A community ecology approach to characterizing how multi-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 multi-pathogen interactions (1). The study of infectious disease is primarily concerned with the transmission, dissemination and clinical signs of a pathogen, while ecologists focus on species interactions. My dissertation work aims to bridge these two areas of study, as a disease ecologist, and ask how multiple pathogens interact, and how those interactions affect host health. Considering coinfection is of vital importance as multiple infection is common and the repercussions are poorly understood (2). 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 (1).

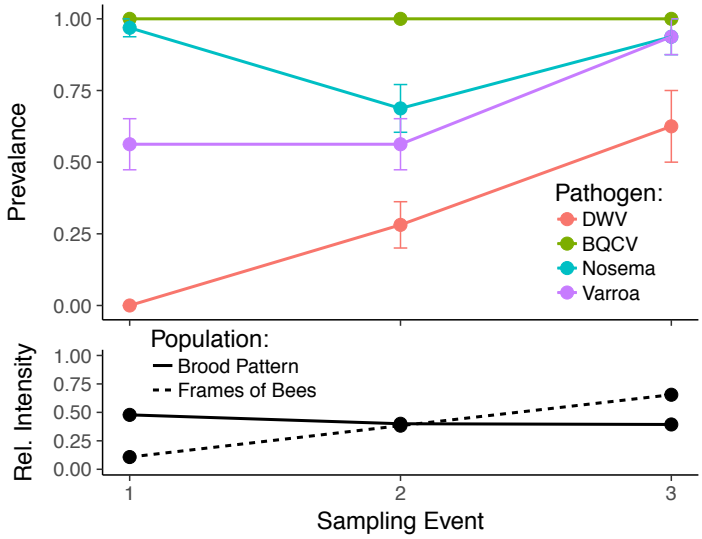

Figure 1: *(above)* Prevalence of four common honeybee pathogens sampled from 32 colonies at three time points every four weeks. *(below)* Scaled (Relative Intensity) proxies for colony population size (frames of bees) and queen health (brood pattern).

Approximately one third of the planet's food is dependent upon animal-mediated pollination, the majority of which is provided by bees (3). Given their agricultural and ecological importance, documented declines of bee species across the globe has garnered much attention (3). Among the top threats to these important pollinators are pests and pathogens including *Nosema spp*., *Varroa* mites, and numerous RNA viruses (4). Colonies of bees and even individuals are likely to host multiple pathogens at one time and this coinfection is linked to colony collapse disorder in honeybees (5). However, our understanding of coinfection, specifically, how pathogens interact with each other within a host, is severely lacking (2). **My goal is to advance our understanding of the mechanisms and outcomes related to coinfection by applying fundamental concepts of community ecology (i.e. competition theory) to the honeybee disease system.** I will focus on four honeybee pathogens known to adversely affect bee health: *Nosema ceranae* (a microsporidian parasite), *Varroa destructor* (a parasite mite) 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)** The importance of temporal variation in disease prevalence, disease load, and patterns of coinfection; **2)** How pathogens interact with each other once coinfection has occurred; **3)** Synergistic effects due to multi-pathogen interactions that may influence host mortality at both the individual and colony levels. In addition to furthering the study of disease ecology, my proposed research will enhance native and managed pollinator

conservation efforts by improving our understanding of high-risk pathogen combinations that increase bee mortality.

1) Temporal Variation and Patterns of Coinfection: I will determine how four common honeybee pathogens fluctuate in prevalence and load through time, and how these fluctuations influence patterns of coinfection. ***Approach:*** In 2017, I sampled 32 individual colonies spread across three comparable field sites. I inspected and sampled each colony three times at four week intervals and analyzed each for the four previously mention pathogens (**Fig. 1**) using microscopy for *Nosema*, RT-PCR for RNA viruses, and determined *Varroa* mite loads using established methods (6). In addition, I measured colony population size and queen health (brood pattern) at each time point. ***Preliminary findings*** indicate that during times of increased pathogen prevalence, the probability of certain coinfections occurring increase significantly. Most notably, when *Varroa* is highly prevalent, colonies are more likely to have high 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, Varroa* and DWV) have already been isolated. With the help of a collaborating virologist, I will isolate BQCV. I will conduct inoculation studies in the lab using field-realistic pathogen combinations identified from the field study in Obj.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 multi-pathogen interactions affect host health and mortality at both an individual and colony level. ***Approach:*** I will select a subset of pathogen combinations from Obj. 2 and conduct inoculation experiments using small experimental colonies. Individual mortality and colony loss will be recorded. Bees will be assayed for final pathogen loads and sub-lethal health effects such as learning and activity.

BROADER IMPACTS: In discovering how multiple pathogens interact, my work will advance our understanding of the threats to bees and inform efforts to improve honey bee health. My work will be of strong interest and benefit to beekeepers and growers reliant on pollination services. 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) also affect wild bee communities and are thought to spill over from honeybees (7). Understanding how pathogens interact in managed honeybees will allow us to make better recommendations for treatment options and reduce 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. I will aid beekeepers who wish to pursue research projects in experimental design, statistical analysis and publication. In addition to continuing my work co-leading the National Honey Bee Survey in Vermont, I have already begun working with the VT Agency of Agriculture's Apiary Inspection Program on a citizen science project to collect longitudinal data on honeybee pathogens in Vermont.

References: 1) Johnson, P. T. J., et al. 2016. Why infectious disease research needs community ecology. Science. *349*(6252): 1-20. **2)** Rigaud, T. et al. 2010. Parasite and host assemblages: embracing the reality will improve our knowledge of parasite transmission and virulence. *Proc Biol Sci*, *277*(1701), 3693–3702. **3)** Aizen, M. a., et al. 2009. How much does agriculture depend on pollinators? Lessons from long-term trends in crop production. Annals of Botany, 103: 1579–1588. **4)** Van Engelsdorp, D., et al. 2008. A survey of honey bee colony losses in the U.S., Fall 2007 to Spring 2008. PLoS ONE, 3(12): 8–13. **5)** Cox-Foster, D. L., et al. 2007. A metagenomic survey of microbes in honey bee colony collapse disorder. Science 318: 283–287. **6)** Dietemann, V., et al. 2013. Standard methods for varroa research, J. Api. Res. *52*(1): 1–54. **7)** Fürst, M et al. 2014. Disease associations between honeybees and bumblebees as a threat to wild pollinators. Nature. 506: 364-373.