

Final Schedule

3RD ANNUAL
YOSEMITE SYMBIOSIS WORKSHOP
MAY 25-26, 2013



Yosemite Symbiosis Workshop
Sierra Nevada Research Institute
<http://www.medinalab.org/portal/symbiosis-workshop>

Co-organized by Monica Medina and Joel Sachs

Final Schedule

Saturday May 25

Lunch 12:00-1:00pm

Session I **Mediation, regulation and control in symbiosis**

- 1:00-1:15 Rita Auro Bacteriophage adherence to mucus: Protecting the underlying epithelium from cell death
- 1:15-1:30 Ross Cunning Dynamic regulation of endosymbionts in reef corals maximizes interaction benefit in a changing environment
- 1:30-1:45 Katie Barott Mechanisms for pH sensing and regulation in corals
- 1:45-2:00 Malcolm Hill Evolutionary and ecological significance of sponge-*Symbiodinium* symbioses: genetic regulation of uptake and maintenance in sponges
- 2:00-2:15 Esti Larrainzar Deep sequencing of the *Medicago truncatula* root transcriptome reveals a massive early response to Nod factor and ethylene signals

15 minute break for coffee and snacks

Session II **New systems and new paradigms of symbiosis**

- 2:30-2:45 Zareen Khan Poplar and willow endophytes improve growth in crop plants and grasses
- 2:45-3:00 Eunsoo Kim Diversity and transcriptome sequencing of green algae that associate with amphibian embryos
- 3:00-3:15 Carolin Frank Are bacterial endosymbionts the hidden source of nitrogen in forests?
- 3:15-3:30 Justine Garcia Partner specificity in an environmentally-acquired insect-*Burkholderia* symbiosis

30 minute break for coffee and snacks

Session III **Population dynamics and diversification of symbiotic partners**

- 4:00-4:15 D. Coleman-Derr Mining the *Agave* microbiome for adaptations to arid environments
- 4:15-4:30 Patrick O'Grady Hawaiian insects, plants and microbes: Species interactions and adaptive radiation
- 4:30-4:45 Vera Tai The diversity and co-evolution of protistan and bacterial hindgut symbionts
- 4:45-5:00 Chris I. Smith Population genomics of an obligate pollination mutualism

Session IV **Poster Session - 5:00-6:00 (beer, wine and snacks provided)**

- KEYNOTE LECTURE 6:00 – 7:00 John N. Thompson Relentless Coevolutionary Dynamics

Dinner 7:00-8:30pm

Final Schedule

Sunday May 26

Breakfast 8:00-9:00am

Session V **Multiparty interactions: moving beyond the 'host-versus-symbiont' paradigm**

- 9:00-9:15 Seabird McKeon High temperature environment drives cascading fitness effects through multi-party reef coral mutualism
- 9:15-9:30 Lauren A. Paul A role for hypervariable immunoglobulin-like domains on phage: Accumulation and evolution to host surfaces
- 9:30-9:45 Nicole Gerardo Impacts of symbiosis and alternative host plants on the development of the invasive stinkbug, *Megacopta cribraria*
- 9:45-10:00 Jesse Zaneveld Runoff, overfishing, and thermal stress synergistically impact coral reefs by altering microbial interactions
- 10:00-10:15 H. Allen Herre Foliar endophytic fungi induce shifts in host genetic expression coupled to physiological changes and increased pathogen resistance in the tropical tree *Theobroma cacao L.*

30 minute break for coffee and snacks

Session VI **Corals & *Symbiodinium* as a model of symbiosis in a changing world**

- 10:45-11:00 Thomas Oliver Ocean-scale environmental drivers on *Symbiodinium* distributions: Resistant *Symbiodinium* reliably appear in hot, productive habitats
- 11:00-11:15 Steven Quistad Induction of apoptosis in *Acropora yongeei* reveals 550 million years of evolutionary conservation
- 11:15-11:30 Melissa S. Roth Coral fluorescence and its influence on symbiotic algae in mesophotic reef-building corals
- 11:30-11:45 Dan Barshis Genomic signatures of increased stress tolerance in reef corals
- 11:45-12:00 Andrew Baker Creating chimeras: Experimental manipulations of reef coral symbioses for fun and profit (Not the monetary kind)

Lunch 12:00-1:00pm

Final Schedule

Full talk abstracts:

KEYNOTE

John N. Thompson, University of California, Santa Cruz
Relentless Coevolutionary Dynamics

We now know that coevolution is not a slow and stately process. It is often fast and relentless at every temporal and spatial scale. We have evidence of rapid coevolutionary change in environments ranging from relatively simple microcosms to complex communities in nature. The process generates ever-shifting mosaics of adaptation and counter-adaptation across ecosystems. Most coevolutionary changes do not lead to sustained directional change in species, but they are ecologically important and evolutionarily crucial as the means by which populations persist. It is likely that we are still underestimating the rates and importance of ongoing coevolution among interacting species.

Rita Auro, San Diego State University
Rita Auro, Jeremy Barr, and Forest Rohwer

Bacteriophage Adherence to Mucus: Protecting the Underlying Epithelium from Cell Death

Bacteriophage (phage) and bacteria are known to be associated with mucosal surfaces. Mucus is primarily composed of mucin glycoproteins that constitute the protective mucosal surface for the organism. Our research details a mucin-dependent increase in phage abundance on all surfaces that produce and secrete mucus. From these findings, we propose the Bacteriophage Adherence to Mucus (BAM) model, in which phage bind to mucus and provide a specific non-host-derived layer of immunity on mucosal surfaces.

Using *Escherichia coli* (*E. coli*) bacterial host and its respective T4 phage, we performed in vitro studies using tissue culture (TC) cells, both with and without surface mucus. Mucus producing A549 human lung epithelial cells were cultured and grown as monolayers. Further, an A549 shRNA mucus knockdown cell line (MUC-) was created to reduce mucus production in A549 cells. Both cell lines were exposed to *E. coli* (10⁷ ml⁻¹) overnight, either with or without 30 minute pre-treatment with T4 phage (10⁷ ml⁻¹). Subsequent cell death was quantified flow cytometry.

Results show that pretreatment of mucus-producing TC cells with phage reduced A549 cell death from *E. coli* bacterial insult by 10-fold (n=12, ****P<0.0001). Conversely, phage pretreatment of non-mucus producing MUC- cells decreased associated TC cell death by only 2-fold. The presence of a mucus layer, in combination with phage pretreatment, resulted in a 3.6-fold increase in A549 cell survival (n=12, *P=0.0181). Results show significant phage protection of mucus producing A459 cells, and decreased protection for non-mucus producing cells when exposed to bacteria. Phage adherence to mucus reduced bacterial colonization of the mucosal surface and protected the underlying epithelium from cell death.

Andrew Baker, University of Miami

*Creating chimeras: Experimental manipulations of reef coral symbioses for fun and profit** **Not the monetary kind*

Despite considerable research on diversity in coral-algal symbiosis over the last 18+ years there have been comparatively few attempts to experimentally manipulate this system in a controlled laboratory setting. There are several practical reasons for this (corals grow slowly, tend to be very environmentally sensitive, and the symbiotic environment is almost impossible to characterize or regulate in absolute terms). However, compared to field-based observation and monitoring, the ability to control environmental conditions and to work with clonal host lineages (using replicate cores of individual coral colonies) provide many advantages for symbiotic studies. By combining this laboratory approach with a relatively recent actin-based molecular assay that allows changes in symbiont abundance to be accurately quantified (even when symbionts are extremely rare), my lab has been able to address a variety of questions that include (1) how environmental conditions drive symbiont community structure; (2) whether rare symbionts affect host resistance/resilience to stress; (3) how disturbance (i.e., bleaching) disrupts community structure and facilitates change; and (4) how manipulations of the symbiotic milieu influence community dynamics. These studies have helped reveal the remarkable dynamism of these mutualisms, with potential implications for the conservation of these endangered organisms and the critical ecosystems they build.

Final Schedule

Katie Barott, Scripps Institution of Oceanography, University of California San Diego
Katie Barott, Megan Barron, and Martin Tresguerres
Mechanisms for pH sensing and regulation in corals

Maintaining acid/base homeostasis is critical for all forms of life. While it is known that corals increase extracellular pH to promote calcification while undergoing daily fluctuations in intracellular pH as a result of photosynthesis, little is known about how corals sense or regulate these changes. One potential player that we investigate here is the enzyme soluble adenylyl cyclase (sAC), which is directly stimulated by bicarbonate to produce cAMP in many organisms. In sharks, sAC activity modulates proton pumps (V-type H⁺-ATPase [VHA]), which help compensate for changes in pH. Investigation of the *Acropora digitifera* genome revealed two isoforms of sAC, as well as VHA. Bicarbonate-stimulated cAMP production was detected in homogenized coral tissue as well as in purified recombinant coral sAC, supporting the hypothesis that sAC is active in corals. Endogenous cAMP levels in live corals were higher during the day compared to the middle of the night, suggesting a diel cycle. These results suggest that sAC is an important regulator of coral physiology, especially in response to light, acid/base disturbances and inorganic carbon levels. Finally, immunolocalization of VHA found that the protein is most abundant in coral cells hosting zooxanthellae, suggesting that VHA plays a role in regulating intracellular acid/base conditions in response to or in promotion of photosynthesis (e.g. as part of a carbon concentrating mechanism).

Dan Barshis, NOAA Fisheries-UC Santa Cruz
Barshis DJ, Ladner JT, Oliver TA, Seneca FO, Traylor-Knowles N, and SR Palumbi
Genomic signatures of increased stress tolerance in reef corals

Corals have been found to differ substantially in physiological susceptibility to environmental stress. The molecular mechanisms behind these differences in coral stress tolerance are not well known, yet are critical to the persistence of both corals and coral reefs in the future. Here, we compare conspecific thermally sensitive and thermally tolerant corals using advances in DNA sequencing technologies (RNA-Seq) to identify the molecular pathways underlying differential physiological tolerance limits. We subjected these phenotypically distinct corals to simulated bleaching stress and found that tolerant corals had higher pre-stress expression and reduced reaction to thermal stress across 60 coral genes, as compared to the sensitive corals. These “frontloaded” transcripts included not only established thermal tolerance genes such as heat shock proteins and antioxidant enzymes, but also a much broader array of molecular processes such as apoptosis regulation, tumor suppression, innate immune response, and cell-cell adhesion. We propose a hypothesis wherein constitutive “frontloading” of coral stress genes enables an individual to maintain physiological resilience despite frequently encountered environmental stress. In contrast, Symbiodinium gene expression was unaltered by the heat stress, despite visible signs of bleaching. However, up to 36% of orthologous gene pairs showed significant differential expression between clades within control and heated samples, in the same common garden location, and within the same identical host genetic background. These data paint a fundamentally different picture between the transcriptomic response to stress of the cnidarian host and the dinoflagellate symbionts.

Devin Coleman-Derr, Joint Genome Institute
Devin Coleman-Derr, Stephen Gross, Tanja Wojke, Gretchen North, Laila Partida-Martinez, Kristen DeAngelis, Scott Clingenpeel, Susannah Tringe, Axel Visel
Mining the Agave microbiome for adaptations to arid environments

A major challenge facing the biofuels industry is the identification of high-yield plant feedstocks that can be cultivated with minimal resource inputs without competing for land and water supplies with existing food crops. Recent research has demonstrated that the Agave plant, cultivated in Mexico and Southwestern United States for the production of fiber and alcohol, meets these criteria. Agaves grow on non-arable rocky soils in regions characterized by prolonged drought and extreme temperatures, due in part to physiological adaptations that prevent excess water-loss in arid environments. Plant-microbial symbioses can play a role in helping plants adapt to heat and drought stress, increasing the accessibility of soil nutrients, or compete with plant pathogens. Whether agaves have similar beneficial microbe interactions in their native environment is unknown. We aim to provide a comprehensive characterization of the Agave microbiome, with the goal of identifying specific community members that may contribute to Agave biotic and abiotic stress tolerance. We are investigating the microbial communities of both wild and cultivated Agave species in California and in Mexico. For each specimen, microbial samples were collected from the phyllosphere (leaf surface), rhizosphere (root surface) and leaf and root interiors (endospheres). A 16S iTag survey of these samples will be used to select specific microbiome compartments for further characterization, including shotgun metagenomics and single-cell genomics. Our project will expand our understanding of microbial diversity in desert soils, catalog and characterize the microbial factors that contribute to

Final Schedule

Agave's successful adaptation to the extreme environments of its endemic range. Ultimately, we aim to enable microbiome manipulation aimed at improving the suitability of Agave for use in the rapidly growing biofuels industry. known, with the goal of identifying specific community members that contribute to biotic and abiotic stress tolerance in their host. We have investigated and compared the microbial communities of native and cultivated Agave species in California and Mexico under a panel of different environmental conditions. These microbiomes were collected from the root, the leaf, and surrounding soils, and an initial 16S iTag survey across all samples is being used to select a subset for shotgun metagenomics and single-cell genomics. This research will expand our understanding of microbial diversity in desert soils and has the potential to aid research aimed at improving the suitability of Agave for use in the rapidly growing biofuels industry.

Ross Cunning, RSMAS/University of Miami

Ross Cunning, Nathan Vaughan, Phil Gillette, Tom R. Capo, Juan L. Maté, Andrew C. Baker

Dynamic regulation of endosymbionts in reef corals maximizes interaction benefit in a changing environment

The costs and benefits of symbiotic interactions may change as a function of the population density of the interacting partner, with maximum net benefit occurring at some "optimal" density. Density-dependent costs and benefits may shift as changing abiotic conditions affect organismal physiology, thereby shifting the optimal partner density. Symbiotic organisms may respond adaptively by regulating partner density to match this shifting optimum. To explore the capacity for dynamic regulation in a coral-algal endosymbiosis, we studied population dynamics of two different Symbiodinium types in the coral *Pocillopora damicornis* under varying abiotic conditions. We found that symbiont populations were readjusted to different equilibrium densities depending on their genetic type and environment. The observed patterns can be explained by modeling functional responses of the costs and benefits of symbiosis under different conditions, which suggests that dynamic regulation of partner density may allow symbiotic organisms to sustain maximum interaction benefit in a changing environment.

Carolyn Frank, UC Merced

Carolyn Frank, Alyssa Carrell, Emily Wilson

Are bacterial endosymbionts the hidden source of nitrogen in forests?

Nitrogen (N) is the nutrient that most often limits plant growth in natural ecosystems, still the source of N to temperate and boreal forests is a long-standing ecological mystery. In these ecosystems, N accumulation in the soil and plants is much higher than expected given the apparent N inputs. We have circumstantial evidence that bacterial symbionts—called endophytes—living inside the aerial parts of plants—are a hidden input of N in the forest, and that the symbiosis between plants and endophytes may be more intimate than previously thought.

Justine Garcia, Emory University

Justine Garcia, Euisun Chung, Claire Trincot, and Nicole Gerardo

Partner Specificity in an Environmentally-Acquired Insect-Burkholderia Symbiosis

Many organisms acquire beneficial microbes (symbionts) from the environment. These host-symbiont associations can be quite specific and evolutionarily stable, but they are also more prone to lower partner specificity and partner switching. Here, we investigated the partner specificity in an insect-Burkholderia symbiosis. We screened four sympatric broad-headed bug species and their habitat, including soil and food plants, for Burkholderia symbionts using 16S rRNA gene sequences of cultivated and non-cultivated strains. We found a variety of Burkholderia in the insects' midgut crypts, including strains that grouped with a clade of other insect symbionts and strains that grouped with free-living and rhizosphere-associated Burkholderia. Some individual insects were co-infected with two or more strains, but one Burkholderia strain (JRG1) was highly abundant in all four insect species. This strain groups within a clade of insect symbionts and is also the dominant symbiont in other stinkbug species with varying geographic distributions. Consequently, there was no significant difference in symbiont strains by host species or by host geography when our focal species were analyzed separately and together with other Burkholderia-hosting stinkbugs. Finally, we investigated the bacterial diversity in the nitrogen-fixing root nodules of the insects' food plant, *Lespedeza* spp., and in the soil around these plants and found a number of Burkholderia strains. Many Burkholderia strains were found in the soil and some were similar or identical to symbiont strains found in insect guts. Strikingly, though, all of the Burkholderia strains isolated from *Lespedeza* nodules grouped with the clade of insect symbionts and some strains were nearly identical to strain JRG1, the most common symbiont strain in the insects. This finding indicates sympatric plant and insect hosts may harbor the same or similar symbionts and the soil may be a symbiont source for both hosts. We conclude that broad-headed bugs are exposed to a variety of potential Burkholderia symbionts in the environment, but there is one symbiont that is preferred in a number of broad-headed bug species and that the presence of this same symbiont in a food plant may increase its abundance in the environment, specifically the soil.

Final Schedule

Nicole Gerardo, Emory University
Nicole Gerardo, Nelle Couret, Lynn Huynh

Impacts of symbiosis and alternative host plants on the development of the invasive stinkbug, Megacopta cribraria

In October of 2009, an invasive hemipteran bug from Asia, *Megacopta cribraria*, was first identified on kudzu vine in Georgia and has since been observed spreading across the Southeast United States, utilizing both kudzu and soybeans as host plants. In Asia, where *Megacopta* is a devastating pest of soybeans, the ability to utilize soybeans is dependent on infection by a specific strain of obligate bacterial symbiont. Our group is characterizing this symbiotic association from the earliest stages of its symbiotic invasion. We have characterized patterns of *Megacopta* development and survival on alternative hosts plants and in the presence and absence of symbionts; the impact of symbionts on development depends on the host plant on which the bugs are reared. We are coupling this investigation with profiling of host transcriptional profiles, focusing on the symbiont housing region of the gut, to begin to understand how host plants and symbionts impact host metabolism, stress and development.

Malcolm Hill, University of Richmond
Hill M, Hill A, Cotman C, Friday S, Heist T, McCauley M, Peterson K, Richardson C, Riesgo A, Strehlow B
*Evolutionary and ecological significance of sponge-Symbiodinium symbioses:
genetic regulation of uptake and maintenance in sponges.*

Symbioses involving Symbiodinium are arguably the most important ecological interaction on coral reefs because zooxanthellae energetically subsidize the entire community. These algal symbionts also enhance rates of calcification, and thus facilitate the creation of three-dimensional structure for all organisms. Nonetheless, we have a remarkably limited understanding of the symbiont's niche (e.g., why is zooxanthella distribution so restricted among sponge hosts?). We will present two novel hypotheses derived from our work with sponges (the Magnesium Inhibition Hypothesis and the Arrested Phagosome Hypothesis) that help explain well-known aspects of Symbiodinium associations regardless of taxonomic status of the host. We will argue that sponges afford unique methodological opportunities and broad-reaching insights into the associations found in all other Symbiodinium-based symbioses. We will present research examining the genetic regulation, physiological integration, and ecological/evolutionary significance of Symbiodinium symbioses involving sponge hosts. Data from suppressive subtractive hybridization and transcriptomic-based approaches have identified genes differentially regulated during zooxanthella uptake. Gene expression profiles will be correlated with various stages of reinfection as aposymbiotic sponge hosts re-acquire their algal partners. The physiological integration of partners and capacity of Clade G Symbiodinium to tolerate stressful conditions will also be presented. Finally, we will evaluate in hospite "residence time" as a useful heuristic for studying zooxanthella symbioses.

E. Allen Herre, Tulane University

E. Allen Herre, L.C. Mejía, S. Van Bael, J. Stitt, S. Zulkafli, Y. Zhang, Z. Shi, K. Winter, J. Sparks, M.J. Guiltinan, S.N. Maximova
Foliar endophytic fungi induce shifts in host genetic expression coupled to physiological changes and increased pathogen resistance in the tropical tree *Theobroma cacao* L.

Endophytic microbes are increasingly recognized as ubiquitous associates of healthy, apparently asymptomatic plants. Although experiments show that endophyte presence can contribute to host resistance to pathogen damage, mechanisms underlying these benefits and any potential, associated costs are largely unexplored. We compared genetic expression in *Theobroma cacao* L. leaves that were endophyte-free (E-) or inoculated with *Colletotrichum tropicale* (E+), the dominant fungal endophyte in healthy *T. cacao* leaves. We used a cDNA microarray to demonstrate that E+ plants exhibit significant up- and down- regulation of hundreds of genes, relative to E- plants. Many of these genes code for known components of photosynthetic, cell wall, or pathogen and other stress related pathways. In subsequent growth chamber and green house experiments, we showed: a) reduced maximum rates of photosynthesis (Amax), b) heavier stable isotope ratios of both nitrogen and carbon in the leaves, and c) increased lignin and cellulose content of host cell walls in E+ plants. Finally, we demonstrated that one of the genes of previously unknown function (3107) that was up-regulated by inoculation with the endophyte *C. tropicale* conferred increased resistance to pathogen damage. Collectively, these experiments indicate that endophytic fungi can exert profound influences on host gene expression that are often coupled to changes in multiple aspects of the host's anatomy, physiology, and ability to resist pathogen damage. Thus, increased pathogen resistance in E+ plants appears to be at least partially due to enhanced induction of intrinsic host defense pathways, and comes at the potential costs of reduced photosynthetic capacity and endophyte metabolism of host tissues and / or products.

Final Schedule

Zareen Khan, University of Washington

Zareen Khan, Grant Guelich, Ha Phan, Regina Redman, and Sharon Doty
Poplar and willow endophytes improve growth in crop plants and grasses

Several endophytes isolated from poplar and willow were tested for their effects on commercially important crops including corn, tomato, pepper, squash, sunflower, and grasses. Most of these endophytes produce growth hormones such as indoleacetic acid and have the nitrogenase gene essential for nitrogen fixation. In our study we found that certain crops and grasses that were inoculated with these endophytes had better viability, early flowering and fruiting, increased plant growth and fruit yields when grown in nitrogen limited soil. In the grasses, in addition to promoting plant growth there was also more nitrogen incorporated in the plant tissues, indicative of nitrogen fixation. Overall, this study demonstrated the successful colonization, growth enhancement, and increased fruit yields of certain crops by the addition of poplar and willow endophytes. Future studies should be directed towards the potential use of these strains for crop development and fruit yield enhancements under poor nutrient conditions in field studies.

Eunsoo Kim, American Museum of Natural History

Diversity and transcriptome sequencing of green algae that associate with amphibian embryos

Symbiosis between spotted salamander embryos (*Ambystoma maculatum*) and green algae has been known for more than 120 years. While the association was thought to be strictly epibiotic, our recent results have shown that algal cells penetrate both tissues and cells of the host salamander during its embryonic stage. This unexpected algal invasion has led to further questions regarding the diversity of amphibian embryo–algal symbioses and the mechanism of symbiont acquisition and maintenance. Here, I present (1) our initial batch of phylogenetic diversity data for algae that associate with North American *Ambystoma* and *Rana* amphibians and (2) preliminary results based on the analyses of transcriptome data generated from cultured green algal symbionts. I also discuss new co-culturing techniques that we are currently developing for the investigation of the association under controlled laboratory conditions.

Estibaliz Larrainzar, University of California, Davis

Estibaliz Larrainzar, Brendan K. Riely, Alex Greenspan, Hyun-Ju Hwang, Sang-Cheol Kim, Jeong-Hwan Mun, Douglas R. Cook

Deep sequencing of the Medicago truncatula root transcriptome reveals a massive early response to Nod factor and ethylene signals

Legumes are able to establish nitrogen-fixing endosymbiosis with rhizobium bacteria. This interaction is a highly regulated process that involves complex developmental changes in roots. Ethylene is a negative regulator of nodulation and it acts at different levels during symbiotic development but the molecular mechanisms underlying this regulation are not fully understood. Here we have applied RNA-seq technology to analyze the transcriptional changes occurring in roots of the model legume *Medicago truncatula* hours after inoculation with its microsymbiont *Sinorhizobium meliloti* with a two-fold aim: i) to identify symbiosis-specific genes whose transcriptional activation could be related to early NF-signaling events; ii) to characterize the ET biosynthesis and signaling pathway in *M. truncatula* and understand the regulation of these pathways at early symbiotic stages. We carried out a detailed time course (nine time points from uninoculated to 48 h post inoculation) and used four *M. truncatula* genotypes with contrasting responses to NFs: wild-type Jemalong A17, two mutants in the NF-receptors *nfp* and *lyk3*, as well as the ET-insensitive supernodulating mutant *skl*. This work provides an insightful analysis of early changes in the transcriptome of inoculated roots that we hope will contribute towards understanding the complex events occurring during NF-signaling in *M. truncatula*.

Seabird McKeon, Smithsonian Marine Science Network

C. Seabird McKeon, Tom Oliver

High Temperature Environment Drives Cascading Fitness Effects Through Multi-party Reef Coral Mutualism

Reef corals are symbiotic assemblages that can derive up to 95% of their carbon demands from endosymbiotic dinoflagellates in the genus Symbiodinium. The coral-algal symbiosis also relies on other inter-species interactions, including the mutualistic symbiosis between corals in the genus Pocillopora and crabs in the genus Trapezia. The exosymbiotic Trapezia live among branches of the coral, and benefit their host by deterring corallivores and removing sediment and debris. In return Pocillopora not only shelter, but also feed their Trapezia with photosynthetically-derived lipids translocated into tentacle tips, which the crabs clip and consume. As warming seas threaten temperature-sensitive multi-species coral assemblages, we know that genetically distinct Symbiodinium can confer distinct thermal tolerance upon the coral-algal symbiosis, but little work has highlighted the cascading effects of such symbionts on other coral associates. Here we show that in a high-temperature coral reef habitat, physiological distinctions between Symbiodinium genotypes carry effects beyond the coral-algal symbiosis and affect the fitness of associated fauna, including Trapezia. We show that in high temperature back reef pools with high proportions of heat-resistant Symbiodinium D, those Trapezia living

Final Schedule

with Pocillopora hosting the more thermally sensitive Symbiodinium C1 are smaller and less fecund than those associated with corals hosting the more heat-resistant Symbiodinium D. As smaller Trapezia are less effective at fending off corallivores, by not hosting thermally robust Symbiodinium in this high-temperature habitat a coral not only increases its risk of direct physiological effects from thermal stress, but also undermines its 'defense force', thus increasing its risk of predation.

Patrick O'Grady, UC Berkeley

Patrick O'Grady, Brian Ort

Hawaiian Insects, Plants and Microbes: Species Interactions and Adaptive Radiation

Hawaiian Drosophila depend primarily, sometimes exclusively, on specific host plants for oviposition and larval development, and most specialize further on a particular decomposing part of that plant. Differences in fungal community between host plants and substrate types may establish the basis for host specificity in Hawaiian Drosophila. Fungi mediate decomposition, releasing plant micronutrients and volatiles that can indicate high quality substrates and serve as cues to stimulate oviposition. Our results show very little sharing of fungal taxa among the substrates, and tests of community composition confirmed that the structure of the fungal community differed significantly among the substrates and host plants. Fungal community structure plays a central role in the establishment of host preference in the Hawaiian Drosophila radiation.

Thomas Oliver, University of Hawaii

Thomas Oliver, Kevin Arrigo, Stephen Palumbi

Ocean-scale environmental drivers on Symbiodinium distributions:

Resistant Symbiodinium reliably appear in hot, productive habitats

As oceans warm, high-temperature bleaching threatens reef-building corals. By hosting more temperature resistant algal symbionts, some common reef corals may elevate their thermal tolerances. Because the environmental correlates of temperature-resistant symbionts remain unclear, judging a site's likelihood of supporting such symbionts, now or in the future, is difficult. Here we gather distribution data of symbiont types from 143 studies including 80 scleractinian coral genera across 202 global localities and analyze the resulting database of Symbiodinium communities for correlations to a wide range of environmental variables. On the scale of the Indo-Pacific basin, we show strong correlations between more thermally resistant Symbiodinium genotypes and a site's long-term temperature, but no strong trend with a site's bleaching history nor history of episodic heating events. We further predict potential shifts in environmental suitability of distinct Symbiodinium genotypes by building multiple linear regression models of environmental correlates to current Symbiodinium genotype distributions and applying climate projection models. These models of future Indo-Pacific climate predict that areas now unsuitable for these thermally resistant genotypes will rapidly shift to favor their dominance. However, many of these areas currently host no such thermally resistant genotypes, raising the concern that environmental change will sweep over areas faster than dispersal and population growth can add these Symbiodinium types to reefs.

Lauren A. Paul, San Diego State University

Lauren A. Paul, Jeremy J. Barr, Forest Rohwer

A Role for Hypervariable Immunoglobulin-like Domains on Phage: Accumulation and Evolution to Host Surfaces

Bacteriophage (phage) drive microbial diversity and regulate bacterial communities throughout environmental reservoirs and the human microbiome. Previous research has established that phage accumulate in mucosal surfaces via interactions between Immunoglobulin-like (Ig-like) domains, displayed on the phage capsid, and glycan residues in mucus. This interaction reduces bacterial colonization of the mucus and protects underlying epithelia from bacterial pathology. Here, we hypothesize that mutations within hypervariable phage capsid proteins, such as the Ig-like domains, result in an enhanced phage adherence to environmental surfaces, such as mucus. Using the T4 phage model system, we positively selected for mucus-adherent phage through successive rounds of replication. These 'evolved' T4 phage demonstrated increased mucus adherence when compared to a naïve T4 phage. T4 phage genomes were sequenced and functionally profiled for glycan binding, to elucidate if changes in Ig-like domains were responsible for the increased adherence of phage to mucus. Based on the plasticity of Ig-folds and the ubiquity of phage in nature, we further explored phage adherence to alternative biological surfaces. We observed similar patterns of phage enrichment to aqueous plant surfaces, which produce a high-molecular weight polysaccharide, compared to the adjacent environment. We propose the Bacteriophage Adherence to "X" (BAX) model, demonstrating phage accumulation to any glycan-coated, host-environmental interface through adaptable Ig-like domains.

Final Schedule

Steven Quistad, San Diego State University

*Induction of apoptosis in *Acropora yongeei* reveals 550 million years of evolutionary conservation*

Coral reef health is in rapid decline worldwide yet the molecular mechanisms behind coral death remain poorly understood. The Tumor Necrosis Factor (TNF) receptor-ligand superfamily (TNFRSF/TNFSF) is a central mediator of apoptosis and it is hypothesized that the expansion of the TNFRSF/TNFSF occurred following the divergence of invertebrates and vertebrates. Here we challenge this hypothesis and identify more putative coral TNFRSF members than any organism described thus far, including humans. We then predicted Human TNF α (HuTNF α), a known inducer of apoptosis in humans, would also cause apoptosis in coral. Upon HuTNF α stimulation the coral proteome underwent an acidic shift, suggesting the induction signaling cascades. Stimulation of coral with HuTNF α also induced apoptotic blebbing, caspase activation and coral bleaching. This work identifies the first ligand/receptor system to be directly involved with apoptosis and bleaching in coral, and provides evidence for an ancient origin of the TNFRSF/TNFSF that has been functionally maintained for over 550 million years.

Melissa S. Roth, Lawrence Berkeley National Laboratory

Melissa S. Roth, Jacqueline L. Padilla-Gamiño, Xavier Pochon, Heather L. Spalding

Coral fluorescence and its influence on symbiotic algae in mesophotic reef-building corals

Mesophotic reef-building coral photophysiology is poorly understood. Similar to shallow-water coral reefs, coral fluorescence is widespread in mesophotic reef ecosystems. Coral fluorescence is produced by fluorescent proteins, which absorb high-energy light and re-emit lower energy light. We investigated patterns of coral fluorescence and its relationship with endosymbiotic dinoflagellates in the mesophotic reef-building coral *Leptoseris hawaiiensis*. In the Au'au Channel of the Hawaiian Archipelago, we observed corals expressing green fluorescence along side nonfluorescent corals over the depth range of *L. hawaiiensis* (65-125m). The coral fluorescence was attributed to two green fluorescent proteins that were depth correlated and overlapping at middle depths. Fluorescent patterns were highly variable between individuals and common fluorescence expression patterns included fluorescence in the coenosarc and/or the oral disk, and rare fluorescence phenotypes included random or striated spots in the coenosarc. Coral fluorescence was also compared with endosymbiotic dinoflagellate genotype, density, photosynthetic pigments, and photophysiology to determine the influence of coral fluorescence on algal symbionts. Studying coral fluorescence in light-limited environments like the mesophotic zone, in contrast to shallow-water high-light environments, may provide unique insights into the function of fluorescent proteins in corals.

Christopher Smith, Willamette University

Christopher Smith, Dylan Goldade, Ramona Flatz

Population genomics of an obligate pollination mutualism:

Using RAD sequencing to study coevolution of yuccas and yucca moths

The yucca - yucca moth pollination system is perhaps one of the best known of the 'nursery pollination' systems, in which insects both deliberately pollinate the flowers of their host plant and lay their eggs in the developing ovules. Despite nearly a century of research on this system, and despite much circumstantial evidence that is suggestive of coevolution, reciprocal selection has never been empirically demonstrated. We combined RAD sequencing with an FST-outliers approach to identify regions of the genome that appear to be experiencing positive selection. Using association studies, we show that these regions of the genome are also associated with variation in floral morphology, suggesting pollinator-mediated selection acting on the hosts.

Vera Tai, University of British Columbia

Vera Tai, Steve Perlman, Patrick Keeling

The diversity and co-evolution of protistan and bacterial hindgut symbionts across the lower termites as assessed using 454 pyrosequencing

Lower termites and the closely related wood-eating *Cryptocercus* cockroaches harbour a diverse community of protists and bacteria that aid in their digestion of ligno-cellulose. Many of these symbionts are the only representatives of major lineages of life and are endemic to these hindgut environments. The symbionts have also co-evolved with their host due to the vertical transmission of symbionts via proctodeal feeding. The diversity and ecology of these communities are poorly understood largely because these microorganisms are difficult to cultivate. To more accurately describe the diversity of the protist and bacterial symbionts from hindgut communities, we used 454 technology to sequence molecular taxonomic markers (the V4 region of the 18S rRNA gene for protists and the V1 region of the 16S rRNA gene for bacteria) from the hindgut communities spanning the diversity of lower termites and *Cryptocercus* cockroaches. Based on these data, the diversity of the protist symbionts is significantly higher than morphology-based observations. The bacterial diversity is approximately 10-fold higher than the protist diversity and is described for the first

Final Schedule

time for many of the hindgut communities. The diversity of symbionts from hindgut communities of related hosts are more similar to each other than to more evolutionarily distant hosts, confirming the expectation that many of the symbionts have co-evolved. Through closer examination, we identify the taxa that are more strictly co-evolving, endemic, or widely distributed. Protists tended to be more endemic than the bacteria (i.e. reflecting stronger co-evolution). We also used the sequence data to generate phylogenetic trees and identify lineages where co-evolution and diversification have resulted in the currently observed diversity in the hindguts. Because the occurrence of symbiont taxa in different hindguts can be explained relatively simply compared to most taxa from microbial communities, these hindgut communities can serve as models to understand the diversification, speciation, and evolution of microorganisms in natural ecosystems.

Jesse Zaneveld, Oregon State University, Microbiology

Jesse Zaneveld, A. Shantz, R. McMinds, C. Pritchard, R. Welsh, S. Rosales, A. Correa, C. Fuchs, K. Carver, J. Payet, A. Duran, M. Ladd, M. Kelley, B. Gunn, L. Bhatti, J. Tepper, D. Burkepile, Rebecca Vega Thurber

Runoff, overfishing, and thermal stress synergistically impact coral reefs by altering microbial interactions

We are investigating the complex microbial community associated with corals, which includes both photosynthetic algal Symbiodinium symbionts, and a variety of bacteria, archaea, fungi, viruses and phage. We hypothesize that this coral microbiota plays an important role in mediating the effects of human impacts such as agricultural runoff and overfishing on coral health. To test this hypothesis, we have so far analyzed 336 16S rRNA samples selected from a large (>1300 sample) library of coral mucus DNA samples using 454 pyrosequencing. The samples were collected during a multi-year in situ experimental intervention in which the top-down ecological effects of herbivore removal (e.g. from overfishing) were tested by enclosing coral subplots with cages, while the bottom-up effects of inorganic nitrogen and phosphorous addition (e.g. from agricultural runoff) were tested by addition of a nail carrying a fertilizer diffuser. The results thus far suggest: 1) that thermal stress, along with the treatment effects of herbivore removal and fertilizer addition have species-specific but often synergistic effects on the coral microbiota; 2) Antibiotic-producing actinobacteria may contain outbreaks of opportunistic coral pathogens including *Vibrio* spp ; 3) long-term fertilizer addition treatments significantly increases the susceptibility of *Siderastrea siderea* corals to dark spot disease. Finally, we have collaborated to develop a new software tool for estimating community metagenomes from 16S rRNA sequences (PICRUST; picrust.github.com), and are currently applying this tool to understand how the functional capabilities of the coral microbiota shift during exposure to anthropogenic stressors.

Posters

Rebecca Batstone, University of Toronto
Batstone, R.T., and Frederickson, M.E.

A preliminary investigation of nitrogen foraging and rhizobia sanctioning within Medicago roots: does a positive genetic correlation exist?

Angus Chandler, University of California, Davis
Composition and Assembly of the Drosophila microbiota

Scott Clingenpeel, DOE Joint Genome Institute
Scott Clingenpeel, Derek Lundberg, Jeff Dangl, Tanja Woyke
Investigating Arabidopsis thaliana root endophyte communities by single cell genomics

Elizabeth Green, University of California, Merced
Elizabeth Green, Sarah Davies, Mikhail Matz, Monica Medina
Using 454 sequencing to genotype Symbiodinium diversity in Montastraea species at Flower Garden Banks

Aki Ohdera, UC Merced
Ohdera, Aki, Fitt, William, Medina, Monica
Microbial community of degrading mangrove leaves and its involvement in settlement and metamorphosis of the upside-down jellyfish Cassiopea xamachana

Rita Rachmawati, UCLA
Rita Rachmawati, Paul H. Barber
Variation in bleaching susceptibility of Indonesian corals

Megan A Rua, The University of Mississippi
Megan A. Rúa, Bridgett D. Piculell, Jason D. Hoeksema
The effect of local adaptation on mycorrhizae-host relationships

Arthur Weiss Silva-Lima, Laboratório de Microbiologia Marinha
Silva-Lima, A. W.; Salomon, P.S.; Moura, R.L. & Thompson, F.L.
Diversity of Symbiodinium isolated from scleractinian corals of the Abrolhos Reef Bank, Brazil

Michele Weber, University of California, Merced

Michele Weber, Monica Medina

Robustness in coral-algal symbiosis

Emily C. Wilson, University of California, Merced

Emily C. Wilson, E. Dan Hirlleman, A. Carolin Frank

Efficient isolation of endophytic bacteria from bud tissue

Ashley Yang, American Museum of Natural History

Final Schedule

Ashley Yang, Apurva Narechania, Eunsoo Kim

A rickettsiales symbiont in the green algal flagellate *Mesostigma viride*