

**CORNELL INSTITUTE OF HOST-MICROBE
INTERACTIONS AND DISEASE**



SUMMER SYMPOSIUM 2018

“Host-Microbe Interactions at the Interface”

July 26, 2018

9:10 – 5:00

coffee at 8:30, reception to follow

Baker Institute

Hungerford Road; parking is free but limited so please carpool

Register at cihmid.cornell.edu by July 19

*no registration fee, **lunch included***



Cornell University

Schedule of Events

8:30 – 9:10 Coffee and Welcome Reception

9:10 – 9:15 Opening Remarks (Brian Lazzaro, CIHMID Director)

9:15 – 9:45 Maria Harrison (Boyce Thompson Institute)
“Reprogramming root cells for endosymbiosis with AM fungi”

9:45 – 10:15 Jeongmin Song (Microbiology and Immunology)
“Typhoid Toxin and Salmonella Typhi Pathogenesis”

10:15 – 10:30 Weishan Huang (Microbiology and Immunology)
“Regulatory T cells in the mucosal interface of host-pathogen interactions”

10:30 – 10:45 Anna Weaver (Microbiology)
“Lytic transglycosylases assist in V. cholerae daughter cell separation”

10:45 – 11:05 Coffee Break

11:05 – 11:20 Noam Eckshtain-Levi (School of Integrative Plant Science; BTI)
“Pseudomonas floridensis: a new threat to tomato production in Florida?”

11:20 – 11:35 Mia Howard (School of Integrative Plant Science)
“Soil microbes mediate shifts in plant herbivore resistance over oldfield succession”

11:35 – 12:05 Tory Hendry (Microbiology)
“Life and death in the phyllosphere: plant-associated bacteria impact aphid survival and behavior”

12:05 – 1:30 Lunch and Poster Viewing

1:30 – 2:00 Cynthia Leifer (Microbiology and Immunology)
“Regulation Innate Immune Receptor Signaling”

2:00 – 2:30 Angela Poole (Nutritional Sciences)
“The impact of host salivary amylase gene copy number on oral and gut microbiomes”

2:30 – 2:45 Maria Teresa Reinoso-Perez (Ecology and Evolutionary Biology)
“Effect of Mycoplasma gallisepticum on the parasitaemia in birds infected with Haemosporidian”

2:45 – 3:00 Allison Tracy (Ecology and Evolutionary Biology)
“Coral co-infection: The role of host immunity in parasite interactions”

3:00 – 3:20 Coffee Break

3:20 – 3:35 Nicole Andre (Microbiology and Immunology)

“Detection of feline coronavirus from the respiratory tract in a cat with feline infectious peritonitis”

3:35 – 3:50 Karen Barnard (Baker Institute)

“The use of glycoengineered cell lines for investigating influenza A interactions with modified sialic acids”

3:50 – 4:05 John Frank (Molecular Biology and Genetics)

“Human endogenous envelope-derived proteins offer potential protection from retroviral infection”

4:05 – 4:35 Adam Bogdanove (School of Integrative Plant Science)

“Direct transcriptional activation of host disease susceptibility genes by the plant pathogen Xanthomonas and what to do about it”

4:35 – 5:05 David Russell (Microbiology and Immunology)

“Mycobacterium tuberculosis: The metabolic interface of host and pathogen”

5:05 – Closing Reception

SUBMITTED ABSTRACTS

Talks

Regulatory T cells in the mucosal interface of host-pathogen interactions

Weishan Huang^{1,2} and Avery August¹

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The mammalian immune system has evolved both the effector and regulatory immune axes, and T cells are the key players in both arms. The immune system sorts, responds to and clears invading pathogens through pro-inflammatory activity, which can cause damage to the affected tissues, and will eventually have to resolve to allow tissue remodeling and resume physiological homeostasis. The balance between pro-inflammatory and suppressive immune functions are critical for health. T cells with regulatory function play pivotal roles in controlling pro-inflammatory responses to self- and foreign antigens, as well as to pathogenic and commensal microorganisms. During the initiation, progress and resolution of inflammation, the host-microbe interaction creates a changing microenvironment for immune cell development. T cells are activated through their antigen receptor (T cell receptor, TCR), and can differentiate according to their local microenvironment to become effectors and/or regulators. While effector T cells such as the IL-17-producing Th17 cells can contribute to inflammation in a chronic manner regulatory T cells that express the regulatory cytokine IL-10 play important roles in preventing excessive inflammation. We have found multiple subsets of T cells capable of producing IL-10 in the mucosal interface of host-pathogen interactions. The composition of these cells varies in different anatomical locations and co-relate to the presence of antigenic stimulation spatiotemporally. The nutritional condition in the local microenvironment can modulate the counter-balance of effector and regulatory T cell development and may serve to transition inflammation into tolerance to prevent excessive tissue damage during pathogenic invasion. The TCR and nutrient sensing signaling pathways may be promising venues for strategic development of approaches to modulate regulatory T cell development and function to regulate mucosal inflammation.

Lytic transglycosylases assist in *V. cholerae* daughter cell separation

Anna Weaver and Tobias Doerr

Department of Microbiology, Cornell University

The rigidity of the peptidoglycan (PG) cell wall is necessary for containing the bacterial cell's high internal pressure. However, it restricts other essential processes such as cell growth, division, and even motility which is why "autolysins," which break down PG material thus allowing for cell wall remodeling and PG recycling, are so important to bacterial survival. The specific physiological roles of each autolysin have been historically difficult to assign because they are numerous and exhibit high functional redundancy. *Vibrio cholerae*, for example, encodes 8 lytic transglycosylases (LTGs) that cleave the sugar backbone of PG, none of which appear to be individually essential. However, we have found that a pair of LTGs, RlpA and MltC, is integral for separation of daughter cells after cytokinesis as a deletion of both LTGs causes cells to form long chains. This is contrary to popular dogma that amidases are the primary autolysins required for septal PG cleavage. Despite the ability of RlpA or MltC to complement the chaining phenotype on their own, details about their mechanisms of localization and substrate specificities reveal that both LTGs make unique contributions to cell separation that cannot be performed by *V. cholerae*'s only and highly regulated amidase, AmiB.

***Pseudomonas floridensis*: a new threat to tomato production in Florida?**

Noam Eckshtain-Levi^{1,2}, Magdalen Lindeberg¹, and Gregory Martin^{1,2}

¹Plant Pathology and Plant-Microbe Biology Section, School of Integrative Plant Science, Cornell University and ²Boyce Thompson Institute for Plant Research

A disease outbreak was observed in a tomato field in central Florida in 2010 and 2011 with the symptoms being similar to bacterial speck caused by *Pseudomonas syringae* pv. tomato (*Pst*) [1]. Subsequently, eight UV-fluorescent strains belonging to the genus *Pseudomonas* were isolated from eight different symptomatic plants in the field [1]. Whole genome sequence together with other evidence demonstrated that this group represents a novel species in the genus *Pseudomonas*, and the name *P. floridensis* was proposed [1]. The genome sequence of the type strain revealed only nine type III effector genes. A previously generated *Pst* strain carrying just eight effectors was found to be virulent on *Nicotiana benthamiana* but not on tomato [2]. Interestingly, five of the effectors in *P. floridensis* are contained within this eight 'minimal effector' set. We hypothesized that the additional effectors present in *P. floridensis* allow it to become a pathogen of tomato. We will present our results testing this hypothesis.

- 1) Timilsina S., Minsavage G.V., Preston J., Newberry E.A., Paret M.L., Goss E.M., Jones J.B. and Vallad G.E. (2018). *Pseudomonas floridensis* sp. nov., a bacterial pathogen isolated from tomato. *Int. J. Syst. Evol. Microbiol.* 68:64-70.
- 2) Cunnac S., Chakravarthy S., Kvitko B.H., Russell A.B., Martin G.B. and Collmer A. (2011). Genetic disassembly and combinatorial reassembly identify a minimal functional repertoire of type III effectors in *Pseudomonas syringae*. *Proc. Natl. Acad. Sci. USA* 108:2975-80

Soil microbes mediate shifts in plant herbivore resistance over oldfield succession

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Plant defenses typically escalate over ecological succession, paralleling mounting pressure from herbivores. We observed drastic increases in the herbivore resistance of goldenrod, *Solidago altissima* (Asteraceae), over the first 15 years of oldfield succession (after conventional maize) in a large-scale field experiment. While our previous work has demonstrated that this increase in resistance is partially due to rapid microevolutionary shifts in *S. altissima* populations, plastic phenotypic shifts in response to the changing soil environment are also likely to contribute. Field surveys revealed significant shifts in soil microbial communities in the *S. altissima* rhizosphere over succession, which are likely to have important functional effects on plant defense phenotypes. In a 3-way feeding choice experiment, the specialist beetle, *Trirhabda virgata*, strongly preferred to eat leaves from *S. altissima* plants grown in soil media inoculated with earlier succession (2 yrs) soil microbiomes over their later succession (6 and 15 yrs) counterparts, indicating that the soil microbiome affects resistance to aboveground herbivores. Likewise, the concentrations of several leaf secondary metabolites, particularly diterpenes, differed significantly with successional inoculant age. Our findings indicate that soil microbial shifts over succession enhance plant herbivore resistance, likely contributing to the pattern of decreased herbivory at later successional stages.

Effect of MG on the parasitaemia in birds infected with Haemosporidian

Maria Teresa Reinoso-Perez and André A. Dhondt

Department of Ecology and Evolutionary Biology, Cornell University

Wildlife hosts typically deal with multiple pathogens, which could generate complex interactions. Co-infecting pathogens interact among themselves and with the host, in a positive or negative way. Co-infection therefore may provide an advantage to pathogens and facilitate the emergence of diseases. *Mycoplasma gallisepticum* (MG) emerged in 1994 in house finches in the eastern USA and rapidly spread across the whole country, reaching epidemic levels and reducing house finch population sizes. The presence of another pathogen could compromise the immune system of the host and thereby facilitate the establishment of a new pathogen. We found that birds with *Plasmodium spp.* developed infections with higher MG load and tended to develop more severe eye lesions. Alternatively, corticosterone levels increased after an MG infection and returned to pre-infection levels once the infection was cleared. Parasitaemia increased in House sparrows infected with *Plasmodium relictum* after a corticosterone treatment. The aim of this study is to test the hypothesis that because corticosterone levels increase after an MG infection and because *Plasmodium* parasitaemia increases after a corticosterone treatment, haemosporidian parasitaemia should increase following an MG infection. We tested this hypothesis by comparing the number of circulating haemosporidian parasites between individuals that were inoculated with MG and control birds. If a positive feedback exists between the haemosporidian and MG pathogens, the success of transmission of both pathogens would be enhanced by the prior infection with one of them.

Coral co-infection: The role of host immunity in parasite interactions

Allison M. Tracy and C. Drew Harvell

Department of Ecology and Evolutionary Biology, Cornell University

Co-infection can change the outcome of host-parasite interactions and is remarkably common in nature. During co-infection, the parasites' resource use and the host immune response are critical for determining whether there is an antagonistic or facilitative interaction between the parasites. The nature of this interaction can have important consequences for the host and both parasites. We conducted a laboratory experiment to explore how two parasites of the Caribbean sea fan, *Gorgonia ventalina*, interact indirectly through the host immune response. The parasites include a fungus implicated in an important disease outbreak in sea fans in the early 2000s, as well as a recently emerging copepod parasite. The fungus infects the skeletal axis of the host and the copepod infects polyps, suggesting that the two parasites do not compete for resources. We measured several metrics of host immunity in a fully factorial, clonally replicated experiment. Both parasites led to increased expression of candidate immune genes, confirming the immune relevance of these genes. The immune genes were not differentially expressed in single infections of the two parasites, or in single infections vs. co-infections. However, the presence of the copepod led to the induction of a cellular immune response that was not present with the fungus, supporting a one-way antagonism where co-infection is disadvantageous for the fungus. Paired field surveys of immunity and parasite prevalence in the natural sea fan population in Puerto Rico will indicate how this potential immune-mediated antagonism translates to host immune profiles and parasite co-occurrence. The field surveys will also provide an important perspective on the role of environmental and ecological factors in co-infection. A combined approach using laboratory and field studies is critical for advancing research on the factors that structure co-infection patterns in the wild.

Detection of feline coronavirus from the respiratory tract in a cat with feline infectious peritonitis

Nicole M. André¹, Andrew D. Miller² and Gary R. Whittaker¹

¹Department of Microbiology & Immunology, ²Department of Biomedical Sciences, Section of Anatomic Pathology, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853

Coronaviruses have been implicated in respiratory and gastrointestinal disease in many animal species. In cats, feline coronavirus (FCoV) is associated with a mild, typically self-limiting gastrointestinal infection. In some cases, mild respiratory signs have been noted; however, FCoV is not routinely considered a common respiratory pathogen in cats. Feline upper respiratory infection (URI) is an important infectious disease complex that affects the feline population, particularly those in densely-housed populations such as shelter and rescue facilities. Clinical signs include conjunctivitis, corneal ulcers, and ocular discharge, as well as sneezing or coughing. Feline URIs are associated with numerous pathogens including feline herpesvirus-1 (FHV-1), feline calicivirus (FCV), pneumovirus, *Chlamydophila felis*, *Mycoplasma* sp., *Bordetella bronchiseptica*, and *Streptococcus equi* subsp. *zooepidemicus*. The role that feline coronavirus (FCoV) has as a primary causative agent of feline URI is not entirely clear. Here, we collected swabs from the conjunctiva, an array of visceral tissues, and samples from the nasal turbinates from a cat diagnosed with feline infectious peritonitis (FIP) at necropsy. Clinically, the cat had a history of mild respiratory disease including conjunctivitis, sneezing, and coughing. Histological examination revealed pyogranulomatous inflammation in the nasal cavity, especially affecting the cribriform plate region. Immunohistochemistry, using the FIP 3-70 antibody, localized virus to macrophages in the areas of inflammation. This supports that FCoV is a pathogen to be considered in feline respiratory infection in cats and should be considered an agent associated with the feline respiratory disease complex.

The use of glycoengineered cell lines for investigating influenza A interactions with modified sialic acids

Karen Barnard [1], Brian Wasik [1], Brynn Lawrence [1], Colin Parrish [1]

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Influenza A viruses (IAV) use sialic acids (Sia) as the primary receptor for infection via the hemagglutinin (HA) and neuraminidase (NA) glycoproteins. Sias are found in large amounts in mucus that protect respiratory tract cells. Sias may be chemically modified (4-O-, 7,9-O-, 9-O-acetyl, 5-N-glycolyl), and are attached to glycan chains through different linkages, which vary between hosts and tissues. While the roles of Sia linkages have been explored in IAV tropism and evolution, the roles of modified Sias are not well known. Modified Sias have been identified as inhibitors of NA and HA, but their role during infection is unclear. We have shown that 7,9-O- and 9-O-Ac Sias are highly expressed in the respiratory tissue of humans, embryonated chicken eggs, and on MDCK, HEK293, and A549 cells. Neu5Gc is known to be highly expressed in pigs and horses, and in mice and guinea pigs (model species for IAV), but is not found in humans, chickens, and ferrets. In this presented work, we are creating glycoengineered cell lines using CRISPR/Cas9 and expression plasmids that lack/overexpress 7,9-O- and 9-O-Ac or Neu5Gc to determine their impact on IAV enzyme function, growth, and evolution.

Human endogenous envelope-derived proteins offer potential protection from retroviral infection

John Frank, Harrison Cullen, Cedric Feschotte

Department of Molecular Biology and Genetics, Cornell University

Endogenous retroviruses (ERV) are derived from exogenous retroviruses that have entered the genome through past infection of the host germline. Like exogenous retroviruses, ERV proviruses minimally encode *gag*, *pol* and *env* genes. These ERV-derived sequences provide a reservoir of protein-coding material with the potential to be preserved by natural selection and domesticated to serve host cellular functions. ERV-encoded *env* proteins have been reported to confer resistance to exogenous retroviral infection in several vertebrates. While recent studies suggest human ERV (HERV) *env* may have antiviral activity, no systematic analysis testing whether ERV *env* confer resistance to retroviral infection in primates has been performed. We hypothesize that a subset of human ERV (HERV) *env* may function as inducible antiviral factors against diverse exogenous and potentially zoonotic viruses. As a proof of principle, we chose two known HERV-derived *env* candidates, *Syncytin-1* (*syn1*) and *Suppressyn* (*sup*). Both *syn1* and *sup* have been implicated in placental development and bind ASCT2, a known target receptor of the RDR superinfection interference group. Using phylogenetic and codon selection analyses, we found that *sup* experienced a selective regime of purifying selection in apes indicating this protein may have been preserved for some function independent of its role in placental development. In support of our hypothesis, we show that overexpression of *syn1* and *sup* in HEK-293T cells, drastically reduces infection by HIV-GFP particles pseudotyped with envelopes of the RDR superinfection interference group. We further test the antiviral activity of endogenously expressed Syncytin-1 and Suppressyn in placenta-derived trophoblast cell lines using our restriction assay. In order to analyze the potential pool of HERV *env* with antiviral activity, we employed two complementary computational pipelines to establish a catalog of HERV *env* sequences bearing the signature of functional activity and/or selective constraint. Notably, we identified multiple *env*-derived genes that have experienced purifying selection during primate evolution, which we are now testing for evidence of infection-inducible expression and retroviral restriction. Our data not only indicate that *syn1* and *sup* are capable of restricting infection mediated by RDR interference group *env* but also suggest multiple HERV *env* may have been domesticated as antiviral factors against a wide range of exogenous viruses.

POSTERS (CIHMID Undergraduate Research Experience)**

Characterizing mobile genetic elements in farm animal microbiomes within and between species

****Diana Balint & Ilana Brito**

Biomedical Engineering, Cornell University

Background/Question/Methods

Genetically engineered bacteria have the potential to be used within living hosts for long-lasting desired effects. One such effect is increasing muscle and fat growth of farm animals without the use of antibiotics, which are commonly used in low doses as a growth promotant. The gut microbiome plays a significant role in metabolism, so introducing specific genetically engineered bacteria to farm animals' microbiomes can potentially replace the need for antibiotics by similarly acting as a growth promotant. Before introducing any genetically engineered bacteria, it is important to understand how genes are mobilized in the gut microbiomes of these animals. This is to predict and prevent the movement of introduced genes from microbe to microbe in the microbiome, as well as from animal to animal within and between species. To understand genetic mobility, we are currently characterizing mobile genetic elements using stool samples from goats, pigs, and cows. Plasmid extraction and IPCR to identify transposons are methods currently being explored to understand mobile genetic elements in the microbiome.

Research plan

We plan to continue exploring the best methods to identify mobile genetic elements in the microbiome, including plasmids, transposons, and potentially phage. After characterizing these mobile genetic elements, we plan to experiment with the mobility of an introduced gene within a bacterial community, identifying mobile genetic elements and bacterial species that facilitate horizontal gene transfer of the introduced gene. What we find may be useful in the design and management of genetically engineered bacteria used in farm animals.

Typhoid vaccines targeting both *Salmonella* Typhi and its secreted virulence factor

**Alexander Chong & Jeongmin Song

Microbiology and Immunology, Cornell University

Typhoid fever is a major global health concern, with ongoing outbreaks occurring in many low and middle-income countries. The causative agent of typhoid fever is the gram-negative bacterium, *Salmonella enterica* serovar Typhi (*S. Typhi*). It has been recently discovered that *S. Typhi* produces an unusual toxin known as typhoid toxin that plays a significant role in both acute and chronic infection. The goal of this research project is to generate typhoid fever vaccines targeting both the bacterium and its secreted virulence factor typhoid toxin. To reach this goal, we are engineering the Ty21a vaccine strain to make it express either inactive typhoid toxin or the toxin's receptor binding subunit without its two enzymatic subunits. The modified Ty21a vaccines generated as part of this study are expected to be more effective and may have potential applications for use in humans.

The effect of mating and Juvenile Hormone exposure on the immunity of male *Drosophila melanogaster*

******Sayyed Hussain, Vanika Gupta, Brian P. Lazzaro

Department of Entomology, Cornell University

The role of Juvenile Hormone (JH) in life-history tradeoffs of *Drosophila melanogaster* is much better understood in females than in males. Previously, we reported endogenous production of JH upon mating in females results in the suppression of immunity. This time, we studied the effects of mating on immunity in males, by analyzing the survival rate of virgin and mated males systemically infected with bacteria of varying virulence. We also exposed virgin and mated males to artificial JH followed by infection with bacterium *Providencia rettgeri* to assay if JH causes immunosuppression in males as well. We infected flies using a thin needle (0.1mm) which was dipped in fresh bacterial suspension ($OD_{600} = 1$) each time a fly was infected (pricked). Data were collected by counting fly survivorship for 96 hours post-infection, with the first set of observations at six hours to estimate fly mortality due to injury rather than infection. Kaplan-Meier plots were constructed using the survivorship data and differences between the treatments were tested using the log-rank test. We found that virgin males survived significantly better than mated males in most of the bacterial infections. Our results from JH exposure showed that males continuously exposed to higher JH levels suffered worse outcomes than unexposed controls and males continuously exposed to lower JH levels. We also found transient exposure to JH to be less reliable in producing outcomes significantly different from controls. We suggest that mating and JH can be immunosuppressive in males as observed in females previously.

Genetic Influences on Intestinal Homeostasis

**Miranda Martinez, Alessandro Bonfini, Nicolas Buchon

Department of Entomology, Cornell University

The gut is one of the primary interfaces with the external environment, exposing it to variety of challenges from toxic compounds to infection. These factors may lead to loss of cells, which need to be replenished to maintain homeostasis. This is maintained in the intestine through a stem cell population, which acts to replenish lost cells. Stem cell regulation is one of the key components in maintaining homeostasis within the intestines. Our project aims to study the genetic network that controls stem cells and its effect on homeostasis within the intestines, using *Drosophila melanogaster* as the model organism. To do so, we will first infect flies with a certain type of bacteria, *Erwinia carotovora carotovora 15 (Ecc15)*, which induces loss of around 50% of the gut. We will block the activity of genes enriched in the stem cell population through RNAi interference and assay the regenerative capacity of these guts upon infection through immune staining of dissected fruit flies' intestines and various methods such as counting PH3 during the proliferative peak, measuring gut lengths at the end of the process, and assaying the shape of stem cells in both infected and unchallenged conditions. This research could potentially help expand our knowledge on homeostasis of the digestive system, leading to new advancements in treatment options for many types of diseases, such as Crohn's and inflammatory bowel disease.

The Effect of Opioid Abuse on the rate of Influenza through Statistical Modeling

****Stephenie Ojilere & Renata Ivanek**

Department of Population Medicine and Diagnostic Sciences, Cornell University

Influenza, commonly known as the seasonal flu, has been infecting humans worldwide for many years. Although the flu vaccine can be used to decrease the risk of transmitting the virus, the 2017 Influenza Epidemic has proven to us that the actual vaccines are not always effective by itself. Thus, my research consists of using statistical modeling to predict the prevalence of those infected by simulating a typical influenza season with controlled factors. The risk factor that I decided to focus my research on is opioid abuse. There has been research on opioids having an immunosuppression characteristic on mammals, and I wanted to know if it specifically affects the rate of influenza cases in humans. I used data from varying New York State counties to construct models and data tables that concluded that there is in fact a positive correlation between the Opioid Related Emergency Department Admissions and Influenza Cases. My next step is incorporating my findings into the computer simulation to predict how will affect a simulated population.

For the symposium, I will be presenting general information about the drug and its ability to weaken the immune system of a host. I will also present my models and data tables concluding that opioids can increase the overall risk of influenza in humans. Although my research is not completed, the overall goal is to simulate specific scenarios with opioid use as the main risk factor, and answer the question of what strategies can we introduce to society that can hopefully decrease the prevalence of influenza in the future.

Characterization of a novel manganese homeostasis protein in *Bacillus subtilis*

**Srinand Paruthiyil & John Helmann

Department of Microbiology, Cornell University

Background/Questions/Methods

Manganese (Mn) is an essential element throughout most of the tree of life and regulates the virulence of many Gram-positive pathogens. We study the Mn homeostasis systems of *Bacillus subtilis*, a model organism for such pathogens. Recently, we discovered a new protein of unknown function, YceF. Although it is predicted to be involved in tellurium (Te) resistance, our research shows that YceF greatly contributes to Mn homeostasis in *B. subtilis*. Here, we used various classic microbiological approaches to investigate the transcriptional and physiological characteristics of YceF and its operon.

Results/Conclusion

A knockout strain of *yceF* has significant Mn sensitivity in *B. subtilis*. This sensitivity is further exacerbated by knocking out the known Mn efflux pumps MneP and MneS. A plasmid carrying a *lacZ* fusion of the *yce* operon's promoter region was constructed to serve as a transcriptional reporter. Upon introduction of the plasmid into *B. subtilis*, promoter activity increased as a function of Mn concentration. Based on bioinformatic analysis and previously published microarray data, activation of this promoter by Mn seems to be independent of the known dedicated Mn detection protein, MntR. Instead this operon seems to be regulated by a complex array of sigma factors including the ECF sigma factors W, M, and X, as well as the general stress response sigma factor B. We hypothesize that the operon's response to Mn is mediated through sigma B. Our research is expanding our understanding of Mn homeostasis in *B. subtilis* through the identification of a Mn responsive gene predicted to be involved in Te resistance, which is regulated independently of MntR. Furthermore, YceF homologs exist in other bacteria, including pathogens. Information about YceF may be useful for future studies investigating mechanisms of metal related nutritional immunity in host-pathogen interactions.

Equine Coronavirus: An Emerging Disease of Horses

**Hannah Pambianchi¹, Nicole M. André², Javier James³ Erin Goodrich⁴, and Gary R. Whittaker²

¹College of Arts and Sciences,²Department of Microbiology and Immunology College of Veterinary Medicine, ³College of Agriculture and Life Sciences, ⁴Animal Health Diagnostic Center, Department of Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, NY 14853

Coronaviruses are single stranded RNA viruses that infect a wide variety of mammalian and avian hosts. While some coronavirus species are known to cause serious respiratory or gastrointestinal disease, others may present with only mild enteric signs, or are subclinical in nature. Equine coronavirus (ECoV) is a poorly understood virus of horses. It is thought to be of low virulence, but difficulties are encountered in its isolation and limited surveillance has left gaps in our knowledge of its pathogenesis and diversity. Here we characterize ECoV with both genomic analysis and viral isolation. Molecular analysis was performed by comparing the spike gene sequences of ECoV positive clinical samples with NCBI database entries. Using 7 sets of overlapping primers, we were able to sequence the full spike gene of one clinical sample. Due to sequence heterogeneity of S1/S2 and S2' region in other samples, experiments are still undergoing optimization. Virus isolation was carried out in HRT-18 cells, using the prototypic ECoV-NC99 strain. Each infection passage was monitored for cytopathic effect (CPE), and assayed using PCR and antibody-conjugated fluorescent staining. One of the most striking results of the cell-culture infections was the lack of observed CPE with and without the addition of trypsin. Although this suggested lack of infection, increased PCR product amplification was demonstrated using RNA extracted from the supernatant of each subsequent infection passage. The immunofluorescence (IFAT) assay was positive in passages 7 and 8 onwards, suggesting amplification and adaptation of the virus to cell culture. From these results, we conclude that equine coronavirus is able to infect a monolayer without causing CPE. Further studies are in progress to determine adaptive changes that may have occurred in the virus during propagation in cell culture in the absence of trypsin.

Evolutionary trajectory of the *Rhizopus-Burkholderia* symbiosis

**Evaniya Shakya, Olga Lastovestky, Jackson Waite-Himmelwright, and Teresa Pawlowska

School of Integrative Plant Science, Cornell University

Rhizopus microsporus, a saprotrophic fungus, is commonly found in molding food products and plants. For example, it can cause rice seedling blight. Some of the isolates of *R. microsporus* harbor heritable endobacteria such as *Burkholderia rhizoxinica*, *Burkholderia endofungorum*, and *Burkholderia* sp. that control fungal sporulation. This mutualism between *Rhizopus* and its endobacteria *Burkholderia* has emerged as a model for understanding fungal-bacterial symbioses, including mechanisms of endosymbiont genome contraction. To explore the co-evolution of *Rhizopus* and *Burkholderia*, we genotyped a collection of bacterial and fungal isolates, reconstructed their phylogenies, and looked for evidence of recombination. Comparisons of partner phylogenies using revealed a history of partner codivergence punctuated by host switches. Based on fossil record, we dated the divergence of fungal isolates ATCC 56028 and DUKE 8560 that have features intermediate between *Rhizopus delemar* and *R. microsporus* to ~160 MYA. We will further expand this investigation to determine whether genome contraction in these heritable endobacteria is adaptive or degenerative.

Assessing the Role of Motility in *Pseudomonas syringae* Virulence in Insects

**John Tawil & Tory Hendry

Department of Microbiology, Cornell University

Pseudomonas syringae is a bacterium that is commonly known as a plant pathogen, but it has also been known to infect certain insects. The role of virulence genes utilized by *P. syringae* is well characterized in plant pathogenesis, but is widely unknown in insect pathogenesis. Motility is known to play an important role in the infection of plants, but its role in insects is unknown. Motility knockouts have shown less virulence in aphids, but the mechanism for this reduced virulence is in question. My project seeks to understand the role of motility in the infection of aphids and to determine if swimming or swarming motility are necessary upon infection. Using Gateway cloning I will make motility knockouts and test them for their ability to cause disease in aphids. By comparing the mutant strains to the wild type strains, I hope to understand motility's role in *P. syringae* infection of aphids.

Influence of extra-pair copulations on gut and cloacal microbiome diversity in local Ithaca tree swallows

****Jason Yeung, Conor Taff, & Maren Vitousek**

Department of Ecology and Evolutionary Biology, Cornell University

Extra-pair copulation, mating outside of a monogamous pair, is commonly observed in bird species and tree swallows (*Tachycineta bicolor*) here in Ithaca are no different. EPC between birds obviously carries the risk of pathogen transfer, and that risk only gets higher the more social a bird is. However, extra-pair copulation still occurs frequently in a population. A less studied but significant aspect of these copulations is their effect on gut and cloacal microbiome diversity. Just like pathogens, other microbes found in tree swallows can also be transmitted during the mating process. High gut microbe diversity has been attributed to better health and fitness in many animals, so if tree swallows that engage in EPC do demonstrate increased levels of microbial diversity, it is possible that extra-pair copulations actually have a positive impact on their health and social activity.

Data collection for the project is still ongoing; DNA is being extracted from nestling bird blood collected during the field season, which will then be sequenced for cases of extra-pair paternity in relation to blood samples collected from adult male and female birds. Microbiome samples were obtained by performing cloacal swabs on adult birds in the field, which DNA was then extracted from in the lab. That DNA is now undergoing PCR to amplify the 16s ribosomal RNA gene and will be then sent for sequencing to differentiate and identify groups of microorganisms. While the data is still in its early stages, there is some data on paternity and social interactions from previous years that are relevant to this project. For example, in a previous study that looked at the effect of corticosteroids and predator exposure on tree swallows, there was a positive trend between number of social visits between bird boxes and microbiome diversity (via the Faith's D index) for the control group.

If we find support for the hypothesis that tree swallows do gain the benefit of microbial diversity through extra-pair copulation, this study could have applications to other animal species where EPC has been observed and provide another lens through which to study their health and fitness.

A highly prevalent densovirus discovered among sea stars from the East Coast of the United States

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Viral metagenomic analysis of tissues from *Asterias forbesii* led to the discovery of a novel densovirus (AfDV). The genome organization of AfDV and phylogenetic analysis place this virus among the *Ambidensovirus* genus in the subfamily *Densoviridae*, family *Parvoviridae*.

A qPCR Taq-man assay was designed to assess tissue tropism, host specificity, and prevalence among wild populations of sea stars along the East Coast of the United States. AfDV could be detected among wild populations of sea stars along the New England region and was highly prevalent, 80-100%, among populations. AfDV could be detected in the body wall, gonads, and digestive gland exceeding 15,000 viral copies/mg tissue but was not detected in the coelomic fluid of the animals. RT-PCR was performed on virus qPCR positive tissue to measure active replication but no amplification was detected in tissues. To establish if the virus is persisting in a purely genomic state or as a virus particle, the putative capsid ORF was cloned into the pFastBac Dual vector to create a recombinant baculovirus (AcMNPV-AfDV VP1) for subsequent protein expression, purification, and antibody production. Future work entails a western blot analysis with sea star tissue homogenate to corroborate molecular approaches. Taken together, AfDV is a highly persistent densovirus that is commonly found among healthy populations of sea stars along the East Coast of the United States.

Associations between Afrotropical bats, parasites, and microbial symbionts

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Bats are among the most diverse mammals on the planet and harbor numerous bacterial, viral, and eukaryotic symbionts. The interplay between bacterial community composition and parasitism in bats is not well understood and may have important implications for studies of similar systems. Here we present a comprehensive survey of 495 Afrotropical bats (representing eight families and nineteen genera) and their symbionts, including dipteran and malarial parasites, as well as gut, oral, and skin microbiota. We identify significant correlations between bacterial community composition of the skin and dipteran ectoparasite prevalence across four major bat lineages, as well as links between the oral microbiome and malarial parasitism, suggesting a potential mechanism for host selection and vector-borne disease transmission in bats. Mirroring recent studies of host-microbiome co-speciation in mammals, we find a weak correlation between chiropteran phylogenetic distances and bacterial community dissimilarity across the three anatomical sites, suggesting that host environment is more important than shared ancestry in shaping the composition of associated bacterial communities. This study provides a framework for future approaches to systems biology of host-symbiont interactions across broad taxonomic scales, which may have important implications for wildlife health.

***Vibrio cholerae* encodes a cell wall hydrolase adapted for low zinc conditions**

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The bacterial cell wall is a rigid, but flexible, meshwork of peptidoglycan (PG) that gives the cell structural integrity. The cell wall undergoes continuous remodeling to maintain cell shape during growth: a process that requires a fine balance of both PG synthesis and degradation. An improved understanding of how these cell wall mechanics respond to stress, especially in non-traditional model organisms like *Vibrio cholerae*, can help to identify exploitable drug targets in other gram-negative pathogens. One class of autolysins responsible for PG breakdown are the endopeptidases (EPs), which cleave the peptide cross-links that hold together adjacent PG strands. *V. cholerae* possesses a group of three nearly identical EP's that are implicated in cell wall expansion. Two of these EP's (*shyA* and *shyC*) are housekeeping genes and form synthetic lethal pair, while the third (*shyB*) is not expressed under standard laboratory conditions. To explore the potential role of *shyB*, we conducted a transposon screen to identify mutations that activate *shyB* transcription. We found that *shyB* is upregulated during zinc-starvation and is repressed by Zur in zinc-rich conditions, a mode of regulation not previously reported among autolysins. *shyAB* were shown to form a synthetic lethal pair under In zinc-chelated conditions and, since ShyB and ShyC show similar localization patterns, this suggests that ShyB may substitute for ShyC in low zinc-conditions, a stress that may be encountered within environmental reservoirs or a human host. Furthermore, bioinformatics indicate that Zur-controlled *shyB* homologs are widespread in the *Vibrio* genus, likely due to *shyB*'s position on a mobilizable genomic island.

Trait-based modeling of multi-host transmission: New insights from plant-pollinator networks

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Community ecologists are making great strides predicting multi-species interactions using a trait-based rather than taxonomic approach, identifying key functional attributes of organisms that are important to understanding the system. At the same time, disease ecologists often use network modeling to understand pathogen transmission in complex communities. Yet the merging of a trait-based approach with network modeling is in its infancy. Our main objective is to merge a trait-based approach with network modeling to understand disease spread in diverse communities of bees that transmit parasites at flowers. We are testing whether bee-flower visitation patterns predict parasite prevalence in communities of bee hosts and flower transmission venues, and whether grouping species according to traits can improve parasite prevalence predictions and potentially simplify disease spread models.

First demonstration of equid gammaherpesviruses within the gastric mucosal epithelium of horses

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Horses commonly develop gastric mucosal ulcers, similar to humans, a condition known as equine gastric ulcer syndrome (EGUS) that can lead to poor performance and lost training time and care expenses. Unlike humans, however, an infectious bacterial cause of ulcers has not been conclusively identified. Herpesviruses, while well-established causative agents of diseases such as cold sores, genital lesions, and certain types of cancer, have also been implicated in the development of a subset of gastric ulcers in humans. The presence of equid herpesviruses in the gastrointestinal tract and their potential contribution to EGUS has not been evaluated. Here, we provide the first evidence of equid gammaherpesviruses 2 and 5 (EHV-2 and -5) within the epithelium of the gastric mucosa of horses. These viruses were initially detected by a nested PCR screen of gastric tissue samples obtained from client- and university-owned horses with and without ulcers; however, no association with EGUS was found in this limited sample set. We then validated a highly sensitive *in situ* hybridization (ISH) assay and used this assay to characterize the distribution of these viruses in necropsy gastric tissue samples from five racehorses. Analyses revealed frequent EHV-2 and EHV-5 co-infections within the gastric mucosal epithelium, regardless of the ulcer status. These results are the first to demonstrate the presence of equid gammaherpesviruses in the gastric mucosa of horses and warrants further investigation to determine the contribution of these viruses to the development of EGUS and/or other gastrointestinal diseases.

Investigating the impacts of immune priming by a virulence attenuated *Listeria monocytogenes* strain on the incidence of listeriosis through use of a compartmental mathematical model

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Listeria monocytogenes is a gram positive, food-borne pathogen, and the causative agent of listeriosis. In susceptible population, listeriosis can cause encephalitis, septicemia, and meningitis and death. Additionally, pregnant women may experience late-term abortions or stillbirths. While stringent food safety regulations exist to limit exposures of *L. monocytogenes*, there has been less than expected decline in the incidence of listeriosis in the United States. The likelihood that this pattern is due to improved diagnostics, however, is only minimal, giving the serious medical conditions that occur due to listeriosis. Among contaminated food samples, two distinct strains of *L. monocytogenes* exist, a virulence attenuated (VA) strain and a fully virulent strain. Up to 45% of samples are virulence attenuated, however, the VA strain accounts for less than 5% of clinical cases. Additionally, exposure to virulence attenuated strains appears to have a protective immune effect in mice that are later exposed to the fully virulent strain. Thus, exposures to VA may result in minimal disease and be protective against more serious listeriosis. The objective of this ongoing study is to develop a compartmental mathematical model to evaluate the public health effects, positive and negative, of increased exposure to VA *L. monocytogenes* strains in a population, either through a natural, foodborne exposure or a hypothetical vaccine.

Under the compartmental model, the general population may be exposed to either the VA or fully virulent strains, upon which they have a chance of developing clinical illness or simply being colonized with the bacteria without clinical disease. Both the individuals who become colonized or ill mount an immune response that is protective for a length of period. While protected, individuals can again be exposed to *L. monocytogenes*, but at this point not become ill due to previous immunity. This essentially is an immunity booster. Results of this modeling experiment may indicate the need to rethink current policies regarding *L. monocytogenes* contaminations, or prompt further research into the development of a vaccine using the virulence attenuated strain.

Environment, dosage and pathogen isolate moderate virulence in eelgrass wasting disease

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Eelgrass wasting disease, caused by the marine pathogen *Labyrinthula zosterae*, has the potential to devastate important eelgrass habitats worldwide. Despite this, little is known about the host-pathogen interaction or how the disease will be impacted by projected changes in the environment. In this study, we investigate the effects of variation in distinct *L. zosterae* isolates, pathogen dosage, temperature, and light on virulence of infections. Severity of lesions on eelgrass varied among the three different isolates inoculated in laboratory trials. Our methods to control dosage of inoculum showed that disease severity increased with pathogen dosage from 10^4 to 10^6 cells ml⁻¹. In a dosage-controlled, light and temperature two-way factorial experiment consisting of two light regimes (diel light cycle and complete darkness) and two temperatures (11°C and 18°C), *L. zosterae* cell growth rate *in vitro* was higher at the warmer temperature. In a companion experiment that tested the effects of light and temperature in *in vivo* inoculations, disease severity was higher in dark treatments and temperature marginally significant. We suggest that the much greater impact of light in the *in vivo* inoculation experiment indicates an important role for plant physiology and the need for photosynthesis in slowing severity of infections. Our work with controlled inoculation of distinct *L. zosterae* isolates shows that pathogen isolate, dosage of inoculum, temperature and light impact disease severity, suggesting *L. zosterae* will cause increased damage to eelgrass beds with shifting environmental conditions.