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

**PRIORITY AREA FOCI:**

**1)** Patterns of coinfections between two species of *Nosema* and three RNA viruses

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

**3)** Creating a general model for how time of year might predict pathogen abundance and probability of coinfection.

**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.

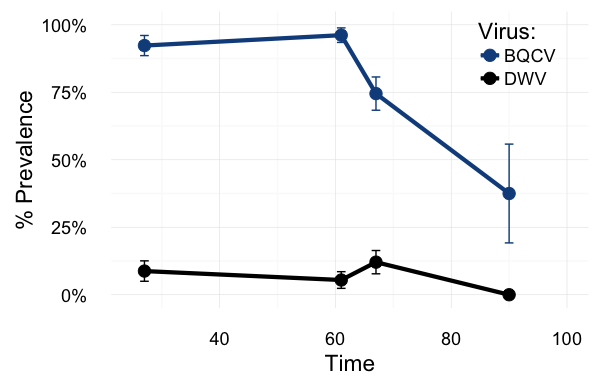
There are many threats that are thought to be causing bumblebee declines including habitat loss, pesticide use and climate change, bumble bee pathogens and the interactions that occur between them are relatively under studied. Two groups of pathogens that affect bumble bees are the microsporidian parasite *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. 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: **(I)** look for patterns of coinfection between *N. ceranae*and *N. bombi*, **(II)** examine if coinfection between *Nosema spp.* and RNA viruses (DWV, BQCV and LSV) is common in bumble bees, **(III)** and to examine and model temporal variation in pathogen load between these five infectious agents.

**OBJECTIVES:**

**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, I reanalyzed these data and found seasonal differences between one virus of interest, Black Queen Cell Virus (BQCV). In the bumble bees, this virus peaked etc. (Fig. 1). To my knowledge, this became the first evidence of seasonal variation in RNA viruses among bumble bees. To fully understand this variation, more work needed to be done. 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.

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

In 2017, I will isolate RNA and DNA 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*) on the DNA isolations using RT-qPCR to look for patterns of coinfection between the two species of *Nosema*. Using the RNA isolations I will assay the same bees using RT-qPCR for three RNA viruses: Deformed Wing Virus (DWV), Lake Sinai Virus (LSV) and Black Queen Cell Virus (BQCV). 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. Using these data as well as data from 2015, I will be able to construct a deterministic ODE (Ordinary Differential Equations) model to predict pathogen load and coinfection probability through time (R statistical package and Python programming language).

**REFERENCES:**

Arneberg, P., Skorping, A., Grenfell, B., & Read, A. F. (1998). Host densities as determinants of abundance in parasite communities. *Proceedings of the Royal Society B: Biological Sciences*, *265*(1403), 1283–1289.

Bourgeois, A. L., Rinderer, T. E., Beaman, L. D., & Danka, R. G. (2010). Genetic detection and quantification of Nosema apis and N . ceranae in the honey bee. *Journal of Invertebrate Pathology*, *103*(1), 53–58.

Colla, S. R., Gadallah, F., Richardson, L., Wagner, D., & Gall, L. (2012). Assessing declines of North American bumble bees (Bombus spp.) using museum specimens. *Biodiversity and Conservation*, *21*(14), 3585–3595.

Fries, I., Chauzat, M.-P., Chen, Y.-P. P., Doublet, V., Genersch, E., Gisder, S., … Gisder, S. (2013). Standard methods for nosema research. *Journal of Apicultural Research*, *52*(1), 1–28.

Fürst, M. A., Mcmahon, D. P., Osborne, J. L., Paxton, R. J., & Brown, M. J. F. (2014). Europe PMC Funders Group Disease associations between honeybees and bumblebees as a threat to wild pollinators, *506*(7488), 364–366.

Graystock, P., Meeus, I., Smagghe, G. U. Y., Goulson, D., & Hughes, W. O. H. (2015). The e ff ects of single and mixed infections of Apicystis bombi and deformed wing virus in Bombus terrestris.

Imhoof, B., & Schmid-Hempel, P. (1999). Colony success of the bumble bee, Bombus terrestris, in relation to infections by two protozoan parasites, Crithidia bombi and Nosema bombi. *Insectes Sociaux*, *46*(3), 233–238. .

Otterstatter, M. C., Gegear, R. J., Colla, S. R., & Thomson, J. D. (2005). Effects of parasitic mites and protozoa on the flower constancy and foraging rate of bumble bees. *Behavioral Ecology and Sociobiology*, *58*(4), 383–389.

Schroeder, D. C., Martin, S. J., Hill, C., & Manchester, G. (2012). Virulence News & Views Deformed wing virus: The main suspect in unexplained honeybee deaths, 589–598.

Strange, J. P. (2015). Bombus huntii, Bombus impatiens, and Bombus vosnesenskii (Hymenoptera: Apidae) Pollinate Greenhouse-Grown Tomatoes in Western North America. *Journal of Economic Entomology*.

Natsopoulou, M. E., Mcmahon, D. P., Doublet, V., Bryden, J., & Paxton, R. J. (2014). Interspecific competition in honeybee intracellular gut parasites is asymmetric and favours the spread of an emerging infectious disease.

Thornberry, S., and A. Jerardo. 2012. Vegetables and Pulses Outlook. USDA, Economic Research Service, p. 52.

Van Engelsdorp, D., Hayes, J., Underwood, R. M., & Pettis, J. (2008). A survey of honey bee colony losses in the U.S., Fall 2007 to Spring 2008. *PLoS ONE*, *3*(12), 8–13.

Vermont adds nine species to threatened and endangered list. (2015) Vermont Fish

and Wildlife Department. Retrieved 9/5/15 from http://www.vtfishandwildlife.com

/cms/One.aspx?portalId=73163&pageId=269142