*Nosema* at large: A survey and epidemiological model of a microsporidan parasite in Vermont bumble bees.

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**ABTRACT:**

Pollinators contribute more than $250 billion annually in pollination services. Bumble bees are important native pollinators, however, their decline has not been as well studied as that of honeybees. In 2015, Vermont added *Bombus affinis* and *B. ashtoni* to the endangered species list and *B. terricola* as threatened. Pathogens, like the microsporidian parasite *Nosema,* are thought to be a factor in their decline. *Nosema* lives in the ventriculus of its host and is known to cause sub-lethal effects such as dysentery and decreased forging efficiency as well as increase mortality in heavily infected individuals. To examine the prevalence of *Nosema* in Vermont bumblebees, and examine patterns of infection across species and geography, we conducted a survey. In 2014, 350 bumblebees were randomly netted at 13 different field sights across Northern Vermont. Flowering vegetation transects and bee abundance surveys were conducted at each site. The ventriculus was dissected out of each bee and was homogenized. Spores were counted for each bee using a hemocytometer. These data were analyzed by species, site and caste and honeybee presence. No significant relationships were found, however, the parasite exhibited a prevalence of 20%. The ubiquitous nature of *Nosema* makes looking at interactions between other pathogens relevant to bumblebee decline due to the high probability of coinfection. In future studies I intend to look for synergistic interactions between different species of *Nosema* and RNA viruses in order to better understand the part this parasite is playing in the decline of some of our most important pollinators.

**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 bumble bees as honeybees are poor pollinators of these plants (Strange, 2015; Thornsbury and Jerardo, 2012). Bumble bee 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 bumble bee declines. One parasite in particular, the microsporidian *Nosema sp*., is considered to be an important detrimental parasite to bumble bees. *Nosema* lives in the ventriculus of its host and seems to peak in prevalence in the spring. It has been shown to cause dysentery and adversely affects forging efficiency (Otterstatter et al., 2005).Spores can be transferred when a bumble bee visits a flower that has already been visited by an infected bee (Imhoof and Schmid-Hempel, 1999). The two species that affect bumble bees are *N. bombi* (the native species) and *N. ceranae* (an introduced species). *N. ceranae* has become ubiquitous in the European honeybee (*A. mellifera*), outcompetes *A. mellifera’s* unique species of *Nosema*, *N. apis*,which has only recently been found in bumble bee populations. It has been hypothesized that N. ceranae spilled over into bumble bee populations from honeybees (A. Bourgeois et al., 2010; M. Natsopoulou et al., 2014).

In this study we examine the prevalence of Nosema spp. in Vermont’s bumble bee populations. We ask whether various bumble bee life history traits such as species or caste influence the prevalence of this parasite. In addition, we look at how proximity to honeybee apiaries affects parasite prevalence. **(I)** We hypothesize that differences in species phenology and morphology will create variation in parasite prevalence between species. **(II)** We hypothesize that there will be differences in prevalence between caste (queens, workers and males) due to differences in exposure to floral reservoirs. **(III)** We also hypothesize that bees caught adjacent to honeybee apiaries will exhibit a higher prevalence do to the pathogen spillover hypothesis. Using the parameters derived from the empirical study we conducted, we created and parameterized an epidemiological model to describe the dynamics of this understudied system.

**METHODS:**

***Data Collection and Analysis:***

Over 350 bumble bees were collected in northern Vermont from 13 different field sites during the summer of 2014. The bees were netted randomly while foraging on flowers. Queens and males were caught as well as workers. The bees were put on dry ice in the field and were transferred to a -80oC freezer within 12 hours of being captured. At each site, bee abundance and vegetation surveys were performed on 100m transects. In addition, forging honeybees were netted and pollinator friendly flowers collected at each site. GPS coordinates, elevation, weather conditions, and nearest town were also logged at each location.

In order to assay each bee for *Nosema*, the ventriculus was dissected from the bee by pulling on the last segment (terga) of the abdomen. The ventriculus for each bee was then homogenized in 500uL of GITC buffer with a polypropylene pestle for 1 minute. These were then vortexed and 10 uL of the homogenized bee gut were put into each chamber of a hemocytometer. Counts were made of the Nosema spores present using a traditional Neubauer® counting grid and the two chambers were averaged together resulting in the total count.

These data were analyzed in R using a contingency table with a Pearson's Chi-squared test. Tables of the independent variables (species, caste and honeybee proximity) by the presences/absences Nosema data (binary) were created. Infected and Uninfected vectors were created and bound into a matrix. A the tests were performed using the "chi.sq" function and mosaic plots using the "mosaicplot" function.

Figure 1: The prevalence of \*Nosema\* (nominal dependent variable) plotted against the five most common \*Bombus spp.\* in this data set (\*B. bimaculatus, B. borealis, B. impatiens, B. ternarius and B. vagans\*). This graphical representation of a contingency table shows the percentage of bees in each category. A Chi-squared test yielded and insignificant result (p<0.05).

Figure 4: The infection rate through time beginning in the early spring and ending in the early fall. The susceptible population is shown in green and the infection rate in blue. The critically infected (diseased) rate is shown in red. The parameters for this model are: beta=0.202 (data), gamma=0.05 (data), I1=0.05 (estimated).

***Epidemiological Modeling***

**RESULTS:**

***Data Collection and Analysis***

***Epidemiological Modeling***

**DISCUSSION:**

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