

Friday April 19th

7:00pm - 10:00pm Arrival & Welcome Party Landsnaes House 2667 English Lane, Wawona CA

Saturday April 20th

Breakfast provided in SNRI Cabins

Lunch 12:00pm – 12:55pm, Wawona Community Center (see attached map)

12:55 Welcome to the 2023 Yosemite Symbiosis Workshop (Carolin Frank, Joel Sachs)

Section I

- 1. **1:00** Sophia MacVittie Microbiome recovery dynamics and host fitness consequences of antibiotic exposure in *Exaiptasia diaphana*
- 2. **1:15** Christopher Bivins Are mycoheterotrophs actually parasitic? Investigating proximitybased abundance of *Tomentella fuscocinerea* relative to *Corallorhiza striata*
- 3. **1:30** Daniel Olivares-Zambrano Shifting symbiont communities in a vertically transmitting coral at different life stages
- 4. **1:45** Ruiqi Li Comparative transcriptomics revealed parallel evolution and innovation of photosymbiosis molecular mechanisms in marine bivalves

45 minute break for coffee and snacks

Session II

- 5. **2:45** Scott Roy Parasitic-symbiotic gray areas in so-called selfish genes
- 6. **3:00** Malcolm Thieme Is the Relationship Kraken? How Climate Stress Influences Symbiotic Dynamics in the Squid-*Vibrio* Mutualism
- 7. 3:15 Joel Sachs The genomics of ecological specialization in a rhizobia metacommunity
- 8. 3:30 Emily Neff Examining biogeographic bariation in a bioluminescent fish-bacteria symbiosis
- 9. 3:45 Alison Gould Flexibility in an otherwise highly specific bioluminescent symbiosis
- 10. **4:00** Steph Varghese Modeling the endosymbiotic origins of eukaryotes

45 minute break for snacks, beer and wine

5:00-6:00 KEYNOTE: Kabir Peay Plant-fungal symbiosis in a changing world

6:00-7:30 Dinner

7:30-8:30pm Poster Session

Sunday April 21st

Breakfast 8:00-9:00am Wawona Community Center (see attached map)

Session III

- 11. **9:00 Bailey Crowley** Temperature-mediated effects on the solitary bee provision microbiota: implications for larval fitness
- 12. 9:15 Johana Rotterova Population genomic insights into syntrophic symbiosis between marine anaerobic ciliates and intracellular methanogens
- 13. 9:30 Lucas Nell Interactions between pollinator behavior and nectar microbial communities across a landscape
- 14. 9:45 Joshua Fowler Microbial symbionts buffer hosts from the demographic costs of environmental stochasticity
- 15. **10:00** Sonia Vargas Microbial diversity and community composition in deep sea sediments underlying the ocean's largest oxygen minimum zone

30 minute break for coffee and snacks

Session IV

- 16. **10:45** Younghwan Kwak *Liberibacter psyllaurous* infection shapes the metabolic dynamics of its insect vector and microbiome
- 17. 11:00 Usha Lingappa Chlamydomonas reinhardtii from biological model to ecological subject
- 18. **11:15 Amaury Payelleville** Priority effects and coexistence in insect-feeding microorganisms in the oak woodland soil
- 19. **11:30** Kaden Muffett Snapshot physiological changes to temperature stress in the symbiotic anemone *Exaiptasia*
- 20. 11:45 Peter Pellitier Fungal Communities in the Boreal Forest Influence Carbon Sequestration

Carolin Frank, Joel Sachs Closing remarks

12:00-1:00 Lunch

End of conference

Talks

1. Sophia MacVittie smacvittie@ucmerced.edu University of California Merced Microbiome recovery dynamics and host fitness consequences of antibiotic exposure in Exaiptasia diaphana

Sophia MacVittie, Saam Doorodian, Aaron Alberto, Maggie Sogin

Microbial communities play an important role in supporting the health and resilience of their hosts, but demonstrating the functional roles of microbes in supporting host health is an ongoing challenge. The use of laboratory generated gnotobiotic individuals is a powerful tool to study the impacts of microbial species on host fitness in both terrestrial and marine systems. Exaiptasia diaphana, or Aiptasia, is a small sea anemone commonly used as a model to study mechanisms of coral algal symbiosis and more recently to study the relationship between Cnidarians and their associated bacterial communities. In this study, we investigated how reducing Aiptasia's microbial load via antibiotic dosage impacted host fitness and monitored microbial community dynamics during and after antibiotic exposure. Individuals were exposed to one of two different antibiotic cocktails for three weeks, then held in sterilized artificial seawater and allowed to recover for an additional three weeks. Both treatments successfully reduced microbial load up to one week after antibiotic exposure. During and after exposure asexual reproduction was halted, and individuals showed a decrease in biomass, demonstrating fitness consequences for the host. Additionally, one of the treatments resulted in reduction of algal symbionts during antibiotic exposure. While microbial load was reduced during antibiotic exposure, 16S rRNA sequencing data revealed limited changes to microbial community composition. It was only during the recovery phase that we observed significant reductions in community diversity and shifts in community composition. Our data demonstrates a link between microbial communities and host fitness, additionally, exposure to antibiotics yields a stable but less diverse microbial community. This is a promising step in the use of Aiptasia as a gnotobiotic model, and towards elucidating the functional roles of cnidarians in supporting host-fitness.

2. Christopher Bivins cbivins@ucmerced.edu UC Merced

Are mycoheterotrophs actually parasitic? Investigating proximity-based abundance of *Tomentella fuscocinerea* relative to *Corallorhiza striata*.

Christopher Bivins, Carolin Frank

Mycoheterotrophs are non-photosynthetic plants that engage in symbiotic relationships with fungi, and are typically described as parasitic on their fungal hosts. Our research

focuses on two distinct mycoheterotrophic plants, Sarcodes sanguinea from the Eudicot lineage and Corallorhiza striata from the Monocot lineage, and their respective ectomycorrhizal fungal hosts, Rhizopogon ellenae and Tomentella fuscocinerea. Research by Bidartondo et al. (2000) showed that S. sanguinea may enhance the abundance R. ellenae, potentially indicating a mutualistic relationship. Our study extends this investigation to C. striata, comparing its interactions with, T. fuscocinerea, to assess whether such potentially mutualistic associations are more widespread across mycoheterotrophs. Utilizing environmental DNA (eDNA) metabarcoding, we investigated the relative abundance of these fungi in proximity to their mycoheterotrophic partners. Through soil eDNA fungal metabarcoding from rhizosphere soil and ectomycorrhizal root tips, we aimed to replicate Bidartondo et al.'s findings. We then extended this investigation to assess the proximity-based abundance of T. fuscocinerea relative to C. striata. Our findings suggest that the relationship between mycoheterotrophs and their fungal hosts may not be solely parasitic but context-dependent, with potential mutualistic interactions varying across species. This study contributes to a nuanced understanding of mycoheterotrophs, highlighting the complexity of these plants' ecological interactions.

3. Daniel Olivares-Zambrano danielo7@usc.edu University of Southern California Shifting symbiont communities in a vertically transmitting coral at different life stages

Daniel Olivares-Zambrano, Courtney Timmons, Carly D. Kenkel, Kate M. Quigley Coral reefs worldwide are threatened by increasing ocean temperatures. All reef building corals form obligate, mutualistic symbiotic relationships with dinoflagellates (family Symbiodiniaceae) with roughly 25% of species acquiring their initial symbiont complement from their parents through vertical transmission. Symbiodiniaceae vary in their thermal tolerance thresholds and the host corals' ability to tolerate stress is therefore significantly influenced by their endosymbionts. Corals may tolerate a wider range of] temperatures through changing symbiont abundances, known as shuffling, and altered abundance ratios can be transgenerationally inherited. To understand the physiological consequences of changes in symbiont communities in early life stages, we exposed larvae and juveniles of the widespread, vertically transmitting coral Montipora digitata to heat stress (32°C). Symbiont communities before and after heat stress, and in control conditions (27°C) were characterized using amplicon sequencing of the ITS2rDNA region. Percent survival, size and photosynthetic efficiency of larvae and juveniles was also measured. All fitness-related traits varied significantly across larvae, juveniles, and temperature treatments and could be attributed to variation in relative abundances of symbiont communities that they contained in heat and ambient conditions. Larvae

were able to increase the relative abundance of rare, potentially thermally tolerant strains of Symbiodiniaceae (shuffling) in response to thermal stress while juveniles showed limited ability to do so. Through the tracking of larvae from three crossing family groups, we were able to identify that larva had increased symbiont diversity from a maternal source, but this group of larvae performed the worst when compared to the two other family crosses. Moreover, we observed differences in the ability for rare and dominant symbiont communities to be inherited. These findings suggest that early life stages have the capacity to alter symbiont communities before metamorphosis into the recruitment stage and hints at the possibility that this larval plasticity supports increased ability to survive a warming climate at this stage.

4. Ruiqi Li ruiqi.li@colorado.edu University of Colorado Boulder

Comparative transcriptomics revealed parallel evolution and innovation of photosymbiosis molecular mechanisms in marine bivalves

Ruiqi Li, Daniel Zarate, Viridiana Avila- Magaña, Jingchun Li

Photosymbioses between heterotrophic hosts and autotrophic symbionts are evolutionarily prevalent and ecologically significant. However, the molecular mechanisms behind such symbioses remain less elucidated, which hinders our understanding of their origin and adaptive evolution. This study compared gene expression patterns in a photosymbiotic bivalve (Fragum sueziense) and a closely related non-symbiotic species (Trigoniocardia granifera) under different light conditions to detect potential molecular pathways involved in mollusk photosymbiosis. We discovered that the presence of algal symbionts greatly impacted host gene expression in symbiont-containing tissues. We found that the host immune functions were suppressed under normal light compared to in the dark. In addition, we found that cilia in the symbiont-containing tissues play important roles in symbiont regulation or photoreception. Interestingly, many potential photosymbiosis genes could not be annotated or do not exhibit orthologs in T. granifera transcriptomes, indicating unique molecular functions in photosymbiotic bivalves. Overall, we found both novel and known molecular mechanisms involved in animal-algal photosymbiosis within bivalves. Given that many of the molecular pathways are shared among distantly related host lineages, such as mollusks and cnidarians, it indicates that parallel and/or convergent evolution is instrumental in shaping host-symbiont interactions and responses in diverse organisms.

5. Scott Roy roy@sfsu.edu San Francisco State University Parasitic-symbiotic gray areas in so-called selfish genes

The parasitic-symbiotic spectrum is well acknowledged in the case of organismorganism interactions. Less well explored are the dynamics when they pertain to interactions between genes in an organism, that is, whether genes often regarded as selfish may have overlooked effects that render them symbiotic. Drawing from the literature, I will discuss several classes of elements, with a goal of clarifying the question through examples. I will offer some thoughts on a host of cases of so-called selfish genes and their symbiotic potential, from masculinizing B chromosomes to maternallytransmitted symbionts to paternally-inherited mitochondria to transposable elements.

6. Malcolm Thieme mthieme2@ucmerced.edu University of California Merced

Is the Relationship Kraken? – How Climate Stress Influences Symbiotic Dynamics in the Squid-Vibrio Mutualism

M. Thieme, Valdez, B. Z., Hobbs, A., D. Real, D. Vakil, M. K. Nishiguchi Symbiotic associations are found in all organisms on Earth, where microbes contribute to the overall health and well-being of their host. These associations are stable, yet recent global warming has jeopardized the survival of these organisms. Determining whether these microbes can be used as a proxy for predicting host fitness in the race against climate change will allow us to predict future outcomes that may be important for preserving biodiversity. One symbiotic model that can be used to measure this proxy is the beneficial association between bobtail squids (Cephalopoda: Sepiolidae) and their Vibrio bacterial symbionts. This model can be used as a vardstick to understand how temperature influences host-microbe associations. Vibrio bacteria were isolated from Euprymna scolopes (Hawaii=ES114), E. tasmanica (Australia=ETBB1-C), E. albatrossae (Philippines=EAS005), and E. morsei (Japan=EM17), and adapted under three temperatures: high (32°C), moderate (25°C) and low (18°C) conditions. All strains were experimentally evolved over the course of 1400 generations. Symbiotic phenotypes such as growth rate, bioluminescence, motility, and biofilm formation were measured throughout the evolved lines. We observed that bioluminescence decreases under both heat and cold stress conditions and show significant reduction in motility. These results indicate that strains evolved at higher temperatures are able to combat severe stress, but may convey less fitness to their host's ability to counterilluminate. Thus, disentangling gain/loss of symbiotic traits due to increasing temperature will provide clues on whether beneficial associations are able to mitigate climate change scenarios, and provide a broader perspective on the ability of symbioses persist and maintain biodiversity on a changing planet.

7. Joel Sachsjoels@ucr.eduUC RiversideThe genomics of ecological specialization in a rhizobia metacommunity

Lorena Torres-Martínez, Alexandra J. Weisberg, Stephanie Porter, Fizzah Khairi, Jeff H. Chang, and Joel L. Sachs

Most symbiotic bacteria have multipartite lifestyles, thriving in complex environments and forming intimate associations with multiple host species. But it is unclear how these features and their variation shape the realized niche and genome architecture of bacteria. We studied the genomic bases of ecological specialization in a metacommunity of rhizobia, focusing on populations that associate with sixteen related species of legumes in the Acmispon and Lotus genera across a set of diverse habitats in California, ranging from woodlands to grassland to desert. Ecological specialization in rhizobia is structured in parallel by adaptation to local geographic sites and by host specificity, each shaped by independently evolving genomic modules. Local adaptation of rhizobia appears encoded by sets of chromosomal loci whose presence and absence are structured by local environmental parameters. Specialization of rhizobia to Acmispon host species is defined by signature symbiosis genes that cluster to groups of ecologically similar host species, defined as guilds, suggesting that the host niche of rhizobia is shaped by symbiotically equivalent sets of sympatric hosts upon which communities of rhizobia acquire and reshuffle host-guild specific genes. Symbiotic associations with Acmispon spp. have evolved independently in both Bradyrhizobium and Mesorhizobium, diverged genera of proteobacteria that have each acquired integrative and conjugative elements (ICEs) that carry independent sets of signature symbiosis genes. The parallel patterning of local adaptation and host-guild specialization in diverged bacterial genera suggests that these are common niche axes that shape these environmentally acquired bacterial symbionts.

8. Emily Neff eneff@calacademy.org California Academy of Sciences **Examining Biogeographic Variation in a Bioluminescent Fish-Bacteria Symbiosis** Emily Neff, Alison Gould

The marine environment is a highly connected ecosystem where populations often remain interconnected. The genus Siphamia is comprised of 25 cardinalfish species, which are characterized by their light organ and large geographic range that spans the Indo-Pacific. Based on previous studies, S. tubifer, which has the largest range of the genus, appears to have high specificity for their bioluminescent symbiont, Photobacterium mandapamensis. However, only specimens from a small region of Japan were examined. It remains unknown whether this high specificity is upheld throughout other parts of their range and among other Siphamia species. Moreso, it is not known how this relationship persists through time. To further characterize the specificity of this symbiotic association, I analyzed genetic variation in S. tubifer and its bioluminescent symbiont from two distinct time points in Okinawa, Japan and from

Caubyan, Philippines. I then compared the genetic variability of the symbionts of S. tubifer to that of S. fuscolineata from Verde Island, Philippines and S. mossambica from Zanzibar, Tanzania. To do so, I applied shotgun sequencing to the DNA from the fish light organs to recover whole genome information for both the host and symbiont. With this approach I characterized symbiont diversity within and between host species and defined the degree of specificity of the association. All symbionts were identified as P. mandapamensis from a core gene tree including those from all three Siphamia populations signifying symbiotic specificity extends across host species. Based on 290 consensus SNPs, symbiotic populations of P. mandapamensis in the Philippines and Japan were found to be genetically distinct from each other indicating host populations are acquiring symbionts from different regions. When characterizing the hosts based on 81,296 SNPs, a comparison of S. tubifer between Japan and the Philippines resulted in an Fst value of 0.03 suggesting some genetic differentiation between these populations. Surprisingly, we found that Japan populations had very low genetic diversity compared to the Philippines population. By characterizing the genetic diversity between Siphamia and their symbiont, we can gain further insight regarding symbiotic associations. Overall, this binary symbiosis can help us understand how specificity and stability of host-microbe associations are maintained across space and time.

9. Alison Gould agould@calacademy.org California Academy of Sciences Flexibility in an Otherwise Highly Specific Bioluminescent Symbiosis

The cryptic cardinal fish genus Siphamia is comprised of 25 species, all of which are symbiotically luminous. Most Siphamia species inhabit tropical coral reefs throughout the Indo-Pacific, with the exception of two temperate species, both endemic to Australia. While the tropical species appear to form a highly specific, binary association with the bioluminescent bacterium Photobacterium mandapamensis, the identity of the temperate and sub-tropical species' symbionts remain unknown. The goal of this study is to characterize and compare the bioluminescent symbionts of two species with overlapping distributions in Australia, S. cephalotes, a temperate species, and S. roseigaster, a sub-tropical species, to that of S. tubifer, to determine whether the specificity with P. mandapamensis extends across hosts from these various climactic zones. Additionally, the pangenomes of the symbionts from these different hosts were characterized and compared to identify any distinct genomic signatures associated with their host. Both species were collected from neighboring sites in the Sydney area, representing the northern-most range of S. cephalotes and the southern-most range of S. roseigaster. Bacteria from the light organs of eight S. cephalotes and three S. roseigaster specimens were isolated in culture, and unique strain types were identified using a PCR-fingerprinting technique. The unique strains were then sequenced using

Oxford Nanopore sequencing technology and their genomes were assembled and compared within and between hosts. Results indicate that S. roseigaster, the more subtropical host, associates with P. mandapamensis, while the temperate species, S. cephalotes, might have the ability to associate with other species of luminous bacteria in addition to P. mandapamensis, including Vibrio species. This flexibility in the association could reflect a unique characteristic of these temperate species' partnerships, which experience more seasonality in their environment, and presumably the symbiont community as well. An additional analysis of the symbiotic P. mandapamensis strains across species revealed distinct clades corresponding to their host of origin, suggesting that despite their overlapping ranges, symbiont acquisition could be occurring in different locations. Alternatively, these results suggest that different hosts have preferences for specific symbiont genotypes, indicating extreme intra-specific symbiont specificity in the system. More extensive investigation into the light organ symbionts of these temperate Siphamia species will reveal the extent of flexibility of the symbiosis and the potential role of seasonality in shaping the association.

10. Steph Varghese steph-v@umn.edu University of Minnesota

Modeling the endosymbiotic origins of eukaryotes

Steph Varghese, Allison Shaw, Michael Travisano

The emergence of eukaryotic life represents a major turning point in evolutionary history. From the material of ancient, free-living prokaryotes sprung an entirely new lineage of cells, with membrane-bound nuclei and other specialized organelles now operating as one unit. Many contemporary theories of eukaryogenesis involve: (1) a prokaryotic host engulfing a mitochondrion that then became its endosymbiont; (2) nucleogenesis from this prokaryotic material; and (3) reproduction wherein copies of both organelles were passed down to clones. Navigating this multistep pipeline required surmounting (or averting) numerous obstacles, from host cells digesting their mitochondria to clones lacking copies of them. Herein, we explore the circumstances that might have enabled eukaryotic cells to emerge despite such obstacles. We built a model where an archaealderived host cell is "susceptible" to "infection" by an alphaproteobacterial-derived endosymbiont. Unlike in the traditional Susceptible-Infected-Recovered (SIR) framework, this endosymbiont benefits its host (metabolically as a proto-mitochondrion). Cells in the final "recovered" class have succeeded in nucleogenesis after acquiring materials during the "infection" stage and are thus considered eukaryotic. Through simulations of this model, we show that eukaryotic cells can overcome the bottleneck due to increasing fitness along the endosymbiotic pipeline. This higher fitness begins with mutualistic interactions and is sustained as partners coevolve greater complementarity to maximize cooperative gains. For example, a host can access chemical energy from its

mitochondrion without having to synthesize it itself, and the mitochondrion can transfer the bulk of its reproductive load onto its host. With one partner specializing in energy synthesis and their other in reproduction, both are able to streamline their genomes, conserve energy, reproduce more efficiently, and operate as one unit. We plan to expand on this model to help reconcile competing hypotheses of eukaryogenesis, such as mitochondrion-early versus -late. In doing so, we hope to contribute to theoretical understandings of eukaryotic emergence via endosymbiotic processes.

11. Bailey Crowley b.crowley@usu.edu Utah State University

Temperature-mediated effects on the solitary bee provision microbiota: implications for larval fitness

Bailey L. Crowley & Robert N. Schaeffer

Increasing evidence suggests that solitary bees rely upon the pollen provision microbiota for successful development. Climate warming has the potential to alter bee-microbe interactions, yet remains unexamined. We performed an in vitro study to investigate the effects of thermal environment on provision microbiome composition and the measured fitness outcomes of developing Osmia lignaria larvae. Contrary to previous research, larvae reared on sterile provisions weighed more and had higher total fat content than counterparts reared on microbe-rich provisions. These effects were significant in the warming treatment, but marginal and inconsequential in treatments emulating present day and historical microclimates, respectively. Despite these findings, provision microbiome diversity and composition were not significantly different between treatments. The most dominant bacterial genus, however, was Arsenophonus, a symbiont with many recorded phenotypes, including reproductive parasitism. The presence of Arsenophonus across samples may explain why larval survivorship and mortality did not differ between treatments. We predict that the abundance of Arsenophonus will be highest in samples at the warm microclimate compared to the other thermal environments. We conclude that the activity of the provision microbiota is influenced by the thermal environment, including those with detrimental consequences for bee health.

12. Johana Rotterova johana.rotterova@upr.edu Department of Marine Sciences, University of Puerto Rico

Population genomic insights into syntrophic symbiosis between marine anaerobic ciliates and intracellular methanogens

Johana Rotterová, Corinna Breusing, Ivan Čepička, Roxanne Beinart

Multi-domain symbioses between microbial eukaryotes and intracellular methanogenic archaea are crucial to our understanding of the origins and mechanisms of eukaryotic

anaerobiosis. Nearly all anaerobic ciliates, ecologically important protists commonly found in diverse oxygen-depleted environments, host methanogenic endosymbionts, sometimes with additional bacterial partners, that facilitate their anaerobic metabolism. The methanogenic endosymbionts of anaerobic protists represent the only known intracellular archaea. Though it is known that vertical symbiont transmission occurs over the short-term via synchronous host-symbiont division, there is also evidence that symbiont-switching occurs. However, the factors influencing patterns of host-symbiont specificity and intraspecific variability remain mostly unknown. Here, we present the first intra-specific genomic analysis of both host and symbionts in such partnerships, providing key insights into the fidelity of eukaryotic-prokaryotic liaisons in anoxia. We assessed the symbiont-host co-diversification and genetic variation by analyzing symbiont genomes (Methanocorpusculum sp.) and host mitochondrial genomes from 78 holobiont cells from 12 populations of a marine anaerobic ciliate Metopus sp. (Armophorea, SAL), across various geographic scales. Symbiont comparative and population genomics enable us to further comprehend the complex nature of these multi-partner syntrophic symbioses, which are crucial to our perception of cell-cell interactions across domains of life.

13. Lucas Nell Inell@stanford.edu Stanford University

Interactions between pollinator behavior and nectar microbial communities across a landscape

Lucas A. Nell, Tadashi Fukami

Animals often act as dispersal agents for microbes. Because animals modify their behavior based on cues from the local environment, this form of dispersal is likely to have interactive effects with conditions in the various habitat patches across which animals move. Despite these interactions having the potential to qualitatively change ecological dynamics (e.g., from coexistence to exclusion), little is known about how interactions between local conditions and animal-mediated dispersal affect microbial communities. We constructed a mathematical model of nectar microbes in hummingbird-pollinated sticky monkeyflower (Diplacus aurantiacus) flowers across a landscape of interconnected nectar communities (metacommunities). In the model, single flowers are either yeast- or bacteria-dominated, pollinators are repelled by the sour taste of bacteria, following empirical observations. Through simulation of this model, we found that hummingbird activity could dictate outcomes of competition between yeast and bacteria, and that microbial communities can likewise change patterns of hummingbird movement, with clear effects on pollination for plants. Our results highlight

the importance of interactions between the conditions of local communities and the behavior of dispersal agents in shaping ecological outcomes at landscape scales.

14. Joshua Fowler jcf221@miami.edu University of Miami

Microbial symbionts buffer hosts from the demographic costs of environmental stochasticity

Joshua C. Fowler, Shaun Ziegler, Kenneth D. Whitney, Jennifer A. Rudgers, Tom E.X. Miller Species' persistence in increasingly variable climates will depend on resilience against the fitness costs of environmental stochasticity. Most organisms host microbiota that shield against stressors. Here, we test the hypothesis that, by limiting exposure to environmental extremes, microbial symbionts reduce hosts' demographic variance. We parameterized stochastic models using data from a 14-year symbiont-removal experiment including seven grass species that host Epichloë fungal endophytes. Endophytes reduced variance in fitness by > 10\%, on average. Hosts with ``fast" life history traits that lacked longevity as an intrinsic buffer benefited most from symbiontmediated variance buffering. Under current climate conditions, contributions of variance buffering were modest compared to symbiont benefits to mean fitness. However, simulations of increased stochasticity amplified benefits of variance buffering and made it the more important pathway of host-symbiont mutualism than elevated mean fitness. Microbial-mediated variance buffering is likely an important, yet cryptic, mechanism of resilience in an increasingly variable world.

15. Sonia Vargas <u>svargas28@ucmerced.edu</u> UC Merced

Microbial diversity and community composition in deep sea sediments underlying the ocean's largest oxygen minimum zone

Sonia Vargas, Samantha Vazquez, Jesse Wilson, José Q. García-Maldonado, J. Michael Beman

Ocean sediments cover over two-thirds of Earth's surface and are consequently one of Earth's largest habitats. Despite their global importance and relevance, sediment microbial diversity and spatial distribution remain poorly explored and understood. This is particularly true for sediments underlying oceanic oxygen minimum zones (OMZs): OMZs play pivotal roles in biogeochemical cycling due to the range of microbial processes that take place within them, and how OMZ sediment microbial communities assemble and respond to environmental variation is important for our overall understanding of OMZ functioning and ocean biogeochemistry. Sampling sediment cores at multiple depths across five stations in water depths >3000 m in the eastern tropical North Pacific Ocean (ETNP), we captured systematic variations in sediment microbial community composition and diversity. Chloroflexi, Caladatribacteriota,

Proteobacteria, Planctomycetota, Acidobacteriota, Crenarchaeota, unclassified Bacteria, Nanoarchaeota, Asgardarchaeota, and Desulfobacterota were the most abundant phyla across all sediment samples, together constituting between 69-87% of 16S rRNA sequences. We identified significant variations in these and less abundant microbial groups both with sediment depth (PERMANOVA p=0.005) and between stations (PERMANOVA p=0.001). Microbial communities also displayed significant variations in overall community richness and evenness with depth, as deeper sediment communities were less diverse. In addition to these patterns in composition and diversity related to sediment core depth, we found evidence that distance from shore, overlying water column productivity, and oxygen exert strong influences on community composition—providing new insight into microbial diversity and community composition in marine sediments underlying oceanic OMZs.

16. Younghwan Kwak ykwak@ucmerced.edu UC Merced Liberibacter psyllaurous infection shapes the metabolic dynamics of its insect vector and microbiome

Younghwan Kwak, Jacob A. Argandona, Sen Miao, and Allison K. Hansen

Despite the prevalence of bacterial symbionts in insects, our understanding of the impact that cohabiting symbionts have on the metabolic dynamics within insect hosts and among members of their microbiome remains incomplete. Psyllids, which are plant sap-sucking insects, possess the obligate nutritional endosymbiont Carsonella that plays a crucial role in synthesizing essential amino acids and vitamins for the host. Due to Carsonella's small genome and extensive gene loss, the psyllid host complements Carsonella's essential amino acid pathways with a subset of psyllid encoded genes, which includes horizontally acquired genes from bacteria. Some psyllids also harbor additional symbionts including Wolbachia and Candidatus Liberibacter that has dual roles as a plant pathogen. However, how these additional symbionts influence metabolic interactions of their insect hosts and the obligate endosymbiont Carsonella is largely unclear. Here, we investigate the impact of Liberibacter psyllaurous on the psyllid Bactericera cockerelli and its microbiome to unravel the dynamics of host-microbiome interactions. We have reconstructed the metabolic capabilities of the psyllid's microbiome by sequencing the genomes of Carsonella, Wolbachia, and Liberibacter, all of which are abundant and co-localize within the insect tissues. Through RNA-seq analysis, we found that the presence of Liberibacter dramatically shifts the psyllid's gene expression underlying its nutritional metabolism with Carsonella in addition to the repression of genes in the Toll immune pathway. We further propose host and microbe genomic mechanisms that may contribute to these metabolic and immune alterations. Overall, our findings suggest that

the addition of an economically important microbial player, Liberibacter psyllaurous, dramatically shapes shared host-symbiont nutritional pathways and immune regulation in their insect host.

17. Usha Lingappa ufl@berkeley.edu University of California Berkeley **Chlamydomonas reinhardtii from biological model to ecological subject** Usha Lingappa & Dupuia L Chastain L Edwarda & Sindarmann P. Craig P. Stu

Usha Lingappa, S Dupuis, J Chastain, J Edwards, E Sindermann, R Craig, R Stuart, P Weber, X Mayali, S Merchant

Here we leverage our extensive laboratory knowledge of the model unicellular chlorophyte Chlamydomonas reinhardtii for a new window into symbioses between algae and bacteria. Depending on the availability of light and different carbon sources, C. reinhardtii can employ photosynthetic, respiratory, or fermentative metabolism, each of which carries different implications for contributions to carbon and oxygen cycling. When grown under a diel light cycle, C. reinhardtii exhibits highly rhythmic metabolic patterns and therefore contributes to ecosystem processes with a rhythmic cadence. We are investigating how this dynamic and variable biology controls C. reinhardtii's interactions with bacterial partners. We began with an established model coculture system between C. reinhardtii and Mesorhizobium loti, and expanded to cocultures with putative natural partners that are more specifically adapted to growth with C. reinhardtii and exhibit far more dynamic interactions in coculture. This work provides a new perspective on algal-bacterial symbioses in soil ecosystems and helps apply our knowledge of C. reinhardtii's biology from decades of study as a reference organism towards an understanding of its ecology.

18. Amaury Payelleville amauryp@stanford.edu Stanford

Priority effects and coexistence in insect-feeding microorganisms in the oak woodland soil

Payelleville A., Fukami T.

In the soil, a diversity of nematodes, fungi, and bacteria use insect larvae as a source of nutrients. Many of these organisms are used as biocontrol agents and are therefore studied in depth, but independently of one another. How they interact with one other in their natural environment largely remains to be investigated, despite their ecological significance in terrestrial ecosystems. We focused our study on insect-feeding microorganisms in the soil of oak woodland at Stanford University's Jasper Ridge Biological Preserve – 'Ootchamin 'Ooyakma, located in the Santa Cruz Mountains of California. In repeated soil sampling, we frequently found two fungal genera (Beauveria and Metarhizium) and four species of nematodes (Oscheius tipulae, Mesorhabditis simplex, Rhabditis terricola, and Steinernema kraussei), all of which were confirmed to

use larvae of acorn-feeding weevils and moths to complete their life cycle. Our yearlong survey showed that these fungi and nematodes were prevalent particularly during winter, when the acorn-feeding larva were abundant in the soil. We also found that the fungi and the nematodes strongly compete against one other, often taking over the insect larvae completely. Many of these competitive interactions appeared to involve priority effects, where the first species to arrive at the insect has a competitive advantage against latearriving species. However, all nematode species except S. kraussei were unable to kill a larva by themselves, indicating that they need S. kraussei or one of the two fungi to kill the insect first before they can enter and grow in it. Thus they can be grouped into two categories: (1) primary arrivers, which can kill a larva by themselves and therefore can arrive first and take advantage of priority effects (Beauveria, Metarhizium, and S. kraussei), and (2) secondary arrivers, which need a primary arriver to kill the larva before being able to use the cadaver as a nutrient source (O. tipulae, M. simplex, and R. terricola). We also found that some secondary arrivers can compete against primary arrivers because their symbiotic bacteria grow rapidly once released from the secondary arriver into the insect cadaver and inhibit the growth of the early-arriving primary arriver under certain conditions. We are now characterizing these competitive relationships more thoroughly to draw a comprehensive interaction network among the nematodes, the fungi, and the bacteria. Once elucidated, this network information will be used to understand how species coexistence in this microbial community is realized both in spite of and thanks to the strongly inhibitory priority effects found in this system.

19. Kaden Muffett <u>kadenmuffett@ucmerced.edu</u> University of California Merced Snapshot physiological changes to temperature stress in the symbiotic anemone Exaiptasia

Exaiptasia diaphana, the glass anemone, provides unique insights into the interactions between host, algal endosymbiont, and microbial consortium. To examine the ways in which dynamics of both host development and symbiotic function are perturbed with temperature, we assayed both a highly tolerant anemone strain and a supposedly intolerant strain to four temperatures from baseline (26.5C) to near-fatal (34C). Strains responded differently to each temperature, providing insights into where temperature "breaking points" in function are found.

20. Peter Pellitier ptpell@stanford.edu Stanford University

Fungal Communities in the Boreal Forest Influence Carbon Sequestration

Forest soils harbor hyper-diverse fungal communities which fundamentally regulate carbon and nutrient cycling across the globe. But, the extent to which soil fungi influence carbon storage is poorly understood. Both saprotrophic fungi and ectomycorrhizal fungi

are key fungal guilds in boreal forest ecosystems. We studied saprotrophic and ectomycorrhzial fungal taxa in soil and on Picea glauca root-tips across a broad climatic gradient in interior Alaska. Using high-throughput molecular sequencing, we characterized fungal communities. We identified that root-associated fungi were more strongly correlated with soil carbon stocks than were fungi in the soil compartment. This effect was independent of climatic variables and suggests a novel linkage between fungal community composition and soil carbon cycling. Ongoing work is aimed at understanding the functional attributes of these fungal communities that influence soil carbon stocks and tree-growth.

Posters

21. Shayna Bennett sbennett3@ucmerced.edu University of California, Merced **Mobile Genetic Elements and CRISPR Spacers in the Conifer Needle Microbiome** Shayna Bennett, Robert M. Bowers

The phyllosphere, or the aerial surface of plants, hosts a diverse community of microorganisms. Phyllosphere community members play an important role in plant health and ecosystem function, but little is known about the role of Mobile Genetic Elements (MGEs), such as viruses and plasmids, in community composition and function. To better understand the viral community of the conifer needle microbiome, an understudied phyllosphere community, we identified viral contigs and CRISPR spacers from metagenomic data of three conifer species found across the western United States. CRISPR spacers were blasted against identified viral contigs and the IMG/VR database to link viral host interactions. Our results indicate a viral community dominated by Caudoviricetes and Revtraviricetes. Viral contigs were found predominantly in prokaryotic or fungal MAGs, with the most common being Hymenobacteraceae. More than 70k CRISPR spacer clusters were identified and over 99.99% remain unlinked to a known viral sequence. Half of successful CRISPR spacer matches across viral hits in the conifer needle microbiome and IMG/VR returned low abundance taxa, suggesting diverse bacterial defense pathways exist within the conifer needle microbiome. CRISPR spacers assigned to MAGs made up less than 0.01% of total spacers identified and nearly a guarter were found in Hymenobacteraceae, consistent with the taxonomic composition of MAGs with viral hits. In addition, metadata from CRISPR matched in the IMG/VR database suggest spacers from the conifer needle microbiome are found in a broad range of habitats including marine, freshwater, and soil environments. Our findings

suggest a dynamic and complex viral community within the conifer needle microbiome and highlights the potential role of viral-host interactions in shaping interactions within the phyllopshere.

22. Joshua Garcia joagarcia@ucdavis.edu University of California, Davis Transcriptome of tomato (Solanum lycopersicum L.) reveals impacts of root microbiomes on host interactions

Joshua Garcia, Micaela Moravek, Tara Fish, Ted Thannhauser, Zhangjun Fei, Jed Sparks, Jim Giovannoni, Jenny Kao-Kniffin

Although rhizosphere microbiomes have a tremendous influence on plant growth and development, their effects on the expression of specific genes and regulatory pathways of plant hosts remain largely unknown. In this experiment, we inoculated tomato (Solanum lycopersicum L.) with rhizosphere microbiomes from vermicompost to study plant transcriptome responses to root microbiomes. We hypothesized that tomatoes grown with living vermicompost microbiomes in their rhizospheres would display transcriptome changes in key nutrient pathways compared to sterile vermicompost and control (i.e., no compost) inoculant treatments. Illumina DNA sequencing indicated the rhizosphere bacterial community profile was distinct between the three treatments at harvest, with a higher abundance of gammaproteobacteria in the vermicompost treatment compared to the control, while fungal communities in the rhizosphere remained unchanged between all treatments. Additionally, we observed increases in above ground biomass production and foliar $\delta 15N$ values in the vermicompost and sterile vermicompost treatments compared to the control at harvest. Our analysis of foliar tissue RNA sequences revealed distinct changes in gene expression in the vermicompost treatment, including the upregulation of key nutrient transporter genes, compared to both the sterile vermicompost and control treatments. While plants from the vermicompost treatment had phenotypes similar to plants from the sterile vermicompost treatment, plant transcriptome analyses indicate rhizosphere microbiomes derived from vermicompost promoted the expression of tomato genes and regulatory pathways associated with enhanced plant growth. Broadly, these results highlight discrete molecular effects that rhizosphere microbiomes can have on tomato gene expression and the usefulness of tools such as RNA sequencing in identifying molecular responses in phytobiome studies.

23. Ariadne Castaneda ariadne.j.castaneda@gmail.comUniversity of California Merced Helvella paeloris - Describing a novel fungal species from the Sierra Nevada Foothills

Christopher Bivins, Ariadne Castaneda, Carolin Frank

The Sierra Foothills is an extremely understudied region when it comes to mycology and symbiotic relationships between Sierra Nevada trees and fungal species. In this study, we undertake the formal scientific description of a novel species within the fungal genus Helvella, a specimen collected by Ph.D. student Christopher Bivins. Our research encompasses molecular systematics and a comprehensive examination of the fungus, including an analysis of its macro/micromorphological structures and a preliminary exploration of its ecology. Molecular systematics has involved the sequencing of two short genomic regions, ITS and LSU which will be followed by the production of a phylogenetic tree to align its evolutionary relationship to other closely related fungi. Lastly, the results will be published in a scientific paper along with the proposal of a name for the new species, tentatively identified as H. paeloris which upon completion will become the official name.

24. Emma Roman eroman@calacademy.org California Academy of Sciences On your Mark, Get Set, Go!: Priority Effects and Strain-Level Differences on Light Organ Community Assembly of a Coral Reef Fish

Emma D. Román, Tadashi Fukami, Alison L. Gould

Binary microbial symbioses, characterized by a host species associating with a singular bacterial species, are used as simple study systems to examine host-symbiont interactions. Nonetheless, even these single-species symbionts can form complex multi-strain communities within their hosts, and the composition and function of these communities can be highly variable. Although host selection can explain some of this variation, communities may develop differently depending on colonization history. When the initial relative abundance of organisms determines how other organisms interact with one another, this is called a priority effect and could be a strong determinant of how multi-strain communities form. However, experimental documentation of priority effects in symbiotic communities remains rare. We studied the effect that strain-level differences have on symbiont community assembly, using the luminous bacterium Photobacterium mandapamensis isolated from the light organ of the coral reef fish Siphamia tubifer. While the symbiosis between P. mandapamensis and S. tubifer is binary, each individual fish hosts an average of 5-6 strains in the light organ with few strains shared across individuals. Furthermore, strains differ in luminosity and growth rate, suggesting that they could differ functionally as symbiotic partners. In previous in vitro experiments, we observed strong priority effects when the initial abundance of two strains was manipulated and measured using plasmid-integrated fluorescent tags. Despite limitations from high plasmid loss, leading to misidentification of tagged strains as wild type, priority effects remained evident in our study. Here we expanded our investigation to include three strains tagged with genome-integrated fluorescent markers

and found that strong priority effects persisted. Specifically, the strain with the higher initial abundance always outcompeted the strain(s) with a low initial abundance. In contrast, if all strains had equal initial abundances, they grew equally well regardless of initial abundance being collectively high or low. We confirmed this result using plating and flow cytometry. Interestingly, when all three strains had equal, low initial concentrations, variations in final abundance were observed among them. However, this discrepancy did not occur when their initial concentrations were high. These results suggest that while priority effects remain strong, intra-specific competition from strain-level differences may contribute to maintaining high strain diversity in community composition. In future work, we aim to characterize potential differences in strain tolerances to varying pH, temperature, and salinity conditions. Additionally, we will extend these experiments to study interactions within a naturally occurring five-member community and also conduct experiments in vivo using captive-reared fish.

25. Hailey Hatch hhatch@ucmerced.edu UC Merced

Exploring host-bacteria interactions through microbial community manipulation in Aiptasia

Hailey Hatch and Maggie Sogin

Exaiptasia diaphana, commonly known as Aiptasia, is a well established model for understanding coral-algal symbioses. However, we lack critical data that allows us to interpret the role of other microbial partners within the Aiptasia metaorganism. Here, we seek to better understand the function and role of the microbial community within Aiptasia. To this end, we will present an experimental overview of how we plan to quantify the presence and abundance of microbial species and define the influence of single inoculants on the fitness of the host. Using a collection of bacteria isolated from Aiptasia, we will first identify potentially beneficial bacterial partners through annotation of whole genome sequences and functional assays. We will then design bacteria-species specific primers to track bacterial abundance after inoculation in order to determine uptake rate and how quickly increased association returns to pre-inoculation levels. Using a tagsequencing approach of the 16S rRNA gene, we will determine how the inoculation of a single bacterial isolate shifts the composition of the host microbiome. We predict to see an increase in individual inoculant abundance in the short term and a gradual reduction as the host only maintains the abundance necessary. Additionally, using 16S rRNA community analysis we expect to see clear microbial community shifts not seen in control treatments. Finally, we predict an increase in host fitness within Aiptasia that retain a higher abundance of the bacterial treatment, suggesting an important functional role of the bacterial symbiont. Increased knowledge of host-bacteria interactions will

ultimately lead to improved methods for identifying potential probiotics in cnidarian biology.

26. Jay Yeam jyeam@stanford.edu Stanford University

Population genomic differentiation of the ectomycorrhizal fungus Suillus pungens along a climate gradient

Jay J. Yeam, Peter T. Pellitier, Mark C. Bitter, Terry T. Henkel, Kabir G. Peay

Dispersal limitations and geographic barriers can influence microbial population structure and gene flow at the landscape level, resulting in divergent genotypes and regional endemism. However, the drivers and genetic basis of population differentiation across large spatial scales, particularly among mycorrhizal fungi, remains poorly understood. In this study, we investigate the population structure of Suillus pungens, an ectomycorrhizal fungus endemic to the coast of California and a host-specialist to Pinus muricata and Pinus radiata. We performed whole-genome sequencing on 70 individuals collected across a latitudinal and 4-fold precipitation gradient. We found significant population genetic structure among Northern and Southern populations as well as highly differentiated host-associated genotypes. We identified a strong functional signature of adaptation, with southern populations enriched in genes involved in cell signaling and membrane fluidity, a potential adaptation to drought stress. Our results provide some of the first genomic evidence for local adaptation within ectomycorrhizal species and show that barriers to gene flow can develop over relatively small spatial scales. Future work will explore how this degree of local adaptation by ectomycorrhizal fungi contributes to host stress tolerance and affects ecosystem function.

27. Ethan VanValkenburg evanvalk@stanford.edu Stanford University Intraspecific variation and tradeoffs in nectar-inhabiting yeast

Ethan Van Valkenburg, Lucas Nell, Donovan Giang, Tadashi Fukami In communities of microbes inhabiting the nectar of the sticky monkeyflower (Diplacus auriantiacus), arrival order dictates outcomes of community assembly. Early-arriving bacteria inhibit late-arriving yeast by lowering pH, while early-arriving yeast rapidly consume the amino acids that are a limiting resource for bacterial growth. Although individual flowers are usually dominated by one or few species, greater species diversity exists in the metacommunity of flowers where microbes are dispersed by pollinators, wind, and other insects. Theory suggests that intraspecific trait variation and a tradeoff between inter- and intraspecific interference can contribute to the maintenance of diversity at the metacommunity scale. Yet, this theoretical prediction remains largely untested. We quantified potential tradeoffs using laboratory experiments with 12 genetically and phenotypically diverse yeast strains of Metschnikowia reukaufii isolated

from D. aurantiacus flowers. We conducted growth assays in synthetic nectar treatments with varying pH, sugar concentration, and amino acid concentration. We found evidence of tradeoffs between yeast growth rates in normal nectar compared to growth in nectar with multiple stressors, but no tradeoffs between growth in normal versus single-stressor nectar. Our results illustrate the factors shaping yeast evolution across a heterogeneous nectar landscape and suggest eco-evolutionary dynamics in nectar microbe communities that affect yeast-bacteria interactions.

28. Sarif Morningstar smtr@ucdavis.edu University of California, Davis

Betting on Fungi: Metabolic Hedging May Confer Higher Fitness and Explain Ectomychorrizal Fungal Diversity

Sarif Morningstar, Laura Bogar

A tree may host a diverse range of ectomycorrhizal species in symbiotic relationships. However, not all relationships are equal, and resource exchange of photosynthate for fungal benefit varies between one tree and many fungal partners. We hypothesize that low-quality fungal partners in one context may become high-quality in a different context. Hence, a host tree hedges their bet by maintaining low-quality fungal partners in the event that said partner becomes high-quality, resulting in higher fitness through the maintenance of a diverse community in the long run. We will test this hypothesis using alternating cycles of temperature stress on Douglass fir seedlings associated with various ectomycorrhizal fungal partners and observing plant fitness. The temperature cycle will be controlled through a water bath with the plant root zone in containers submerged but spatially separated from the water. The temperature will cycle through medium and high heat stress in each 12-hour period. The Douglas Fir will be divided into four treatments, each containing five plants. These groups are plants associated with: 1) no fungal partners, 2) fungal partners with thermal niches at medium heat stress, 3) fungal partners with thermal niches at high heat stress, and 4) plants associated with both types of fungal partners. After 30 days, we will measure plant performance, such as height, biomass, and chlorophyll concentration. We hypothesize that plants with both medium and high heat stress thermal niche partners will perform better than other treatments because each partner provides a buffer to the varying levels of heat stress.

29. Shari Larsen slarsen 5@ucmerced.edu UC Merced

Microbial Metropolis

Wastewater treatment plants are akin to complex microbial metropolises, wherein diverse microbial communities play crucial roles in the bioremediation of pollutants, showcasing an exemplary model of ecological efficiency and sustainability. This article delves into the intricate interactions and indispensable functions of these microbial

inhabitants, focusing on their specialized contributions to nutrient cycling, particularly nitrogen and phosphorus removal. Bacteria engaged in nitrification and denitrification processes orchestrate the conversion of harmful ammonia into benign nitrogen gas, effectively mitigating nitrogen accumulation in aquatic ecosystems. Similarly, phosphorus-accumulating organisms capture and internalize excess phosphorus, preventing eutrophication and enabling resource recovery. The exploration of novel supports like natural zeolite for microbial attachment underscores the ongoing innovations aimed at enhancing treatment efficacy and microbial community stability. However, challenges such as antibiotic resistance and pathogen persistence highlight the necessity for continuous research and development in treatment technologies. Advancements in algal-bacterial symbioses offer promising prospects for improved nutrient and micropollutant removal, emphasizing the dynamic interplay and adaptability of microbial communities in wastewater treatment. Through a deeper understanding of these microbial dynamics, we unlock potential avenues for environmental protection and resource conservation, propelled by the unseen yet impactful forces within the microbial metropolis of wastewater treatment plants.

30. Jamie Burgess <u>J.burgess.4@warwick.ac.uk</u> University of Warwick

How the Root Microbiome Influences Nodulation and Host Phenotype in Legumes and Parasponia

Jamie Burgess, Alice Coppock, Beatriz Lagunas, Gary Bending, Miriam Gifford Legumes are an important family of plants worldwide, forming the second most cultivated group of crops. They are well known for their ability to fixate nitrogen gas into a form that can be assimilated by utilising an almost unique symbiosis with a group of bacteria known as rhizobia. These rhizobia are not uniform in their ability to fixate nitrogen however and exist on a spectrum between from mutualistic partners to commensalists to pathogens. A diverse ecosystem of plant-associated bacteria also modulates this symbiosis, and the root microbiome represents a large resource of potentially plant growth promoting microorganisms that can be harnessed to reduce dependence on nitrogen fertilisers. To study these interactions, it is important to know how nodulation is influenced by these root microorganisms. The tropical tree genus Parasponia can also act as an evolutionary comparison to studying nodulation in legumes, as they are the only non-legume genus to form a symbiotic interaction with rhizobia. This project focuses on finding root microorganisms that can promote plant growth by testing if they increase biomass after inoculation into the root system. This also involves characterising key species in the root microbiome and studying the mechanism of action behind how they enhance plant growth. It also explores how P. andersonii interacts with rhizobia, to glean more information about how its nodulation system

differs from that of legumes and to increase our understanding in how the process of nodulation can be utilised in other species to enhance plant growth.

31. Ellen Simms esimms@berkeley.edu UC Berkeley

Interactions between European and Californian Bradyrhizobium strains and rhizobiophages

Ellen Simms, Jannick Van Cauwenberghe

We will present the current results from the third year of a large-scale cross-inoculation study involving Bradyrhizobium strains and rhizobiophages trapped from soils collected from replicate populations of the California native Lupinus arboreus and replicate populations of Genista monspessulana, including both native European and invasive California populations.

32. Jude Edwards judith.edwards_23@berkeley.edu UC Berkeley

Variable modes of algal-bacterial interactions

Judith L. Edwards, Usha F. Lingappa, Jordan L. Chastain, Eve S. Sindermann, Sunnyjoy Dupuis, Sabeeha S. Merchant

Microalgae contribute significantly to global primary production, but little is known about their roles in soil ecology. To explore contributions to terrestrial carbon cycling by microalgae, we grew lab-adapted and field strains of the soil chlorophyte Chlamydomonas reinhardtii, with and without organic carbon sources for mixotrophic growth and in coculture with heterotrophic bacterial symbionts. We demonstrate distinct carbon transfer dynamics in cocultures of C. reinhardtii with different bacteria, including a model bacterial partner (Mezorhizobium loti) and members of the natural C. reinhardtii phycosphere community. These results highlight the heterogeneity of algal-bacterial interactions, further complicated by metabolic flexibility and physiologies that follow the day-night cycle.

33. Christopher Bivins cbivins@ucmerced.edu UC Merced

Impact of Cynipid Wasp Galling on Fungal Endophyte Communities in Blue Oak Leaves

Christopher Bivins, Carolin Frank

This study aims to elucidate the influence of leaf galling, induced by two Cynipid wasp species - the urchin gall wasp (Cynips quercusechinus) and the saucer gall wasp (Andricus gigas), on the fungal endophyte communities within blue oak (Quercus douglasii) leaves. Fungal endophytes play crucial roles in plant health and ecosystem functioning, yet their dynamics in response to insect-induced galling remain poorly

understood. This research hypothesizes that the galls formed by different wasp species will harbor distinct fungal endophyte communities. Additionally, it posits that galled leaves, in comparison to ungalled leaves, will exhibit more impoverished (i.e., less diverse) fungal endophyte communities. This study offers insights into the complex interplay between plant-insect-fungal interactions and contributes to a deeper understanding of ecosystem dynamics, with potential implications for forest health and management strategies.

34. Spencer Peterman spete045@ucr.edu University of California Riverside

Effects of host species and location on symbiosis variation in communities of rhizobia Joel Sachs

Spencer Peterman, Raymundo Lopez-Amaya, Max Porter, and Joel Sachs Little is known about the relative importance of factors that shape the composition and symbiotic function of communities of rhizobia. Legume hosts place strong selection on rhizobia that may lead to local enrichment of compatible symbionts, but rhizobia also face selection from the soil environment, which may weaken selection for host specialization and cause rhizobial communities to be structured by (e.g.) local edaphic factors. This project aimed to experimentally dissect the roles of host species and geographic location on the symbiotic function and diversity of whole communities of rhizobia associated with legume root microbiomes, focusing on plants in the genus Acmispon, which is widely distributed across western North America and associates with rhizobia in the genera Bradyrhizobium and Mesorhizobium. Pairs of root-associated soil samples were taken, one each from sympatric Acmispon strigosus and A. americanus, from four locations spanning an ecological gradient in Southern California. Soil filtrates were prepared from these samples and inoculated onto A. strigosus, A. americanus, and two of their congeners in a greenhouse experiment. All Acmispon species formed root nodules in response to live soil filtrates and generally received significant growth benefits compared to controls that were inoculated with autoclavesterilized filtrates. Acmispon growth responses were structured much more strongly by host factors (i.e., host of soil origin, host of inoculation, and their interaction) than by location of soil origin, suggesting that sympatric Acmispon species have evolved differing symbiotic specificity traits and harbor differing soil microbiota, including rhizobia that vary at specificity loci. A. strigosus and A. americanus also benefitted significantly more from their own microbes than from those of the other species, indicating that soil microbiota are specialized to their respective hosts. Planned genotyping work using amplicon sequencing of the rhizobia-specific nifD locus will investigate (1) the diversity of rhizobia in soil samples and nodules, (2) how this diversity is structured by location, selection from the host, and competition between rhizobia, and (3) whether and to what

extent host fitness is driven by individual strains or by emergent effects of rhizobial communities.

35. Joaquin Lucero jlucero25@ucmerced.edu University of California Merced Biofilm Formation and Strain Distribution in the Symbiotic Association of *Vibrio fischeri* with Sepiolid Squids

Vibrio fischeri is a cosmopolitan bioluminescent bacterium that is a beneficial symbiont with sepiolid squids (Cephalopoda: Sepiolidae). The association is a tractable model to study the interactions and molecular crosstalk between host and symbiont. V. fischeri are environmentally transmitted from the surrounding seawater to newly hatched aposymbiotic juvenile squid through colonization of a specialized light organ. These bacteria provide squids with bioluminescence, which aids in a behavior termed counterillumination or silhouette reduction. Recently, there has been a surge in tools for modifying the genome of these symbionts, enabling visualization and quantification of bacterial abundance within the host light organ during colonization and persistence. Considering the critical role of biofilm formation in shaping bacterial colonization dynamics, particularly within the host's intricate light organ environment. Our investigation delves into the significance of this attribute in guiding the spatial distribution of V. fischeri strains, complementing our aim to understand the infection and colonization dynamics in this symbiotic association . Although these tools have been exemplar in discovering the specificity and mechanisms by which this symbiosis is established and persists, little is known about the spatial distribution and strain diversity inside the squid light organ. Therefore, the focus of our study is to develop a plasmid that integrates into the genome of V. fischeri, allowing differentiate between various populations within the host light organ. Currently, we are employing two different strains of V. fischeri for plasmid insertion at the Tn7 site for genome integration. Two symbiontic strains, ES4G1 from Euprymna scolopes and ETBB1-C from Euprymna tasmanica, were selected for their ability to form biofilms . Investigating the localization of these dominant strains will help bridge the gap in our understanding of how V. fischeri select specific locations within the host light organ during infection and colonization. Results from our study will contribute to an increased understanding of competition among strains inside the host, as well as how spatial distribution affects the ability of dominant strains to outcompete other non-native strains. Alternatively, it may facilitate co-infections of less dominant strains, thus shedding light on the persistence dynamics within the light organs of certain hosts.

36. Rosa McGuire rmcguire@stanford.edu Stanford University

Temperature effects on community assembly using nectar microbes of Sticky Monkeyflower

Rosa M. McGuire and Tadashi Fukami

Community assembly is an integral process to the development and maintenance of ecological communities.

Priority effects, whereby early arriving species outcompete late-arriving ones, are key in shaping community composition. In this context, nectar-inhabiting microbes are a suitable study system for community assembly because they are lab and field amenable and influence plant/pollinator interactions. Moreover, the strength of priority effects can be altered by environmental variation through the effects of temperature on each individual species' growth rate. As a result, climate change may affect nectar microbial communities with possible subsequent effects on pollination services and plant fitness.

In a project recently started, we seek to address how environmental variability alters community assembly by studying the temporal variation in microbial communities of sticky monkeyflower (Diplacus aurantiacus). We are conducting inoculation experiments using the yeast Metschnikowia reukaufii and the bacteria Acinetobacter nectaris, two nectar microbes associated with D. aurantiacus. Priority effects are introduced by changing the initial population densities of the competing species, with all treatments repeated on 20 experimental plants. Our plant locations span a wide variety of microclimate diversity, and daily temperature will be recorded. Inoculations will occur throughout the whole flowering season (April-June). A subset of inoculated flowers will be harvested to characterize nectar community composition. We will also examine flowers will be allowed to form seed capsules to quantify seed set as our proxy for plant fitness. Our results will help unveil the relationship between priority effects, community composition, and temperature variation in monkeyflower as well as make mechanistic predictions about the effects of climate change in other communities.

37. Stephen Williams <u>swilliam64@ucmerced.edu</u> UC Merced

Exploring the fluid dynamics of symbiosis establishment in the bobtail squid

Stephen Williams, Elizabeth Heath-Heckman, Erica Rutter, Shilpa Khatri The bobtail squid Euprymna Scolopes employs counter-illumination to protect itself from predation, a process crucial to its survival. For this, it must successfully extract the bioluminescent bacteria, Aliivibrio fischeri, from its environment. This capture process takes place largely on the surface of a specialised organ within the squid, known as the light organ, which co-evolved to support the bacterial colonisation of the squid. Fluid-structure interactions at the surface of the light organ and bacterial chemotaxis facilitate this process. By combining techniques from low Reynolds number fluid dynamics and

sensitivity analysis within numerical simulations, we will explore how the geometry of the light organ impacts the ability of the squid to capture and select its bacterial partners.

38. Lynn Breithaupt <u>lbreithaupt@ucmerced.edu</u> UC Merced

The temporal variation of a Vernal Pool plant-pollinator community

Lynn Breithaupt, Dr. Jay Sexton, Dr. Marilia Gaiarsa

Vernal pool ecosystems have suffered > 90% habitat loss in California1, and recent studies indicate global pollinator declines are occurring at an alarming rate. Both have been negatively impacted by habitat loss, pollution, and global climate change2. Although vulnerable, vernal pool ecosystems serve as biodiversity hot spots dominated by native flowering species. Since 85% of flowering species depend on animals for reproduction , pollinator declines should be regarded as a high concern for maintaining ecosystem biodiversity and functionality. Little is known of what pollinator species are interacting with vernal pool plants in California, so we are investigating how the pollinator community changes as different vernal pool plants flower within the Merced Vernal Pool and Grassland Reserve.

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