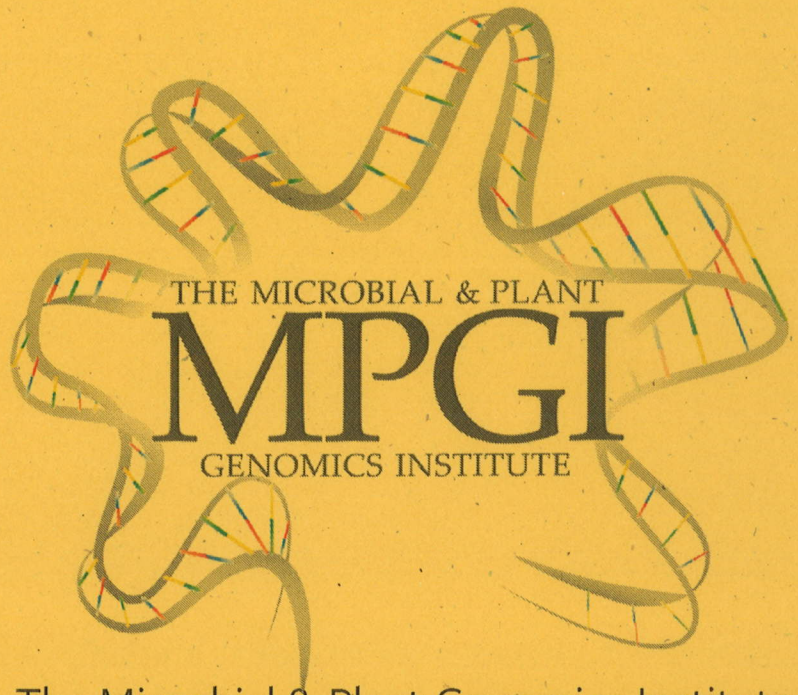


The Microbial & Plant Genomics Institute

ANNUAL RETREAT

January 12, 2017
Cargill Room 105

RETREAT SCHEDULE

8:30-9:00	Breakfast
9:00-9:10	Opening remarks
9:10-9:30	Molly Tillmann MPGI Travel Grant Recipient
9:30-9:50	Rebecca Breuer MPGI Travel Grant Recipient
9:50-10:10	Andrzej Noyszewski MPGI Travel Grant Recipient
10:10-10:30	Jonathan Schilling New MPGI Member
10:30-10:50	Break
10:50-11:10	Peter Intile
11:10-11:30	Leon Van Eck MPGI Travel Grant Recipient
11:30-11:50	Weiming Hu
11:50-12:10	Felipe Avila MPGI Travel Grant Recipient
12:10-1:20	Lunch (Atrium)
1:20-1:50	Melania Figueroa MPGI Seed Grant Recipient
1:50-2:10	Carolann Knutson MPGI Travel Grant Recipient
2:10-2:30	Amanda Waters PepsiCo Global R&D Agro Discovery
2:30-2:40	Closing Remarks
2:40	Reception

MOLLY TILLMANN
Graduate Student, MPGI Travel Grant Recipient
UofM Dept. Horticultural Science

Light-induced adventitious rooting is increased in Arabidopsis phytochrome B (phyB) mutant seedlings and is associated with elevated auxin levels

Although many horticulturally important plants are propagated by auxin application to induce adventitious rooting, the specific changes in auxin metabolism during adventitious root development are not well known. Adventitious rooting can also be stimulated without exogenous hormones in *Arabidopsis thaliana* by transferring etiolated seedlings into red or white light. We are nvestigating the role of light quality, auxin, and plant photoreceptors in this process. Etiolated wild type Arabidopsis seedlings exposed to red, white, or lue light for one week produced significantly more adventitious roots than seedlings that were kept in continuous darkness or exposed to far red light only. *Phytochrome B (phyB)* mutants produced significantly more adventitious roots than wild type in response to red or white light treatments, but also produced very few adventitious roots under dark conditions. Preliminary results uggest higher concentrations of indole-3- acetic acid (IAA) are correlated with higher levels of adventitious rooting in response to light. *PhyB* hypocotyls contained higher levels of IAA upon transition to red light compared to wild type, and both genotypes maintained higher IAA levels under red light than in darkness.

REBECCA BREUER
Graduate Student, MPGI Travel Grant Recipient
UofM Dept. of Microbiology and Immunology

Single cell analysis of Enterococcus faecalis transcription reveals heterogeneity in response to communication required for gene transfer

Population-level quantification of gene expression via transcriptomics and proteomics provides an incomplete picture of microbial behaviors. In reality, stochasticity and differing microenvironments can cause gene expression by individual cells within isogenic populations to differ considerably from the population mean. This can result in wide-ranging responses to stimuli, diverse phenotypes, and unexpected properties. In *Enterococcus faecalis,* gene transfer by conjugation is enabled by cell-to-cell communication through signal peptides and transfer rates vary based on the transcriptional response to signaling. Development of hybridization chain reaction fluorescence in situ hybridization (HCR-FISH) has enabled us to experimentally analyze the gene expression response to signal peptides within individual cells. This provides direct evidence for variability such that per cell, the response is variable in timing and magnitude, and rapidly shuts down after reaching maximum. HCR-FISH data were validated using fluorescent reporter fusion constructs and also agreed with stochastic mathematical modeling predictions. Use of the HCR-FISH method allows characterization of single-cell gene expression ithout the need to create reporters or antibodies and may potentiate future lucidation of important transcriptional regulatory mechanisms in bacteria.

ANDRZEJ NOYSZEWSKI
Research Associate, MPGI Travel Grant Recipient
UofM Dept. of Horticultural Science

Polymorphism and structure of style-specific arabinogalactan proteins of Nicotiana as determinants of pollen tube growth

Pollen tube growth and fertilization are key processes in angiosperm sexual reproduction. The stylar transmitting tract (TT) of *Nicotiana tabacum* controls pollen tube growth in part by secreting pistil extensin-like protein III (PELPIII), transmitting-tract-specific (TTS) and 120 kDa glycoprotein (120K) into the style extracellular matrix. The N-terminal domain (NTD) of stylar arabinogalactan proteins (AGPs) is proline rich and polymorphic among *Nicotiana spp.* The NTD was predicted to be mainly an intrinsically disordered region (IDR), making it a candidate for protein-protein interactions. The NTD is also the location for the majority of the predicted O-glycosylation patterns that were variable among *Nicotiana spp.* The C-terminal domain (CTD) contains an Ole e 1-like domain, that was predicted to form beta-sheets that are similar in position and length among *Nicotiana spp.,* and among stylar AGPs. The TTS protein had the greatest amino acid and predicted O-glycosylation conservation among *Nicotiana spp.* relative to the PELPIII and 120K. The PELPIII, TTS and 120K genes undergo negative selection, with dN/dS ratios of 0.59, 0.29 and 0.38 for each gene, respectively. The dN/dS ratio for individual species, based on the same group of Nicotiana species ranged from 0.4 to 0.9, and from 0.1 to 0.8, for PELPIII and TTS genes, respectively. These data indicate that PELPIII and TTS genes are under different selective pressures. A new gene, *Nt*PRP, was found with a similar intron-exon configuration and structure resembling the other stylar AGPs, particularly TTS. Further studies of NtPRP gene are necessary to elucidate its biological role. Due to its high similarity to the TTS gene, *Nt*PRP may possibly be involved in pollen tube guidance and growth, as recognized function of TTS. In contrast to TTS, both PELPIII and 120K genes are more diverse and this would indicate their possible role in speciation or mating preference of *Nicotiana spp.*. We hypothesize that the stylar AGPs and *Nt*PRP share a common origin from a single gene that duplicated and diversified into four distinct genes involved in pollen-style interactions.

JONATHAN SCHILLING
Associate Professor, New MPGI Member
UofM Dept. of Bioproducts and Biosystems Engineering

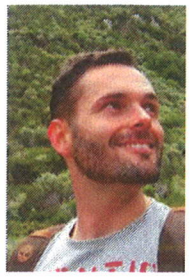
Wood-degrading fungi that make a radical entrance.

A recent addition to MPGI, the Schilling group is focused on fungal biology, how fungal mechanisms might be harnessed in biotechnology, and how these same mechanisms underpin, as 'traits,' their ecological roles in nature. In particular, we have been focusing on a group of carbohydrate-selective wood degrading fungi known as 'brown rot' fungi. These fungi deploy an initial oxidative step that loosens the plant cell wall, prior to upregulation of a host of familiar carbohydrate-active genes and products. This is a true accomplishment, given that the oxidative radicals deployed at the hyphal front are highly reactive with energetically-expensive fungal metabolites and hyphae, along with the intended plant cell wall target. How do they do make this 'radical entrance' without annihilating themselves? In what type of 'crowd' will this bold entrance work? Using molecular tools as well as some traditional wood physiochemical techniques, we are beginning to answer these questions, with help from collaborators and access to some remarkable new tools.

PETER INTILE
Postdoctoral Associate
UofM BioTechnology Institute

Development of a zebrafish intestinal microbiome model.

The zebrafish (*Danio rerio*) and its associated intestinal microbiota serve as an excellent model system to investigate both host/microbiome interactions as well as environmental effects upon microbiome diversity and establishment.Gammaproteobacteria are common residents of the zebrafish intestinal microbiome including *Shewanella, Vibrio,* and *Aeromonas* species that have the ability to conduct extracellular electron transfer (EET) and secrete flavins. EET and flavin secretion are two mechanisms of great interest in bioremediation and energy production fields as exported electrons can reduce toxic metals to an insoluble state or be collected via an anode, respectively. The roles of EET and flavin secretion, however, have yet to be investigated within the context of host/microbiome interactions. Towards this goal, we have acquired multiple *Shewanella* isolates directly from the zebrafish intestine and surrounding environments and have demonstrated that these isolates are capable of EET, flavin secretion, and are amenable to the genetic techniques available for the common laboratory strain *Shewanella oneidensis* MR-1. Additionally, we have been successful in generating axenic zebrafish larvae absent of intestinal microbiota, and can subsequently inoculate axenic fish with our genetically modified *Shewanella* isolates. Finally, we have initiated studies in collaboration with the College of Biological Sciences Foundations in Biology course towards investigating how common environmental contaminants can alter Gammaproteobacteria diversity within the zebrafish microbiome. Together, our studies provide a foundation towards better understanding the role of Gammaproteobacteria and EET in the context of a host microbiome.



LEON VAN ECK
Postdoctoral Associate, MPGI Travel Grant Recipient
UofM Dept. of Plant Pathology

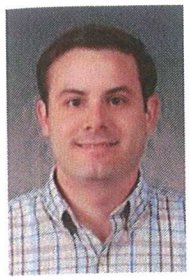
A Family-Wide Analysis of Disease Resistance Genes in the Rosaceae Using a Custom Bioinformatic Pipeline.

Efficiently targeted mining of genomic data benefits plant breeding. We developed a Python-based bioinformatics pipeline for the discovery, characterization, and phylogenetic analysis of disease resistance (R) genes from public genome databases. We conducted a comparative analysis of R genes from the high-quality genome sequences of eleven species within the Rosaceae, including important fruit crops like apple, pear, peach, and strawberry. From these eleven genomes, a total of 2,489 putative R genes encoding full-length NB-ARC domains were discovered. The pipeline classified 793 putative R genes as TIR, and 1,696 as non-TIR subtypes. The pipeline additionally clustered putative R protein sequences into the RosaR80 Framework, a set of 651 homology groups based on an 80% amino acid identity threshold for the NB-ARC domain. Maximum Likelihood analysis of RosaR80 homology groups revealed idiosyncratic patterns of diversification. Groups present in all genomes represented highly conserved R gene lineages, with preserved gene copy number and syntenic chromosomal positions between *Rosaceous* species. RosaR80 homology groups derived from a single species represented R gene lineages with species-specific patterns of diversification. For example, in *Malus* × *domestica*, cloned and candidate genes affording resistance to apple scab (*Venturia inaequalis*) were derived from large, highly diversified,species-specificlineages,oftenlocatedinchromosomalgeneclusters, suggesting a recent co-evolutionary arms race with the pathogen. In addition to being a tool for generating new evolutionary hypotheses, our pipeline facilitates the identification of agronomically useful R gene lineages for plant improvement, and may inform evaluation of gene bank collections for targeted allele mining.

WEIMING HU
Postdoctoral Associate
UofM Dept. of Plant and Microbial Biology

Microbial communities in the cysts of soybean cyst nematode in a soybean-corn rotation system.

The soybean cyst nematode (SCN) is a major pathogen of soybean that decreases soybean yield both through direct infection of roots and also by vectoring or promoting other microbial diseases of soybean. There is great need for development of effective and environmentally sustainable management strategies for the SCN, but development of effective biocontrol is hampered by a lack of knowledge of nematode control microorganisms. The goal of this research is to characterize key genetic, functional and taxonomic groups of fungi colonizing the cysts under different crop rotation systems. The research utilizes a long-term crop rotation experiment of corn and soybean that includes annual rotation, 5-year rotation, and continuous monoculture. Fifty intact cysts of SCN from each plot were collected at planting, mid-season and harvest in 2015 and the fungal communities were investigated by metabarcoding of the fungal ITS1 region. Although the overall alpha fungal community diversity did not differ across the soybean-corn rotation treatments and among sampling seasons, the fungal community composition of first year soybean and soybean monoculture was distinguished from other rotation treatments. Further investigation of the relative abundance of fungal genera indicated that potential biological agents were shifted by different years of soybean-corn rotation. *Exophiala sp.* and *Clonostachys sp.* had greater relative abundance in fifth-year soybean than first-year soybean, while Pochonia sp. had higher abundance in soybean monoculture than second-year soybean. Our results suggest that soybean-corn rotation shifted the fungal community colonizing the cysts of SCN and that some nematode biocontrol fungi were reduced when soybean was rotated with five years of corn.



FELIPE AVILA
Postdoctoral Fellow, MPGI Travel Grant Recipient
UofM Dept. of Veterinary Population Medicine

Genomic Signatures of Selection in the Arabian Horse

The most identifiable feature of Arabian horses is their finely chiseled head. Moreover, some individuals have 5 lumbar vertebrae instead of 6, and 17 pairs of ribs instead of the usual 18. Because of that, Arabians have a shorter back and a more horizontal pelvis, which increases their capacity to carry weight and contributes to their unique pattern of locomotion. The objectives of this study were to 1) identify genomic regions undergoing selection in Arabians; 2) investigate haplotype structure and ancestry within the breed; 3) identify candidate genes for cranial and body morphology. For that, 36 Arabians were genotyped at 2 million SNP markers across the genome. Regions harboring signatures of selection were identified using EHH and two FST -based statistics (di and hapFLK); local haplotype sharing among individuals was calculated with hapQTL. A total of 361 genes were retrieved within signatures of selection from 28 autosomes using BioMart. Candidate genes were prioritized using Endeavour-GW and functional and enrichment analyses were performed using DAVID and FunRich3. A statistical overrepresentation of genes present in pathways involved in skull and spinal cord development (such as Wnt and Notch pathways) was detected. Genes associated with cellular migration and development of osseous tissue were also identified. Examples include ATF2, ALX1, and NKD1. WGS data from 14 Arabians will be used for the annotation of biologically relevant alleles within these candidate genes and to investigate their association with the Arabian's distinctive cranial morphology and body conformation.

MELANIA FIGUEROA
Assistant Professor, MPGI Seed Grant Recipient
UofM Dept. of Plant Pathology

Cereal rust diseases: walking a fine line between resistance and susceptibility

Rust fungi are some of the most destructive pathogens of cereals affecting grain production in many areas of the world, especially in developing countries. Breeding with resistance genes has been a major focus for disease control, but the pathogen evolves rapidly to overcome resistance genes, undermining this effort. Understanding the molecular mechanisms underlying susceptibility could lead to the development of more durable approaches to protect crops from rust disease. Rust fungi are biotrophic pathogens that manipulate living cells to support their growth, and so must both suppress host immunity mechanisms and alter the host physiology to deliver nutrients. These complex parasitic interactions are the result of long co-evolutionary relationships between these organisms and their host, which are generally highly species specific. Because of these characteristics rust fungi are ideal systems to investigate factors controlling plant-pathogen compatibility. To investigate how rust fungi establish a compatible interaction with a host we have selected two rust species *Puccinia graminis* f. sp. *tritici* (*Pgt*) and *Puccinia coronata* f. sp. avenae (*Pca*), which cause wheat stem rust and oat crown rust, respectively, and their host plants wheat and oat. Leveraging recently developed genomic resources in both rust fungi and these crops, we are employing imaging and transcriptional profiling techniques for a multi-species comparative study. Our analysis involves compatible and incompatible host-pathogen species pairs as well as the related grass model species *Brachypodium distachyon*, which is an intermediate host for *Pgt* and *Pca*. This study will address the basis of *Pgt* and *Pca* host-specialization and uncover core susceptibility factors and gene networks associated with disease susceptibility.

CAROLANN KNUTSON
Graduate Student, MPGI Travel Grant Recipient
UofM Dept. Bioproducts and Biosystems Engineering

Using hydrogen production as an in vivo indicator of nitrogenase activity

In photosynthetic organisms, nitrogen is often the most expensive and limiting nutrient input. Traditionally, energy intensive methods such as the Haber-Bosch process have produced the nitrogen fertilizer required. Our research aims to provide alternative means of producing the necessary nitrogen for photosynthetic crops. *Azotobacter vinelandii* DJ is a model diazotrophic and aerobic bacterium which can be genetically manipulated to increase its extracellular nitrogen production. Here we present the use of hydrogen production as a way to quantify *in vivo* responses of nitrogenase and associated hydrogenases. These assays have provided a snapshot of the internal flow of metabolites and reduction potential within this species, and are being used to develop a better understanding the complex system of nitrogen fixation regulation present in *A. vinelandii* DJ.

AMANDA WATERS
Postdoctoral Fellow
Trait Discovery & Crop Data Sciences
PepsiCo

PepsiCo Agro Discovery: Introduction to the team and UMN partnership

PepsiCo is a global leader in the food and beverage market. The requirements for maintaining high quality products, supplying novel products to customers with improved nutritional value, and delivering new solutions to ever changing and diverse markets relies heavily on raw material stability and innovation. The Agro Discovery team is driven to identify, test, adopt, and improve raw materials from agriculture for PepsiCo products through the use of cutting edge genetic, physiological, computational, and breeding technologies. Agro Discovery is currently focusing on several crops including potato, oat, oranges, stevia, sweet potato, and corn. The collaboration between PepsiCo and the University of Minnesota was formed from a united strategy to take on the challenges of global food security, improved nutrition, and sustainability. Agro Discovery is driven to impact the entire value chain through raw material innovation by implementing new models of collaboration that equally benefit both partners and society as a whole. A large amount of the current research being done in Agro Discovery is pre-competitive and ideal for building collaborations and resources for the scientific community. For example, PepsiCo is collaborating with researchers to advance genomics for the oat community as well as the oat breeding program at the University of Minnesota. The partnership between PepsiCo and the University of Minnesota has and will continue to combine innovation and commercialization to achieve common goals of food security, improved nutrition, and environmental sustainability.