Fourth Nemasym Meeting

"Origins and Evolution of Nematode-Bacteria Associations"

April 3rd 2012
Cold Spring Harbor
Laboratory, NY

PROGRAM

Hawkins Conference Room, Wendt Laboratory Building
Cold Spring Harbor Laboratory

Monday April 3rd	
Morning	
8:30 - 8:45	Registration
9:00 - 9:10	Opening Remarks
9.10- 10.00	*Invited Keynote Speaker,* ***Mark Blaxter, University of Edinburgh***
10:00-10.15	Coffee break
10:15- 12.30	Oral presentations
12:30 - 1:50	Lunch
Afternoon	
2:00 - 2:45	Round Panel Discussion
2:45 - 3:00	Coffee break
3:00 - 4.30	Round Panel Discussion
4.30- 5.00	Closing Remarks. Plans for 2013 activities.
5.00	Meeting Adjourned
Thursday April 5, 2012	
9.00-12.00. NEMASYM- sponsored Symposium. **" Parasitism, Pathogenesis & Symbiosis"**	
Wednesday April 4, 2012 **2.00 PM**	
NEMASYM sponsored poster presentations	

ABSTRACTS

Entomopathogenic Rhabditid Nematodes and their symbiotic bacteria

Almenara, Daniela Peres[1].; Yoshida, Nídia Cristiane[2].; Kato, Massuo Jorge.[2]; Kamitani, Fernando Luiz.1; Neves, Maira Rodrigues de Camargo[1] and Winter, Carlos Eduardo[1].
[1]Dep. Parasitologia, ICB, USP, SP, Brazil; [2]Dep. de Química Fundamental, IQ, USP, SP, Brazil. [3] CPqRR- Fiocruz –MG, Brazil.

Despite massive losses of primary forest, the Amazonian rainforest remains an extremely rich source of biodiversity. In recent years, entomopathogenic nematodes (EPNs) have been isolated from soil in different landscapes of the world and used successfully as biological control agents against numerous arthropod pests. Therefore, a sampling in the rainforest of Monte Negro, Rondônia, Brazil was conducted with the aim of discovering new strains and/or species of EPNs for future development as biological control agents. The strain named LPP7 was identified as *Heterorhabditis baujardi*. The enterobacteria *Photorhabdus* spp. are symbionts of the entomopathogenic nematodes *Heterorhabditis* spp. These gram-negative γ-proteobacteria undergo a complex life cycle involving a symbiotic stage in which bacteria colonize the digestive tract of the nematodes and a pathogenic stage in which several species of insect larvae are killed by both the nematode and the bacteria. *Photorhabdus* spp. can be grown in the absence of their nematode partner, under standard laboratory conditions used for other enterobacteria. *Photorhabdus* spp. synthesize and secrete several extracellular products, including a broad spectrum of bioactive secondary metabolites (Clarke, Cell Microbiol. 10:2159-2167, 2008). Here we present the taxonomic and biochemical characterization of *Photorhabdus sp.* (strain MN7), isolated from *H. baujardi* LPP7 (Dolinski et al., Mem. IOC, 103:150-159, 2008).

Nematode genomics and bacterial symbioses

Mark Blaxter[1], Sujai Kumar[1], Georgios Koutsovoulos[1], Gaganjot Kaur[1], Benjamin Makepeace[2], Alistair Darby[3]

[1] Institute of Evolutionary Biology, University of Edinburgh, Edinburgh UK, [2] Institute of Infection & Global Health and [3] Institute of Integrative Biology, The University of Liverpool, Liverpool UK

The nematode *Caenorhabditis elegans* was the first animal for which a whole genome sequence was determined, and this model continues to serve as a touchstone for complete, high quality genomics. In collaboration with an international consortium of nematode biologists, we are involved in genome sequencing of a number of other nematode species using second-generation technologies to infer robust phylogenetic hypotheses for the evolution of the Nematoda, and of particular phenotypes such as parasitism. Within a focus on animal parasites, we have draft genome data for several filarial nematode parasites of humans and other animals, and thus have also been

identifying and assembling their endosymbiotic *Wolbachia* genomes. We will present the current state of play in filarial nematode and filarial *Wolbachia* genomics, examining gene retention and loss across *Wolbachia* clades C and D, horizontal gene transfers to the host nucleus and inferences of the likely functional underpinnings of the nematode-bacterial symbiosis.

Molecular characterization of two LipoPolysaccharide-Binding (LBP)/Bactericidal Permeability Increasing (BPI) proteins from the marine nematode *Laxus oneistus*

Lisa Bauer[1,2], Harald Gruber-Vodicka[1] and Silvia Bulgheresi[2,3]

[1]Department of Marine Biology, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria
[2]Department of Genetics in Ecology, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria
[3]Center for Anatomy and Cell Biology, Laboratories of Genome Dynamics, Medical University of Vienna, Währingerstrasse 10, 1090 Vienna, Austria

Laxus oneistus nematodes are coated with sulphur-oxidizing Gammaproteobacteria. These belong to a single 16S rRNA-gene phylotype and are restricted to the posterior region of the nematode cuticle. The anterior part, instead, is left uncolonized. We characterized two nematode genes encoding for lipopolysaccharide-binding/bactericidal permeability increasing (LBP/BPI) proteins, Lo-BPI1 and Lo-BPI2. Based on transcriptomic data, these are expressed by adult nematodes. Cloning of their full-length cDNAs revealed that they encode for secreted proteins, each having an N-terminal and a C-terminal BPI domain. Mature BPI1 and 2 are 24-30% identical to other invertebrate BPIs and 22-24% identical to human BPI. Localization pattern analysis via immunostaining revealed that the hypodermal glands of the nematode secrete BPI1 and 2 throughout its anterior-posterior axis, and that these proteins bind the ectosymbiont. This suggests that, instead of affecting the ectosymbiont, the predicted antimicrobial action of BPI1 and 2 may prevent cuticle colonization by unwanted Gram-negative bacteria. In order to prove this hypothesis, recombinant expression and functional analysis of BPI1 and 2 are ongoing.

***Candidatus* Thiosymbion' spp., a genus of thiotrophic *Gammaproteobacteria* associated with two groups of nematodes as ecto- and endosymbionts.**

Harald R. Gruber-Vodicka[1*†], Manuel Kleiner[3†], Cecilia Wentrup[3], Nikolaus Leisch[2], Nicole Dubilier[3], Silvia Bulgheresi[2] and Jörg A. Ott[1]

[1]University of Vienna - Department of Marine Biology, Althanstrasse 14, A-1090 Vienna, Austria
[2]University of Vienna - Department of Genetics in Ecology, Althanstrasse 14, A-1090 Vienna, Austria
[3]Max Planck Institute for Marine Microbiology - Symbiosis Group, Celsiusstrasse 1, D-28359 Bremen, Germany.

Animals from six phyla are known to harbor chemoautotrophic sulfur-oxidizing (thiotrophic) bacteria that enable their hosts to live on inorganic sources of carbon and energy. Most of the numerous thiotrophic symbiont groups are associated with a single host group. In contrast, here we characterize '*Candidatus* Thiosymbion', *Gammaproteobacteria* related to the *Chromatiaceae*, which are associated with at least three host groups. Two of these groups belong to the nematodes – the Stilbonematinae (Desmodoridae) and the genus *Astomonema* (Siphonolaimidae), the third host group are gutless oligochaetes of the subfamily Phallodrilinae (Tubificidae). On the cuticle of stilbonematin nematodes, *Candidatus* Thiosymbion forms an ectosymbiotic coat, whereas in the mouthless genus *Astomonema* they are intracellular endosymbionts in a gut relict and in the gutless oligochaetes, *Candidatus* Thiosymbion is part of a bacterial consortium of extracellular endosymbionts between the cuticle and the epidermis. As the different host groups are not closely related and each descended from a non-symbiotic ancestor, the symbiotic associations with *Candidatus* Thiosymbion evolved independently in convergent evolution. Based on 16S rRNA as well as morphological analyses of 24 *Candidatus* Thiosymbion symbioses, we detected significant differences between the symbionts from different hosts. This provides clear evidence that each host species is associated with its own *Candidatus* Thiosymbion species in a highly stable and specific manner. Several of the 24 characterized *Candidatus* Thiosymbion symbioses are highly amendable for experimental fieldwork and offer a unique opportunity to study topics such as host-symbiont recognition or the effects of the different hosts on symbiont genome evolution.

A local isolate of the opportunistic pathogen *Stenotrophomonas maltophilia* evades a major *Caenorhabditis elegans* innate immune pathway

Corin V. White, Vinod Mony, Brian Darby* and Michael Herman.

Ecological Genomics Institute, Division of Biology, Kansas State University, Manhattan, KS.
*Current address: Department of Biological Sciences, University of North Dakota, Grand Forks, ND.

We have been investigating the interaction between *C. elegans* and St*enotrophomonas maltophilia,* a ubiquitous bacterium that can cause nosocomial and community-acquired infections, to dissect the impact of one important driver that serves to shape nematode

communities. We have discovered a pathogenic interaction between *C. elegans* and a local *S. maltophilia* environmental isolate, *JCMS.* Based on several analyses, we find *JCMS* to be more virulent than the reference environmental and clinical isolates. In addition, live GFP-labeled *JCMS* accumulated in the intestine significantly more than *E. coli OP50* or other *S. maltophilia* stains. Heat or UV-killed *JCMS* kills *C. elegans* less effectively than live bacteria, suggesting that the accumulation of living bacteria contributes significantly to JCMS virulence. Several innate immune pathways that serve to protect *C. elegans* from various pathogenic bacteria have been discovered, including the p38 MAP kinase, DAF-2/16 insulin-like and Sma/Mab TGFβ-related pathways. Mutants that disrupt numerous components of these pathways are hypersensitive to *JCMS* and *OP50*, suggesting minor involvement of these genes in a specific immune response. However, a *daf-2/*insulin receptor mutant that is resistant to almost all pathogens tested to date, was not resistant to *S. maltophilia JCMS,* suggesting the involvement of other undiscovered pathways. We are currently using a forward genetic approach to identify these genes and pathways and have identified hyper-susceptible and resistant mutants. Our combined studies might provide insight on the mechanisms of adaptation employed by *C. elegans* in response to *S. maltophilia* isolates encountered in the wild.

An interesting facet of the potential role of the *Wolbachia* surface proteins in the endosymbiotic relationship with *Brugia malayi* host

Elena Melnikow1*, Thomas Unnasch2, Elodie Ghedin3, Sara Lustigman1
1 Lindsley F. Kimball Research Institute, New York Blood Center, New York, NY 10065 USA
2 Department of Global Health, University of South Florida, Tampa, FL 33612 USA
3 Center for Vaccine Research, University of Pittsburgh School of Medicine, 3501 5th Ave, Pittsburgh,
Pennsylvania 15261 USA

Over 138 million individuals worldwide, mainly in the tropics, are infected with the parasitic nematode *Brugia malayi*, a lymphatic-dwelling filarial nematode, which causes Lymphatic Filariasis. *B. malayi* harbors the endosymbiotic intracellular bacterium *Wolbachia*, which is required for its development and reproduction. The molecular basis for this symbiotic relationship remains unknown. Thus we aim to identify and characterize the essential interactions of these endosymbiotic partners. Our initial studies indicate that members of the *Wolbachia* surface protein (WSP) family potentially play a role in this symbiotic relationship. The present study was designed to determine whether two members of this family, wBm0152 and wBm0432, which were also identified in *B. malayi* excretory-secretory products, are involved in the symbiosis. First, utilizing an ELISA-based method, we demonstrated that both proteins bound specifically to *B. malayi* crude extracts. Second, immunoelectron localization studies of these two WSPs indicated that these proteins were present not only on the surface of *Wolbachia* but also in the various host tissues. Finally, to indirectly identify the putative interacting *B. malayi* host protein(s) of these WSPs we used an immunoprecipitation pull-down

assay followed by mass spectrometry. Interestingly, these data suggest that wBm0432 interacts with several key enzymes involved in the host glycolytic pathway, while wBm0152 interacts with host cell's cytoskeletal proteins. Our ongoing studies aim to verify the bindings of WSPs to their putative interacting host proteins, providing a better

Comprehensive determination of the microbial influences on host health

Buck S. Samuel[1], Tim Durfee[2], Holli Rowedder[1], Chris Carr[1], Justine Melo[1], Jeremy Glasner[2], Sean Sykes[3], Sarah Young[3], Carsten Russ[3], Guy Plunkett[2], Chad Nusbaum[3], Gary Ruvkun[1]

[1] Dept. of Molecular Biology, Mass. General Hospital and Dept. of Genetics, Harvard Medical School, Boston, MA; [2] Department of Genetics and Genome Center of Wisconsin, University of Wisconsin, Madison, WI; [3] Genome Sequencing and Analysis Program, Broad Institute of MIT and Harvard, Cambridge, MA

Like other metazoans, *C. elegans* fitness (successful expansion) within its microbe-rich habitats depends on a tight balance of energy acquisition and expenditure. Thus, it is also highly tuned to microbial cues that allow it to separate potential food or friend from foe. Accordingly, some microbial signals have been postulated to influence fat storage in parallel to endogenous endocrine cues. Several studies also show that the *E. coli*-adapted N2-Bristol strain is especially sensitive to 'minor' differences in *E. coli* strains: faster growth rates, increased progeny delivery rates, and less fat retention is seen when worms consume HB101 compared to OP50. Perhaps due to this fitness benefit, worms also exhibit increased satiety and a behavioral preference for HB101.

Thus, we have sought to identify the *E. coli* gene products that modulate *C. elegans* fitness. To this end, we have sequenced *E. coli* genomes routinely used in *C. elegans* cultivation: HB101 (2 isolates), OP50 (2 isolates) and HT115. Despite little variation among strain isolates, 350 and 412 genes are 'unique' to OP50 and HB101, respectively. Many are organized into clusters, and represent a range of gene functions: e.g., carbohydrate utilization (96), cell wall/LPS modification (42), amino acid metabolism (21), regulation (41), the Cascade system (6) and fatty acid metabolism (5). Phenotype microarrays were also used to confirm the metabolic defects.

In order to systematically test the impact of these microbial gene products on *C. elegans'* fitness, we assembled nearly 200 single gene mutants with defined function in a 'neutral' and consistent genetic background (*E. coli* K12). We then used a number of assays to test a mutant's impact on N2 growth, broods, body size and fat storage. Our analyses indicate that both genes in core metabolism and transport/biosynthesis of conserved mediators of host interaction—autoinducers, biogenic amines, short-chain fatty acids and LPS—influence N2 fitness. Studies of these small molecules as sensory or nutritive cues to *C. elegans* directly or via regulation of *E. coli* metabolism are ongoing. However, results so far indicate that the microbial milieu of signals may be just as important of a determinant of *C. elegans'* fitness as the nutritional potential for supporting growth of a population within a given habitat.

Targeted Genome Enrichment as a Method of Purifying Endosymbiont DNA from Host DNA

Sandrine Geniez, Jeremy Foster, Sanjay Kumar and Barton E. Slatko[1]

New England Biolabs, Inc. Ipswich MA 01938 USA

Wolbachia endosymbionts are widespread in arthropods and are considered reproductive parasites, inducing a variety of phenotypes including cytoplasmic incompatibility, parthenogenesis, feminization and male killing, which serve to promote their spread through populations. In contrast, *Wolbachia* infecting filarial nematodes that cause human diseases, including elephantiasis and river blindness, are obligate mutualists. These *Wolbachia* are subjects of drug discovery initiatives, but purification methods for efficient sequencing of these unculturable bacteria have proven difficult using a variety of techniques including chemical gradients, PFG purification, library construction followed by gene walking, etc. To examine the biology of symbiosis in worldwide natural populations, we have created a set of SureSelect™ (Agilent) 120-mer target enrichment RNA oligonucleotides ("baits") for solution hybrid selection. These were identified from *Wolbachia* complete and partial genome sequences in Genbank, and were tiled across each genomic sequence with 60bp overlap. Baits were filtered for homology against host genomes that contain *Wolbachia* using BLAT, and sequences with significant host homology were removed from the bait pool. Filarial parasite *Brugia malayi* DNA was used as a test case, as the complete *Wolbachia* sequence is known. DNA eluted from capture was size selected (200-bp fragments) and sequencing samples were prepared using the NEBNext® Sample Preparation Kit. One-third of a 50nt paired-end sequencing lane on the HiSeq™ 2000 (Illumina) yielded 53 million reads and the entirety of the *Wolbachia* genome was captured. We then used the baits to isolate >95% *Wolbachia* DNA from *Armadillidium vulgare,* a distantly related *Wolbachia*, demonstrating that the method can be used to enrich target DNA from unculturable microbes over large evolutionary distances.

NEMASYM SPONOSORED ORAL AND POSTER PRESENTATIONS
C. ELEGANS CONFERENCE

Characterization of a bacterial symbiont from the Amazonian entomopathogenic nematode *Heterorhabditis baujardi*

Daniela P Almenara[1], Nidia C Yoshida[2], Massuo J Kato[2], Fernando L Kamitani[3], Maira R Neves[1], Carlos E Winter[1]
[1]Universidade de Sao Paulo, Departmento de Parasitologia, Sao Paulo, 05508-900, Brazil, [2]Universidade de Sao Paulo, Departmento de Quimica Fundamental, Sao Paulo, 05508-900, Brazil, 3Fundacao Oswaldo Cruz, Centro de Pesquisas Rene Rachou, Belo Horizonte, 30190-002, Brazil.

Enterobacteria of the genus *Photorhabdus* are symbionts of *Heterorhabditis* entomopathogenic nematodes.*Photorhabdus* undergoes a complex life cycle involving a mutualistic stage in which bacteria colonize the digestive tract of the nematodes and a

pathogenic stage in which several species of insect larvae are killed by the symbiotic partners. *Photorhabdus* spp. synthesize and secrete several extracellular products, including a broad spectrum of bioactive secondary metabolites (Clarke, Cell Microbiol. **10:**2159-2167, 2008). Some of these molecules are probably involved with communication between bacteria and nematode during symbiosis and pathogenesis. In this study, we intend to elucidate how the secondary metabolites biosynthesized by *Photorhabdus* affect the survival and physiology of *Caenorhabditis elegans*. Here we present the taxonomic and biochemical characterization of *Photorhabdus* sp. (strain MN7), isolated from *Heterorhabditis baujardi* collected in Monte Negro, Rondonia, Brazil (Dolinski et al., Mem. IOC, **103:**150-159, 2008). Taxonomic analysis was made using GyrB, GlnA and Rec-A gene sequences with both Maximum Likelihood and Minimum Evolution methods. MN7 is provisionally identified as a new strain in the *Photorhabdus luminescens luminescens* group. MN7 is resistant to ampicillin at 10µg/ml and other β-lactamic antibiotics. Secondary metabolites were extracted with ethyl acetate from LB culture media 48h after bacterial inoculum. The organic phase compounds were then analyzed by thin-layer chromatography (TLC). Major bands were purified in preparative plates, followed by high-performance liquid chromatography (HPLC) and submitted to nuclear magnetic resonance (NMR) analysis. Chemical structures were elucidated for two major compounds (D and F). Compound F, with a Retention Factor (RF) of 0.65 in TLC, is an stilbene, more exactly 2-isopropyl-5-[(Z)-2-phenylvinyl]benzene-1,3-diol. Compound D has a RF of 0.5 in TLC and preliminary results suggest it is an anthraquinone pigment. MN7 conditioned broth also presents a proteolytic activity against gelatin. Taken together, these results are the first reported for a Brazilian strain of the entomopathogenic genus *Photorhabdus*

Genetical Genomics of nematode parasitism

David M Bird[1,2], Valerie M Williamson3, Elizabeth H Scholl[2], Peter M DiGennaro[2], Dahlia Nielsen[1]

[1]NC State University, Bioinformatics Res. Cntr., Raleigh, NC, 27695-7566, [2]NC State University, Plant Pathology, Raleigh, NC, 27695, [3]UC-Davis, Nematology, Davis, CA, 95616

Host-parasite interactions can be viewed as the co-evolved interaction between genomes. Given suitable genetic and genomic resources for both partners, it should be possible to directly examine the parasitic interaction in a comprehensive, gene-by-gene manner. We have initiated such a project for the plant-parasitic nematode, *Meloidogyne hapla*, and the plant host, *Medicago truncatula*, to address the broad question: “How does the genetic makeup of the pathogen influence host gene expression?” More specifically, we consider the influence of allelic variation at each individual nematode locus on the expression of each and every plant gene. Natural genetic and phenotypic variation in field isolates of *M. hapla* has been captured in several, highly inbred nematode parental lines, and 140 recombinant inbred progeny lines (RIL) developed as a mapping population. Our broad approach is to perform a cross-species expression quantitative trait locus (eQTL) mapping experiment, followed by regulatory network

inference to characterize the cross-talk between organisms. Replicate pools of host plants have been
individually infected with the 140 nematode RILs and the combined transcriptomes of each individual determined by Illumina-based RNA-Seq. Deconvoluting the data allows us to determine expression levels of each plant and each pathogen gene, and also to genotype the pathogen line (based on SNPs) in one assay. Although this long-term experiment remains in progress, analysis of an initial 30 RILs has yielded an unprecedented insight into *M. hapla* genome organization, and both nematode and host expression profiles.

A Phylogenetic Analysis of the Genus *Photorhabdus*: 'til death do us part

Dana Blackburn1, Byron Adams[1]
[1]Brigham Young University, Biology, Provo, UT, 84602

Photorhabdus is a genus of Gram-negative bacteria belonging to the Enterobacteriaceae family. In addition to forming a mutualistic relationship with the Heterorhabditidae family of nematodes, these bacteria are virulent toward the insects that are hosts to these nematodes. Together, these organisms are used as biological control agents as analternative to harmful chemical pesticides. *Photorhabdus* virulence is dependent on various toxins and other virulence factors common to this family of bacteria such as type three secretion systems. There are three described species of *Photorhabdus*; *luminescens* and *temperata*, which are strictly entomopathogens, and *asymbiotica*, which has been isolated from wound infections in humans. Phylogenetic relationships were investigated using parsimony and maximum likelihood analyses with 62 taxa and three genes, 16s rRNA, gyrB, and glnA. Species formed strong monophyletic groups; however, subspecies placement was not as resolved. To investigate how virulence has evolved in this genus, bacterial cells were injected into *Galleria mellonella* larvae, and the LT50 was calculated for each strain. These values were mapped onto the phylogeny using ancestral reconstruction methods. Future research will investigate LT50 of *Photorhabdus* with various insect hosts to determine if the trends seen in *G. mellonella* are universal for this genus. Conversely, different species may more or less virulent toward different insect hosts. This study provides further insight into how virulence has evolved in *Photorhabdus*, which may aid inthe selection of nematode-bacterium complexes for biological control.

Then role and origin of nematode-encoded plant peptide hormone mimics in the host-parasite interaction

Peter M DiGennaro[1], Nijat Imin[2], Elizabeth H Scholl[1], Michael A Djordjevic[2], David M Bird[1,3]

[1]NC State University, Plant Pathology, Raleigh, NC, 27695, [2]The Australian National University, Plant Science Division, Canberra, ACT 0200, Australia, [3]NC State University, Bioinformatics Research Center, Raleigh, NC, 27695

Establishment of the parasitic interaction between root-knot nematode (RKN: *Meloidogyne* spp.) and its plant host is predicated on the ability of the nematode to not only accurately assess host physiological status, but also to effect cellular changes in the host. Like animals, plants deploy a suite of local and systemic hormones, including peptides, to regulate physiology and development. We recently discovered a new family of plant-peptide hormone implicated in relaying nutritional and environmental cues to regulate root developmental phenotypes, including root branching, hair development, and rhizobia-induced nodules. Based on this role, we named this family RAR: Root Architecture Regulators. Native RAR proteins are secreted into the extracellular domain (the apoplast) and processed into a 15 residue, bioactive peptide that presumably binds a surface receptor. Remarkably, RKN also encode RAR peptides, and we hypothesize that these hormone mimics are central to the ability of these parasites to subvert their host. Over-expression of plant RARs in the model legume *Medicago truncatula* induces the formation of galls with anatomical similarity to RKN feeding sites. Both gall types result from cellular proliferation of the root cortex. Exogenous application of a synthetic plant or nematode RAR 15-mer phenocopies gall formation. Endogenous RAR expression is up-regulated in RKN feeding sites as well as rhizobia-induced nodules. Although the origin of RAR loci in RKN is speculative, we favor the hypothesis that RKN acquired them from a plant by an ancienthorizontal gene transfer event; phylogenetic models are consistent with this interpretation.

Genomic analysis of Steinernema: insights into insect parasitism and intagenus evolution

Adler R Dillman[1,2], Kelley Fracchia3,4, Igor Antoshechkin[2], Ali Mortazavi3,4, Paul W Sternberg[1,2]
[1]Howard Hughes Medical Institute, Biology Division, Pasadena, CA, 91125, [2]California Insitute of Technology, Biology Division, Pasadena, CA, 91125, [3]University of California, Irvine, Development and Cell Biology, Irvine, CA, 92697, 4 University of California, Irvine, Center for Complex Biological Systems, Irvine, CA, 92697

The new generation of DNA sequencers have opened up the entire nematode phylum to whole-genome analysis, thus allowing us to explore the evolution of different genera. The genus *Steinernema* comprises over 70 characterized species that are lethal parasites of insects with differing foraging strategies and host ranges. We have sequenced and assembled the genomes and staged transcriptomes (set of expressed mRNAs) of five whole genomes spanning the *Steinernema* genus (*S. carpocapsae*, *S. scapterisci*, *S. monticolum*, *S. glaseri*, and *S. feltiae* using the Illumina DNA sequencing platform. Steinernematid genomes prove amenable to Illumina sequencing due to their size (~95 Mb) and high G+C content (~45%). The combination of multiple closely related genomes in a non-*Caenorhabidtis* clade and accompanying deeply sequenced transcriptomes allows for powerful comparisons to other genera such as *Caenorhabditis*. In particular, comparisons in expression at defined stages shows significant plasticity of timing across one-to-one orthologous genes in the 5 genomes plus *C. elegans*. Using available ecological and molecular data we explore genomic

differences likely to be involved in insect parasitism, particularly in host-range and specificity of these five species. We also examine the utility of these five genomes by orthology analysis within Nematoda, assessing the conservation of biological pathways, analyzing regulatory regions, and evaluating the established relationships within *Steinernema*.

Differential gene expression of rhabditid soil nematodes to diverse soil bacteria

Michael Herman[1], Brian Darby[2], David Wheeler[3]
[1]Kansas State University, Ecological Genomics Institute, Division of Biology, Manhattan, KS, 66506, 2University of North Dakota, Biology, Grand Forks, ND, 58202, [3]University of Rochester, Biology, Rochester, NY, 14627

Soil bacteria are an important part of the biotic environment for bacterial feeding soil nematodes, serving not only as food sources but also as potential pathogens. Interactions between nematodes and bacteria, therefore, are of significant importance to understand the ecological dynamics of the soil community but teasing out the underlying biology of these interactions remains a challenge. To begin to address this question we have isolated four rhabditidnematodes (*Oscheius tipulae, Oscheius sp. 2, Rhabditis* sp. and *Mesorhabditis* sp.) from soils collected at Konza Prairie Biological Station and used transcriptome sequencing to investigate their responses to a diverse set of soil bacteria. First, we co-assembled sequences from two RNA-seq libraries, one that was normalized and sequenced using the Roche 454 sequencing platform and another that was not normalized and sequenced using the Illumina Genome Analyzer IIx. Second, we used digital gene expression analysis to test the genomic response of nematodes to feeding on certain bacteria. Specifically, duplicate sequencing libraries were prepared from nematodes that were grown on one of six bacteria: two that were isolated from Konza prairie soils (*Pseudomonas sp., Bacillus megaterium*), two that were pathogenic (*Stenotrophomonas maltophilia, B. thuringiensis*), and two lab strains *(E. coli OP50, B. subtilis*). Short Illumina reads from each library were mapped against the hybrid assemblies as a digital estimate of transcript abundance (i.e. "gene expression"). Approximately 7000 genes that were shared by all four *de novo* transcriptomes and had a clear *C. elegans* ortholog were used in the preliminary analyses. This approach reveals that the genomic response of nematodes to bacteria appears to be more a function of taxonomic grouping than of pathogenicity or origin of isolation. In addition, there was very little overlap in the genes that are differentially expressed between species. This suggests that responses of different nematodes to different bacteria might be quite specific. In addition to understanding nematode responses to soil bacteria, these transcriptome sequences will complement genomic information available within the Rhabditid family and be useful in comparative genome studies. Ultimately, our studies will help us understand the gene functions involved in the formation and maintenance of dynamic soil nematode communities in changing environments.

Genome resident elements across 20 nematode genomes

Sujai Kumar[1], Mark L Blaxter1

[1]University of Edinburgh, Institute of Evolutionary Biology, Edinburgh, EH9 3JT, United Kingdom

We use the term Genome Resident Elements (GREs) to describe functional DNA elements that do not make an expressed product. GREs are identified by first performing whole-genome alignments of multiple species and then looking for conserved elements that are unlikely to code for proteins or RNA.Previous studies on such elements in vertebrates have concluded that they are highly conserved cis-regulatory elements located near developmental genes. Similar elements have been found near analogous developmental genes in alignments of five insect and three Caenorhabditis genomes. The elements did not show sequence similarity across phyla, leading to the speculation that such elements may somehow be related to the definition of the phylum body plan. In this study, we expand the study of GREs from three *Caenorhabditis* species to the breadth of the phylum Nematoda using 20 complete genomes. Preliminary results show that no GREs are shared across all nematode clades, and are therefore unlikely to be related to the phylum body plan. We will explore (a) whether the associations between GREs and genes or gene classes remain consistent across clades, (b) the gain and loss of GREs within each major nematode clade, and (c) the relationships between GREs and sequence-based functional elements identified by modENCODE. Armed with these analyses, we will be able to test specific hypotheses regarding the evolution and function of GREs.

Host mating system influences the degree of pathogen local adaptation in populations of *Caenorhabditis elegans* experimentally coevolved with a virulent bacterial pathogen

Levi T Morran[1], Raymond C Parrish[1], Ian A Gelarden[1], Curtis M Lively[1]
[1]Indiana University, Biology, Bloomington, IN, 47405

Under most circumstances, prolonged antagonistic coevolution is predicted to generate pathogens that can infect sympatric hosts with greater success than allopatric hosts. This form of local adaptation has been observed in several host-pathogen systems known to coevolve antagonistically. However, these results are not universal. Some pathogen populations within these systems have also been found to exhibit either local maladaptation or no difference in infectivity between sympatric and allopatric hosts. Together these seemingly opposing results show no clear pattern to distinguish the factors contributing to pathogen local adaptation or the lack thereof. We experimentally coevolved obligately outcrossing and mixed mating host populations of *C. elegans* with the virulent bacterial pathogen *S. marcescens*. This antagonistic coevolution generated several locally adapted populations of *S. marcescens*. However, the frequency and degree of local adaptation was largely determined by the host mating system. Local adaptation was more prevalent and more extensive in pathogen populations that

coevolved with obligately outcrossing hosts relative to those that coevolved with mixed mating hosts. We found that obligate outcrossing facilitated greater rates of host adaptation to the coevolving pathogens than did mixed mating. Greater rates of evolutionary change may have resulted in greater levels of divergence between obligately outcrossing populations than mixed mating populations, and therefore explain the relative differences in local adaptation. Although many factors may contribute to patterns of local adaptation, here we have identified host mating system as one factor that can influence the degree of pathogen local adaptation under antagonistic coevolution.

Bioactivity of secondary metabolites from *Photorhabdus luminescens sonorensis*, the bacterial symbiont of *Heterorhabdistis sonorensis*

Rousel A Orozco[1] and S. P. Stock[1].

[1] Department of Entomology, University of Arizona. Forbes Bldg. Rm 410. 1140 E. South Campus Dr., Tucson, Arizona, USA.

Symbiotic microorganisms represent an abundant and accessible source of novel bioactive compounds with potential applications in agriculture. Entomopathogenic bacteria in the genus *Photorhabdus* represent a rich source of such molecules, which possess insecticidal, nematicidal, antibiotic and antimycotic properties. The use of bacterial natural products in agriculture as environment friendly alternatives to harmful chemicals reduces pesticide application as part of integrated pest management. The bioactivity of secondary metabolites produced by the nematode symbiont *Photorhabdus luminescens subsp. sonorensis* was evaluated by fermenting bacterial cultures and extracting crude extracts, using Triptic soy broth fermentations as controls. The extract's composition was analyzed by TLC, HPLC-UV, and MS. Bioactivity of the metabolites was evaluated on the following plant pest and pathogens: *Helicoverpa zea*, *Meloidogine incognita*, *Fusarium oxysporum*, and *Pseudomonas syringae*. Significant levels of bioactivity where detected on all test organisms, which suggest the presence of multiple compounds with potential for agricultural bioprospecting. The most promising compounds for practical applications will be characterized by isolation and analysis of various fractions from the crude extracts, by elucidation of their structure and chemical nature, and by evaluating their bioactivity.

Microbial determinants of *C. elegans* health

Buck S Samuel1, Christian Braendle2, Marie-Anne Felix3, Gary Ruvkun[1]

[1]Massachussets General Hospital, Department of Molecular Biology, Boston, MA, 02114, [2]University of Nice
Sophia-Antipolis, CNRS, Nice, 06108, France, 3Ecole Normale Supérieure, Institute of Biology (IBENS), Paris, 75230, France.

Animals have evolved intimate symbiotic relationships with a consortium of gut microbes (microbiota) that represent a functional extension of the host genome and exert influence upon host health. The microbiota represents a 'microbial organ' that is likely to produce hormone-like molecules that affect the endocrine regulation of host energy balance and the ability to resist ecological pressures, such as pathogens and harmful chemicals (xenobiotics). Our studies demonstrate that the already genetically tractable, high-throughput and microbially 'tuned' nematode *Caenorhabditis elegans* is a useful system for interrogating these beneficial microbe-host interactions. By extensively sampling their natural habitats of rotting fruits and vegetation, we show that wild *C. elegans* adults harbor associated intestinal microbes characteristic of a microbiota. Through metagenomic sequencing efforts, we define the composition of this community with respect to the surrounding habitats both at the genomic and organismal level. Finally, we have found that these wild *C. elegans* strains are also highly adapted to respond to their native microbes, and are exploring the genetics of these interaction circuits. Using a combination of sequencing, comparative genomics, and biochemical approaches, we have demonstrated in parallel that *C. elegans* 'tunes' its metabolism to both evolutionarily conserved and novel microbial products. This natural system is well suited to expedite the identification and further characterization of the host and microbial signals and pathways that affect host health.

The Evolutionary History of Entomopathogenic Nematodes and their Bacterial Symbionts: Promiscuity or Fidelity?

S.Patricia Stock[1], Ming-Min Lee[1]

[1]University of Arizona, Entomology, Tucson, AZ, 85721

Entomopathogenic nematodes, *Steinernema* and *Heterorhabditis* have a specialized, mutualistic relationship with gram-negative bacteria (γ-Proteobacteria, Enterobacteriaceae) that are lethal to a wide array of insect hosts. This partnership functions as a result of combining both lifestyles of nematode host and bacterial symbiont enabling the entire association to take advantage of a broader ecological niche than either one could accomplish independently. Indeed, like in many primary endosymbionts pairs, each partner has an important function in the life history of the symbiosis. For example, nematodes help disseminate the bacteria from one insect host to another and provide a secure environment for the bacteria when they live outside the insect hosts. As bacteria proliferate inside the infected cadaver, they act both as a direct food source for the nematodes and actively degrade the host carcass to form secondary metabolites that nourish the nematodes. The bacteria perform also a protective role for their nematode hosts inside the infected insect cadaver, by creating a near-exclusive environment for themselves and their specific nematode via the production of antibiotics and antimicrobialcompounds.In this presentation we summarize the evolutionary histories of *Steinernema* and *Heterorhabditis* nematodes and their bacterial symbionts, *Xenorhabdus* spp. and *Photorhabdus* spp., respectively. Topological and likelihood based testing methods were employed to reconstruct the history of association between

host-symbiont pairs, and to gauge the level of similarity between their inferred phylogenetic patterns. The possible scenarios for historical association in the form of a cophylogenetic hypothesis will be presented and discussed.

Highly specific ectosymbionts of nematodes from the North Sea and their role in host nutrition

Judith Zimmermann[1], Jillian M Petersen[1], Jörg Ott[2], Niculina Musat[1], Nicole Dubilier[1]

[1]Max Planck Institute for Marine Microbiology, Symbiosis Group, Bremen, 28359, Germany, [2]University of Vienna, Department of Marine Biology, Vienna, 1090, Austria

Ectosymbiotic bacteria are widespread on marine organisms but the specificity of these associations and the beneficial role of the symbionts are still poorly understood. Stilbonematid nematodes occur worldwide in coastal sediments and carry a characteristic coat of sulfur-oxidizing ectosymbionts on their cuticle. Our aim was to investigate the specificity of these symbiotic associations and the role of these ectosymbionts for their nematodehost. For this, we investigated several closely related stilbonematid nematodes from the genus *Leptonemella* that co-occur in intertidal sandy sediments of the North Sea island of Sylt. Three *Leptonemella* species from Sylt have been described so far based on their morphology, but nothing is known about their ectosymbiotic bacteria. Our phylogenetic analyses, based on the 18S rRNA gene of the nematodes, revealed an unexpectedly high diversity of at least five *Leptonemella* species (95- 97% sequence identity) that co-occur in Sylt sediments. Our analyses of the bacterial 16S rRNA gene and the ribosomal intergenic spacer region (ITS) showed that the *Leptonemella* ectosymbionts are closely related to the gammaproteobacterial sulfur-oxidizing ectosymbionts of other nematode species and the endosymbionts of gutless marine oligochaetes. Remarkably, each of the five host species has its own distinct 16S-ITS rRNA symbiont phylotype (99.1- 97.2% sequence identity), indicating that these ectosymbioses are highly specific, despite the fact that the hosts co-occur and acquire their symbionts from the environment. It is widely believed that ectosymbiotic bacteria of stilbonematid nematodes provide nutrition to their hosts. As no clear evidence has been provided so far, a further aim of this study was to investigate this hypothesis by incubating the *Leptonemella* worms and their symbionts with radiolabelled bicarbonate. After developing a method to separate the ectosymbionts from the worms, we measured radioactive label incorporation separately in both fractions. With this method we showed that the ectosymbionts fix inorganic carbon, which is then transferred to the host tissue. We are currently using nanoscale secondary ion mass spectrometry (NanoSIMS) on *Leptonemella* tissue sections to examine the transfer of carbon in more detail. In summary, our findings show that there is a high degree of specificity in the ectosymbiotic associations of these very closely related co-occurring host species and that the hosts benefit nutritionally from their symbionts.

LIST OF PARTICIPANTS

Last name	First Name	Country	Email
Almenara	Daniela	Brazil	danisorocaba@gmail.com
Bauer	Lisa	Austria	lbauer@gmx.net
Bird	David	USA	david_bird@ncsu.edu
Blackburn	Dana	USA	Blackburn84@gmail.com
Blaxter	Mark	UK	mark.blaxter@ed.ac.uk
Denver	Dee	USA	denvedee@cgrb.oregonstate.edu
Di Genaro	Peter	USA	pmdigenn@ncsu.edu
Dillman	Adler	USA	adlerdillman@gmail.com
Ghedin	Elodie	USA	elg21@pitt.edu
Goodrich-Blair	Heidi	USA	hgblair@bact.wisc.edu
Gruber-Vodicka	Harald	Austria	harald.gruber@univie.ac.at
Herman	Michael	USA	mherman@ksu.edu
Koutsovoulos	Georgios	UK	G.D.Koutsovoulos@sms.ed.ac.uk
Kumar	Sujai	UK	sujai.kumar@ed.ac.uk
Lustigman	Sara	USA	slustigman@nybloodcenter.org
Melnikov	Helena		EMelnikow@NYBloodCenter.org
Morran	Levi	USA	lmorran@indiana.edu
Orozco	Rousel	USA	rouselo@email.arizona.edu
Rogers	Matthew	USA	rogersm@pitt.edu
Samuel	Buck	USA	bsamuel@molbio.mgh.harvard.edu
Schwartz	Hillel	USA	hillels@caltech.edu
Slatko	Barton	USA	slatko@neb.com
Stock	Patricia	USA	spstock@email.arizona.edu
Zimmermmann	Judith	Germany	jzimmern@mpi-bremen.de

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