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Information and Abstract Booklet

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UNIVERSITY of WISCONSIN
UWMILWAUKEE

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Schedule of Events

Virtual Poster Session April 24th 2020
UWM Biological Sciences Website

2020 Biological Sciences Award Announcements
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Abstracts

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Social Experience and Reproductive Isolation in a Vibrational Insect

Nour Abuomar*, Rafael Rodriguez, Camille Desjonqueres

Enchenopa binotata treehoppers are host plant-feeding insects that communicate through plant-borne vibrational signals. Typically, each species has a distinctive host plant species and male mating signal frequency (with the females of each species preferring the signal frequency of their males). At some sites, however, more than one species may be found on the same host plant. We chose to investigate whether developing in mixed- or single-species aggregations will affect an individual's communicational behavior. Does developing in a mixed aggregation make communication systems more similar or more distinct? The consequences may weaken of strengthen reproductive isolation between the species. We raised two species of *Enchenopa* in treatments of either mixed or single-species aggregations from nymphs to adulthood. Once they reached adulthood, we assessed female preference with vibrational playbacks of male signals that ranged from high to low frequencies. When females heard an attractive call, they responded with their own mating call in return. By comparing the responses to the playbacks, we were able to determine which of the playback frequencies were preferred. We discuss our findings in terms of species reproductive isolation and the consequences for insect behavior in the wild.

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Allelopathic Interactions of *Chlamydomonas reinhardti* and *Microcystis aeruginosa*.

Sierra Aguirre*, Lauren Simmons, Dr. John Berges

The goal of the experiment was to observe the influence two aquatic species had on one an others population and photosynthetic capability due to possible toxins they excrete. The experiment began by growing *Chlamydomonas reinhardit* (labeled Species A) and *Microcystis aeruginosa* (labeled Species B) in separate flasks of DY-V media to ensure the same level of nutrients were provided. I then transferred the newly grown cells to multiple co-culturing apparatuses that housed Species A with itself, Species B with itself and finally Species A and B paired together. The strains were separated in the apparatuses by a membrane filter to preventing cells from diffusing and allow only possible toxins to move across the equipment. I took samples daily from each arm of the equipment and ran them through a Flow Cytometer with either a Sytox Green stain, Annexin V stain, and unstained samples in order to observe cell size, the fluctuation in population, and cell decay. Photosynthetic capability was measured using a TD-700 Fluorometer. I allowed the cells to acclimate to a dark environment and proceeded to record their fluorescence (Fv) They were then exposed to DCMU, a photosynthetic inhibitor, and measured again (F_m).

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Regulation of c-di-GMP, Type II and Type III Secretion Systems in *Dickeya dadantii* by the Vfm Quorum Sensing System

Biswarup Banerjee*, Ching-Hong Yang

Dickeya dadantii is a phytopathogenic bacterium which causes diseases on a wide range of host plants. It secretes PelD, an endo-pectate lyase enzyme, through the Type II Secretion System that degrades the cell wall in host plants. This pathogen utilizes the Type III Secretion System to suppress host defense responses. The second messenger, cyclic-diguanylate-monophosphate (c-di-GMP), produced by diguanylate-cyclases and degraded by phosphodiesterases has been reported to regulate the expression of virulence genes in *D. dadantii*. EcpC, a phosphodiesterase, was found to regulate multiple cellular behaviors and virulence gene expressions. High c-di-GMP represses *pelD* and *hrpA* expression. The HrpA is the major component of the T3SS pilus. The VfmE (AraC family regulator), belonging to the Vfm quorum sensing system, was reported to regulate cell-wall degrading enzymes, protease, and pectinase in *D. dadantii*. VfmE was observed to positively regulate *pelD* and *hrpA*. Interestingly, under high c-di-GMP background, the *pelD* promoter activity was restored to WT level but the *hrpA* was further repressed. These regulatory effects of VfmE in PelD and HrpA were found to be associated with regulators SlyA, RsmA, GcpA (diguanylate-cyclase), EgcpB (phosphodiesterase), H-NS, HrpS, and HrpL. Our results show that the Vfm quorum sensing system plays an important role in regulation of *pel* and *hrp* gene expression through *slyA* and *rsm* regulatory pathways. It also controls the level of c-di-GMP through *gcpA* and *egcpB* in *D. dadantii*. We propose that Vfm quorum sensing system controls pectate lyase and *hrp* gene expression through multiple regulatory networks that include *vfmE-slyA-pelD* and *vfmE-slyA-hrpS-hrpA*.

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Novel Regulators of Yeast AMPK

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Members of the AMP-activated protein kinase (AMPK) family sense energy limitation and act to restore the energy balance in eukaryotes from yeast to humans. Mammalian AMPK has been implicated in diseases from diabetes to cancer. The yeast *Saccharomyces cerevisiae* (baker's yeast) serves as an advantageous model for studying AMPK regulation. Yeast AMPK is called Snf1 (sucrose non-fermenting 1). Snf1 works to adapt to energy stress caused by glucose limitation and promotes utilization of alternate carbon sources. Like human AMPK, the yeast Snf1 kinase complex contains three subunits: catalytic α subunit (Snf1), targeting β subunit (Sip1, Sip2, or Gal83), and regulatory γ subunit (Snf4). Furthermore, like human AMPK, yeast Snf1 is upregulated by two main factors: 1) catalytic activation that depends on phosphorylation of the conserved threonine residue (Thr210) in the activation loop of Snf1; 2) enrichment of the activated Snf1 complex in the nucleus. These important mechanisms are not fully understood even in such a simple system as baker's yeast. Here, we present evidence for two novel regulators of Snf1, provisionally dubbed Snf13 and Snf14. Until now, the functions of Snf13 and Snf14 have remained unknown, but proteomic studies suggest that they both physically interact with Snf1 and other components of the Snf1 pathway. We show that while neither the *snf13*Δ nor *snf14*Δ single mutations affect the Snf1 pathway, the *snf13*Δ *snf14*Δ double mutant shows a significant defect in Snf1 nuclear localization. We suggest that Snf13 and Snf14 work redundantly to promote Snf1 nuclear localization in response to energy stress.

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Role of Mitochondrial Function in Controlling the Yeast AMPK Homolog Snf1

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Snf1 (sucrose non-fermenting 1) is the yeast homolog of mammalian AMP-activated protein kinase (AMPK). It is essential for responses to many stress signals, notably energy stress. Under conditions of energy stress caused by glucose deprivation, Snf1 becomes catalytically activated and enriches in the nucleus to up-regulate stress-induced genes, including genes required for mitochondrial respiration.

Mitochondria play an important role in energy metabolism and, in turn, are poised to regulate the Snf1 pathway. The yeast Saccharomyces cerevisiae is an advantageous model system to study the effects of mitochondrial dysfunction because this organism can survive with its mitochondrial respiration completely disrupted, such as in rho0 cells lacking the mitochondrial genome.

Preliminary studies have found several regulatory defects in the Snf1 pathway in respiratory-deficient rho0 cells. Here, we explore the connection between respiratory function and its role in the regulation of Snf1.

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Iron Acquisition Systems of the Fish Pathogen *Flavobacterium columnare*

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Flavobacterium columnare is a Gram-negative, aquatic bacterium that causes columnaris disease in wild and aquaculture-reared freshwater fish. The mechanisms responsible for columnaris disease are not understood and little is known regarding *F. columnare* virulence. Iron acquisition from the host during infection is important for pathogenicity and virulence of many bacterial pathogens. *F. columnare* genes predicted to function in iron uptake were identified by genome analyses. The genes encoding a heme-binding protein HmuY, siderophore synthesis proteins, the outer membrane iron receptor FhuA, and an iron related cytoplasmic membrane ABC transporter were deleted. The mutants were examined for growth defects in iron-limited conditions and for virulence defects in zebrafish. The ΔABC transporter, Δsiderophore synthesis and Δ*fhuA* mutants exhibited the greatest growth defects. We suspected a growth defect in the iron-limited conditions would lead to an inability to grow in a fish host where iron is expected to be sequestered. Preliminary results indicate that mutants targeting single uptake mechanisms were as virulent as wild type cells. These data suggest that the individual iron acquisition mechanisms targeted thus far are not essential for virulence in zebrafish. We constructed a deletion strain lacking the ABC transporter and siderophore synthesis genes. This mutant displayed a greater growth defect in iron-limited conditions and reduced virulence in zebrafish. These data suggest possible redundancy among iron acquisition mechanisms with respect to virulence. Future experiments will aim to construct further multiple deletion strains and to examine the role of iron uptake in *F. columnare* virulence.

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Broad-Scale Mechanisms Generating Variation in Genes Under Selection

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Climate change is causing selective pressures in natural habitats to fluctuate. Changing environments may impact a population's fitness through mechanisms like altering resource availability, reproductive behavior, and parasite dynamics. Species may respond to these new pressures by adapting to their current environment or migrating to a more suitable habitat. The adaptive potential is maintained by higher genetic variability, especially in genes under selection. For example, to combat a changing parasite load, it is favorable for a population to have variability in their immune-related genes, such as those in the Major Histocompatibility Complex (MHC). To assess adaptive potential, it is crucial to understand the selective mechanisms affecting adaptive genes like those in the MHC. In this study, geographic patterns of allelic richness in mule deer were analyzed using 9 neutral microsatellite loci and the MHC class II DRB exon 2 locus. This allowed us to compare variation at an adaptive locus, the MHC, to that at neutral loci, which aids in detangling the evolutionary mechanisms acting on the different genes. 32 alleles were found at the MHC locus, with each of the 16 mule deer populations containing 6-17 alleles. A pattern of isolation by distance was observed in the MHC, but not in the microsatellite loci, suggesting that selection is occurring at the MHC. These results along with future work will help us determine which selective pressures act on the MHC in mule deer and will provide insight into adaptive and evolutionary mechanisms in natural systems.

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Negative Regulation of the Protein Kinase Raf

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Activating mutations in the Ser/Thr protein kinase Raf are common in cancers, especially melanomas. Raf is activated by the EGF Receptor and Ras, and signaling transduction occurs through activation of a kinase cascade involving Raf, MEK, and ERK. However, relatively little is known about cellular regulators that inhibit signaling by activated Raf. Using the nematode *Caenorhabditis elegans*, we performed a genetic screen to identify candidate negative regulators of Raf. In wild-type *C. elegans*, Raf protein degradation is triggered by activation of the downstream kinase ERK in an epithelial cell type called vulval precursor cells (VPCs). Raf degradation is mediated by the E3 Ubiquitin ligase SEL-10 and requires a conserved Cdc4-phosphodegron (CPD) sequence located in Raf protein. In our genetic screen, we identified a mutation, *cov19*, that causes stabilization of Raf protein in VPCs. We will present evidence that *cov19* is a loss-of-function mutation in *ufd-2*, which encodes a conserved ubiquitination factor UFD-2/UBE4B. We find that UFD-2 acts cell-autonomously to promote Raf protein degradation. However, unlike SEL-10, UFD-2 may not regulate Raf protein via the CPD motif. Because UFD-2 is known to act in a chaperone-mediated cytoplasmic unfolded protein response, we are now using a candidate mutant approach to determine whether Raf is regulated by this mechanism. Finally, we find that loss of *ufd-2* enhances phenotypes caused by a mutant, activated form of Raf, strongly suggesting that a UFD-2-mediated mechanism may be important to inhibit mutant forms of Raf found in human cancers.

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Elucidating The Role of Jun Regulatory Elements in Promoting Optic Nerve Regeneration in Zebrafish

Sumona P. Dhara*, Andrea Rau, Heather L. Leskinen, Paul WL. Auer, Ava J. Udvadia

In adult humans, damage to the optic nerve has a poor prognosis because the retinal neurons fail to reinitiate expression of genes that are important for nerve regeneration. Unlike mammals, adult zebrafish can turn on gene expression in response to optic nerve injury that will eventually lead to restoration of visual function. Our recent work describes a hierarchical gene regulatory circuitry that controls successful regeneration in the zebrafish optic nerve (Dhara et al., 2019). We have identified specific changes in the structure of the genetic material (chromatin) at early stages of regenerative nerve growth that enables expression of key transcription factor, Jun. Failure to activate Jun after nerve injury leads to reduced neuronal outgrowth in cultured neurons and diminished nerve regeneration in injured animals. To date, it's unclear how Jun is transcriptionally regulated in response to injury and the downstream Jun-regulated genes that contribute to initiating and sustaining nerve regeneration. Our central hypothesis is that regeneration-specific DNA elements (enhancers) regulate Jun expression, which in turn drive downstream microtubule reorganization in the early stages of injury-induced nerve growth in zebrafish. Our approach will use chromatin conformation capture-qPCR and CRISPR-mediated interference to elucidate the interaction between regeneration-associated enhancers that initiate the expression of Jun following nerve injury in zebrafish. Subsequently, we will determine the effect of Jun knockout in regulating microtubule organization that promotes optic nerve regeneration in zebrafish. We expect that the outcomes of these studies will delineate early key transcription factor driving the regenerative gene regulatory network in fish.

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Coast to Coast: the Importance of the Nearshore for Lake Michigan Si Cycling

***Driskill AM, Girard SR, Berges JA, Young EB**

Spring dissolved silicate (dSi) concentrations in pelagic Lake Michigan have increased ~15 to 34 µM from 1983 to 2017. A decline in spring diatom blooms has been suggested as contributing, but this is based on sampling in the pelagic zone, despite historical diatom blooms starting in the nearshore. Nearshore Lake Michigan has seen an increase in the benthic, filamentous alga *Cladophora* sp., which supports heavy loads of epiphytic diatoms, so is a potential but poorly defined Si sink. The contributions of nearshore algae along with riverine inputs to changing lake Si are not well understood. We aimed to better define nearshore Si fluxes to understand significance for the recent dSi increases. Nearshore dissolved and particulate Si were examined in western Lake Michigan beaches in 2018 and 2019, and drift *Cladophora* filaments were collected and analyzed for biogenic Si (bSi) content. Riverine inputs of dSi and particulate Si were examined in Milwaukee rivers during base flow and high discharge events. Riverine particulate Si concentrations were positively correlated with discharge rates ($p < 0.01$, $R^2 = 0.53$). Nearshore particulate Si concentrations were highly variable (range 0.094-1.9 mg Si L^{-1}), while dSi concentrations increased from April to December ($p < 0.01$, $R^2 = 0.29$). By comparison, the east side of the lake, which does not experience *Cladophora* blooms, showed significantly lower particulate Si concentrations than the Wisconsin side in September 2018 ($p < 0.01$). Incorporating the productive nearshore into a lake-wide Si budget could be important for understanding recent dSi changes.

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The Role of NEKL-3 In the Regulation of Axon Termination

***Cody J. Drozd and Christopher C. Quinn**

Formation of a healthy and stable nervous system requires precise regulation of neuronal development. Failures in axon targeting could lead to a neurodevelopmental disease, such as Bipolar Disorder (BD). Genetic analysis of genes associated with BD has developed a better understanding of the disease. The NEK Kinase protein family has been linked to roles in mitosis, molting, and BD. However, the molecular mechanisms of NEK Kinases in neuron axons are unknown. Our research utilizes the model *C. elegans* to explore the role of a specific NEK kinase (NEKL-3) in the (Posterior Lateral Microtubule) PLM neuron. We observed that a complete loss of NEKL-3 function leads to axon termination defects. The null *nekl-3* mutation, *gk506*, produced a PLM overextension in 36% of 200 PLM axons observed in *C. elegans*. Additionally, fluorescently labelled NEKL-3 was observed in clusters moving bidirectionally along the axon. These observations suggest that NEKL-3 regulates axon termination in neurons. Furthermore, we observed that NEKL-3 does not alter axon termination defects in RPM-1 pathway mutants, suggesting NEKL-3 works with components of the RPM-1 pathway. Further preliminary data indicate that motor protein UNC-116 causes axon overextension. To understand the movement of NEKL-3 as well as its role in axon termination, we are analyzing fluorescent NEKL-3 movement in motor protein mutants along with genetic analysis between NEKL-3 and mutations in other genes that regulate axon development. We aspire for our research to aide in understanding the role of NEKL-3 in axon termination and to help explain abnormal axon formation in BD.

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The Role of the Basement Membrane in Mediating Basal Tissue Folding

Elizabeth J. Falat*, Jennifer L. Wendlick, Michael Stoneman, Valerica Raicu, and Jennifer H. Gutzman

Morphogenesis requires proper cell shape changes and mechanical force, such as tension. Both of these regulators are known to be mediated by intracellular components, such as the actin and microtubule cytoskeletal networks; however, there is a significant gap in our understanding of how interactions between cells and their extracellular environment, specifically with the extracellular matrix (ECM), function to mediate these morphogenetic processes. The basement membrane is a specific type of ECM found along the basal surface of epithelia and is primarily composed of laminin, collagen, and proteoglycans such as agrin. This matrix was previously regarded as a static scaffold, functioning as a passive structure to mediate cell adhesion and polarity. Now, the basement membrane is emerging as a dynamic network critical for regulating mechanical forces required during morphogenesis. There remains a critical gap in our fundamental knowledge of how cell shape changes and mechanical forces, such as tension, are mediated by the basement membrane to ensure proper tissue morphogenesis. Using the zebrafish midbrain-hindbrain boundary (MHB) as a model system, our investigation focuses on the individual proteins of the basement membrane to determine their role in modulating cell shape changes and tensile forces required for basal tissue folding.

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Mate Preferences and Choosiness Vary Independently According to Different Causes of Variation: A Case Study with Eastern Gray Treefrogs (*Hyla versicolor*)

Olivia Feagles* and Gerlinde Höbel

Mate choice is an important cause of natural and sexual selection, driving the evolution of male displays and promoting diversification and speciation. Mate choice decisions arise from the interaction of several components, and knowledge of each individual component is crucial for understanding of the evolutionary consequences of mate choice. Here we focus on the relationship between preference functions (attractiveness ranking of prospective mates) and choosiness (effort invested in obtaining the preferred mate), testing the hypothesis that they are independent components of mate choice decisions. This predicts that preference functions and choosiness are not correlated with one other, and that each is either influenced by different modifying factors, or differently by the same factors. We examine individual variation in female preference functions and choosiness in Eastern Gray Treefrogs (*Hyla versicolor*) and show a lack of correlation between components and influences by separate factors. We also tested the subsidiary hypothesis that choosiness is more malleable than mate preferences, particularly by factors related to energy availability (body size, body condition, reproductive investment) and hormonal states (testosterone, corticosterone). We indeed found that choosiness was influenced by more factors: choosier females had lower concentrations of testosterone and higher concentrations of corticosterone.

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The Importance of Protein Interactions in Chemosensory Array Formation and Signal Transduction for Safe Biofilm Dispersal of Non-Motile *Pseudomonas aeruginosa*

Meredith Frank*, Sonia Bardy

Afflicting more than 700,000 people worldwide, Cystic Fibrosis (CF) is a disorder characterized by the buildup of mucus within the airways of affected individuals. Particles such as bacteria get trapped in the airways of CF patients and increase the risk of infection, respiratory failure, and other complications. One such bacteria, *Pseudomonas aeruginosa*, forms biofilms within the lungs of CF patients. These biofilms have increased antibiotic resistance which hinders treatment of *P. aeruginosa* infection; biofilm dispersal is proposed as a critical part of treatment of *P. aeruginosa* infection. However, biofilm dispersal of swimming bacteria can trigger satellite infections within the airways. Safe biofilm dispersion would require non-motile bacteria. Swimming motility, powered by a rotating flagellum, is controlled by a chemotaxis system *of P. aeruginosa*. Proper swimming motility relies on the formation and localization of unipolar chemosensory arrays. It was recently discovered that interrupting the stability and/or localization of these chemosensory arrays has negative effects on swimming motility. I am seeking to understand the level of interdependence between chemosensory proteins that form these arrays and are essential for swimming motility. Specifically, my results will focus on protein stability in the absence of an interacting partner. I have created fluorescent fusion proteins and will use FACS analysis to determine if protein expression is altered in the absence of an interacting partner. These results will help better model the protein interactions in array formation and signal transduction in *P. aeruginosa* and allow us to target swimming motility to limit satellite infections during biofilm dispersal.

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Going with the Flow: Silicate Influxes from Soil and Grasses to the Milwaukee River

Girard SR*, Driskill AM & Young EB.

Silicate is a common mineral compound of silicon and oxygen and is an important nutrient for some algae in aquatic ecosystems. One algal group, the diatoms, have strict silicate requirements for growth as they form protective silicified cell walls called frustules using biogenic (biologically transformed) silicate. Though silicates are common minerals, in aquatic ecosystems the dissolved bio-available silicic acid (H_4SiO_4) is often limiting for diatoms. Inputs of Si to lakes include from rivers. Preliminary data from 2018 and 2019 suggests that biogenic silicate inputs to the Milwaukee river increases with river discharge ($P < 0.01$, $R_2 = 0.53$). Some higher plants have biogenic silicate deposits, called phytoliths, which can break down and dissolve into silicic acid, contributing to riverine Si inputs. This project aimed to determine silicate content of river grasses and nearby soil, and the significance of phytoliths and plant Si released into the Milwaukee river during snowmelt or rainfall. Samples of grass, soil, and river water were collected from alongside the Milwaukee River during winter. Samples were analyzed for dissolved Si and for total biogenic silicate content using a hot, alkaline extraction. The methodology for extraction of Si from soil was tested using a range of extraction intensities to ensure extractions were representing total biogenic silicate with minimal contribution from mineral Si. To examine conversion of biogenic silicate in grasses to dissolved silicate under natural conditions, samples were incubated in river water and silicic acid content of the water measured over time. This work contributes to ongoing research on the Lake Michigan Si budget, characterizing riverine sources of Si to the lake, especially in the productive nearshore zones.

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Characterizing symbiotic interactions between *Rhizobium* sp. IRBG74 and *Sesbania* in aquatic conditions

Karli Kai Hess* and Prasad Gyaneshwar

The nitrogen-fixing symbiosis between *Rhizobium* and legumes plays a significant role not only in the environment but also in agriculture sustainability. *Rhizobium*-legume communication is mediated by plant-secreted flavonoids and *Rhizobium* secreted Nod factors. Once recognized by its legume host, rhizobia form a root hair infection thread that lead to formation of nodules in which the rhizobia fix nitrogen. However, in aquatic legumes rhizobia infect aquatic through natural cracks during the formation of lateral roots (crack entry). The role of Nod factors in root hair infection is well established but very little is known about rhizobial-legume communication during crack entry. This study was aimed at determining the role of Nod factor in crack entry of aquatic legume *Sesbania* by *Rhizobium* sp. IRBG74. *Sesbania* was inoculated with different nod mutants of *Rhizobium* sp. IRBG74 marked with GUS and the colonization and nodulation was studied using GUS staining and microscopy. We show that the Δ*nodB*, that makes only a part of Nod factor, forms nodule primordial-like structures at the junction of the lateral root and main root. While these structures do not form a full nodule or fix nitrogen, bacterial were observed inside the lateral root junctions. No colonization or root deformation was observed with Δ*nodA or* Δ*nodC* strains. The results of this study and it's implication on Nod factor, nodulation, and nitrogen fixation will be discussed.

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Analysis of non-ribosomal peptide synthetase genes involved in antibiotic production in *Xenorhabdus szentirmaii*

Kimia Jazayeri*, Shane Wesener and Steven Forst

Xenorhabdus species are symbiotic with soil nematodes and pathogenic towards insect hosts. The nematodes invade susceptible insects, perforate the insect gut and release *Xenorhabdus* into the insect hemocoel where they encounter bacterial competitors originating from the insect gut. *Staphylococcus saprophyticus* derived from the gut of the model insect *Manduca sexta* is present in the insect hemocoel soon after nematode invasion. *Xenorhabdus szentirmaii* produces strong antibiotic activity against *S. saprophyticus*. Antibiotics of *X. szentirmai* are produced by nonribosomal peptide synthetase (NRPS) operons. We identified 12 different NRPS gene clusters in the *X. szentirmaii* genome that are potentially involved in antibiotic production. All NRPS enzymes require a phosphopantetheinyl group added by the enzyme encoded by the *ngrA* gene. Thus, a *ngrA* mutant strain lacks antibiotic activity. To determine which NRPS gene clusters are involved in antibiotic production the 12 different NRPS gene clusters were disrupted creating 12 different NRPS mutant strains. In this study, the antibiotic activity of the individual NRPS mutant strains against *S. saprophyticus* was analyzed by both overlay assays and in vitro competition assays. Four mutant strains (Xsz3, 7, 8 and 11) displayed dramatic reduction of antibiotic activity in the overlay assay. Wild-type *X. szentirmaii* eliminated *S. saprophyticus* in competition assays while *S. saprophyticus* grew in competition with the *ngrA* strain. *S. saprophyticus* also grew in competition with the Xsz3, Xse8 and 11 strains indicating that the NRPS clusters inactivated in these strains are involved in producing antibiotics.

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Investigation of The Regulatory Mechanism of CpxA-CpxR Two-component System on The Type III Secretion System of *Dickeya dadantii*

Daqing Jiang*, Ching-Hong Yang

Type III secretion system (T3SS) is an essential virulence determinant for the plant pathogen *Dicekya dadantii* and is regulated by multiple signaling systems. Previously, two-component systems (TCS) HrpX/HrpY and GacS/GacA were reported to regulate T3SS expression of *D. dadantii*. The HrpX/HrpY TCS activates the T3SS by increasing the transcription of *hrpS*, which encodes a σ^{54} enhancer binding protein. HrpS facilitates the binding of RpoN (σ^{54}) to RNA polymerase. The RNA polymerase recognizes specific DNA sequences and facilitate the transcription of *hrpL*. HrpL, an alternative σ factor, is the master regulator of T3SS in *D. dadantii*. It activates the transcription of the *hrp* gene clusters which encode the T3SS structural and functional proteins, such as *hrpA, hrpN* and *dspE*. HrpL is subjected to post-transcriptional regulation by a second TCS GacS/GacA. In the presence of RsmA, *hrpL* mRNA is subject to the RsmA-mediated degradation. Upon activation, GacS/GacA TCS activates the transcription of a small regulatory RNA encoding gene *rsmB*. RsmB binds to RsmA and neutralizes its negative effect on *hrpL* mRNA. Recently, the regulation of T3SS expression was shown to be regulated by a ubiquitous bacterial second messenger cyclic diguanylate monophosphate (c-di-GMP). In this study, we uncover an envelope stress response TCS, CpxA-CpxR, that regulates the T3SS of *D. dadantii*. It influences the T3SS through controlling the expression of *hrpL* and c-di-GMP level.

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Searching for JUN Dimerization Partners that Promote Regeneration-Associated Gene Expression after CNS Injury

Heather L. Leskinen*, Sumona P. Dhara, Shama P. Mirza, Ava J. Udvadia

In humans and other mammals, injury to the central nervous system (CNS) can cause a permanent loss of neuronal function, leading to cognitive defects, limb paralysis, and other neurological disabilities. In contrast, some non-mammalian vertebrates, like zebrafish, have the remarkable ability to functionally regenerate axons after CNS injury by reactivating and sustaining the expression of regeneration-associated genes. Our recently published work identified the Jun gene as a putative target of regeneration-specific enhancers (Dhara et al, 2019). Our combined RNA- and ATAC-seq analyses revealed the JUN protein as a potential master regulator of stage-specific regeneration after optic nerve injury in zebrafish. JUN functions as an obligate dimer and is known to both homo- and hetero-dimerize to regulate gene expression in response to injury of the peripheral nervous system. However, it is not known if these same binding partners interact with JUN during CNS regeneration. To investigate this, we will use the zebrafish optic nerve crush as a CNS injury model to identify JUN-interacting proteins over the course of regeneration using proximity labeling assays, followed by mass spectrometry. Once candidate binding partners are identified, we will determine functional significance of specific JUN interactions *in vivo* with forced expression of heterodimers by examining axon growth in larval zebrafish optic nerve transection models. Elucidating the zebrafish gene regulatory program will aid in developing new therapeutic approaches in human CNS regeneration.

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O Cricket, Where Art Thou: Do Black Widow Spiders Remember the Site of Prey Capture in a Complex Web?

Margaret Marshall*, Clinton Sergi, Rafael Lucas Rodríguez

Animals vary in how they form memories about their environment. There is evidence that web spiders form memories about the layout and content of their webs. However, most of this evidence comes from spiders that form two-dimensional webs. I analyzed the memory capability of Black Widow spiders, which build three-dimensional webs (consisting of a sheet and gum-footed lines). I tested the hypothesis that spiders form memories of the sites at which they have captured their prey. This hypothesis makes the prediction that spiders will predominantly search the sheet of their webs when looking for prey that was captured in the sheet, and the gum-footed lines when looking for prey that was captured in a gum-footed line. I tested this prediction by offering prey to spiders in either the sheet or gum-footed lines of their webs, then removing the prey after the spiders had captured the prey. Spiders in control groups were either subject to damage to the web (equivalent to the damage spiders cause when removing prey from the web) or given prey and left to consume it after capture. Spiders searched for prey they had captured and lost. The site of prey capture had no overall effect on where they searched. However, spiders were more likely to search at the gum-footed lines for larger prey. I conclude that the spiders' memories of captures prey include details about the site of prey capture and the prey features, which they use to regulate prey re-acquisition efforts.

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Rational Transitive Mate Choice In *Enchenopa binotata* Treehoppers (Hemiptera: Membracidae)

Vladislav Melnikov*, Rafael Rodríguez, Bretta Speck

Mate choice is a crucial decision for females. Mate choice decisions are based on mate preference functions, which are curves that describe the relationship between the attractiveness of sexual ornaments and variation in their features. One possibility for how mate choice decisions relate to preference functions involves rationality, whereby females always select the male with the preferred traits when in the presence of less-preferred males. A competing alternative — with support from humans and some vertebrates — involves irrationality, whereby the presence of very low or very high quality males can cause females to alter their preferences in the available mates. We test whether treehoppers use rational choice to select mates, and predict that responsiveness will be higher for preferred male signals regardless whether a decoy was presented. Using vibrational playback stimuli that varied in a single variable, frequency, we presented females with preferred and less-preferred males in the presence or absence of attractive and unattractive decoy males. We found that females selected the preferred males over the less-preferred males regardless of the presence of a decoy or the quality of the decoy. These findings show that *Enchenopa* treehoppers use rational mate choice.

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Does Size-Assortative Mating Improve Fertilization Success in Grey Treefrogs?

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Success of external fertilization is often optimized by the closeness of egg and sperm release. In frogs, males and females perform amplexus, mating embrace, during egg laying. We hypothesized that size-assortative mating improves fertilization success. This hypothesis makes two predictions: (1) fertilization success should be correlated with a particular within-pair size difference; and (2) size differences observed in nature should align with the optimal size ratio. To test prediction (1) we collected 20 pairs of Eastern Grey Treefrogs (*Hyla versicolor*), and allowed them to oviposit into separate marked containers. Fertilized embryos, at four days post-oviposition, were distinguishable from unfertilized eggs. Proportions of fertilized eggs were then calculated using photo analysis. The body length of each adult frog was measured using calipers. Size ratios for each adult pair was compared to the fertilization success of their eggs. We also collected additional mated pairs and measured their size ratios. We found that there is indeed a size ratio that optimizes fertilization success, but that about 50% of the breeding population does not mate with optimally size-matched partners.

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Visual Mate Choice in Eastern Gray Tree Frogs: Mated and Unmated Males Look Different

Samuel Murray*, Gerlinde Hoebel

Eastern Gray Tree Frogs (*Hyla versicolor*) are dichromatic, and the body area showing the strongest difference between the sexes is the throat. Males have gray throats whereas females have white throats. Other areas of the body (the white bellies and bright-yellow colored inner thighs) are similar between the sexes. We hypothesized that throat coloration was significant to mate choice and predicted that this area of the body would be the most different between mated and unmated males. Because belly and thigh color are not sexually dimorphic, we predicted them to be similar between both groups of males. We collected 40 mated males and 37 unmated males from the UWM field station in Saukville and brought them to the lab at UWM. Using a photo spectrometer, we measured brightness, saturation, and hue on the inner thigh, belly, and throats. As predicted, throats of mated males were different – they were darker. This may indicate that throat color is significant to mate choice. Whether females directly prefer darker throats, or whether the association between throat color and mating success is indirect because color indicates a better-fed and more fit partner remains to be studied in the future.

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Understanding the Genetic Underpinnings of sexual development in a Giant Kelp Breeding Program

Shanice Piango*, Rachael Wade, Filipe Alberto

Aquaculture of giant kelp, *Macrocystis pyrifera* gained commercial interest in America during the early 20th century. This interest was primarily in production of alginates, but also for food, organic fertilizer, biofuels and other sustainable products. While production continues to increase, the costs and efficiency required to support these industries have not. Therefore, success of seaweed aquaculture depends on the development of sustainable production strategies. One of which is the capacity to preserve seaweed strains via a breeding program. In-vitro culturing allows selection of desirable morphological and physiological traits to enhance production, but not without its own challenges. Species specific conditions required for long term culturing, and cryo-preservation methods with deleterious thawing effects leave germplasm preservation of liquid cultures under dormant conditions as the best solution. Successful cultivation requires understanding the species life history. Kelps have heteromorphic life histories which alter between diploid macroscopic and haploid microscopic generations. Little is known about the microscopic generations. S**tudies showed that male and female gametophytes differed phenotypically, physiologically and u**se their genome differently, which could suggest differential expression among sexes. Genes associated with growth are highly conserved between sexes but differentiate under sexual conditions, however this is species specific. To address this unknown we will use transcriptomic analysis on male and female gametophytes to observe gene expression changes from vegetative to reproductive stages. RNA will be extracted at three time points at different growth stages. We expect differences in expression between males and females and a better understanding of the genetic conditions facilitating these.

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Is yellow leg coloration of Gray Tree Frogs (*Hyla versicolor*) involved in mate choice?

Richard Pulkowski* & Gerlinde Höbel

Eastern Gray Tree Frogs are unique in the yellow coloration on their legs and groin. We know that the yellow coloration cannot be generated within the body, and diet is important to creating this coloration. Our hypothesis is that the increased yellow coloration on males will lead to a more favorable mating selection. Larger males, males in a better condition, will have more yellow coloration. Secondly, males collected later in the season will be less yellow due to the time spent calling for a mate rather than foraging. Finally, males found in amplexus will have more yellow coloration than unmated males. We caught 76 male frogs (39 mated and 37 unmated) in a pond located near the UWM Field station in Saukville, WI. They were then photographed for lateral and ventral measurement. These measurements were then analyzed using software called ImageJ. The measurements were then used to compare yellow coloration in the lateral body length and ventral leg area. This was the basis of validity for studying both lateral and ventral as these measurements could have presented a strong correlation. The purpose of this was to ensure that all possibilities of mate selection were considered for reasoning of yellow coloration. Diet, age, frog size, time during mating season, and likelihood of previous mating success were all factored into the conclusion. This allowed for a definitive result that leads to predation aversion as a future area of study.

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Zebrafish as a model for *MYH9*-related disease

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This study aims to develop vertebrate models to elucidate molecular mechanisms by which known mutations in *MYH9* disrupt development. There are five clinical disorders that result from mutations in *MYH9* that are classified as *MYH9*-related diseases: May-Hegglin anomaly, Sebastian, Fetchner, and Epstein syndrome; and non-syndromic deafness DFNA17. These diseases are characterized by symptoms including platelet abnormalities, nephritis, visual defects, and hearing loss. *MYH9* encodes for the highly conserved non-muscle myosin IIA protein (NMIIA), which has essential roles in cell division, cell migration, and cell shape changes. However, there is a critical gap in understanding how *MYH9* mutations found in the human population contribute to the etiology of *MYH9*-related diseases. Our current studies are investigating a zebrafish mutant line we generated containing a stop codon in Ex12Δ37, which results in a truncated NMIIA protein. This mutation leads to heart edema between 4-6dpf (days post fertilization) and phenotypes are consistent with previously reported abnormal kidney development. Our preliminary results indicate that these homozygous mutants die before 14dpf. In addition, we are developing zebrafish models of the most common human *MYH9* mutations, specifically at the conserved Arginine 702/705 locus, to examine development of organs affected in MYH9-related disease through CRISPR/Cas genome editing.

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Motility is important for competitive nodulation of *Mimosa pudica* by *Paraburkholderia phymatum*

Sara Saleh*, Shahsini Welmillage and Prasad Gyaneshwar

Nitrogen (N) is the most limiting nutrients for plants and plant growth requires supplementation with N-fertilizer that results in environmental degradation. However, many bacteria can fix atmospheric nitrogen and some plants, such as legumes, form a symbiotic relationship with N-fixing bacteria, giving them a steady supply of nitrogen. The important mechanisms of rhizobial-legume symbiosis are extensively studied. However, these studies have been performed in sterile, defined conditions in the laboratory. In contrast, very little is known about the mechanisms that mediate this symbiosis in soil with other microorganisms. To study legume nodulation in soils, we are utilizing the symbiosis between *Mimosa pudica* and *Paraburkholderia phymatum*. M. pudica is native to Brazil and the soils of Midwestern USA lack *P. phymatum*. Random transposon insertion mutants in P. pymatum were screened for defects in motility as compared to the wild type strain. Two mutants were significantly less motile than the wild type and were selected and evaluated for their ability to nodulate *M. pudica*. Both nodulated M. pudica similarly to the wild type in both axenic and soil conditions. This indicates that motility is not required for nodulation. To determine if motility can confer competitive advantage, the mutnats and WT where co-innoculated in different ratios and their nodulation ability was determined by reisolating mutants from the nodules. The mutants showed defect in symbiosis when co-inoculated with the wild type. Only ~25% of the nodules contained the mutants. This shows that rhizobial motility is not essential but is important for colonization of legumes.

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Lifetime Changes in Mate Choosiness in *Enchenopa binotata* Treehoppers (Hemiptera: Membracidae)

Sara Seidita*, Rafael Rodríguez, Bretta Speck

Mate choice decisions are influenced by choosiness (the effort individuals are prepared to invest in securing their preferred mate types). We tested the hypothesis that females adjust choosiness as they age, because they are selected to attempt to obtain preferred mate types while ensuring that mating occurs. This hypothesis predicts that choosiness will decrease over a female's lifetime (a pattern expected due to the diminishing availability of males over the course of the mating season). We tested this prediction with 60 female *Enchenopa* treehoppers, herbivorous insects that communicate with plant-borne vibrational signals. We assessed female responses from first sexual maturity until death using vibrational playbacks and laser vibrometry. We measured choosiness as the difference in female duetting effort with attractive versus unattractive vibrational playbacks. We found that female choosiness decreased with age, while their mass did not, indicating that the change in choosiness was not simply due to decreasing condition. Choosiness plays a significant role in mate choice decisions throughout the female treehoppers' lifetime. The decrease in choosiness gives an increased availability in mate selection which is important for biological fitness in nature.

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Sizing Up the Memory of Western Black Widow Spiders, Latrodectus hesperus (Araneae: Theridiidae)

Audrey Schlais*, Clint Sergi, Rafael Rodriguez

Animals differ in memory ability. To date, research regarding spider memory abilities has been limited to species that build two-dimensional orb webs. I analyzed the memory capability of a spider that builds more complex, three-dimensional webs: the Western Black Widow Spider, *Latrodectus hesperus*. I first tested the hypothesis that *L. hesperus* form a memory about having captured prey. This hypothesis makes the prediction that the spiders will search for prey that they have captured and then lost. I then tested the hypothesis that *L. hesperus* memories of captured prey include details about the size of the prey. This hypothesis makes the prediction that search effort (e.g., search duration, search bouts) will vary with prey size. I offered *L. hesperus* spiders prey items of varying mass and then removed that prey after capture was complete. The control spiders either experienced damage to the web (holes equivalent to what the spiders create when removing prey from the web), or no damage to the web, and were left to consume the prey after capture. Spiders that captured and lost prey were more likely to search, and searched for much longer, than control spiders. However, relative prey size had no effect on whether spiders searched, or for how long. I conclude that L. hesperus form memories of having captured prey, and exert effort in re-acquiring them when lost. Those efforts do not seem to be adjusted to overall prey size.

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Genomic Analysis of Migration in Warblers

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The genetic mechanisms by which complex migratory behavior has frequently evolved remain poorly understood. This study used the presence of both migratory and resident populations of the common yellowthroat and a close congener, the gray-crowned yellowthroat of Belize, to investigate the genetic basis of migratory behavior. We sequenced the whole genomes (41x coverage) of pools of 36-40 individuals from each of two migratory yellowthroat (New York & Wisconsin) and three resident populations (Arizona, Belize, New York). We identified genes under strong purifying selection using the bottom 0.5% of values of Tajima's D, three genes (*ARNTL, FAXC, LY75),* which had been previously described in migratory studies, differed consistently in selection between the migratory and resident populations. In addition, we also identified four genes (*BFSP1, FCGBP, HA1F, SUSD4*) that differed in selection between migratory and resident populations that had not been previously identified. The genes above are related to circadian rhythms (ARNTL) and immune response (SUSD4 & HA1F). Further study will be needed to determine the adaptive significance of these differences, but this is the first study to find genetic differences between migratory and resident populations of the same species.

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A bivariate exploration of among-female variation in mate preference functions (Hyla versicolor)

Kane D. Stratman & Gerlinde Höbel

Female choice is a widely researched topic of sexual selection. Biologists have characterized both the variation in male mating displays and shape of female preferences for such behaviors across a variety of taxa. A female's *reference functions* represents both the most preferred male trait value and her willingness to accept non-ideal values. In the wild, females are expected to choose based on preferences for multiple aspects of a male display, however the previous research has only measured female-by-female preferences for single traits. In this study we built two preference functions *per individual* (Eastern gray treefrogs); one exploring responses to calling duration, and another calling rate. There was considerable variation in general preference shapes, implying that population-level measures in fact hide strongly divergent behaviors. In particular, we found that females are nearly evenly split in their assessment of very rapid calls; many prefer the most extreme rates, while the rest strongly prefer an intermediate value. Knowledge of a female's preference for one trait did not predict her preference for the second. Comparing these bivariate functions to the acoustic distributions of actual recorded males, we found support for directional selection, however the wide range of trait preferences predicts mating success for nearly any signaling male in the wild.

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Growth Rates and Genetic Content of Pah-Degrading Bacterial Communities from a Contaminated Urban Watershed

Nathaniel Thorngate-Rein, Marit Bastian, John Berges

Polycyclic aromatic hydrocarbons (PAHs) are organic pollutants common in urban watershed sediments. PAH-degrading bacteria could facilitate bioremediation of contaminated environments (vs. dredging and disposal), but mechanistic knowledge of PAH-degrading pathways in natural communities is limited. We examined PAH degradation by assessing the ability of bacterial communities to use PAHs as carbon sources and identifying genes associated with PAH degradation in samples from two sites in the Milwaukee River Area of Concern: the lower Milwaukee River and the Grand Trunk wetland. Sediment dilutions were inoculated into carbon-free Bushnell-Haas media (BHM) saturated with either naphthalene or phenanthrene, and growth was monitored by measuring absorbance (600 nm). Genomic DNA and RNA were extracted from sediments and log-phase PAH cultures. DNA extracts were used to amplify 16S rRNA V3/V4 hypervariable regions and Rieske-type dioxygenase genes (catalyzing the initial step of PAH degradation) for use in Illumina sequencing. Preliminary analysis of dioxygenase gene sequences suggests that *Rhodococcus* is an important PAH-degrading genus in in Milwaukee River sediments. Additionally, *Pseudomonas* sp. were isolated from PAH microcosm cultures; these bacteria were unable to utilize PAHs when grown in pure culture, implying that PAH degradation in these sediments may be a community effort. We plan to use 16S data from environmental and cultured samples in order to identify taxonomic shifts and infer metabolic function *in silico* (e.g. PICRUSt). We will also compare the transcriptomes of cultures grown in nutrient-rich media and BHM+PAH cultures to identify changes in gene expression in response to PAH stress and nutrient limitation.

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The Impact of Interspecies Interaction on Adult Male Treehoppers Signaling Behavior

Nathan Wagner*, Jak Maliszewski, and Rafael Rodriguez

We focused on *Enchenopa binotata*, a species of treehopper that communicates through vibrational signals on a plant medium. While most species of treehoppers each inhabit a specific host plant, some species may share the same niche. However, while species may live on the same plant, different species communicate at different frequencies which discourages interspecies interaction. We decided to study the impact that mixed versus single species groups have on their communication signals as adults. Does being exposed to signals of other species affect the calls of other individuals and if so, how? By creating mixed and single species groups of nymphs, and then recording their signals with laser vibrometry when they reach adulthood, we were able to measure any change in their signaling behavior due to interspecies interactions. We focused on measuring duration, frequency, pulse rate, and quantity of calls, and compared the two treatments. We expect that being raised in multi species groups will have an impact on signaling behavior of adults. Our findings give us more information on how separate species of treehoppers communicate and interact in the wild and how that interaction may affect their signaling behaviors.

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Rhizobial colonization of legume host in soils: Bacterial community analysis upon inoculation of *Mimosa pudica* by *Paraburkholderia phymatum*

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Nitrogen fixing symbiotic association between rhizobia and legumes contribute significant amounts of fixed nitrogen to agricultural and natural environments. Mechanisms of nodulation and N2-fixation are well defined using axenic laboratory conditions. Although, mechanisms important for rhizobia to survive and colonize legumes in natural soil conditions are not well understood. The presence of natural nodulating strains hamper efforts to determine the mechanisms of rhizobial-legume interactions in soils. As a first step in determining rhizobial symbiosis in non-sterile soils, we have used the symbiotic interactions of *Mimosa pudica* and *Paraburkholderia phymatum* as a model. *M. pudica* is not nodulated by native bacteria present in agricultural soil of Wisconsin, USA. Inoculation of *M. pudica* with *P. phymatum* in these soils lead to nodule formation and nitrogen fixation. The effect of symbiont inoculation on native bacterial community was studied using 16S rDNA sequence analysis. The results reveal that inoculation did not significantly alter the bacterial community of bulk soil compared to the non-inoculated plants. Relative abundance of *P. phymatum* declined after inoculation in soil but increased in the root over time. In contrast to the soil and root samples that comprised of wide diversity, the nodules were colonized predominantly by *P. phymatum*. These results suggest that success of the inoculated symbiont likely depend on its ability for root attachment by outcompeting other soil bacteria on the root. Better understanding of mechanisms that allow the inoculated strain to colonize its plant host is crucial for realizing the full potential of microbial inoculants in sustainable agriculture.

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The starvation sensing protein *rspB* alters *pel* expression in high c-di-GMP condition in *D. dadantii*

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Dickeya dadantii is a phytopathogenic bacterium with a wide host range. PelD, an endopectatelyase enzyme secreted by *D. dadantii* through the type II secretion system, degrades the cell wall in host plants. The second messenger cyclic diguanylate monophosphate (c-di-GMP) has been reported to regulate the expression of virulence genes in *D. dadantii*. EcpC is a phosphodiesterase that hydrolyzes c-di-GMP in *D. dadantii*, thus lowering the intracellular c-di-GMP concentrations. A low *pelD* expression is observed under the *ecpC* mutant background (high c-di-GMP). In order to elucidate the c-di-GMP effectors that regulate PelD production, a transposon library was made under *ecpC* mutant background. Mutants were screened by analyzing the expression of PelD. Those with an increased *pelD* promoter activity were selected for further investigation. One of the genes mutated by the transposon was identified to be *rspB* by sequencing. The *rspB* supposedly is a starvation sensing protein as per annotation. Probably *rspA-rspB* operon functions together for regulating downstream gene expressions. In *Escherichia coli* K12 strain this gene has been reported to regulate multiple virulence factor genes. Future work will explore the role of RspB in regulating type II, type III secretion systems and connect the role of c-di-GMP in these regulatory pathways.

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Does deletion of *chpC* cause a loss of swimming phenotype or is there a hidden secondary mutation?

Tamia Williams*, Sonia Bardy

Pseudomonas aeruginosa is an opportunistic pathogen that is able to live in a wide range of environments. The capacity to live in this breadth of habitats is aided by its swimming motility and its twitching motility. These motility structures are controlled by chemosensory systems that allow bacteria to respond to their outside environment. The Chp system is the chemosensory system in *P. aeruginosa* that controls twitching motility and regulates intracellular levels of adenosine 3', 5'-cyclic monophosphate (cAMP). This system allows response to the cell's outside environment based on communication with methyl-accepting chemotaxis proteins (MCPs). ChpC is an adaptor protein involved in twitching motility environmental response. ChpC connects with the MCP and is a homolog of CheW protein. Deletion of *chpC* (Δ*chpC*) reduces twitching motility response to environmental signals but was not expected to have an effect on swimming motility. Recent studies in our lab have unexpectedly shown that Δ*chpC* might be affecting flagellum formation and function. I verified this phenotype by testing a lineage of our strains to determine when this altered phenotype first occurred. I generated a fresh *chpC* deletion to determine if this deletion is truly responsible for loss of flagellation biogenesis and/or function, resulting in swimming motility flaws. If Δ*chpC* is responsible, complementation of *chpC* should restore swimming motility. Understanding the relationship between *chpC* and swimming capability can either confirm or deny relations between twitching and swimming motility systems in *P. aeruginosa*, leading to better understanding of the Chp system and regulation of different motility systems.

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Nuclear Phylogeography and Distribution Modeling of the Widespread Species Big Brown Bat

Xueling Yi* and Emily K. Latch

Big brown bat (*Eptesicus fuscus*) is a common hibernating bat distributed across most of North America, several Caribbean Islands, and northern South America. A previous range-wide study of big brown bat populations identified mitochondrial phylogeographic patterns that roughly corresponded to the morphological subspecies and indicated clear geographic divergence. However, the previously generated nuclear structure showed a lack of differentiation, reflecting either sex-biased gene flow or insufficient power of the markers used. Further clarification of nuclear divergence using more powerful markers is thus important for the understanding of population structure and patterns of gene flow. Here we hypothesize that mitochondrial and nuclear genomes have similar population structures shaped by both unbiased contemporary gene flow and historical vicariance among glacial refugia. We used the more powerful SNP data generated by bestRAD sequencing, and we did species distribution modeling (SDM) in MaxEnt to identify historical refugia using Bioclimatic variables on WorldClim and occurrence records on the Global Biodiversity Information Facility. Preliminary results from 96 samples confirmed nuclear divergence among populations in the western US, eastern US, and Caribbean Islands, similar to the patterns of mitochondrial phylogeography. The SDM reconstruction showed that Central America host the glacial refugia but is faced with a loss of suitable habitats under future climate change scenarios. Our data supported the geographic nuclear divergence and particularly suggested genetic distinction of populations on the Caribbean Islands, which are proposed to be evolutionarily significant units (ESUs) and a focus of conservation under impacts of climate change.

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