Final Schedule of Invited Talks

Final Schedule
4th ANNUAL
YOSEMITE SYMBIOSIS WORKSHOP
MAY 3-4, 2014

Yosemite Symbiosis Workshop
Sierra Nevada Research Institute
http://www.sachslab.com/symbiosis-2014.php
Co-organized by Monica Medina, Joel Sachs, and Becca Fenwick

Final Schedule of Invited Talks

INVITED TALKS

Saturday May 3

Lunch 12:00-1:00pm

Session I Coadaptation between species: partner control, immunity, defense, and specificity

• 1:00-1:15	Peter Tiffin	*Identifying the genetic basis and selective history of symbiosis in Medicago truncatula and Ensifer rhizobia*
• 1:15-1:30	Debbie Brock	*Is your immunity compromised by cooperative bacterial interactions? Insights from a social amoeba.*
• 1:30-1:45	Amelia Lindsey	*Experimental Evolution of Wolbachia in Recombinant Hosts*
• 1:45-2:00	Rachelle Adams	*Chemically armed mercenary ants protect fungus-farming societies*

15 minute break for coffee and snacks

Session II Molecular mechanisms that mediate onset and maintenance of symbiosis

• 2:15-2:30	Jianmin Zhong	*Biochemical Characterization of the Folate Biosynthetic Pathway in a Rickettsial Endosymbiont of Ixodes pacificus*
• 2:30-2:45	Lisl Esherick	*Possible Role for C-Type Lectins in the Establishment of Cnidarian-Dinoflagellate Symbiosis*
• 2:45-3:00	Joel Griffitts	*Upon the edge of a knife: Legume-rhizobium negotiations near the onset of nitrogen fixation*

30 minute break for coffee and snacks

Session III Origins, evolution, and diversification of symbioses

• 3:30-3:45	Joel Sachs	Epidemic population distributions of wild *Bradyrhizobium* across a landscape
• 3:45-4:00	J. Angus Chandler	*Characterization of the Aedes aegypti microbiota using RNA metagenomics*
• 4:00-4:15	Jingchun Li	*The fast and the various: free-living and commensal marine clams differ in modes of evolution*
• 4:15-4:30	Marie Braad Lund	G*enome evolution of vertically transmitted, extra-cellular symbionts of earthworms*
• 4:30-4:45	Alejandro Grajales	*Patterns of Symbiodinium spp. associations within the family Aiptasiidae, a monophyletic lineage of symbiotic of sea anemones*
• 4:45-5:00	Alyssa Carrell	*Potential for aboveground endophytic N2 fixation in subalpine conifers revealed by 16S rRNA gene analysis*

Session IV Poster Session

• 5:00-6:00 Poster presenters see below *(beer, wine and snacks provided)*

• KEYNOTE LECTURE

• 6:00-7:00 John Pringle *Aiptasia*, a model system for dinoflagellate-cnidarian symbiosis

Dinner 700-8:30pm

Final Schedule of Invited Talks

INVITED TALKS

Sunday May 4

Breakfast 8:00-9:00am

Session V Host development and reproduction: experimental systems

- 9:00-9:15 Monica Medina Sym*biosis-driven development in the upside-down jellyfish Cassiopea xamachana*
- 9:15-9:30 Jacqueline Padilla-Gamino *From Parent to Gamete: Vertical Transmission of Symbiodinium (Dinophyceae) ITS2 Sequence Assemblages in the Reef Building Coral*
- 9:30-9:45 Janja Ceh *Living in a nutrient-poor world: bacteria released with coral offspring provide nitrogen to Symbiodinium in coral larvae*
- 9:45-10:00 Thomas Oliver *Sex and the Single Gonochore: A Transcriptomic Perspective on Sex Deterimination in Porites lobata, a Gonochoristic Coral*

30 minute break for coffee and snacks

Session VI Cnidarian holobionts- Models of symbiosis in a changing world

- ~~10:30-10:45~~ **CANCELLED**~~Shaker H. Alhazeem Kuwait Artificial Reef Fisheries and Benthic Cover~~ **CANCELLED**
- 10:45-11:00 Tamar L Goulet *Ontogenetic, temporal, and spatial shifts in the symbiotic continuum*
- 11:00-11:15 Charles J. Walton *Acropora cervicornis microbial symbionts under healthy and diseased conditions*
- 11:15-11:30 Shanna D. Hanes *Measuring differential expression in Aiptasia pallida during heat and light stress using RNA-Seq*
- 11:30-11:45 Johanna Holm A *description of bacteria and a polyp endophyte associated with southern California species of Muricea*
- 11:45-12:00 Cory J. Krediet *Thermal tolerance and acclimation potential of a model symbiotic cnidarian*

Lunch 12:00-1:00pm

Symbiosis Workshop 2014

Abstracts of invited talks

1 Peter Tiffin, University of Minnesota ptiffin@umn.edu
Dept. Plant Biology Univ. of MN, 250 Biosciences, Saint Paul, MN 55108"
Identifying the genetic basis and selective history of symbiosis in Medicago truncatula and Ensifer rhizobia
Population genomic data provide opportunities to identify the genes that contribute to naturally occurring variation in symbiosis as well as characterize how selection has shaped the evolution of those genes. I will present results from genome-wide association mapping, using phenotype data and full-genome sequence data from ~ 250 accessions of the model legume M. truncatula, that identified genes that contribute to naturally occurring variation in the symbiosis. The candidates include genes previously identified through forward genetic screens as well as novel candidates, these novel candidates are now being investigated through functional studies. I also will present results from analyses that characterize the selective history of Medicago genes identified as being involved in mediating interactions with rhizobia. The results from these analyses should provide insight into the stability of symbiotic interactions."

2 Debbie Brock, (W. Éamon Callison, David C. Queller and Joan E. Strassmann) Washington University, dbrock@wustl.edu
Is your immunity compromised by cooperative bacterial interactions? Insights from a social amoeba.
Eukaryotes are dependent on beneficial microbes, but can be killed by harmful ones. How have they evolved responses that protect themselves from harmful bacteria while coddling the beneficial ones? An ideal system for investigating this relationship is the eukaryote social amoeba Dictyostelium discoideum because some clones carry commensal bacteria through the social state to use as future seed corn, while others do not. Most of its life, D. discoideum amoeba consume bacteria and divide by binary fission. Under starvation, amoebae aggregate into a multicellular body which crawls to light, then forms a fruiting body of 20% dead stalk cells and 80% living spore cells. During the crawling stage, some cells known as sentinel cells pass from front to back of the slug picking up toxins and bacteria, as a combined liver and innate immune system. We hypothesized that the farmer clones might be more vulnerable to harmful bacteria because they had to retain their helpful ones. In support of our hypothesis, we found that farmers have considerably fewer sentinel cells compared to non-farmers, indicating a potential trade-off between transporting bacteria for agricultural purposes and defense against harm from toxins and/or pathogenic bacteria. However further examination revealed that non-farmer reproductive output was significantly diminished under toxic conditions but farmer reproductive output remained unchanged under the same conditions. One possible explanation for the disparity between robustness of the innate immune system as measured by sentinel cell number and reproductive output could be farmer-associated bacteria are effectively replacing the role of sentinel cells in regards to toxic chemicals for farmers.

3 Amelia Lindsey, University of California, Riverside alind005@ucr.edu
Experimental Evolution of Wolbachia in Recombinant Hosts "
Amelia Lindsey, Richard Stouthamer, Department of Entomology, University of California, Riverside
Wolbachia is a common maternally inherited symbiont of insects, well known for altering host reproduction to select for female offspring. In the fields of vector- and biological control, there is great interest in introducing Wolbachia with particularly favorable phenotypes, into a variety of target hosts. Unfortunately, nothing is known about how Wolbachia adapts to a new host, and experimental attempts at horizontal transfer have proven largely unsuccessful, resulting in the loss of bacterial titers or failure to modify host reproduction. Failure is likely heavily influenced by a lack of co-evolution between host and symbiont. Instead of trying to infect a previously uninfected host with Wolbachia, the approach taken here is to manipulate the nuclear genetic background of a host that has long co-evolved with Wolbachia, and then observe how the symbiont adapts to its ""new"" host background. To explore Wolbachia evolution we are using haplodiploid parasitoid wasps of the genus Trichogramma as hosts. We took females from a population of T. pretiosum infected with Wolbachia for over 100 years and mated them with males from an uninfected population, and as a result of fertilization (rather than Wolbachia induced parthenogenesis), created a series of genetically unique, recombinant, infected daughters. Each daughter was used to initiate an experimental, parthenogenetic, homozygous, iso-female line. At generation three, the Wolbachia performance of each line relative to the original infected population, was determined by measuring symbiont titer, host fecundity, and host sex ratio. Recombinant wasps produced fewer female offspring, and a significant percentage of intersexes, a result of incomplete modification of host reproduction by Wolbachia. We conclude that Wolbachia is less adapted to recombinant Trichogramma. This system will be used as a model for studying the evolution of symbiosis, hopefully identifying key genomic changes that occur during this process.

4 Rachelle M.M. Adams, University of Copenhagen RAdams@bio.ku.dk

Chemically armed mercenary ants protect fungus-farming societies

Mutualisms are attractive targets for parasites, because resources freely exchanged between partners can be intercepted and stolen. This leads to complex species networks that present a formidable challenge, when considering hypotheses to elucidate the coevolution and relations between associates. The fungus-growing ant mutualism is a model system, recognized as a species network that spans kingdoms, and where the roles of the different symbionts are only now becoming clear. The ants are farmers of a fungal cultivar and protect their crop from devastating pathogens with weeding behavior and by housing an active bacterial community on their exoskeleton. They, however, are unable to deter the chemical warriors - social parasites of the ant genus Megalomyrmex (Solenopsidini) - that infiltrate the protective fortress of their nest. The most derived Megalomyrmex species infiltrate, then integrate into the host nest, consuming the fungus garden and offspring of the host colony for years. However, when a Sericomyrmex amabilis host colony is threatened by a marauding agro-predator from the Gnamptogenys ant genus, Megalomyrmex symmetochus ants use their toxic chemical weaponry to protect the host ants, farm and ultimately their shared home. The mere presence of the parasite discourages the fierce ant predator from even attempting a raid, thus serving as an effective prophylactic. Counter to expectation, the symbiotic relationship between M. symmetochus and S. amabilis may be beneficial rather than costly under certain ecological conditions.

5 Jianmin Zhong, Humboldt State University, jz15@humboldt.edu

Biochemical Characterization of the Folate Biosynthetic Pathway in a Rickettsial Endosymbiont of Ixodes pacificus

Nonpathogenic nutrient-providing bacterial endosymbionts have been shown to contribute to their arthropod hosts' fitness by supplying them with essential vitamins and amino acids. Little is known about the nutritional basis for the symbiotic relationship of endosymbionts in ticks. Recent metabolic reconstructions in our laboratory have shown that Rickettsia species phylotype G021 in Ixodes pacificus carries all six genes for de novo folate synthesis. In this study, sequence annotations and recombinant protein expressions were used to study de novo folate biosynthesis genes of the Rickettsia species. The rickettsial folate genes (folA, folC, folE, folKP, and ptpS) were amplified by PCR. Sequencing and bioinformatic analyses have identified putative promoter and ribosome binding site of open reading frame for each folate gene. In contrast to other folate genes, the folA and folKP gene locus is located in a multi-cistronic operon; BLAST searches confirmed that several species of Rickettsia and Wolbachia have the same folA-folKP gene organization. The amplified folate genes were amplified, digested, and cloned into the pET-41a(+) expression vector, and transformed into competent non-expressive host cells. The clones were verified by digestion, and the correct open reading frame for each gene was verified by sequencing. SDS-PAGE results showed that all five recombinant rickettsial proteins were overexpressed in BL21(DE3) E. coli. Future in vitro enzymatic assays will be performed to access the functions of the five recombinant rickettsial folate proteins. This study has shown that folate genes exist in the genome of Rickettsia species phylotype G021 of I. pacificus and may be capable producing functional enzymes, providing fitness advantages to the host.

6 Lisl Esherick, (Erik Lehnert, John R Pringle) Stanford University, lisl.esherick@stanford.edu

Possible Role for C-Type Lectins in the Establishment of Cnidarian-Dinoflagellate Symbiosis

Coral reefs are among the world's most diverse ecosystems and have enormous ecological and economic importance. The symbiosis between coral animals and their intracellular algal symbionts (dinoflagellates in the genus Symbiodinium) is key to the growth and health of coral reefs because most of the animal's energy supply is derived from algal photosynthesis. It is well documented that the cnidarian-dinoflagellate symbiosis is highly specific: a given host is capable of forming a stable symbiosis with only some types within the diverse Symbiodinium genus. However, the cellular and molecular mechanisms underlying this specificity are poorly understood.

During the initial establishment of symbiosis in larvae, or during its reestablishment in adults that have lost their symbionts, hosts must recognize compatible dinoflagellates among a diversity of microbes in the environment. Recent research has raised the hypothesis that recognition depends on the binding of extracellular host lectins to oligosaccharide glycans on the dinoflagellate cell surface. The importance of Symbiodinium glycans in recognition and symbiosis establishment is comparatively well characterized, whereas the specific lectins involved in this process have yet to be definitively identified in any cnidarian species.

Using the small symbiotic sea anemone Aiptasia as a model system, we have identified by transcriptome sequencing and RT-PCR several genes encoding lectin-like proteins that are differentially expressed between symbiotic and aposymbiotic (without symbionts) anemones. Notably, a majority of the putative lectins that were differentially expressed showed increased expression in aposymbiotic anemones, suggesting that hosts may up-regulate genes involved in pattern-recognition when lacking symbionts. One of these differentially expressed genes, encoding the C-type lectin-like protein Ctl-1, is expressed eight-fold higher in aposymbiotic animals. We have cloned and expressed a recombinant GST-tagged Ctl1-1 and assessed its binding capabilities by pull-down assays using four axenic Symbiodinium strains as "bait" followed by Western blotting, immunofluorescence, and flow

cytometry. Ctl1-1 appears to bind strongly to the incompatible strain SSE01, weakly to the incompatible strain SSA03 and compatible strain SSB01, and not at all to the compatible strain SSA02. Although it is encouraging to find that Ctl1-1 can distinguish between different strains of Symbiodinium, we cannot say that it exclusively binds either compatible or incompatible algae.

7 Joel Griffitts, Brigham Young University, joelg@byu.edu

Upon the edge of a knife: Legume-rhizobium negotiations near the onset of nitrogen fixation

The legume-rhizobium symbiosis involves the permissive invasion of rhizobial cells into nodule tissue, where a reciprocal exchange of metabolites typically enhances the fitness of both organisms. During nodule development, a large rhizobial subpopulation is endocytosed into specialized plant cells, where the bacteria differentiate into nitrogen-fixing bacteroids. In many legume-rhizobium symbioses, bacteroid differentiation is induced when the host secretes hundreds of different nodule-specific cysteine-rich (NCR) peptides. This salvo of antimicrobial peptides is directed to developing bacteroids at sub-lethal doses through a specialized symbiotic secretion system. NCR peptide-induced bacteroid differentiation appears to be terminal, so that only the undifferentiated rhizobial clonemates ever escape to proliferate after nodule senescence. We are investigating with great interest a plasmid-encoded rhizobial protein that may turn the tables on hosts that use this peptide-induced terminal differentiation strategy. This protein (HrrP, for host range restriction protease) blocks nitrogen fixation on certain host plants, and it appears to do so by obstructing precisely the phase of terminal bacteroid differentiation that is induced by NCR peptides. The HrrP protein belongs to a family of well-characterized peptide-specific metalloproteases that exhibit broad peptide substrate specificity. Whether HrrP is an NCR cleavage protease is still not clear, but I will present accumulating evidence in support of this model. I will also describe efforts to map host genes that predispose a plant to the effects of rhizobial HrrP. Whatever the outome of these experiments, HrrP is a fascinating anti-differentiation factor that essentially gives rhizobia a bargaining chip during symbiotic negotiations near the onset of nitrogen fixation. While its normal function might be to modulate symbiotic committment in subtle ways, this peptide protease has the potential to completely disrupt end-stage symbiotic transitions, with a strikingly exploitative outcome.

8 Joel Sachs, UC Riverside , joels@ucr.edu

Epidemic population distributions of wild *Bradyrhizobium* across a landscape

Host-associated bacteria encounter a world where their habitat can rapidly shift from living on or inside of a host, to free living phases between host infection. But the degree to which each life stage shapes bacterial adaptation remains poorly understood. To address this question, we analyzed population genomic structure of *Bradyrhizobium*, a nitrogen fixing symbiont of the wild legume *Lotus strigosus*. We sequenced loci distributed across the *Bradyrhizobium* genome, including four loci in the chromosome and four in the genomic island that encodes symbiotic traits (symbiosis island). Our study uncovered multiple epidemic haplotypes from locations across the host plant range. Strikingly, a third of all isolates are comprised of a single chromosomal haplotype, which was isolated at sites separated by >500 kilometers. These epidemic distributions suggest intense natural selection for certain haplotypes. But epidemicity varies substantially across genome regions suggesting that symbiosis-island genotypes are much more likely to be locally adapted, compared to chromosomal genotypes.

9 James Angus Chandler, California Academy of Sciences, jamesanguschandler@gmail.com

Characterization of the Aedes aegypti microbiota using RNA metagenomics

The animal microbiota is composed of many interconnected groups. Explorations into the identity and composition of these groups has mainly focused on just a single group at a time, for example through 16S sequencing to determine the bacterial community or with physical filtering methods to enrich for viral particles. Because the interplay between different components of the microbiota is well established, we are seeking to develop methods to interrogate the entire community simultaneously. We present data from RNA metagenomes from Aedes aegypti mosquitoes collected in Thailand and discuss whether the diversity of the viral, bacterial, fungal, and protistan communities can be evaluated from short-read Illumina data. Depth of coverage is sufficient to construct the complete genome of a recently discovered insect-specific Phlebovirus. We also evaluate different methods of ribosomal RNA meta-assembly from mixed datasets and discuss their applicability to different sequencing technologies. By comparing the metagenomic dataset to 16S and 18S amplicon libraries generated from the exact same pool of mosquitoes, we are able to evaluate the feasibility of using RNA metagenomic sequencing for characterization of the insect microbiota. Finally, we discuss how this work fits into an ongoing study that links mosquito and habitat diversity to the diversity of the mosquito microbiota.

10 Jingchun Li, University of Michigan, jingchun@umich.edu

The fast and the various: free-living and commensal marine clams differ in modes of evolution

The interplay between biotic and abiotic factors has shaped the planet's biodiversity through time. However, studies of contemporary marine lineage diversification are typically framed within abiotic hypothesis-testing contexts only and have collectively lagged behind terrestrial studies in developing an integrated framework that includes a meaningful biotic perspective. Here, we demonstrated how biotic and abiotic factors differentially affected marine taxa evolution using the hyperdiverse bivalve superfamily Galeommatoidea as a study system. This superfamily contains large numbers of obligate commensal as well as free-living species and is therefore amenable to comparative approaches. We constructed a global, multi-gene molecular phylogeny of Galeommatoidea incorporating hundreds of species and quantified their overall shell morphologies. We showed that the commensal lifestyle is likely to be the ancestral trait of galeommatoidean clams and secondary invasions to hard-bottom habitats are coupled with loss of commensalism. Macroevolutionary analyses indicated that the free-living lineages collectively have a higher diversification rate compared to the commensal clades. But the commensal clades exhibit much higher within-clade morphological disparity and intercladal convergence. Given that the commensal lifestyle in Galeommatoidea is an adaptation to living in sediments and that the free-living lifestyle is tightly associated with living in hard-bottom habitats, these findings imply that by colonizing heterogenous hard-bottom habitats, galeommatoideans were able to escape a``host-commensal'' evolutionary constraint and undergo an accelerated diversification as free-living species. However, intimate clam-host associations have promoted rapid morphological divergence in the commensal lineages.

11 Marie Braad Lund, Andreas Schramm, Aarhus University, Denmark mblu@aias.au.dk
Genome evolution of vertically transmitted, extra-cellular symbionts of earthworms
Almost all Lumbricid earthworms (Oligochaeta: Lumbricidae) harbor extracellular species-specific bacterial symbionts of the genus Verminephrobacter (Betaproteobacteria) in their nephridia (excretory organs). The symbionts have a beneficial effect on host reproduction and likely live on their host's waste products. They are vertically transmitted and have co-evolved with earthworms since the origin of Lumbricidae 62 – 136 million years ago. Interestingly, the Verminephrobacter have escaped the AT bias (65% GC), size reduction (5.6 Mb), and pseudogenization, which are the common hallmarks of genome evolution in vertically transmitted intracellular symbionts of similar age. Meanwhile, a comparative genome analysis of two symbiotic Verminephrobacter species and two free-living representatives of the sister genus Acidovorax have shown that the Verminephrobacter genomes do carry signs of genetic drift, such as accelerated evolutionary rates, low codon usage bias, and extensive genome shuffling. Thus, the transition to an obligate symbiotic lifestyle was indeed accompanied by moderate but genome-wide relaxed purifying selection. Genes involved in membrane/cell-wall biogenesis and signal transduction exhibited most instances of relaxed selection.
Unlike intracellular symbionts, Verminephrobacter encounter other bacteria during the host life cycle. We propose that the opportunity for genetic mixing (within and outside the symbiont population) during part of the host – symbiont life cycle is the key to evade drift-induced genome erosion. We furthermore suggest the earthworm-Verminephrobacter association as new experimental system for investigating host-microbe interactions, and especially for understanding genome evolution of vertically transmitted symbionts in the presence of genetic mixing.

12 Alejandro Grajales, (Daniel Thornhill, Estefania Rodriguez) American Museum of Natural History, agrajales@amnh.org
Patterns of Symbiodinium spp. associations within the family Aiptasiidae, a monophyletic lineage of symbiotic of sea anemones
Although the symbiotic relationships between dinoflagellates and cnidarians are well recognized, few studies have examined these associations from an evolutionary perspective. This is especially true for symbiotic sea anemones, where many reports consist of an approximate species identification of the host, followed by the identification of the dinoflagellate symbiont using molecular genetic markers. To further explore the evolutionary history of sea anemone-dinoflagellate associations, we documented the diversity of Symbiodinum spp. in a monophyletic clade of sea anemones, the family Aiptasiidae. We combined information from several molecular genetic markers, including nuclear ITS2 and plastid cp23S-rDNA, to evaluate the patterns of evolution and diversification of Symbiodinum in the light of an existing phylogenetic framework for the host. At the host family level, we found no evidence for coevolution or reciprocal phylogenies between host and endosymbiont. However, within some individual host species, Symbiodinium spp. exhibited patterns of host specialization and cladogenesis. This pattern suggests that coevolution between host and symbiont occurred within species and genera lineages, but that this process was regularly disrupted and symbiotic partners were recombined during the longer-term evolutionary history of the Aiptasiidae. Furthermore, we observed independent cases of phylogeographic partitioning of Symbiodinium within a single host species, suggesting ecological speciation along an environmental gradient contributed to the diversity of associations found in nature.

13 Alyssa Carrell, University of California, Merced, acarrell@ucmerced.edu
Potential for aboveground endophytic N2 fixation in subalpine conifers revealed by 16S rRNA gene analysis

Bacterial endophytes, which colonize the interior of plants, are emerging as an important component of the plant microbiome. Their roles in plant growth, nutrient acquisition, and protection against disease and abiotic stress are increasingly being harnessed in agricultural ecosystems, yet, not much is known about how such properties influence plants in the wild. Their ability to colonize harsh and nutrient-poor environments make conifers ideal species for research on microbes that support plants in terrestrial ecosystems. Our objective here was to identify potentially significant aboveground conifer-bacteria symbioses. Our approach was to examine the variation in the needle endophyte communities within and among individual trees of Pinus flexilis (limber pine) and Picea engelmanni (Engelmann spruce), growing in subalpine forest at Niwot Ridge, CO. We found remarkably low variability in the endophyte community species composition among and within individuals and conifer species, with a few phylotypes in the family Acetobacteraceae dominating both conifers. The most prominent phylotype in all samples of both conifer species (36 and 21 % of the total sequences from P. flexilis and P. engelmani, respectively) is similar to Gluconacetobacter diaztrophicus, an N2 fixing endophyte in sugar cane and other agricultural crops, and may represent a novel, habitat-adapted N2-fixing symbiosis in conifers. The presence of such symbioses could help explain why boreal and temperate ecosystems, which are dominated by conifers, have been found to accumulate more nitrogen than can be accounted for by known nitrogen input pathways.

14 Monica Medina, Pennsylvania State University momedinamunoz@gmail.com

Sym*biosis-driven development in the upside-down jellyfish Cassiopea xamachana*

There is increasing awareness of how critical microbial symbionts are in the life cycle, and survival in general, of multicellular hosts. In cnidarian systems, there has been a major focus on photosymbionts from a nutritional perspective however microbial interactions are known to have other functions. In the upside-down jellyfish, Cassiopea xamachana, microbial symbionts play key roles in development inducing two metamorphic stages: during larvae settlement and metamorphose into benthic scyphistomae in response to cues from bacterial biofilms, and when scyphistomae strobilate into swimming juvenile jellyfish (ephyrae) exclusively when infected by competent symbiotic dinoflagellates (Symbiodinium spp.). We have utilized these two life history transitions to investigate the influence of microbes on metazoan development and place symbiosis in the context of embryonic development and morphogenesis."

15 Jacqueline Padilla-Gamino, UC Santa Barbara, jacquelinelpg@gmail.com

From Parent to Gamete: Vertical Transmission of Symbiodinium (Dinophyceae) ITS2 Sequence Assemblages in the Reef Building Coral "Xavier Pochon, Christopher Bird, Gregory T. Concepcion, Ruth D. Gates

Parental effects are ubiquitous in nature and in many organisms play a particularly critical role in the transfer of symbionts across generations; however, their influence and relative importance in the marine environment has rarely been considered. Coral reefs are biologically diverse and productive marine ecosystems, whose success is framed by symbiosis between reefbuilding corals and unicellular dinoflagellates in the genus Symbiodinium. Many corals produce aposymbiotic larvae that are infected by Symbiodinium from the environment (horizontal transmission), which allows for the acquisition of new endosymbionts (different from their parents) each generation. In the remaining species, Symbiodinium are transmitted directly from parent to offspring via eggs (vertical transmission), a mechanism that perpetuates the relationship between some or all of the Symbiodinium diversity found in the parent through multiple generations. Here we examine vertical transmission in the Hawaiian coral Montipora capitata by comparing the Symbiodinium ITS2 sequence assemblages in parent colonies and the eggs they produce. Parental effects on sequence assemblages in eggs are explored in the context of the coral genotype, colony morphology, and the environment of parent colonies. Our results indicate that ITS2 sequence assemblages in eggs are generally similar to their parents, and patterns in parental assemblages are different, and reflect environmental conditions, but not colony morphology or coral genotype. We conclude that eggs released by parent colonies during mass spawning events are seeded with different ITS2 sequence assemblages, which encompass phylogenetic variability that may have profound implications for the development, settlement and survival of coral offspring.

16 Janja Ceh, Universidad de Antofagasta, Chile, janja.m.ceh@gmail.com

Living in a nutrient-poor world: bacteria released with coral offspring provide nitrogen to Symbiodinium in coral larvae

Corals and microbes live in close and complex relationships and it is widely accepted that some microbial partners increase the animals' fitness. The inheritance of specific bacteria from mother colonies to their offspring and the initial establishment of coral-microbial associations in the early life stages of corals have received little scrutiny and remain unclear however. A pyrosequencing approach assessed bacterial diversity and identity in planulation water from P. damicornis colonies to investigate whether specific bacteria were released with their offspring. Through advanced nano-scale secondary ion mass spectrometry (NanoSIMS) the translocation of extracellular nitrogen into the coral-symbiotic partner Symbiodinium by two highly abundant bacteria strains, isolated from the planulation water and the planulae, was visualized. These findings reveal intergenerational transfer of specific

beneficial bacteria in brooding corals, show the early onset of coral-bacterial partnerships and demonstrate the important role of coral associated bacteria providing an essential but limited nutrient to coral larvae.

17 Thomas Oliver, (Rob Toonen, Erik Franklin) University of Hawai'i, at Manoa, taoliver@hawaii.edu
Sex and the Single Gonochore: A Transcriptomic Perspective on Sex Deterimination in Porites lobata, a Gonochoristic Coral
In scleractinian corals, hermaphrodites outnumber those that maintain separate sexes roughly three to one. For those relative few gonochores, sex is largely a cryptic phenotype, as a researcher can only identify males and females by waiting for a spawn, which frequently occurs only once a year, or by examining ripe gametes microscopically. The challenge of assaying this critical character has led to gonochores being largely treated with the same methods as their hermaphroditic cousin, even for population models that rely local densities to estimate likely population growth parameters. A few coral gonochores have been closely studied, and in multiple cases these 'gonochores' have been shown to be sequential hermaphrodites. No one has yet examined these issues in one of the most common, and more stress tolerant, genera of corals in the Pacific, Porites. To assess to what degree Porites showed (a) fixed genetic differences, (b) hemizygosity, and (c) sexually bias expression, we sampled 100 massive Porites in the week before spawning in two populations on Oahu, Hawai'i. We sectioned and histologically stained, each of the 100 samples, and identified males and females. We then prepared 10 males and 10 females for mRNAseq sequencing on the Illumina platform. Through de-novo assembly, SNP calling, and differential expression analysis we evaluated (a) to what degree sex in Porites is determined by fixed genetic differences, (b) whether genomic regions appear hemizygous, and (c) if there was evidence of sex-biased expression.

18 Shaker H. Alhazeem, Kuwait Institute for Scientific Research salhazeem@yahoo.com
Kuwait Artificial Reef Fisheries and Benthic Cover
The fish assemblage and epibenthic organisms associated with the Kuwait Oil Company's (KOC's) artificial reef (established using reef ball clusters), adjacent non-reef area and Qit'at Uraifjan natural reef were surveyed by scuba divers twice a month from January 2011 through December 2012. Fish and epibenthic species, frequencies of occurrence, individual numbers for each species and percentages of coverage were assessed. Forty-four fish and other motile aquatic species were identified in the three areas. Reef coverage include 42 epibenthic species as well as sand. The Qit'at Uraifjan natural reef had the most fish and epibenthic species as well as the highest numbers of each species, followed by KOC's artificial reef and then by the adjacent non-reef area. The results indicate that KOC's artificial reef has created new habitats for a range of marine life including commercially important fish species. However, recruitment of corals and other reef-associated benthos has been limited. The low abundance of coral recruits in KOC's artificial reef area is probably due to the deployment of the reef ball clusters in a relatively turbid inshore environment at depths where the light is insufficient and sedimentation rates are too high for successful coral recruitment.

19 Tamar L Goulet, University of MIssissippi, tlgoulet@olemiss.edu
Ontogenetic, temporal, and spatial shifts in the symbiotic continuum
Mutualism is not idealism. It is a coexistence that is beneficial to both parties under specific conditions. In addition, the physiological performance of the host-symbiont genotypic combination (the holobiont) is not the sum of its symbiotic consortium. Rather, it is its own physiological entity. On coral reefs, numerous hosts, representing several phyla, form symbiotic combinations with unicellular dinoflagellates belonging to the genus Symbiodinium. The phototrophic Symbiodinium can contribute their photosynthetic products to their host. But, the cost to the host, and its ability to withstand the symbionts' metabolic demands, varies depending on the host's genetic identity, host ontogeny and environmental conditions. Furthermore, the holobiont's robustness in changing environmental conditions is contingent on the physiological integrity of the holobiont unit. Even though the host or symbiont may be detrimentally affected in a new environment, if one partner offsets this deficit, the holobiont may withstand the changing environment. Multiple examples of host-Symbiodinium associations on coral reefs will be presented to illustrate the concepts above.

20 Charles J. Walton, Nova Southeastern University Oceanographic Center, cw808@nova.edu
Acropora cervicornis microbial symbionts under healthy and diseased conditions
Scleractinian corals play host to numerous symbiotic organisms known collectively as the coral holobiont. A primary component of the coral holobiont is the bacterial community, as it plays a role in the defenses of the coral as well as having been linked to compromised health conditions. One compromised health condition, known as rapid tissue loss (RTL), has impacted Acropora cervicornis populations off Southeast Florida, which are still found in relatively high abundance. This study examines the bacterial and archaeal components of the holobiont of A. cervicornis colonies in Southeast Florida, both healthy and those affected by RTL. Using 454 high throughput sequencing techniques, we described the tissue and mucus communities of RTL affected A. cervicornis. Additionally, we performed a comparative analysis of the bacterial communities associated with the healthy, visually healthy on

diseased colonies, and diseased margins of A. cervicornis colonies. Initial analysis of approximately 70,000 16S rRNA sequences show bacterial diversity among tissue samples to be greatest in the diseased tissue margins. Proteobacteria, primarily alpha- and gamma, dominated all tissue samples in relative 16S rRNA gene abundance. Additionally, distinct communities were observed between the mucus and the tissue and all mucus communities were similar regardless of condition. Preliminary results indicate two phyla, Bacteroidetes and Planctomycetes, were present at higher relative abundance in diseased samples compared to the healthy on diseased and healthy samples. These differences indicate avenues for further investigation into the pathogen(s) responsible for RTL in A. cervicornis. In addition, the results from this study have important implications for population restoration efforts which utilize nursery propagated colonies and the associated risk of disease transmission.

21 Shanna D. Hanes, Auburn University, shanna.hanes@gmail.com

Measuring differential expression in Aiptasia pallida during heat and light stress using RNA-Seq

Coral reefs have dramatically declined over the past few decades as a result of mass mortality bleaching events. Bleaching functions as a stress response to elevated temperature and/or light conditions resulting in the loss of dinoflagellates (Symbiodinium) from host cnidarian tissues. This process involves a complex series of events that occur throughout the duration of the bleaching episode and involve cellular interactions between both symbiotic members. However, few studies have investigated the early host stress response when symbiotic breakdown is initiated. Recent development of high-throughput next-generation sequencing techniques have allowed rapid advancements in expression profiling of non-model organisms. In this study, RNA-Seq was employed to measure differential gene expression during the first 48 hours of heat and light stress in Aiptasia pallida. Both symbiotic and aposymbiotic anemones were exposed to stress conditions of ~32.5oC at 140 µmols irradiance for 12 hours daily followed by 12 hours of darkness at~24^{o}C. RNA was extracted at various time points (0, 3, and 48 hours) and sequenced using an Illumina platform. Results from this investigation indicate that the gene expression profile of A. pallida changes during early stages of bleaching, and several key genes were identified that are involved in the host stress response and protein degradation/synthesis as well as other cellular activities. Validation of these expression profiles using qRT-PCR on a subset of genes is currently underway. This study provides a better understanding of the genetic determinants of stress tolerance in a host anthozoan, and offers further insight into the cellular processes that underlie coral bleaching.

22 Johanna Holm (P. Webster, W. Ziebis, K. Heidelberg), USC, jholm@usc.edu

A *description of bacteria and a polyp endophyte associated with southern California species of Muricea*

Marine ecosystems of southern California are irrefutably going to experience the effects of our changing climatic conditions due to nearby coastal upwelling systems. There is a body of evidence indicating that changes in oceanic chemistry and temperature significantly alters scleractinian coral-associated microbial diversity and metabolism, which in turn affects coral health. Far less information regarding the baseline associations of non-tropical octocorals with their resident microbial communities is available. The octocorals, Muricea californica and M. fruticosa, are essential contributors to overall benthic heterogeneity and a habitat for small invertebrates in kelp forests of southern California. Additionally, they have been recorded as azooxanthellate. Here, I will discuss bacterial diversity and a novel polyp endophyte associated with our local gorgonians, Muricea californica and M. fruticosa using multiple tools including next-generation sequencing, fluorescence and electron microscopy, oxygen microsensors, and pigment analyses.

23 Cory J. Krediet (Erik Lehnert , John Pringle) Stanford University , ckrediet@stanford.edu

Thermal tolerance and acclimation potential of a model symbiotic cnidarian

Despite its ecological importance for coral-reef ecosystems, the cnidarian-dinoflagellate symbiosis remains poorly understood at the molecular and cellular levels. To further our understanding of the mechanisms that underlie this symbiosis, we are using RNA-Seq to identify genes that are differentially expressed under thermal stress in the Aiptasia model system. Clonal stocks of symbiotic and aposymbiotic Aiptasia were thermally stressed at 34°C for 10 days in either the light or dark and sampled at 0, 3, 12, 24, 48, 96, and 240 h. The comparison of symbiotic to aposymbiotic anemones allows us to determine if the differentially expressed genes are host-specific or mediated by the presence of an endosymbiont. Preliminary analyses indicate a strong upregulation in the mRNAs encoding heat-shock and other stress-response proteins at early time points (0-12 h) and distinct expression patterns at the onset of bleaching (96 h). In addition to identifying the specific genes expressed differentially during stress, we wish to determine how prior thermal history influences the tolerance of Aiptasia to subsequent thermal stress. Acclimation of Aiptasia at elevated but sub-bleaching temperatures (30-32°C) decreases the rate and severity of bleaching during subsequent thermal stress at 34°C. Future studies will vary the acclimation temperatures and durations, as well as analyze gene expression in thermally acclimated animals, thus detecting genes that influence thermal tolerance.

INVITED POSTERS

Melissa Whitaker, Harvard University, Naomi Pierce melliwhitaker@gmail.com TBD

Nathan Kirk, Oregon State University, kirkn@science.oregonstate.edu

Using light to enumerate symbionts in a cnidarian system

Cnidarians form symbioses with numerous symbiotic dinoflagellates with the genus Symbiodinium. These symbionts provide these hosts with photosynthetically-fixed carbon in exchange for nutrients and shelter. However, these symbionts can be lost in the process of bleaching, which can have deleterious impacts on host physiology and health. Therefore, there is interest in rapidly enumerating Symbiodinium in vivo and this study assesses Symbiodinium autofluorescence as a proxy for cell counting. This method is preferable to traditional cell counts as it is rapid, easy to assay, and non-destructive allowing enumeration without killing or harming individuals (i.e. tentacle clips). The sea anemone Aiptasia pulchella was utilized in this study because it is amenable to laboratory culturing, doesn't express fluorescent proteins that could potentially mask signal, and harbors the same Symbiodinium species (S. minutum=type "B1" sensu LaJeunesse). Anemones were illuminated with 2400 µmol/m2/s of 470 nM blue light, viewed through a long pass filter attached to a Zeiss Stemi 1000 stereo microscope and photographed using standardized camera settings. Mean fluorescence intensity relative to background was ascertained in ImageJ for each individual. These animals were then sacrificed, homogenized in seawater, and split for Symbiodinium cell and chlorophyll counts as well as qPCR. Aiptasia were then standardized for size to oral disc diameter and total protein content. In preliminary experiments, cell counts were correlated to Symbiodinium autofluorescence ($r = 0.868$, $n=14$ $p= 5.0 \times 10\text{-}6$), although there was higher precision and sensitivity using mean fluorescence intensity at detecting changes during laboratory-induced bleaching events. This technique can be modified to work with corals by changing the filter set to remove emission wavelengths from fluorescent proteins and is currently being pursued.

Angela Poole, Oregon State University, poolea@science.oregonstate.edu

Laser capture microdissection (LCM) as a tool for examining tissue specific expression differences in the anemone Aiptasia palli

Laser capture microdissection (LCM) is a technique used to isolate single cells or groups of cells from histological preparations containing multiple tissue types. The resulting material can be used for a variety of downstream applications including DNA, RNA, and protein analysis. While LCM has primarily been used in mammalian and model systems, the application of this technique to study cnidarian-dinoflagellate symbiosis could provide the ability to quantitatively examine gene expression differences between different cell types and tissue layers. Cnidarians have two tissue layers, an outer epidermis and an inner gastrodermis, but their symbiotic dinoflagellates reside specifically within gastodermal cells. Therefore, the ability to isolate cells from these two host tissue layers will promote discovery of symbiosis specific genes and an understanding of how symbiotic state influences each tissue layer. The research presented here will focus on the development of the LCM technique in the anemone Aiptasia pallida as a tool for comparing gene expression between the epidermis and the gastrodermis. Preliminary results show that ectoderm and gastroderm tissue can be successfully isolated using LCM. Data presented will include the development of tissue specific markers to confirm this tissue separation and demonstration of tissue specific expression differences using qPCR.

Emily C. Wilson University of California, ewilson3@ucmerced.edu

Culture Identification of Bacterial Endophytes in California Conifers

Endophytes—bacteria and fungi that live inside healthy plant tissue—are emerging as an important part of the plant microbiome, with roles in host health and development. While endophytes have been studied extensively in agricultural ecosystems, our understanding of endophytes in natural ecosystems such as forests is limited. Some evidence of nitrogen fixation and host growth promotion has been linked to conifer endophytes, but very little is known about the bacteria associated with these ecologically and economically important trees. This study encompasses a culture-based study of *Pinus contorta, Pinus flexilis*, and *Pinus radiata*. Conifer needle and shoot (bud) tissue have natural microbial inhibitors (phenolics) so characterizing bacterial endophytes through culture isolation can be challenging. The objective of this study was to maximize recovery of bacterial endophytes from a variety of conifer species, tissue types, tissue ages across a range of geographic locations. Innovations in tissue preparation, culture media optimization, culture condition customization, and rapid identification of isolates will be presented.

Melissa Roth, UC Berkeley, melissa.s.roth@gmail.com

Changes in coral fluorescence provide early warning of declining coral health

Monitoring coral health is increasingly important because of the worldwide pressure on coral reefs and their subsequent decline. Widespread temperature stress has caused catastrophic coral bleaching events that have been devastating for coral reefs. Here, we evaluate whether coral fluorescence could be utilized as a noninvasive assessment for coral health. We conducted cold and heat stress treatments on the branching coral Acropora yongei, and found that green fluorescent protein (GFP) concentration and fluorescence decreased with declining coral health, prior to initiation of bleaching. Ultimately, cold-treated corals acclimated and GFP concentration and fluorescence recovered. In contrast, heat-treated corals eventually bleached but showed strong fluorescence despite reduced GFP concentration, likely resulting from the large reduction in shading from decreased endosymbiotic dinoflagellate density. Consequently, GFP concentration and fluorescence showed distinct correlations in non-bleached and bleached corals. Green fluorescence was positively correlated with endosymbiotic dinoflagellate photobiology, but its closest correlation was with coral growth suggesting that green fluorescence could be used as a physiological proxy for health in some corals.

Elizabeth Green , University of California-Merced, ewindmeyer@gmail.com

High-throughput molecular genotyping detects Symbiodinium diversity patterns across a depth gradient within Orbicella Montastra

A suite of molecular genotyping techniques and markers has been utilized to detect fine scale Symbiodinium diversity within coral hosts. Available databases cumulating published Symbiodinium types encompass an array of genotyping techniques. The diverse nature of the methods used reduces our abilities to make worldwide correlations and distribution patterns of species diversity. We are using next-generation sequencing (NGS) platforms to assess Symbiodinium diversity. Using our previously optimized 454 barcodes we pooled 15 individuals to one MiSeq index, reducing sequencing costs while increasing coverage. Here, we assessed Symbiodinium species diversity within coral host Orbicella faveolata collected from a depth gradient (~7 meters, ~12 meters and ~16 meters) off Curaçao in the eastern Caribbean using the Illumina MiSeq platform with a conversion library preparation. We show species diversity decreases with depth and detect Symbiodinium type G3, a type not typically seen in scleractinian hosts or in the Caribbean. Fourteen Symbiodinium types, haplotypes from clades A, B, C, D and G, were detected as well as non-specific primer amplification of host DNA. A comparison between bioinformatics pipelines for cd-hit versus UPARSE yielded reduce species diversity from cd-hit showing the significance of clustering algorithms. Identifying cryptic Symbiodinium species diversity within hosts will contribute to understanding the plasticity of symbiosis and pave the way for future studies assessing roles of other coral holobiont members

Rita Rachmawati, UCLA, rrachma@ucla.edu

Symbionts associated with Porites bleaching

Among corals, Porites, especially massive Porites, is one of groups that less sensitive to bleaching associate with temperature stress. From experiment, Porites in area with low susceptibility to bleaching has high adaptation to high temperature anomaly up to almost 5oC above maximum temperature average. On the other, Porites in area that experienced severe bleaching bleached at very low temperature, even the bleaching started at temperature that lower than starting bleaching temperature of Acropora and Pocillopora, which are from coral groups that relatively more sensitive to bleaching. Therefore, the bleaching sensitivity of Porites has broader temperature range. In addition to its association with Symbiodinium, as other hard corals in general, Porites is also associated with specific bleaching-related bacteria. This study reveals if bacteria is a stronger bleaching-associated symbiont than Symbiodinium in Porites."

Ryan Guillemette, Scripps Institution of Oceanography, rpguille@ucsd.edu

Micro-scale Interactions between Symbiodinium & Heterotrophic Bacteria

Ryan Guillemette*, Yanyan Zhou*, Farooq Azam (*Equal Contributions

Tamaki Bieri Stanford Genetics, tamakib@stanford.edu

Cellular Mechanisms of Cnidarian Bleaching

Several mechanisms of bleaching (loss of symbiotic dinoflagellates) have been reported in corals and sea anemones. These include in situ degradation of algal cells, exocytosis of algal cells, detachment of host cells, and host-cell death (by either apoptosis or necrosis). However, these mechanisms have not been studied in parallel and were observed in different species under different stress conditions. In addition, the molecular mechanisms underlying the four possible bleaching mechanisms remain unclear.

We are developing assays to monitor all four possible bleaching mechanisms in parallel, using animals from a clonal population of the small sea anemone Aiptasia that have been exposed to a variety of precisely controlled temperature-stress and light-stress conditions. The overall bleaching responses are assessed by counting the number of algae remaining in the host using a

flow cytometer that allows precise and rapid counting of a large number of samples. To distinguish exocytosis from host-cell detachment, cells in the seawater surrounding the stressed anemones are collected and examined by fluorescence and electron microscopy. To look at host-cell death and in situ degradation, we are using western blotting, immunohistochemistry, protease-activity assays, and qPCR to study the possible roles of different cellular pathways such as apoptosis and autophagy.

We are also attempting to develop transgenesis as a genetic tool in Aiptasia, as this would allow us to study the expression patterns of proteins of interest during bleaching and other cellular processes of interest. We are attempting to stably integrate GFP-tagged copies of several genes of interest, using microinjection of zygotes to deliver DNA (as done successfully in Hydra and Nematostella)."

Cawa Tran John Pringle, Stanford cawa@stanford.edu

Induction of Aiptasia larval settlement in the laboratory

Recapitulating the full sexual cycle of Aiptasia in the laboratory is of central importance for the further development of this critical model system for coral biology and symbiosis studies. This will allow development of classical and molecular genetic analyses, as well as germ-line transformation with gene-disruption and gene-tagging constructs. We have focused both on improving the efficiency and predictability of laboratory spawning and on achieving the settlement and metamorphosis of the larvae produced in the lab. This has led to field studies at sites in Florida that have large natural populations of Aiptasia to identify natural cues and surfaces that induce settlement and metamorphosis of Aiptasia larvae. Some anemones were found on mollusc shells, rocks, and crustose coralline algae, while larger populations dominate mangrove roots in the Florida Keys. Glass microscope slides and ceramic tiles were deployed at these sites to collect microbial biofilms over time. Settlement assays were conducted on natural substrata, biofilmed slides and tiles placed in the field for 2 months, and individual bacterial strains isolated from these surfaces. The small, translucent Aiptasia larvae were stained with neutral red to improve visualization of larvae and potential recruits on dark surfaces. Larvae from 5 to 14 days old were able to attach to a biofilmed surface as early as 8 h after initial exposure and to settle by 24 h. To our knowledge, this is the first documented observation of successful settlement of Aiptasia larvae within a laboratory setting. Efforts are continuing to identify the exact cues associated with the surfaces that induce settlement and metamorphosis.

Mahmoud Ghayyem Ashrafi, Islamic Azad University, Tehran m.gh.ashrafi@gmail.com

Identification of Symbiotic Zooxanthellae on Scleractinian Corals of Hendourabi Island-Persian Gulf

Zooxanthellae is the common name of a group Dinoflagellae that may occur as symbiont in other marine invertebrates such as corals. These are mainly belonging to the genus Symbiodinium. So far, 11 species belong to Symbiodinium have been identified based on morphological characteristics. Using molecular method 9 clades of Symbiodinium so called A to I have been identified. In the present study, specimens of Acropora sp. and Porites sp. were collected off Hendourabi Island from the northern part of the Persian Gulf. Zooxanthellae were removed using air brush and the slurry was preserved in DMSO buffer (20% Dimethyl Sulfoxide in NaCl saturated). DNA was extracted using CTAB (Cethyl Trimethyl Ammonium Bromide) method. ITS2 region was amplified by polymerase chain reaction (PCR). The gene sequences showed that Acropora sp. harbored Symbiodinium clade D and Porites sp. harbored Symbiodinium clade C.

Dana Carper, UC Merced, dcarper@ucmerced.edu

Bacterial Endophytes within the Needle and Bud Tissues of Lodgepole Pine (Pinus contorta ssp. murrayana)

Bacterial endophytes are symbiont organisms that live within the tissues of healthy plants. Primarily studied in agricultural crops, these organisms have been shown to provide their hosts with resources that may be limited within the environment. Some of these resources include nitrogen (via nitrogen fixation), phosphorus (phosphorus recruitment) and the production of growth promoting factors. Little is known about the role of bacterial endophytes in conifers, but their ability to maintain relatively high rates of productivity in low fertility soil suggests symbioses with bacteria. Pinus contorta ssp. murrayana (Lodgepole pine) is a long-lived species of plant that occurs across California, Oregon and Washington. This species is usually found within subalpine forests, like Tuolumne Meadows, which have cold wet winters and dry warm summers. The wide spread distribution and ability to survive in multiple environments makes this species an ideal candidate for studying endophytes. Some studies have shown differences in endophyte communities when examining the differences between root and leaf or needle tissue, though few have looked at differences between the bud and needle tissue, which is an undeveloped shoot that will develop into new stems, and needle tissue. The needle and bud tissues could possibly host different endophytic communities due to the differences in the nutrient needs in those tissues. For this study bacterial DNA was isolated from the needle and bud tissues of Lodgepole pines growing within four quadrants of Tuolumne Meadows. Six trees from each quadrant that contained both bud and needle tissues were used for DNA isolation after surface sterilization. The DNA was then amplified using 16s Illumina primers targeting the V4 region. Sequences will be processed through QIIME and analyzed for differences between trees and between the tissue types.

Carolin Frank, (Anna-Maria Pirttila, Janne Koskimakki) UC Merced, cfrank3@ucmerced.edu

Genome sequence of an intracellular, growth-promoting conifer meristem endophyte

"Conifers tolerate harsh and nutrient poor sites where few other trees can survive, and dominate many of the world's temperate and boreal forest ecosystems. This is generally thought to be the result of adaptations in the conifer genome and associations with mycorrhizae, but other microbial symbionts could be involved in conifer growth and stress adaptation. Our group studies bacterial endophytes, which colonize the interior of healthy plant tissue. Endophytes are generally thought to colonize plants through the roots and reside in the intercellular spaces. Intracellular colonization without specialized structures appears either extremely unusual or understudied

Methylobacterium extorquens DSM 13060 is an endophyte that colonizes the meristematic cells of Scots pine shoot tips (buds) in association with the plant nucleus. The strain was first discovered in bud-derived tissue cultures of Scots pine, which could not be grown in the absence of these bacteria. M. extorquens is likely involved in normal host development and growth. The bacterium is present in buds of every tree examined, and is most abundant/active just prior to bud elongation and differentiation. Scots pine seedlings inoculated with M. extorquens in vitro display significantly increased root and needle growth compared to control seedlings.

We sequenced and analyzed the M. extorquens genome with the aim to reveal some of the mechanisms by which this bacterium affects host growth and development. M. extorquens does not encode any of the genes known to be involved in endophytic plant growth stimulation (e.g. plant hormone biosynthesis). Given the intracellular location close to the plant nucleus, we hypothesized that this strain encodes nucleomodulins, i.e. proteins that some pathogens (e.g. Chlamydia) use to modify host processes inside the nucleus. Nucleomodulins are often more eukaryotic- than bacterial-like. By searching for eukaryote-like proteins, we identified several putative nucleomodulins as well as other proteins with potential roles in plant growth stimulation. Our results provide insights into novel bacteria-plant symbioses.