

TENTH ANNUAL

YOSEMITE  
SYMBIOSIS  
WORKSHOP



**Sierra Nevada Research Institute**

**Yosemite National Park, May 13-15 2022**

## Friday May 13, 2022

Arrival & Welcome Party 7:00pm – 10:00pm

Landsnaes House 2667 English Lane, Wawona CA

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## Saturday May 14

Wawona Community Center (SEE MAP)

Lunch 11:30pm – 12:25pm

12:25 Welcome to the 2022 Yosemite Symbiosis Workshop (Carolin Frank, Joel Sachs)

### Session I: Evolution of symbiotic partnerships

- 12:30 Tobin Hammer, UC Irvine  
**Mystery of the missing microbes: why do bees keep losing their symbionts?**
- 12:45 Yumary Vasquez, UC Merced  
**Leveraging a Hawaiian leafhopper adaptive radiation to understand bacterial symbiont genome evolution**
- 1:00 Joel L. Sachs, UC Riverside  
**Pangenome evolution reconciles robustness and instability of rhizobial symbiosis**

30 minute break for coffee and snacks

### Session II: Coadaptation between host & symbiont

- 1:45 Kyle Meyer UC, Berkeley  
**The impact of transmission within versus among host plant species on microbiome diversity and adaptation**
- 2:00 Jorge Armando Montiel Molina, UC Merced,  
**Amphibious plants present a gigantic shift in root microbiome community across life cycles**
- 2:15 Joseph Russo, UC Riverside  
**Cnidarian–Symbiodiniaceae symbiosis establishment is independent of photosynthesis**
- 2:30 David Fronk, UC Riverside  
**Symbiotic Organs: common motifs and evolutionary pressures**

30 minute break for coffee and snacks

### Session III: Evolutionary genetics of host-symbiont adaptation

- 3:15 Gordon M. Bennett, UC Merced  
**Evolving beneficial symbioses between leafhoppers and bacteria**
- 3:30 Alison Gould, California Academy of Sciences  
**Symbiosis-induced changes in gene expression of a bioluminescent vertebrate-bacteria association**
- 3:45 Brendan Cornwell, Hopkins Marine Station of Stanford University  
**Using population genomic patterns in *Acropora* sp. and their symbionts to identify and characterize optimal partnerships**
- 4:00 Tingting Xiang, University of North Carolina at Charlotte  
**Euplotes-Symbiodinium, a unicellular model system for coral-algal symbiosis**

Beer, Wine & Snacks 4:15-5:00

### **Keynote Lecture Michele Nishiguchi 5:00-6:00**

Interpreting the road map between ecological and molecular boundaries using a squid-bacterial mutualism

Dinner 6:30-800

**Poster Session 8:00-9:30pm**

## Sunday May 15

### Breakfast in your Cabins

Wawona Community Center (SEE MAP)

#### Session IV: *Ecological mediators of symbiosis*

- 9:00 Daravuth Cheam, UC Merced  
**Vibrio fischeri** biofilms respond differentially to protozoan predation
- 9:15 Jakob Kaare-Rasmussen, UC Santa Barbara,  
**Modeling Food Dependent Symbiosis in *Exaiptasia pallida***
- 9:30 Gabriel Santiago Ortiz, UC Riverside  
***Lotus japonicus* regulates root nodulation and nitrogen fixation dependent on the molecular form of nitrogen**
- 9:45 Arafat Rahman, UC Riverside  
**Interstrain competition among Bradyrhizobium reduces plant performance in *Acmispon strigosus***
- 10:00 Gabe Runte, UC Santa Barbara  
**Ectomycorrhizal neighbors: Fungal community assembly in a mixed oak/fir system**

### 30 minute break for coffee and snacks

#### Session V: *Population biology of symbiotic partnerships*

- 10:45 Jason Chen, Emory University  
**Stochastic transmission and symbiont population structure in an insect-microbial symbiosis**
- 11:00 Caitlin MacGregor, CSU Northridge  
**Climate adaptation in the obligate moth pollinators of Joshua tree**
- 11:15 Jeremy B. Yoder, CSU Northridge  
**Species-wide genomic resequencing in a model of mutualism in extreme environments**

#### Session VI: *Anthropogenic changes and symbiosis*

- 11:30 Veronica Iriart, University of Pittsburgh  
**Plant-rhizobia symbiosis is negatively impacted by pollution from herbicide drift**
- 11:45 Max Porter, UC Riverside  
**Nodulation does not equal causation: context-dependent cowpea growth benefits from seed-coat rhizobial inoculation**
- 12:00 Maggie Sogin, UC Merced  
**Insights into the chemical milieu of the seagrass rhizosphere**

### Lunch 12:30-1:30

### End of conference

### Talk Abstracts

1. Michele Nishiguchi, UC Merced, [nish@ucmerced.edu](mailto:nish@ucmerced.edu)

#### Keynote lecture

#### **Interpreting the road map between ecological and molecular boundaries using a squid-bacterial mutualism**

The mutualistic association between sepiolid squids (Mollusca: Cephalopoda) and their *Vibrio* symbionts is an experimentally tractable model to study the evolution of animal-bacterial associations through both wild-caught and experimentally evolved populations. Since *Vibrio* bacteria are environmentally transmitted to new hosts with every generation, it provides a unique opportunity to resolve how changing environmental conditions may effect bacterial infection, colonization, and persistence in different host species. *Vibrio* bacteria encounter potentially conflicting selective pressures, competing with one another to colonize the sepiolid light organ, but also vying for resources in the environment outside the squid. Both abiotic and biotic factors contribute to the fitness of individual strains of *Vibrio* bacteria, but which of these factors are amenable to adaptation and eventually lead to a successful beneficial association has yet to be elucidated. This seminar will cover how environmental conditions and host specificity lead to the development of symbiotically adapted *Vibrio* bacteria, generating new ideas on the evolution of beneficial associations.

**Bio:** Dr. Michele “Nish” Nishiguchi is presently a Professor in the Department of Molecular and Cell Biology at UC Merced. Most recently she was a Regents professor, the Sundt Honors College Endowed Chair, and the Academic Department Head (2016-20), and faculty in the Department of Biology at New Mexico State University from 1999-2020. She obtained her B.S. in Biochemistry/Theatre Arts at the University of California, Davis, M.S. in Marine Biology at Scripps Institution of Oceanography, University of California, San Diego, and Ph.D. in Biology at the University of California, Santa Cruz. She has focused her work on the evolutionary ecology between marine organisms and their symbiotic bacteria. Despite being a marine biologist in the middle of the Central Valley, Nish manages to chase after squid and their bioluminescent bacteria in places such as the Indo-West Pacific, Australia, and the Mediterranean Sea, and manages to eat some calamari along the way. Her work spans the bridge between microbial ecology and disease evolution, and is best known for deciphering the mechanisms for environmental transmission of beneficial bacteria that occur in animal hosts. She has been passionate about reaching out to students in the US Southwest and now Central California, and introducing them to marine biology and microbial ecology. She has trained a number of students from underrepresented groups (4 postdocs-2 URG/4 women, graduate students-28-13 URG/14 women and undergraduate students-103-64 URG/58 women), and continues her commitment to increasing diversity through research, teaching, service, and outreach. Her hobbies include marathons/ultras, ironman triathlons, ballroom dancing, cooking/eating, and trail running with her rescue dog, Saupa.

2. Gordon M. Bennett, UC Merced, [gbennett2@ucmerced.edu](mailto:gbennett2@ucmerced.edu)

#### **Evolving beneficial symbioses between leafhoppers and bacteria**

Many insects depend on bacterial symbionts for essential nutrition. However, bacteria pose challenges for their hosts as they lose genes involved in most essential cellular functions. The evolutionary strategies hosts use to maintain these symbionts, and then replace them when benefits end, remains poorly understood. Leafhopper insects offer a key model to address these questions. They generally rely on two intracellular symbionts for complete nutrition. These symbionts are among the most ancient and genomically degraded known, leading to their occasional replacements. To sustain and support symbionts, leafhopper hosts evolve novel organs and functional traits that involve thousands of genes. These genes are derived from horizontal gene transfers to the host genome, reassignment of mitochondrial support genes, and gene-family duplications. These host-encoded genes enter the bacterial cell matrix to support fundamental cellular processes.

3. Daravuth Cheam, UC Merced, [dcheam@ucmerced.edu](mailto:dcheam@ucmerced.edu)

#### ***Vibrio fischeri* biofilms respond differentially to protozoan predation**

*Vibrio fischeri* are bioluminescent bacteria that can exist as an aggregate known as a biofilm. They form symbiotic relationships with sepiolid squids, which utilize their bioluminescence to avoid predation through counterillumination.

Although *V. fischeri* form biofilms inside the host squids to persist in the light organ, additional biotic factors such as protozoan grazing outside the host can shape the structure and function of biofilms. In order to determine whether biotic factors “outside the squid” effect the ability of *V. fischeri* to successfully colonize and persist in sepiolid squids, we used an experimental evolution approach to evaluate which factors are important for *V. fischeri* biofilm fitness. Assays were performed using various strains of *V. fischeri* and two different protozoans, *Acanthamoeba castellanii* and *Tetrahymena pyriformis*. Biofilms were exposed to both grazers over generations to determine whether *V. fischeri* biofilms were able to adapt to grazing pressure. Hawaiian *V. fischeri* strain ES114 had reduced biofilms when exposed to *A. castellanii* over generations. Conversely, Australian *V. fischeri* strain ETBB-1C increased biofilm growth in response to *A. castellanii* over generations. Interestingly, both strains increased the amount of biofilm when grazed by *T. pyriformis*. Our results demonstrate that response to protozoan grazing pressure may be strain-specific. Given that biofilms are necessary for a successful symbiotic colonization, predatory-prey interactions between protozoans and *V. fischeri* outside the host light organ may provide novel characteristics that benefit symbiosis and optimize *V. fischeri* association with sepiolid squids. Such interactions are crucial in understanding how environmentally transmitted symbioses have evolved between animals and their bacterial counterparts.

4. Jason Chen, Emory University, [jche226@emory.edu](mailto:jche226@emory.edu)

**Stochastic transmission and symbiont population structure in an insect-microbial symbiosis**

Many multicellular hosts rely on specific microbial symbionts for essential functions such as nutritional supplementation and defense against pathogens and parasites. Because of these benefits, some hosts and symbionts have evolved mechanisms that facilitate symbiont colonization every generation. Furthermore, symbionts are also expected to compete for opportunities to colonize the limited number of hosts in their environment. However, transmission is also inherently a stochastic process, due in part to transmission bottlenecks in which symbionts undergo severe reductions in population size as they infect new hosts. These bottlenecks can generate patterns in microbial population structure at multiple scales that can affect the evolution of symbiosis and maintain genetic diversity in symbiont populations.

The squash bug, *Anasa tristis*, is a major pest of cucurbit crops in North America. *A. tristis* acquires  $\beta$ -proteobacterial symbionts of the genus *Caballeronia* from the environment with high specificity. Previous work has demonstrated that symbiotic infections associated with wild squash bugs tend to be dominated by one or a few *Caballeronia* taxa, but individual insects vary in the identity of their dominant strain. Here, using symbiont strains isolated from wild insects, we artificially modulate bottleneck size during colonization to demonstrate the significance of stochasticity in generating this pattern. Although equally host-beneficial symbionts do compete during colonization, stochasticity could also explain low within-host diversity in symbiont populations while maintaining diversity between hosts. Furthermore, symbiont populations also exhibit high spatial structure within the symbiotic organ, and unexpected gradients in spatial structure along its length suggest patterns of symbiont migration in nascent infections. Our findings illuminate the processes that might affect symbiont transmission and population structure in nature, which can drive the evolution of microbial cooperation and host-microbe symbiosis.

5. Brendan Cornwell, Hopkins Marine Station of Stanford University, [bcornwel@stanford.edu](mailto:bcornwel@stanford.edu)

**Using population genomic patterns in *Acropora* sp. and their symbionts to identify and characterize optimal partnerships**

Partner quality in coral-dinoflagellate endosymbioses impacts the performance of the holobiont, shaping ecologically important phenotypes such as local adaptation and stress-induced bleaching. Here we investigate population genetic patterns in *Cladocopium* spp. across many *Acropora* species inhabiting the same reef environment and then examine how these patterns correspond to bleaching within a single species, the tabletop coral *Acropora hyacinthus*. We sequenced the genomes of 28 *Acropora* colonies from five morphological groups, yielding approximately 740,000 and 50,000 SNPs from hosts and symbionts, respectively. Symbionts are differentiated into genetic groups but not by host species. By contrast, we found significant associations between ca. 300 pairs of host and symbiont SNPs, showing that certain alleles in host loci are associated with certain alleles in the symbionts. These associations suggest coevolution or interactions between coral and symbiont variants. To get a more fine-grained view of these possible co-evolutionary patterns, we analyzed host and symbiont alleles from ~200 *Acropora hyacinthus* colonies across Palau. We found that 31 pairs loci are strongly associated between host and symbiont genomes. Furthermore, to assess possible functional importance of these allele matches, we found that individuals which show more coral-symbiont matching at these loci

show decreased bleaching in experimental assays for 29 of 31 loci. These data suggest a constellation of genetic interactions between corals and symbionts tunes the nature of their interactions. Such genetic correlations within and between species have implications for the establishment of symbiosis, the breakdown of the partnership when temperatures rise, and the consequences of symbionts interacting with multiple host species across generations.

6. David Fronk, UC Riverside, [dfron001@ucr.edu](mailto:dfron001@ucr.edu)

**Symbiotic Organs: common motifs and evolutionary pressures**

Mutualistic relationships between microbial symbionts and multicellular hosts are dispersed throughout the tree of life, with symbionts providing varied benefits, including nitrogen fixation, nutrition, bioluminescence, and chemical defense against pathogens. Across these symbioses, hosts have developed specialized structures, symbiotic organs, which serve as the nexus of interaction between partners. While these symbiotic organs differ between species, they often share common structural and functional motifs. These motifs can lead to similar evolutionary pressures on different hosts and symbionts, as each partner navigates their formation and maintenance. Working to examine phylogenetically and functionally diverse symbiotic organs, we have created a framework for examining these systems that we hope will be useful to the wider scientific community.

7. Alison Gould, California Academy of Sciences, [agould@calacademy.org](mailto:agould@calacademy.org)

**Symbiosis-induced changes in gene expression of a bioluminescent vertebrate-bacteria association**

Describing the molecular conversation between hosts and their microbial symbionts can provide valuable insight into the mechanisms governing these essential partnerships. With the advancement of sequencing technologies and emergence of new non-traditional model systems, our understanding of microbial symbioses across the tree of life is rapidly expanding. The experimentally tractable, binary symbiosis involving the coral reef fish *Siphamia tubifer* and the luminous bacterium *Photobacterium mandapamensis* provides the opportunity to characterize changes in gene expression in both the host and symbiont induced by the symbiosis. Applying dualRNA-seq methods, gene expression was compared between symbiotic and aposymbiotic hosts as well as between the luminous bacteria in its symbiotic and planktonic states. Host genes involved in cell signaling were significantly upregulated in the symbiosis, whereas several immune function genes, including adaptive (T-cell) immune responses, were significantly downregulated. An outer membrane protein (*ompA*) was significantly upregulated by the symbiont inside the host, as were genes involved in light production (the *lux* operon). Additionally, symbiont genes involved in various metabolic pathways were either up- or down-regulated in symbiosis, providing novel insight into the metabolic shift in the bacterium between its planktonic and symbiotic states. Several motility and chemotaxis genes were also upregulated by the bacterium while in the plankton. This study provides a window into the biochemical conversation that occurs between *S. tubifer* and *P. mandapamensis*, revealing candidate genes involved in the symbiosis that will be the targets of future studies, and highlights the value of this association as a vertebrate-bacteria model for symbiosis research.

8. Tobin Hammer, UC Irvine, [hammert@uci.edu](mailto:hammert@uci.edu)

**Mystery of the missing microbes: why do bees keep losing their symbionts?**

Host-microbe symbioses are often mutually beneficial, and sometimes ancient. And yet, the more we survey microbiomes, the more common it appears for them to fall apart. Social bees provide an opportunity to address the extent, causes, and consequences of symbiont loss. The common ancestor of these bees acquired a few species of gut bacteria that subsequently co-diversified. In honeybees, the most well-studied group, these symbionts are highly stable across individuals and over time. However, our work on other bee taxa indicates that stability may be the exception rather than the rule. In the stingless bee *Melipona*, we recently discovered a genus-wide extinction of two ancient bee symbionts. These bacteria are thought to provide crucial benefits to honeybees; their absence in stingless bees raises questions about the ubiquity of these benefits. Bumblebees exhibit another form of symbiont loss: across four continents, wild populations contain numerous visibly healthy bees whose core symbionts have been lost and replaced with environmental microbes. We hypothesized parallels with coral bleaching, but using *in vitro* and *in vivo* assays, we found that the core symbionts are quite robust to heat stress. We then tested aging as an intrinsic driver of symbiont loss. In lab-reared bumblebees, both microbiomes and gut immunity were highly dynamic in early “adulthood”, but then stabilized, and did not senesce in old age. I will attempt to reconcile these results in the context of bee life history and symbiont evolution, and will outline our next efforts to understand why bee microbes go missing.



9. Veronica Iriart, University of Pittsburgh, [vei2@pitt.edu](mailto:vei2@pitt.edu)

**Plant-rhizobia symbiosis is negatively impacted by pollution from herbicide drift**

The symbiosis between plants and rhizobia bacteria that occurs within root nodules provides critical ecosystem services via plant growth promotion and nutrient cycling. However, anthropogenic herbicide pollution can impose stress on wild plants, their rhizobial partners, and potentially the quantity and quality of their interactions; but these impacts are largely undescribed. Accordingly, we conducted a greenhouse study where we grew *Trifolium pratense* plants from 17 full-sibling families either alone (uninoculated) or in symbiosis with one of two rhizobial strains, and exposed plants to 1) the auxinic herbicide dicamba at a rate relevant to 'drift' pollution (off-target movement via wind) or 2) a control solution. We evaluated how herbicide exposure affected the plant-rhizobia symbiosis and whether this varied with plant or rhizobial genotype. We found that, while rhizobia significantly enhanced plant growth in the control environment, they did not mitigate the negative growth effects of herbicide drift: drift-exposed inoculated plants grew to the same size as drift-exposed, uninoculated plants. Furthermore, although plant and rhizobial genetic variation influenced results slightly, herbicide drift exposure had overwhelmingly negative impacts on key components of the plant-rhizobia symbiosis, such as the number of nodules per plant and the amount of symbiotically fixed nitrogen. Altogether, these findings suggest that herbicide pollution may be a major selective force impacting the plant-rhizobia symbiosis in agricultural-ecological environments, with consequences for the beneficial services these interactions provide for the broader ecosystem.

10. Jakob Kaare-Rasmussen, UC Santa Barbara, [jkaare-rasmussen@ucsb.edu](mailto:jkaare-rasmussen@ucsb.edu)

**Modeling Food Dependent Symbiosis in *Exaiptasia pallida***

The delicate mutualism between Cnidarians and their symbionts is rooted in nutrient sharing. Cnidarian hosts provide their symbionts with a variety of nutrients including nitrogen while symbionts can photosynthesize, providing the host with carbohydrates. Symbionts can either be costly or beneficial to the host, depending on the food available to the host. Here, we use a Dynamic Energy Budget (DEB) model to quantify the impact of food availability on *Exaiptasia pallida*, a common model organism for Cnidarian symbiosis. Previously published work by Peng et al. 2020 on this anemone showed a surprising amplification effect of symbionts on anemone performance. While well-fed anemones grow faster with symbionts than without them, starved anemones suffer from malnourishment and die much faster when they are inoculated with symbionts. We sought to model this food dependence of symbiont outcomes by approximating the anemone's sharing of nutrients as the symbiont actively consumes ("eating") the host. This model accurately captures the intensifying effect symbionts have on anemones and quantifies the food availability that anemones need to maintain mutualism with their symbionts. Similarly, it furthers our understanding of how other factors in the model, like the photosynthesis rate of the symbionts, impact this delicate balance. Lastly, since *E. pallida* are model organisms for other Cnidarians, this model helps us better understand the cost-benefit balance in coral mutualism.

11. Caitlin MacGregor, CSU Northridge, [caitlin.macgregor.404@my.csun.edu](mailto:caitlin.macgregor.404@my.csun.edu)

**Climate adaptation in the obligate moth pollinators of Joshua tree**

Rapid environmental changes are beginning to outpace the ability of species to adapt, and there is evidence that these changes can disrupt critical symbioses such as plant-pollinator mutualisms. One such specialized interaction expected to experience severe impacts of climate change is the obligate mutualism between yucca moths (*Tegeticula synthetica* and *Tegeticula antithetica*) and the two species of Joshua trees (*Yucca brevifolia* and *Yucca jaegeriana*) they exclusively pollinate. Studies have examined how Joshua trees might adapt to predicted changes in their Mojave Desert habitat, but little has been done to study climate adaptation in their pollinator moths. I used a landscape genomics approach to identify locally adapted yucca moth loci and quantify the degree to which moth populations experience ecological isolation due to climate. I assembled a RADSeq SNP dataset from pollinator populations found across the Mojave and population-specific environmental data to ask whether any SNPs exhibit significant correlations to environmental variables. I identify and characterize a number of candidate SNPs indicative of local adaptation in each species, and I find SNPs shared between pollinators that exhibit strong correlations to the same environmental factor. BEDASSLE analysis of the relative contributions of environmental and geographic distance reveals that in both pollinator species, environmental distance between populations has a larger effect on observed genetic differentiation. These results suggest that yucca moth populations are adapted to their local climate conditions to some extent, and this may

ultimately impact both their mutualistic relationship with Joshua trees and their ability to adapt to ongoing climate change.

12. Kyle Meyer UC, Berkeley, [kmmeyer@berkeley.edu](mailto:kmmeyer@berkeley.edu)

**The impact of transmission within versus among host plant species on microbiome diversity and adaptation**

Plants often occur in complex multispecies assemblages and hence are subject to the effects of microbial transmission between conspecific (same species) and heterospecific (different species) hosts. Here we disentangle the effects microbiome transmission using experimental passaging of plant microbiomes over 6 generations using 4 species of plants (tomato, ancestral tomato, pepper, and bean). 42 microbiome lines were derived from a common inoculum and passaged among conspecific hosts or alternating repeatedly between heterospecific hosts. The effects of host microbiome filtering were persistently strong over the six passages for the conspecific lines. In contrast, filtering effects decreased for the heterospecific transmission lines, suggesting a cost to host specialization. Using community coalescence we further demonstrate that conspecifically transmitted tomato microbiomes outcompete conspecifically transmitted bean and pepper microbiomes on tomato hosts, suggesting host specialization at the microbiome level. Overall our work shows that transmission mode and host-association history can drive microbiomes down divergent paths.

13. Jorge Armando Montiel Molina, UC Merced, [jmontielmolina@ucmerced.edu](mailto:jmontielmolina@ucmerced.edu)

**Amphibious plants present a gigantic shift in root microbiome community across life cycles.**

The relationship between plants and microbial communities living inside plants—endophytes, is complex, especially for those plants inhabiting ecosystems that are subject to strong environmental shifts. Amphibious plants in ephemeral wetlands—vernal pools, are defined by their ability to tolerate a wide range of hydrologic conditions, from completely saturated soils to progressively drier conditions. These plants serve as shelter for microbial communities that in turn may influence the host's environmental tolerance. This research aims to investigate for the first time the diversity and community composition of microbial endophytes associated with the California amphibious native plant *Eryngium castrense* inhabiting vernal pools. Whether microbial communities remain the same across plant life cycles, aquatic and terrestrial, and the environmental constraints when soils go from saturated to dry is unknown. On the other hand, is also of interest to investigate if the microbiome of plants in more pristine vernal pools differs from those inhabiting pools under anthropogenic pressures. Roots and shoots were collected according to their aquatic and terrestrial morphological stage, across a transect of five kilometers spanning from urban to more pristine habitat, at the University of California Vernal Pools and Grassland Reserve, located in the Central Valley of California. High-throughput sequencing of the 16S rRNA region was employed to analyze the microbiome of the plant specimens. To our knowledge, this study is the first of its kind in exploring amphibious plants inhabiting ephemeral wetlands known as vernal pools. Preliminary results showed a gigantic shift in root microbial communities in comparison with the microbes in the shoots, suggesting that plants might choose the associated microbiome, according to the environmental conditions of the soil, water saturation, or desiccation.

14. Gabriel Santiago Ortiz, UC Riverside, [gorti010@ucr.edu](mailto:gorti010@ucr.edu)

**Lotus japonicus regulates root nodulation and nitrogen fixation dependent on the molecular form of nitrogen**

Legumes form root nodules to gain fixed nitrogen from rhizobia and also access nitrogen in soil, and appear to adaptively discriminate among these sources. We investigated how root nodulation and nitrogen fixation varies depending on the molecular form and concentration of extrinsic nitrogen. *Lotus japonicus* host plants were inoculated with their compatible nitrogen-fixing symbiont, *Mesorhizobium loti*, and fertilized with organic nitrogen molecules, predicted to be products of symbiotic nitrogen fixation excreted to host cells, as well as inorganic molecules that can be accessed in the soil. By varying sources and concentrations of nitrogen for the host, these experiments investigate how host benefit from and regulate symbiosis depending on the sources of nitrogen available. Growth benefits of fertilization varied with nitrogen source, suggesting differences in catabolism efficiency. Net benefits of nodulation were reduced or eliminated under extrinsic fertilization. Even when costly, however, hosts did not reduce investment into nodulation or nitrogen fixation when exposed to organic sole amino acid fertilizers, unlike with inorganic fertilization. Root nodulation by rhizobia involves costs that are often outweighed by the benefits of nitrogen fixation. However, when extrinsic nitrogen is available, hosts can downregulate investment into symbiosis to save energy. Our



data here suggest that legumes can discriminate among forms of nitrogen that are received from symbionts from those that are acquired in the soil.

15. Max Porter, UC Riverside, [aport008@ucr.edu](mailto:aport008@ucr.edu)

**Nodulation does not equal causation: context-dependent cowpea growth benefits from seed-coat rhizobial inoculation**

Cowpea (*Vigna unguiculata*) is a legume crop host that associates with rhizobia, proteobacteria which can trigger the formation of symbiotic nodules on cowpea roots and fix nitrogen for the host plants in exchange for photosynthates. Harnessing the symbiotic relationship between cowpeas and rhizobia could reduce the usage of nitrogen fertilizers, which are environmentally and economically costly. Fields without established communities of beneficial rhizobia (usually those without a history of legume growth) are often amended with rhizobial inoculants with the goal of inducing nodulation, providing fixed nitrogen for the host, and improving crop yields. These touted benefits are tenuous, however, as inoculated strains can be out-competed by local bacterial populations or fail to survive diverse field conditions. One strategy to improve the availability of rhizobial inoculants to plant roots is to coat seeds with inoculant prior to planting. To test the varied efficacy of seed-coat inoculation, we planted cowpeas in two adjacent fields: one with a long history of cowpea growth (and an established beneficial rhizobial community), and one with no prior history of legume cultivation. Seeds were treated with one of three seed-coat inoculants: (1) soil from the respective field, (2) a synthetic community of cowpea-associating rhizobial strains, and (3) a commercially-available rhizobial inoculant with widespread usage across California. We found significant differences in root nodulation among inoculation treatments in the field without a prior history of legume growth, with the synthetic rhizobial community promoting the highest number of nodules. However, in both fields, increased nodulation was negatively correlated with cowpea growth benefits. These findings are of critical interest for cowpea growers, who might observe increases in nodulation from rhizobial inoculants and infer that they are “working” to improve yields, when this may not be the case. Additionally, we found that the commercially-available inoculant did not induce nodulation nor improve yields in either field, despite stimulating nodulation in a greenhouse setting, suggesting its efficacy may be greatly reduced in field settings.

16. Arafat Rahman, UC Riverside, [arahm010@ucr.edu](mailto:arahm010@ucr.edu)

**Interstrain competition among Bradyrhizobium reduces plant performance in *Acmispon strigosus***

The major challenge to the successful application of rhizobia is that nitrogen fixing strains are often outcompeted by ineffective strains that fix little nitrogen. Here, we investigated fitness effects of inter-strain competition, using eight wild Bradyrhizobium isolates which had been previously characterized for root nodulation and nitrogen fixation traits on their native host, *Acmispon strigosus*. These strains ranged from highly beneficial to ineffective for nitrogen fixation on the target host. Both clonal infections and coinfections were conducted, using 28 pairwise factorial combinations. Nodulation patterns and host growth benefit were quantified, and 1125 nodules were genotyped for bacterial occupancy using a dual-indexed amplicon sequencing of the *nifD* gene on the MiSeq platform. Growth patterns were compared between single and co-inoculation treatments using a null model which predicted effects of combining strains. Genotyping showed that beneficial strains on average had higher occupancy when co-inoculated with ineffective strains, however one strain that provided very low benefits was highly competitive. Co-inoculation treatments with at least one beneficial strain provided significant benefit to the host. The benefit received in co-inoculation treatments were significantly less in comparison to the benefit from strains in single-inoculation. The difference in expected and observed relative growth and observed nodule area was tightly correlated with the difference in expected and observed nodule numbers. These data indicate that pre-nodulation inter-strain competition leads to sub-optimal benefit received from the rhizobial community. This study provides an understanding of rhizobial dominance hierarchy and shows how this competitive dominance can affect host benefit under competition.

17. Gabe Runte, UC Santa Barbara, [gabe.runte@lifesci.ucsb.edu](mailto:gabe.runte@lifesci.ucsb.edu)

**Ectomycorrhizal neighbors: Fungal community assembly in a mixed oak/fir system**

In this greenhouse study, we work to understand how soil conditioning and neighboring plant species interact to influence the ectomycorrhizal portfolios of sympatric oak (*Quercus chrysolepis*) and fir (*Pseudotsuga macrocarpa*) seedlings. Seedlings of both species were grown up in each of three soil conditions: a sterile potting mix, potting mix

with 20% field soil from under an oak, or potting mix with 20% field soil from under a fir (6 total species x soil cohorts). After a year, seedlings were uprooted, washed clean of soil, observed for fungal colonization and community composition, and repotted with a “neighbor” into a larger pot. Neighbors were co-potted from nearly all (except for a few ecologically irrelevant) combinations of cohorts. This step was designed to bring only fungi that were in symbiosis with either seedling into the shared pot, excluding any additional spores from the prior pots.

Over the next year, seedlings were tracked for differences in growth related to soil and neighbor conditioning. After spring leaf-out, seedlings have proceeded to the final phase of this experiment, wherein half of the pots have been subjected to a simulated drought. This drought will maintain a consistent soil moisture, using frequent, brief drip irrigation for half the duration of the control pots’ watering. In the fall, we will harvest these seedlings to assess differences in the size, health, and mycorrhizal communities of the seedlings. We will analyze our community data to assess the role of fungal communities in seedling growth and drought adaptation.

18. Joseph Russo, UC Riverside, [jrusso012@ucr.edu](mailto:jrusso012@ucr.edu)

**Cnidarian–Symbiodiniaceae symbiosis establishment is independent of photosynthesis**

Photosynthesis shapes the symbiotic relationships between cnidarians and Symbiodiniaceae algae—with many cnidarian hosts requiring symbiont photosynthate for survival—but little is known about how photosynthesis impacts symbiosis establishment. Here we show that during symbiosis establishment infection, proliferation, and maintenance can proceed without photosynthesis, but the ability to do so is dependent on specific cnidarian–Symbiodiniaceae relationships. Evaluation of 31 pairs of symbiotic relationships (five species of Symbiodiniaceae in sea anemone, coral, and jellyfish hosts), revealed that infection can occur without photosynthesis. A UV mutagenesis method for Symbiodiniaceae was established and used to generate six photosynthesis mutants which can infect these hosts. Without photosynthesis, Symbiodiniaceae cannot proliferate in the sea anemone *Aiptasia* or jellyfish *Cassiopea*, but can in juvenile polyps of the coral *Acropora*. After six-months of darkness, *Breviolum minutum* is maintained within *Aiptasia* indicating Symbiodiniaceae maintenance can be independent of photosynthesis. Manipulating photosynthesis provides insights into cnidarian–Symbiodiniaceae symbiosis.

19. Joel L. Sachs, UC Riverside, [joels@ucr.edu](mailto:joels@ucr.edu)

**Pangenome evolution reconciles robustness and instability of rhizobial symbiosis**

Joel Sachs, Alexandra Weisberg, Arafat Rahman, Jeff H. Chang

Variation in the services of microbial mutualists is considered paradoxical given host mechanisms to select beneficial genotypes. Rhizobia strains vary markedly in the magnitude of fixed nitrogen provided to hosts, despite host legumes having mechanisms to selectively reward beneficial strains and to punish ones that do not fix sufficient nitrogen. Moreover, the recurrent evolution of non-fixing symbiont genotypes is predicted to destabilize symbiosis, but breakdown has rarely been observed. Here, we deconstructed hundreds of genome sequences from genotypically and phenotypically diverse *Bradyrhizobium* strains and revealed mechanisms that generate variation in symbiotic nitrogen fixation. We show that this trait is conferred by a modular system consisting of many extremely large integrative conjugative elements and few conjugative plasmids. Their transmissibility and propensity to reshuffle genes generate new combinations that lead to uncooperative genotypes and make individual partnerships unstable. We also demonstrate that these same properties extend beneficial associations to diverse host species and transfer symbiotic capacity among diverse strains. Hence, symbiotic nitrogen fixation is underpinned by modularity, which engenders flexibility, a feature that reconciles evolutionary robustness and instability.

20. Maggie Sogin, UC Merced, [esogin@ucmerced.edu](mailto:esogin@ucmerced.edu)

**Insights into the chemical milieu of the seagrass rhizosphere**

Seagrasses are among the most efficient sinks of carbon dioxide on Earth. They can bury organic carbon up to 35 times faster per unit area than tropical rainforests and sink up to 10% of the total organic carbon in the ocean. While carbon sequestration in terrestrial plants is linked to the microorganisms living in their soils, the interactions that enable carbon burial in seagrasses and within their rhizospheres are poorly understood. Here, I will present data that shows seagrasses release organic carbon into their rhizosphere sediments primarily in the form of sucrose. Sucrose persists in this anoxic environment because the sediments also contain phenolic compounds that limit microbial metabolism of simple sugars. Using this system as a model for exploring the chemical interactions between a marine plant and its

microbial associated, I will outline experimental approaches for furthering our understanding of the role of this complex symbiotic system in carbon sequestration.

21. Yumary Vasquez, UC Merced, [vasquez8@ucmerced.edu](mailto:vasquez8@ucmerced.edu)  
**Leveraging a Hawaiian leafhopper adaptive radiation to understand bacterial symbiont genome evolution**  
 Many insects depend on ancient associations with intracellular bacteria for essential nutrition. The genomes of these bacteria are often highly reduced. Although drift is a major driver of symbiont evolution, other evolutionary forces continue to influence them. To understand how ongoing molecular evolution and gene loss shape symbiont genomes, we sequenced two of the most ancient symbionts known, *Sulcia* and *Nasuia*, from 20 Hawaiian *Nesophrosyne* leafhoppers. We leveraged the parallel divergence of *Nesophrosyne* lineages throughout Hawaii as a natural experimental framework. To investigate the fine-scale evolutionary processes acting upon symbiont genes, we used genomic analyses including ancestral state reconstruction of lost symbiont genes, estimation of evolutionary rates in absolute time of divergence and tests of positive selection within functional categories. *Sulcia* and *Nasuia* experience ongoing, but divergent, gene loss often in a predictable fashion. Many genes are under relaxed selection. However, purifying and positive selection are also important drivers of genome evolution, particularly in maintaining certain functions. Finally, our results demonstrate that symbionts experience complex and dramatically different evolutionary environments—as evidenced by the finding that *Sulcia* and *Nasuia* have one of the slowest and fastest rates of molecular evolution yet identified.
  
22. Tingting Xiang, University of North Carolina at Charlotte, [txiang@uncc.edu](mailto:txiang@uncc.edu)  
**Euplotes-Symbiodinium, a unicellular model system for coral-algal symbiosis**  
 The symbiosis between dinoflagellate algae in the family Symbiodiniaceae and their hosts are cornerstones of marine ecosystems. This symbiosis is based on metabolic exchange between the cnidarian host and symbiotic algae, but has been increasingly threatened by environmental perturbations and stress. The break-down of this relationship, or ‘coral bleaching’ can ultimately lead to coral death and destruction of the reef ecosystem. Despite the critical nature of Symbiodiniaceae symbiosis, the underlying genes and molecular mechanisms that govern this symbiotic interaction remain largely unknown. Genomics and transcriptomics have provided some correlative insights into gene function but causal mechanisms remain elusive. The main challenge is that current model systems to study Symbiodiniaceae symbiosis are not ideal for functional genomic evaluation. Current models are complex multicellular hosts that are for the most part genetically intractable. Because these hosts also can be large, slow to grow, and multicellular organisms, high-throughput molecular and cellular experimental approaches to study this symbiosis are limited. To overcome these limitations, we have established the single-celled model ciliate *Euplotes*, which hosts Symbiodiniaceae endosymbionts, as a novel model to study genes involved in endosymbiosis. *Euplotes* is a microorganism, grows rapidly in multi-well plates, and is amenable to RNA interference (RNAi), making it a powerful system for functional genomic studies like genetic screens and high-throughput protein localizations.
  
23. Jeremy B. Yoder, California State University Northridge, [jeremy.yoder@csun.edu](mailto:jeremy.yoder@csun.edu)  
**Species-wide genomic resequencing in a model of mutualism in extreme environments**  
 Joshua trees' divergence into two sister taxa (*Yucca brevifolia* and *Y. jaegeriana*) has been understood to be driven by their specialized, mutually dependent interaction with sister species of pollinating yucca moths (*Tegeticula synthetica* and *T. antithetica*). However, this model mutualism plays out across the harsh conditions of the Mojave Desert, and the trees' adaptation to climate variation across their range has been much less studied than their coevolution with yucca moths. I analyze whole-genome resequencing data collected for hundreds of geographically dispersed Joshua trees to test for evidence of local adaptation to climate, and to compare population divergence driven by climate variation to differentiation attributable to pollinators.

## POSTERS

1. Bolivar Aponte, Tulane University, Bolivar Aponte, [baponterolon@tulane.edu](mailto:baponterolon@tulane.edu)  
**The role of host genotype and leaf trait plasticity in foliar endophytic fungal colonization of yellow monkeyflowers**  
 Phenotypic plasticity and genetic adaptation are key drivers of evolution, and both processes may aid in plant adaptation to a warming climate. Plants have evolved alongside fungi and bacteria; however, little is known about

how they influence or are influenced by their host's adaptation or plasticity. Foliar endophytic fungi (FEF) are a significant component of the plant microbiome as studies have suggested that they can alter plant traits under stressful conditions. The interaction between leaf traits (phenotype) and host genetics (genotype) may shape the foliar microbiomes of plants. In yellow monkeyflowers, the evolution of leaf shape for 3 edaphic species is controlled by overlapping genetic regions, making it an ideal model system to explore the interactions between leaf traits and FEF. We hypothesize that host phenotypic plasticity drives FEF colonization in monkeyflowers, regardless of genotype. Wherein, FEF communities in plants with similar phenotype (i.e., leaf traits) will be more similar than those compared across *Mimulus guttatus* and *Mimulus laciniatus* species. To test this hypothesis, we designed a reciprocal transplant experiment along Tioga Road at Yosemite National Park. In the growing season of 2021, we planted parental inbred lines of *M. guttatus* and *M. laciniatus* and their F2 hybrid crosses at two meadows where *M. guttatus* is native and two granite outcrops where *M. laciniatus* is native. We present our preliminary findings and discuss expected results.

2. Jacob Francis, UC Davis, [jsfranci@ucdavis.edu](mailto:jsfranci@ucdavis.edu)

**Intraspecific variation in nectar microbiome driven by plant traits and dispersal limitation**

Flowers host communities of bacteria and fungi that can influence pollinators and plant reproduction. While intraspecific variation in floral traits has well documented effects on plants' interactions with macroorganisms, much less is known about how individual and population level variation in floral traits impacts interactions with floral microbes. We used a combination of surveys and nectar microbial inoculations of *Epilobium canum* (Onagraceae) to investigate sub-species level variation in nectar microbiome. In a common garden of four distinct genotypes of *E. canum*, we used a culture-based approach to characterize the density of yeasts and bacteria in 320 open-visited flowers across 8 plants of each genotype. Genotypes, and individual plants within a genotype, differed in culturable bacterial and fungal densities. To investigate whether these differences were driven by animal-mediated dispersal limitation of microbes or differences in plant traits, we limited animal access to 172 flowers across 6-7 individual plants per genotype. We inoculated these flowers with the nectar yeast, *Metschnikowia koreensis*, and bacteria, *Acinetobacter pollinis*, isolated from flowers at the site. There was no difference in *A. pollinis* growth among plants or cultivars. Alternatively, there were strong significant differences in *M. koreensis* densities among plants, but not among cultivars, driven by a small subset of plants that had particularly high or low yeast growth. However plants that hosted high microbial growth when openly visited were not those with the highest growth after inoculation. This suggests that both floral traits and dispersal limitation might play a role in shaping a plants' nectar microbiome.

3. Perla Gonzalez Moreno, UC Merced, [pgonzalezmoreno@ucmerced.edu](mailto:pgonzalezmoreno@ucmerced.edu)

**Influence of the type VI secretion system on host colonization in the Euprymna-Vibrio symbiosis** Bacterial symbionts can often engage in combative behaviors by deploying antibacterial killing mechanisms to warrant their dominance over the host niche. In the Euprymna-Vibrio symbiosis, *Vibrio fischeri* colonize individual crypts within the light organ of the bobtail squid, Euprymna (Cephalopoda: Sepiolidae). The *Vibrio* symbionts are environmentally transmitted, and although many *V. fischeri* strains are naturally present in seawater, only a few strains are successful in colonizing the light organ of host squid. Previous studies have shown that only certain strains of *V. fischeri* harbor a Type VI Secretion System (T6SS). The T6SS is a contact-dependent, molecular weapon that delivers toxic effector proteins to a targeted prey cell. Therefore, we wanted to examine whether T6SS-facilitated intraspecific competition may regulate the diversity and spatial distribution of *V. fischeri* strains found within the light organ of Euprymna. To determine if the T6SS plays an important role in symbiont specificity among host, ten different *V. fischeri* strains encoding a T6SS were analyzed to determine if they exhibited a killing phenotype against various competitor strains. Candidate T6SS encoding strains were coinoculated with T6SS-deficient *V. fischeri* strains isolated from within and among host populations. Five T6SS encoding strains exhibited a lethal killing phenotype against the competitor strains, which suggests that these strains may potentially obliterate T6SS-deficient competitor strains during the initial stages of host colonization. Thus, the T6SS may serve as a major driving force in regulating the diversity and spatial distribution of symbiont strains found within the light organ of various host populations.

4. Andres Hobbs, UC Merced, [ahobbs6@ucmerced.edu](mailto:ahobbs6@ucmerced.edu)

**Transferring the GFP Gene by Triparental Mating to Visualize Vibrio fischeri Biofilms**

Green Fluorescent Protein (GFP) is a hydrophobic protein that fluoresces under UV light and emits green when analysed under a microscope. GFP can be transferred from a donor *Escherichia coli* strain to another bacterium of

interest, which in this case is *Vibrio fischeri*. This is done through the process of conjugation with plasmids containing the *gfp* gene. In particular, a triparental mating was performed where three different bacteria were combined together. This included the donor strain *E. coli* pVSV102 which has a plasmid containing the *gfp* gene, the helper strain *E. coli* pMJM534 which contains *tra* genes for conjugation, and the recipient strain *V. fischeri* ES114. These three bacteria were grown and mixed together, and fluorescent *V. fischeri* colonies were isolated. Transparent colonies that were also fluorescing green were isolated after the triparental mating. This suggests that the procedure was successful as *V. fischeri* colonies are transparent, in contrast to the donor and helper *E. coli* strains which have opaque colonies. The green fluorescing *V. fischeri* colonies were inoculated in liquid media and were subsequently frozen at -80°C for long-term storage. This experiment shows that plasmids can be transferred into *V. fischeri* using triparental matings. These green fluorescent *V. fischeri* strains will be used in experiments involving visualising biofilms treated with protozoan predators. Predation may affect the topography of *V. fischeri* biofilms. And the green fluorescing biofilms can be imaged by confocal microscopy. Red and blue fluorescent genes will also be conjugated into other *V. fischeri* strains for comparative visualisation.

5. Sydney Metternich, Illinois State University, [skmette@ilstu.edu](mailto:skmette@ilstu.edu)

**Adaptation of *Lobelia spicata* to Soil Microbial Communities Across the North American Tallgrass Prairie**

Plant-soil feedbacks have been well described as interactions between plants and soil microbes; soil microbes associated with plant roots, acquire nutrients for plants that are otherwise unavailable. Plants quickly differentiate a soil microbial community by attracting additional conspecific microbes, and while these microbes are often mutualistic, species-specific microbial pathogens can accumulate in the soil as well, generating negative plant soil-feedbacks. Biotic factors, such as soil history, in combination with abiotic factors, such as rainfall, determine the diversity of the soil microbial community. The North American Tallgrass Prairie ecosystem has been subjected to severe habitat fragmentation. The rainfall gradient (increasing eastward) which crosses where native prairie plant populations are found, provides a unique opportunity to study the effects of varying rainfall on soil microbial communities and how these communities subsequently influence the fitness of geographically constrained plant populations. To test for plant-soil feedbacks we performed a greenhouse study that subjected *Lobelia spicata*, a perennial prairie forb, sampled from populations in Illinois and Kansas to local and novel conspecific soil microbes. Additionally, they were treated with heterospecific soil microbes differentiated by native Illinois and Kansas *Andropogon gerardii* and *Lespedeza capitata*, grown in both states. Rosette diameter and biomass are used as estimates of plant fitness. Local adaptation is expected when *L. spicata* fitness is correlated positively with the locality of soil microbes; this would provide support for positive plant-soil feedback. Negative plant-soil feedback is suspected if *L. spicata* incurs a fitness advantage when freed from conspecific pathogens.

6. Pedro Antonio Pérez Ferrer, UC Merced, [pperez40@ucmerced.edu](mailto:pperez40@ucmerced.edu)

**Genetic variation in the Atlantic bobtail squid-*Vibrio* symbiosis from the Galician Rías**

Symbiotic marine bacteria that are transmitted through the environment are susceptible to possible abiotic factors (salinity, temperature, physical barriers) that separates them from their hosts. Given that many symbioses are driven by host specificity, environmentally transmitted symbionts can alter symbiont specificity depending on conditions over space and time. In order to determine whether the population structure of environmentally transmitted beneficial associations reflect host specificity or biogeography, we analyzed the genetic structure of *Sepiola atlantica* (Cephalopoda: Sepiolidae) and their *Vibrio* symbionts (*V. fischeri* and *V. logei*) in four Galician Rías (Spain). This geographical location is characterized by a jagged coastline with a deep-sea entrance into the land, ideal for testing whether such population barriers exist due to genetic isolation. We used haplotype estimates combined with nested clade analysis to determine the genetic relatedness for both *S. atlantica* and *Vibrio* bacteria. Analyses of molecular variance (AMOVA) were used to estimate variation within and between populations for both host and symbiont genetic data. Preliminary analyses exhibit a low percentage of variation among host populations, suggesting that these populations are panmictic, whereas between host populations variation does exist due to geographical distance. In contrast, *Vibrio* symbionts are panmictic among and between populations, demonstrating that the hydrology of the Rías are driving bacterial distribution (and not host specificity). Thus, for environmentally transmitted symbioses such as the sepiolid squid-*Vibrio* association, abiotic factors can be major selective force for determining population structure for one of the partners.

7. Ferdinand Pfab, UC Santa Barbara, [ferdinand.pfab@gmail.com](mailto:ferdinand.pfab@gmail.com)

**Modeling heat stress on coral-algae symbiosis**

Corals live in a close relation with endosymbiotic algae. The corals supply the algae with nutrients (such as nitrogen), while the algae in turn share carbohydrates they acquire through photosynthesis. Worldwide corals are losing their symbionts, which makes them susceptible to starvation. This process is known as coral bleaching. The main trigger for coral bleaching is thought to be increased sea temperature. Different mechanisms for why increased temperature leads to loss of symbionts have been proposed. In the poster we present a bioenergetic model to shed light into one of the leading hypotheses on coral bleaching. According to this hypothesis, heat stress leads to a cascade of processes in the holobiont. The primary site of heat stress lies within the chloroplasts of the symbionts. Particularly, heat is thought to damage proteins involved in the light reaction of photosynthesis. This damage then renders the photosynthesis less efficient so that when exposed to light it produces less photosynthate and more reactive oxygen species (ROS). The ROS then further damages the algae and the corals, and triggers the expulsion of symbionts by the host as a defense mechanism. This loss of symbionts is observed as coral bleaching. Our model captures the process well and helps to qualitatively and quantitatively understand a series of processes that lead to coral bleaching. Beyond understanding coral bleaching, our hope is that similar models can be used to understand the symbiosis (and its breakdown) between different types of organisms."

8. Brian Pipes, UC Merced, [bpipes@ucmerced.edu](mailto:bpipes@ucmerced.edu)

**CRISPRi: A novel system for gene regulation in *Vibrio fischeri***

The mutualistic symbioses between the marine-proteobacterium *Vibrio fischeri* and squids in the genus *Euprymna* (Cephalopoda: Sepiolidae), are ideal model systems to examine how animal hosts accommodate and interact with their beneficial microbes. While gene knockout strains have provided insight into the processes of juvenile host colonization and maintenance of the symbiosis in mature hosts, limitations of this genetic mutational technique preclude the investigation of specific aspects of the symbiosis, such as temporal and spatial gene expression patterns. To address these limitations, we are developing an engineered orthogonal CRISPRi (Clustered Regularly Interspaced Short Palindromic Repeats, interfering) system for inducible targeted repression of gene expression in *V. fischeri*. Using CRISPRi, the expression of any gene(s) of interest can be repressed in an inducible, titratable, and reversible manner. This system can be used to regulate the timing and strength of gene expression of single and multiple *V. fischeri* genes of interest, during both the free-living planktonic and symbiotic stages within the host. Here, we report on differences in the utility and efficiency of the CRISPRi strains at repression of both reporter (RFP) and endogenous (*lux*) genes during cultured *V. fischeri* growth. Monitoring these strains during symbiosis will provide a window into the timing and power of *lux*-based bioluminescence in *V. fischeri* colonized hosts during the successful establishment and maintenance of the association.

**Attendees**

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