Symbiosis Workshop 2011
Symposium Schedule
Sierra Nevada Research Institute


Organized by Monica Medina & Joel Sachs
Symbiosis Workshop Schedule April 30, 2011 – May 1, 2011

Schedule of invited talks

April 30, 2011

Session 1. Mutualism, parasitism and abandonment of symbiosis

- 12:50-1:00 PM Introduction and welcome to meeting (Monica Medina or Joel Sachs)
- 1:10-1:30 PM Jason Stajich  Fungal encounters with the amphibian kind: Comparative and population genomics of the amphibian pathogen Batrachochytrium dendrobatidis (Bd)
- 1:30-1:50 PM Mónica Medina  Recently uncovered intricacies of coral-Symbiodinium interactions.
- 1:50-2:10 PM Richard Stouthamer  Host-symbiont intragenomic conflict in Parthenogenesis Inducing Wolbachia infected wasps
- 2:10-2:30 PM Michal Segoli  The effect of Wolbachia on realized lifetime reproductive success of parasitoid wasps
- 2:30-2:50 PM Joel L. Sachs  Evolutionary transitions in bacterial symbiosis
- 2:50-3:20 PM Break for coffee and snacks

Session 2. Host adaptation, specificity and phylogeography

- 3:20-3:40 PM Ellen L. Simms  Meso-phylogeography of a native population of nodulated Bradyrhizobium
- 3:40-4:00 PM Anthony S. Amend  Community Structure, Transcriptomics and Phylogenetics of Coral-Associated Fungi Indicate Novel Nutritional Roles and Deep-Branching Lineages
- 4:00-4:20 PM Nicole A. Hynson  Partners in Crime: non-pathogenic microbial invasions and the functional ecology of plant-fungal symbioses
- 4:40 Closing remarks (Monica Medina or Joel Sachs)
- 5:00-7:30 PM Dinner and poster session
- 7:30 Roundtable Discussion of Research Coordination Network NSF grant

May 1, 2011

Session 3. Mutualist services

- 9:00-9:20 AM Rodolfo Dirzo  Biotic anti-herbivore defense in plants: examples from the tropics.
- 9:40-10:00 AM Steven Lindow  Interactions between bacteria and fungi and with the plants on which they live
- 10:00-10:30am Break for coffee and snacks

4. Symbiont consortia and multispecies interactions

- 10:30-10:50 AM Scott C. Dawson  Anaerobic protists are microbial communities: ecto- and endo-symbiotic bacteria and archaea in rumen protists
- 10:50-11:10 AM M. Pilar Francino  Host-microbial symbiosis in the human gastrointestinal tract
- 11:10-11:30 PM Forest Rohwer  Revisiting the coral holobiont

- Closing Remarks (Monica Medina or Joel Sachs)
Jason Stajich
Fungal encounters with the amphibian kind: Comparative and population genomics of the amphibian pathogen *Batrachochytrium dendrobatidis* (Bd)

*Bd* is an emerging infectious disease linked to worldwide amphibian decline. It is the only known Chytrid fungus that infects animals. Motile zoospores land on frog skin, encyst, and develop into sporangia which mitotically divide and produce zoospores. Open questions in the field include whether these fungi reproduce sexually, what are the means and range of dispersal, and the recent evolution that lead to its success as a pathogen.

Global genotypic variation appears to be low, but some private geographic-specific alleles are present suggesting genetic isolation of strains. Using the whole genome sequence of reference strains and Illumina-based re-sequencing of additional strains we identified polymorphisms to compare variation among isolates from the Western & Eastern US and Central & South America. The *Bd* genome is diploid and previous work (James et al, 2009) has identified regions of loss of heterozygosity (LOH) from Multi Locus Sequencing markers. Analysis of whole genomes of multiple strains now identifies precise genomic locations of independent and shared LOH events. We have found that strains from Central and South America have lost alleles that are observed in North America due to reciprocal crossover of chromosome arms. The resequencing data from geographically and genetically diverse strains allow us to build a high-resolution inventory of genetic variation useful for future developments of high throughput sample genotyping and tracing origins of *Bd* outbreaks.

In addition to population genetics of this emerging pathogen we also have compared the genomes of it and closest available relatives and identified sets of lineage-specific gene family expansions. These expansions include gene families involved in protease activity, carbohydrate binding modules, and secreted peptides. Our genomic comparisons shed light into recent genomic changes suggesting adaptations that may have been important for transitions to pathogenicity.

Mónica Medina
**Recently uncovered intricacies of coral-*Symbiodinium* interactions.**

Coral-algal symbioses are essential for the existence of coral reef ecosystems. *Coral*-*Symbiodinium* symbioses are tightly coupled interactions susceptible to imminent threats associated with global climate change. Increasing sea surface temperatures in particular, are posing mounting pressure on coral reefs. Thermally induced bleaching is linked with the disruption of the symbiosis. I will discuss the genomic (gene expression) information that is providing insight into the cellular processes involved in the onset and breakdown of coral-*Symbiodinium* symbiosis. We have found that physiological performance is not always coupled with transcriptome responses. We are now trying to elucidate how much of this uncoupling is associated to host-algal genotype combinations, environmental conditions, and/or potentially other holobiont members.

Richard Stouthamer
**Host-symbiont intragenomic conflict in Parthenogenesis Inducing *Wolbachia* infected wasps**

Bacteria of the genus *Wolbachia* are very common symbionts in insects and nematodes. In insects these bacteria are generally classified as reproductive parasites, manipulating the hosts reproduction to further their own transmission, while in nematodes they appear to be nutritive. *Wolbachia* have evolved several different strategies to manipulate their hosts reproduction to their own advantages, these include: cytoplasmic incompatibility, male killing, feminization and induction of parthenogenesis. Because these symbionts are exclusively maternally inherited males are a "dead end" for the transmission of *Wolbachia*. The parthenogenesis inducing (PI) *Wolbachia* have come up with the ultimate adaptation by inducing females to produce female offspring from unfertilized eggs. This is accomplished by a modification of the first mitotic division leading to a failure of separation of the two identical sets of chromosomes in the first mitotic division, thus doubling the number of chromosomes in the unfertilized eggs, and this results in the development of females in these haplo-diploid wasps. If a PI *Wolbachia* infected egg is fertilized then the *Wolbachia* does not appear to have any influence on the chromosome behavior and the diploidy of the egg is accomplished by the sperm chromosomes. The resulting egg will develop into an infected female. When a population is initially infected with PI-*Wolbachia* there is a clear conflict over the offspring sex ratio between the nuclear genes, that have some optimal sex-ratio involving males, and the cytoplasmic genes (PI-*Wolbachia*) whose optimal sex ratio consists of only females. This conflict can be resolved by either the loss of the infection through the action of nuclear suppressor alleles or by fixation of the infection in the population with a loss of sexual reproduction. In some cases a stalemate is reached where the infection remains at an intermediate level. Examples of these outcomes will be discussed.
Michal Segoli, Jay Rosenheim\textsuperscript{1}, Corinne Stouthamer\textsuperscript{1} and Richard Stouthamer\textsuperscript{2}

\textbf{The effect of Wolbachia on realized lifetime reproductive success of parasitoid wasps}

The bacterium \textit{Wolbachia} is found in a wide range of invertebrates. To enhance its transmission, \textit{Wolbachia} has evolved a variety of reproductive manipulations of its hosts. Due to its wide distribution and diverse phenotypes, \textit{Wolbachia} is considered important in the evolution of sex determination and speciation. A fundamental question is what is the effect of \textit{Wolbachia} on the fitness of their host? Laboratory studies showed that \textit{Wolbachia} may have positive, negative or no effect on the reproductive success of their hosts. However, it is not certain whether these results pertain to natural conditions. The parasitoid wasp \textit{Anagrus sophiae} that parasitizes the eggs of planthoppers is a good model to address these questions. Some wasp populations consist almost entirely of females because nearly all wasps carry a \textit{Wolbachia} strain that induces asexual reproduction (i.e. parthenogenesis), while in other populations sex ratio is close to normal. We have developed a method to estimate the lifetime reproductive success of these wasps by collecting females soon after they die and counting the number of eggs left in their ovaries. We had microsatellites markers developed for these wasps to control the genetic background of females from the field. Finally, we established a protocol to extract DNA from wasps of known eggloads to determine \textit{Wolbachia} titers from individual females. We intend to use these methods to study the effect of \textit{Wolbachia} on the realized fitness of wasps from different populations. According to theory, we predict that \textit{Wolbachia} will be less virulent in highly infected populations than in those of intermediate prevalences. We will present a literature survey on the effect of \textit{Wolbachia} on host fitness, our detailed proposal and preliminary results.

\textbf{Joel Sachs}

\textbf{Evolutionary transitions in bacterial symbiosis}

Diverse bacterial lineages form beneficial infections with eukaryotic hosts. The origins, evolution and breakdown of these mutualisms represent important evolutionary transitions. To examine these key events, we synthesize data from diverse interactions between bacteria and eukaryote hosts. Five key transitions are investigated, including the origins of bacterial associations with eukaryotes, the origins and subsequent stable maintenance of bacterial mutualism with hosts, the capture of beneficial symbionts via the evolution of strict vertical transmission within host lineages and the evolutionary breakdown of bacterial mutualism. Each of these transitions has occurred many times in the history of bacterial-eukaryote symbiosis. We investigate these transitions across the bacterial domain and also among a focal set of well-studied bacterial mutualist lineages. Our goals are to generate a framework for examining evolutionary transitions in bacterial symbiosis and to test hypotheses about the selective, ecological and genomic forces that shape these events.

\textbf{SESSION 2. HOST ADAPTATION, SPECIFICITY AND PHYLOGEOGRAPHY}

\textbf{Ellen Simms, Martine Ehinger and Toni Mohr}

\textbf{Meso-phylogeography of a native population of nodulated Bradyrhizobium}

The Bradyrhizobium population of the Bodega Dunes interacts with several species of native legumes, including both lupines and lotus. Trapping experiments have revealed spatial structure in the population, which corresponds to host distributions and possibly to soil types. Current experiments are attempting to determine the degree to which hosts and physical factors are responsible for this structure.

\textbf{Anthony S. Amend, Daniel J. Barshis, Thomas A. Oliver}

\textbf{Community Structure, Transcriptomics and Phylogenetics of Coral-Associated Fungi Indicate Novel Nutritional Roles and Deep-Branching Lineages}

Scleractinian coral survivorship and stress tolerance are intricately tied to their association with microbial symbionts. While much is known about the mutualistic symbiosis between corals and their dinoflagellate photobionts (\textit{Symbiodinium} spp.), little is known about the taxonomy, stability or function of their symbiotic fungal communities. Using a combination of targeted gene sequencing and meta-transcriptomics we examine how fungal communities, \textit{in situ}, respond to differences in environmental stability and to subglade identity of the \textit{Symbiodinium}. Phylogenetic analysis of coral-associated fungi shows a high diversity of Dikarya, including
symbionts, but establishment outside pine groves is extremely rare, and is likely limited in part by EM fungal inoculum. This pattern may differ however in drier settings.

This collaborative research project seeks to determine the role of cooperation between unrelated organisms in species invasions. To achieve this, we are using the spread of pine trees on the islands of Hawaii as a model system to test plant-microbe community feedbacks in invaded ecosystems. We hypothesize that pines’ interaction with non-native ectomycorrhizal fungi enables them to occupy a unique niche within their new habitats; thus increasing their ability to invade. To test this hypothesis we are using a combination of molecular and emerging nanotechnological techniques in a series of field and greenhouse experiments. Thus far we have identified the dominant ectomycorrhizal fungi responsible for pine establishment on the islands of Maui and Hawaii. As a next step in this research we are using quantum dots as nutrient tracers to compare the potentially disparate physiologies of pine associate ectomycorrhizal fungi with those of native Hawaiian arbuscular mycorrhizal fungi. From this study we will gain new information on the functional ecology of plant-fungal symbioses, which will in-turn provide a better predictive framework for the factors that determine plant-microbe community assemblies and distributions. In addition, this project will add valuable information to the currently small body of research regarding ectomycorrhizal plant and fungal invasions in the tropics.

Spore dispersal and behavior of ectomycorrhizal fungi and its relationship to pine seedling establishment. Following the 1995 Mt. Vision fire at Pt Reyes National Seashore, bishop pine forest expanded dramatically into areas that were previous non-forested. The symbiosis between pines and ectomycorrhizal (EM) fungi is obligatory for both partners, but pine seeds and EM fungal spores disperse independently. When pines invaded the scrub and grassland communities they were expanding into area that lacked EM plant species. Thus a chicken-egg scenario exists with this symbiosis, in which successful pine establishment requires ectomycorrhizal fungi, but establishment of mycorrhizal fungi requires pine. To explore this conundrum we have studied mycorrhizal spore dispersal patterns at Pt Reyes using bioassays, trap seedlings, spore burial experiments, qPCR, and pyrosequencing. Our results show that dense, long-lived spore banks exist within a few hundred meters of the pine forest border, and fungal species dominating this zone have animal dispersed spores. Beyond that distance, spore dispersal is dominated by aerially dispersed species, especially Suillus, but aerial spore dispersal tapers off rapidly and becomes limiting for pine inoculation by about 1 km. These distances appear to be concordant with wind-dispersed post-fire pine seed, so these pines may not be limited by mycorrhizal inoculum except at the edges of their own dispersal curves. This pattern may differ however in drier settings where aerial spore dispersal is less frequent. For example earlier work with bristlecone pine in the White Mountains showed that mycorrhizal spore banks stop within meters of the edge of established pine groves, and in these settings above ground fungal fruiting is very infrequent. In this case the bird-dispersed pine seed, should have much greater dispersal ability than its fungal symbionts, but establishment outside pine groves is extremely rare, and is likely limited in part by EM fungal inoculum.
SESSION 3. MUTUALIST SERVICES

Rodolfo Dirzo
Biotic anti-herbivore defense in plants: examples from the tropics.
Biotic defense is a prominent plant-animal mutualism among tropical plants. Such mutualisms typically involve plants that offer rewards to insects (commonly ants) that operate as anti-herbivore defense agents. This interaction is further complicated by the presence, in some cases, of additional partners, such as scale insects, that provide food for the ants while at the same time take plant resources (sap). In other cases, defending partners seem to take advantage of the rewards plants offer to pollinators (floral nectar) and operate as mutualists via their dual role as pollinators and defenders. I will discuss the natural history and ecological consequences of these complex reward-defense mutualisms.

Ann Hirsch
The amazing below ground world of plant roots and their microbiome: symbiosis on a grand scale.
A.M. Hirsch1,2, A.A. Angus1, R. Hessabi1, I. Ortil1, A.R. Schwartz1, and D.A. DeMason3. 1Dept. of Molecular, Cell, and Developmental Biology, 2Molecular Biology Institute, UCLA, Los Angeles, CA 90095, and 3Dept. of Botany and Plant Sciences, UC–Riverside, Riverside, CA 91521.
The rhizosphere is an eclectic and integrated ecosystem closely associated with the plant root. This environment is very much influenced by the diverse organisms that reside in it and the edaphic factors (soil, pH, water) that shape it. The plant microbiome consists of both bacteria and fungi that exhibit many positive effects on plant growth. They do this by secreting phytohormones or by degrading the inhibitor hormone ethylene via accD, by exploiting biocontrol mechanisms (antibiotic secretion, induced systemic resistance, competition for nutrients), by providing hard -to-get nutrients (phosphorous, nitrogen, iron, among others), and by stimulating bioremediation via increasing the numbers of biodegradative bacteria that improve plant health overall. We have initiated studies on a number of Gram-positive and Gram-negative bacteria that mediate plant growth. Gram-–negative Burkholderia species have been isolated from legume nodules and have been shown to fix atmospheric nitrogen into ammonia, solubilize phosphate, and convert Fe3+ to Fe2+. Gram-positive Bacillus species secrete the plant hormone auxin and increase plant biomass in a number of ways. Data on these species and how they influence plant growth will be presented. The genomes of four nitrogen-fixing Burkholderia species have been sequenced and they are currently being annotated. The Bacillus species genome has also been sequenced.

Steven Lindow
Interactions between bacteria and fungi and with the plants on which they live
Large and diverse bacterial communities occur on the surface of healthy plants. These communities are limited in size by the amount of carbon compounds that are provided by the plant. While it has long been thought that the plant resources were provided directly to the bacterial colonists, much information now suggests that at least some of these resources are provided indirectly via fungi that also colonize plants. Neotyphodium endophytes are asymptomatic fungal colonizers of the aerial tissues of cool season grasses, and have a mutualistic relationship with their hosts that is in part supported by the production of loline (saturated 1-aminopyrrolizidine) alkaloids. While lolines are known for inhibiting herbivory by insects, we show that N-formylLoline on the surface of tall fescue (Festuca arundinacea) and meadow fescue (Festuca pratensis) leaves selects for a unique epiphytic bacterial microflora. Population sizes achieved by a loline-catabolizing Burkholderia sp., are about 10-fold higher on fescues infected with a loline-producing Neotyphodium strain than on loline-free plants. Likewise, a Burkholderia mutant incapable of using loline as a sole Carbon and Nitrogen source attained similar population sizes when inoculated onto loline-containing and loline-free plants and which were about 10-fold lower than that achieved by the WT loline-catabolizing strain. Additionally, Burkholderia sp. constitutes a large proportion (>70%) of the culturable tall fescue phyllosphere bacterial community while they are rare or absent from the surfaces of plant species which are not infected with Neotyphodium endophytes. Terminal restriction fragment length polymorphisms of loline enrichment cultures inoculated with bacteria recovered from tall fescue revealed a simple community of strains capable of using loline as a sole carbon source. While less than 6% of the bacterial taxa recovered from tall fescue could consume lolines, they comprised over 70% of the total population size. These results indicate that loline catabolism is specialized amongst bacterial colonists of loline-producing grass-fungal mutualists and that loline derivatives are the major source of carbon and energy on such plants. Therefore, fungi play a role in determining the composition of the bacterial community in the phylosphere of Neotyphodium-infected grasses. The selectivity of loline catabolism might be exploited to establish particular bacteria on plants for beneficial purposes. Many Pseudomonas syringae strains harbor a protein, which we term Phc, that has significant homology to
fungal Het-c proteins involved in heterokaryon incompatibility (HI). Transformation of phc from P. syringae B728a or DC3000 into wild type Neurospora crassa strains resulted in transformants with the classic HI phenotypes of drastically reduced growth rates lack of conidial production and cells undergoing a process known as hyphal compartmentation and death. P. syringae strains are able to colonize and move along fungal hyphae and multiply on the surface of such fungi, particularly at sites of dead hyphal cells. P. syringae may thus induce HI in fungi via delivery of Phc to overcome nutrient limitations on leaves.

**SESSION 4. SYMBIONT CONSORTIA AND MULTISPECIES INTERACTIONS**

Scott C. Dawson
Anaerobic protists are microbial communities: ecto- and endo-symbiotic bacteria and archaea in rumen protists
Perhaps without exception, anaerobic protists act within the larger rumen microbial ecosystem as self-contained microbial communities with intimately associated symbiotic bacteria and archaea. Rumen protists and associated symbionts contribute significantly to degradation of plant material with the concomitant generation of methane and carbon dioxide. Such microbial interactions between anaerobic protists and endosymbionts are poorly characterized, yet these intracellular elemental cycling and symbiont-protist genetic have likely shaped the ecophysiology of the rumen and the evolution the eukaryotic cell.

M. Pilar Francino
Host-microbial symbiosis in the human gastrointestinal tract
The gastrointestinal tract (GIT) is host to the most abundant and diverse microbial community in the human body. This microbiota plays essential roles for gut maturation, food digestion, vitamin synthesis, modulation of the immune system, and protection from pathogens. As a consequence, a variety of health problems may ensue from alterations in this community. Metagenomic and other “omic” approaches are revealing the large compositional diversity, individual variability and functional complexity of the GIT microbiota. This talk will explore what these approaches can tell us about the symbiotic host-microbial interactions in the GIT. We will focus on microbiota development in infancy, and will investigate the functional, ecological and evolutionary consequences that may result from the particular dynamics of this process.

Forest Rohwer
Revisiting the coral holobiont
The coral holobiont was originally conceived as an adaptable ecological unit with inter-changeable microbial, viral, and algal components. Over a decade later, we still do not have strong evidence that this is true. In this talk, I will revisit the holobiont concept and ask if we need a new theoretical context to understand coral-microbial interactions.
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