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

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**Intellectual Merit:** The documented decline of important pollinators has garnered much attention and concern in recent years. 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). Honeybees alone account for an estimated 14 billion USD a year in national revenue (2). Honeybees’ importance to agriculture, as well as their roles in ecological communities, makes their increasing losses of grave concern. Colony losses have been approximately 40% a year since 2012, up from 10% twenty years ago (3). Among the many threats facing bees are a series of pathogens including microsporidians, ectoparasites, RNA viruses and various bacteria. Coinfections might play an important role in colony collapse disorder (CCD) and are understudied(4).

Rplot01.pdfThe importance of understanding the outcome of coinfection is of vital importance as multiple infections are common and their repercussions poorly understood (5). There has been a recent call for experts in infectious disease to take a community ecology approach when thinking about host-parasite and parasite-parasite interactions (6). If one thinks of the colony or even the individual bee as an environment inhabited by a community of pathogens, the advantages of this way of thinking become apparent. The vetted models and methods of the field of community ecology can be readily applied to the relatively understudied field of coinfection.

**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).

**My goals are to:** **A)** advance our understanding of the mechanisms and outcomes related to coinfection using the tools of epidemiology and community ecology **B)** aid in native and managed pollinator conservation by using the results of this study to inform the beekeeping community of the importance of treating for high-risk pathogen combinations that increase bee mortality. To accomplish these goals, I will use four notable honeybee pathogens: *Nosema ceranae* (a microsporidian parasite), *Varroa destructor* (an arthropod ectoparasite) and two RNA viruses, Deformed Wing Virus (DWV) and Black Queen Cell Virus (BQCV) to address **3 main research objectives**: **1)** How temporal variation in disease prevalence and load affects patterns of coinfection. **2)** How pathogens interact with each other once coinfection has occurred. **3)** How synergistic effects, attributable to pathogen-pathogen interactions influence host mortality at the individual and colony levels.

**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 North Carolina in the winter of 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, *N. ceranae* was counted using a hemocytometer, and *V. destructor* loads determined using standardized methods (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 high BQCV and DWV loads. further

**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 be working on isolating BQCV and perfecting inoculation techniques in 2018. . I will conduct inoculation studies in the lab using field-realistic pathogen combinations identified from the field study in objective 1. By measuring disease loads after coinfection, I will characterize how the Primary infection reacts to secondary infection.

**3) Synergistic Effects and Host Mortality:** I will determine how these pathogen-pathogen interactions affect host mortality at both an individual and colony setting. ***Approach:*** I will select a subset of pathogen combinations from research objective 2 and conduct inoculation experiments using small experimental colonies (micro-colonies). Mortality of individuals as well as colony loss will be recorded. Surviving bees will be assayed for pathogen loads.

**Broader Impacts: A) *Community Ecology Approach to Infectious Disease*.** Coinfection describes pathogen-pathogen and pathogen-host interactions. Infectious disease researchers need look no farther than the well-vetted field of community ecology for both methodology and expertise. By imagining a community of parasites populating a host environment, the ideas of competition, mutualism and resource-depletion come readily to mind. By bringing fundamental principles of community ecology into the infectious disease arena, I hope to begin to think about coinfection from a novel angle, bringing the top minds in each field together. **B) *Pollinator Conservation*.** Increased mortality due to coinfection poses a risk to the native bee community as well as managed honeybees. These pathogens, especially *N. ceranae* and various RNA viruses (including DWV and BQCV) have been shown to be spilling over into native 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 decreasing honeybee mortality as well as native pollinator declines due to spillover benefitting beekeepers, growers, bee communities and consumers alike.

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