FINAL SCHEDULE
22 April 2017

Final Schedule -- 7th Annual Yosemite Symbiosis Workshop – May 6-7th, 2017

SCHEDULE OF INVITED TALKS

SATURDAY, MAY 6

Meeting Welcome and Lunch

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

SESSION 1. Experimental approaches unraveling host-symbiont interactions

- 1:00 **Quinn McFrederick** Microbiome selection protects bees from pesticides
- 1:15 **Christine Dodge** Sans specificity: loose mutualisms between two ambrosia beetles (Euwallacea spp.) and their fungal symbionts
- 1:30 **Sharon L. Doty** Drought Tolerance in Poplar, Rice, Maize, and Conifers with Endophytes of Wild Salicaceae
- 1:45 **Tobin Hammer** Independence from microbial symbionts in leaf-feeding caterpillars
- 2:00 **Joshua Harrison** A heritable symbiont and host-associated factors shape fungal endophyte communities across spatial scales
- 2:15 **Matt Jones** Development of an in-vitro culture system for studying the interactions between a host, resident bacteria and an invading fungus

Coffee Break for 30 min

SESSION 2. Genetic & genomic mechanisms of symbiont adaptation to hosts and the environment

- 3:00 **Benjamin Cole** Genome-wide Identification of Bacterial Plant Colonization Genes
- 3:15 **Brendan Cornwell** Both abiotic and biotic conditions shape the population genomic structure of a Cnidarian-Dinoflagellate symbiosis
- 3:30 **Jun Gu** The function of type III secretion system of Bradyrhizobium sp. HY4 isolated from peanut nodule

Coffee Break for 30 min

SESSION 3. Mechanistic bases of host regulation of associated symbionts

- 4:15 **Honglin Feng** microRNA regulation in an ancient obligate endosymbiosis
- 4:30 **Kenjiro Quides** Lotus japonicus alters the in planta fitness of Mesorhizobium loti dependent on symbiotic nitrogen fixation
- 4:45 **Joel Sachs** Cell autonomous sanctions in legumes target ineffective rhizobia during mixed infections

SESSION 4. Poster Presentations 5:00-6:00 Poster Presenters see below (*beer, wine, and snacks provided)*

KEYNOTE LECTURE 6:00-7:00 **Corrie Moreau,** Field Museum,

Ants, Plants, and Bacteria: Symbiosis as a Driver of Evolutionary Diversification

DINNER – 7:00-8:30

SUNDAY, MAY 7

BREAKFAST – 9:00-10:00AM

SESSION 5 The ecology & evolution of host associated microbial communities

- 10:00 **Elizabeth A Bowman** Ectomycorrhizal and foliar endophytic fungal communities of *Pinus ponderosa* along a spatially constrained elevation gradient
- 10:15 **Alison Gould** Shedding light on symbioses: lessons from a bioluminescent vertebrate-microbe association
- 10:30 **Will Ludington** Stochastic colonization underlies gut microbiome stability
- 10:45 **Dan Naylor** The Effect of Drought and Host Genotype on the Grass Root Microbiome
- 11:00 **Kaleigh Russell** Effects of climate change on nectar microbes and pollinator preference
- 11:15 **Sabah Ul-Hasan** Searching for Microbes in the Uncharted Venomous Territories of Marine Cone Snails
- 11:30 **Chris Wall** Patterns of Symbiodinium diversity in the coral Montipora capitata from shallow reefs of the remote Northwestern Hawaiian Island
- 11:45 **Su'ad Yoon** Examining the role of maternal microbes in the insect immune response: an example of wild immunology

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

1 Elizabeth A Bowman, A. Elizabeth Arnold **-** University of Arizona

Ectomycorrhizal and foliar endophytic fungal communities of Pinus ponderosa along a spatially constrained elevation gradient

Understanding factors that influence the distributions of plant-symbiotic fungi is important for projecting community-level responses to environmental change. Members of the genus Pinus simultaneously host diverse fungal communities that include foliar endophytes (FE) in leaves and ectomycorrhizal (EM) fungi in association with roots. Relationships of these communities to one another and to the environmental context of their shared hosts have not been examined previously for forest trees. We characterized FE and EM associated with Pinus ponderosa along a spatially constrained elevation gradient corresponding to differences in soil chemistry, plant communities, and climate (a change in 4°C in mean annual temperature and 15 cm in mean annual precipitation). Community measures were examined in the context of soil chemistry and plant community structure, ruling out spatial autocorrelation and other potential artifacts of sampling. Abundance of FE isolated in culture decreased with elevation, whereas abundance of EM increased. Community composition of cultured FE did not differ with elevation, but EM fungal communities differed as a function of three major factors relevant to elevation (climate, plant community composition, and to a lesser degree, soil chemistry). Particular EM fungi were distinctly associated with low- and high-elevation sites, suggesting that effects of climate change may be observed more clearly among EM fungi than FE fungi in these trees. Together these data provide a simultaneous perspective on fungal communities associating with leaves and roots of a dominant forest tree. Future work includes manipulative experiments to test the relative importance of climate-related factors in shaping communities of symbionts."

2 Benjamin Cole

DOE-Joint Genome Institute

Genome-wide Identification of Bacterial Plant Colonization Genes

Diverse soil-resident bacteria can contribute to plant growth and health, but the molecular mechanisms enabling them to effectively colonize their plant hosts remain poorly understood. We used randomly barcoded transposon mutagenesis sequencing (RB-TnSeq) in Pseudomonas simiae, a model root-colonizing bacterium, to establish a genome-wide map of bacterial genes required for colonization of the Arabidopsis thaliana root system. We identified 115 genes (2% of all P. simiae genes) whose function is required for maximal competitive colonization of the root system. Among the genes we identified were some with obvious colonization-related roles in motility and carbon metabolism, as well as forty-four other genes that had no or vague functional predictions. Independent validation assays of individual genes confirmed colonization functions for 20 of 22 (91%) cases tested. To further characterize genes identified by our screen, we compared the functional contributions of P. simiae genes to growth in 90 distinct in vitro conditions by RB-TnSeq, highlighting specific metabolic functions associated with root colonization genes. Our analysis of bacterial genes by sequence-driven saturation mutagenesis revealed a genome-wide map of the genetic determinants of plant root colonization and offers a starting point for targeted improvement of the colonization capabilities of plant-beneficial microbes.

3 Brendan Cornwell

Both abiotic and biotic conditions shape the population genomic structure of a Cnidarian-Dinoflagellate symbiosis

Cnidarian-dinoflagellate symbioses are the foundation for some of the most productive and diverse ecosystems on the planet. These relationships can persist across highly variable abiotic conditions, and optimal host-symbiont combinations can change over the scale of centimeters to kilometers. Critical gaps in our knowledge remain concerning the strategies (e.g. local adaptation or phenotypic plasticity) that cnidarians and dinoflagellates employ to match local conditions, and how these strategies impact the formation and performance of their partnerships. Here, I dissect the role abiotic and biotic conditions play in structuring cnidarian-dinoflagellate symbioses using three species of anemones in the genus Anthopleura and their endosymbionts Symbiodinium sp. (all clade B) that inhabit the intertidal zone of the Pacific coast of North America. In particular, I use population genomic tools to characterize levels of gene flow and signatures of selection in host and symbiont populations across three upwelling (and interspersed downwelling) zones along the coast of California and Baja California, Mexico. These analyses reveal that all three species of Anthopleura are largely panmictic across this region, while the symbionts exhibit high levels of genetic differentiation between the geographic sites I sampled. Furthermore, within each geographic location, symbiont populations are genetically differentiated by host species. These contrasting patterns suggest that the symbionts might be able to more easily adapt to local conditions, including the internal (and intracellular) environment of a single host species.

4 Christine Dodge, Joseph Carrillo, Akif Eskalen, Richard Stouthamer
UC Riverside
Sans specificity: loose mutualisms between two ambrosia beetles (Euwallacea spp.) and their fungal symbionts
Ambrosia beetles in the tribe Xyleborini (Coleoptera: Scolytinae) are proficient colonizers of naïve areas, due in part to their obligate mutualisms with specific fungi. This tribe includes members of the invasive Euwallacea fornicatus species complex, a recently discovered group revealing several genera of fungal symbionts. Two members of this group, the polyphagous shot hole borer and Kuroshio shot hole borer (Euwallacea spp.), are invasive to Southern California, where they vector distinct phytopathogenic Fusarium symbionts that are damaging natural, urban, and agricultural environments. The beetles each harbor a Graphium symbiont as well, and the polyphagous shot hole borer is additionally associated with a Paracremonium species. Symbioses with specific ambrosia fungi may inform ambrosia beetle host preference, geographic range, or ecological role in a particular environment. We performed reciprocal in vitro rearing experiments to determine the extent to which each shot hole borer could utilize their congener's Fusarium and Graphium symbionts. Sterile adult females were introduced to different fungal treatments, upon which they were allowed to feed and reproduce. From the offspring we isolated mycangial contents of adult females and gut contents of adult males and larvae, using a qPCR assay to identify fungal species. We also quantified relative gut and mycangial loads of each fungus from dilution plating. Our results paired with recent phylogeographic data indicate that fungal symbionts in this complex are capable of host switching, the implications of which are discussed. We also make inferences about the roles of each fungal symbiont in the development of its beetle host.

5 Sharon L. Doty, Tony Rho, Zareen Khan, Matthew Aghai, Andrea Firrincieli, Carolyn Hartman, Evora Glenn, Pierre Joubert, Greg Ettl, and Soo-Hyung Kim
University of Washington
Drought Tolerance in Poplar, Rice, Maize, and Conifers with Endophytes of Wild Salicaceae
With the relatively long life cycles of trees, symbiosis with microorganisms may allow plants to more rapidly overcome environmental challenges. Endophytes, the bacteria and fungi that live in intimate association within plants, provide numerous benefits to the host plant including N-fixation, phytohormone production, reduced stress responses, anti-microbial production, and pollutant degradation. A consortium of endophyte strains from wild Salicaceae plants (poplar and willow) were shown to enhance the drought tolerance of hybrid poplar. The same consortium was then tested on the evolutionarily distant monocots, rice and maize, and the gymnosperms, Douglas-fir and western red cedar. Water use efficiency was significantly improved in the grasses and mortality was reduced in the conifers subjected to drought stress. Genomics analysis, microbial assays, and plant physiological tests implicated multiple potential mechanisms for the endophyte-conferred drought tolerance on the host plant. With the increasing frequency of regional droughts, it is imperative that a deeper understanding of the symbiotic traits required for these broad host range benefits is achieved.

6 Honglin Feng, Stefan Wuchty, Lingyu Wang, Alex C. C. Wilson

University of Miami
microRNA regulation in an ancient obligate endosymbiosis
Many insects are associated with obligate bacterial endosymbionts. Yet, the mechanisms by which these host/endosymbiont interactions are regulated remain mysterious. Recently, microRNAs (miRNAs) have emerged as key regulators mediating host-microbe interactions, including host-facultative symbiont interactions. However, the role of miRNAs in host-obligate endosymbiont interactions is yet to be studied. Here, using the aphid and its obligate endosymbiont Buchnera aphidicola as a model of host-obligate endosymbiosis, we identified de novo miRNAs that potentially regulate symbiotic interactions. In the green peach aphid, Myzus persicae's bacteriocyte cells, the specialized aphid cells that house Buchnera, we identified 14 miRNAs that are enriched and/or differentially expressed between bacteriocytes and a non-Buchnera housing tissue (gut). Notably expression of these 14 miRNAs is conserved in the pea aphid, Acyrthosiphon pisum; an aphid that diverged from M. persicae 30 million years ago. Using a computational pipeline we predicted 47 targets of the 14 conserved miRNAs and found that all 47 targets are differentially expressed between Buchnera-housing (bacteriocytes) and non-housing (gut) tissues. Strikingly, many of the 47 targets have previously been reported to be involved in biology important to the symbiosis; targets whose functions include metabolic and biosynthetic processes, membrane structure and transport. Our results suggest that miRNAs are potentially crucial regulators of aphid/Buchnera interactions and that such patterns may be generalizable across other host-obligate endosymbioses.

7 Alison Gould, Paul Dunlap
UC Berkeley
Shedding light on symbioses: lessons from a bioluminescent vertebrate-microbe association
Virtually all organisms are dependent on symbioses with microorganisms for their success, yet the processes by which these essential interactions are established and maintained over time remain largely unknown. I examined a pairwise symbiosis involving a coral reef cardinalfish and a luminous bacterium as a model association to define the ecological mechanisms that help to maintain symbiont specificity over host generations. To do so, I integrated field studies to define key attributes of the host's behavioral ecology and life history in Okinawa, Japan with recently developed genomic methods (restriction site-associated sequencing, "RAD-Seq") to test the hypothesis that the fish's ecology genetically structures populations of its facultative symbiont over time and space, consequently promoting the specificity of the association. Results indicate that resident populations of adult fish at a reef enrich the surrounding water daily with excess luminous symbionts and that larval fish disperse significant distances as a cohort to non-natal reefs, acquiring a symbiont from the locally enriched water near their settlement site. Ultimately this study highlights the role of a host animal in structuring natural populations of its bacterial symbiont, thereby promoting the specificity of its symbiosis over host generations.

8 Jun Gu, Meijuan Hu, Ting Li, Jingyu Chen, Yongxiu Lai, Huaqin Ruan, Entao Wang
South China Normal University, Guangzhou
The function of type III secretion system of Bradyrhizobium sp. HY4 isolated from peanut nodule
Type III secretion system had different effects on the rhizobia-legume interaction, especially for the classic mode of root hair infection. Bradyrhizobia nodulated with the host in the Aeschynomeneae tribe or the Dalbergieae tribe of the Leguminosae such as the genera of Aeschynomene, Arachis, Stylosanthes and Zornia can use an intercellular infection mode that is considered more ancestral way than the root hair infection. The function of type III secretion system was unclear in the intercellular infection mode. Here we isolated one strain Bradyrhizobium sp. HY4 from the peanut nodule, which closely related to B. japonicum/B.diazoefficiens symbiotic clade based on nodA gene phylogenic analysis but was relative with B. arachidis in the ITS phylogenic tree. The strain Bradyrhizobium sp. HY4 can nodulated with soybean Zhonghuang 57, peanut Shanyou 523 and Yueyou 45, and purple bush-bean. When we constructed the ttsI and rhcJ mutants of its type III secretion

system, respectively, and the nodulation ability were tested. We found that two different mutants of the type III secretion system both lost the nodulation ability on the soybean Zhonghuang 57, but could nodulate with peanut Shanyou 523 and Yueyou 45, and purple bush-bean. Our results indicated that the type III secretion system of Bradyrhizobium sp. HY4 had a negative role for its interaction with soybean Zhonghuang 57. The function of the type III secretion system in the intercellular infection mode was needed to be revealed in the future.

9 Tobin Hammer, Daniel Janzen, Winnifred Hallwachs, Samuel Jaffe, Noah Fierer
University of Colorado
Independence from microbial symbionts in leaf-feeding caterpillars
A current paradigm holds that animals are invariably associated with microbial symbionts that influence their hosts' development, ecological interactions, and evolutionary diversification. However, firm evidence for the existence and functional importance of such microbiomes in larval Lepidoptera (caterpillars) is lacking, despite the fact that these insects are enormously diverse and major herbivores in terrestrial ecosystems and agriculture. Using 16S rDNA sequencing and quantitative PCR, we characterized the gut microbiomes of wild caterpillars in the United States and Costa Rica, representing 112 species from 16 families. As compared with a range of non-lepidopteran insects and vertebrates assayed with the same methods, caterpillar gut microbiomes are unusually low in abundance and highly variable among conspecific individuals. Furthermore, the abundance and composition of microbes sampled from leaves were largely reflected in the feces of caterpillars consuming the same plant. These patterns parallel findings from decades-old microscopy-based studies: diet-derived microbes may be present (though likely inactive) in the gut, but resident and growing microbial populations are largely absent. To test whether a low number of transient microbes might still contribute to caterpillar feeding and development, we conducted an experiment on field-collected tobacco hornworms (Manduca sexta). Suppressing the M. sexta gut microbiome using antibiotics had no detectable effect on performance, development time, or survival. The high pH, simple gut structure, and fast transit that typify caterpillar digestive physiology may prevent the establishment of a functional gut microbiome. Moreover, caterpillars have long been known to possess endogenous digestive and detoxification mechanisms, and these may render microbial symbionts unnecessary for herbivory. At the Symbiosis Workshop, I hope to stimulate discussion over the ecological and macroevolutionary tradeoffs inherent in hosting symbionts, whether caterpillars represent one of potentially many groups of ""free-living"" animals, and what approaches can be used to test for animal independence from microbial mutualism.

10 Joshua Harrison, T. L. Parchman, D. Cook, R. R. Gardner, M. L. Forister
University of Nevada, Reno
A heritable symbiont and host-associated factors shape fungal endophyte communities across spatial scales
Plants host numerous fungi that live asymptomatically within their tissues. These fungal endophytes have attracted a great deal of interest because of their putative ecological importance, however biogeographic patterns in endophyte diversity remain understudied. To investigate how host plant genetic structure, population proximity, and habitat variation together structure endophyte communities, we assayed endophyte communities occurring in spotted locoweed (Astragalus lentiginosus) across the arid West. Spotted locoweed is a tremendously phenotypically variable plant, with approximately forty nominal varieties that occur in a wide variety of habitat types. Additionally, spotted locoweed hosts a seed-borne, and thus heritable, fungal endophyte that synthesizes a bioactive alkaloid which causes millions of dollars in damages to livestock annually. We took a genotyping-by-sequencing approach to characterize the population genetic structure of spotted locoweed that allowed us to investigate the link between host plant genetic variation and associated fungal endophyte communities. We observed that differences between endophyte communities, and overall endophyte diversity, were shaped by host genetics, population proximity, and variation in abiotic habitat. However, the strongest influence on endophyte community structure was the relative abundance of the

heritable fungal endophyte of spotted locoweed. Interestingly, the prevalence, or even occurrence, of this fungal endophyte varied across the West, as did the concentration of the bioactive alkaloid it synthesized. These results bring up important questions about the ecological and evolutionary consequences of geographically variable interactions between plants and their endophytes. "

11 Matt Jones, Jones Claudia Wierzbicki, Lorraine Lawrence, Kieran Bates, Thomas Bell, Vania Braga, Matthew C. Fisher, Silwood Park Campus, Ascot, Berkshire
Development of an in-vitro culture system for studying the interactions between a host, resident bacteria and an invading fungus

Tissue culture systems that allow the co-cultivation of host tissues with both resident and invading microbes are highly desirable for understanding how symbiotic relations affect pathogenesis. Since the discovery of Janthinobacterium lividum's association with amphibian skin and role in reducing the infectivity of the amphibian chytrid fungus, Batrachochytrium dendrobatidis (Bd), there has been a surge of interest in finding similar symbiotic associations between skin bacteria and this emerging fungal pathogen. However, this research has been hindered by the lack of techniques available for studying the interactions between Bd and skin bacteria in situ. Consequently, most interactions are inferred from the microbiomes of amphibian hosts (as they relate to infection prevalence) or from Bd inhibition assays conducted outside the host in high nutrient laboratory growth media. We present the ongoing development of a novel skin explant culture system that allows the culture of host tissue alongside their resident microbiomes. Our system opens up new possibilities both for finding new skin symbionts beneficial in amphibian hosts' fight against chytridiomycosis and for delving further into the exact nature of the interactions between host, bacteria and pathogen.

12 Will Ludington, Benjamin Obadia, Vivian Zhang, Tuzun Guvener, Javier Ceja-Navarro, Eoin Brodie, Bill Ja
UC Berkeley
Stochastic colonization underlies gut microbiome stability
The species composition of the gut microbiome impacts host health, but the processes determining this composition are largely unknown. An unexplained observation is that gut species composition varies widely between individuals but is largely stable within an individual over time. Stochastic factors during establishment may drive these alternative stable states, which can influence susceptibility to pathogens such as Clostridium difficile. Here we sought to quantify and model the dose response, dynamics, and stability of bacterial colonization of the gut using germ-free Drosophila melanogaster (fruit fly). Our precise, high-throughput technique based on the capillary feeder assay revealed stable between-fly variation in colonization even when individual germ-free flies were fed natural commensal bacteria (n = 17 strains representing 13 species, including Lactobacillus plantarum, a probiotic of flies and humans). Some flies were colonized while others remained germ-free even when fed extremely high bacterial doses. Thus, alternative stable states of colonization exist even in the simple germ-free fly. These alternative states are driven by a fundamental asymmetry between the inoculum population and the stable population that is mediated by spatial localization and a bottleneck. Prior colonization with other bacteria reduced the chances of subsequent colonization, thus increasing the stability of higher diversity guts. Therefore, stable gut diversity may be driven by inherently stochastic processes, which has important implications for combatting infectious diseases, such as Clostridium difficile, and for stably establishing probiotics, such as Lactobacillus plantarum, in the gut.

13 Quinn McFrederick, Peter Graystock
UC Riverside
Microbiome selection protects bees from pesticides
Determining microbiome function remains a major challenge. By adapting methods first used in plants, we have enhanced the protective function of the bumble bee microbiome against pesticide stress. We challenged bees with the pesticide imidacloprid at the LC50 at 11 days dose, then passaged the microbiomes of the surviving

bees to newly emerged, sterile bees. As a control, we inoculated sterile bees with microbiomes of bees never exposed to imidacloprid. After two passaging rounds, survivorship of the selected microbiome treatment bees increased significantly compared to control bees. This effect persisted until the end of the experiment, and 16S rRNA gene profiling showed that microbiomes in treatment versus control bees significantly differed. To our knowledge, this is the first example of differential microbiome propagation working in animals. This is also the first successful application of a method for increasing bee survival when the bees are challenged with one of the most-used pesticides. We are therefore further pursuing both basic and applied aspects of this research

14 Dan Naylor, Stephanie DeGraaf, Elizabeth Purdom, Devin Coleman-Derr
University of California, Berkeley, "
The Effect of Drought and Host Genotype on the Grass Root Microbiome
Plant health is intimately intertwined with the communities of microbes that live on and within their roots. However, the processes that influence root microbiome composition are poorly understood. Community composition might be expected to vary by a number of factors, including proximity to the root, water availability, and plant host genotype. We hypothesized that microbiome dissimilarity would positively correlate with host phylogenetic distance, and furthermore that imposing drought would provoke conserved drought stress responses in plants that would lessen the strength of this correlation. To test this hypothesis, we examined root microbial communities within bulk soil, rhizosphere, and root endosphere samples, for 18 distinct Poaceae grass lineages for both drought and well-watered conditions. Host phylogenetic distances based on chloroplast sequences correlated with microbiome dissimilarity in most root and rhizosphere samples, though the effect size of this correlation was less in drought samples than control. We also found a significant enrichment of certain bacteria lineages by watering regime, most notably a universal enrichment of class Actinobacteria in drought that was enhanced in roots compared to soil. Our results suggest evolutionary history might be a predictor of root microbiome composition, and that drought provokes common responses in the root microbiome that might serve as a basis for microbial-based soil amendment strategies for drought tolerance in crops.

15 Kenjiro Quides, Glenna M. Stomackin, Hsu-Han Lee, Jeff H. Chang, Joel L. Sachs
University of California, Riverside
Lotus japonicus alters the in planta fitness of Mesorhizobium loti dependent on symbiotic nitrogen fixation
To maintain the costly service of nitrogen fixation in rhizobial symbiosis, legumes must impose selection that favors symbiotically effective rhizobial genotypes. But it is unclear what stimuli legumes use to trigger control over rhizobia or which stages of the symbiosis can be manipulated by the host. We used Lotus japonicus and Mesorhizobium loti to test hypotheses about how and when legume hosts bias plant investment towards effective symbionts. L. japonicus hosts were inoculated with nearly isogenic M. loti strains that only differed in nitrogen fixation function, including the wildtype (MAFF303099), a mutant with mediocre nitrogen fixation (STM30), and a nonfixing mutant (STM6). In both single and mixed inoculation experiments the less effective rhizobial strains exhibited reduced fitness in planta relative to the wildtype. Coinoculated hosts consistently formed fewer nodules with the less-effective mutants, and the less effective strains were found at lower population sizes within host nodules. Moreover, we found a linear relationship between symbiotic effectiveness and rhizobial fitness. Our results suggest that L. japonicas can adaptively modulate the fitness of symbionts in single and coinoculation settings, and that is does so relative to the symbiont effectiveness.

16 Kaleigh Russell, Quinn McFrederick
UC Riverside
Effects of climate change on nectar microbes and pollinator preference
The study of plant-pollinator networks is beginning to incorporate the interaction of microbial communities on and within flowers and their pollinator. Nectar, an important resource to many pollinators, is inhabited by many

microbes such as yeasts and bacteria, which have been shown to influence pollinator preference. This dynamic and complex network is even more influenced by the changing climate. Warming caused by climate change is already responsible for 1C increase in global surface temperatures, making 2016 the hottest year on record. To understand how this warming is influencing nectar microbial communities we performed a choice assay with the common eastern bumble bee, Bombus impatiens. Fifty bees were given the choice between synthetic nectar with an environmental microbial community incubated at the average high temperature in Riverside, Ca, and synthetic nectar with the same microbial community, but incubated at a climate change predicted temperature. Bumble bees significantly preferred nectar that had microbial communities incubated at current average temperatures over climate change predicted temperatures. Temperature directly influenced the microbial community in nectar, which caused changes to nectar chemistry and ultimately, influenced bumble bee preference. Further work is being done looking at the implications of the influence of temperature change on nectar microbial communities in the field.

17 Joel Sachs, John Regus, Kenjiro Quides, Jeff Chang
UC Riverside
Cell autonomous sanctions in legumes target ineffective rhizobia during mixed infections
To maximize benefits from symbiosis legumes must sanction ineffective rhizobia that nodulate hosts without fixing nitrogen. The capacity of legumes decrease relative fitness of ineffective rhizobia – known as sanctions – is predicted to work at the whole-nodule level. However, whole nodule sanctions would make the host vulnerable to mixed nodule infections, which are a common feature of this symbiosis. Here, we present and test a cell-autonomous model of legume sanctions that can resolve this dilemma . We investigated histological evidence in two diverged legume species, Acmispon strigosus and Lotus japonicus. We used clonal and mixed genotype rhizobial treatments of rhizobia that naturally vary in nitrogen fixation as well as Fix- mutants with fluorescence-protein tags. Both hosts exhibited evidence of cell autonomous sanctions. Only plant cells harboring ineffective rhizobia exhibited features of accelerated nodule senescence, including collapsed vacuoles, ruptured symbiosomes, and bacteroids that are released into the cytosol. Our data implicate an elegant mechanism by which legumes can detect and defend against ineffective rhizobia even when nodules harbor a mix of effective and ineffective rhizobial genotypes. The evidence for cell autonomous sanctioning resolves the dilemma between empirical evidence of coinfected nodules and the whole-nodule model of legume sanctions.

18 Sabah Ul-Hasan, Robert M Bowers, Tanja Woyke, Mark Sistrom
UC Merced
Searching for Microbes in the Uncharted Venomous Territories of Marine Cone Snails
Californiconus californicus is one of over 800 members of Conidae, a family of venomous marine gastropods. The California cone snail is a Conidae outgroup in (1) being the only species found along the California-Baja coast and (2) exhibiting extremely generalist predatory behavior. Conidae venom chemistry and phylogenetics are extensively studied, but surprisingly little is known about C. californicus ecology or microbes within venomous animals. Found across three marine ecoregions and possessing an easily dissectible venom duct, C. californicus serves as a dynamic model for bridging macroecology and microbiology. We ask if microbes influence venom production in their hosts, focusing on C. californicus as an underutilized, cross-disciplinary model. We initially examined host microbial community and venom chemistry variation in one of the three marine ecoregions (Puerto Nuevo, MX). DNA was extracted from the foot, hepatopancreas, venom duct, and eggs of five animals for 16S and 18S iTag sequencing. Animals were collected at an arbitrary coastal point during low tide, with second (0.5 km) and third locations (1.5 km) northward. A total of twenty animals were collected for corresponding venom chemistry characterization. Water, sediment, and captive C. californicus were also sequenced as referenced controls. We hypothesize microbial communities differ according to anatomy, life stage, medium, time of day, and year, but maintain conservation between individuals in a given location. A

conserved microbial community in the venom duct may infer previously unidentified symbiotic relationships, which we aim to further investigate via antibiotic treatments in a laboratory setting.

19 Chris Wall, RD Gates
Hawai'i Institute of Marine BIology
Patterns of Symbiodinium diversity in the coral Montipora capitata from shallow reefs of the remote Northwestern Hawaiian Island
Reef corals form symbiotic relationships with a diversity of dinoflagellates in the genus Symbiodinium. The composition of a coral's symbiont community differs according to coral species, geography, and environmental conditions. The coral Montipora capitata is found across the Hawaiian archipelago and associates with clade C Symbiodinium (namely, C31) and D1a symbionts, with the presence of the latter often correlating with abiotic stress. In the remote Northwestern Hawaiian Islands (NWHI) D1a has been reported at French Frigate Shoals, but constrained sampling efforts and targeted depth zones (>10 m) have left our understanding of the distribution of coral-host assemblages in shallow habitats in this area incomplete. To address this gap, we used qPCR to examine the Symbiodinium communities in M. capitata (N= 348) collected at <2-14 m depth from 40 sites spanning 7 island/atolls and tested for the presence of clades A, C and D. Clade C was the only symbiont detected in > 80% of corals at each site. Clade D was observed in corals from French Frigate Shoals and Laysan Island (4% and 11% of corals, respectively). Corals associating with clade D were in mixed communities with clade C symbionts, although D was always the dominant member when detected (89–98% of the community). Fourteen and 20% of the corals found in symbiosis with clade C at Lisianski and Laysan Island respectively, also harbored clade A symbionts. Clade A was also found in 2–3% of corals from Kure Atoll and Maro Reef. Our findings support the conclusion that M. capitata host-symbiont assemblages that show stability at geographic scales, suggesting local adaptation of either the host or symbiont. While, alternative host-symbiont assemblages exist in low numbers, recent environmental challenges (archipelagic bleaching) at some sites may have allowed more opportunistic clades (clades D, A) to infect corals. The stability or long term implications of these associations for the health of the coral host is unknown.

20 Su'ad Yoon, Josh Harrison, Matthew Forister, Angela Smilanich
University of Nevada, Reno
Examining the role of maternal microbes in the insect immune response: an example of wild immunology
In recent years, the burgeoning field of ecological immunology has gained attention due to the realization amongst both immunologists and ecologists that immune response is a major component of fitness in natural populations, and that variation in immune response is often linked to variation in ecological factors. Broadly, the goal of ecological immunology is to investigate the internal and external factors regulating immune function, and how these factors may affect the spread of disease in wild populations. The examination of natural microbiome variation and its relationship to the immune system in wild populations also represents a gap in our current knowledge of ecological immunology. I examined the role of maternal microbes in mediating the immune response of the specialist butterfly, Lycaeides melissa. L. melissa has colonized the exotic legume Medicago sativa in the past 200 years; L. melissa frequently utilizes M. sativa throughout its range despite the fact that L. melissa larvae suffer both reduced survival and decreased adult fecundity on this host, compared to the native host Astragalus canadensis. Maternal effects are widespread throughout biology, however, only recently have biologists become aware of how important vertically transmitted microbes can be for the regulation of health. In insects, a few well-studied vertically transmitted bacteria are known to have profound effects on health and pathogen resistance. However, what is less well understood is how the microbial community as a whole affects performance and immune response, especially in the context of novel host use. In this study, I conducted a comparative rearing experiment examining host by site interactions, using gravid adult females collected from one A. canadensis site (Washoe Lake, NV) and one M. sativa site (Verdi, NV). After acquiring eggs from females collected at these sites, I removed maternal microbes from these eggs using a series

of anti-microbial washes (PVP-I, contact solution, and distilled water) for each host by site treatment group, leaving a subset of unwashed eggs for each treatment as a control. Thus, the goal of this project was to the compare the relative importance of maternal microbes versus host plant microbes in regulating the immune system of L. melissa. Larvae were reared until 4th instar and then assayed for both standing immune response (prophenoloxidase) and induced immune response (melanization). Larval performance was also measured as final weight. Finally, larvae were sequenced for bacteria using Illumina MiSeq sequencing of 16S rRNA in the V4 region.

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