS)
University of Tsukuba

Organizers
Isao Inoue (University of Tsukuba)
Hiroshi Kouchi (National Institute of Agrobiological Sciences)
Takema Fukatsu (National Institute of Advanced Industrial Science and Technology)

Symposium URL
http://staff.aist.go.jp/t-fukatsu/IPBSympHome.html