CORNELL INSTITUTE OF HOST-MICROBE INTERACTIONS AND DISEASE

SUMMER SYMPOSIUM

July 26, 2017

10:00 – 4:30

Baker Institute

Register at cihmid.cornell.edu by July 19

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

Cornell University

Schedule of Events

9:30 – 10:10 Coffee and Welcome Reception

10:10 – 10:30 Brian Lazzaro (CIHMID Director)
Opening Remarks and Updates on CIHMID for 2017-2018

10:30 – 11:00 Luis Schang (Baker Institute)
"In search of commonalities among unrelated viruses"

11:00 – 11:15 Brian Wasik (Parrish Lab, Baker Institute)
"Modified Sialic Acids and Influenza-Host Interactions"

11:15 – 11:30 Jose Vargas (Perry Lab, PPPMB)
"Virus infection effects on gene expression regulatory networks"

11:30 – 11:45 Garrett League (Harrington Lab, Entomology)
"*The metamorphosis of the mosquito circulatory and immune systems: comparative studies in larval and adult* Anopheles gambiae*"*

11:45 – 12:15 Nicolas Buchon (Entomology)
"Bugs in bugs: Host-microbe interactions in Drosophila *and beyond"*

12:15 – 1:45 **Lunch and Poster Viewing**

1:45 – 2:15 Tobias Dörr (Microbiology)
"Cell envelope maintenance pathways in Vibrio cholerae*"*

2:15 – 2:30 André Dhondt (Lab of Ornithology)
"*Evolutionary changes following a successful host jump: the case of* Mycoplasma gallisepticum*"*

2:30 – 2:45 Phoebe Dawkins (Harvell Lab, EEB)
"*Three's a crowd: Strain, dosage, and environment increase virulence of eelgrass wasting disease"*

2:45 – 3:00 Peter Graystock (McArt Lab, Entomology)
"Insights from network inference of wild bee microbiomes during single and mixed infections of Nosema *and* Crithidia*"*

3:00 – 3:30 Teresa Pawlowska (PPPMB)
"Evolutionary stability in fungal-bacterial symbioses"

3:30 – 4:00 Chris Myers (Physics)
"The dynamics of infection: networks and interactions across scales"

4:30 – Closing Reception

SUBMITTED ABSTRACTS

Talks

Modified Sialic Acids and Influenza-Host Interactions

Brian R Wasik, Karen N Barnard, Brynn K Lawrence, Colin R Parrish

Baker Institute for Animal Health, Cornell University, Ithaca, NY, USA

Sialic acids (Sias) are found in many modified forms in nature, but their relative distribution and display across species and tissues is not well understood. Influenza viruses (and many other pathogens) utilize Sias as primary receptors for cell binding and infection. The specific Sia chemistry can influence influenza infection and host tropism, either through effects on Hemagglutinin (HA) binding or the sialidase activity of Neuraminidase (NA). Historically, unique Sia structures have been seen as inhibitors of some human influenza viruses – notable in horse or guinea pig serum. However, the roles (if any) of modified Sias in the host-specific interactions of the viruses are not well understood. We are developing a variety of pathogen-origin sialoglycan-recognizing probes (SGRPs) for assessing the expression of modified Sias on influenza host cells and tissues, including Neu5Ac and Neu5Gc, the 9-O-acetyl, 7,9-O-acetyl and 4-O-acetyl variants, and α2-3- and α2-6-linked Sia. The SGRPs have been used to identify Sia forms in tissues from different influenza host animals to assess the presence and abundance *in situ*. Confirming the presence of modified Sias in host respiratory tissues justifies the need for experimental systems to understand their effects on influenza. Having identified modified Sias in major cell culture lines used for influenza studies, we are currently developing 'glyco-engineered' cell lines for the direct investigation of modified Sias and influenza infection. The impacts of diverse Sia receptor forms may underlie host ranges and emergence, transmission potential, in addition to population effects on HA/NA molecular evolution.

Virus infection effects on gene expression regulatory networks

Jose Asencio Vargas and Keith Perry

Department of Plant Pathology and Plant Microbe Biology, Cornell University, Ithaca, NY, USA

Virus infection in plants leads to an alteration of gene expression patterns. These differences can be attributed to two main effects: a) induction due to detection of the pathogen by cellular surveillance mechanisms, b) pathogen's interference of gene expression regulatory pathways. In plants, RNA silencing operates as a mechanism of defense against viral pathogens by targeting and degrading pathogen nucleic acids. RNA silencing also acts as one of the most important mechanisms regulating host gene expression, including a coordination of the transcriptional response to pathogen infection. Most plant viruses have evolved proteins that are able to interfere with host RNA silencing, hindering the plant's ability to counter the infection. In this work, we make use of a newly identified, experimentally validated plant gene expression regulatory network that encompasses the regulatory activities of RNA silencing and transcription factors. This networks provides a framework to evaluate observed changes in host gene expression upon virus infection. Structural analysis is used to evaluate virus-induced changes in the expression of regulatory molecules from a network level.

The metamorphosis of the mosquito circulatory and immune systems: comparative studies in larval and adult *Anopheles gambiae*

Garrett P. League[1,2] and Julián F. Hillyer[2]

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[2]Department of Biological Sciences, Vanderbilt University

As holometabolous insects, mosquitoes differ both morphologically and physiologically between the larval and adult life stages. Furthermore, both stages occupy distinct ecological niches, as mosquito larvae live in aquatic habitats while adults live in terrestrial and aerial habitats. Because of these differences, we conducted a series of comparative analyses on the circulatory and immune systems of *Anopheles gambiae* larvae and adults to see how these closely intertwined systems change across metamorphosis. Using intravital imaging in live mosquitoes, we found that the larval and adult heart differs with respect to contraction directionality and acceptance of hemolymph (insect blood) into its ostia (valves), leading to distinct patterns of hemolymph flow into the heart in each stage. By infecting larvae and adults with fluorescently-labeled bacteria, we also showed that these distinct flow patterns result in differing patterns of hemocyte-mediated pathogen sequestration in the areas of highest hemolymph flow in each stage. Lastly, we showed that these changes correlate with differences in the strength and composition of various cellular and humoral immune responses between larvae and adults. Together, these findings highlight important differences in the functional coordination of the larval and adult circulatory and immune systems and suggest that adaptive decoupling, or the independent evolution of larval and adult traits made possible by metamorphosis, has occurred in the mosquito lineage.

Evolutionary changes following a successful host jump: the case of *Mycoplasma gallisepticum*

André Dhondt[1,2], Wesley Hochachka[1], Keila Dhondt[3], Andrew Dobson[4], Steven Geary[5], Edan Tulman[5], Dana Hawley[6] and David Ley[7]

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[2]Department of Ecology and Evolutionary Biology, Cornell University,
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[4]Princeton University, [5]University of Connecticut, [6]University of Vermont, [7]North Carolina State University

In the early nineteen-nineties the poultry bacterium *Mycoplasma gallisepticum* successfully jumped to free-living house finches and started an epidemic that is still ongoing. The epidemic rapidly spread across eastern North America, and after a 5-year pause also made it across to the West.
We have been able to study this system for the last twenty years and collected bacterial samples from infected finches throughout this epidemic. We found (1) a gradual increase in virulence once the pathogen had become established in an area; (2) a change in initial pathogen survival in that eastern isolates barely survive in the host after inoculation during the first few days after inoculation (after which they do grow), while the western isolates survive quite well following inoculation; and (3) a change in severity of disease caused by a given bacterial load between eastern and western strains, in that the house finches are more tolerant to western than to eastern strains. We will discuss possible mechanisms driving these evolutionary changes.

Three's a crowd: Strain, dosage, and environment increase virulence of eelgrass wasting disease

Phoebe Dawkins, Drew Harvell

Department of Ecology and Evolutionary Biology, Cornell University

Eelgrass wasting disease, caused by the opportunistic marine pathogen *Labyrinthula zosterae*, has the potential to devastate important eelgrass habitats worldwide, yet little is known about the host-pathogen interaction and how the disease will be impacted by changes in the environment. In this study we develop methods for dose-controlled inoculation experiments of *L. zosterae* in pure culture and in the intact pathosystem, to investigate the effect of *L. zosterae* strain, pathogen dosage, temperature and light on pathogen virulence. Disease severity increased with pathogen dosage and temperature. Under controlled dosage and temperature *in vivo* conditions, disease severity is affected by the strain of pathogen inoculated. In *in vivo* controlled temperature and strain experiments, disease severity was increased in the dark treatments. The rapid increase in severity of infection in the dark may be a sign that under normal light conditions, plant defenses mediate damage levels. Pathogen cell growth rate was higher at 18C than 12C temperatures in our *in vitro* trials. Disease severity is highly influenced by strain of *L. zosterae* and varies with dosage, temperature and light. *L. zosterae* is unusually sensitive to changes in the environment and is expected to cause increased damage to eelgrass beds in a changing ocean.

Insights from network inference of wild bee microbiomes during single and mixed infections of *Nosema* and *Crithidia*.

Peter Graystock[1,‡], Quinn McFrederick, Claire Carvell[2], Andrew Bourke[3], Matt Heard[2], Mark Beaumont[1], Seirian Sumner[1]

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Within the digestive tract of animals, rich and diverse microbial communities persist. Often referred to as microbiomes, these gut microbial communities are typically composed of a multitude of interacting bacterial species, in addition to viruses, archea and protists. Within these diverse communities, possible interactions range from parasitism and commensalism to predation and mutualism. In such complex, interconnected communities, changes in the presence or abundance of an individual taxa may have widespread, cascading consequences across the entire community. Here, I utilise network inference tools on the bacterial microbiome of wild bumblebees to infer the relationships within core microbes. In addition, by incorporating the data on the presence and spore loadings of 2 eukaryotic parasites into the microbial network, I infer the relationships between and within the microbiome-parasite dynamic during single *Crithidia* infection and mixed *Crithidia*/*Nosema* infections.

POSTERS

Interactions of influenza A viruses with O-acetylated sialic acids

Karen N. Barnard[1], Brian R. Wasik[1], Brynn K. Lawrence[1], Ajit Varki[2], Colin R. Parrish[1]

[1]Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University
[2]Department of Cellular and Molecular Medicine, University of California-San Diego

Influenza A viruses (IAV) use sialic acids (Sias) as primary receptors for cell entry during infection via the hemagglutinin (HA) and neuraminidase (NA) glycoproteins. Sias are found in large amounts in mucus that protects respiratory tract cells, as well as on cell membrane glycoproteins and glycolipids. Sias may be modified by addition of numerous chemical groups (9-O-, 7,9-O-, 4-O-acetyl, 5-N-glycolyl among others), and are attached to underlying glycans through different linkages, which vary between different hosts and tissues. While the roles of Sias linkages have been explored in IAV tropism and evolution, the roles of chemical modifications to Sias are not well understood. While some O-acetyl modified Sias have been identified as infection inhibitors (horse or guinea pig serum, Neu4,5Ac) or as inhibitors of NA efficiency (Neu5,9Ac), details of these interactions and their roles in tropism are not well understood. We have previously shown that 9-O-acetyl Sias are expressed at high levels in the respiratory tissue of humans and other species, on the membranes of embryonated chicken eggs, as well as on MDCK, HEK293, and A549 cells. To determine how acetyl modifications influence virus-Sias interactions, soluble forms of IAV HA and NA were produced and evaluated along with whole virus. HA proteins from human subtype H1 (three strains) and subtype H3 (four strains) were synthesized and expressed as fusions with the Fc domains of human IgG1 (HA-Fc). Binding of HA-Fcs to glyco-engineered cell variants of HEK293, A549, MDCK that lack or overexpress 7,9-*O*- and 9-*O*-Ac was evaluated via flow cytometry. Additionally, we are examining the effects of the modified Sias on NA activity, using both viruses and expressed NA molecules. These molecular tests and tools provide a solid platform for understanding the effects of Sias modifications in influenza infection and host tropisms.

Dynamic interplay between bacterial growth and the host immune response generates a stochastic outcome of infection

David Duneau[1,2], Jean-Baptiste Ferdy[2], Jonathan Revah[1], Hannah Kondolf[1], Gerardo Ortiz[1], Brian P. Lazzaro[1], Nicolas Buchon[1]

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A central problem in infection biology is understanding why two individuals exposed to apparently identical infections may have dramatically different clinical outcomes. One individual may recover fairly easily while the other suffers devastating illness or even death. We have developed an experimental model where genetically identical, co-housed fruit flies given identical systemic infections experience dramatically different outcomes, with some individuals succumbing to lethal acute infection while others control the pathogen as a fairly asymptomatic persistent infection. We found that flies die at a fixed bacterial burden regardless of the time post-infection at which death occurs, and that the lethal burden varies across distinct bacteria. Using multiple experimental and genetic manipulations, we identify 3 key factors that predict whether the infection proceeds to this lethal burden. The first is the effective rate of bacterial proliferation, which is the difference between the rate of cell division and the rate of clearance by the immune system. The second is the establishment of effective immunological control. In particular, we have found that the activation of the Imd pathway can vary dramatically between individuals, suggesting that the Imd pathway is intrinsically noisy and that inter-individual stochasticity early in infection might lead to differences in the probability of ultimate survival. Third, we infer a threshold pathogen density that must be reached to enable the switch to a lethally acute infection instead of a chronically persistent one. Inter-host variation in survival must therefore originate in the ability of the pathogen to reach that threshold before effective immune control is established. Altogether, our results illustrate the mechanisms underlying the variable nature of infection outcome and provide a framework for studying the individual host-pathogen parameters that govern the progression of infection and lead ultimately to life or death.

Genes involved in internalizing pathogens in *Drosophila* are shaped by recent and recurrent positive selection

Joo Hyun Im, Brian Lazzaro

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Autophagy and phagocytosis are cellular mechanisms that internalize and eliminate intracellular and extracellular pathogens. The dynamic conflict between the host and pathogens that evolve to escape, resist or compromise host immunity can result in co-evolution, leading to a recurrent positive directional selection on host genes. We hypothesized that host phagocytosis and autophagy genes may experience such co-evolutionary pressure and therefore may show molecular evidence of adaptation. We performed population genetic analyses on phagocytosis and autophagy genes, as well as matching control genes, using previously published *Drosophila melanogaster* and *D. simulans* genome sequences. First, we detected a strong, recent selection on the genes involved in expansion of autophagosome and recognition and internalization of extracellular pathogens. For instance, we see a strong signature of selection at the *Atg8a* gene. *Atg8a* encodes a protein whose human homolog is known to be a target of an inhibitory effector in *Legionella pneumophila*. Nevertheless, we see distinct gene sets showing evidence of recent adaptation in *D. melanogaster* versus *D. simulans*, indicating that these two species may have faced unique challenges. Next, we observed several cases of an adaptive evolution in phagocytosis genes involved in particle recognition and degradation in both species. Although we see evidence of recent adaptation in individual genes, we do not find evidence that recent or recurrent positive selection is pervasive throughout entire functional classes of genes, which the exception of genes involved in degradation of phagocytized pathogens.

Community metabolism of Drosophila resident microbiota

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Microbial fermentation products have been demonstrated to have profound impacts on animal physiology, development, and behavior. Much emphasis has been put on how a suite of metabolites from an individual microbe can influence animal health; while few studies have elucidated the impact of a suite of metabolites on an animal host that are produced by co-occurring microbes in a community. To better understand the impact of microbial communities on animal health, we compared the metabolite production of Drosophila resident gut microbiota when they are grown in isolation or in a community. This system provides a superb model to address this question because we can readily culture many of the gut microbiota and assess the dynamics of metabolite production. We hypothesized that increased complexity of the Drosophila resident microbiota will result in emergent metabolites, which individual members of this community would not be capable of producing when in isolation. Preliminary results from our study indicate that we obtain distinct profiles between our treatments. One particular compound identified is acetic acid, which acetic acid bacteria produce this metabolite when they are in the presence of yeast-derived ethanol. Our data indicates that acetic acid impacts both Drosophila physiology (e.g. stored lipid availability, delayed development) and behavior (e.g. increased oviposition). These results provide insight into how community metabolites from a microbial community can influence animal health and development, which would be lost if we merely investigated individual microbes and their metabolites.

Compartmentalization of the translational machinery within mammalian orthoreovirus replication sites is mediated by two viral nonstructural proteins

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Mammalian orthoreoviruses (REOV) replicate and assemble in the cytoplasm of infected cells. The sites of viral replication and assembly are well-defined, virally-derived, cytoplasmic structures called viral factories (VFs). Active translation occurs within viral factories and translation factors and ribosomal components are present within viral factories (Desmet et al. mBio, 2014, 5, e01463-14). To determine the mechanism underlying the recruitment of the translational machinery to VFs, we examined the capacity of viral proteins to recruit and interact with translational factors and ribosomes. We found that all the translation factors recruited to VFs in infected cells are recruited to VF-like structures formed when μNS and σNS are co-expressed. Most translation factors were recruited to VF-like structures only when σNS was expressed with μNS. However, expression of μNS alone led to recruitment of translational factors eIF2 and eIF4E to VF-like structures. These findings indicate that reovirus nonstructural proteins μNS and σNS modify the location and function of the cellular translational machinery, presumably to optimize translation of viral mRNAs. When expressed alone, σNS localized to ER membranes and co-localized with polyadenylated RNA, areas of active translation and proteins synthesis, and ribosomal proteins. Polysome analysis of infected cells revealed that viral proteins μNS and σNS co-fractionate with small and large ribosomal subunits and with 80S monosomes and polysomes. Ectopically-expressed σNS also co-fractionated with 40 and 60S ribosomal subunits and with monosomes and polysomes. Ongoing studies are focused on the role of the σNS protein in viral translation and the specific interactions that drive recruitment of the translational machinery to reovirus replication sites.

Physiological and Molecular Triggers for SARS-Coronavirus Membrane Fusion and Entry into Host Cells

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Coronaviruses are a major infectious disease threat, and include the pathogenic human pathogens of zoonotic origin: SARS-CoV and MERS-CoV. Entry of coronaviruses into host cells is mediated by the viral spike (S) protein, which is structurally categorized as a class I viral fusion protein, within the same group as influenza virus HA and HIV Env. However, S proteins have distinct features compared to HA or Env, including the presence of two proteolytic cleavage sites—at the interface of the receptor binding and fusion domains (S1/S2) and within the fusion domain (S2'). Unusually, these sites can be cleaved by a wide range of cellular proteases. The exact location of the coronavirus fusion peptide (FP) has been disputed. However, most evidence suggests that the domain immediately downstream of the S2' cleavage site is the bone fide fusion peptide (amino acids 798-818 SFIEDLLFNKVTLADAGFMKQY for SARS-CoV, FP1). Here we characterize the SARS-CoV fusion peptide. Our results indicate that FP1 and also a second region immediately downstream (amino acids 816-835 KQYGECLGDINARDLICAQKF, FP2) induce significant membrane ordering by electron spin resonance (ESR) spectroscopy, dictated by conserved hydrophobic residues. Furthermore, the effects are calcium-dependent, which is consistent with the presence of conserved acidic residues and a predicted calcium binding site within the fusion peptide. Isothermal titration calorimetry (ITC) showed a direct interaction between calcium cations and both FP1 and FP2, indicating that the coronavirus fusion peptide exhibits a mechanistically novel behavior. SARS-CoV entry was found to be sensitive to calcium chelators and ionophores, confirming a role for calcium in vivo. We hypothesize that the requirement for calcium ions explains the known behavior of coronaviruses, which can be "early-" or "late-" entering viruses.