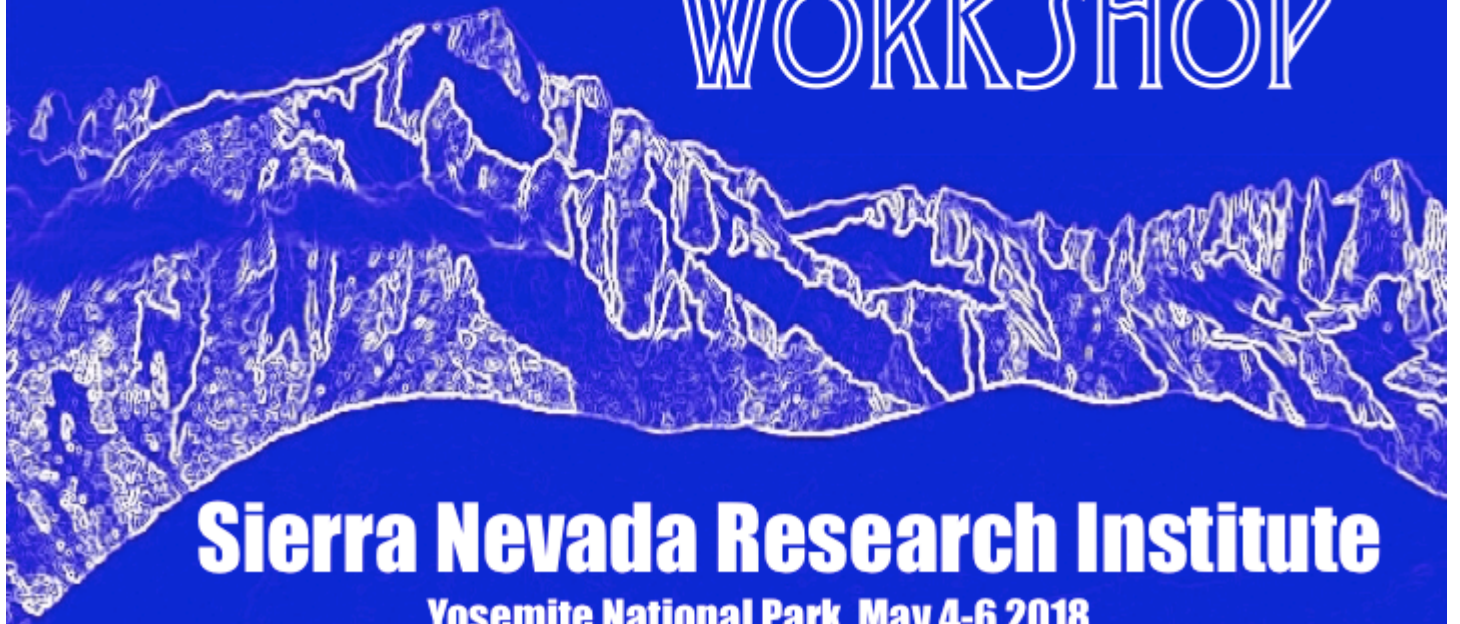


EIGHTH ANNUAL

# YOSEMITE SYMBIOSIS WORKSHOP



**Sierra Nevada Research Institute**

**Yosemite National Park, May 4-6 2018**

## Friday May 4, 2018

Arrival & Welcome Party 7:00pm – 10:00pm  
Landsneas House 2667 English Lane, Wawona CA (see attached map)

---

## Saturday May 5

Wawona Community Center (see attached map)

### Lunch 11:30pm – 12:25pm

12:25 Welcome to the 2018 Yosemite Symbiosis Workshop (Carolyn Frank, Joel Sachs)

#### **Session I: Host and symbiont adaptations to manage their partnership**

---

- 12:30 Meng Mao Tailored host adaptation to support two ancient bacterial symbionts with differentially degraded genomes in a leafhopper host  
12:45 Camille Wendlandt Host investment varies among populations of the legume *Acemispum strigosus*, but host sanctions are invariant  
1:00 Kenji Quides Experimental Evolution of *Lotus japonicus* symbionts  
1:15 Joel Sachs Multiple evolutionary losses of beneficial symbiosis in *Bradyrhizobium*

### 30 minute break for coffee and snacks

#### **Session II: Drivers of diversity, specificity, and stability**

---

- 2:00 Austen Apigo Host specificity may predict a reversed latitudinal diversity gradient pattern in foliar fungal endophytes  
2:15 Ryoko Oono Effect of foliar nutrients on species diversity of endophytic fungi depends on host specificity  
2:30 David Weston Determining the genetic and environmental factors underlying mutualism within a peatmoss –N fixing bacterial association  
2:45 Dana Carper Variations in the foliar microbial communities in *Pinus flexilis* (limber pine) across its native range  
3:00 Andrew Brooks Phylosymbiosis: Ecological Assembly of Microbial Communities across Host Evolutionary History  
3:15 Lupita Ruiz-Jones Investigating patterns of coral clonality across an environmental mosaic

### 30 minute break for coffee and snacks

#### **Session III: Multipartner interactions and networks**

---

- 4:00 Lauren Ponisio Understanding interaction flexibility in mutualistic networks  
4:15 Mohsin Tariq Occupancy of non-nodulating endophytic plant growth promoting bacteria in the root nodules of pea (*Pisum sativum* L.)  
4:30 Kane Keller Tri-trophic symbiotic interactions affected by warming  
4:45 Kaleigh A Russell The effects of temperature on nectar-inhabiting microorganisms

### Beer, Wine & Snacks 5:00-5:30

Keynote Lecture Tadashi Fukami 5:30-6:30

## "Historical contingency in symbiont community assembly"

### Dinner 6:30-8:00

Poster Session 8:00-9:30pm

---

## Sunday May 6

### Breakfast in your Cabins

#### **Session IV: Metabolism and biochemistry of symbiosis functions**

---

- 9:00 Jenifer Walke Stability of amphibian skin symbionts and their metabolites across seasons and disturbance  
9:15 Jack C Koch Low carbonic anhydrase activity in *Elliptochloris*-containing *Anthopleura elegantissima* and the negative correlation between diameter and carbonic anhydrase activity  
9:30 Trevor R Tivey Manipulation of surface glycan biosynthesis decreases colonization success during onset of cnidarian-dinoflagellate symbiosis.  
9:45 Christopher S Ward Chytrid parasitism in mass algal culture and the potential significance of the algal-chytrid-bacterial tripartite interaction  
10:00 Jason Rothman Microbe-Mediated Protection Against Selenium Toxicity and Effects of Selenium and Cadmium on the Bee Microbiome

### 30 minute break for coffee and snacks

#### **Session V: Genomic tools to uncovering functions**

---

- 10:45 Carly Kenkel Using comparative transcriptomics to identify genes involved in the evolution of coral symbiont transmission mode  
11:00 Hoang Vuong Comparative Genomics of *Lactobacillus* Associated with Wild Bees Reveal Potential Adaptation to Hosts  
11:15 Gordon M. Bennett A parallel universe: obligate bacterial symbiosis in a Hawaiian planthopper Hemiptera: Cixiidae) reveals rearranged nutritional responsibilities in anciently diverged partners

#### **Session VI: Understanding fitness and function in symbiosis**

---

- 11:30 Sharon L. Doty Mechanisms for Conferring Tolerance to Abiotic and Biotic Stresses by the Plant Microbiome  
11:45 Holly V. Moeller Tree-ectomycorrhizal symbioses: Does diversity confer function?  
12:00 Carolyn Frank Diversity and function of the conifer endophyte microbiome

### Lunch 12:15-1:30, End of conference.

## Symbiosis Workshop 2018 Talk Abstracts (alpha-order)

### 1. Austen Apigo, University of California, Santa Barbara;

#### Host specificity may predict a reversed latitudinal diversity gradient pattern in foliar fungal endophytes

Austen Apigo, Rodolfo Salas-Lizana, Jose Rubén Montés, Edward Allen Herre, Luis Mejía, Ryoko Oono

Foliar fungal endophytes asymptotically live within the tissues of all plant phyla and are distributed worldwide across every terrestrial ecosystem that supports plant life. These cryptic fungal endosymbionts have been suggested as an indicator group for global fungal biodiversity due to their ubiquity, but their apparent 'hyperdiversity' has yet to be quantified (1) across the diversity of ecosystems in which they occur, (2) with culture-independent methods (i.e., high-throughput sequencing) that capture rare and unculturable fungal species and (3) with analyses that explicitly account for the structure of the host community. To understand how the structure of these complex symbioses vary as a function of climate and the plant host community, we surveyed foliar fungal endophyte communities from 21 temperate and tropical forests (5°N - 64°N) by intensively sampling all co-occurring plant host species within five 50 m<sup>2</sup> quadrats per site during the summers of 2016 and 2017. We then sequenced the internal transcribed spacer 1 region on the Illumina MiSeq platform to directly characterize fungal community structure from host leaf tissue (n = 2,424 plant samples). Previous culture-dependent studies suggest foliar fungal endophytes are highly abundant and diverse in the tropics and follow the widely documented pattern in many plants and animals where species richness increases towards the equator - the latitudinal diversity gradient. However, our preliminary high-throughput data (6 of 21 sites) strongly suggest that foliar fungal endophyte diversity increases with increasing latitude, the opposite expectation of the latitudinal diversity gradient. I will discuss (1) how plant community structure and foliar fungal endophyte host specificity may contribute to these unexpected diversity patterns, (2) how contrasting patterns between previous studies and our preliminary data may be explained by detection technique, (3) the implications of foliar fungal endophyte diversity on our understanding of global fungal biodiversity and (4) outline future directions with phylogeographic and quantitative approaches.

### 2. Gordon M. Bennett, University of California Merced

#### A parallel universe: obligate bacterial symbiosis in a Hawaiian planthopper Hemiptera: Cixiidae) reveals rearranged nutritional responsibilities in anciently diverged partners

Gordon M. Bennett, Meng Mao

Auchenorrhyncha insects (Hemiptera: Suborder) have engaged in nutritional symbioses with bacteria for ~300 million years [MYA]. The suborder split early during its diversification (~250 MYA) into the Fulgoroidea (planthoppers) and Cicadomorpha (leafhoppers and cicadas). The two lineages may share symbionts, including *Sulcia* and a *Betaproteobacteria* that provide their hosts with ten essential amino acids (EAA). Some hosts additionally harbor three bacteria, as is common among planthoppers. However, genomic studies are currently restricted to the dual-bacterial symbioses found in Cicadomorpha, leaving the broad origins and functions of these symbionts unclear. To address this question, we sequenced the genomes of "*Candidatus Sulcia muelleri*" (*Bacteroidetes*), "*Ca. Vidania fulgoroidea*" (*Betaproteobacteria*), and "*Ca. Purcelliella pentastirinorum*" (*Gammaproteobacteria*) from a Hawaiian planthopper (Cixiidae: *Oliarus*). Remarkably, although *Sulcia* lineages have highly conserved genomes, its nutritional responsibilities are reduced in cixiids. In contrast to the Cicadomorpha, *Sulcia* synthesizes three EAAs, *Vidania* contributes seven, and *Purcelliella* appears to provide only b-vitamins and sulfur-based metabolites. Phylogenomic evidence suggests that *Vidania* is ancient and related to *Betaproteobacteria* found throughout the suborder. *Purcelliella* is more recently derived from plant and insect associated bacteria (e.g., *Erwinia* and *Pantoea*). Thus, the Cixiidae provide a valuable comparative model to understand the co-evolution of these ancient symbioses.

### 3. Andrew Brooks, Vanderbilt Genetics Institute

#### Phylosymbiosis: Ecological Assembly of Microbial Communities across Host Evolutionary History

Andrew Brooks, Seth Bordenstein, Robert Brucker, Kevin Kohl, Edward van Opstal, Brittany Leigh

Our ongoing research explores whether microbiota assembly is generally random with respect to host relatedness or demonstrates the recently described term "phylosymbiosis," an eco- evolutionary pattern where ecological relatedness of host-associated microbial communities parallels the phylogeny of related host species (see figure, Brooks *et al.* 2016. *PLOS Biology*). We previously examined the prevalence of phylosymbiosis and its functional consequence under controlled conditions by characterizing the microbiota of 24 animal species from four different groups (*Peromyscus* deer mice, *Drosophila* flies, mosquitoes, and *Nasonia* wasps), and reevaluated the phylosymbiotic relationships of seven species of wild hominids. We demonstrated three key findings. First, within species microbiota variation is consistently less than for between species, and microbiota-based models predict host species origin with high accuracy across the dataset. Interestingly, the age of host clade divergence positively associated with the degree of microbial community distinguishability between species within the host clades, spanning recent host speciation events (~1 million y ago) to more distantly related host genera (~108 million y ago). Second, topological congruence analyses of each group's complete phylogeny and microbiota dendrogram reveal significant degrees of phylosymbiosis, irrespective of host clade age and regardless of diverse taxonomy. Third, consistent with selection on host-microbiota interactions driving phylosymbiosis, there are survival and performance reductions when interspecific microbiota transplants are conducted between even closely related *Nasonia* and more divergent *Peromyscus* host species pairs respectively. In unpublished work within the *Nasonia* model system we have metagenomically sequenced the virome for the first time, and revealed that the pattern of phylosymbiosis extends to the community of bacteriophages. Finally, recent investigation utilizing dozens of wild species suggests limits in detecting phylosymbiosis among more physiologically and environmentally diverged organisms. Overall, these findings indicate that the composition and functional consequences of animals' microbial communities can be closely allied with host evolution, even across wide-ranging timescales, diverse animal systems, and when controlled conditions disentangle environmental from host influences.

### 4. Dana Carper, University of California Merced

#### Variations in the foliar microbial communities in *Pinus flexilis* (limber pine) across its native range

Dana Carper, A. Carolin Frank

The microbiome of plants plays a key role in the health and survival of its host. Host plants can benefit through activities, such as, the production of growth promoting hormones, phosphorus acquisition, nitrogen fixation and protection against pathogens. Through the activities of the microbiome plants are able to flourish in harsh environments with limited resources. *Pinus flexilis* is a high elevation conifer species that grows in nutrient poor environments across the western United States. Previous work on *P. flexilis* foliar tissue from two sites (Colorado and California) have shown a simple and consistent bacterial community dominated potential nitrogen fixing bacteria in the family Acetobacteraceae. In this study we ask if the core community previously documented in *P. flexilis* is consistent across its entire native range.

Needle samples from 10 *P. flexilis* trees were sampled from 15 sites across the native *P. flexilis* range. Sonication was used to separate bacterial communities from the needle surface and endosphere compartments. DNA was extracted from both compartments and used as a template to amplify the 16S rRNA gene. Samples were sequenced on the Illumina HiSeq platform using a dual indexing approach. Sequences were processed through the Qiime2 bioinformatics pipeline using the DADA2 package to infer amplicon sequence variants.

Preliminary results suggest that both needle surface and interior communities are primarily comprised of Proteobacteria, specifically Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria, but that the two compartments host different proportions of each. Surface communities had a greater proportion of Alphaproteobacteria comprised mainly of a single family the Acetobacteraceae. The endosphere compartment had a greater proportion of Gammaproteobacteria from the family Pseudomonadaceae. Our results also suggest that geography structures the *P. flexilis* foliar microbiome.

The core of Acetobacteraceae that was previously seen in *P. flexilis* was also found in these samples but within the surface communities instead of the endosphere communities. The endosphere communities were dominated by Pseudomonadaceae which have been found to rapidly colonize root tissue in other plant systems. Some strains of Pseudomonadaceae are also capable of nitrogen fixation and may be responsible for the nitrogenase activity previously documented in the foliar tissues of *P. flexilis*.

#### 5. Sharon L. Doty, University of Washington

##### **Mechanisms for Conferring Tolerance to Abiotic and Biotic Stresses by the Plant Microbiome**

Endophytes, the bacteria and fungi that live in intimate association within plants, provide numerous benefits to the host plant including N-fixation, phytohormone production, reduced abiotic stress responses, anti-microbial production, and pollutant degradation. A consortium of endophyte strains from Salicaceae plants (poplar and willow) improved the growth of a wide range of evolutionarily distant plant species in nutrient-limited and water-limited conditions. Some of these endophyte strains were shown to fix N<sub>2</sub> and solubilize phosphate *in vitro*, thus directly providing necessary macronutrients for plant growth. In contrast, multiple mechanisms may be involved in conferring drought tolerance including phytohormone production and impacts on plant physiology including stomatal conductance and photosynthesis. Direct mechanisms may be involved in the degradation of specific pollutants that result in profound increases in growth, health, and effectiveness of inoculated trees in contaminated areas. Some of the endophyte strains also have strong anti-microbial activity against a broad suite of pathogens. With the increasing interest in more environmentally-sustainable agriculture and forestry, manipulation of the plant microbiome offers a widely applicable and easily deployable solution.

#### 6. Carolin Frank University of California Merced

##### **Diversity and function of the conifer endophyte microbiome**

Lara Koppers, Jennifer Pett-Ridge, Tanja Woyke, Dana Carper, Paola Saldierna, James Kuyper

High-latitude and-altitude ecosystems are typically nitrogen (N)-limited due to low rates of N input and rapid microbial immobilization of inorganic N, and temperate and boreal forest trees must use a combination of sources and pathways to meet their N demand. Recent findings suggest that both evergreen and deciduous trees have the potential to directly access atmospheric N via N<sub>2</sub>-fixing bacteria in foliage and roots, and that in conifers, the bacteria responsible may be conserved across sites species. To better understand the role of endophytic N fixation for trees and ecosystems, our lab is asking what host- and environmental factors control the variation in N<sub>2</sub>-fixation rates, what taxa are responsible for N<sub>2</sub>-fixation, and how the endophyte community varies across species and sites. We used the acetylene reduction assay to measure nitrogenase activity in pines growing pine growing across a soil age chronosequence of marine terraces in coastal California, and found higher rates in bishop- than Bolander pine, and higher rates at the lowest terrace. These difference were also reflected in the endophyte community as characterized via 16S rRNA sequencing. To identify N<sub>2</sub>-fixing taxa, we are using single cell sorting and genome sequencing, but genome coverage has so far been too low to identify nitrogen-fixation genes. However, single cell sorting recovers the same bacterial groups as 16S rRNA sequencing, and the partial genomes encode host-interaction traits, including secretion systems, plant domain mimics, and carbohydrate metabolism, suggesting that these conserved taxa are adapted to colonize conifers.

#### 7. Kane Keller, California State University Bakersfield

##### **Tri-trophic symbiotic interactions affected by warming**

Kane Keller, Jennifer Lau, Mark Hammond

Climate change may alter the strength and importance of symbiotic interactions to plants. While some mutualists could mediate plant responses to climate change, changes in parasitic symbionts may exacerbate negative effects. Moreover, many species in natural systems are involved in multiple simultaneous symbiotic interactions providing unique or overlapping host benefits. To more fully understand plant responses to ongoing climate change, it is increasingly important to study how these changes may alter resource allocation to multiple mutualists as a result of differential availability associated with physiological changes, and the subsequent interplay between species. By manipulating nitrogen-fixing rhizobia, extrafloral nectar-tending ants, and temperature, here we explore how tri-trophic interactions between multiple mutualists and the plant host are affected by warming as well as how these potential mutualists may independently and interactively influence plant traits, affect fitness responses to climate change, and even cascade to alter the herbivore community. We find that spatial and temporal variation in selective pressures influence the effects of multiple mutualisms differentially across warming treatments. In particular, ants increased plant fitness while rhizobia increased fitness in elevated temperature and surprisingly decreased fitness in ambient conditions. Moreover, interactions between ants and rhizobia mediated through the plant vary with warming, as well, potentially due to shifts in carbon allocation with changing environmental conditions. Taken together, warming associated with climate change not only has the potential to affect individual species responses, but shifts in mutualistic interactions may further exacerbate these changes.

#### 8. Carly Kenkel, University of Southern California

##### **Using comparative transcriptomics to identify genes involved in the evolution of coral symbiont transmission mode**

Groves Dixon, Carly Kenkel

The endosymbiosis between tropical reef-building corals and *Symbiodinium* spp. forms the basis of one of the most biodiverse and productive ecosystems on the planet, yet much remains unknown about the cellular and molecular mechanisms governing this relationship. The coral- algal symbiosis is canonically defined as 'horizontal'; and most corals do acquire their endosymbionts from the environment. However, vertical transmission has repeatedly evolved in at least four independent lineages. We sequenced a set of coral transcriptomes for species representing these different transitions, in addition to mining other publicly available genomic resources, and used a comparative genomics approach to identify genes showing repeated signatures of non-neutral mutation rates specific to vertically transmitting lineages. Contigs for each transcriptome were converted into candidate protein coding sequences and orthologs were inferred using a reciprocal best BLAST match in combination with the program FastOrtho. dN/dS ratios were calculated for each ortholog by comparing vertical transmitters with their closest horizontally transmitting relatives, while accounting for other co-varying life-history traits. We also examined the nature of the inferred amino acid substitutions. Top candidate genes were required to exhibit both signatures of positive selection (i.e. dN/dS > 1) as well as convergent amino acid changes specific to vertically transmitting lineages. Perhaps unsurprisingly, these analyses identified uncharacterized proteins, but cathepsin and a copine- 7-like protein, which has previously been shown to play a role in membrane trafficking, were also highlighted. Future aims include cellular characterization of these candidates to determine their functional role in the vertical transmission of symbionts.

#### 9. Jack C Koch, Oregon State University

Low carbonic anhydrase activity in ellipsochloris-containing Anthopleura elegantissima and the negative correlation between diameter and carbonic anhydrase activity.

Jack C. Koch, E. Alan Verde, Virginia M. Weis

The sea anemone *Anthopleura elegantissima* is a prominent member of the intertidal zone on the west coast of North America and can exist in three symbiotic states: a white, brown, or green state where anemones lack symbionts, contain predominantly *Symbiodinium muscatinei*, or *Ellipsochloris marina*, respectively. The enzyme carbonic anhydrase (CA) catalyzes the interconversion between CO<sub>2</sub> and HCO<sub>3</sub><sup>-1</sup> and is known to play a dynamic role in delivering CO<sub>2</sub> to symbionts embedded deep within host gastroderm. To test the effect of symbiotic state and anemone size on CA activity in hospite, *A. elegantissima* were collected near Anacortes, WA, flash frozen in liquid nitrogen in the field, and subsequently processed for CA. Brown anemones displayed significantly higher CA activity than either green or white anemones and green anemones unexpectedly displayed CA activity equal to that of white anemones. CA activity, when normalized to anemone protein, was highest in small anemones and lowest in large anemones; furthermore,

regression analysis showed a significant inverse correlation between anemone size and CA activity. Symbiodinium has much greater rates of primary productivity than does *Elliptochloris* so higher CA activity in brown anemones is expected in order to provide their algae with unrestricted amounts of CO<sub>2</sub> to satisfy photosynthetic carbon demand. The negative correlation between anemone size and CA activity is hypothesized to be driven primarily by the surface area to algal density relationship. We propose that small sea anemones have smaller photosynthetically active surface area yet have similar algal densities to large anemones, necessitating higher CA activity to provide adequate CO<sub>2</sub> for photosynthetic activity.

**10. Meng Mao, University of California Merced;**

**Tailored host adaptation to support two ancient bacterial symbionts with differentially degraded genomes in a leafhopper host**

Xiushuai Yang, Gordon Bennett

Many plant sap-feeding insects rely on bacterial symbionts for essential nutrition absent in their diets. These bacteria experience extreme genome streamlining and ongoing gene losses, requiring cellular resources from their hosts. However, the co-evolutionary mechanisms that shape the cellular and metabolic integration of host-symbiont systems remain poorly understood, particularly when a host relies on multiple bacterial partners. The leafhopper *Macrostelus quadrilineatus* (Hemiptera: Cicadellidae: ALF) harbors two ancient symbionts that have the smallest known bacterial genomes: *Nasuia* (112 kilobases [kb]) and *Sulcia* (190 kb). They vary dramatically in their basic cellular and metabolic capabilities and they are housed separately in discrete host cells (bacteriocytes). To understand how ALF has co-adapted to maintain a stable symbiosis with *Sulcia* and *Nasuia*, we investigated the cell-specific gene expression of the distinct bacteriocytes and traced the origins of the differentially expressed genes in the host genome. We found that ALF has differentially reprogrammed the expression of thousands of genes in the bacteriocytes to support the cellular and metabolic requirements of each bacterium. These genes are involved in a range of bacterial cell functions, including the synthesis of essential nutrients. Several of these support mechanisms are derived from the evolution of novel functional traits via horizontal gene transfer from other bacteria to the host genome, reassignment of mitochondrial support genes, and host gene duplications with bacteriocyte-specific expression. Thus, the segregation of symbionts into discrete bacteriocytes likely permits the host to adaptively meet the needs of each symbiont.

**11. Holly V. Moeller, University of California Santa Barbara**

**Tree-ectomycorrhizal symbioses: Does diversity confer function?**

Michael G. Neubert, Ian A. Dickie, Kabir G. Peay, and Tadashi Fukami

Many trees form mutualistic relationships with ectomycorrhizal fungi, exchanging photosynthetically derived sugars for belowground pathogen defense, nutrients, and water. These host-associated communities are strikingly speciose. For example, a single tree can simultaneously host dozens of fungal partners, and some tree species are known to associate with thousands of fungal species. Alongside this species diversity, accumulating evidence suggests that fungi vary in their functional traits (e.g., water transport and nutrient acquisition strategies) and, as a consequence, their partner quality. Thus, tree-ectomycorrhizal symbioses serve as a model system for the multi-species mutualisms that abound in nature. In this talk, I explore the role that fungal diversity may play in facilitating tree establishment and growth in varied environmental contexts. I also highlight the challenges that partner diversity presents to the maintenance and stability of mutualistic relationships, and consider mechanisms host trees may evolve to regulate their fungal partners.

**12. Ryoko Oono, University of California Santa Barbara**

**Effect of foliar nutrients on species diversity of endophytic fungi depends on host specificity**

Eric Slessarev, Amanda Strom, Burton Sickler, Danielle Black, Austen Apigo

Endophytes are one of the most diverse guilds of symbiotic fungi that are found in the photosynthetic tissues of every major plant lineage in the world. A single leaf can be inhabited by dozens of different species and a single plant can host hundreds. To understand how fungal endophytes maintain such high species diversity, we set to understand what host leaf characteristics correlated with their diversity. When resource availability decreases, species diversity typically increases due to the release of competitive exclusion by a small number of dominating species. Hence, we hypothesized that lower nutrient availability in plant leaves would cause endophyte species diversity to increase. We took advantage of a natural edaphic gradient along a California coast where multiple marine terraces had been uplifted over hundreds of thousands of years, leading to a gradient of soil nutrient availability for the plants. The youngest terrace is relatively fertile but exposed to salt deposition by coastal winds. The terraces farther from the coast are progressively weathered and depleted in nitrogen, plant-available phosphorus, and other nutrients, supporting dwarfed 'pygmy' plant populations. We sampled the fungal endophytes from the leaves of two plant species that could be found across the entire gradient: *Pinus muricata* and *Vaccinium ovatum*. We found that species richness or evenness of fungal endophytes can both increase with lower nutrient availability, as predicted, but the strength of the correlations depended on host species. While the endophyte species evenness in *P. muricata* was strongly negatively correlated with foliar nitrogen levels, richness was only slightly negatively correlated. The endophyte species richness in *V. ovatum* was strongly negatively correlated with foliar nitrogen levels, but evenness remained high across terraces regardless of nitrogen levels. *P. muricata* is host to a smaller and more specific pool of endophytes than *V. ovatum*, suggesting that the endophytes in *P. muricata* are significantly more host-specific than the endophytes in *V. ovatum*. This crucial difference in host-specificity can explain why endophyte evenness was already relatively high in terraces with high nitrogen levels and changed little across terraces for *V. ovatum*. Endophyte richness in *P. muricata* was highest in the terrace with the lowest level of nitrogen, but remained similarly low in the other terraces, most likely because of the smaller host-specific pool of endophytes that can inhabit pines even under competitive exclusion.

**13. Lauren Ponisio, University of California Riverside**

**Understanding interaction flexibility in mutualistic networks**

Lauren Ponisio, Marilia Gaiarsa

Species that are able to change their interaction partners may be more resilient to global change. A species can be flexible in their interactions by changing: 1) the identity of their interaction partners; 2) their role in a network; and 3) their importance to network organization. Though these ways to be flexible can overlap, it is possible, for example, for a species to change interaction partners without changing their network role if they are swapping out similar species. We examine whether species traits are related to interaction flexibility using a 9-year dataset of over 20,000 pollinator specimens and 1500 unique interactions between plants and pollinators in native plant hedgerows in the Central Valley of CA. Specifically, we examine whether pollinator and plant abundance, diet breadth and phenology are related to interaction flexibility. We find that partner identity variability was significantly positively related to abundance, while network role variability was significantly related to phenology. Given that species interactions are responsible for many ecosystem functions, understanding how species traits are related to interaction flexibility will help to predict how communities will respond to changes in species composition.

**14. Kenjiro Quides, University of California Riverside**

**Experimental Evolution of *Lotus japonicus* symbionts**

Fathi Salaheldine, Ruchi Jarwala, Hsu-Han Lee, Jerry Trinh, Paola Cardenas, Jeff Chang, Joel Sachs

The legume-rhizobium symbiosis has become a model interaction to study the evolutionary implications of interacting partners across the mutualism-parasitism spectrum. Rhizobial bacteria are housed in legume root nodules where they provide costly fixed atmospheric nitrogen in return for host derived carbon. However, there is variation in the amount of fixed nitrogen a rhizobium species provides on a given host. One way host legumes limit their interactions with rhizobia that fix less nitrogen compared to other symbiotic rhizobia is through sanctions. This host control system adaptively

modulates *in planta* symbiont fitness based on the amount of fixed nitrogen a symbiont provides. Furthermore, host legumes can control the number of nodules they form dependent on nitrogen demand. But, this leaves the host susceptible to exploitation because if nodule number gets too high the host suffers a fitness consequence while the rhizobia continue to gain a fitness benefit. Here, I performed *in vitro-in planta* passages of two mediocre rhizobial symbionts on two host genotypes of *Lotus japonicus*, the wildtype host MG-20 and a hypernodulating mutant genotype *har1*. *Rhizobium etli* (CE3) is a symbiont that forms prematurely senescing nodules on *L. japonicus*, while *Ensifer fredii* (NGR234) forms nodules that are delayed to begin fixing nitrogen. After passaging symbionts a minimum of 10 times I conducted a greenhouse experiment to compare ancestral and derived symbionts on their respective hosts to assess evolved fitness differences.

15. **Lupita Ruiz-Jones**, Stanford University

**Investigating patterns of coral clonality across an environmental mosaic**

Carlo Caruso, Mariana Rocha de Souza, & Ruth Gates

**ABSTRACT TBA**

16. **Jason Rothman**, University of California Riverside

**Microbe-Mediated Protection Against Selenium Toxicity and Effects of Selenium and Cadmium on the Bee Microbiome**

Jason Rothman, Quinn McFrederick

Significant effort has been invested into understanding the complex interactions that bees have with their environments. One aspect that has been overlooked is the detrimental effects of metal pollutants on bee health. Plants growing in contaminated areas can accumulate these pollutants in their pollen and nectar, which in turn negatively affects the foraging bees. Social bees harbor a simple set of microbial symbionts which are known to have functional characteristics that are beneficial to bee health. Until now, nothing has been known about the effects of environmental metal/metalloid pollutants on the bee microbiome. Our work is tripartite: We harvested bumble bee cocoons from commercially-purchased hives and exposed either sterile or microbe-inoculated bees to 0.75 mg/kg selenium. We assayed mortality and established that bees with a microbiome live significantly longer than sterile bees. Secondly, we exposed bumble bees and honey bees to either 0.5 mg/kg selenium or cadmium in their diets. We used Illumina MiSeq sequencing of the 16S rRNA gene to investigate the effects of these toxins on the bees' microbiomes. We found that both selenium and cadmium significantly alter the bee microbiome. Lastly, we exposed the major core bacteria *Snodgrassella*, *Gilliamella* and *Lactobacillus* to these toxins *in vitro*. Our research is the first to show that the bee microbiome is protective against environmental metalloid pollution. We also show that the bee microbial community is robust against cadmium and selenium exposure and that individual members are resistant *in vitro* against these insults.

17. **Kaleigh A Russell**, University of California Riverside

**The effects of temperature on nectar-inhabiting microorganisms**

Kaleigh A Russell, Quinn McFrederick

The study of plant-pollinator networks is beginning to incorporate the interaction of microbial communities associated flowers and their pollinator. Nectar, an important resource to many pollinators, is inhabited by many microbes such as yeasts and bacteria, which have been shown to influence pollinator preference. This dynamic and complex network is even more influenced by the changing climate. To understand how global warming is influencing nectar microbial communities we performed a choice assay with the common eastern bumble bee, *Bombus impatiens*. Fifty bees were given the choice between synthetic nectar with an environmental microbial community incubated at the average Spring high temperature in Riverside, Ca, and synthetic nectar with the same microbial community, but incubated at a climate change predicted temperature. Bumble bees significantly preferred nectar that had microbial communities incubated at current average temperatures over climate change predicted temperatures. Temperature directly influenced the microbial community in nectar with an increase of *Fructobacillus* in the warmer treatment. Further work is being done looking at the implications of the influence of temperature change on nectar microbial communities in the field as well as the effects of temperature change on microbial biology such as volatile emissions.

18. **Joel L. Sachs**, University of California Riverside

**Multiple evolutionary losses of beneficial symbiosis in *Bradyrhizobium***

Bacterial mutualists are often acquired from the environment by eukaryotic hosts. However, both theory and empirical work suggest that this bacterial lifestyle is evolutionarily unstable. Here, we investigate the evolutionary erosion of nitrogen fixation in *Bradyrhizobium*, a nodulating root symbiont of legumes. We phenotyped *Bradyrhizobium* isolates across a metapopulation of *Acmispon strigosus* hosts, and reconstructed phylogenetic relationships in the bacterial population to infer loss events of nitrogen fixation capability. We tested hypotheses about the drivers of mutualism erosion in this system. The drivers of loss-of-nitrogen fixation events in natural *Bradyrhizobium* populations appears to be at least partially driven by adaptive evolution to exploit the host.

19. **Mohsin Tariq**, University of California Berkeley,

**Occupancy of non-nodulating endophytic plant growth promoting bacteria in the root nodules of pea (*Pisum sativum* L.)**

Root nodule accommodates various non-nodulating bacteria at varying densities. Present study was planned to identify and characterize the non-nodulating bacteria from the pea plant. Ten fast growing bacteria were isolated from the root nodules of cultivated pea plants. These bacterial isolates were unable to nodulate pea plants in nodulation assay, which indicate the non-rhizobial nature of these bacteria. Bacterial isolates were tested *in vitro* for plant growth promoting properties including indole acetic acid (IAA) production, nitrogen fixation, phosphate solubilization, root colonization and bio-film formation. Six isolates were able to produce IAA at varying level from 0.86 to 16.16  $\mu\text{l ml}^{-1}$ , with the isolate MSP9 being most efficient. Only two isolates, MSP2 and MSP10, were able to fix nitrogen. All isolates were able to solubilize inorganic phosphorus ranging from 5.57 to 11.73  $\mu\text{l ml}^{-1}$ , except MSP4. Bacterial isolates showed considerably better potential for colonization on pea roots. Isolates MSP9 and MSP10 were most efficient in biofilm formation on polyvinyl chloride, which indicated their potential to withstand various biotic and abiotic stresses, whereas the remaining isolates showed a very poor biofilm formation ability. The most efficient plant growth promoting agents, MSP9 and MSP10, were phylogenetically identified by 16S rRNA gene sequence analysis as *Ochrobactrum* and *Enterobacter*, respectively, with 99 % similarity. It is suggested the potential endophytic bacterial strains, *Ochrobactrum* sp. MSP9 and *Enterobacter* sp. MSP10, can be used as biofertilizers for various legume and non-legume crops after studying their interaction with the host crop and field evaluation.

20. **Trevor R Tivey**, Oregon State University

**Manipulation of surface glycan biosynthesis decreases colonization success during onset of cnidarian-dinoflagellate symbiosis.**

John E Parkinson, Paige E Mandelare, Donovan A Adpressa, Sandra Loesgen, Virginia M Weis

The relationships between hosts and microbes often rely on successful interpartner communication. In many of these symbioses, a fundamental mechanism of this communication is the interaction between glycans and lectins. During onset of cnidarian-dinoflagellate symbiosis, cnidarian gastrodermal cells bind sugar residues on their algal partners. These algal glycans are important for recognition and may in part mediate host-symbiont specificity. To determine the effect of specific glycans on symbiont colonization success, we manipulated the *Symbiodinium minutum* glycan surface through direct enzymatic cleavage and small molecule enzymatic inhibitors that simplify and enrich for specific glycans. We first characterized the

response and recovery of *S. minutum* cultures to glycan reduction by N-glycosidase and O-glycosidase treatment. To test for efficacy, cultures were incubated with lectin-conjugated fluorophores that labeled N-linked, high-mannose containing glycans. Flow cytometric analysis revealed a decrease in glycans for cultures under enzymatic treatment. After three days of recovery from N-glycan cleavage, there was an increase in overall glycans compared to the surface of untreated cultures, indicating robust glycan biosynthesis that may have been overstimulated by prior glycan removal. After confirming glycan reduction and enhancement in treated and recovered cells, we compared colonization success of each culture in the symbiotic sea anemone *Aiptasia*. Colonized hosts were imaged three days after inoculation to quantify symbiont density. Declines in colonization were found with glycosidase-treated cultures compared to untreated cultures, measured by symbiont density. No difference in colonization was found between cultures with recovered glycans and untreated cultures. To finely examine specific glycan effects on symbiosis, cultures were treated with two different mannosidase inhibitors, kifunensine and swainsonine, which directly inhibit endogenous glycan biosynthesis pathways in the ER and Golgi, respectively. Kifunensine- and swainsonine-treated cultures had significant enrichment for high-mannose compared to untreated cultures, as expected as kifunensine treatment should interrupt N-glycan biosynthesis at the high-mannoside stage, while swainsonine allows further diversification. In a pattern that mirrored glycosidase-treated cultures, kifunensine-treated cultures had significantly lower colonization compared to untreated cultures. Together, these results suggest that the number and type of surface glycans influences host-symbiont recognition. Simplified N-glycan *Symbiodinium* had decreased success in colonization, which may highlight the importance of complex glycans in symbiosis and coral-algae recognition.

**21. Hoang Vuong**, University of California Riverside

#### **Comparative Genomics of *Lactobacillus* Associated with Wild Bees Reveal Potential Adaptation to Hosts**

Hoang Vuong, Quinn S. McFrederick

*Lactobacillus micheneri*, *L. timberlakei*, and *L. quenuiae* are novel bacterial symbionts found abundantly in wild bee pollen provisions and adults. These bacterial species form a clade that we refer to as the *L. micheneri* clade. Though not found in honey or bumble bees, these bacteria are associated with a rich diversity of wild bees and managed bees that pollinate crops. We sequenced and assembled the genomes of 30 *L. micheneri* clade isolates to identify their possible ecological functions in flower and bee hosts. We completed gene gain and loss content analysis with Count, detected genes with amino acid sites under positive selection with PAML, and analyzed allele frequency for balancing selection and selective sweeps. A large loss of genes occurred in common ancestors of the *L. micheneri* clade and their relatives. Many lost genes were involved in carbohydrate transport, amino-acid biosynthesis, transcription, and coenzyme metabolism, suggestive of adaptation to a nutrient-rich niche. Outside of our gene gain and loss analyses, we have found several genes involved in colonization and metabolism that may be important for *L. micheneri* survival and proliferation in both flowers and bees. Finally, we found other genes involved in metabolism and animal tissue colonization that suggest that *L. micheneri* is more adapted to bee guts than to flowers or other plant tissue. We plan to use these findings as possible hypotheses of mechanisms of host colonization, nutritional exchange, defense in the *L. micheneri*-wild bee symbioses.

**22. Jenifer Walke**, Eastern Washington University

#### **Stability of amphibian skin symbionts and their metabolites across seasons and disturbance**

Matthew H. Becker, Elizabeth A. Burzynski, Celina Santiago, Thomas P. Umile, Kevin P. C. Minbiole, Lisa K. Belden

Amphibian populations are experiencing unprecedented declines and extinctions globally, largely due to the skin disease, chytridiomycosis, caused by the fungal pathogen, *Batrachochytrium dendrobatidis*. Amphibian skin hosts diverse bacterial communities that have an important role in host defense against this pathogen, as some skin symbionts can inhibit pathogen growth via secondary metabolites. However, it is unclear how stable the skin microbiome of individual amphibians is over time and in response to disturbance in natural environments. Here, we monitored the skin microbiome and metabolite profiles of individual Eastern newts (*Notophthalmus viridescens*) for two years in large field enclosures within a permanent pond and assessed the effects of season and disturbance on skin microbial community dynamics. Environmental perturbation had a dramatic effect on skin bacterial community composition, richness, phylogenetic diversity, evenness, and metabolite profiles. Season also had a significant effect on the skin bacterial community composition and metabolites, but there were no effects of season on richness, phylogenetic diversity, and evenness. There were seven core OTUs that were found on all newts in all seasons, and before and after perturbation, with the most abundant OTU being a Comamonadaceae OTU that represented, on average, 44% of the bacterial relative abundance on newt skin. The results from this long-term study suggest that single environmental perturbations can have lasting effects on the skin microbiome and that these effects can be stronger than temperate seasonality, but the core symbionts remain relatively stable through both season and disturbance. It will be important to further understand how these changes might influence pathogen defense and other functions of amphibian skin symbionts.

**23. Christopher S Ward**, Lawrence Livermore National Laboratory

#### **Chytrid parasitism in mass algal culture and the potential significance of the algal-chytrid-bacterial tripartite interaction**

Laura T. Carney, Joyce E. Longcore, Michael P. Thelen, Rhona K. Stuart

Chytrids can cause rapid, severe declines in microalgal populations in both natural water bodies and industrial production ponds; as such, they are key regulators of primary production and carbon cycling in aquatic ecosystems. Despite their ecological and economic impact, surprisingly little is understood about how and under what conditions chytrids infect microalgae. To address this, we studied chytrid infection both in field and laboratory settings. From extensive data monitoring of algal production ponds, we determined that chytrid infection rates are correlated with water temperature and daily irradiance, though only when algal motility is low (i.e., in cyst stage). Additionally, heterotrophic bacterial community dynamics were tightly coupled to algal physiology and culture age. Although there was no apparent link between chytrid infections and bacterial community composition, bacterial abundance and secondary production are typically correlated with water temperature in aquatic systems. To further investigate biotic and abiotic drivers of chytrid infection, we isolated two chytrid strains – *Rhizophydiales* sp. (Chytridiomycota) and *Paraphysoderma sedebokerense* (Blastocladiomycota) – from crashed ponds. Culture-based assays supported the dependence of chytrid infectivity on algal growth stage, with highest infectivity of both strains on cyst-stage algae. Furthermore, unexpectedly we observed that the chytrids can switch between infective (i.e., feeding directly on algal cells) and non-infective (i.e., feeding on algal exudate) lifestyles. We found that the chytrid's trophic strategy appears to be regulated by dissolved organic carbon (DOC) availability, which in turn is determined by algal exudation and competition with heterotrophic bacteria. Ongoing work will address the mechanistic underpinnings of the algal-fungal-bacterial tripartite interaction, including the molecular changes underlying the switch in trophic strategies and subsequent physiologies, using metatranscriptomics and exometabolomics. While the full significance of multiple chytrid trophic strategies in outdoor algal ponds is still unclear, identifying the molecular mechanisms and ecological drivers controlling chytrid metabolism is critical for deciphering their complex environmental roles, and will ultimately benefit our understanding of aquatic carbon cycling and algal bioenergy production.

**24. Camille Wendlandt**, University of California Riverside

#### **Host investment varies among populations of the legume *Acmispon strigosus*, but host sanctions are invariant**

Camille Wendlandt, Joel Sachs

To maintain effective symbiosis, hosts must bear traits to selectively reward beneficial partners and punish ineffective partners. Under efficient host control, ineffective symbionts are predicted to be extirpated, but symbionts that provide no benefit to hosts are nonetheless widespread in nature. Here, we tested three hypotheses for the maintenance of symbiotic variation in rhizobia that associate with native legume populations: 1) partner mismatch between host and symbiont, such that symbiont effectiveness varies with host genotype, 2) resource satiation, whereby extrinsic sources of nutrients relax host control, and 3) genetic variation in host control among host populations. We inoculated *Acmispon strigosus* sourced from six populations with three root nodule-forming *Bradyrhizobium* strains that vary in symbiotic effectiveness on sympatric hosts. We measured proxies of host



and symbiont fitness in single and co-inoculations under fertilization treatments of zero added nitrogen and near-growth-saturating nitrogen. We examined two components of host control: 'host investment' was measured as mean nodule size during single- and co-inoculations, and 'host sanctions' was measured as relative *in planta* abundance of the most effective strain during co-inoculations. The *Bradyrhizobium* strains exhibited conserved growth effects on hosts, and host control was not attenuated under experimental fertilization, inconsistent with the partner mismatch and resource satiation hypotheses, respectively. Host sanctions were robust in all host populations, but host populations varied significantly in measures of host investment in both single- and co-inoculation experiments. Our data suggest that host investment traits, rather than sanctions, vary significantly among *A. strigosus* populations. Segregating variation in host investment could promote variation in symbiotic effectiveness and prevent the extinction of low-quality *Bradyrhizobium* from natural populations.

**25. David Weston**, Oak Ridge National Lab.

#### **Determining the genetic and environmental factors underlying mutualism within a peatmoss - N<sub>2</sub> fixing bacterial association**

DJ Weston, A Carrel, D Pelletier, D Velickovic, R Chu, C Anderton, K Louie, T Louie, T Northen

The importance of plant-microbiome systems on terrestrial carbon and nitrogen processes is perhaps most pronounced in *Sphagnum* dominated ecosystems, which occupy 3% of the Earth's land surface yet store approximately 25% of terrestrial carbon as recalcitrant organic matter (i.e., peat). The foundation plant genus *Sphagnum* is responsible for much of the primary production in peatland ecosystems and produces recalcitrant dead organic matter. Together with associated N<sub>2</sub>-fixing microorganisms, *Sphagnum* contributes to substantial peatland nitrogen inputs. *Sphagnum* growth and production (carbon gain) depends, in part, on a symbiotic association with N<sub>2</sub>-fixing, diazotrophic microbes. Under changing environmental conditions, a central question about these ecosystems is whether the *Sphagnum*-diazotroph symbiosis will maintain its beneficial interaction, or will it shift to neutral or even antagonistic interactions that ultimately influence peatland carbon gain and storage. To begin to address this question, we are initiating a 5-year project using field-scale warming manipulations, synthetic communities, genotype-to-phenotype associations, and metabolic characterization to address two overarching hypotheses, 1) *Sphagnum* host and diazotroph genetic variations play a key role in determining the environmental tipping point of beneficial symbiosis (i.e., environmental disruption), and 2) the surrounding microbiome can further adjust the tipping point through facilitation, competition, and antagonism. Results from our field manipulation study show that warming decreased *Sphagnum* associated microbial diversity (p<0.05), and that N<sub>2</sub> fixing diazotrophs shifted from diverse communities to those dominated by *Nostocaceae* (from 25% in unheated samples to 99% in warmed samples).

To provide a fundamental understanding of the field results, we are developing resources for synthetic communities. We now have draft genomes of 15 *Sphagnum* species and (re)sequencing of a 200-member pedigree was recently completed. On the microbial side, 72 *Sphagnum* associated heterotrophic bacteria strains, along with 12 cyanobacteria and 30 putative methanotrophs have been isolated on multiple medium types. A pilot optimization experiment confirms that our synthetic community approach is amenable to spatial characterization of target metabolites using matrix assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) along with liquid extraction surface analysis (LESA). Equipped with these resources, our team is now initiating experimentation to address the quantitative genetics of symbiosis, metabolite exchange and codependency, and ultimately how environmental perturbations interact with plant and microbial genetics to form and break symbiosis.

---

#### Posters

**Samuel Bedgood**, UC Irvine,

#### **Drivers of Diversity in the Sea Anemone Genus Anthopleura**

M. E. S. Bracken, Samuel Bedgood,

Three congeneric species of sea anemone hosting algal symbionts are found along the west coast of North America. *Anthopleura elegantissima*, *Anthopleura sola*, and *Anthopleura xanthogrammica* commonly co-occur in the California intertidal zone. How these closely related anemones co-exist despite substantial overlap in resources remains unknown. Diet and microhabitat have been proposed as niche axes, but nutrient acquisition strategies related to symbiont-to-host carbon translocation could vary as well. The highly flexible relationship between anemones and symbionts is key to understanding niche differentiation. Possible drivers of diversity were explored via surveys at nine sites, and stable isotope analysis of both anemone tissue and symbionts. We recorded species, microhabitat, size, and tidal elevation. The stable isotope analysis compared anemone species, tidal elevation, and tissue type. Preliminary survey results reveal little to no complementarity in tidal zonation, but microhabitat use is different among species.

**Megan Gable**, Oak Ridge National Lab

#### **Utilizing Quantitative Trait Loci analysis in a Sphagnum-diazotroph symbiosis to determine underlying genetic factors**

Megan Gable, Alyssa Carrell, Sara Jawdy, Lee Gunter, Jeremy Schmutz, Jonathan Shaw, David Weston

*Sphagnum*-dominated peatlands sequester more carbon in temperate and boreal ecosystems than any other plant group. *Sphagnum* derive much of their nitrogen budget from an intimate symbiosis with nitrogen-fixing bacteria, but little is known about the factors mediating this symbiotic relationship. Our research is guided by the overarching question: What are the genetic and physiological controls that shape *Sphagnum*-diazotroph symbiosis and function? Partnered with DOE JGI, we are (re)sequencing the genomes of 200 individuals from an *S. fallax* pedigree and genomes from *Sphagnum* isolated diazotroph to create a QTL analysis to identify moss genes controlling the initiation of symbiosis. Here we will present preliminary results from a pH gradient experiment study that demonstrated diazotroph colonization was both pH and *Sphagnum* genotype dependent. Next, we will use this information to guide *Sphagnum* genotype and diazotroph strain selection to thoroughly explore the genetic controls on the *Sphagnum*-diazotroph initiation and maintenance. Our research will provide a better understanding of plant-microbe interactions, ecological genomics, and peatland carbon and nitrogen cycling.

**Diler Haji**, University of Connecticut

#### **Poster: Distinct gut-associated bacteria in the symbiont-host consortium of subterranean *Magicalicada septendecim* nymphs**

Susan Janton, Russ Meister, Joerg Graf, Chris Simon

Patterns of insect gut microbial community compositions show that the microbiome is often host-specific and implicated in putative nutritional and immunological benefits that are of interest in understanding the adaptive evolution of their hosts. These communities are also dynamic and subject to both the constraints of host-specific internal environments and the interactions among microbial taxa. Periodical cicadas (Genus *Magicalicada*) are unique among insects because of their prolonged and primarily subterranean 13- and 17-year life cycles and their reliance on nutrient-poor plant sap-based diets, which require intimate symbioses with endosymbiotic bacteria that provision missing vitamins and amino acids. Despite ongoing work on this endosymbiont-host consortium, no studies have explored patterns and diversity of microbial inhabitants within the guts of periodical cicadas. We collected *M. septendecim* nymphs and associated soil samples from emergence tunnels in North Carolina, Connecticut, and Ohio and adults from North Carolina for 16S- and ITS2-amplicon community profiling of bacteria and fungi, respectively. We were unable to amplify or sequence fungus from periodical cicadas in contrast to previous studies on Asian cicadas. Our bacterial community profiles showed an overwhelming relative abundance of the cicada-specific secondary endosymbiont *Candidatus Hodgkinia cicadicola* thought to be present only within the bacteriocytes of the host. After removal of endosymbiont sequences, we confirmed that bacterial communities within periodical cicada guts are distinct and represent a subset of the diversity of microbes from associated soil samples. We also found a bacterium within the genus *Cupriavidus* at high abundance consistently within cicada nymphs possibly representing a symbiotic microbe important in the biology of periodical cicadas. We cloned and sequenced the full-length 16S rRNA of this



*Cupriavidus* strain to better understand the relationship of this bacterium to other strains of the genus, members of which are sometimes associated with root nodules. We conducted FISH on gut sections in an attempt to confirm the presence of *Hodgkinia cicadicola* and localize the position of the *Cupriavidus* strain in the alimentary canal.

**Travis J. Lawrence**, UC Merced

#### **tRNA Interaction Network Sheds Light on the Origin of Chloroplast**

Travis J. Lawrence, Katherine C. H. Amrine, Wesley D. Swingley, and David H. Ardell

The acquisition of a cyanobacterial endosymbiont by the last common ancestor of Archaeplastida transferred the trait of oxygenic photosynthesis to eukaryotes. The subsequent radiation of eukaryotic photoautotrophs profoundly influenced the history of life on Earth. Despite consensus on the cyanobacterial tree of life, the precise location of plastids within Cyanobacteria remains controversial. Previous phylogenomic studies yielded strong support for two incompatible hypotheses for the origin of plastids: as an early diverging lineage or alternatively within a later radiation of Cyanobacteria. Phylogenetic inference concerning the origin of plastids is complicated by at least one billion years of vertical descent and lineages with extremely reduced genomes in plastids and Cyanobacteria. In addition to smaller datasets, reduced genomes are associated with non-stationary composition of genomes, violating the assumptions of many phylogenetic models.

Recently we introduced a novel signature-based approach to genome phylogeny based on our bioinformatic estimates of the evolving functionally critical features in tRNA gene complements, which appears robust to non-stationary compositions of genes and genomes. Our method exploits unique aspects of tRNA structural biology for accurate biogenesis and function. Based on our estimates of the evolving functionally critical features in tRNA gene complements we implemented an Artificial Neural Network-based phyloclassification algorithm and applied it to test competing phylogenetic hypotheses regarding the origin of plastids within Cyanobacteria. Using 5,476 tRNA gene sequences from 113 cyanobacterial genomes, we found support that plastids originated within a more recently diverging lineage of Cyanobacteria, within the starch-producing clade containing marine/freshwater single-cell diazotrophic species.

**Jorge Armando Montiel-Molina**, University of California Merced

#### **Do fungal endophytes have a mutualistic role on vernal pool plants?**

Jorge Armando Montiel-Molina, **Carolyn Frank, Jason Sexton, Michael Beman.**

Amphibious behavior displayed by vernal pool plants might be mediated by a stress tolerance-symbiosis mechanism involving fungal endophytes. The proposed research explores the functional role of fungal endophytes living in symbiosis with two vernal pool plant specialists. Vernal pools are temporary wetlands encompassing radical desiccation and flooding scenarios, harmful for any other terrestrial or aquatic plant species. To test the hypothesis that symbionts confer stress tolerance to amphibious vernal pool plants, a greenhouse experiment will be performed, where vernal pool environmental conditions (desiccation and flooding) will be tested on symbiotic and non symbiotic vernal pool plants; specifically, I will be evaluating lifetime plant fitness by examining seed germination, growth, flowering (reproduction) and survival rate. Our result will answer whether fungal endophytes are associated positively with the host plants. A successful establishment of the vernal pool plant under extreme conditions can be seen as further adaptation process driven by symbiosis.

**Jake Sarver**, UCSB,

#### **Poster: Exploring Foliar Fungal Endophyte Assemblage, Diversity, and Host Specialization in Pine**

Jake Sarver, Rodolfo Salas-Lizana, Austen Apigo, David S. Gernandt, and Ryoko Oono

Host specialization of foliar fungal endophytes (FFE's) remains a cryptic and uncommon phenomena. Since patterns of host specificity are sensitive to host taxonomic and spatial scales, a field study that investigates the fungal endophytic community of conifers, a taxonomically well-defined and diverse group, across a wide geographic range (spanning much of North America) was conducted. Conifer trees have a high incidence of FFE infection, likely due to the longevity of their evergreen foliage as well as their dominance in some ecosystems. Furthermore, *Lophodermium* (Rhytismataceae), a well-studied FFE genus that seems to be common within needles of the Pinaceae family, indicates high phylogenetic host specificity that is rarely documented for other FFE's. In total, 51 species of conifers were sampled from 69 localities across the United States and Mexico. Illumina MiSeq sequences were collected on the community assemblage of FFE's with a closer investigation of *Lophodermium* OTUs. Host specificity of common OTUs were analyzed and compared across geographic and taxonomic groups of conifers. These findings will be crucial for furthering our understanding of the evolutionary and ecological nature of these mysterious microfungi on their hosts.

**Kaoru Tsuji**, Stanford University

#### **Community-wide consequences of sexual dimorphism: evidence from nectar microbes in dioecious plants**

Kaoru Tsuji, Tadashi Fukami

Intra-specific trait variation is receiving renewed interest as a factor affecting the structure of multi-species communities within and across trophic levels. One pervasive form of intra-specific trait variation is sexual dimorphism in animals and plants, which might exert large effects particularly on the communities of host-associated organisms, but the extent of these effects is not well understood. We investigated whether host-associated microbial communities developed differently in the floral nectar of female and male individuals of the dioecious shrubs, *Eurya emarginata* and *E. japonica*. We found that nectar-colonizing microbes such as bacteria and fungi were more than twice as prevalent and, overall, more than ten times as abundant in male flowers as in female flowers. Microbial species composition also differed between flower sexes. To examine potential mechanisms behind these differences, we manipulated the frequency of flower visitation by animals and the order of arrival of microbial species to nectar. Animal visitation frequency affected microbial communities more greatly in male flowers, while arrival order affected them more in female flowers. These sex-specific effects appeared attributable to differences in how animals and microbes altered the level of osmotic stress that limits microbial growth in nectar. Taken together, our results provide evidence that sexual dimorphism can have large effects on the structure of host-associated communities.

**Marriam Zafar**, University of California Berkeley

#### **Effect of single and co-inoculation of rhizobia on peas (*Pisum sativum*)**

Nisar Ahmed, Ellen L. Simms and Mohsin Tariq

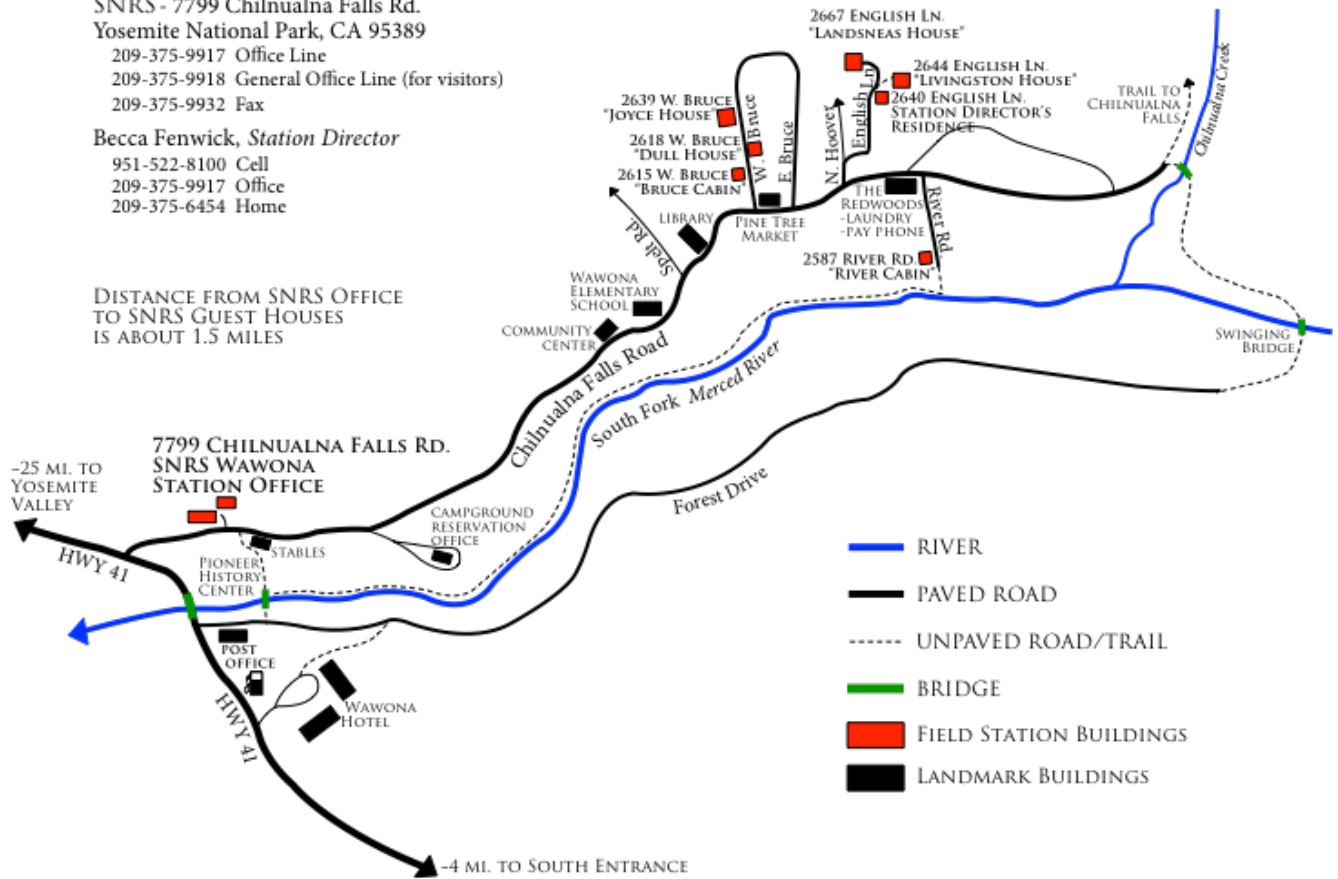
Plant growth promoting rhizobacteria (PGPR) represent soil and plant associated bacteria that promote growth and yield of plants by various mechanisms. In agriculture, PGPR can replace chemical fertilizers, pesticides and other supplements. Rhizosphere microorganisms produce large quantities of substances that promote plant growth and indirectly affect plant morphology. In this study, root nodulating bacteria were isolated from pea. We observed their nodulation activity, biofilm formation, and root colonization and characterized their solubilization of phosphate, production of indole acetic acid, and fixation of nitrogen. Potential root nodulating bacteria were identified using 16S rRNA, ITS and *nodC* gene sequence analysis and were evaluated for their ability to enhance growth and yield of peas. *Rhizobium bangladeshense* was isolated from pea nodules for the first time. We also confirmed that *Rhizobium bangladeshense* nodulates pea. *Rhizobium bangladeshense* PM3 and *Rhizobium leguminosarum* strains PM1 best improved pea growth. Co-inoculating *Rhizobium bangladeshense* PM3 and *Rhizobium leguminosarum* PM2 strains improved pea growth more than when singly inoculated.

## SIERRA NEVADA RESEARCH STATION- YOSEMITE, UC MERCED

SNRS - 7799 Chilnualna Falls Rd.  
 Yosemite National Park, CA 95389  
 209-375-9917 Office Line  
 209-375-9918 General Office Line (for visitors)  
 209-375-9932 Fax

Becca Fenwick, *Station Director*  
 951-522-8100 Cell  
 209-375-9917 Office  
 209-375-6454 Home

DISTANCE FROM SNRS OFFICE  
 TO SNRS GUEST HOUSES  
 IS ABOUT 1.5 MILES



## DIRECTIONS TO SNRI WAWONA STATION:

### ***Important Notes:***

- \* Wawona is near the *South* Entrance of Yosemite. From the west, going south to Oakhurst and then north into the park is quicker than taking Hwy 140 or 120 into Yosemite Valley.
- \* Google directions sometimes will take you on tiny roads thru Raymond, CA. It is easy to get lost this way, especially at night. I don't recommend it.

### *From the Bay Area (From the South – Skip to Oakhurst, CA):*

- \* 580 East to I5 South (do not take 205 to Stockton/Tracy)
- \* Interstate 5 South to 132 East (to Modesto)
- \* 132 East to 99 South
  - \* stop and eat a taco at Las Gruillenses (on the right just before the jct. with Hwy 99)-homemade corn tortillas and great carnitas
- \* 99 South to 140 East (direction Yosemite/Mariposa)
- \* 140 East to Mariposa, CA
  - \* stop and eat another taco at Ramon's Taco Truck in Planada (a few miles after you leave Merced).
- \* 49 South to **Oakhurst, CA 93644**
- \* Go North on Hwy 41 about 15 Miles to the South Entrance of Yosemite National Park
- \* At the entrance gate, tell them that you are working with me at UC Merced's Sierra Nevada Research Institute. They will give you a "Gate Pass" that I must sign so you can get out for free.
- \* Continue North about 4 miles to the village of Wawona.
- \* Pass the big white historic Wawona Hotel. Cross the small bridge with stone sides.
- \* Take an immediate right after the bridge on Chilnualna Falls Rd.
- \* The **SNRI Wawona Station office building** is the second white house on the left. It has a covered porch. (SNRI address: 7799 Chilnualna Falls Rd. )

### *From the north:*

- \* Google yourself to Groveland, CA 95321 and follow Hwy 120 to Yosemite's north entrance.
- \* Follow signs to Yosemite Valley (do not take 120 over Tioga Pass)
- \* From Yosemite Valley, take Hwy 41 South towards Wawona (and Badger Pass)
- \* Continue on Hwy 41 South about 25 miles until you reach Wawona (you will pass the Wawona Campground on your left)
- \* Turn left on Chilnualna Falls Rd immediately BEFORE the bridge. (If you've reached the Wawona Hotel you've gone too far)
- \* The **SNRI Wawona Station office building** is the second white house on the left. It has a covered porch.
- \* SNRI address: 7799 Chilnualna Falls Rd.

### *From the east:*

- \* Google yourself to Lee Vining, CA 93541 and take Hwy 120 west to Yosemite's east entrance and over Tioga Pass.
- \* Turn Left on Big Oak Flat Rd and Follow signs to Yosemite Valley
- \* From Yosemite Valley, take Hwy 41 South towards Wawona (and Badger Pass)

## ***What to Expect at the UC Merced SNRI Wawona Field Station, and What the SNRI Expects from You***

The Sierra Nevada Research Institute Wawona Field Station and its related facilities are inside the south boundary of Yosemite National Park. A "Gate Pass" can be requested at the entry for all official use of the SNRI field station. Gate Passes must be signed by SNRI staff before you leave to validate the fee waiver. Longer term fee waivers may be obtained from the field station director. At the entry, you will also be provided with brochures listing rules and regulations governing behavior in the park. Pay particularly close attention to garbage and trash disposal regulations. Feeding the wildlife is a serious offense. Speed limits are equally important because of the wildlife in the park. Many bears, hundreds of deer, and thousands of small mammals die every year after being hit by speeding cars. Please be sure that you are never in violation of any of the park guidelines or regulations. As a guest of the SNRI, your conduct will reflect on all of us and on the UC Merced.

### ***Office:***

There are T1 Internet connections available, but no extra computers. For a fast connection for your own equipment, bring an Ethernet cable. Wireless is also available.

There is poor or no cell phone access in Wawona depending on your carrier. Don't count on it. There is a general use phone – 209-375-9918 – but bring a *calling card* for long distance calls. In an emergency, dial 911. Operators will notify Yosemite law enforcement or fire personnel.

The office has a small kitchen with dishes, cookware, a small oven, hot plates, coffee maker, electric tea kettle, refrigerator, etc. There is also a shower.

Our custodial services are provided by the same person who does all the maintenance for all seven SNRI buildings. Please keep this in mind and pitch in. Please also pack out your recyclables, or put them in the bins near the dumpsters in the maintenance area up the road (see map).

If you're the last to leave the office, make sure that all 3 doors are locked.

### ***Houses:***

All SNRI housing is supplied at minimal charge for the convenience of those who otherwise would not be able to conduct research so far from home or campus. There is no housekeeping staff, nor anyone available to take care of your day to day needs. In almost all situations, you are expected to take care of yourself. In the event of a serious electrical, plumbing, law enforcement or other problem, there is a white board in the office with emergency contact information.

Please treat the houses with care and respect. They are owned by the National Park Service and are under permit to the UC Merced. It has been a long, arduous, and expensive process to get them assigned, functional, and livable. Please be careful not to damage either the houses or our relationship with the surrounding community.