RETREAT SCHEDULE

9:00-9:30  Breakfast

9:30-9:40  Opening remarks
Gerit Bethke, Research Associate, Plant Biology, Glazebrook Lab

9:40-10:00  Will Harcombe
Assistant Professor, Ecology, Evolution, and Behavior

10:00-10:20  Yuan Xu
Graduate Student, Horticulture, Cohen Lab

10:20-10:40  Tonya Ward
Post-Doctoral Associate, Biotechnology Institute, Knights Lab

10:40-11:00  Ben Bonis
Graduate Student, Biochemistry, Gradnick Lab

11:00-11:20  Break

11:20-11:40  Tony Schmitt
Graduate Student, Plant Biology, Carter Lab

11:40-12:00  Michelle Riehle
Senior Research Associate, Microbiology, Vernick Lab

12:00-12:20  Ryan Briscoe-Ruquist
Post-Doctoral Associate, Plant Biology, Moeller Lab

12:20-12:50  Nils Stein
Head of Genomics of Genetic Resources, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany

12:50-1:50  Lunch (Atrium)

1:50-2:10  Jonathan Badalamenti
Post-Doctoral Associate
Biotechnology Institute, Bond Lab

2:10-2:30  Candice Hirsch
Assistant Professor, Agronomy & Plant Genetics

2:30-2:50  Eli Krumholz
Graduate Student, Plant Biology, Libourel Lab

2:50-3:10  Caleb Lever
Assistant Brewer, Fair State Brewing Cooperative

3:10-3:20  Closing Remarks
Anke Reinders, Senior Research Associate, Plant Biology, Ward Lab

3:30  Refreshments and social hour, Cargill atrium
System biology and eco-evolutionary feedbacks in microbial communities

There has been a great deal of work on both the robustness of genomes and the stability of communities, but little work connecting the two. We used genome-scale metabolic modeling to computationally analyze the impact of genetic changes when two species of bacteria (E. coli and Salmonella enterica) are competing versus when they are involved in an obligate mutualism. We computationally generated metabolic models of all possible single knock out mutants of E. coli. Then, using a recently developed multi-scale computational framework, we simulated the growth of each mutant E. coli in the presence of S. enterica. We found that the community was most robust to genetic perturbations when the organisms were cooperating. Species ratios were more stable in the cooperative community, and community biomass had equal variance in the two contexts. These results highlight the utility of connecting metabolic mechanisms and studies of ecological stability. Additionally, the work demonstrated that genomic robustness is significantly influenced by ecological context.

Using metabolomics to define common stress responses across abiotic challenges and determine unique signatures for specific stress events

Plant abiotic stress responses lead to modified gene expression that result in changes of metabolism, the direct signature of biochemical activity. A mass spectrometry-based untargeted metabolomics approach was used to study whole plant metabolic changes in Arabidopsis thaliana that were induced by different abiotic stresses including drought, heat, cold, and high light. Arabidopsis thaliana wild type seeds were grown vertically on agar plates at 22°C under a 16-h-light/8-h-dark photoperiod of 80 µmolm-2s-1 cool-white fluorescent for 11 days. Seedlings were treated with drought stress (desiccation for 2h), basal heat stress (45°C for 5h), acquired heat stress (38°C for 1.5h, 22°C for 2h, 45°C for 5h), basal cold stress (3°C for 3h), acquired cold stress (3°C for 3h, -20°C for 1h), and high light stress (902 µmolm-2s-1 high light for 1h). Each stress group has a corresponding recovery group where the plants were moved to non-stress conditions for 2 days after the stress treatment. Metabolic profiles of control, stress groups, and stress recovery groups were acquired using ultra performance liquid chromatography high resolution mass spectrometry (UPLC-HRMS) on a hybrid quadrupole orbitrap instrument. Thousands of metabolic features [m/z, retention time, intensity] were analyzed by SIEVE™ software (Thermo Scientific) for principal component (PCA) and single metabolite t-test analyses. Hundreds of metabolites were significantly altered in stress groups or recovery groups compared with the control group (p-value < 0.05 from t-test). Among them, 30 metabolites of interests were confidently identified by comparison to authentic standards. These included amino acids, tricarboxylic acid cycle intermediates, sugars, and other plant metabolites, indicating that significant aspects of their metabolism were modified by exposure to different abiotic stress conditions.
BugBase: A tool for predicting community-wide phenotypes

With the rapid expansion of microbiome studies, the complexity of microbial communities and the intricacy of host-microbiome interactions have become apparent. Many microbiome studies rely on amplicon sequencing of marker genes to determine the taxonomic composition of a microbiome. Although useful tools such as PICRUSt now allow us to estimate the total genomic content of microbes using marker gene surveys, functional characterizations at the level of metabolic pathways can still be challenging to interpret. We hypothesized that we could predict several biologically relevant microbiome-wide traits like Gram staining, oxygen tolerance, the ability to form biofilms, pathogenicity, stress tolerance and mobile element content at the microbiome-wide level based on existing annotations and the presence of open reading frames within the genomes of community members. By combining a curated annotation database with the PICRUSt framework we have developed BugBase, a user-friendly tool that characterizes microbiome phenotypes using marker gene survey data. BugBase, which is available as a web-application or open-source package, requires users to input an OTU table and mapping file, which are used to generate phenotype predictions in the form of publication quality plots with corresponding statistical analyses. Using published data we have predicted microbiome-wide traits to vary significantly across a variety of host and environmental parameters, including bodysite, antibiotic exposure, disease status, geographical location and pH. We believe BugBase will provide researchers with useful, broad, phenotypic descriptions of microbiomes that will simplify the interpretation of complex microbial biomarkers and serve as a jumping off point for further hypothesis driven in-depth analysis.

Genetic Interrogation of an Fe(II)-Oxidizing Marinobacter

Iron is one of the most abundant redox active elements on Earth, and as such strongly influences the cycling of biologically relevant elements. As widespread agents that affect solubility and reactivity of iron, Fe(II)-oxidizing bacteria are a phylogenetically diverse group that mediate this influence. The costs associated with accelerated weathering of iron-bearing constructs facilitated by the biological activity of Fe(II)-oxidizing bacteria is significant, as are the benefits to be gained by harnessing this metabolism for industry and biotechnology. Despite the economic and industrial advantages of a mechanistic understanding of microaerophilic Fe(II)-oxidizing bacteria, the genetics of these organisms remain entirely uncharacterized due to the particular requirements for their cultivation. Genetic interrogation of a microaerophilic Fe(II)-oxidizing strain of Marinobacter, isolated from the Soudan iron mine in northern Minnesota is conducted using high-throughput methods. Tn-seq is used to assign a fitness cost to genes under heterotrophic conditions, establishing a background against which future Tn-seq results under Fe(II)-oxidizing conditions can be compared. DEATHseq, a modified application of Tn-seq, allows for a similar assessment genetic fitness cost independent of growth. The availability of a genetically tractable Fe(II)-oxidizing strain capable of heterotrophic growth is a powerful tool in the study of biologically mediated Fe(II)-oxidation, and will provide a foundation by which to study the role these organisms play in the environment. Marinobacter are prevalent in marine and subsurface environments, and a greater understanding of this organisms metabolic capabilities will grant insight into the nature and magnitude of this contribution.
Tony Schmitt
Graduate Student, Plant Biology, Carter Lab

The role of the octadecanoid pathway in regulating nectar secretion in Arabidopsis thaliana

Floral nectar is the major energetic reward for a majority of pollinators. While over 75% of crop species depend on pollinators in order to achieve maximum seed set, very little is known about the mechanisms regulating nectar secretion. The phytohormone jasmonic acid (JA) is recognized to be involved in several plant processes including plant development, reproduction, and defense. Recently, JA has been shown to positively influence nectar secretion in both floral nectaries and extrafloral nectaries. For example, endogenous JA levels appear to peak in flowers just prior to floral nectar secretion, but the details of how JA regulates nectar secretion have yet to be elucidated. We have found that the octadecanoid pathway does indeed play a role in the production and regulation of floral nectar in Arabidopsis thaliana. Null alleles for several JA biosynthesis and response genes have significantly reduced amounts of nectar, as well as altered expression of genes known to be involved in nectar production. Interestingly, a T-DNA knockout for 12-oxophytodienoate reductase 3 (OPR3) produced no nectar in newly opened flowers, but it did secrete nectar in older flowers. OPR3 encodes an enzyme further down the JA biosynthetic pathway involved in reducing 12-oxo phytodienoic acid (OPDA) into 3-oxo-2/12(2)-pentenylocyclopentane-1-octanoic acid (OPOC8). Furthermore, a similar phenotype was observed in col1-1, a mutant for the JA receptor CORONATINE INSENSITIVE PROTEIN 1 (COI1). These observations strongly suggest a potential role for a JA- and COI1-independent pathway in regulating nectar production in Arabidopsis. Finally, we also have identified crosstalk between the JA and auxin response pathways in Arabidopsis nectaries, as well as a role for the JA-responsive transcription factor MYB21. Cumulatively, our findings indicate an essential role for octadecanoid biosynthesis and response pathway in regulating nectar secretion.

Michelle Riehl
Sr. Research Associate, Microbiology, Vernick Lab

Large segregating chromosomal inversion in Anopheles gambiæ is associated with differential malaria susceptibility

Malaria remains a prominent public health problem as 40% of the world’s population is at risk. Despite being both preventable and treatable, malaria remains responsible for 15% of all childhood deaths in Africa. The mosquito vector, Anopheles gambiae, is the primary vector for malaria transmission across West Africa. Recent increases in genomics data have helped to decipher how genetic variation within and across the A. gambiae species complex expands malaria transmission over both space and time. Here we explore one of the sources of ecological and behavioral plasticity in this insect vector, chromosomal inversions. We combine the power of mosquito field collections with next generation sequencing to explore the association between the inversion karyotype of a large ~22MB paracentric inversion, 2La, and susceptibility to infection by Plasmodium falciparum. The non-inverted 2La+ form of the inversion is significantly more susceptible to malaria infection, but has no effects on mosquito longevity or susceptibility to another eukaryotic pathogen, entomopathogenic fungus. Whole genome sequencing of mosquitoes sampled across the African continent bolsters support for the single inversion event as individuals of the same karyotype collected across the African continent are more genetically similar to one another within and flanking the inverted region than they are to sympatric individuals collected at the same time, yet differing in karyotype. This pattern breaks down away from the inversion and on other chromosomes. Association of infection phenotype with the 2La chromosomal inversion can have profound effects on disease epidemiology as rates of the inversion vary widely across geography.

CONGRATULATIONS to Jan - June 2016 Travel Award Recipients:
Felipe Avila, Marike Boenisch, Alex Brohammer, Clairessa Brown, Liana Burghardt, Benjamin Campbell, Tomas Cermak, Emily Conley, Shaun Curtin, Dana Freund, Liangliang Gao, Amanda Gorton, Alex Harkness, Adam Herman, Cory Hirsch, Mark Holmes, Eva Konecna, Praveen Kumar, Tung Sy Le, Lotus Lofgren, Marisa Miller, Claire Milsted, Andrzej Noysewski, Anke Reinders, Nichol Schultz, Sara Li The, Diana Trujillo, Leon van Eck, Tonya Ward, Yang Yang, and Yu Lu

Science on the Spot
Thursday, 4th - 5th PM
Room 105 Cargill
February 4th, March 3rd, April 14th

Special Thanks to:
Fair State Brewing Cooperative
https://fairstate.coop/2506a Central Ave NE
Studies of reproductive isolation often find that prezygotic barriers evolve more rapidly than postzygotic barriers between incipient species. However, it has been challenging to determine whether selection has directly caused elevated isolation (reinforcement) or whether it has occurred as a by-product of adaptation to alternative environments. In *Clarkia xantiana*, there is a pronounced pattern of reproductive character displacement (RCD) between incipient plant species that remain cross-compatible, a key signature of reinforcement. The self-fertilizing taxon is recently derived from the primarily outcrossing taxon (~65,000 yrs) and phylogeographic studies show that they have come into secondary sympatry in a narrow zone. Here, we test whether reinforcement selection has directly caused the evolution of RCD in floral traits using a series of large field experiments. Our results indicate that hybridization between incipient species is strongly reduced between sympatric genotypes but is approximately twice as likely when allopatric genotypes of the two taxa are paired, consistent with the reinforcement hypothesis. By contrast, we found no evidence that floral evolution has occurred as by-product of adaptation to contrasting pollination environments in the allopatric and sympatric regions. These results are novel in demonstrating a contribution of both reinforcement selection and mating system divergence to the speciation process.

**New opportunities for G2P analysis in barley facilitated by a reference genome sequence**

Barley is one of the most important cereal crop species. It is a close relative to wheat and rye. Its haploid genome of 5 Gbp is almost twice the size of any fully sequenced organism or crop species. Recently, the International Barley Sequencing Consortium (IBSC) established access to a gene-centric view of the barley genome: a physical map densely integrated with the genetic map and substantiated by ~400 megabases of assembled whole genome shotgun sequence containing more than 20,000 transcriptionally active genes. Although strongly enabling research and application in barley improvement, the resource is limited due to the low physical resolution at centromeres and due to the lack of sequence contiguity. Thus, IBSC continued to work towards a complete genome sequence based on sequencing a minimal tiling path (MTP) of overlapping BAC clones provided by the established physical map. Sequencing data of all seven barley chromosomes has been accumulated and assembly and annotation have been accomplished. This step-changing resource of genomic sequence information is enabling now true genome scale analysis in barley and lays the foundation for genomics based breeding, crop improvement and comparative / evolutionary analyses within the genus *Hordeum* and between Triticeae species. State-of-the-art examples of barley genomic research and applications exploiting the potential of the new resource will be presented.
Deep sequencing in the deep subsurface: ‘De novo’ long read metagenomic assembly to recover complete genomes from the Soudan Iron Mine

Reaching a depth of 713 m (2,341 ft) below the surface, the Soudan Iron Mine transects massive veins of hematite and Archaean (2.7 Gy) banded iron formations embedded within northern Minnesota’s expansive Iron Range. On its lowest level, abandoned exploratory boreholes act as low-flow conduits for anoxic, calcium chloride brines with ionic strengths up to three times saltier than seawater, low oxidation-reduction potentials, circumneutral pH, and low concentrations of organic electron donors. These factors predict low biomass concentrations, in agreement with 16S rRNA-based surveys showing low abundances of putative metal reducers in situ. To enrich these microorganisms and understand their role in metal reduction in the deep subsurface, we inoculated anoxic brines into saline, acetate-fed bioreactors containing poised graphite electrodes (+0.24 V vs. SHE) as the sole electron acceptor. Shotgun metagenomics of electrode-attached cells yielded high-coverage reconstructions of four dominant genomes, with two Desulfuromonas spp. among the most abundant (>90% of reads). Incorporating just two SMRT cells of PacBio long read data completely resolved the 3,924,648-bp circular genome of a novel halophilic Deltaproteobacterium, ‘Ca. Desulfuromonas biwabikus DDH964,’ from the electrode-enriched metagenome. This long read assembly revealed several repetitive, physiologically important features missed by short reads, including a 5-kbp tandem CRISPR repeat, a putative 63-heme c-type cytochrome, and a near perfect 4-kbp duplication of the C-terminal region of a putative extracellular 16-heme c-type cytochrome. Notably, metabolic capabilities predicted by the ‘Ca. D. biwabikus’ genome (e.g., degradation of aromatics, glyoxylate shunt) were distinct from another cultured isolate from Soudan, ‘D. soudanensis WTL,’ underscoring the power of metagenomics in characterizing microbes that might otherwise elude laboratory cultivation. When compared against metagenomes of native, un-enriched samples, genomes of putative metal reducers were rare, indicating that poised electrodes are highly selective for extracellular electron transfer. Our results underscore the potential for metagenomics, particularly using long reads, in bypassing culture isolation to explore the microbial diversity of metal reducers from the deep biosphere.

Impacts of genotypic variation on allelic variation in elite maize inbred lines

Maize is a species with extensive diversity from the genome to the phenome, and as such is an excellent model system to study natural variation and the relationship between various levels of natural variation such as the genome versus the transcriptome. We recently developed a second maize genome assembly to complement the existing B73 reference genome assembly. Using these assemblies and transcriptome profiling throughout development we have been able to extensively mine the variation that exists between elite inbred lines at the genome and transcriptome levels. The relationship and impact of promoter variation, transcribed allelic variation, structural variation, and spatial and temporal transcriptional variation will be discussed.
**Rational relaxation of thermodynamic constraints in metabolic networks improves network quality**

Genome-scale metabolic networks rigorously describe the chemical reactions available to organisms and provide a useful scaffold for integrating and analyzing biological data. An ideal metabolic network can describe all possible metabolic states an organism can exist in while simultaneously disallowing infeasible states by defining constraints that the network must obey, such as directionality constraints that enforce the irreversibility of certain reactions. In reality metabolic networks contain erroneous constraints, leading to errors of both excessive and insufficient constraints that ultimately degrade the quality of networks. A network completion algorithm is presented that uses weighted linear programming to modify metabolic networks by relaxing constraints and adding necessary reactions to a metabolic network to model the synthesis of biomass metabolites (DNA, RNA, protein, essential small molecules, etc). This approach combines estimates of Gibbs free energy and a requirement of biomass synthesis to rationally select specific network modifications from a large space of possible solutions. Randomized metabolic networks and existing network completion algorithms are used as controls to demonstrate that this algorithm significantly improves the quality of metabolic networks. Three out of four metabolic networks achieved significantly more accurate predictions of gene essentiality after application of the network completion algorithm.

**Better Beer Through Microbiology**

Microbes alter the environments in which they live in a variety of ways. Perhaps most important is the ability of bacteria and eukaryotes to drive the conversion of nutrient rich agricultural feedstocks into value added consumables. This process, less romantically termed “making beer”, represents a 300 billion dollar industry worldwide, with craft beer and sour beer production becoming an ever increasing portion of the market share. Specific interactions between lactic acid bacteria and “wild” yeast in the context of sour beer production are important for the development of pleasing flavor and aroma compounds that typify these beverages. An increased understanding of these organisms’ physiology, metabolism, and interaction in fermenting wort may lead to more efficient and consistent beer production methods and provides a unique opportunity for scientific outreach to the craft brewing community at large.
MPGI'S NEW INITIATIVES

2016 Seed Grant

The Microbial and Plant Genome Institute is pleased to announce a seed grant competition for a single $50,000 award in 2016. Successful proposals must have an emphasis on genomics, with preference given to new areas of research and new collaborations. Proposals will only be accepted from full MPGI members and will be evaluated by a committee including the Director and members of the MPGI Steering Committee.

Submission materials must include:
- A cover letter from the PI (1 page limit)
- Proposal (2 page limit)
- Brief Budget and Timeline (1 page limit)
- Biographies for PI and Co-PIs (if any)

Please submit all materials as a single .pdf to mleffler@umn.edu by **Thursday, February 18th**. Award notification is anticipated within two weeks of the submission deadline.

2016 Graduate Student Recruitment Award

In an effort to recruit top candidates in the area of genomics, the Microbial and Plant Genome Institute is pleased to announce graduate student recruitment awards for the entering class of 2016. This one time award of $5000 will be available to recipients when they arrive on campus in the Fall of 2016 to begin their graduate studies and may be used at their own discretion to enhance educational opportunities at the University of Minnesota.

Students may only be nominated by full MPGI members. The nominated student must be either i) accepted into or ii) in the process of interviewing for a PhD-granting graduate program at the University of Minnesota. The nomination materials must include a brief letter from an MPGI member and the student’s application materials from the Graduate School. Please send nomination materials to mleffler@umn.edu. For full consideration, nominations must be submitted by **Tuesday, February 12**. Nominations submitted past this deadline may be considered, pending availability of funds.

A committee consisting of the Director of MPGI and several members of the MPGI Steering Committee will make selections and provide the corresponding student with a notification of award. An emphasis on utilization of genomics and/or bioinformatics in their graduate work will be a major consideration for selection along with academic performance and experience.