Plight of the bumble bee: Patterns of temporal variation and coinfection between *Nosema spp.* and three RNA viruses

**PRIORITY AREA FOCI:**

**1)** Patterns of coinfection between two species of *Nosema* (*N. bombi* and *N. ceranae*)

**2)** Patterns of coinfection between *Nosema spp.* and three RNA viruses

**3)** Examining temporal variation in pathogen load between these 5 infectious agents

**INTRODUCTION:**

The documented decline of important pollinators has garnered much attention and concern in recent years. Bumblebees (*Bombus spp.*) in particular are important native pollinators whose decline has been understudied in light of managed honeybee losses (van Engelsdorp et al., 2008). Certain plants, most notably of the genus *Solanum* (tomatoes, potatoes, eggplant etc.), primarily rely on pollination provided by bumblebees as honeybees are poor pollinators of these plants (Strange, 2015; Thornsbury and Jerardo, 2012). Bumblebee declines in recent years have the potential to drastically disrupt the pollination services they provide and the industries that rely on them. Species such as *B. affinis, B. borealis, B. ashtoni, B. fervidus, B. pensylvanicus, and B. sandersoni* (all species that can be found in Vermont) have decreased in abundance since the 1960s (Colla et al., 2012). In 2015, the state of Vermont listed two species of bumblebees as endangered (*B. affinis and B. ashtoni*) and one as threatened (*B. terricola*) (Vermont Fish and Wildlife Department, 2015). In 2017, *B. affinis* was the first bumble bee to listed as federally endangered.

There are many threats that are thought to be causing bumblebee declines including habitat loss, pesticide use and climate change, bumble bee pathogens and the interactions that occur between them are relatively under studied. Two groups of pathogens that affect bumble bees are the microsporidian parasite *Nosema spp*. as well as a number of RNA viruses. *Nosema* lives in the midgut of its host. It has been shown to cause dysentery and adversely affects forging efficiency (Otterstatter et al., 2005).The two species that affect bumblebees are *N. bombi* (the native species) and *N. ceranae* (an invasive species). *N. ceranae* has become ubiquitous in the European honeybee (*A. mellifera*), and outcompetes *A. mellifera’s* unique species of *Nosema*, *N. apis* (A. Bourgeois et al., 2010; M. Natsopoulou et al., 2014). In addition to *Nosema*, RNA viruses originally discovered in honeybees have been found in bumblebee populations (M.A. Fürst et al., 2014). Viruses like Deformed Wing Virus (DWV), Lake Sinai Virus (LSV) and Black Queen Cell Virus (BQCV) cause behavioral abnormalities, inefficient foraging, wing deformities, abnormal queen cells and death (D. Schroeder and S. Martin, 2012; P. Graystock et al., 2015).

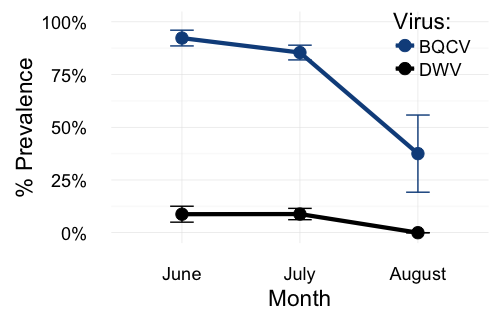
Although *Nosema* and RNA viruses have already been documented in bumblebees, interactions between these pathogens and their host have been understudied and not much is known about temporal variation in their pathogen loads. This is important as differential fluctuations in disease load between pathogens might result in certain pathogens peaking in abundance at the same time. This could increase the probability of coinfection. Coinfections (multiple pathogens in one host) might play an important role in colony collapse disorder (CCD) in honeybees (Cox-Foster et al., 2007). Understanding how these patterns work in native bee populations will allow us to make better recommendations for their conservation.

**OBJECTIVES:**

I propose to conduct assays (viral and fungal) on 440 bumble bees caught at four time points in a Vermont survey I conducted in 2016 to: **(1)** look for patterns of coinfection between *N. ceranae*and *N. bombi*, **(2)** examine if coinfection between *Nosema spp.* and RNA viruses (DWV, BQCV and LSV) is common in bumble bees, **(3)** and to examine and model temporal variation in pathogen load between these five infectious agents.

**METHODS:**

***What has already been done:***

****In 2014 and 2015, I assisted in a survey of RNA viruses in native bumble bees across Vermont. This work was funded by a Centennial Pollinator Fellowship awarded to Samantha Alger. This survey provided the first documentation of deformed wing virus (DWV) and black queen cell virus (BQCV) in Vermont bumble bees. Through this work, we found evidence for disease spillover from managed honey bees into wild bumble bees: bumble bees were more likely to be infected and had higher viral loads when they were caught near a honey bee apiary. We also found differences in viral prevalence between bee species. Most interesting to me, when I reanalyzed these data by grouping sampling events by month, I found seasonal differences prevalence for one virus of interest, Black Queen Cell Virus (*x*32 = 70.05, p < 0.0001). In bumble bees, this virus had a high prevalence in June, but dropped below 50% prevalence in August (**Fig. 1**). To my knowledge, this became the first evidence of seasonal variation in RNA viruses among bumble bees. To fully understand this variation and remove the confounding variable of site variation, a survey designed to repeatedly measure pathogen loads for the same sites at discrete time points needed to be conducted. In the summer of 2016, I revisited 5 of the field sites at 4 different time points throughout the summer. For each time point, I collected over 100 bumble bees of two focal species and three castes, as well as conducted species abundance surveys. These specimens are now safely stored in our -80 freezer awaiting analysis.

**Figure 1** Prevalence for 2 viruses (Black Queen Cell Virus and Deformed Wing Virus) by month. There is evidence for seasonal variation in virus abundance for BQCV (x32 = 70.05, p < 0.0001) but not for Deformed Wing Virus. In sites sampled in June and July, high prevalences were recorded for BQCV but prevalence dipped below 50% in August.

***What will be done during 2017:***

**(1)** In 2017, I will isolate RNA (for RNA viruses) and DNA (for *Nosema*) from the 440 bumble bees caught in the 2016 survey. Using primers I have already designed, I will conduct *Nosema* assays for both species (*N. ceranae* and *N. bombi*) using molecular techniques (RT-qPCR). This will give me *Nosema* loads and prevalence data for both species of the parasite allowing me to determine if there are patterns of coinfection between the two. **(2)** I will assay the same bees for three RNA viruses: Deformed Wing Virus (DWV), Lake Sinai Virus (LSV) and Black Queen Cell Virus (BQCV) again using the same molecular methods. By using statistical analysis (repeated measures ANOVA and generalized linear models) I will be able to look for patterns in viral and parasite load between these five pathogens. **(3)** Using these data as well as data from 2015, I will be able to look at how the prevalence and pathogen load fluctuates throughout the course of the growing season. This information will allow me to construct a deterministic model that predicts pathogen load and coinfection probability through time.

**IMPLICATIONS:**

In general, native bee decline is difficult to document due to the lack of historic records. However, Vermont is unique in that it has one of the best historic collections of bumble bee species dating back to 1915 (Vermont Center for Ecostudies unpub. data). Surveys conducted 2012-2014 by the Vermont Center of Ecostudies confirmed that over half of our state's native bumble bee species are in decline. Among the many threats to our native bees, pathogens are a serious concern. Many have recognized the gap in our knowledge of the interactions of multiple pathogens (CITE). The study I propose will examine this understudied area and contribute to our knowledge on the multiple threats affecting our native pollinators.

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