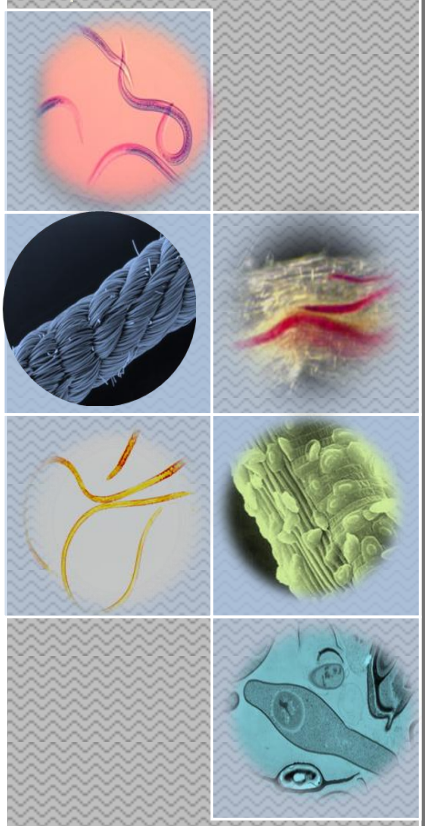
August 12, 2009



1st NEMASYM Research Coordination Network Meeting

University of Wisconsin, Madison, Wisconsin

NemaSym
A Research Coordination Network for the Study of Nematode-Bacterium Symbioses

National Science Foundation
WHERE DISCOVERIES BEGIN

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MEETING SCHEDULE

Room 1520 Microbial Sciences Building (MSB)

9.00-9.15 Opening remarks [Patricia Stock]

9.15-9.30 Clayton Cook, National Science Foundation, USA. The RCN program and other funding opportunities

9.30-9.50 NEMASYM background and goals. [Patricia Stock]

9.50-10.10 NEMASYM's role in education. [Heidi Goodrich-Blair]

10.10-10.30. Research interfaces, cross-disciplinary interactions and databasing over the course of the coming years. [David Bird]

10.30-10.45 Coffee Break

10.45-11.00 Upcoming meetings and events [Barton Slatko and Elodie Ghedin]

11.00-11.45 Introduction of participants. Core participants attending the meeting will give a 10 min summary of their research programs.

12.00-1.00 Lunch (on your own)

1.00-5.00 Oral presentations

1.00-1.10 David McK. Bird, North Carolina State University, NC, USA. *Signatures of Horizontal Gene Transfer and Convergent Evolution in the Root-Knot Nematode Genome.*

1.10-1.20 Kristina Fontanez, Harvard University, USA. *Bacterial diversity associated with* Caenorhabditis elegans

1.20-1.30 Buck S. Samuel, Harvard Medical School, Boston, MA,' USA. *More than just food: effect of wild microbes on* C. elegans' *metabolism*

1.30-1.40 Michael A. 6253 DESICCATOR CABINET, Medium - BelArt, Kansas State University, USA. *Ecological Genomics of Soil Nematode Community Responses: Model and Non-model Approaches.*

1.40-1.50 Louis S. Tisa. University of New Hampshire, USA.*A tale of two nematodes and their symbionts.*

1.50-2.00 Silvia Bulgheresi, University of Edinburgh, UK. *A first glance into the transcriptome of an ectosymbiotic marine nematode*

2.00-2.10 Ming-Min Lee, University of Arizona, AZ, USA. *A multi-gene perspective on the evolutionary history of* Xenorhabdus *spp., the bacterial symbionts of* Steinernema *nematodes.*

2.10-2.20 Susan M. Bornstein-Forst, Marian University, WI, USA. *Comparing Prokaryotic and Eukaryotic Stress Responses.*

2.20-2.30 John M. Chaston, University of Wisconsin, Madison, WI, USA. *Mutational analysis yields insights into the role of a bacterial host-association factor in a model animal- bacterial mutualism.*

2.30-2.40 *Regina C.M. Whitemars,* University of Wisconsin, Madison, WI, USA. *Investigation of the roles of nilb and nilc, host-association factors in a model animal-bacterial mutualism, using homologs in diverse Gram-negative bacteria.*

2.40-2.50 Steven Forst, University of Wisconsin, Milwaukee, WI, USA *Genetic analysis of xenocoumacin antibiotic production in the mutualistic bacterium* Xenorhabdus nematophila.

2.50-3.00 Mathieu Sicard, University of Poitiers*, France. On the different sides of specificity in* Steinernema-Xenorhabdus *symbioses*

3.00-3.10 Farrah-Bashey-Visser. Indiana University, IN, USA. *Evolution of life-history and social strategies using* Steinernema/Xenorhabdus.

3.10-3.20 Shelton,.G. Hurst IV, Valdosta State University, GA, USA. *Bacteriocin activity of* Xenorhabdus nematophila*, the bacterial symbiont of the entomopathogenic nematode* Steinernema carpocapsae*.*

3.20-3.30 Archna Bhasin, Valdosta State University, Valdosta, GA, USA. *Xenorhabdus bovienii* narrow-spectrum antimicrobial activity varies between strains.

3.30-3.50 Coffee break

4.00-4.10 Rousel A. Orozco, University of Arizona, AZ, USA. *Characterization and phylogenetic relationships of Photorhabdus sp. (Gamma-Proteobacteria: Enterobacteriaceae) the bacterial symbiont of Heterorhabditis sonorensis (Nematoda: Heterorhabditidae).*

4.10-4.20 Todd Ciche, Michigan State University, MI, USA. *A phenotypic switch is key to* Photorhabdus *symbiosis.*

4.20-4.30 Anthony Heidt, Michigan State University, MI, USA.Role of Type VI secretion system in symbiosis of *Photorhabdus luminescens* and *Heterorhabditis bacteriophora*

4.30-4.40 Holli Rowedder, University of New Hampshire, NH, USA. Identification of *Photorhabdus temperata* Mutants with Altered Symbiosis and Pathogenic Characteristics

4.40-4.50 Barton Slatko, New England Biolabs, MA, USA. *The* Wolbachia *Endosymbiont as a Potential Anti-filarial Nematode Target.*

4.50-5.00 Sara Lustigman, New York Blood Center, NY, USA. Brugia malayi *gene expression in response to the targeting of the Wolbachia endosymbiont elimination by treatment with tetracycline.*

5.00-5.10 Tiruneh Hailemariam, Lindsley F. Kimball Research Institute, NY, USA. *Investigation of ankyrin repeat domain containing* Wolbachia *proteins as a potential mediators of endosymbiosis with* Brugia malayi*.*

5.10-5.20 Joanne P. Odden. Metropolitan State College of Denver, CO, USA. U*ndergraduate Research and Education: Prevalence of* Wolbachia *Infections in Colorado Mosquitoes.*

5.20-5.30 Closing remark. Adjourn of meeting [Patricia Stock]

ABSTRACTS

Nematode-Bacteria Symbioses Research Coordination Network: Promoting Multidisciplinary Research and Expanding Educational Curricula

S. Patricia Stock[1], David McK. Bird[2], Elodie Ghedin[3] and Heidi Goodrich-Blair[4].[1]Department of Entomology, University of Arizona, [2]Center for the Biology of Nematode Parasitism, NC State University, [3]Department of Medicine, Division of Infectious Diseases, University of Pittsburgh, [4]Department of Bacteriology, University of Wisconsin-Madison, USA

Associations between nematodes and bacteria are ubiquitous, diverse and occur in all habitats. These symbioses range from fortuitous to obligate and from beneficial to pathogenic, making them an excellent model to understand many key questions in symbiosis (i.e. how microbes move between host species, how host and microbe adapt to each other physiologically and genetically, and what evolutionary consequences result from microbial-host associations) and leading numerous researchers worldwide to study them. However, few of these scientists interact with each other. A major reason for this lack of interaction is an artificial disciplinary division based primarily on nematode trophic groups. This inherent separation of medical/veterinary, agricultural and basic biological disciplines have inhibited interactions, communication and data sharing between scientists in these research areas. This, in turn, precludes emergence of fundamental themes of symbiosis and prevents technical and intellectual advances made in one system from being rapidly applied to others. To address this critical need for crossing disciplinary lines and to promote intellectual discourse among scientists studying bacteria-nematode associations, we have organized a Research Coordination Network on 'Nematode-Bacteria Symbioses.' Our intent is to provide a venue for setting common and comparative research goals and create new research collaborations and directions. Specifically, our main goals are: 1) Foster interdisciplinary collaborations between scientists; 2) Encourage scientists engaged in basic and applied research to explore how cross-talk and networking can enhance and advance science in this field: 3) Develop and distribute educational materials to nematologists, microbiologists and educators to consider and promote the study of nematode-bacteria symbioses as biological model systems in science and education. There is no other group in the United States or elsewhere that is similar to this research work group in its broad scope of nematode-bacteria interactions.

Signatures of Horizontal Gene Transfer and Convergent Evolution in the Root-Knot Nematode Genome.

David McK. Bird, Elizabeth Scholl, John Cromer, Peter DiGennaro Charles H. Opperman. North Carolina State University, North Carolina, USA.

Root-knot nematodes (RKN: *Meloidogyne* spp.) are widely distributed throughout temperate and tropical regions and are responsible for major yield losses on food and fiber crops. They render plants more susceptible to drought stress and are a significant contributing factor to a looming world food crisis. Our recently completed genome sequence of the 54 Mbp diploid RKN, *M. hapla*, provides a research platform to study

the genetic and biochemical basis for parasitism. One key feature the *M. hapla* genome is that it encodes a large cadre of genes apparently acquired during evolutionary history from bacteria, perhaps representing the legacy of ancient nematode-bacterial symbioses. These genes, many of which have seemingly obvious roles in plant-parasitism, have been amplified into families with diverse sub-specialties and at least partial redundancy. RKN also appear to encode a set of genes whose role is to mimic plant regulatory functions. Based on initial computational analyses, it appears that these functions evolved by convergent evolution. Collectively, RKN genes with strong inter-kingdom analogues appear to lie at the core of the parasitic armory.

***Bacterial diversity associated with* Caenorhabditis elegans**

Kristina Fontanez, Guus Roeselers, Adam Bahrami, Oleg Dmytrenko, Daniel Schott, C.R. Young, Erin Cram, Craig Hunter, Yun Zhang and Colleen Cavanaugh. Harvard University, Cambridge, MA.

Caenorhabditis elegans, the workhorse of modern molecular biology, is arguably one of the most well-studied model organisms, and yet very little is known about its associated microbial community. While laboratory-cultivated *C. elegans* has historically been considered to be germ-free due to the alkaline bleach method used to clean up strains, this assertion has not been rigorously tested. Further, the natural bacterial diversity associated with *C. elegans* isolated from the environment has not been well characterized. Using 454 pyrosequencing of the 16S rRNA gene and fluorescent in situ hybridization, we examined the bacterial diversity associated with laboratory-cultivated strains of *C. elegans* N2 from three different laboratories and a newly isolated environmental strain. Over 30 distinct genera, including those typically found in soils (e.g., *Burkholderia* and *Bradyrhizobium*), were detected in both laboratory and environmental *C.elegans* strains. These genera included representatives of diverse phyla such as Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria. This microbiome extends the genetic and metabolic landscape of its invertebrate host, raising questions regarding the microbial contribution to *C. elegans* biology including development, behavior, and innate immunity.

More than just food: effect of wild microbes on* C. elegans' *metabolism

Buck S. Samuel[1], Christian Braendle[2], Marie-Anne Felix[3], Gary Ruvkun[1]. [1]Dept. of Molecular Biology, Mass. General Hospital and Dept. of Genetics, Harvard Medical School,Boston, MA; [2]Institute of Developmental Biology and Cancer, CNRS, University of Nice Sophia-Antipolis, Nice, France; [3]Institut Jacques Monod, CNRS, Universities of Paris 7 and 6, Paris, France.

Animals have evolved in a microbial world. Microbes exert influence on a broad range of physiologic processes, from shear survival to development to regulation of energy balance. *C. elegans* is highly tuned to microbial cues as potential food or pathogen for its survival. Indeed, culture-based assessments of natural C. elegans habitats indicate the presence of a broad range of bacterial and phylotypes. Further, wild C. elegans adults harbor a population of undigested microbes in their intestines. To more broadly examine the natural microbiota of wild C. elegans populations, we performed culture-independent sequencing of bacterial (and fungal) SSU rDNA from habitats harboring wild *C. elegans* populations. The data indicates that these animals most commonly encounter bacteria belonging to four phylogenetic divisions (phyla), Actinobacteria, Bacteroidetes,

Firmicutes and Proteobacteria. Our goal is to use this natural association to explore conserved endocrine responses to microbes in *C. elegans.* To this end, we selected a panel of bacteria that were identified in at least three independent habitats and assayed various measures of energy balance. Growth rates, brood size and feeding rates of *C. elegans* are coarse assessments of the food quality and potential modulation of energy store partitioning. We found that *C. elegans* exhibited a spectrum of growth rates on the wild microbes compared to *E. coli*. Decreased brood sizes were also observed in animals grown on three diverse bacteria. Feeding rates were diminished on four microbes, with a Bacteroidetes being the most dramatic. Two of these microbes exhibit decreased Nile Red staining, though biochemical analyses of lipid content are ongoing. To allow for better classification of these growth responses by food quality-based measures, we are employing additional assays of *C. elegans'* activity and behavior. These results suggest that exposure to commonly encountered wild microbes distinctly alters *C. elegans* energy metabolism, which may represent co-evolved endocrine responses to microbes

Ecological Genomics of Soil Nematode Community Responses: Model and Non-model Approaches.

Michael A. Herman[1], Joseph D. Coolon[1], Kenneth Jones[1], Timothy Todd[2] and Vinod K. Mony[1]. KSU Ecological Genomics Institute, [1]Division of Biology, [2]Department of Plant Pathology, Kansas State University, Manhattan, KS 66502

Determining the genetic mechanisms involved in organismal response to environmental change is essential for understanding the effects of anthropogenic disturbance. This can be challenging, as well developed genomic tools exist for only a few organisms. We are using resident soil nematode populations sampled from the Konza Prairie Biological Station, near Manhattan, Kansas, to link the responses of organisms to environmental change at the genetic level. As nematodes are among the most abundant invertebrates in soils and respond quickly to changing environmental conditions, they are ideal organisms to assess the potential impacts of environmental change on soil communities. We have focused on microbial-feeding nematodes because of their important role in soil communities and to take advantage of the genomic tools available in the model soil nematode *Caenorhabditis elegans*. Both the microbial-feeding nematode and bacterial communities differentially respond to altered disturbance regimes and nutrient enrichment. We then used transcriptional profiling in *C. elegans* to identify candidate genes regulated in response to bacteria isolated in association with grassland nematodes or from grassland soils. Many of the regulated candidate genes are predicted to effect metabolism and innate immunity suggesting similar genes could influence nematode community dynamics in natural systems. Using mutations that inactivate many of the identified genes in *C. elegans*, we showed that most contribute to fitness and/or defense in a given bacterial environment. Although these bacteria may not be natural food sources for *C. elegans*, we show that changes in food source, as can occur in environmental disturbance, can have a large effect on gene expression, with important consequences for fitness. Moreover, we used regression analysis to demonstrate that for many genes the degree of differential gene expression between two bacterial environments predicted the magnitude of the effect of the loss of gene function on life history traits in those environments. This observation has important implications for interpreting the results of transcriptional profiling experiments of nematode populations in native environments, where in many cases the genetic tools to disrupt gene function have not yet been fully developed or that interfering with gene functions in nature may

not be feasible. We have also investigated nematode response to *Stenotrophmonas maltophilia*, a ubiquitous bacterium that we isolated in association with grassland soil Rhabditid nematodes that can cause nosocommial infections in immuno-compromised individuals. We have observed that, *daf-2* mutants, which have been shown to extend *C. elegans* lifespan on all bacteria tested to date, have a reduced lifespan in response to *S. maltophilia*. This suggests that there are still unknown immunity pathways and components that are involved in *C. elegans* defense pathways that can be explored by studying interactions with other nematode-associated bacteria.

A tale of two nematodes and their symbionts

Louis S. Tisa. University of New Hampshire, Durham, NH, USA

My research group is interested in the use of genome-wide approaches toward studying host-microbe interactions in symbiosis and pathogenesis. The roles of microbial behavior, signal molecules, and signal transduction in host-microbe interactions are areas of current focus. We study two different microbial-nematode systems that of interest to the NEMASYM network. The first system involves the entomopathogenic nematode *Heterorhabditis bacteriophora* which forms a specific mutualistic association with its bacterial partner *Photorhabdus temperata*. We are interested in identifying genes involved in insect pathogenesis and nematode symbiosis and have used a genetic approach to address this question. The *P. temperata* genome was sequenced to help facilitate the identification of these genes. A collection of 10,000 transposon mutants was screened and several mutant classes were recognized. From a pool of 86 motility mutants, preliminary screening identified 14 and 8 mutants with altered insect pathogenesis and symbiosis, respectively. In Collaboration with Kelley Thomas and Vaughn Cooper at UNH, we are also investigating a new microbe-nematode association. The *Serratia-Caenorhabditis briggsae* association forms an entomopathogenic complex. We have learned that *Serratia* strain SCB1 will form a symbiosis with other *Caenorhabditis* nematodes, including *C. elegans*. Like the *Photorhabdus*/*Heterorhabditis* system, this bacterial-nematode symbiosis is a potent insect pathogen and potential biological control agent. We have completely sequenced the genome of *Serratia* SCB1 and developed transposon mutant library to studying these interactions.

A first glance into the transcriptome of an ectosymbiotic marine nematode

Silvia Bulgheresi[1], Mark Blaxter[2], and Joerg A. Ott[1]. University of Vienna, [2] Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, UK

Stilbonematids are marine nematodes which are obligatorily coated by sulfur-oxidizing bacteria. They live few centimetres below the sea bottom, where they migrate between superficial sand layers and deeper ones. These migrations allow the bacteria to alternatively obtain oxygen and sulfide. In turn, the symbionts are the major components of their host diet. In order to discover genes involved in symbiosis establishment and maintanance, we generated an Expressed Sequence Tags (ESTs) collection from a Laxus oneistus cDNA library and focused on the most abundantly expressed transcripts. Among them are two novel highly hydrophilic proteins, a metallopeptidase, a thrombospondin, and an mRNA stabilizing protein. We are now in the process of analysing their localization pattern and their function.

A multi-gene perspective on the evolutionary history of* Xenorhabdus *spp., the bacterial symbionts of* Steinernema *nematodes.

Ming-Min Lee and S. Patricia Stock. Department of Entomology, University of Arizona, Tucson, AZ, USA

Bacteria in the genus *Xenorhabdus* (Enterobacteriaceae) belong to the gamma subdivision of Proteobacteria, and are found in symbiosis with entomopathogenic nematodes of the genus *Steinernema*. The two partners form a mutualistic association, lethal to insects, wherein the bacteria provides nourishment and chemical defense for its host nematode in exchange for protection and transportation between hosts. Although the evolutionary relationships between *Steinernema* have been studied quite extensively, relatively little molecular phylogenetic data exists for *Xenorhabdus*. To date, only one phylogenetic framework based on partial 16S rDNA sequences has been developed to estimate evolutionary relationships of *Xenorhabdus* spp. The following presents the results of several phylogenetic analyses methods based on 16s rDNA and three house keeping genes survey of 31 *Xenorhabdus* species.
Datasets were analyzed together and concatenated, considering various methods of phylogenetic analysis. Results of these analyses will be presented and discussed.

Comparing Prokaryotic and Eukaryotic Stress Responses

Susan M. Bornstein-Forst, Marian University, Fond du Lac, WI, USA

This report summarizes data on the conservation of metabolic choices made by prokaryotes and eukaryotes under environmental stress. Outcomes for exposure of entomopathogenic nematodes to temperature, and desiccation stress will be compared with those of the bacterium *Escherichia coli*. Results indicate conservation of cross-protection responses, induction and modulation of specific metabolic pathways, and increases in virulence capabilities for both representative organisms. The implications for use of EPN/EPB as a symbiotic system for biocontrol will be opened for discussion.

Mutational analysis yields insights into the role of a bacterial host-association factor in an model animal- bacterial mutualism

John M. Chaston and Heidi Goodrich-Blair. University of Wisconsin, Madison, Madison, WI, USA

The mutualistic monospecific association between *Steinernema spp.* (Nematoda) and enterobacteria in the genus *Xenorhabdus* is a model for studying animal-microbe associations and host range specificity. Colonization of a specialized region of the *S. carpocapsae* intestine by several hundred *X. nematophila* bacteria requires a 446-residue nematode intestine localization factor NilB. The role of NilB in colonization is unknown, and it has no homologs of known function in national sequence databases from which to infer function. NilB is a surface-exposed outer membrane protein that is predicted to adopt a beta-barrel structure within the membrane, suggesting a possible role as a porin or as a ligand for interactions with host molecules. To gain insight into the function of this novel host-association factor in nematode colonization, we employed a site-directed mutagenesis approach. Six regions of NilB that are predicted to be exposed to the surface of the cell were individually deleted in-frame. We also created seven in-frame deletions of portions of the amino terminal domain of NilB, a region of ~ 90 amino acids that is predicted to be localized to the periplasm and may function as a cork to the

predicted beta-barrel structure formed by the remaining residues of the protein. Deletion of some regions resulted in attenuated nematode colonization even though NilB was still detected in the membrane in most cases, suggesting a role for these residues in NilB biological function. Using epifluorescent microscopy and bacterial cells expressing green fluorescent protein, we investigated the effect of *nilB* mutations on three known stages of nematode colonization: initiation of colonization by a small founder population (one or a few cells), outgrowth of the founder population to a fully colonized population (several hundred cells), and persistence of the bacteria within the nematode intestine over time. *nilB* mutants were identified with defects in each of these stages of colonization, suggesting that NilB function is necessary for all three stages of host association. This work highlights the importance of NilB in colonization. Current studies are underway to further probe the structure and function of NilB in nematode colonization. Susceptibility tests comparing wild-type *X. nematophila* cells with the amino-terminal deletion mutants are being used to determine whether NilB forms a pore within the membrane, and NilB topology is being investigated using cysteine scanning.

Investigation of the roles of nilb and nilc, host-association factors in a model animal-bacterial mutualism, using homologs in diverse Gram-negative bacteria

Regina C.M. Whitemarsh, John M. Chaston, Dr. Heidi Goodrich-Blair.University of Wisconsin-Madison, Madison, WI, USA

The association between the nematode *Steinernema carpocapsae* and *Xenorhabdus nematophila* is a model to explore the molecular characteristics of mutualistic relationships between animals and bacteria. *X. nematophila* is specialized for colonizing the nematode intestine, in a region called the receptacle, at 30-60 colony forming units per nematode (CFU/IJ). Two bacterial genes, *nilB* and *nilC,* encode membrane localized factors required for *X. nematophila* to colonize the receptacle. Mutants lacking these factors have a nematode colonization defect. Although the specific roles of these factors in the colonization process are unknown, NilC may be an accessory factor to NilB since cellular levels of NilB are lower in a *nilC* mutant than in wild type bacteria. NilB is predicted to adopt an outer membrane β-barrel structure, making it a candidate for symbiont-host interaction through transport of important signaling or nutritional molecules, or by binding to host surfaces or structures. Online sequence repositories have only revealed sequences of unknown function with full-length similarity to NilB in a variety of ovine, bovine, porcine and human bacterial pathogens. To determine if NilB-like proteins are a general class of host interaction proteins across a wide variety of bacterial species, I sought to identify whether or not the homologs of NilB have a similar function as NilB. I complemented the colonization defect of an *X. nematophila nilB* mutant with *nilB* homologs from *Actinobacillus pleuropneumoniae*, *Dichelobacter nodosus*, *Haemophilus somnus*, and *Salmonella enterica arizonae. apl* and *dno* partially recovered colonization at ~0.01-3 CFU/IJ. An *X. nematophila nilB* mutant expressing *hso* and *sen* did not recover colonization, suggesting that these homologs were unable to complement a colonization defect, or that functional protein was not made. To differentiate among these possibilities, I inserted a FLAG epitope into each homolog and assessed protein levels via Western blot. Western-detectable homologs will act as tools I will use in continued research to gain insights into the role of NilB and NilC in colonization. Using the Western-detectable homologs I will determine if NilC has a specific or general effect on NilB on outer membrane proteins, if NilC is directly required for colonization or is an accessory factor to NilB, identify whether the amount of NilB-like

protein produced affects the level of nematode colonization, and characterize the colonization phenotype of each complementing NilB homolog.

***Genetic analysis of xenocoumacin antibiotic production in the mutualistic bacterium* Xenorhabdus nematophila**

Dongjin Park[1], Kristin Ciezki[1], Ransome van der Hoeven[1], Swati Singh[1], Daniela Reimer[2], Helge B. Bode[2] and Steven Forst[1]. [1]Department of Biological Sciences, University of Wisconsin, Milwaukee, WI 53201.[2] Institut für Molekulare Biowissenschaften, Goethe Universität Frankfurt, Germany.

Xenocoumacin 1 (Xcn1) and xenocoumacin 2 (Xcn2) are the major antimicrobial compounds produced by *Xenorhabdus nematophila*. To study the role of Xcn1 and Xcn2 in the life cycle of *X. nematophila* the 14 gene cluster (*xcnA-N*) required for their synthesis was identified. Overlap RT-PCR analysis identified 6 major *xcn* transcripts. Individual inactivation of the non-ribosomal peptide synthetase genes, *xcnA* and *xcnK*, and polyketide synthetase genes, *xcnF*, *xcnH* and *xcnL*, eliminated Xcn1 production. Xcn1 levels and expression of *xcnA-L* were increased in an *ompR* strain while Xcn2 levels and *xcnMN* expression were reduced. Xcn1 production was also increased in a strain lacking acetyl phosphate that can donate phosphate groups to OmpR. Together these findings suggest that OmpR-phosphate negatively regulates *xcnA-L* gene expression while positively regulating *xcnMN* expression. HPLC-MS analysis revealed that Xcn1 was produced first and was subsequently converted to Xcn2. Inactivation of *xcnM* and *xcnN* eliminated conversion of Xcn1 to Xcn2 resulting in elevated Xcn1 production. The viability of the *xcnM* strain was reduced 20-fold relative to the wild-type strain supporting the idea that conversion of Xcn1 to Xcn2 provides a mechanism to avoid self-toxicity. Interestingly, inactivation of *ompR* enhanced cell viability during prolonged culturing.

On the different sides of specificity in* Steinernema-Xenorhabdus *symbioses

M. Sicard. Laboratoire Ecologie, Evolution, Symbiose, University of Poitiers, 40 Av. du recteur Pineau, Poitiers, France.

One of the most investigated issue in symbiosis evolution is that of their specificity. Being specialized host might be a way to select the symbionts with which the interaction is the most efficient. If this is correct, one expects specificity to be detected on all traits which are determined by the interaction between the hosts and the symbionts. We investigated this issue in symbiotic systems where nematodes (*Steinernema*) and bacteria (*Xenorhabdus*) reproduce in insects they have both contributed to kill. Newborn infective juveniles (IJs) that carry bacteria in their intestine then disperse from the insect cadaver in the search of a new host to infect. In this work, we show that specificity can be detected on three different stages of the nematodes' life cycle: first insect exploitation, second re-association of IJs with bacteria and third IJs survival during dispersal. We studied these different types of specificity in two symbioses, *S. carpocapsae/X. nematophila* and *S. feltiae/X. bovienii*, which differ in their ecology. We found that they display the same overall specificity pattern even though specificity is not detected during the same life stage.

***Evolution of life-history and social strategies using* Steinernema/Xenorhabdus**
Farrah-Bashey-Visser. Indiana University, IN, USA

Reproductive decisions of parasites may affect within host success and between host transmission. We have used manipulative experiments to explore the evolution of these strategies in laboratory populations of the insect-parasitic nematode *Steinernema carpocapsae* and its bacterial symbiont *Xenorhabdus nematophila*. We have found that by selecting among insect hosts, it is possible to change the timing, number, and size of emerging nematodes. Additionally, varying nematode migration among insects affects bacterial antagonisms, the speed of insect death, and the timing of nematode emergence. Further we have begun to characterize the nematode life-history patterns, interspecific competitive abilities, and virulence in of three locally sympatric species. Thus far we have found that the trade-off between the size and number of nematodes emerging from a host varies across species. We have also found that high virulence is correlated with competitive dominance across species and that bacterial antagonisms are common in these natural isolates.

Bacteriocin activity of* Xenorhabdus nematophila*, the bacterial symbiont of the entomopathogenic nematode* Steinernema carpocapsae*.
Shelton,.G. Hurst IV, A. Bhasin. Valdosta State University, Valdosta, GA, USA.

Bacteria have evolved to produce various antimicrobial compounds to compete with one another. Specifically, bacteriocins are proteinaceous secondary metabolites that target specific species of bacteria, unlike the broader spectrum antibiotics. A bacterial symbiont of the nematode *Steinernema carpocapsae, Xenorhabdus nematophila* produces bacteriocins in response to the environment. The focus of the following experiment was to determine which related *Xenorhabdus* and *Photorhabdus* species *X. nematophila* inhibits along with the genes responsible for the bacteriocin production. To determine this, an antibiotic overlay assay was used. *X. nematophila* was spotted on tryptic soy agar for approximately 5-7 days at 30°C, then were chloroform killed for thirty minutes. Lastly, indicator strains were overlaid on the plates. Bacteriocin activity was evaluated by observing a clearing around the original spotted cultures. Results show that *X. nematophila* ATCC19061 inhibits the growth of *B. subtilis, P. luminescens* TT01, *X. szentermaii, X. japonicus, X. beddingii , X. budapestensis, X. ehlersii* and, *X. poinarii.* ATCC19061 does not inhibit *X. innexi.* The results also show that *X. nematophila* A24 inhibits growth of *B. subtilis, X. japonicus, X. beddingii, X. ehlersii, P. luminescens* TT01, *X. budapestensis, X. innexi,* and *X. szentermaii.* Lastly, a rifampicin resistant strain of *X. nematophila* ATCC19061 inhibits growth of *B. subtilis, X. japonicus, X. beddingii, P. luminescens* TT01, *X. szentermaii, X. budapestensis, X. ehlersii* and, *X. poinarii.* It does not inhibit *X. innexi.* The experiment yielded reproducible and consistent results. Currently, determination of the genes responsible for the bacteriocin and immunity proteins in *X. nematophila* A24 and *X. nematophila* ATCC19061 are the focus. Production of a knockout of the xenocin bacteriocin and immunity genes, found by N. Banerjee, is underway to determine if this xenocin gene is required for the antimicrobial activity observed. Secondly, a transposon mutagenesis screen for bacteriocin mutants is necessary as other genes may be involved in inhibition activity. A *himar* transposon has been used in the initial conjugation and mutagenesis efforts with a rifampicin-resistant strain of *X. nematophila*. An attempt to rescue-clone the transposons from exconjugants to verify that transposition occurred was unsuccessful. Continuation of the rescue-

cloning, as well as southern blotting, is being carried out to verify *himar* transposon mutagenesis.

Xenorhabdus bovienii **narrow-spectrum antimicrobial activity varies between strains**

Vidushi Gupta, Neha Gupta, Archna Bhasin, Valdosta State University, Valdosta, GA, USA.

Xenorhabdus bacteria are known to produce numerous secondary metabolites including an array of wide-spectrum and narrow-spectrum antimicrobials. Although the role of these antimicrobials is largely unclear, it is presumed that the wide-spectrum antimicrobials keep the host insect cadaver clean and free from other microbes while the narrow-spectrum antimicrobials may play a role in competition between *Xenorhabdus* spp. for host nematode association. To elucidate the function of *Xenorhabdus bovienii* narrow-spectrum antimicrobials, a growth inhibition survey was undertaken with multiple *X. bovienii* strains. The orange variant of the sequenced *X. bovienii* subsp. Jollieti strain (HGB1055) has the strongest growth inhibition phenotype; this strain produces 15-17.5mm radius halos when *X. nematophila*, *B. subtilis*, *P. luminescens* and *X. poinarii* are used as indicator strains. In addition, HGB1055 inhibits the growth of itself (4.6mm halo radius) as well as all other *X. bovienii* strains tested (with 8-14mm halo radii). The cream variant of the sequenced *X. bovienii* subsp. Jollieti strain (HGB1054) is approximately half as effective as the orange variant in growth inhibition. HGB1054 produces 6-9mm radius halos when *X. nematophila*, *X. poinarii*, *X. bovienii* HGB1055, *P. luminescens* and *B. subtilis* are used as indicator strains. Like HGB1055, HGB 1054 also inhibits itself (3.35mm halo radius) as well as all other *X. bovienii* strains tested (with 4.5-7mm halo radii). The primary and secondary forms of *X. bovienii* ATCC35271 were also tested for their inhibition profile. Although the secondary form (HGB004) did not grow well on TSA plates, the primary form (HGB003) grew and demonstrated inhibitory activities. The inhibition profile of HGB003 varied from the *X. bovienii* subsp. Jollieti profiles in that HGB003 inhibited *X. japonicus* the most with a halo radius of 5mm but hardly inhibited *P. luminescens* (halo radius of 2mm). HGB003 did inhibit itself (2mm halo radius) as well as all other *X. bovienii* strains tested (with 4-5mm halo radii). Prior to these results, we hypothesized that *Xenorhabdus* produced narrow-spectrum antimicrobials to compete with related species rather than with related strains and self-strains. In light of the self-killing and inter-strain-killing phenotypes observed, it is likely that at least some of the antimicrobials produced do not have/express cognate immunity proteins under the conditions used in this study. Also, since *X. bovienii* can colonize multiple Steinernematid nematodes (*S. jollieti*, *S. feltiae*, *S. intermedium*, *S. oregonense*), it is possible that different strains prefer different hosts and compete for their preferred host. It is also possible that these antimicrobials play a role unrelated to competition for nematode host association. Future identification of the gene(s) that encode the narrow-spectrum antimicrobial activity will shed light on the function(s) of these secondary metabolites.

Characterization and phylogenetic relationships of Photorhabdus sp. (Gamma-Proteobacteria: Enterobacteriaceae) the bacterial symbiont of Heterorhabditis sonorensis (Nematoda: Heterorhabditidae).

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Photorhabdus (Enterobacteriaceae) is a gram-negative bacterium that has a mutualistic association with entomopathogenic nematodes in the family Heterorhabditidae. Bacterial symbionts are harbored inside the intestinal lumen of the nematode of the third-stage infective juveniles, which vector the bacteria from one insect host to another. The bacteria are released by the nematodes into the insect hemocoel and kill insect host by septicemia in 24-48 h. The bacterium produces toxins and secondary compounds which provide an ideal environment for nematode reproduction and growth. Accurate identification and characterization of bacterial symbionts and their nematode hosts is essential to develop a more efficient utilization of this nematode-bacteria complex in insect pest management, as well to understanding of symbiotic interactions between these two mutualistic partners. In this study, we characterized the bacterial symbiont of *Heterorhabditis sonorensis* (strains CH35 and Caborca) a recently discovered entomopathogenic nematode species from the Sonoran desert. Molecular techniques considering a multigene approach (16S rRNA, gyrB, SerC, RecA sequences) were considered to characterize and assess evolutionary relationships of the new *Photorhabdus* strains with other members of this genus. Additionally, phenotypic tests were carried out to characterize these two bacterial strains.

A phenotypic switch is key to* Photorhabdus *symbiosis.

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The insect pathogenic nematode, *Heterorhabditis bacteriophora*, must transmit *Photorhabdus* symbionts to infect insects and to reproduce. The symbiont is responsible for insect killing unleashing an arsenal of insect toxins in the insect hemolymph, producing metabolites essential for nematode reproduction and producing antibiotics that inhibit competing microbes from the insect niche. The nematode serves primarily as a vector transmitting the symbiont between insect hosts. Because this symbiotic association is essential for each organism to reproduce in nature, infectious processes that increase the fidelity of symbiont transmission are likely to be canalized. We previously determined that symbiont transmission occurs maternally and involves infection and invasion of the maternal intestine before these processes are repeated on different cells in nematode offspring developing inside the maternal pseudocoelom casing matricide. Screening 8000 symbiont mutants individually for those unable to colonize offspring nematode intestines revealed that one fimbrial locus is required for colonization. The mutants are defective in initiation at the step of the transmission process unable to adhere to the maternal intestine and to establish a persistent infection. Upstream of the fimbrial locus is an invertible promoter switch that we determined to be predominantly in the OFF orientation in the primary phenotypic (wild type) variants and ON in small colony variants (SCV) by 5'-RACE and reporter fusions. Persistent symbiont biofilm cells in the maternal intestine were found to be SCV while transient symbiont cells present in the maternal intestine were primary variants. The differentiated SCV revert to the primary form at high frequency; papillae of primary colonies arise from SCV. Reversion of the SCV to the primary variant also occurs in the nematode since the primary and not the SCV were again isolated from the nematode offspring. From these

results we conclude that symbiont differentiation from the primary variant cells to SCV cells is essential for expression of adhesive organelles and initiation of symbiosis in the maternal intestine. In sum, symbiont transmission by insect parasitic nematodes requires cell differentiation, phenotypic and phase variation and production of an cell-surface adhesin organelle.

Role of Type VI secretion system in symbiosis of *Photorhabdus luminescens* and *Heterorhabditis bacteriophora*

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The nematode *Heterorhabditis bacteriophora* forms a mutualistic relationship with the enteric bacterium *Photorhabdus luminescens.* This interaction is useful for modelling the process of beneficial bacterial infection of a eukaryotic host. Following a transposon mutant screen, a mutant unable to colonize nematodes was isolated with an insertion in gene plu2287 (*tssH)* predicted to encode a ClpV-like protein. This locus resembles the type VI secretion system (T6SS) in *Edwardsiella tarda* and other related bacteria. The predicted protein is essential for T6S in *E. tarda* and is thought to function as a translocase instead of a protease. This recently discovered secretion system could play a role in cell-to-cell recognition between the nematode and the bacteria. Another gene of interest in this locus is Plu2299, which is predicted to encode a secreted Hcp-like protein. To validate the requirement of *tssH* for symbiont transmission we are creating markerless non-polar mutations in the genes using recombineering. The genetic manipulations will be verified by PCR and the ability of the mutants to be transmitted by nematodes. With these knockout mutants we hope to verify and determine the role of these genes in symbiotic transmission.

Identification of *Photorhabdus temperata* Mutants with Altered Symbiosis and Pathogenic Characteristics

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The entomopathogenic nematode *Heterorhabditis bacteriophora* forms an obligate association with its bacterial partner *Photorhabdus temperata*. The growth and development of this nematode requires a microbial symbiont, and each nematode species is very selective in associating with a specific bacterial strain. Joined with the bacteria the nematode actively seeks out insects to infect. After penetrating the insect, the bacteria are released within the hemocoel, where *P. temperata* rapidly replicates and produces a multitude of virulence factors, including several potent insect toxins and broad antibiotics. The purpose of these experiments was to determine the mechanisms involved with this symbiosis and the virulence factors that affect insect death. A random transposon library, pre-screened for motility defects were tested for altered pathogenic interactions with host model, *Galleria mellonella,* and for their ability to support growth and development of nematode host. Mutants were screened for pathogenesis by direct inoculation into the model insect *G. mellonella* for comparison to the wild-type. Two mutants, UNH5832 and UNH8309, were found to kill the insect more quickly than the wild-type, while still maintaining many other wild-type characteristics. In contrast, two mutants, UNH1307 and UNH6441, were found to cause delayed insect death when compared to wild-type; these two mutants also maintained similar growth rates.

Symbiotic interactions were tested by plating axenic nematodes onto lawns of mutant bacteria, and observed for 21 days. This screen yielded two mutants with alternative symbiosis characteristics, UNH6427 which cause delayed nematode growth, and UNH5832, which caused nematodes to develop faster than wild-type. Arbitrary PCR is currently be used to determine the sequence of the transposon-interrupted genes.

The* Wolbachia *Endosymbiont as a Potential Anti-filarial Nematode Target

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Over 1 billion people in more than 90 countries are at risk from filarial nematode infections, with 150 million people infected. The parasitic nematodes are insect-borne and are responsible for lymphatic (Elephantiasis) or cutaneous filariasis (Onchocerciasis /African River Blindness). Lymphatic filariasis is caused predominantly by *Wuchereria bancrofti* and *Brugia malayi* and affects 120 million individuals, a third of whom show disfigurement, while onchocerciasis, caused by *Onchocerca volvulus*, affects 18 million people of whom 500,000 have visual impairment and 270,000 are blind. Within these filarial parasites are intracellular alpha-proteobacteria, *Wolbachia*, that were first observed almost 30 years ago.Current anti-filarial chemotherapy can interrupt transmission by killing larvae, but is less effective on adult worms, which can live 10-15 years in humans. There is an urgent need to develop adulticidal drugs. Over the last several years, the obligate endosymbiont *Wolbachia* has been recognized as a potential target for filarial nematode life cycle intervention as evidenced by the loss of worm fertility and viability upon antibiotic treatment, including in human trials. However, current drug treatments are not practical due to the dosages and length of required treatments. Nevertheless, anti-*Wolbachia* targeting appears promising for filariasis control. The symbiotic relationship between *Wolbachia* and its nematode host remains elusive. Comparative genomics and bioinfomatic analysis has identified a number of potential interactions which may be drug targets, of which one is *de novo* heme biosynthesis, due to its absence in the host *B. malayi* genome sequence but presence in the *Wolbachia* genome sequence and its potential role in worm molting and reproduction. We will describe our worm viability assays which suggest that both female and male *B. malayi* adult worms are killed by heme biosynthesis-specific inhibitors. We will also describe our cloning, over-expression and analysis of the enzymes of the heme biosynthetic pathway for preparing proteins for drug targeting and our development of an *E. coli* functional complementation drug targeting strategy. Finally, we will describe our approaches for understanding the symbiotic relationship and identifying potential drug targets by analysis of lateral gene transfer events and initial protein-protein interaction studies.My goals for this presentation are two-fold: 1) I will briefly describe my undergraduate research project to survey *Wolbachia* bacterial infection prevalence in two genera of mosquitoes collected in Colorado and 2) I will briefly introduce my website with my teaching resources on mutualistic symbioses. A goal for many four-year teaching institutions is to bring undergraduates into research. A goal for many faculty members with full-time teaching loads is to find complementary approaches to teaching and research. My presentation will describe my foray into these fields. First, the focus of my undergraduate research project is studying *Wolbachia,* a maternally inherited endosymbiont,that has been estimated to infect between 20 and 76% of all arthropod species, and at least 15% of insect species. The phenomenon of cytoplasmic incompatability is associated with *Wolbachia* infections, and confers a reproductive advantage to *Wolbachia* infected females. This phenomenon may be applied to develop

methods of bio-control for vector-borne diseases, such as dengue fever and yellow fever, by introducing disease-blocking genes through transgenic *Wolbachia*. Data of biogeographic *Wolbachia* infection levels are necessary to determine the potential success of introduced transgenes. For this study, we have obtained *Aedes vexans* and *Culex tarsalis* mosquito samples collected by the Colorado Mosquito Control. Hundreds of insects from these genera (as well as smaller numbers of other genera, including *Culex pipiens*) were collected in numerous sites in the greater metropolitan Denver area. We plan to compare *Wolbachia* infection levels of these two mosquito genera found in similar habitats but with different life cycles.

Brugia malayi *gene expression in response to the targeting of the Wolbachia endosymbiont elimination by treatment with tetracycline*

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Brugia malayi, like most human filarial parasite species, harbors an endosymbiotic bacterium of the genus *Wolbachia*. Elimination of the endosymbiont leads to sterilization of the adult female. Previous biochemical and genetic studies have established that communication with its endobacterium is essential for survival of the worm. As a first step in identifying proteins involved in this process, we characterized by microscopy the effects of antibiotic treatment on *Wolbachia* cell structure and on the regulation of *B. malayi* transcripts altered in response to the anti-*Wolbachia* treatment. Using a microarray of the *B. malayi* coding sequences, we observe primarily upregulation of transcripts encoding proteins and enzymes involved in amino acid synthesis and protein translation and downregulation of transcripts involved in cuticle biosynthesis. Notably, we observe a bimodal pattern of regulation for most of the genes tested further by *in vitro* treatment and quantitative reverse transcriptase PCR: signaling genes and cysteine proteases were shown to be initially upregulated during the early phase of antibiotic treatment but are quickly downregulated in the following days, to then be once more upregulated by 6 days post-treatment. While the upregulation of protein translation and amino acid synthesis may indicate a generalized stress response induced in *B. malayi* due to a shortage of essential nutrients/factors which are otherwise supplied by *Wolbachia*, the downregulation of transcripts involved in cuticle biosynthesis perhaps reflects a disruption in the normal embryogenic program. This is confirmed by the expression pattern of transcripts which may be representative of the worms' response to *Wolbachia* targeted in different tissues; the early peak potentially reflects the effect of bacteria death on the embryogenic program while the second peak may be a manifestation of the adult worm response to the affected bacteria within the hypodermis.

Investigation of ankyrin repeat domain containing* Wolbachia *proteins as a potential mediators of endosymbiosis with* Brugia malayi*.

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Most human filarial parasites harbor an endosymbiotic bacterium of the genus *Wolbachia*. Several evidences suggest that the elimination of endosymbiotic *Wolbachia* is detrimental to the fertility and viability of the host Brugia malayi (Bm) worm. Hence *Wolbachia* endosymbiont might be an attractive new chemotherapeutic target for the treatment of human filarial infections. The overall objective of this project is to identify proteins that are involved in the endosymbiotic relationship. Among *Wolbachia* secreted proteins are those containing ankyrin repeat domains, known to mediate protein-protein interaction and implicated in host-pathogen interactions in other bacteria. To investigate weather wBm ankyrins are involved in host-bacterium interaction and to identify their binding partners in Bm extract, we expressed and purified three recombinant proteins containing ankyrin repeat domains within the wolbachia proteins wbm_0287, wbm_0394 and wbm_0447. We conducted a modified ELISA assay for interaction of the ankyrin domain of, wbm_0394, with varying concentration of Bm crude extracts. Our preliminary data indicates that this recombinant ankyrin domain binds to Bm extract in a concentration dependent manner. The binding of the recombinant ankyrin protein levels off at higher concentrations suggesting a possible saturation, a characteristic of specific interaction. We are in the process of panning of a cDNA phage display library and using proteomic approaches to identify specific interacting target in the filarial host.

Undergraduate Research and Education: Prevalence of* Wolbachia *Infections in Colorado Mosquitoes

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We will use previously described methods using polymerase chain reaction (PCR) primers specific to *Wolbachia* DNA to assay if individual mosquitoes are infected with *Wolbachia*. We will also use PCR to amplify insect DNA to determine DNA template quality. Results of prevalence of *Wolbachia* infection rates of collected *A. vexans* and *C. tarsalis*, should they be available at the time of the conference, will bepresented. Completion of the proposed research plan would provide the first data, to the best of our knowledge, of prevalence of *Wolbachia* in Colorado collected mosquitoes. Such data have been published for select mosquito genera in many sites worldwide, including California, Europe, Southeast Asia, and Africa. Together these data contribute to an understanding of the biogeographic distribution of *Wolbachia* in mosquitoes, and may also suggest the suitability of mosquito genera or species for bio-control mechanisms.
Secondly, I will introduce my website with mutualistic teaching resources. This website focuses predominantly on selected mutualisms featuring photosynthetic, chemosynthetic, luminescent, cellulose degrading, nutritional, or nitrogen fixation associations. A section on shared mechanisms of mutualisms and parasitisms and a

section on endosymbiosis theory are also included. To allow co-evolution of my online resources and my teaching, I welcome feedback and additions to this website.

ABSTRACTS OF POSTERS AND ORAL PRSENATATIONS OF NEMASYM MEBERS PRESENTED AT THE 6TH ISS CONGRESS, MADISON WI, August 9-15, 2009

Antagonistic Interactions among Bacterial Symbionts of Nematodes

Farrah Bashey-Visser, Hadas Hawlena, Curt Lively. Indiana University, Bloomington, IN, USA

Antagonistic interactions among bacteria are thought to be a major mechanism affecting population and community dynamics of microbes. Nevertheless, the actual distribution and variability of these interactions among bacteria in nature is largely unknown. We have examined a community of three entomopathogenic nematode species (genus *Steinernema*) and their symbiotic bacteria (genus *Xenorhabdus*). We find intraspecific antagonism among bacterial isolates of each species. Moreover, the spatial scale of these interactions suggests that symbiont antagonism may be important to the competitive success of their nematode hosts. We examine the ability of repetitive-DNA fingerprints to explain patterns of antagonism. Finally, we discuss whether intraspecific genetic diversity can be maintained via nontransitive interactions (A>B, B>C, and C>A) among bacterial symbionts and the potential consequences for their nematode hosts.

Biosynthesis and function of secondary metabolites from Photorhabdus and Xenorhabdus

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Photorhabdus and *Xenorhabdus* live in symbiosis with *Heterorhabditis* and *Steinernema* nematodes, respectively. The bacteria-nematode complex is highly entomopathogenic and is used in organic farming to kill different insect pests. Moreover, the difference between symbiosis (towards the nematode) and pathogenesis (towards the insects) can be studies using these bacteria and we have started to look in detail into the role of bacterial secondary metabolites, which might play a role in both processes. In the last few years we could identify (i) new secondary metabolites, (ii) the corresponding biosynthesis gene clusters, and (iii) could also propose functions to some of these compounds within the complex life cycle. One example is the biosynthesis of isopropylstilbenes: *Photorhabdus* is the only non-plant stilbene producer identified so far and makes stilbenes via a different pathway than the well-known plant pathway. Stilbenes are also important virulence factors against insects as they inhibit the insect phenoloxidase, are required for nematode development, and act as antibiotics to kill food competitors. Other examples are cyclic and linear peptides, anthraquinones, simple amides, unusual lipids, as well as more complex secondary metabolites, which have been identified in *Photorhabdus* and/or *Xenorhabdus*. In my talk I will present the identification and biosynthesis of these and other compounds and would like to suggest functions for these compounds within the complex bacteria-nematode-insect relationship.

Mobile Elements in Symbiotic Bacteria

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Any theory of bacterial symbiont evolution must account for an inflow and outflow of

genes that can rapidly shift the nature of the symbiosis. The inflow of new genes often occurs via the acquisition of DNA from unrelated bacteria through horizontal gene transfer. Some of the primary agents of horizontal gene transfer in bacteria are mobile genetic elements including bacteriophages, transposons and plasmids. Through their endogenous capacity to confer symbiont adaptations as well as kill the symbiont, these elements may profoundly shape symbiont interactions with eukaryotic hosts.
Here I interconnect recent information in microbial genomics, mobile genetic elements, and bacterial lifestyles to stimulate hypotheses on mobile elements in symbiotic bacteria. I will draw upon recent comparative sequence analyses of full bacterial genomes and experimental work from the widespread phage-*Wolbachia*-arthropod tripartite system to discuss the complexities of multi-layered symbioses. I will discuss two primary findings: (1) First, lifestyle and host range significantly affect the composition of mobile DNA in Eubacteria, and (2) Second, the host-switching *Wolbachia* parasite harbors an inducible, lytic bacteriophage whose activity is affected by abiotic and biotic factors important to the ecology of this widespread, animal-microbe symbiosis.

Life on the edge of Stilbonematid symbionts

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Stilbonematids are marine nematodes that may establish monospecific ectosymbioses with sulfur-oxidizing Gammaproteobacteria. They thrive a few centimetres below the sea bottom, where they migrate between superficial sand layers and deeper ones. In particular, Robbea sp.1 and sp.3 inhabit the shallow water sediment off Corsica (Mediterranean Sea) and off Carrie Bow Cay (Caribbean Sea), respectively. Their migrations allow the bacteria to alternatively obtain oxygen and sulfide. In turn, the symbionts are the major components of their host diet. Because the worms must acquire their symbionts from the environment at each generation and each time their cuticle is replaced by a new one, environmental transmission has long been hypothesized. Here, we could detect the 16S rDNAs of the Robbea symbionts not only in their respective habitats, but also in offshore surface seawater. Surprisingly, we could even identify the symbionts of Robbea sp.1 in Caribbean offshore seawater and Robbea sp.3 symbiont 16S rDNA in Mediterranean offshore seawater. Fluorescence In Situ Hybridization revealed that, for each species, the planktonic and symbiotic bacteria are morphologically similar. Moreover, the former are metabolically active, as they divide by binary fission. Intriguingly, the Robbea sp.3-associated bacterial rods, can undergo two different kinds of binary fission: while sticking on the host, they set their division plan parallel to their long axis (longitudinal fission); while free-living, they undergo a typical, transversal binary fission. This outstanding cytoengeneering feat was confirmed by immunostaining with antibodies against the E. coli tubulin homologue FtsZ. FtsZ forms the contractile rings, which ultimately cause the bacteria to divide: these were differently oriented in planktonic versus symbiotic bacteria. We are now analyzing the localization pattern of the Robbea sp.3 symbiont homologues of a family of proteins (MinCDE) that mediate the spatial localization of E. coli FtsZ: is their localization also influenced by the life-style of the Robbea sp.3-associated bacteria? Furthermore, we are attempting to induce planktonic Robbea sp.3 to divide longitudinally by incubating them with worm extracts and/or worm-secreted proteins. Our data not only support environmental transmission of the Robbea symbionts, but also highlight their striking cytological and metabolic flexibility.

Shedding Light on Chemosynthetic Symbioses: Comparative Genomics, Phylogeny, and Evolution

Cavanaugh, Colleen. Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts, USA

Chemosynthetic bacteria, living in symbiosis with marine invertebrates, are widespread in nature with such associations found in diverse habitats ranging from coastal reducing sediments to deepsea hydrothermal vents. Reduced inorganic sulfur compounds serve as the energy source fueling symbiont chemosynthesis. The intracellular equivalent to chloroplasts, the bacteria "feed" their hosts, using sulfur instead of sunlight for carbon fixation. Chemosynthetic symbioses act like "plants" at deep-sea vents where they are they are the major source of primary productivity. Although not yet cultured, insights into symbiont metabolism and roles in their hosts' nutrition have come from comparative genomic studies. Here, carbon, sulfur, and nitrogen metabolism by intracellular bacterial symbionts, inferred from comparative genomics, will be presented. The impact of vertical transmission vs. lateral acquisition on symbiont metabolism and phylogeny, genome evolution, and recombination will be discussed.

Genetic evidence for symbiotic trade-off in a nematode by experimental selection

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In any organism, life history traits are linked to each other by tradeoffs which shape the adaptation and even speciation processes. One of the best studied trade-offs is that between competitive aptitude and resistance to predators, herbivores or parasites. Costs of resistance have been investigated in various biological interactions : insect–parasitoid, plant–pathogen, and virus–bacteria. Here we have studied a symbiotic interaction where a nematode, Steinernema carpocapsae, associated to a bacterial endosymbiont, Xenorhabdus nematophila, parasitizes insect larvae. We have previously shown that nematodes that carry many bacteria are successful in parasitizing insects but die faster during their dispersal phase. Here we tried to change this benefits and cost to being associated to bacteria by imposing a selective pressure on bacteria to increase their division rate. We first show that bacteria have responded to selection by changing their growth curve, although some aspects of this response were not expected. We also demonstrate that, following this response, the virulence of bacteria toward an insect host has been increased. This increase in bacteria virulence should correlate to an increase of nematodes parasitic success. Nematodes that carry those virulent bacteria might conversely die faster.

Bacterial Symbiont in the Spirocercosis System

Yuval Gottlieb, Itamar Aroch, and Eran Lavy. The Hebrew University of Jerusalem, Rehovot, Israel

Spirocercosis is an emerging canine disease which has a variety of clinical presentations. The disease agent is the canine esophageal worm, *Spirocerca lupi* (Spirurida: Thelaziidae), that is found worldwide especially in tropical and subtropical regions. This worm is transmitted by coleopteran species intermediate hosts to the final canine host. Transmission may also involve a variety of paratenic hosts. In the final host,

the worm larvae migrate to the esophagus via the caudal thoracic aorta where they live in nodules; these nodules can transform to esophageal sarcomas. Diagnosis of spirocercosis in the early stages can be challenging and most animals are diagnosed only in the advanced stage of the disease, once nodules are already present in the esophagus. To find better means to diagnose and control the canine spirocercosis, presence of bacterial symbionts in the worm and the beetle was assessed. Using molecular methods a novel symbiont has been detected in *S. lupi* which is closely related to *Comamonas* spp. (Brukholderiales: Comamonadaceae) of the β-proteobacteria. This bacterium appeared to be located in the gut epithelial cells of the worm larvae. In the dung beetle, the presence of *Comamonas* spp. was detected sporadically, thus may reflect the infection status of the beetle. Other specific symbionts tested were not found either in the beetle or in the worm. Finding a stable infection of symbiont in *S. lupi* has implications in further understanding spircocercosis. Moreover, resolution of the complex interactions among the different organisms involved in this system may potentially lead to novel and simple methods for diagnosis, prevention and treatment of this disease.

A phylogenetic hypothesis on the evolution and interactions of Xenorhabdus spp. (γ-Proteobacteria) and their Steinernema hosts (Nematoda: Steinernematidae)

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Entomopathogenic nematodes of the genus *Steinernema* and their gram negative bacterial symbionts, *Xenorhadus spp*., are a tractable model system ideal for the study of mutualism. A specialized and intimate relationship exists between nematode and bacteria, affecting many of their life history traits, such as nutrition, dispersal, host-finding, foraging and defense from biotic and abiotic factors. Despite ease of culture in the laboratory and their commercial availability as biological pest control species, relatively little is known about the evolutionary relationships of each symbiotic partner and between each other. In this respect, phylogenetic approaches provide powerful tools for inferring the stability of host–symbiont associations. In this study, the congruency of host and symbiont phylogenetic topologies was assessed using a multigene approach to 1) develop phylogenetic histories for host and symbiont lineages and, 2) test the hypothesis of co-evolutionary histories and diversification of these two partners. With a thorough understanding of the co-phylogenetic patterns between *Steinernema* and *Xenorhabdus* we will be able to hypothesize and make predictions regarding genetic, physiological and ecological aspects of this intimate association.

Evolution of A College Teaching Resource: Mutualistic Symbiosis Website

Joanne Odden. Metropolitan State College of Denver, Denver, CO, USA

A fundamental concept in biology is symbiosis. This topic is presented in courses including general biology, botany, mycology, zoology, and parasitology, as well as numerous specialty courses. As a specialty course, symbioses and/or mutualisms have applications to many subdivisions of biology. The breadth and depth of symbiosis information lends itself to using multiple teaching resources beyond a single textbook. I have assembled my teaching resources on mutualistic symbioses in the website: http://www.mscd.edu/~biology/facstaff/faculty/odden/docs/Symbiosis%20 Teaching%20Resources%20Website.htm This website includes resources relevant to both lower division General Biology courses and

upper division specialty courses, such as my Symbiosis and the Environment course. My website includes: 1) Websites and Animation Links for Selected Mutualisms and Symbiosis Topics, 2) Selected Readings for Upper Division and Lower Division College Courses and 3) General Background Information Links. All are designed to supplement textbook resources. Selected Readings span ecosystem relevance and provide perspective. Journal article Reviews and non-traditional readings, for example naturalist essays, are included on this website. This website focuses predominantly on selected mutualisms featuring photosynthetic, chemosynthetic, luminescent, cellulose degrading, nutritional, or nitrogen fixation associations. A section on shared mechanisms of mutualisms and parasitisms and a section on endosymbiosis theory are also included. To allow co-evolution of my online resources and my teaching, I welcome feedback and additions to this website.

The Wolbachia Project: Discover the Microbes Within

William Reznikoff[1], Seth Bordenstein[2].[1]Marine Biological Laboratory, Woods Hole, MA, USA, [2]Vanderbilt University, Nashville, TN, USA

The Wolbachia project is designed to bring discovery-based laboratory exercises into high school curricula. The project introduces students to insect classification and molecular biology and is designed around an analysis of Wolbachia endosymbiosis in insects. Each year 25 teachers are invited to a short workshop at the MBL during which they are introduced to bacterial endosymbiosis and they develop proficiency in the insect identification and molecular biology techniques that their student shall use. The program also offers continuing support to teachers including the provision of critical supplies and loaner equipment, and provides the sequence analysis of amplified Wolbachia DNA. The program also funds teachers who wish to participate in summer research experiences in laboratories studying Wolbachia.

ENTO 310-Living in Symbiosis: Bridging Knowledge in Undergraduate Curricula

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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. Under this scope, ENTO 310 “Living in Symbiosis”, a recently developed undergraduate course at University of Arizona, focuses on symbiotic interactions with an integrated approach. Through this course, students gain knowledge and in depth understanding of microbial-host interactions. This course takes a broad view of the biodiversity, ecology and evolution of symbiotic associations. Spanning from highly integrated obligatory symbioses to loose associations, students learn how symbionts have adapted to their hosts with astounding sophistication, being able, in many cases, to control their reproduction, behavior and overall physiology. Although this course is mostly theoretical, laboratory demonstrations on a number of well defined model systems involving microbes (bacteria, fungi, protozoa etc) with their animal and plant hosts have been incorporated into is curriculum. In this presentation I will summarize the goals and dynamics of this course as a model for bridging knowledge in undergraduate education in the field of biological sciences.

Frankia Genomics, Signaling, and Natural Products: A New Light on an Old

Symbiosis

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Frankia are nitrogen-fixing actinobacteria that form root nodules with dicotyledonous plants in 8 families of angiosperms that are only distantly related to each other. Symbiotic interactions between *Frankia* and the host plant are not well understood. The nature of the chemical signals exchanged between the two partners of actinorhizal symbioses is still unknown due to the lack of genetic tools in *Frankia* and of specific molecular markers of the symbiotic interaction. While we have focused on resolving this situation by developing genetic tools, we have also pursued new genomic approaches toward studying these bacteria. Three *Frankia* genomes were sequenced and five more genomes are currently in the pipeline being sequenced. In the absence of genetic tools, the availability of these genome sequences also provides opportunities to use bioinformatic approaches and other new technologies. The three completely sequenced *Frankia* genomes were analyzed for known and novel natural product biosynthetic systems. Although the *Frankia* are not currently known as prolific natural product producers, a total of 60 (20 per genome) polyketide synthase, non-ribosomal peptide synthetase, terpenoid, specialized lipid or other biosynthetic gene clusters were discovered in numbers comparable to *Streptomyces* and *Salinispora* genomes. In many cases, chemical structures of the compounds could be predicted, and these include presumed antibiotics, siderophores, lipids, pigments and signaling molecules. Despite the high variability of chromosome size in these three species, the numbers of biosynthetic clusters were roughly equivalent. Proteomic experiments have validated the presence of these enzymes while preliminary MS studies have confirmed the production of some of these products. Besides the *Frankia* natural products study, we have use other genome guided approaches to search for marker genes of symbiotic interaction to identify symbiotic signals emitted by actinorhizal plant roots. A molecule present in root exudates from *Casuarina glauca* plants induced molecular and physiological changes in *Frankia*. These results and the similarities of *Frankia* to *Mycobacteria*, an intercellular pathogen, will be discussed.

Underground Communication Between Roots and Soil Organisms

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The underground world is a lively place, with plant roots, soil microbes, and other underground organisms waging a continual battle for resources. Plant roots take an active role in this conflict through the exudation of various chemicals, yet key areas of this process remain mysterious: what genes and gene networks control exudation? How do plant root exudates influence the microbial community of the soil, and how far does this influence extend from the plant root? Finally, if plant roots do play a large role in soil microbial composition, what effect does plant biodiversity have on soil microbial biodiversity?

Our studies in this area have proceeded on three fronts. On the first front, we've looked at how a specific set of genes, the ATP-binding cassette (ABC) transporter genes, influences root exudation (1,2). On another front, we've examined how plant root exudates influence the soil microbial community. We looked at two model plant species,

Arabidopsis thaliana and *Medicago truncatula*, and found that when each was transplanted to new soils with no history of growing its species, the diversity and biomass of that soil's fungal community plunged; the converse was also true (3,4). Through a related study on the effect of the root exudates of an invasive weed we found that the weed decimated soil fungal populations in its own rhizosphere and that of neighbouring plants (5). Current studies involve the analysis of how specific Arabidopsis ABC transporter mutants differently influence native soil microbes depending on their root exudate profile.

Finally, we've delved into the role plant root exudates play in soil microbial diversity. Our findings related to how plants directly determine the soil microbial community led us to question a recent study that found soil microbial diversity to be lowest in areas where the aboveground biodiversity was very high, such as the rainforests of South America (6). Armed with a new generation of microbial census techniques, we have found that the Amazon rainforest in fact has the highest level of microbial diversity of all soils examined, and this finding was confirmed by analyzing the soil microbes present in long term plant biodiversity plots. Finally, preliminary data will be presented on the ability of Rainforest microbes to effectively degrade lignin.

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ADDEDUM

Phylogenomics of Parasitism and Mutualism in Wolbachia

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Ecological and evolutionary theories predict that parasitism and mutualism are not fixed endpoints of the symbiotic spectrum. However, for obligate intracellular bacteria whose genomes are highly reduced, studies specify that discrete symbiotic associations can be evolutionarily stable for hundreds of millions of years. *Wolbachia* is an inherited obligate, intracellular infection of invertebrates containing taxa that act broadly as both parasites in arthropods, and mutualists in certain roundworms. Here I will touch on our current understanding of the differences in ecology, molecular evolution, and genome evolution that underlie these two symbiotic lifestyles. I will also highlight the importance of phylogenomic accuracy in understanding the ancestry of these differences.

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