A community ecology approach to characterizing how pathogen-pathogen interactions affect honeybee mortality

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**Intellectual Merit:** There has been a recent call for experts in infectious disease to apply a community ecology approach to studying host-parasite and parasite-parasite interactions (6). The study of infectious disease is primarily concerned with the transmission, dissemination and clinical signs of a pathogen, while disease ecology focuses on organismal interactions. My work aims to bridge these two areas of study by asking how multiple pathogens interact with each other, and how those interactions affect host health. The importance of considering coinfection is of vital importance as multiple infection is common and the repercussions are poorly understood (5). By bringing fundamental principles of competition theory into the infectious disease arena and testing the classic Lotka-Voltera competition models in the context of coinfection (competing pathogens), my work will further an understudied area of disease ecology (9).

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It is estimated that around one third of the world’s food is dependent upon animal-mediated pollination, the majority of which is provided by bees (1). Given their agricultural and ecological importance, documented declines of bee species across the globe has garnered much attention (1). Among the top threats to these important pollinators (3) are pests and pathogens including *Nosema spp*., *Varroa* mites, and numerous RNA viruses. Colonies of bees and even individuals are likely to host multiple pathogens at one time and this coinfection is linked to colony collapse disorder (4). However, our understanding of coinfection in general, specifically, how pathogens interact with each other within a host is severely lacking (9). **My goal is to advance our understanding of the mechanisms and outcomes related to coinfection by applying fundamental concepts in community ecology (i.e. competition theory) to the honey bee disease system.** I will focus on four honeybee pathogens known to adversely affect bee health: *Nosema ceranae* (a microsporidian parasite), *Varroa destructor* (an arthropod ectoparasite) and two RNA viruses, deformed wing virus (DWV) and black queen cell virus (BQCV). **Using a combination of field surveys, laboratory experiments, and epidemiological modeling, I will examine: 1)** Temporal variation in disease prevalence and load and resulting patterns of coinfection **2)** How pathogens interact with each other once coinfection has occurred and **3)** synergistic effects due to pathogen-pathogen interactions that may influence host mortality at both the individual and colony levels. In addition to furthering study of disease ecology, my proposed research will improve native and managed pollinator conservation by improving our understanding of high-risk pathogen combinations that increase bee mortality.

**Figure 1:** *(above)*Prevalence data for 4 common honeybee pathogens sampled from 32 colonies at 3 time points every 4 weeks. *(below)* Scaled and normalized (Relative Intensity) proxies for colony population size (frames of bees) and queen quality (brood pattern).

**1) Temporal Variation and Patterns of Coinfection:** I will determine how four common honeybee pathogens fluctuate in load and prevalence through time, and how these fluctuations influence patterns of coinfection. ***Approach:*** In 2017, 32 induvial colonies spread across 3 comparable field sites were sampled for the 4 previously mention pathogens of interest. Samples were conducted at 3 time points every 4 weeks (**Fig. 1**). RNA viruses were quantified using qPCR, *Nosema spp.* was counted using a hemocytometer, and *Varroa* mite loads determined using standardized methods from the honeybee research guide (7). In addition, standardized colony population and quality measurements were taken at each point. ***Preliminary findings*** indicate that during times of increased pathogen prevalence, the probability of certain coinfections occurring increase significantly. Most notably, when *V. destructor* is in high prevalence, colonies are more likely to have significantly higher BQCV and DWV loads.

**2) Pathogen Interactions:** I will determine how pathogens interact with each other in a coinfected host. ***Approach:*** Three of the four pathogens (*N. ceranae, V. destructor* and DWV) have already been isolated. I will work on perfecting inoculation techniques and isolating BQCV in 2018.Inoculation studies will be conducted in a lab setting using field-realistic pathogen combinations identified from the field study in objective 1. By measuring disease loads after coinfection, and comparing to known competition models, I will characterize how the primary infection reacts to secondary infection.

**3) Synergistic Effects and Host Mortality:** I will determine how pathogen-pathogen interactions affect host health and mortality at both an individual and colony level. ***Approach:*** I will select a subset of pathogen combinations from research objective 2 and conduct inoculation experiments using small experimental colonies (micro-colonies). Individual mortality and colony loss will be recorded. Bees will be assayed for sub-lethal health effects and final pathogen loads.

**Broader Impacts:** In discovering how multiple pathogens interact to affect bee health, my work will advance efforts to understand the threats to bees and could lead to treatment recommendations to improve honey bee health. This will directly benefit beekeepers and growers reliant on the pollination services of bees.Coinfection also poses a risk to the native bee community as well as managed honeybees. Many pathogens, especially *Nosema. Spp.* and several RNA viruses (including DWV) have been shown to be spilling over into wild bee communities (8). Understanding how pathogens interact with each other and their host in managed honeybees will allow us to make better recommendations for treatment options, potentially reducing the risk of spillover to wild bees. To reach beekeepers and growers, I will continue to hold workshops and lectures to spread awareness of bee disease and management options to reduce coinfection and the risk of spillover. I will publish my results in academic journals and mentor 1-3 undergraduate students each semester.

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