CORNELL INSTITUTE OF HOST-MICROBE INTERACTIONS AND DISEASE

RESEACH SYMPOSIUM 2024

July 11, 2024

9:00 – 3:30

coffee at 8:30, lunch provided

Stocking Hall's PepsiCo Auditorium

Cornell University

8:30 – 9:00 **Coffee and Pastries**

9:05 – 9:15 **Opening Remarks (Tory Hendry, Microbiology)**

9:15 – 9:45 Mandy McGeachy (M&I, CVM)
"Small species-specific genetic changes have beg effects on immunity"

9:45 – 10:05 Camille Holmes (Population Medicine & Diagnostic Sciences)
"Winning by a nose: mucosal immunity in the race against Equine herpesvirus type 1"

10:05 – 10:35 Daniel Sprockett (EEB, CIHMID Postdoc)
"Discovery of ancient rodent-bacterial symbioses reveals recent genetic drift in laboratory-mouse gut microbiota"

10:35 – 11:00 **Coffee Break**

11:00 – 11:30 Jenny Kao-Kniffin (Horticulture)
"Assembly of highly connected microbiomes that modify plant host traits"

11:30 – 11:50 Kathryn Herr (Microbiology)
"The symphony of symbiosis: Ecological and evolutionary impacts of microbial interactions with insects"

11:50 – 12:20 Brian Lovett (Entomology)
"The inspiring potential of arthropod-killing fungi"

12:20 – 1:40 **Lunch**

1:40 – 2:10 Brooks Crickard (MBG)
"Achaea-ology in the pursuit of the evolution of meiosis"

2:10 – 2:30 Trevor Tivey (BTI)
"Arbuscular mycorrhizal symbiosis up close: the spatial landscape of plant and fungal transcriptomes"

2:30 – 3:30 **Poster Session, Reception**

ABSTRACTS – TALKS:

Winning by a nose: mucosal immunity in the race against Equine herpesvirus type 1

Camille M. Holmes, Bettina Wagner

Department of Population Medicine & Diagnostic Sciences, Cornell University College of Veterinary Medicine

Equine herpesvirus type 1 (EHV-1) is a highly prevalent respiratory pathogen of the horse, with yearly outbreaks impacting the equine industry. EHV-1 enters through the upper respiratory tract (URT), where it locally replicates in the epithelial cells of the URT before the establishment of cell-associated viremia. This facilitates systemic transmission and further replication in the endothelial cells of the vasculature, which can result in severe clinical outcomes, including abortions and/or equine herpesvirus myeloencephalopathy. The URT consists of a heterogenous population of immune and structural cells which contribute to mucosal barrier defense. Thus, the mucosal immune response is essential in limiting infection and mediating protection against EHV-1. Here we analyzed the nasal transcriptome during early infection to identify mucosal immune pathways contributing to immunity against EHV-1. Immune (n=4) and non-immune horses (n=4) were experimentally infected with EHV-1, and clinical signs, virus shedding, viremia and mucosal immunity were evaluated. RNA sequencing was performed on nasopharyngeal swabs, and bead-based assays were used to measure protein concentrations in the same samples for confirmation of the top RNAseq results during infection. Non-immune horses had a type I interferon driven inflammatory response and delayed upregulation of adaptive immune pathways, occurring at day 8 to 10 post-infection. A group of interferon stimulated genes (ISGs) were upregulated in non-immune horses, including IFN-induced protein with tetratricopeptide repeats 2 (IFIT2, $p<0.0001$, immune: 1,586±491, non-immune: 6,067±2,617 reads). Protein concentrations of IFN-α were in concert with gene expression. Meanwhile, immune horses had rapid upregulation of innate immunity genes related to homeostatic regulation, including antileukoproteinase (SLPI, $p=0.045$, immune: 829±730, non-immune: 108±39 reads). They also upregulated adaptive immune pathways within the first days of infection, including both T cell and B cell activation pathways. Follow-up on each arm demonstrated a lack of inflammatory response, homing of cytotoxic T cells, and secretion of neutralizing antibodies. In addition to the host response, viral RNA expression was significantly altered between groups. Viral replication was wholly interrupted in immune horses, resulting in no live virus being recovered from the nose. Together this emphasizes the importance of the mucosal immune response for protection against EHV-1 infection.

Discovery of ancient rodent-bacterial symbioses reveals recent genetic drift in laboratory-mouse gut microbiota

Daniel D. Sprockett

Department of Ecology and Evolutionary Biology, Cornell University, CIHMID Postdoc

Laboratory mice (*Mus musculus domesticus*) harbor gut bacterial strains that are distinct from those of wild mice but whose evolutionary histories are poorly understood. Understanding the divergence of laboratory mouse–gut microbiota (LGM) from wild mouse–gut microbiota (WGM) is critical, because LGM and WGM have been previously shown to differentially affect mouse immune-cell proliferation, infection resistance, cancer progression, and ability to model drug outcomes for humans. Here, we show that laboratory mice have retained 24 gut bacterial symbiont lineages that diversified in parallel (co-diversified) with rodent species for > 25 million years, but that LGM strains of these ancestral symbionts have experienced accelerated accumulation of genetic load during the past ~ 120 years of captivity. Compared to closely related WGM strains, co-diversified LGM strains displayed significantly faster genome-wide rates of fixation of nonsynonymous mutations, indicating elevated genetic drift, a pattern that was absent in non-co-diversified LGM strains. Competition experiments in germ-free mice further indicated that LGM strains within co-diversified clades displayed significantly reduced fitness in vivo compared to WGM relatives to an extent not observed within non-co-diversified clades. Thus, stochastic processes (*e.g.*, bottlenecks), not natural selection in the laboratory, have been the predominant evolutionary forces underlying divergence of ancestral symbiont strains between laboratory and wild house mice. Our results show that gut bacterial lineages conserved in diverse rodent species have acquired novel mutational burdens in laboratory mice, providing an evolutionary rationale for restoring laboratory mice with wild gut bacterial strain diversity.

Assembly of highly connected microbiomes that modify plant host traits

Jenny T. Kao-Kniffin

Department of Horticulture, Cornell University

Directed evolution provides a system to study rapid changes in microbial interactions associated with a specific host trait. We share results from two separate experiments involving microbiome assembly through selective pressure on plant phenotypes—flowering time in *Arabidopsis thaliana* and plant productivity in *Brassica rapa*. Microbiomes associated with a specific plant trait were continuously patched across nine to ten successive plantings by collecting soil from the root zone of plants exhibiting the greatest modifications to the targeted trait. In both studies, similar properties of the microbiome emerged. The density of associations across microbial taxa were enhanced, with more edges and connectivity, in plant systems of greater productivity. Multiple models were used to characterize the interactions across taxa. As theoretical systems involving microbial adaptation to a host or ecosystem trait becomes more widely adopted, it may become feasible to identify microbial group behaviors and composition that are closely linked to host function.

The Symphony of Symbiosis: Ecological and Evolutionary Impacts of Microbial Interactions with Insects

Kathryn Herr

Department of Microbiology, Cornell University

Over evolutionary time, repeated interactions between hosts and microbes within the environment can lead to stable symbiotic associations. Environmental microbes can profoundly influence host behavior, physiology, and evolutionary trajectories. These interactions can lead to intimate symbiotic relationships often fundamental to the ecological and evolutionary success of many organisms. Such partnerships can enable hosts to exploit new niches and adapt to diverse environments. How interactions with environmental microbes can influence the ecology and evolution of host and microbe is critical to our understanding of symbiosis and the dynamics that shape symbiotic outcomes. This research examines the evolutionary and ecological significance of interactions between hosts and microbes burying beetles (*Nicrophorus* spp.), carrion feedings insects that utilize small vertebrate carcasses in their reproductive ecology.

Through beetle-regulated manipulation of carcass microbial communities, burying beetles 'preserve' small vertebrate carcasses for larval consumption. This manipulation is achieved through the application of the beetle's microbial-laden oral and anal secretions. Previous research has identified several microbial taxa, including *Yarrowia*-like yeasts (YLY), as potential *Nicrophorus*-associated symbionts which may have roles in carcass microbiome manipulation and nutrient biosynthesis. However, this previous work has focused solely on the model European species, *Nicrophorus vespilloides.* Here, I determine if YLYs and other potential symbionts are associated with additional species of *Nicrophorus*, and if an evolutionary relationship exists between beetle hosts and YLY symbionts. First, I survey the fungal and bacterial communities of hindguts and secretions from across five *Nicrophorus* species and one *Necrophila* species that does not participate in carcass preservation. Then, I use phylogenetic approaches to understand the diversity and evolution of *Yarrowia* symbionts in various *Nicrophorus* species. Together, these investigations provide insights into how environmental microbes impact host behavior and evolutionary dynamics, from initial ecological interactions to symbiotic associations.

The inspiring potential of arthropod-killing fungi

Brian Lovett

Department of Entomology, Cornell University

Arthropod-killing fungi are a diverse assemblage spanning the breadth of the fungal kingdom. These fungi have evolved complex mechanisms to overcome arthropods and, in some interesting cases, manipulate the behavior of their hosts. To understand the evolutionary history of fungi along the mutualist-parasite continuum it is critical to consider arthropod host biology. Transgenic fungi expressing insect-specific toxins have shown great promise as biocontrol. This technology is versatile and is only strengthened by molecular tools in our age of genomics.

Achaea-ology in the pursuit of the evolution of meiosis

Brooks Crickard

Department of Microbiology Biology and Genetics, Cornell University

RecA-type recombinases are essential for genome maintenance in all domains of life, promoting homologous recombination. Their prevalence and conservation across the Tree of Life make them an ideal protein for understanding the evolution of the HR pathway. While the general reaction catalyzed by recombinases is the same in all domains, key differences at the amino acid level inform the history of a critical protein. A missing piece of information in this history is the RecA homologs' protein sequence near the Archaea and Eukarya division. We have analyzed sequence data from RadA protein sequences in the Asgard Archaea superphyla to fill this gap. Our findings include evidence for the evolution of a eukaryotic-specific DNA binding site II within the Asgard lineage. We also identify sequence variants within DNA binding loops L1 and L2 that imply the prevalence of nucleotide mismatch tolerance with the Asgard and DPANN superphylum. These loops are also observed in the meiosis-specific recombinase Dmc1 within Eukarya. Evidence suggests this may be a convergent evolution of mismatch tolerant loops. From this study, we hypothesize that the evolution of mismatch tolerant DNA binding loops may be important for kinetically selecting non-self-DNA during programmed recombination events

Arbuscular mycorrhizal symbiosis up close: the spatial landscape of plant and fungal transcriptomes

Trevor R. Tivey

Boyce-Thompson Institute, Ithaca, NY

Arbuscular mycorrhizal symbiosis (AM) is a widespread nutritional symbiosis between soil fungi and plants in which fixed carbon is provided to fungi in return for limited mineral nutrients such as phosphate. The nutritional exchange occurs within individual plant root cortical cells, in which transitive structures known as arbuscules are formed to facilitate host-microbe nutrient trafficking. This symbiosis occurs along a spatiotemporal gradient, where colonizing fungal hyphae penetrates the plant through the plant cell epidermis, linearly colonizes cortical cells, forms mature arbuscules and then senesces. Although we have a strong understanding of the core genes involved in the symbiotic pathway through plant genetics, we do not fully understand how arbuscular mycorrhizal symbiosis alters transcription at a cell-type resolution. To gain a clearer picture of the transcriptional landscape, spatial and single-nuclei transcriptomics were employed in the model legume *Medicago truncatula* and the AM fungus *Rhizophagus irregularis*. 10X Chromium and Visium libraries from colonized and noncolonized plant roots were generated to support each other's cell-type annotations and transcriptomic findings. Both plant and fungal gene expression showed significant transcriptional differences based on cell-type, location and colonization status. Together, these datasets reveal the dynamics of plant and fungal transcriptional programs during AM colonization.

SUBMITTED ABSTRACTS - POSTERS:

B Bacterial Lifestyles Influence Mobile Genetic Elements in Aquatic Ecosystems

Sophia Aredas, Marian Schmidt

Department of Microbiology, Cornell University, Ithaca, NY

Mobile genetic elements (MGEs) such as plasmids and bacteriophages are significant drivers of horizontal gene transfer (HGT) within microbial communities. However, the role of MGEs in the environment are not well understood. Aquatic bacteria can be categorized into three lifestyles: truly free-living (FL), particle-associated (PA) that form microbial cohorts on particles, or bacteria that alternate between these two lifestyles, known as generalists. Given that particles serve as hotspots of microbial activity and help to facilitate direct cell-to-cell contact, we hypothesized that PA and generalist microbes have enhanced opportunities to engage in HGT and will have higher rates of MGEs. We investigated microbial metagenomes from a freshwater estuary—Muskegon Lake, Michigan. From sixteen samples we first looked at the assembly-level view of assembled short read contigs. Then, we binned the assembled contigs and generated 346 metagenome assembled genomes (MAGs) to identify MGEs using geNomad within these three lifestyles. At the assembly-level, we find the same distribution of conjugation genes, which facilitates the dissemination of plasmids. Additionally, the FL fraction had a significantly higher number of phage-encoded genes. At the MAG-level, the generalist species had the greatest number of conjugation genes, phage-encoded genes, and phage diversity. At both assembly and MAG levels, these results reveal more complex community interactions than originally expected which further highlight the importance of microbial lifestyles. By studying MGEs in aquatic environments, we will be better able to understand the ecology and microbial community dynamics of bacterial lifestyles including the dissemination and maintenance of antibiotic resistance genes.

The role of cell wall remodeling in innate immunity of early divergent Mucoromycotina Fungi

Hana Barrett[1], Maria Laura Gaspar[1], Carlos Lax[2], Victoriano Garre[2], Teresa E. Pawlowska[1]

1. Cornell University, Plant Pathology and Plant Microbe Biology, Ithaca, NY
2. University of Murcia, Spain

The fungal cell wall is the arena for interactions with diverse environmental challenges. However, very little is known about the role of the cell wall in interactions with other microbes. My research focuses on the role of the cell wall in defense against antagonistic bacteria, using the antagonistic interaction between the early divergent fungus *Rhizopus microsporus* (Mucoromycotina) and the bacterium *Mycetohabitans* spp as a model. Previous work from our lab has found significant differential gene expression in fungi cultured with bacteria vs fungi alone. Notably, genes associated with cell wall components chitin, fucose, glucan, mannose, and galactose were differentially expressed in the antagonistic interaction. I quantified and visualized the phenotypic changes in cell wall composition by fluorescently probing cell wall components through microscopy and flow cytometry. I found an increase in chitin concentration in the presence of bacteria. Genes associated with adenylyl cyclase sensors, which have been identified as candidate fungal pattern recognition receptors, were also differentially expressed, so we generated CRISPR/Cas9 disruption mutants of two adenylyl cyclase (*cyr1* and *cyr2*) genes. The chitin increase was still seen in the *cyr2* disruption, but in the *cyr1* disruption there was no increase, suggesting that the *cyr1* sensor plays a role in mediating this response. These results suggest that adenylyl cyclases play a role in detecting bacterial antagonism and initiating defensive cell wall remodeling.

Inferring molecular bases of the *Rhizopus microsporus – Mycetohabitans* symbiosis by genome-wide positive selection analysis

Margaret E. Branine[1], Teresa E. Pawlowska[2]

1. Graduate Field of Microbiology, Cornell University, Ithaca, NY
2. Cornell University, Plant Pathology and Plant Microbe Biology, Ithaca, NY

The early-diverging fungal phylum Mucoromycota displays a high degree of coevolution with bacteria as several lineages harbor highly coevolved and ancient bacterial endosymbionts. The mucoromycete *Rhizopus microsporus* and its bacterial endosymbiont *Mycetohabitans* spp. are emerging as a model system for studying the evolution and molecular bases of such symbioses due to its experimental tractability and genomic resources. In this symbiosis, *Mycetohabitans* provides its host with secondary-metabolite toxins, controls fungal asexual and sexual propagation, and alters the lipid metabolism of the host. We hypothesize that the strong influence of *Mycetohabitans* on the biology of *R. microsporus* has significant consequences for the evolutionary trajectory of host fungi. Importantly, *R. microsporus* isolates naturally free of endosymbionts (i.e., nonhosts) permit comparative analyses into if and how endosymbiotic bacteria influence the evolution of their fungal hosts. To begin addressing this question, I implemented a genome-wide positive selection analysis in host and nonhost strains of *R. microsporus* by calculating the non-synonymous to synonymous substitution rate ratio (dN/dS) of all single-copy orthologs using the CODEML program within the PAML package. Under both branch and branch-site models, I identified genes putatively under positive selection in host *R. microsporus* strains. We hypothesize that many of the genes under positive selection (dN/dS>1) in hosts, but not in nonhosts, are significant within symbiosis maintenance and establishment. To provide functional support for these results, we will next use transcriptomics to characterize the expression profiles of genes putatively under positive selection during symbiosis.

An Investigation of the Gut and Fecal Microbiome of the Eastern Oyster, *Crassostrea virginica*

Alexandra Carabetta[1], Jacob Millspaugh[1], Tyler W. Grrifin[2], J. Evan Ward[2], Lisa Nigro[3]

1. Department of Molecular and Cell Biology, University of Connecticut
2. Department of Marine Sciences, University of Connecticut
3. Institute for Systems Genomics, University of Connecticut and Department of Biology, Central Connecticut State University

The early-diverging fungal phylum Mucoromycota displays a high degree of coevolution with bacteria as several lineages harbor highly coevolved and ancient bacterial endosymbionts. The mucoromycete *Rhizopus microsporus* and its bacterial endosymbiont *Mycetohabitans* spp. are emerging as a model system for studying the evolution and molecular bases of such symbioses due to its experimental tractability and genomic resources. In this symbiosis, *Mycetohabitans* provides its host with secondary-metabolite toxins, controls fungal asexual and sexual propagation, and alters the lipid metabolism of the host. We hypothesize that the strong influence of *Mycetohabitans* on the biology of *R. microsporus* has significant consequences for the evolutionary trajectory of host fungi. Importantly, *R. microsporus* isolates naturally free of endosymbionts (i.e., nonhosts) permit comparative analyses into if and how endosymbiotic bacteria influence the evolution of their fungal hosts. To begin addressing this question, I implemented a genome-wide positive selection analysis in host and nonhost strains of *R. microsporus* by calculating the non-synonymous to synonymous substitution rate ratio (dN/dS) of all single-copy orthologs using the CODEML program within the PAML package. Under both branch and branch-site models, I identified genes putatively under positive selection in host *R. microsporus* strains. We hypothesize that many of the genes under positive selection (dN/dS>1) in hosts, but not in nonhosts, are significant within symbiosis maintenance and establishment. To provide functional support for these results, we will next use transcriptomics to characterize the expression profiles of genes putatively under positive selection during symbiosis.

Mathematical modeling of the transmission dynamics of β-coronaviruses in Australian flying fox fruit bats (*Pteropus spp.*)

Brooklin Hunt[1], Sebastian Llanos-Soto[2], Raina K. Plowright[3], Renata Ivanek[2]

1. Department of Biological and Biomedical Sciences, Cornell University College of Veterinary Medicine
2. Department of Population Medicine & Diagnostic Sciences, Cornell University College of Veterinary Medicine
3. Department of Public & Ecosystem Health, Cornell University College of Veterinary Medicine

Despite the recent pandemic circulation of three zoonotic bat-borne coronaviruses in humans (SARS-CoV-1, SARS-CoV-2, and MERS-CoV), the mechanisms underlying coronavirus transmission in wild bat populations are poorly understood. This knowledge gap prevents the design of effective interventions to reduce the risk of future coronavirus spillovers from bats to humans or domestic animals. In this study, we aim to determine which transmission dynamics are most likely to be involved in the spread of nobecovirus (a sub-genus of *Betacoronavirus*) amongst flying fox fruit bats (*Pteropus spp.*) of Australia. To this end, we are analyzing a series of nested compartmental models developed using ordinary differential equations in R software. The structures of individual models reflect the hypotheses from previous studies examining the dynamics of coronavirus shedding in bats, including: (a) transmission occurs via direct contact or fomites and is density-dependent at the individual level, (b) juvenile bats have a greater risk of infection than adult bats, (c) heterogeneity in the duration of infectiousness increases the risk of viral persistence, and (d) breeding behavior and parturition drive seasonal increases in the prevalence of coronavirus shedding. An additional hypothesis that has yet to be investigated is that bats may experience latency during chronic coronavirus infections, as has been observed with some henipaviruses in their bat hosts. The individual model that best represents the seasonal shedding pulses observed in field data will be validated using bimonthly repeat cross-sectional data gathered from a multi-species flying fox roost in Queensland, Australia, in 2018-2020. Our models are expected to provide new knowledge on the mechanisms underlying coronavirus transmission in wild bat populations, thus contributing knowledge needed to develop countermeasures to prevent future coronavirus spillovers and pandemics.

Drosophila melanogaster genotype impacts metabolic response to chronic bacterial infection

Ananda Kalukin

Department of Entomology, Cornell University

Chronic bacterial infection has significant metabolic consequences on host organisms as energy is diverted to immune requirements and tissue repair. The fruit fly *Drosophila melanogaster* is an excellent model organism for studying chronic infection due to the ease of performing experiments. This project is investigating whether variance in host *D. melanogaster* genetics affects the severity and metabolic outcome of chronic infection. I used a ring cross design to produce five unique progeny genotypes and subjected each genotype to infection with two gram-negative bacterial species; I expected that each genotype would differ in infection severity and metabolic response. Next, I generated survival curves and chronic pathogen load data after bacterial infection and used molecular assays like triglyceride and glycogen quantification to determine metabolic outcomes. Preliminary data suggest that although there is no difference in chronic bacterial load between crosses, there are differences in acute survival to infection and the depletion of energy storage molecules. I hypothesize that susceptible genotypes cannot use energy storage molecules to sustain their bacterial burden over the course of infection. Additional infection experiments with gram-positive bacteria are needed to see whether these results are confined to gram-negative bacteria; further metabolite and gene expression experiments are necessary to determine the molecular basis of these results. A greater knowledge of the complex interplay between genetics, metabolism, and chronic infection will be valuable to many fields.

Assessing the Effects of Ribosome Collisions on Growth in Bacillus subtilis

Michael Kepko, Kevin England, Heather Feaga

Department of Microbiology, Cornell University

In all living organisms, ribosomes are conserved, vital complexes which enable protein synthesis by translating mRNA into a growing polypeptide chain. However, mRNA damage, difficult-to-translate sequences, ribosome-targeting antibiotics, and many other conditions can promote the stalling of ribosomes, wherein a translating ribosome becomes stuck on the mRNA. Subsequently, another ribosome translating the same mRNA can collide with the stalled ribosome, resulting in a ribosome collision. Eukaryotes and bacteria have both evolved mechanisms to rescue and recycle collided ribosomes. In eukaryotes, it is known that accumulating ribosome collisions that overburden the ribosome rescue mechanisms trigger growth arrest. However, the physiological changes that bacteria undergo when ribosome collisions accumulate have not been determined. Therefore, we hypothesize that excessive ribosome collisions might also arrest growth in bacteria. Using the model bacterium Bacillus subtilis, we investigate tolerance to ribosome collisions by measuring growth on solid and liquid media after inducing expression of a gene known to stall ribosomes. This work shows that a single copy of ribosome-stalling gene, under the control of a xylose-inducible promoter is not strong enough to impede growth. Future work will employ stronger promoters to generate more ribosome collisions. By characterizing how Bacillus subtilis withstands and responds to ribosome collisions, this work will contribute to understanding how bacterial translation is regulated under stress to maintain protein synthesis.

High-throughput functional genomics characterizes the genetic response to fungicides

Joshua D. Kerkaert[1], Renata Carvalho[1], Brandon Reyes-Chavez[1,2], Lori Huberman[2]

1. Cornell University, Plant Pathology & Plant Microbe Biology, Ithaca, NY
2. Department of Microbiology, Cornell University

Fungi are responsible for diseases that result in the deaths of over a million individuals each year and devastating crop infestations that threaten global food supplies. Unfortunately, spraying of certain classes of fungicides on crops has resulted in the emergence of strains of fungi that are cross-resistant to both agricultural fungicides and clinical antifungal drugs. To improve our ability to control fungal infections and infestations, we must understand what cellular processes the fungicides target and whether those mechanisms have the potential to yield cross-resistance with clinical antifungals. We used massively parallel screens in combination with transcriptional profiling to investigate the genetic mode of action of a novel fungicide developed by VM Agritech called Curezin™. Using a library of over 300,000 barcoded *Rhodosporidium toruloides* mutants, we identified genes that are involved in responding to fungicide exposure. Genes that, when mutated, caused a significant growth phenotype in massively parallel screens were often not differentially expressed in transcriptional profiling data. Similarly, genes differentially expressed during exposure to fungicides frequently did not cause growth defects when mutated in massively parallel screens. Molecular genetic analysis of genes identified for their role in responding to Curezin™ in both massively parallel screens and transcriptional profiling demonstrated that genes identified via both methods are important in the genetic response to Curezin™. Our work demonstrates the power of using massively parallel screens in concert with transcriptional profiling to identify a more complete set of genes associated with the genetic mode of action of fungicides. We expect that our work in identifying genes involved in responding to fungicides will enable an improved understanding of fungicide target genes to help in designing strategies to control fungal disease going forward.

Optimizing whole genome sequencing of insect-infecting Entomophthorales epizootics

Alex Lando

Cornell University, Plant Pathology & Plant Microbe Biology, Ithaca, NY

Entomophthorales, the early diverging lineage of insect-infecting fungi, remains a “black box” of molecular information, with some of the largest genomes of any known fungi and only six published in the entire order. In industry, these insect infecting fungi are in the process of being developed as biocontrols to limit insect predation and transfer of disease in some well-known invasives, such as the spotted lanternfly or the spongy moth. However, none of the underlying mechanisms for the infection process of these fungi, as well as their complex culturing requirements or storage methods, are known. In the preliminary work for this project, we were successfully able to use published sightings from iNaturalist to track an epizootic of the Entomophthoralean species *Furia ithacensis,* which infects the common snipe fly, *Rhagio mystaceus*. We then collected and cultured samples as well as optimized high molecular weight DNA extraction methods for PacBio Revio sequencing of this fungus through Cornell Weill. In the future work of this project, we plan to replicate this process across other Entomopthorales genera, and generate novel phylogenetic and comparative genomic data to identify and characterize genes involved in range, host specificity, and pathogenicity of this understudied order.

Investigation of the sporulation pangenome of *Paenibacillus*

Isabella N. Lin, Cassidy R. Prince, Heather Feaga

Department of Microbiology, Cornell University

Primary goals in the dairy industry include improving the quality and safety of milk production and reducing waste post-production. These aims are largely hindered by microbial activity, particularly by bacteria that can sporulate, such as species in the Paenibacillus genus. Bacterial spores can withstand the harsh conditions of milk pasteurization and then germinate back into vegetative bacteria post-pasteurization. Previous studies show that Paenibacillus species exhibit resilient germination capabilities at cold temperatures and are the predominant spoilage microorganism present during refrigerated storage. The objective of our research was to measure the sporulation efficiencies of twenty diverse Paenibacillus isolates and examine their genomes to ultimately identify differences in sporulation mechanism and ability across multiple Paenibacillus species. We determined the sporulation pangenome to consist of 189 genes, 76 of which are completely conserved. The least conserved genes only appear in one genome each. Upon performing a linear regression, we found that genome size did not explain sporulation gene presence (R^2 = 0.34). The phylogenetic relatedness of the samples was surveyed by building a maximum likelihood phylogenetic tree with core genome sequences. This revealed that isolates in the same clade had similar sporulation gene profiles. Improving our understanding of sporulation genes across the genus of Paenibacillus will contribute to the broader goal of reducing dairy spoilage by microbial activity.

Characterization of a transcription factor reveals interconnected carbohydrate sensing pathways in an oleaginous yeast

Brandon Reyes-Chavez[1,2], Joshua D. Kerkaert[1], Lori Huberman1

1. Cornell University, Plant Pathology & Plant Microbe Biology, Ithaca, NY
2. Department of Microbiology, Cornell University

The encroaching impacts of climate change on our daily lives necessitate the introduction of sustainable fuels and bioproducts. Microbial oils can help alleviate the need for fossil fuels as they can be sourced from oleaginous yeast grown on renewable plant biomass (lignocellulose). *Rhodosporidium toruloides* is an oleaginous yeast capable of accumulating over 70% of its biomass as lipids. *R. toruloides* can grow on breakdown products of lignocellulose (lignocellulose hydrolysate), yet the pathways involved in importing and utilizing these carbohydrates are poorly understood. We employed an insertional mutagenesis library, to screen mutants for growth defects on cellobiose and cellobiose + 2-deoxy-D-Glucose and identified a transcription factor, CBR1. When CBR1 was deleted growth defects were seen on not only cellobiose but also tricarboxylic acid (TCA) cycle intermediates. Additionally, we showed that CBR1 inhibits carbon catabolite repression. This work can inform the rational engineering of *R. toruloides* to grow more efficiently on lignocellulose hydrolysate to accumulate more lipids for downstream biofuel and bioproduct production.

Dispersal and biotic filtering structure Mucoromycota fungal communities and their associated bacteria across two different biomes

Nicole K. Reynolds[1], Kevin Amses[2], Jessie Uehling[3], Rasheed Adeleke[4], Margaret Branine[5], Teresa E. Pawlowska[1]

1. School of Integrative Plant Science, Cornell University, Ithaca, NY, USA
2. University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA, USA
3. Oregon State University, Department of Botany and Plant Pathology, Corvallis, OR, USA
4. North-West University, Unit for Environmental Sciences and Management, Potchefstroom, North West Province, SA
5. Cornell University, Graduate Field of Microbiology, Ithaca, NY, USA

Despite the ecological importance of Mucoromycota fungi as mycorrhizal symbionts, opportunistic human and plant pathogens, and post-harvest spoilage agents, they remain understudied compared to Dikarya. Fundamental aspects such as geographical distribution, dispersal patterns, and community structure remain unclear. Furthermore, recent and ongoing discoveries about the endosymbiotic bacteria (EB) that many Mucoromycota species harbor have generated new questions regarding their effects on fungal host evolution. EB have different effects on the host fungi depending on the species, influencing asexual and sexual reproduction and metabolic functioning. Our investigations of Mucoromycota and their associated bacteria communities are focused on testing three main community filtering hypotheses: dispersal (based on geographic distance), biotic (influenced by plant communities), and environmental (incorporating abiotic variables). We collected rhizosphere soils from four total locations in California representing two biomes (Desert and Mediterranean scrub) with three transects and two different plant species sampled from each site. These samples are being analyzed using both culture dependent and culture independent (metabarcoding) methods. We tested a nested PCR approach to facilitate detection of putative EB from soil samples. Metabarcoding data were generated using bacterial (16S rDNA) and fungal (28S rDNA) primers and show desert communities had higher proportions of Zoopagomycota taxa, whereas the coastal samples had more mycorrhizal taxa (Glomerales and Endogonales), with several OTUs unique to each habitat. The 16S nested PCR successfully enriched amplicons assigned to known EB groups, with community differences which corresponded with fungal community patterns. Both biotic filtering and dispersal filtering significantly affected fungal and bacterial communities; however, dispersal filtering was only significant over larger distances (km rather than m scale). In addition, desert samples had a higher proportion of fungal OTUs assigned to opportunistic human pathogenic species not detected from the coast. These results suggest that desert environments are likely reservoirs for these pathogens.

Transcriptional profiling of host and non-host *Rhizopus microspores* interactions with endosymbiotic *Mycetohabitans* sp. 13

Emma Scales, Maria Laura Gaspar, Olga Lastovetsky, Teresa E. Pawlowska

School of Integrative Plant Science, Cornell University, Ithaca, NY, USA

Strains of Rhizopus microsporus differ in their capacity to host the endosymbiotic bacteria Mycetohabitans sp. B13. Host strains exhibit a reliance upon Mycetohabitans for sporulation, and cured host strains can re-establish the endosymbiosis upon exposure after being cured of Mycetohabitans. Conversely, non-host strains exhibit characteristics of an antagonistic interaction when exposed to Mycetohabitans. This work utilizes the analysis of RNA-seq data from both host and non-host strains to describe specific differences in the two strains' transcriptional response to Mycetohabitans and identify putative signaling and regulatory networks driving the distinction between mutualistic and antagonistic interactions in this system.

Plant and fungal cell-type expression differences in arbuscular mycorrhizal symbiosis using spatial and single-nuclei transcriptomics.

Trevor R. Tivey[1*], Amitha Karuppiah[1*], Iwijn De Vlaminck[2], Maria J. Harrison[2]

1. Boyce Thompson Institute, Ithaca, NY
2. Meinig School of Biomedical Engineering, Cornell University
* co-presenters

In the arbuscular mycorrhizal (AM) symbiosis, obligate soil fungi grow inside of plant cells to receive fixed carbon, and in return they provide limiting mineral nutrients such as phosphate to their host. This symbiotic exchange is temporary on an individual cell basis, but the fungal hyphae travel through the root cortex and continue to colonize cells, creating linear colonization gradients within the plant root. Critical pathways involved in the establishment of the symbiosis have been well-studied using targeted genetic strategies, however the spatiotemporal landscape of host-microbe gene expression has only been studied at a broad level. To examine the cellular landscape of AM colonization, we used a combined approach of spatial and single-nuclei transcriptomics. *Medicago truncatula* plants containing a colonization-induced fluorescent reporter were inoculated with *Rhizophagus irregularis* spores. Colonized and noncolonized roots were either cryopreserved and used to generate 10X Visium spatial transcriptomic libraries, or chopped to release nuclei and sorted to generate 10X Chromium single-nuclei transcriptomic libraries. Single-cell and spatial data were subsequently combined and clustered to reveal differences between colonized and noncolonized samples at the plant cell-type level. Fungal transcripts were also captured in both datasets and showed significant transcriptional differences based on location. To validate annotated cell types and compare expression between wildtype and colonization mutant plants, *Medicago* roots were transformed with promoter-GUS fusion constructs, which enables visualization of promoter activity in plant tissue. Altogether, our data provides a more resolved picture of plant-fungal gene expression dynamics, and provides a window into how neighboring cell types respond to AM colonization.

A matter of life and death: characterizing the innate immune response of the mucoromycete *Rhizopus microsporus* to the antagonistically perceived *Mycetohabitans* bacterium

Delia Tota[1], Maria Laura Gaspar[2], Carlos Lax[3], Victoriano Garre[3], Teresa E. Pawlowska[2]

1. Graduate Field of Microbiology, Cornell University, Ithaca, NY
2. Cornell University, Plant Pathology & Plant Microbe Biology, Ithaca, NY
2. University of Murcia, Spain

Like plants and animals, fungi employ innate immunity mechanisms to perceive and regulate interactions with microbes, including bacteria. Regulated cell death (RCD) is one of the many innate immune mechanisms with functional similarities shared by plants and animals. However, the surveillance system and means of innate immune defense in fungi, including the use of RCD, is not well understood. The symbiosis between the mucoromycete *Rhizopus microsporus* and the bacterial endosymbiont *Mycetohabitans* sp. presents a valuable system in which to investigate the innate immune response of early-divergent fungi. In contrast to the accommodations for bacteria made by host *R. microsporus* strains, nonhost strains, which do not naturally harbor endosymbionts, perceive *Mycetohabitans* antagonistically upon interaction. The fungus launches a reactive oxygen species (ROS) response accompanied by elevated lipid peroxidation, decreased levels of the antioxidant glutathione, and death of a subpopulation of fungal cells, as indicated by microscopy, glutathione quantification, and flow cytometry assays. Application of exogenous antioxidants and the iron chelator deferoxamine alleviates lipid peroxidation. We also have preliminary evidence that RCD as a defense response against *Mycetohabitans* is initiated, at least in part, by the fungal adenylyl cyclase 1 (Cyr1) receptor sensing bacterial signals. Ongoing experiments will be aimed at quantifying fungal RCD through differential staining in flow cytometry assays and using fluorescent in situ hybridization (FISH) to discern whether the RCD is mediated by bacterial penetration into fungal cells.

The effect of an endosymbiotic bacterium on the mutualistic behavior of the arbuscular mycorrhizal fungus *Rhizophagus clarus*

Tommy Victor[1], Hana Barrett[1], Maria Laura Gaspar[1], Danyel Yogi[2], Teresa E. Pawlowska[1]

1. Cornell University, Plant Pathology & Plant Microbe Biology, Ithaca, NY
2. Pacific Biosciences Research Center, University of Hawai'i at Manoa, Honolulu, HI

The bacterium '*Candidatus* Moeniiplasma glomeromycotorum' is a recently discovered widespread obligate endosymbiont in many lineages of arbuscular mycorrhizal fungi (Glomeromycotina). However, little is known about the effect this bacterium has on its fungal host or the impact of the endosymbiosis on a plant host. Previous work in our lab found tentative fitness advantages in strains of *Rhizophagus clarus* containing the bacterium. Spore germination and hyphal branching rates were higher in strains containing the bacterium than in strains without the bacterium. This study investigates the impact of the bacterium on host plant growth using the model system *Medicago truncatula*. The objectives are to measure how plant biomass and nutrient uptake are affected, and the impact on gene expression of the plant and fungus when the endobacterium is present. *M. truncatula* will be inoculated with endobacterium-containing and endobacterium-free strains of *R. clarus*. AMF strains are sourced from the International Collection of (Vesicular) Arbuscular Mycorrhizal Fungi, and bacterial presence is confirmed using PCR. Plants are treated with phosphorus-sufficient or phosphorus-deficient fertilizer, since nutrient availability influences the dynamics of the fungal-plant relationship, and plant growth responses are measured. RNA sequencing will be performed on plant roots containing arbuscules to identify differentially expressed genes associated with bacterial endosymbiosis. Preliminary results indicated that in normal nutrient conditions, strains of *R. clarus* with the endobacterium did not confer the same fitness benefits as strains without the bacteria. We hypothesize that the endobacterium may be a conditional mutualist in low phosphorus conditions, and when present may lead to increased plant biomass and nutrient content. This study may contribute to building a predictive framework for applications of arbuscular mycorrhizal fungi in agricultural systems and ultimately reduce the dependency on chemical fertilizers.

YeeI is a translation factor in *Bacillus subtilis* important for translating polyproline tracts

Leitan Wu, Hye-Rim Hong, Heather Feaga

Department of Microbiology, Cornell University

Translating genetic information into proteins is fundamental in all living organisms, from bacteria to eukaryotes. This crucial life-sustaining process is executed by complex macromolecular machinery within the cell known as ribosomes. Ribosomes can become stalled during translation, especially when synthesizing 'hard-to-translate' nascent peptide sequences such as polyproline sequences. Previous studies have shown that the rigid loop structure of consecutive prolines disrupts the ribosome's peptide exit tunnel, causing stalling; In bacteria, the elongation factor EF-P alleviates this by stabilizing tRNA and promoting peptide bond formation through alternative peptide chain geometry (1–4). Through a Tn-seq screen for genetic interactions with EF-P, we identified a gene, *yeeI*, of unknown function, which causes significant growth defects when deleted from *B. subtilis* cells lacking EF-P. In this study, our primary objective is to determine the function and specific roles of the YeeI protein in the translation process of *Bacillus subtilis*. So far, we have several key findings: Firstly, by conducting plate reader assays and colony size measurements across various strains, we have found that deletion of *yeeI* causes severe growth defect in *Δefp* background, and even more severe growth defect in a strain lacking both EF-P and YfmR. Secondly, by overexpressing YeeI in strains that lack both EF-P and YfmR and measuring the growth, we have identified that over-expression of YeeI can rescue synthetic growth defects of *ΔefpΔyfmR* strains. Moreover, we also found that YfmR over-expression could rescue the growth rate of *ΔefpΔyeeI* cells. This suggests that YeeI and YfmR function independently, as the overexpression of each can rescue growth defects in the absence of the other. Next, by using a stalling reporter consisting of 5 consecutive prolines in between RFP and CFP, we found that a strain lacking both EF-P and YeeI exhibits increased ribosome stalling, and such phenotype can be rescued by overexpressing either YeeI and YfmR. Moreover, we show that YeeI associates with ribosomes by sucrose density gradient ultracentrifugation. Altogether, we have shown that YeeI is important for the growth and viability of *B. subtilis* in the absence of *efp* and is likely a translation factor that prevents ribosome stalling at polyprolines.