

-- FINAL SCHEDULE --

# YOSEMITE SYMBIOSIS WORKSHOP



**Sierra Nevada Research Institute**

**Yosemite National Park, May 2-3 2015**

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SCHEDULE OF INVITED TALKS

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SATURDAY, MAY 2

Meeting Welcome and Lunch

12:00PM – Meet at Community Center in ‘Downtown’ Wawona (See map)

SESSION 1. Ecology and diversity of symbiont communities

- 1:00 **Joel Sachs** - Introduction and welcome to the meeting
- 1:15 **Alyssa Carrell**, Taxonomic diversity and functional consistency in the foliar bacterial endophyte community of giant trees
- 1:30 **Ryoko Oono**, Community diversity and structure of foliar fungal endophytes across landscapes and within individual leaves
- 1:45 **Dana Carper**, Bacterial endophytic communities associated with seedlings of high elevation pines *Pinus flexilis* & *Picea engelmannii*
- 2:00 **James Angus Chandler**, Interactions between *Drosophila melanogaster* and its natural yeast symbionts
- 2:15 **Courtney Klepac**, Seasonal stability of coral-*Symbiodinium* associations in the subtropical coral habitat of St. Lucie Reef, FL

Coffee Break for 30 min

SESSION 2. Genetics and genomics of symbiont populations

- 3:00 **Asaf Levy**, Genomic features of plant-associated bacteria
- 3:15 **Taichi A. Suzuki**, Clinal variation of gut microbial composition in natural populations of house mice across the Americas
- 3:30 **Carolyn Frank**, Ecology and Evolution of the Bacterial Endophytes Communities in Conifer Foliage

Coffee Break for 30 min

SESSION 3. Metabolite exchange among species

- 4:15 **Sharon L. Doty**, The plant microbiome: Implications of the ecosystem within
- 4:30 **Harmony Hancock**, Carbon translocation revisited: Symbiont carbon sequestration in the mixotrophic coral *Oculina diffusa*
- 4:45 **Sylvie Estrela**, Microbial interdependencies through loss of metabolic functions

SESSION 4. Poster Presentations

- 5:00-6:00 Poster Presenters see below (*beer, wine, and snacks provided*)

KEYNOTE LECTURE

- 6:00-7:00 **Ellen Simms**, UC Berkeley    Cheating and costs of cooperation in legume-rhizobium interactions

DINNER – 7:00-8:30

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SUNDAY, MAY 3

BREAKFAST – 9:00-10:00AM

SESSION 5 Conflict and cooperation in symbiosis

- 10:00 **Joel L. Sachs**, Clonal Expansion and DNA acquisition of *Bradyrhizobium* Across a 700km *Lotus* Metapopulation
- 10:15 **Kelsey Gano**, Effects of co-inoculation of non-symbiotic *Bradyrhizobium* isolates on *Lotus strigosus* hosts
- 10:30 **Camille Wendlandt**, Variation in Legume control over ineffective rhizobia in an anthropogenic nitrogen gradient
- 10:45 **Margie Kinnersley**, Co-Evolution of *E. coli* and its parasite *Bdellovibrio bacteriovorus*: A model for Eukaryogenesis
- 11:00 **Rita Rachmawati**, Coral symbionts and the host susceptibility to bleaching
- 11:15 **Francois Renoz**, Infection dynamic of symbiotic bacteria in the pea aphid *Acyrtosiphon pisum* gut and host immune response
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- 11:30-12:00 *Discussion of Common Themes*, Led by Joel Sachs

LUNCH – 12:00-1:00PM Completion of meeting.

Ellen Simms, UC Berkeley Keynote Seminar

**Cheating and costs of cooperation in legume-rhizobium interactions**

INVITED TALKS (alphabetical, by last name)

Dana Carper, UC Merced,

**The bacterial endophytic communities associated with seedlings of the high elevation pines *Pinus flexilis* and *Picea engelmannii***

Dana Carper, Alyssa A. Carrell, Lara M. Kueppers and A. Carolin Frank

Bacterial endophytes reside within the tissues of healthy plants. A single acetic acid bacterial (AAB) group possibly involved in nitrogen fixation has been found to consistently dominate adult needle tissue of two subalpine conifers *Pinus flexilis* (limber pine) and *Picea engelmannii* (Engelmann spruce), but it is not known when and how the establishment of this relationship occurs. To investigate when and under which conditions this potential symbiosis is established, we characterized the endophyte communities in 1 year old seedlings from a field experiment.

Thirty-three seedlings were collected in the summer of 2012 from Niwot Ridge, Colorado. *P. flexilis* and *P. engelmannii* seedlings growing at three elevations (lower subalpine, upper subalpine, and alpine) under four treatments (control, heated, watered, heated and watered) were sampled. Above- and below ground tissues were separated and DNA was extracted after surface sterilization. The DNA was then amplified using 16S primers targeting the v3 region and sequenced on an Illumina MiSeq. Sequences were processed using QIIME and UPARSE.

Below ground tissues yielded very few sequences due to the small amount of starting tissue available, they were however significantly more diverse than aboveground tissues. AAB was present in the aboveground tissue of the seedlings but at a reduced relative abundance compared to adult tissue. These results indicate that AAB is a potentially important symbiont that is established at a very early stage of life. The lower abundance of AAB could be the result of the reduced need at this early stage for nitrogen fixation. The most abundant phylotypes were from Betaproteobacteria and were closely related to bacteria found within arctic plants. Treatments had no effect on bacterial communities; instead bacterial communities were structured by elevation.

Alyssa Carrell, Oak Ridge National Lab

**Taxonomic diversity and functional consistency in the foliar bacterial endophyte community of giant trees**

Alyssa Carrell and A. Carolin Frank

Microbial endophytes are an integral part of the biology of all plants, with implications for agricultural and natural ecosystems. Recent culture-independent work has revealed that the bacterial needle endophyte communities of high-elevation pines are marked by low diversity and consistency across individuals and species. The objective here was to test if foliar endophytes communities associated with coast redwood (*Sequoia sempervirens*) and giant sequoia (*Sequoiadendron giganteum*) are similarly consistent or, alternatively, variable within and across individuals and populations. We used 454 pyrosequencing of the 16S rRNA gene to explore the diversity of endophytic bacteria associated with two populations of coast redwood and one of giant sequoia, across three canopy heights. Furthermore, we used PICRUSt to infer a metagenome from each sample. We found that the bacterial endophyte communities of coast redwood and giant sequoia were considerably more diverse and variable than those previously observed in pines. While an operational taxonomic unity (OTU) from an uncultured Rhizobiales lineage (LAR1), dominated most coast redwood samples from one population, no single phylotype or genus dominated all samples of a species. Many dominant endophytic taxa belong to genera known for roles in disease protection (e.g. *Bacillus*, *Pseudomonas*), suggesting a defensive role for the endophytic community. With the exception of 'cell motility', predicted gene functions did not vary across canopy height, site, or tree species. Thus, the giant trees associate with diverse taxa of bacterial endophytes while endophytic community function is maintained across, individuals, sites and host species. Taxonomic shifts in the endophytic community may help long-lived trees overcome ongoing challenges from rapidly changing pests and pathogens.

James Angus Chandler, Microbiology Department, California Academy of Sciences

**Interactions between *Drosophila melanogaster* and its natural yeast symbionts – Is *Saccharomyces cerevisiae* a good model for studying the fly-yeast relationship?**

Don Hoang, Artyom Kopp, and James Angus Chandler

Yeasts play an important role in the biology of the fruit fly, *Drosophila melanogaster*. In addition to being a valuable source of nutrition, yeasts affect *D. melanogaster* behavior and interact with the host immune system. Most experiments investigating the role of yeasts in *D. melanogaster* biology use the baker's yeast, *Saccharomyces cerevisiae*. However, *S. cerevisiae* is rarely found with natural populations of *D. melanogaster* or other *Drosophila* species. Moreover, the strain of *S. cerevisiae* used most often in *D. melanogaster* experiments is a commercially and industrially important strain that, to the best of our knowledge, was not isolated from flies. Since disrupting natural host-microbe interactions can have profound effects on host biology, the results from *D. melanogaster*-*S. cerevisiae* laboratory experiments may not be fully representative of host-microbe interactions in nature. In this study, we explore the *D. melanogaster*-yeast relationship using five different strains of yeast that were isolated from wild *Drosophila* populations. Ingested live yeasts have variable persistence in the *D. melanogaster* gastrointestinal tract. For example, *Hanseniaspora occidentalis* persists relative to *S. cerevisiae*, while *Brettanomyces naardenensis* and *Hanseniaspora uvarum* are removed. Despite these differences in persistence relative to *S. cerevisiae*, we find that all yeasts decrease in total abundance over time. Reactive oxygen species (ROS) are an important component of the *D. melanogaster* anti-microbial response and can inhibit *S. cerevisiae* growth in the intestine. To determine if sensitivity to ROS explains the differences in yeast persistence, we measured yeast growth rates in the presence and absence of hydrogen peroxide. The two yeast strains that are completely inhibited by hydrogen peroxide are among the three strains that are the least persistent *in vivo*. However, we find no differences in sensitivity in the remaining strains, regardless of *in vivo* persistence. We also compared the feeding preference of *D. melanogaster* when given the choice between a naturally associated yeast strain and *S. cerevisiae*. We do not find a correlation between preferred yeasts and those that persist in the intestine. Notably, in no instances is *S. cerevisiae* preferred over the naturally associated strains. Overall, our results show that *D. melanogaster*-yeast interactions are more complex than might be revealed in experiments that use only *S. cerevisiae*. We propose that future research utilize other yeasts, and especially those that are naturally associated with *Drosophila*, to more fully understand the role of yeasts in *Drosophila* biology.

Sharon L. Doty, University of Washington

**The plant microbiome: Implications of the ecosystem within**

Andrew W. Sher, Neil D. Fleck, Mahsa Khorasani, Roger B. Bumgarner, Zareen Khan, Soo-Hyung Kim, Thomas H. DeLuca

The microbiota of plants is diverse, and like that of mammals, is important for overall health and nutrient acquisition. In legumes and actinorhizal plants, nitrogen essential for plant growth is obtained through symbiosis with nodule-inhabiting, N<sub>2</sub>-fixing microorganisms. However, a variety of plant species, despite being incapable of such nodule formation, can still thrive in natural, low-nutrient settings. In some of these plant species, endophytes, microbes that live fully within plants, fix atmospheric N<sub>2</sub> allowing for plant growth without fertilization. Using the 15N<sub>2</sub> incorporation assay, we provided the first direct evidence of N<sub>2</sub> fixation in the early successional tree, Populus. The label was incorporated to high levels, indicating strong N<sub>2</sub> fixation. The microbiome was essential for this high activity as internally sterile *in vitro*-grown plants had little 15N incorporation. Culturable bacteria capable of growth on N-limited medium were present at high densities in the plant tissue. Metagenomic sequencing of the wild poplar samples revealed a diversity of bacterial nitrogenase sequences. Diazotrophic (N-fixing) endophytes can be isolated from within wild poplar and willow plants, and these microbes promoted plant growth not only for poplar but also for a variety of other plant species including corn, rice, tomatoes, peppers, turf grasses, and Douglas-fir. Addition of diazotrophic endophytes from wild poplar to cultivated hybrid poplar resulted in substantially increased biomass and drought tolerance. Many of the strains solubilized phosphate and produced siderophores, potentially providing phosphorous and iron in addition to fixed N for the plant hosts. Since poplar was the first tree to have its genome sequenced and members of the poplar microbiome are undergoing full genomic sequencing, it can serve as a model system for studying N<sub>2</sub> fixation in non-nodulating trees. With the general host specificity of the poplar endophytes to such diverse plants as grasses and conifers, there are opportunities to re-direct bioenergy plantations, forestry, and agriculture towards biological N<sub>2</sub> fixation with reductions in chemical fertilization in light of global climate change.

Sylvie Estrela, University of Washington

**Microbial interdependencies through loss of metabolic functions**

Sylvie Estrela, Ben Kerr

Microbes perform many costly biological functions that benefit themselves, and may as a consequence also benefit neighbouring cells. Losing the ability to perform such functions can be advantageous due to cost savings, but when these functions are essential for growth, organisms become functionally dependent on ecological partners to compensate for the loss of these functions. When multiple functions may be lost, the outcomes of interaction are potentially diverse, including free-living organisms only; one-way dependency, where one partner performs all functions and others none; or mutual interdependency where partners perform

complementary essential functions. But what factors drive these different outcomes? We study this question with a computational model where microbes perform multiple leaky functions that help in detoxifying their environment. Here, I will discuss how the cost of performing the function, the degree of essentiality and spatial structure combine to drive the dynamics and outcome of microbial interdependencies.

Carolin Frank, UC Merced

**Ecology and Evolution of the Bacterial Endophytes Communities in Conifer Foliage**

Carolin Frank, Alyssa Carrell, Dana Carper, Emily Wilson, Lara Kueppers, Andrew Moyes

Conifers tolerate challenging environments and dominate many terrestrial ecosystems in the Northern Hemisphere. The conifer microbiome potentially mediates some of the traits that underlie their ecological success, e.g. growth in limited soils and robustness to biotic stress over centuries to millennia. However, an important part of their microbiome—aboveground endophytes—remain mostly unexplored. Using pyro- and Illumina sequencing of the 16S rRNA gene, we have investigated the foliar endophytic communities associated with multiple conifer genera (*Picea*, *Pinus*, *Abies*, *Sequoia*, *Sequoiadendron*) and sites across the Western US. Our results show that while overall, the endophyte microbiota is both species-specific and structured by the environment, specific dominant phylotypes are conserved across species, genera, and sites, indicating beneficial and long-standing associations. Specifically, we have found that phylotypes in the Alphaproteobacterial family *Acetobacteraceae* consistently dominate the foliar endophyte communities in high-elevation pines. In addition, diverse conifers host taxa belonging to LAR1, a lineage of uncultured Rhizobiales previously found exclusively in lichens, as well as other new Alphaproteobacterial lineages. Using the acetylene reduction assay, we have demonstrated nitrogenase activity in the foliage of multiple high-elevation conifer species. Our results suggest that conifers form ecologically and evolutionarily stable associations with bacterial endophytes. We hypothesize that the conifer endophyte microbiome fixes low but significant amounts of nitrogen in limited environments and contributes to host defense in these long-lived organisms.

Kelsey Gano, University of California Riverside

**Effects of co-inoculation of non-symbiotic *Bradyrhizobium* isolates on *Lotus strigosus* hosts**

Kelsey Gano, Amanda Hollowell, John Regus, Camille Wendlandt, Kenjiro Quides, Peter Stokes, Mia Blanton, Joel Sachs

Bacterial mutualisms with eukaryotic hosts are intimate and reciprocally beneficial interactions. However, the benefits provided to the host from the microbial partner are not fixed and can vary due to extrinsic conditions. A key model for examining the evolution of symbiotic quality is the robust legume-rhizobium mutualism. In addition to symbiotic rhizobia, non-symbiotic rhizobia also exist and these diverse rhizobial genotypes are in constant competition within the dynamic soil environment and can ultimately affect host growth response. This work explores how competition between diverse symbiotic and non-symbiotic *Bradyrhizobium* isolates affects both host fitness and the quality of rhizobial infection. Eighteen co-inoculation pairs were infected onto sympatric *Lotus strigosus* hosts from a high and low nitrogen deposition site. Co-infection rates of non-symbiotic isolates were measured as well as the effects on host fitness.

Harmony Hancock, Old Dominion University

**Carbon translocation revisited: Symbiont carbon sequestration in the mixotrophic coral *Oculina diffusa***

Harmony Hancock, Richard Zimmerman

Reef-building corals are symbiotic cnidarians, deriving much of their carbon budget from their endosymbiotic dinoflagellates. Without the carbon contribution from the Symbiodinium, the coral host suffers, ultimately resulting in the disruption of symbiosis, coral bleaching and subsequent mortality. Estimates of carbon translocation from algal symbiont to host provide a measure of symbiosis and stress. The obligate nature of Scleractinian symbiosis makes accurate measures of carbon translocation extremely difficult without the symbioses intact. Thus, carbon translocation measures for most corals with intact symbioses are not known. Lack of accurate estimates makes measuring the coral physiological response to thermal stress difficult. Respiration and photosynthesis of symbiotic (n=27) and aposymbiotic (n=8) *Oculina diffusa* nubbins were measured with increasing temperature (27°C-35°C). Symbiodinium respiration rates were resolved by subtracting mean values of aposymbiotic nubbins from those of symbiotic nubbins and used to calculate carbon fixation and translocation. Preliminary results of Symbiodinium respiration rates revealed 60% of fixed carbon was translocated to the host at ambient temperature (27°C). Translocation decreased linearly with increase in temperature ( $y = -21.05x + 628.12$ ,  $R^2 = .999$ ). Translocation reached zero at temperatures greater than ~29.5°C, where Symbiodinium respiration rates exceeded net photosynthetic rates. Symbiodinium respiratory demands appeared to be strongly affected by temperature ( $Q_{10} \text{ SYMBIODINIUM} = 7.53$ ). These initial findings require more investigation, but suggest utility in



facultative, symbiotic Oculinids for a better understanding of Scleratinian symbiosis. Future work is aimed at comparison of aposymbiotic and symbiotic nubbins to thermal stress, to better understand proteomic and physiological responses during coral bleaching.

Margie Kinnersley, University of Montana

**Co-Evolution of *Escherichia coli* and its parasite *Bdellovibrio bacteriovorus*: An experimental model for Eukaryogenesis**

Margie Kinnersley, Frank Rosenzweig

The history of Life on Earth has been punctuated by major transitions in cellular form and function that have driven significant increases in cellular complexity. Among these transitions, perhaps the most crucial to understanding extant biodiversity is the transition from prokaryote to eukaryote (eukaryogenesis). Because the timing of eukaryogenesis coincides with the phylogenetic origin of the mitochondrion, it has been hypothesized that acquisition of this organelle heralded the prokaryote-eukaryote transition. While our understanding of mitochondriogenesis is complicated by the fact that it only happened once, key aspects of this evolutionary transition can be addressed using the power of experimental evolution. A simple, straightforward model for the advent of mitochondria using *Escherichia coli* and its intracellular prokaryotic pathogen, *Bdellovibrio bacteriovorus*, is currently being investigated. Guided by the “parasitism” hypothesis of mitochondrial origin and the theoretical literature showing how mutualism can evolve from parasitism, increasingly endosymbiotic *Bdellovibrio* are selected by favoring their increased rate of vertical transmission and their increased metabolic dependence on an *E. coli* host. If successful, this co-evolving system will be used to test hypotheses concerning ecological prerequisites for, early molecular events in, and evolutionary consequences of incipient endosymbiosis based on metabolic niche partitioning and energy generation, exactly the features that define the mitochondrion's role in eukaryotic systems. Understanding these aspects of mitochondriogenesis is essential for truly understanding the prokaryotic/eukaryotic transition and thus is applicable to the study of myriad aspects of cellular and organismal diversity.

Courtney N. Klepac, Old Dominion University

**Seasonal stability of coral-Symbiodinium associations in the subtropical coral habitat of St. Lucie Reef, FL**

Courtney N. Klepac, Jeff Beal, Carly D. Kenkel, Ashley Sproles, Jennifer M. Polinski, Maureen A. Williams, Mikhail V. Matz

The coral community at St. Lucie Reef, Stuart, Florida (27° 8' N 80° 8' W) is found near the northern latitudinal range limit for Florida reefs and persists under environmental variability from freshwater discharges, summer upwelling, and thermal instability. Since aspects of coral physiology can be attributed to the composition of endosymbiotic zooxanthellae (genus *Symbiodinium*), we examined the dynamics of *Symbiodinium* strains in St. Lucie corals to gain insight into the organization of coral-algal symbioses under local stressors. Two scleractinian coral species that dominate the reef, *Montastraea cavernosa* and *Pseudodiploria clivosa* were repeatedly sampled at four reef sites over 17 months, during both wet and dry seasons. *Symbiodinium* cellular density and photosynthetic pigments differed between the two coral hosts, where *Pseudodiploria clivosa* had higher cell densities and chlorophyll concentrations than *Montastraea cavernosa*. Over time, these parameters varied, but were not significantly altered following freshwater discharge events. *Symbiodinium* diversity and abundances were identified by ITS-2 region amplification and next-generation sequencing, which revealed remarkable stability of relative proportions of different *Symbiodinium* genotypes throughout the sampling period. Novel associations with unique *Symbiodinium* strains observed for each coral species as well as the stability of these symbioses could indicate local adaptation of St. Lucie Reef corals to their marginal environmental conditions.

Asaf Levy, Joint Genome Institute

**Genomic features of plant-associated bacteria**

Asaf Levy, Scott Clingenpeel, Susannah G. Tringe, Sur H. Paredes, Derek Lundberg, Sarah Lebeis, Jeffery L. Dangl, and Tanja Woyke

Plants tightly associate with an array of phylogenetically and functionally diverse bacteria, with which they share complex and fascinating interactions. Greatly influencing host fitness, some plant-associated (PA) bacteria supply essential nutrients to plants thereby promoting plant growth, other PA bacteria serve as pathogens causing plant diseases, and some PA bacteria protect the plants from those pathogens. In order to fulfill their mutualistic, parasitic or commensal functions the PA bacteria have evolved a large set of genes that enable them to adapt to the plant environment. Here, we employed a large-scale comparative genomics study in order to characterize the core genome of PA bacteria. This was done by comparing the genomes of 1300 bacterial species from seven prominent taxa (from phyla Proteobacteria, Actinobacteria, and Firmicutes) that were classified as being PA bacteria or non-plant associated (NPA) bacteria based on their site of isolation. This set includes a list of 80 newly sequenced bacterial genomes that were isolated from the root endophytic compartment of *Arabidopsis*. The genes enriched in PA bacteria include genes involved

in nitrogen fixation, antibiotics production, sugar transport and metabolism, plant cell wall degradation, type III and type VI secretion systems, and biosynthesis of plant growth promoting hormones. Interestingly, this analysis also revealed a large set of uncharacterized genes that are enriched in PA bacteria. Many of these latter genes are conserved within and across taxa, clustered in operons, and are upregulated in the plant environment, underscoring their likely roles in plant interactions. Understanding the functions of these genes has enormous potential to improve plant growth efficiency and carbon sequestration by plants via microbiome manipulation.

Ryoko Oono, UC Santa Barbara

**Community diversity and structure of foliar fungal endophytes across landscapes and within individual leaves**

Ryoko Oono, Sara Baguskas, Emilie Lefevre

Fungal endophytes living inside tissues of woody plants are species rich and phylogenetically diverse even within a single leaf. The community assembly and structure of foliar fungal endophytes have been shown to depend on host taxa and geography, but there is relatively little known how they relate to chemical and physical properties within individual leaves. Water content, micronutrients, and carbohydrate concentrations vary among plants across landscapes due to environmental factors but also within individual leaves due to developmental and microclimatic differences, leading to variation in endophyte abundance and diversity from broad to fine spatial scales. We report the effects that chemical and physical environments have on the species diversity and community structure of foliar fungal endophytes using culturing and environmental sequencing techniques.

Rita Rachmawati UCLA,

**Coral symbionts and the host susceptibility to bleaching**

Rita Rachmawati, Amy Apprill, Paul Barber

Corals have different level of susceptibility to elevated temperature conditions, and in some cases exposure to these conditions results in bleaching, where they lose their algal symbionts. Corals also host other types of symbionts, including bacteria and archaea, that might have roles in affecting the hosts' response to elevated temperature events. Additionally, there is little known about the role of a prior bleaching event on bleaching susceptibility and symbiont composition. In order to evaluate the role and response of corals and their symbionts to elevated temperature and subsequent bleaching, we conducted experiments on three species of Indonesian coral, *Acropora millepora*, *Pocillopora verrucosa*, and *Porites lobata*, that had previously experienced very mild bleaching followed by recovery during a mass bleaching event. We exposed them to 4 different temperatures (30°C-control, 32°C, 34°C, 36°C) over the course of 12 hours. This experiment revealed that *A. millepora* and *P. verrucosa* started paling at 34°C and bleaching at 36°C, while *P. lobata* did not show any visual different color change at elevated temperature, compared to the controls. We are currently analyzing the composition of two dominant symbiont groups: Symbiodinium, bacteria and archaea, using a deep-sequencing approach, and will discuss these data in relation to the host's ability to cope with temperature stress.

Francois Renoz, Catholic University of Louvain, ELIB, (PI)

**Infection dynamic of symbiotic bacteria in the pea aphid *Acyrtosiphon pisum* gut and host immune response.**

Francois Renoz, Thierry Hance

Symbiotic associations with microbial partners are quite common in insects. Thanks to their well-studied associations with a wide range of bacterial symbionts, aphids represent a valuable model system to study molecular interaction and immune responses of a host with both beneficial and harmful microorganisms. In addition to its obligatory symbiont *Buchnera aphidicola*, the pea aphid *Acyrtosiphon pisum* can harbor several facultative bacterial symbionts which can be mutualistic in the context of various ecological interactions. Belonging to a genus where many members have been described as pathogen in invertebrates, *Serratia symbiotica* is one of the most common facultative partners found in aphids. The recent discovery of strains able to grow outside their host allowed us to simulate environmental acquisition of symbiotic bacteria by aphids. Here, we performed an experiment to characterize the *A. pisum* response to the ingestion of the free-living *S. symbiotica* CWBI-2.3T in comparison to the ingestion of the pathogenic *Serratia marcescens* Db11 at the early steps in the infection process. We found that, while *S. marcescens* Db11 kill the aphids within a few days, *S. symbiotica* CWBI-2.3T do not affect host survival and colonize the whole digestive tract within a few days. Gene expression analysis of immune genes suggests that *S. symbiotica* CWBI-2.3T does not trigger an immune reaction, while *S. marcescens* Db11 does, and supports the hypothesis of a fine-tuning of the host immune response set-up for fighting pathogens while maintaining mutualistic partners. Our results also suggest that the lysosomal system and the JNK pathway are possibly involved in the regulation of invasive bacteria in aphids and that the activation of the JNK pathway is IMD-independent in the pea

aphid. The absence of pathogenicity of *S. symbiotica* CWBI-2.3T observed in our study is discussed in the light of genomic and proteomic results showing that this *S. symbiotica* strain expresses virulent-associated proteins, chitinases and solitude-binding proteins participating in iron-uptake systems, which are required for an efficient infection.

Joel L. Sachs, UC Riverside

**Clonal Expansion and Horizontal Transfer of Bradyrhizobium Across a 700km Lotus Metapopulation**

A. Hollowell, J. Regus, D. Turissini, K. Gano, R. Bantay, A. Bernardo, D. Moore, J. Pham

Rhizobial bacteria exhibit a dual lifestyle, replicating in soil and also within host tissues whence they differentiate into bacteroids and fix nitrogen for their legume hosts. Here, we genotyped and phenotyped >350 Bradyrhizobium isolates from an 840 km transect of native *Lotus strigosus* host populations. We sequenced chromosomal housekeeping genes key to expression under free-living conditions and symbiosis loci differentially expressed in planta and encoded on a transmissible genomic island. For twenty focal genotypes we quantified utilization of 190 sole-carbon sources relevant to soil and rhizosphere fitness. We uncovered a massive clonal expansion restricted to the Bradyrhizobium chromosome, largely driven by a single epidemic chromosomal haplotype spread over 700km and dominating most sampled populations. The epidemic chromosomal haplotype was found associated with 42 divergent symbiosis island haplotypes, none of which were epidemic. Epidemic chromosomal haplotypes exhibited superior carbon utilization, especially for carbohydrates and carboxylic acids, whereas these parameters did not vary among symbiosis island haplotypes. The population genomic patterns that we uncovered are contrary to the paradigm of genomic island acquisition as the dominant driver of bacterial strain epidemics. Our dataset suggests that selection among chromosomal loci - most likely occurring in soils - can be an important driver of rhizobial population structure. Harnessing natural rhizobial epidemics could represent a solution to the meager success of rhizobial inoculants that cannot compete with native strains.

Taichi A. Suzuki, UC Berkeley

**Clinal variation of gut microbial composition in natural populations of house mice across the Americas**

Taichi A. Suzuki, Felipe M. Martins, M. Phifer-Rixey, Yongjie Zhang, John W. Taylor, Michael W. Nachman

A major goal in evolutionary biology is to link individual processes to macro-ecological and evolutionary patterns. Recent advances in microbial ecology have found previously unknown roles for gut microbes in animals that affect digestion, immunity, behavior, and development. Although this raises an intriguing possibility that gut microbiome play an important role in host ecology and evolution, we know little about how variation in the composition of the gut microbiome is shaped or why such variation exists. Here, I characterized the gut microbiota of 21 populations of wild house mice across North and South America using 16S and ITS sequencing. Carbon and nitrogen stable isotope diet analyses from mouse hair were used to test the associations between gut microbiota and diet. Wild mouse populations from extreme latitudes were raised in the laboratory to test how gut microbiota persist in a common environment. My work to date has established an association between the gut microbiota and latitude in both mice and humans. Although identifying the driver that structure the gut microbial variation in nature is a challenge, manipulative experiments and comparative metagenomics will further test the role of gut microbes in host ecology and evolution.

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POSTER PRESENTERS

Andrew Sher, UC San Diego

Sharon Doty

**Quantification of Nitrogen Fixation by Symbiotic Endophytes Living Within Poplar (*Populus trichocarpa*)**

Previous work has shown that symbiotic microbes live in the interstitial space between plant cells, and are thus endophytic. It has been shown that many of these endophytes are able to mitigate plant stressors, and even degrade toxins thus allowing their host to survive in harsh environments. These are exciting findings but the details of the relationship between plant and microbe have still to be elucidated. Unlike the known symbiosis of leguminous plants with Rhizobia, it has been demonstrated that endophytic microbes are less host specific and do not require any specific host structure. However, just as the relationship between plant and rhizobia is



based upon the exchange of fixed nitrogen with fixed carbon, it seems likely that the symbiosis of some endophytes would be based on a similar exchange.

Many diazotrophic endophytes have been isolated from *Populus trichocarpa* that were growing in a low nitrogen riparian ecosystem. These endophytes were identified through 16s amplification and shown to have the ability to fix nitrogen by amplification of the *nifH* gene. A direct means of quantifying this nitrogen fixation is the <sup>15</sup>N<sub>2</sub> incorporation assay. Using this assay it was shown that the diazotrophic symbionts of poplar fix nitrogen in planta. The high level of this incorporation suggests that endophytes are capable of sustaining plant growth even in conditions that would otherwise be impossible.

Danielle Black     University of California Santa Barbara

**How does fungal endophyte community assemblage change across environmental gradients at Jughandle State Natural Reserve?**

Danielle Black, Ryoko Oono

The ecological staircase at the Jughandle State Natural Reserve in Mendocino, CA, is an extreme example of a soil chronosequence comprised of five uplifted marine terraces varying in age from 100,000-500,000 years old. The older terraces in Jughandle have extremely acidic, nutrient poor soils resulting in highly stressed pygmy vegetation whereas the two younger terraces experience higher pH and much higher nutrient levels. The varying levels of nutrient, water, and light stress experienced by the plants across the terraces also alter the leaf tissue environment in which hundreds of fungal symbionts live. Fungal endophytes are ubiquitous but their functions in the symbiotic relationship are largely unknown. In this study, fungal endophyte community composition is compared across environmental gradients caused by changing levels of nitrogen, phosphorus, carbon, secondary metabolites, and other products of plant host stress. The question I aim to address in this study is: How does plant host stress, resulting in varying foliar conditions, affect fungal endophyte community assemblage across the Jughandle edaphic gradient? Community data gathered from a study in a pygmy forest system that experiences extreme and heterogeneous abiotic conditions allows for the examination of endophytes under multiple degrees of stress in a small spatial area. How variable environmental conditions affect fungal symbiont assemblage and abundance can give us clues to the function and evolution of fungal endophyte communities in plant tissues."

Esther Singer     Joint Genome Institute,

**The switchgrass microbiome**

Esther Singer, Thomas E. Juenger, Tanja Woyke

Less carbon-intensive energy sources are needed to reduce greenhouse gas emissions and their predicted role in climate change. There is growing interest in the potential of biofuels for meeting this need. A critical question is whether large-scale biofuel production can be sustainable over the time scales needed to mitigate our carbon debt from fossil fuel consumption. The carbon balance and ultimately the sustainability of biofuel feedstock production is the result of complex climate-coupled interactions between carbon fixation, sequestration, and release through combustion. Similarly, the long-term productivity of biofuels depends on the environmental factors limiting plant growth. These factors are often related to soil resources which involve complex interactions at the plant-microbe-soil interface impacting their availability and cycling. We have used 16S and ITS iTAG sequencing (with highly successful PNA plastid blocking) in an initial exploration of the bacterial and fungal communities on and within leaf and root surfaces. Our study included 6 biological replicates of 4 genotypes (2 upland [VS16 & Dakota] and 2 lowland ecotypes [AP13 & WBC]) grown in the presence and absence of nitrogen fertilization and sampling 4 compartments (phyllosphere, rhizosphere, leaf and root endophytic compartments). We discovered thousands of fungi and bacterial OTUs composing the microbiome with clear indicator taxa for sampled compartments. Moreover, our analysis suggests that host attributes associated with upland/lowland ecotypic divergence play an especially important role in structuring diversity and abundance in comparison to soil nitrogen abundances.

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**Genetic control of plant root colonization by the biocontrol agent, *Pseudomonas fluorescens***

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Plant growth promoting rhizobacteria (PGPR) are a critical component of plant root ecosystems. PGPR promote plant growth by solubilizing inaccessible minerals, suppressing pathogenic microorganisms in the soil, and directly stimulating growth through

hormone synthesis. *Pseudomonas fluorescens* is a well-established PGPR isolated from wheat roots that can also colonize the root system of the model plant, *Arabidopsis thaliana*. We have created barcoded transposon insertion mutant libraries suitable for genome-wide transposon-mediated mutagenesis followed by sequencing (TnSeq). These libraries consist of over 105 independent insertions, collectively providing loss-of-function mutants for nearly all genes in the *P.fluorescens* genome. Each insertion mutant can be unambiguously identified by a randomized 20 nucleotide sequence (barcode) engineered into the transposon sequence. We used these libraries in a gnotobiotic assay to examine the colonization ability of *P.fluorescens* on *A.thaliana* roots. Taking advantage of the ability to distinguish individual colonization events using barcode sequences, we assessed the timing and microbial concentration dependence of colonization of the rhizoplane niche. These data provide direct insight into the dynamics of plant root colonization in an in vivo system and define baseline parameters for the systematic identification of the bacterial genes and molecular pathways using TnSeq assays. Having determined parameters that facilitate potential colonization of roots by thousands of independent insertion mutants in a single assay, we are currently establishing a genome-wide functional map of genes required for root colonization in *P.fluorescens*. Importantly, the approach developed and optimized here for *P.fluorescens*>*A.thaliana* colonization will be applicable to a wide range of plant-microbe interactions, including biofuel feedstock plants and microbes known or hypothesized to impact on biofuel-relevant traits including biomass productivity and pathogen resistance.

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**Symbiotic relationships among uncultured candidate phyla inferred by global co-occurrence patterns**

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Symbiotic relationships between microbes and higher organisms have been studied extensively and are well-understood in some cases, but very little is known about symbioses among prokaryotes. A handful of examples of prokaryotic partnership have been discovered and characterized with experimental approaches, but these efforts are by their nature low throughput and often serendipitous. We now have access to a vast repository of sequence data from diverse habitats, which likely offers clues to discover many more such symbioses. Here, we utilize a database 16S rRNA sequences derived from PCR amplicons and metagenomes to explore patterns of co-occurrence in prokaryotes around the world. We focus on the enigmatic candidate phyla, which have no cultured representatives, and in many cases little to no genomic information available. It has been speculated that some of these lineages have escaped cultivation efforts because they are symbionts, relying facultatively or obligately on other microbes, perhaps even acting as parasites as in the recently discovered example of candidate phylum TM7. Several small networks of candidate phyla were discovered that were consistently positively correlated with each other across multiple sites over large spatial scales. Single cell genomic sequencing as well as complementary metagenomics and data mining efforts are underway to further explore the genomes of these groups for signatures of symbiosis, and mechanisms for which they may interact with each other. As one of the first studies attempting to detect symbioses computationally, this research demonstrates the utility of this approach and yields insights into the evolution and environmental roles of candidate phyla.