Plight of the Bumblebee: A look at interspecific competition between two species of *Nosema* and their interactions with RNA viruses

**INTRODUCTION:**

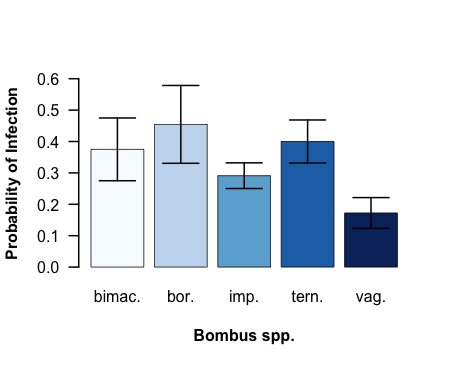
The documented decline of important pollinators has garnered much attention and concern in recent years. Bumblebees (*Bombus spp.*) in particular are important native pollinators whose decline has been understudied in light of managed honeybee losses (van Engelsdorp et al., 2008). Certain plants, most notably of the genus *Solanum* (tomatoes, potatoes and eggplant), 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).

There are many pathogens that are thought to be causing bumblebee declines including the microsporidian parasite *Nosema spp*. as well as a number of RNA viruses. *Nosema* lives in the ventriculus 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) and Black Queen Cell Virus (BQCV) cause behavior 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 been documented in bumblebees, interactions between these pathogens and their host have been understudied. I will examine if the introduction of the invasive parasite *N. ceranae*has led to interspecific competition with *N. bombi*. In addition, I will examine if coinfection between *Nosema spp.* and RNA viruses (DWV and BQCV) leads to synergistic effects that further compromise bee health. **(I)** I predict that *N. ceranae* will outcompete *N. bombi* when both parasites coinfect the host. **(II)** I also predict that the presence of *Nosema spp.* will depress the bees’ immune system, resulting in higher viral loads than when *Nosema* is absent.

**METHODS:**

***What has been done:*** In 2014, 357 *Bombus* specimens were collected haphazardly from 13 different sites in Northern Vermont. The bees were netted while foraging on flowers without regard to species. The bees were put on dry ice in the field and transferred to a -80oC freezer within 12 hours of being captured. In 2015-2016, specimens were assayed for *Nosema spp.* (*both N. ceranae and N. bombi*) by dissection. *Nosema* counts were made for each bee using a hemocytometer. Methods for *Nosema* and viral assays using standard molecular methods (RT-qPCR) were perfected for these experiments.

***What will be done during summer 2016:*  (I)** To look at interspecific competition between *N. ceranae* and *N. bombi*, a controlled lab experiment (using commercial Koppert® bumblebees) will be conducted. *Nosema spp.* will be isolated and cultured using standard methods (I. Fries et al., 2013). The Koppert® bees will be subsampled and assayed for *Nosema* prior to the experiment to ensure no initial infection. There will be four treatments of 30 bees each. They will consist of a control (no *Nosema*), an *N. ceranae* only treatment, an *N. bombi* only treatment, and a treatment with both species in equal parts. Bees will be inoculated orally and kept in a growth chamber.After two weeks, *Nosema* loads will be determined using RT-qPCR. **(II)** To look at interactions between *Nosema* and RNA viruses, a sub sample (n=40) of the 2014 survey bees from both the *Nosema* positive and *Nosema* negative specimens will be assayed for two RNA viruses (DWV and BQCV) and a housekeeping gene (ACTIN) using RT-qPCR. Parasite load will be analyzed as a predictor of viral load**.** The data from both experiments will be analyzed and the interactions modeled using statistical packages in R (a computer programming language popularly used to model ecological systems).

**RESULTS:**

p>0.05

***Preliminary data from 2015-2016:***

The *Nosema* count data collected from the 2014 bumblebee survey demonstrated that the parasite is prevalent on the landscape with a mean probability of infection of 0.249. This increases the risk of coinfection with other pathogens like DWV or BQCV. There were insignificant variations between species (Fig. 1). Caste (queen/worker/male) had no bearing on the probability of infection. These parameters will be used to model the proposed interactions.

**Fig. 1.** The probability of infection (with standard deviation bars) for five common *Bombus* *spp*.: *bimaculatus* (bimac.), *borrealis* (bor.), *impatiens* (imp.), *ternarius* (tern.) and *vagans* (vag.). Variation between species was not significant (Pearson’s Chi-sq). The mean probability of infection for all species was 0.249.

***Future plans:***

Research into the health of both managed and wild pollinators is a lifelong passion of mine. I have kept honeybees since the age of 12 and have become very invested in attempting to understand the forces that are contributing to pollinator decline. Funding from this APLE Summer Stipend will enable me to pursue this research and submit two papers for publication during my senior year. This stipend will provide me with the resources to contribute to the important work in pollinator research that still needs to be completed. The work I propose here will serve as the foundation of my accelerated Master’s thesis, which I will complete in 2018. My long-term goal is to pursue a Ph.D. in ecology and continue my work as a researcher in the field of pollinator conservation. This opportunity will better prepare me to meet my goals while at the same time, provide valuable data on the health of Vermont’s bees.

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