

Symbiosis research, technology, and education: Proceedings of the 6th International Symbiosis Society Congress held in Madison Wisconsin, USA, August 2009

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Abstract Symbiosis, the intimate association between two or more organisms, is a fundamental component of biological systems. Our ability to understand the processes involved in the establishment and function of Symbiosis has critical consequences for the health of humans and the world we live in. For example, a deeper understanding of how legumes and insects have harnessed the nitrogen-fixing capacity of microbes can pave the way toward novel strategies to decrease fertilizer use.

Also, using insect models to elucidate links between diet, gut microbiota, and toxin sensitivity not only has implications for biological control strategies, but also will lend insights into similar links in the human gut ecosystem. These types of ideas were presented and discussed at the 6th International Symbiosis Society Congress held in Madison, Wisconsin August, 2009. Over 300 participants from 20 countries attended the 7-day event, which featured cutting-edge symbiosis research from

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many different perspectives and disciplines. The conference was organized thematically, with oral sessions focused on Evolution, Ecology, Metabolism, the Host-Microbe Interface, Threats to Earth Systems, Symbiosis Models and the Human Microbiome, Viruses and Organelles, and Symbiosis Education. World-renowned scientists, post-doctoral fellows, and students were given the opportunity to describe their most recent discoveries. Session chairs provided overviews of their programs which highlight how the comparative analysis of different systems reveal common trends underlying symbiotic associations, what tools and theory are being developed that may be applied more broadly in symbiosis research, how symbiosis research contributing solutions to

global issues such as emerging antibiotic resistance, a need for alternative energy sources, the pursuit of sustainable agriculture and natural resources, and how symbiotic systems are ideal for educating people about the fascinating natural world around us. The following paragraphs provide an overview of the research and discussions that took place during the congress.

Keywords Evolution · Ecology · Metabolic symbiosis · Host-symbiont interface · Human microbiome · Symbiosis models · Restoration ecology · Viruses · Organelles · Education · Career development

Fig. 1 Participants in the 6th ISS Congress enjoy food and conversation at the Welcome Reception held in the atrium of the Microbial Sciences Building, University of Wisconsin Madison



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Fig. 2 The dinner meeting of the Symbiosis Editorial Board held during the 6th International Symbiosis Society Congress held in Madison, Wisconsin, USA. From left to right, Rusty Rodriguez, Michael Sadowsky, Katharina Pawlowski, Daniele Armaleo, Marc-Andre Selosse, David Richardson, Douglas Zook, Ann Hirsch, the late Gopi Podila (see Symbiosis vol 50(3) for his obituary) and Gavin Kernaghan

1 Focused sessions

1.1 Agents of dependence: from viruses to organelles (Seth Bordenstein)

From the origin of eukaryotes to the formation of entirely new ecosystems, symbioses involving dependent interactions have initiated major evolutionary transitions in the history of Life. The seminars and discussion during this session of the 6th ISS Congress highlighted how dependencies continue to evolve and flourish.

Mary Rumpho (University of Maine) discussed new results on how the marine sea slug *Elysia chlorotica* harnesses energy from the sun as if it were a plant. These green animals eat algal prey, retain the algal chloroplasts in their gut, and use chloroplast and nuclear genes derived from the algae to harvest light energy from the chloroplasts, essentially converting this animal to solar-power overnight.

Serap Aksoy (Yale University) presented data on how symbiont homeostasis can be dramatically altered by single changes in the host gene expression. She reported that the transcription of an ancient immune gene encoding a part of the peptidoglycan recognition protein complex correlates with titers of the tsetse fly's *Wigglesworthia* symbionts that aid nutrition and fitness. Conversely, depletion of this gene's product leads to an increased immunosusceptibility to trypanosome infection and a reduction of *Wigglesworthia*, demonstrating the dynamic interplay between simple

changes in the innate immune system and the resident microbiota.

Seth Bordenstein (Vanderbilt University) presented an investigative framework for how mobile elements—phages, transposons, and plasmids—vary with respect to bacterial lifestyle. Among 384 bacterial genomes analyzed, the mobile element densities did not correlate with phylogeny, but rather with whether bacteria replicate as intracellular symbionts, extracellular organisms, or both, supporting the theory that mobile element evolution is allied closely to bacterial ecology.

1.2 Symbiosis models and human microbiome (Ruth Ley and Justin Sonnenburg)

This session addressed the study of the human microbiome and the role of model systems in dissecting host-microbial interactions in a complex system.

Ruth Ley (Cornell) described the high levels of bacterial diversity inherent to the mammalian gut, and underscored the high levels of variation in gut microbial community composition between people. The diversity within a community can affect host health, as demonstrated, for instance, by its role in obesity: the 'obese microbiome' is more efficient at energy extraction from the diet than the 'lean microbiome'. Model systems allow the mechanisms underlying such associations between microbial species and host to be better understood. This session included three model systems, using three different host species, that dissected the complexity of the host-microbiome interaction in different ways: (a) by focusing on the interactions of the mammalian host with one model microbe, *Toxoplasma gondii*; (b) by controlling the composition of the microbial community using a germ-free vertebrate host that is easily genetically manipulated, the zebrafish, and (c) by studying the interactions in a naturally simple, binary system, the Squid-*Vibrio fischeri* duo.

All three of these model systems revealed remarkably penetrant effects of microbes on their eukaryotic hosts' gene expression. **Laura Knoll** (UW-Madison) discussed her latest findings on the effect of a single organism, *Toxoplasma gondii*, on the host's resistance to other microbes, and on metabolism. Another aspect of microbially-mediated host response was presented by **Karen Guilleman** (University of Oregon): Her group has shown that the microbiota are required for specific aspects of intestinal epithelial differentiation, including intestinal alkaline phosphatase activity, mature patterns of glycan expression, and normal levels of cell proliferation and secretory cell specification. In addition, they have found that the Toll-like receptor adaptor Myd88 is required for transducing bacterial signals that regulate aspects of intestinal epithelial maturation and homeostasis, emphasizing

the importance of innate immune signaling in responses to resident gut bacteria. Finally, **Ned Ruby** (UW-Madison) presented data showing how the microbial symbiont interacts with the host to promote its colonization. In *V. fischeri*, HnoX (a commonly occurring NO-binding protein of unknown function in bacteria) mutants are 10-fold more efficient at initially colonizing the squid host than wild type; however, soon after the mutant fails to persist normally. *V. fischeri* normally senses a host-generated NO signal through HnoX, and modulates the expression of its heme/iron-uptake capacity during the early stages of the light-organ symbiosis. Absence of this signaling capacity may make the bacterium sensitive to subsequent conditions in the tissue.

Together these studies in model systems showed the wide range of host processes directly influenced by the microbiota, from adaptive immunity, metabolism, and development to control of microbial colonization. The challenge going forward will be to understand how microbial diversity, and the specific composition of complex communities, influence host responses.

2 Ecology

Symbioses are increasingly being identified as critical to answering central issues in ecology. Interactions of host and symbionts have been found to contribute to the maintenance of diversity within communities, drive or impede the process of succession and influence the distribution of species. Symbionts have also been shown to contribute to the success, failure and impact of invasive species and the response of communities to anthropogenic environmental change. —James Bever

2.1 Ecology: host defense symbiosis (Keith Clay)

The Host Defense Symbiosis session of ISS6 was stimulated by the growing recognition that a great diversity of symbiotic interactions involving plants, animals, bacteria and/or fungi are based on defense or protection of the host by the microbial symbiont. These associations therefore involve at least three species (host, symbiont and enemy). While the protection of host plants by ants has been known for over 100 years, more recent research focuses on the chemical or immunological defense mechanisms imparted by microbial symbionts.

A broad diversity of systems was described by the speakers but with the common theme of mechanisms for defense against natural enemies. Given that most systems described are characterized by vertical transmission, defense of the host is a self-defense by the symbiont. Microbes are capable of synthesizing a wide range of biologically active compounds that may predispose them to enter into host defense

symbioses. **Nicole Lopanik** (Georgia State University) described the fascinating association between a marine bryozoan with a vertically transmitted bacterial symbiont that produces bryostatins that are most highly concentrated in larvae, which are otherwise vulnerable to fish predation. In a unique case of insect—*Streptomyces* symbiosis, **Martin Kaltenpoth** (University of Regensburg) showed that beeswaxes secrete bacteria-containing fluids from antennal glands and uses them to inoculate subterranean brood cells where they presumably provide protection against pathogenic microorganisms. For the well-studied relationship between grasses and seed-transmitted fungal endophytes, **Keith Clay** (Indiana University) presented experimental evidence demonstrating that fungal alkaloids protect host plants against grazing mammals and insects and that the ecological benefit of protection increases with pest pressure. A very different form of defense by the secondary *Hamiltonella* symbiont of aphids (presented by **Kerry Oliver**, University of Georgia) against parasitoid wasps requires bacteriophage infection of the secondary symbiont, thus involving at least four interacting taxa. **Rumsais Blatrix** (Montpellier) results on host defense symbiosis also had a chemical basis as well but for communication between host plant and ant defender about when active defense (by ants) is required.

Diversity was a second major theme running through the host defense symbiosis session. **Elizabeth Arnold** (University of Arizona) described her research on the staggering diversity of fungal endophytes from higher plants and lichens, and their varied phylogenetic origins. Clearly, host defense symbioses can arise from varied starting points. **Patrick Abbot** (Vanderbilt University) found that ambrosia gall midges associate with galls of diverse morphologies, suggesting a high degree of specialization, and turnover, between insect and fungal partners. Finally, **Barbara Schulz** (University of Braunschweig) discussed the diversity of interactions between a common fungus and a variety of host plants where in some hosts the fungus acted as a pathogen while in others it acted as a benign endophyte.

The overall take-home message of the session was that many symbiotic interactions do not involve nutrition but do involve enemies, and these host defense symbioses may be profitable arenas for the exploration of chemical and biochemical mechanisms of interaction.

2.2 Symbiont impacts on communities and ecosystems (James D. Bever)

While it is commonly noted that direct effects of symbioses on host nutrition can increase community productivity, it has become increasingly clear in recent years that nutritional and defensive symbionts also have cascading effects in communities and ecosystems. The work on ant acacia's in Africa by **Todd Palmer** (University of Florida) beautifully illustrates

this potential. Ants defend acacia trees which provide them with food and shelter and the ant defense has been found to be sufficiently aggressive as to inhibit Elephant herbivory. Acacias without ants are destroyed by elephants thereby converting forested areas to grasslands. The ant symbiosis then proves to be a critical factor in conversion between forest and grassland ecosystems. **Jennifer Rudgers** (Rice University) demonstrated similarly dramatic cascading effects of defensive fungal symbionts of grasses as infection with these symbionts can alter plant-plant interactions and inhibit the processes of succession. However, the nature of the effect of endophytes on plant-plant interactions varies considerably between plant and fungal species.

Understanding the dynamics within a diverse community of symbionts was the central question of **Scott Santos** (Auburn University), who developed molecular methods to demonstrate high diversity of Symbiodinium, nutritional symbiotic dinoflagellates that associate with many residents of coral reefs. Scott finds these symbionts to spatially structured at a fine structure, a pattern consistent with functional significance. **Jim Bever** (Indiana University) found that the high diversity observed within symbiotic arbuscular mycorrhizal fungi can have cascading effects in the terrestrial ecosystem, including a fungal dynamic that contributes to the maintenance of plant diversity, and the degradation or enhancement of mutualism, depending on the strength of preferential allocation by the plant to the most effective mutualist and the spatial mixture within the plant root system. The composition of AM fungi was also shown to influence the vulnerability of soils to erosion.

2.3 Ecology: complex interactions in a changing environment (Kenneth Raffa and Katarzyna Turnau)

This session explored some of the major shifts in how symbioses are researched and interpreted. Collectively, these advances represent increased recognition of the complexity of symbiotic interactions, including in membership, functionality, and variation. Moreover, the environmental template on which these members interact and evolve is constantly changing. These changes include both natural variation in space and time and responses to numerous anthropogenic inputs. The latter, such as climate change, ecosystem management, and invasive species, threaten to be highly destabilizing in some systems. Understanding the complexity of symbiotic interactions also provides enhanced opportunities for conservation biology and sustainable resource management.

Kier Klepzig (United States Forest Service) and coauthors gave an overview of how symbioses could be a key driver in the impacts insects have on terrestrial ecosystems. Examples included multiple levels of spatial and temporal scales, such as physiology, behavior, and population dynamics. The three

subsequent talks addressed how symbioses could be affected, and likewise affect, various components of anthropogenic change. **Katarzyna Turnau** (Jagiellonian University) and her colleagues discussed mycorrhizae from a unique perspective, their roles in endangered plant species, and the potential for considering these symbiotic relationships in conservation projects. **Diana Six** (University of Montana) and her colleagues demonstrated how the predominant fungal symbionts of their host insect can alter with even small shifts in temperature, and how these can affect host fitness and plant mortality. **Simon Dunn** and **Ove Hoegh-Guldberg** (University of Queensland) focused on marine systems, specifically coral reef ecosystems. They showed how current warming trends are having pronounced effects on key symbiotic processes, with emphasis on coral bleaching and dieback. Ocean acidification is likewise exerting additional impacts.

3 Host-symbiont interface

The initiation, function, and transmission of symbiotic associations require complex communication between host and symbiont to ensure that cooperating individuals are selected, non-cooperating symbionts are avoided, symbiotic goods and services are exchanged, and mutually beneficial levels of host and symbiont are maintained. The molecular basis of this communication in diverse systems is being elucidated due in part to the application of new technologies in the study of animal and plant hosts. —Heidi Goodrich-Blair

3.1 Host-symbiont interface: surface molecules and matrices (Karen Visick)

Initial interactions between microbes and their plant or animal hosts occur in the context of surface molecules and matrix components. This session focused on recent advances in our understanding of these molecules and components in diverse symbioses.

One highlight of this session was the talk by **Laurie Comstock** (Channing Laboratory, Brigham and Women's Hospital, Harvard Medical School), in which she described an abundance of glycosylated proteins in the microbial species *Bacteroides fragilis*, a major constituent of the human intestinal microbiota. It was already known that a single strain of *B. fragilis* produces 8 different types of surface capsular polysaccharides whose expression are under complex control, and which are important to bacteria-host interactions. Dr. Comstock showed that a glycosylation locus was necessary for the glycosylation of multiple unrelated proteins. Furthermore, loss of this locus led to an in vitro growth defect and an inability of *B.*

fragilis to compete with wild type for colonization of the gnotobiotic mouse gut. This work demonstrated the complexity of the bacterial surface and showed that protein glycosylation is critical to the bacterium. *B. fragilis* and other *Bacteroides* species are now among the growing list of bacteria that glycosylate proteins—a phenomenon once thought to be limited to eukaryotic cells. It is likely that protein glycosylation will be involved in other bacteria-host interactions in this mutualism.

A second highlight of the session was the talk by **Ann Hirsch** (University of California, Los Angeles). Dr. Hirsch described the development of assays to evaluate biofilm formation by the alfalfa symbiont *Sinorhizobium meliloti* on both abiotic and biotic surfaces. These experiments uncovered a novel role in biofilm formation for Nod factor, a known signaling molecule required for symbiosis. Surprisingly, biofilm formation by a Nod factor mutant could not be complemented with exogenous Nod factor, a result that parallels the long-known inability of exogenous Nod factor to complement the colonization defect of a *nod* mutant. This work thus provides new insight into a well-studied symbiosis determinant.

Graham Walker (MIT) described studies from his lab investigating the *Sinorhizobium meliloti*-plant symbiosis. The protein BacA, found both in *S. meliloti* and in the pathogen *Brucella abortus*, affects the fatty acid content of cell surface lipid A and is required for chronic infection by both organisms. He also described a *S. meliloti* mutant, *bluB*, which has altered surface succinoglycan exopolysaccharide and defects in symbiosis with alfalfa. Dr. Walker's group has established BluB as a key participant in vitamin B12 biosynthesis.

Finally, **Karen Visick** (Loyola University Medical Center) spoke about the role of the symbiosis polysaccharide gene locus in biofilm formation and in symbiotic colonization of the squid *Euprymna scolopes* by *Vibrio fischeri*. She also described the apparent production of outer membrane vesicles by *V. fischeri* by biofilm over-producing cells.

3.2 Host-symbiont interface: host recognition and responses to symbionts (Jean-Michel Ané and Virginia Weis)

This session provided interesting coverage of recent advances in host recognition and responses to symbionts in both animal and plant systems.

The five talks on animal-microbe interactions all focused on the role of the host innate immune response in host-symbiont recognition and maintenance. These talks ranged broadly from presentations on insect-microbiota interactions in the gut (**Won-Jae Lee**, Ehwa Woman's University and National Creative Research Initiative Center for Symbiosystems), to insect-*Wolbachia* interactions (**Laura Serbus**, University of California, Santa Cruz), to cnidarian-dinoflagellate symbioses

typical of reef building corals (**Virginia Weis**, Oregon State University; **Emilie-Fleur Dicks**, Victoria University of Wellington; **Mathieu Pernice**, University of Queensland). That studies from such a broad array of symbioses all featured intense examination of immunity and immune modulation underscores the enormous progress in this area that has been made in the last couple of years. The overall themes emerging from all talks included the ancient and highly conserved nature of the immune response, the complexity of the response, and its modulation that results in cooperative mutual tolerance of the partnership by players. In total the talk included discussions of TLR signaling through NF κ B, TGF β and thrombospondin immunomodulatory pathways, TGF α proliferative pathways and apoptotic pathways in the host.

The three talks focusing on plant-microbe symbioses described functional components in leguminous plants that participate in host-symbiont signaling. Major progress in understanding plant-microbe symbiotic signaling was made over the last decade through the development of two model legumes, *Medicago truncatula* and *Lotus japonicus*. Comparative genetic studies between these two models were an interesting focus of this session. Nod factors are diffusible signals produced by rhizobia and perceived by legumes. **Elena Simona Radutoiu** (The University of Aarhus) gave a detailed account of functional and evolutionary analyses of Nod factor receptors in *Lotus*. Domain swapping between these receptors enabled a unique understanding of the recognition specificity in the rhizobia-legume symbiosis. Dr. Radutoiu succeeded in identifying key residues in these receptors that are necessary for Nod factor recognition. **Jean-Michel Ané** (UW-Madison) discussed the role of other protein kinases and their interacting proteins that participate in downstream signaling. The similarities and sharing of symbiotic signaling components between legume nodulation and arbuscular mycorrhization (AM) were highlighted. **Muthusubramanian Venkateshwaran** (UW-Madison) discussed the evolution of symbiotic ion channels involved in these cascades. It was shown that the function of many symbiotic proteins is extremely well conserved across land plants and especially in early land plants, which suggests that many signaling components of the legume-rhizobium symbiosis have been co-opted from the ancestral mycorrhizal symbiosis. What clearly emerged from these presentations was a call for intensive genetic studies in model legumes as well as in early land plants to shed light on the evolution of plant-microbe symbioses.

3.3 Host-symbiont interface: transmission (Monika Bright and Margaret McFall-Ngai)

This session focused on transmission of symbionts between generations. It opened with an overview of the topic

(**Monika Bright**, University of Vienna) and was followed by a series of case studies in which new data on aspects of transmission in a variety of systems were considered. As transmission is the most critical juncture in the persistence of an association over evolutionary time, it is considered a key facet of the phenomenon of symbiosis. The transmission mode affects all aspects of animal and plant symbioses. The most important concepts coming out of this session that demand further exploration include:

- Mechanisms by which specificity is ensured in horizontally transmitted symbioses—what molecular, biochemical and cellular features of the host and symbiont are operating to ensure transmission with fidelity each generation?
- Ultrastructural, anatomical, and morphological modifications of host and symbiont for the transmission process—how does the precise mode of transmission influence the form and function of host and symbiont cells and tissues?
- Evolutionary consequences—how does transmission mode shape the evolution of host and symbiont? While this aspect is relatively well studied in vertically transmitted symbioses (e.g., reduction of symbiont genome size), it is relatively poorly explored in horizontally transmitted symbioses.
- Ecological significance—how does transmission mode affect the distribution of host animals and plants? This area is particularly important when considering the impact of global climate change—in any given symbiosis, can and do both partners adapt to the changing environment. This aspect is also likely important for the biology of invasive species; e.g., is it possible that some animals and plants are good invaders because they ‘bring’ their symbionts with them, whereas those that are not good invaders do not?

4 Metabolic symbiosis

Metabolism is a defining feature of life. Therefore, it should come as no surprise that metabolism is at the heart of symbiosis. In fact, work on the metabolism of organisms that form symbioses has been instrumental to our understanding of why some symbioses evolved and what each participant gains from the association. —Michael Udvardi

4.1 Metabolic symbiosis: monitoring metabolic flux and potential (Michael Udvardi)

This session covered a wide variety of mutualistic symbioses, in which two or more species benefit from their

interaction with each other, and highlighted the value of novel technologies in advancing our understanding of the metabolic basis of symbiosis.

Victoria Orphan (California Institute of Technology) showed how fluorescence *in-situ* hybridization (FISH) coupled to nano-secondary ion mass spectrometry (nano-SIMS) revealed the identity of un-cultured species of archaea and bacteria in a marine methane-oxidizing consortium, and exactly which species serves as conduit for ^{15}N assimilation by the consortium. This work, and more like it, was made possible by the emergence of a new discipline called metagenomics, which produces sequences of DNA from complex environmental samples, even when the organisms being sequenced cannot be cultured independently in the laboratory. Metagenomics is a boon for symbiosis research because many symbionts cannot be grown in the absence of their co-symbionts.

While metagenomics can help to identify species involved in symbioses and even to predict their metabolic *potential*, it cannot establish what metabolism actually occurs during symbiosis. Proteomics and metabolomics, together with more classical biochemical approaches, can help to flesh out exactly what metabolism occurs during symbiosis. The power of proteomics was nicely illustrated in the talk of **Manuel Kleiner** (Max Planck Institute for Marine Microbiology and University of Greifswald, Germany) who used metaproteomics to identify and quantify over 1,000 proteins, including many enzymes that are expressed by the gutless worm, *Olavius algarvensis*, and its resident microbial community, which includes two gamma proteobacterial sulfur oxidizers and two delta proteobacterial sulfate reducers. The resulting data has fueled hypotheses related to energy conservation and fixation of carbon in this complex symbiosis.

Metabolomics provided a technological basis for the work of **Dieter Strack** (Leibniz Institute of Biochemistry, Germany) who studies the interaction between plants and mycorrhizal fungi. In simple terms, this interaction involves the exchange of sugars from the plant for phosphate and other mineral nutrients from the fungus. However, un-biased metabolite profiling by gas chromatography coupled to mass spectrometry (GC-MS) painted a more complex picture of metabolism, especially secondary metabolism associated with the symbiosis between the legume plant *Medicago truncatula* and the fungus *Glomus intraradices*. RNA interference (RNAi) was used to test the role of two genes/proteins involved in apocarotenoid formation, which was found to be important for sustaining mycorrhizal function.

Legumes were the focus of another talk on a different symbiosis: the nitrogen-fixing symbiosis between plants and bacteria called rhizobia. Built primarily upon the exchange of reduced carbon (e.g. malate) from the plant, for reduced nitrogen (ammonium) from the bacterium, this

is one of the best-characterized symbioses on earth, at least with respect to metabolism. Both partners can be cultured independently of one another, complete genome sequence is available for several legume and rhizobial species, and both are amenable to forward and reverse-genetics. **Michael Udvardi** (Noble Foundation, USA) described how reverse genetics (RNAi) was used to decipher the roles of plant leghemoglobins in maintaining low oxygen and high energy-charge in nodules, both of which are required for symbiotic nitrogen fixation, and how forward genetics was instrumental in identifying a plant sulfate transporter responsible for supplying this nutrient and possibly also molybdate to nitrogen-fixing *Mesorhizobium loti* in *Lotus japonicus* root nodules. He also described the development of a transposon insertion mutant population of the legume, *Medicago truncatula*, which has accelerated reverse genetic studies of nodule metabolism.

Edouard Jurkevitch (Hebrew University, Israel) described another nitrogen-fixing symbiosis, this time between an animal, the Mediterranean fruit fly (*Ceratitidis capitata*) and its gut bacterial community, dominated by *Enterobacteriaceae*. Symbiotic bacteria play important roles at different stages of the fly life cycle, for instance by actively fixing nitrogen both during the larval and the adult stages, and by liberating carbon compounds for larval metabolism. His group also found that the performance of the sterile insect technique used to control this insect pest could be improved by manipulating the gut population of adult flies.

4.2 Metabolic symbiosis: controlling metabolite synthesis and exchange (Maria Harrison)

This session included discussions of nutrient exchange between symbionts, a common feature of many symbioses. In some cases, the nutrients in question have been identified but the mechanisms of their exchange and the regulation of the exchange processes are unknown. In other cases, the metabolites involved have yet to be identified. For example, in the arbuscular mycorrhizal symbiosis, the fungal symbionts deliver phosphate to the plant in return for carbon. Currently, the mechanisms of carbon transport are unknown, but some of the Pi transporters involved in symbiotic Pi transfer have been identified. As described by **Maria Harrison** (Boyce Thompson Institute for Plant Research), analysis of Pi transport mutants indicates that symbiotic Pi transfer is essential to maintain the AM symbiosis. In contrast, in the ectomycorrhizal symbiosis, advances in understanding carbon partitioning between the fungal symbionts and their tree hosts is beginning to be elucidated. **Uwe Nehls** (University of Bremen) presented current studies of the substrate specificities of symbiosis-associated hexose transporters from the plant and fungal

symbionts suggest that the fungal and plant partners focus on glucose and fructose respectively, thus possible sharing sucrose in an equitable manner.

Philip Poole (John Innes Center) described an elegant study of branched chain amino acid transport in the nitrogen-fixing symbiosis formed between rhizobia and their legume host plant revealed an interesting case of symbiotic auxotrophy. While the free living rhizobia are capable of acquiring leucine, valine and isoleucine, the symbiotic form of the rhizobia (bacteroids), are auxotrophic for these amino acids and become dependent on the plant for their supply. In this way, the plant controls development and maintenance of the bacteroids. Moving to symbioses in aquatic ecosystems adds additional challenges to the study of metabolite exchange. Through the use of radiolabeled metabolites, **David Yellowlees** (James Cook University) monitored the transfer of nitrogen and inorganic carbon (CO₂) from corals to their symbionts, but how the supplies are regulated is still unknown as are the mechanisms of photosynthate secretion by their zooxanthellae (genus, *Symbiodinium*). The transcriptome of the coral, *Acropora millepora* and *Symbiodinium* are being deciphered and these additional tools will assist future analyses. **Christian Lott** (Max Planck Institute for Marine Microbiology) discussed the symbiosis formed between gutless marine worms and their sulphur bacteria and highlighted a fascinating chemoautotrophic symbiosis that involves metabolite movement between multiple symbionts. In this symbiosis, both sulphur-oxidizing and sulphur-reducing bacteria inhabit the worm and together they enable carbon-fixation through a chemoautotrophic process that uses sulphide as an energy source. The sulphur-oxidizing bacteria fix CO₂ using sulphide as an energy source and some of this fixed carbon is transferred to the worm. The source of sulphide for this activity is provided by their co-occurring sulphur-reducing symbionts. Thus, a sulphur cycle occurs between the bacterial symbionts within the worm, which ultimately provides the energy for CO₂ fixation and a positive impact for the worm.

5 Evolution

Twenty years ago (June 25–29, 1989), twenty biologists met to discuss “Symbiosis as a Source of Evolutionary Innovation” at a conference organized by Lynn Margulis at the Rockefeller Foundation Conference Center in Bellagio, Italy (see Margulis and Fester, eds. *Symbiosis as a Source of Evolutionary Innovation*, MIT Press, 1991). Of the participants, only two were evolutionary biologists: Richard Law and John Maynard Smith. After a series of speakers recounted examples of plausible symbiosis-engendered plant and animal radiations,

Maynard Smith commented: “Come, come, this is all very interesting, but what does symbiosis explain about REAL evolution?”

Twenty years later, with the application of genomics tools and the development of integrated, interdisciplinary research approaches, symbiosis researchers are starting to address those “real” evolutionary questions in increasingly sophisticated ways. —Mary Beth Saffo

5.1 Evolution: innovation (Mary Beth Saffo)

The “Innovation” session of the International Symbiosis Congress provided some examples of how current symbiosis research is addressing evolutionary questions.

In an elegant blend of natural products chemistry with molecular biology and microbiology, chemist **Christian Hertweck** (Leibniz Inst. for Natural Product Research) outlined the work of his lab in unraveling the evolutionary intricacies of the mutualistic symbiosis between a bacterium, *Burkholderia* sp., and the fungal plant pathogen *Rhizopus microsporus*. *Burkholderia* is central to the biology of *Rhizopus* not only because spore production in *Rhizopus* takes place only in the presence of *Burkholderia*; through its production of the mitosis-blocking toxin rhizoxin, *Burkholderia* is also the direct source of the pathogenicity of its fungal hosts (which are immune to the toxin through felicitous amino acid changes in their beta-tubulin).

In his discussion of lichens (perhaps the ultimate example of symbiosis-engendered ecological and evolutionary innovation), **Francois Lutzoni** (Duke University) demonstrated the importance of integrating theoretical and empirical approaches, and molecular, cellular, ecological, and evolutionary perspectives, and attention to both mutualistic and antagonistic associations, to mature understanding of the evolution of symbiotic associations. Bringing genomic data and thoughtful phylogenetic analysis to bear on the evolution of lichen-forming ascomycete fungi (which comprise 42% of all ascomycetes), Lutzoni noted the ancient origins of lichen-forming ascomycetes, with an estimated origin of 325–454 million years ago, preceding the appearance of ectomycorrhizal basidiomycetes by 100 million years. This ancient origin, and the continued thriving of lichen associations, is all the more stunning considering the physiological variability and apparent evolutionary malleability of lichen associations, with some evidence for conversion of 3 clades of ancestral lichen-forming ascomycete to pathogenic and saprophytic lifestyles over evolutionary time.

Marc-André Selosse (CNRS, Montpellier) showed how molecular tools have revealed new, unexpected diversity in the mycorrhizal fungi associated with the Ericaceae, stimulating revised perspectives on the evolution of mycorrhizae in this important and interesting plant family. Weaving together

physiological, ecological and genomic data, Selosse described the ways in which the development of partnerships with different mycorrhizal fungi over time are correlated both with plant-host phylogeny and also with host niche, including the evolution of parasitic and epiphytic lifestyles, and survival in oligotrophic soils at high latitudes and altitudes.

With an engaging overview of the fascinating and important symbioses between chemosynthetic bacteria and invertebrate hosts in the deep sea, **Robert Vrijenjoek** (Monterey Bay Aquarium Research Institute) raised provocative questions about the differing evolutionary consequences of vertical and horizontal transmission of those symbiotic bacteria to the various marine-invertebrate hosts whose lives depend on them.

Wolfgang J. Miller (Vienna) focused on a question of whose importance to evolutionary biology Maynard Smith would undoubtedly approve: the possible role of *Wolbachia* in speciation in 2 semi-species of the *Drosophila paulistorum* species complex. While the effects of *Wolbachia* on male-female sex ratios (Miller called *Wolbachia* the “ultimate feminist”) and post-mating isolation (through cytoplasmic incompatibility and hybrid inviability) are well known, Miller, working with Daniela Schneider and Prof. Lee Ehrman (SUNY, Purchase), have found that *Wolbachia* also affects mating behavior in Amazonian and Orinocan populations of *D. paulistorum*. In addition to the inviability of Amazonian and Orinocan hybrids (where each semispecies is infected with a different strain of *Wolbachia*, resulting in an overly high *Wolbachia* titers in those hybrids), there also is no mating in nature between these semispecies; laboratory studies confirm that Amazonian females preferentially choose to mate with Amazonian males. After treatment with antibiotics, however, Amazonian females lose all discrimination between Amazonian and Orinocan males, mating randomly, “like a 16-year-old at a bar”, with an isolation index of zero, suggesting that *Wolbachia* may affect the mating behavior of its hosts. Additional evidence that *Wolbachia* influences sex pheromone production, both qualitatively and quantitatively, and the discovery of *Wolbachia* in the mushroom body of the host brain provide intriguing hints for the mechanisms by which that behavior might be affected by *Wolbachia* infection.

5.2 Evolution: stability, conflict and cooperation (Angela Douglas and Cameron Currie)

How and when symbiotic organisms cooperate is a central problem in symbiosis research. The session on Stability, Conflict and Cooperation addressed three broad themes: How are conflict and cooperation related? What organismal traits and environmental conditions promote cooperation/antagonism? Are there predictable evolutionary and ecological consequences of cooperative and antagonistic interactions? This last theme was addressed throughout this session, and

indeed throughout the conference. One talk in this session focused on a specific evolutionary consequence: the impact of associations on speciation rates. **Dale Clayton** (University of Utah) demonstrated how the co-evolutionary relationship between feather lice and birds has an adaptive basis, linked to escape from host preening.

That conflict and cooperation are interdependent processes was exemplified by the presentation of **Michael Poulsen** (UW-Madison), who illustrated that conflict can be the basis for cooperative interactions. Dr. Poulsen described how the association between Attine ants and actinobacteria is based on the ability of the actinobacteria to produce antibiotics against a fungus, *Escovopsis*, which is a pathogen of the ants' garden fungal symbiont. **Angela Douglas** (Cornell University) provided evidence that cooperation can be genetically enforced. Her studies of the pea aphid symbiosis with the *Buchnera* bacterium show that through genome reduction and gene loss, the predicted metabolic network of *Buchnera* is structured to promote nutrient release; for example, sustained synthesis of purines, required for *Buchnera* growth, is dependent on high synthesis rates of histidine, an essential amino acid released to the aphid host.

Research was presented that focused on the impact of partner diversity on the outcome of associations. **Kevin Foster** (Harvard University) described an elegant model system involving the bacterium *Pseudomonas aeruginosa*. This species cooperates by contributing extracellular polysaccharide, which is of communal value, to the production of a community biofilm. Contrary to the expectation from kin selection theory, multiple unrelated strains of *Pseudomonas* produce more biofilm than single strains, and this is related to the involvement of biofilms in mediating competitive interactions among strains, as well as in cooperation. **Alison Bennett** (UW-Madison) has modeled mutualistic networks involving multiple taxa, as a basis to explore the stability and resilience of such networks. As new theories emerge, it will be interesting to test them empirically in model systems of symbiosis.

Two speakers addressed the evolution of mutualisms from antagonistic associations. **Debra Brock** (Rice University) described the carriage of bacteria by the slime mold *Dictyostelium discoideum*, providing a food source for newly hatched slime mould spores. **Catherine Masson-Boivin** (INRA, France) demonstrated early stages in the evolutionary origin of the legume-rhizobial symbiosis by selecting for symbiotic phenotypes in the plant pathogenic bacterium *Ralstonia solanacearum*. This research illustrates that associations can be plastic in ecological and evolutionary contexts.

5.3 Evolution: specificity (Ned Ruby)

In this session of the ISS, the unifying theme was the evolution of a central characteristic of symbioses: host-

microbe species specificity. The range of both the host (hydra, coral, nematode, squid and insects) and the microbes represented among the eight speakers was quite diverse. Nevertheless, several concepts were common to more than one of the speakers: (a) the evolution and function of specificity factors; (b) the creation of new structures and behaviors promoting specificity; (c) the role of diet in driving specific associations; (d) the diversity of evolutionary strategies for maintaining stability; and, (e) the value of developing and applying a combination of inferential and experimental approaches the study of symbiosis.

While all the presentations provided excellent examples of the excitement and novel findings enriching our understanding of how host-microbe specificity evolves, two of them merit special mention. **Thomas Bosch** (Kiel University) described the fascinating relationship between different hydra species and the specific, highly conserved community of microbial partners residing on their epithelia. Developmental disturbance of the tissue has been shown to lead to reproducible changes in the associated microbiota, which can be quantified by molecular approaches. However, most exciting was the discovery that cationic peptides produced by the host, and previously presumed to play an antimicrobial role in other animals, were actually a strong stabilizing influence on the association. That is, changes in the levels of these peptides caused a shift in the community from one dominated by alpha-proteobacteria to one dominated by beta-proteobacteria. Thus, the production of antimicrobial peptides in animals may have initially evolved as a mechanism for the host to select, maintain and modulate its co-evolved natural microbiota.

Margaret McFall-Ngai (UW-Madison) demonstrated that in the Hawaiian bobtail squid the genes, proteins, and physiology normally associated with the visual system are expressed bioluminescence organ. Thus, by developing a mechanism for ectopically expressing these functions, the bioluminescent organ has evolved the capacity to sense its own light-producing activity. Because luminescence is produced by symbiotic bacteria maintained in the organ, the ability to monitor light emission provides a means to sense the presence of 'cheater' symbionts that fail to bioluminesce. Excitingly, the discovery of this functional 'eye' has provided a specificity mechanism that explains a previously mysterious finding: the host squid can specifically sanction non-light-emitting symbionts.

In both these presentations, the evolution of specific chemicals or structures has led to mechanisms that allow the host to maintain a particular symbiotic microbiota. However, these particular findings play a larger role: they impact the broader scientific audience including the fields of pathogenesis and sensory biology, and demonstrate the central importance of symbiosis research to biology.

5.4 Evolution: genomics and coevolution (Nicole Gerardo)

Genomics increasingly serves as a tool to explore species interactions. For many symbioses, it is a particularly powerful tool, as genomic information provides novel insights into non-model host organisms involved in extraordinary symbioses and microbial symbionts that were once challenging to understand because they are not cultivable like their free-living relatives.

Talks in the Evolutionary Genomics section highlighted both of these advantages of genomics approaches. From the perspective of the host genome, **Nicole Gerardo** (Emory University), highlighted recent investigations into the immune gene repertoire of aphids, which are host to Gram-negative bacterial symbionts that provide nutrients not available from aphid diet and that provide protection against pathogens and parasites. Analysis of the recently available aphid genome suggests that this bacterial host is missing genes involved in responding to bacterial invaders that are present in other insects. This could both facilitate the maintenance and acquisition of microbial symbionts.

From the perspective of symbiont genomes, **John McCutcheon** (University of Arizona) highlighted his recent work on bacterial symbionts that co-infect insects. Astoundingly, genome sequences of these bacteria reveal that these symbionts often have highly complementary, non-overlapping capacities to provide nutrients necessary for their hosts' survival. Coevolution of these symbionts, such that one provides what the other does not, likely facilitated the reduction in their genome sizes, leading to some of the smallest bacterial genomes known to date. In a different system, that of chemosynthetic bacteria and their marine invertebrate hosts, **Colleen Cavanaugh's** (Harvard University) work similarly highlighted the diverse metabolic capacities of symbionts that can be revealed through genomic analyses.

The importance of genomics in Symbiosis research was apparent throughout the conference, beyond this single session. For example, other presentations of note include that of **Angela Douglas** (Cornell University), which demonstrated that metabolic exchange between aphid host and symbiont, and those from scientists investigating the squid-vibrio model system who are using genomic tools, like microarrays, to provide insight into how hosts respond to symbiont establishment.

5.5 Teaching symbiosis: bridging knowledge in high school and undergraduate curricula (S. Patricia Stock and Marc-André Selosse)

A fundamental concept in biology is symbiosis. Symbiotic associations are incredibly widespread in nature. Interactions

between microbes and their hosts can be viewed in terms of a continuum between symbiosis, commensalism, and pathogenicity. Thus, studying these relationships should be incorporated into biology classes and outreach programs from the high school to post-graduate levels. At present few educational institutions worldwide have incorporated this topic into their curricula. In this session, presenters discussed their teaching experiences and methods of Symbiosis instruction in a variety of venues. **Marc-André Selosse** (University of Montpellier) introduced the topic then illustrated how cooperative associations are inconsistent with Darwin's theories at first glance, but can be reconciled in light of current research. **William Reznikoff** (UW-Madison and Marine Biological Laboratories) described a lab series for high school students, developed with **Seth Bordenstein** (Vanderbilt University), to detect and sequence *Wolbachia* endosymbionts. **Patricia Stock** (University of Arizona) and **Karen Cloud Hansen** discussed methods to better engage undergraduates, using symbiotic systems and current societal issues. **Joanne Odden** (Metropolitan State College of Denver) presented details about a website for mutualistic symbiosis that serves as a college teaching resource, and encouraged symbiosis researchers to contribute their ideas. In an example of how independent research can be used as a powerful educational tool, **Darby Oldenburg** (UW-Richland) discussed the adaptation of the earthworm-microbial symbiosis model for undergraduate education. Following these talks, a session-wide discussion focused on teaching approaches that could provide undergraduate students and high school students and teachers with a broad view of the biodiversity, ecology and evolution of symbiotic associations. It was agreed that symbiosis is best used as a teaching platform integrated within other courses. Congress participants were then invited to participate in a hands-on lab practicum where they could observe and manipulate living symbiosis materials, including lichens, mycorrhizae, mites, earthworms, root nodules, ants, squid, nematodes, and insects.

5.6 Postdoctoral forum: acquiring a faculty position (John Chaston, Arijit Mukherjee, Bethany Rader, and Garret Suen)

A panel discussion designed to communicate the requirements for the successful application and acquisition of a faculty position in the biological sciences was held at the 6th International Symbiosis Symposium Congress. The panel consisted of senior and junior, male and female, faculty from domestic and foreign academic institutions with varying missions and goals. Areas of discussion were: the post-doctoral experience, networking, the application process, the interview process, post-interview communication, and negotiation. A summary of their insight along with current resources available to

those pursuing a career in academic biological science is being developed.

Panel Members: T. Bosch, N. Gerardo, K. McMahon, R. Rodriguez, S.P. Stock, R. Vega Thurber, and D. Yellowlees

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