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An examination of dilution in bumble bees: Spillover of RNA viruses through shared flowers depends on floral diversity

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Introduction:

In the field of epidemiology, disease outbreaks and the mechanisms related to transmission and dissemination are traditionally studied in isolation (Fang & Casadevall, 2011). While, good fundamental work has been conducted in many fields of epidemiology, it has been brought to the attention of the scientific community in recent years that, as the complexity of natural disease systems unfolds, disease research now requires a community ecology approach (Wood et al., 2012; Zakary, 2015; Altizer et al., 2006; Johnson, et al, 2016). This methodology consists of treating a disease system holistically as a community of pathogens interacting with each other and their host through a series of interconnected mechanisms. In addition, the influence of biodiversity on disease transmission (hotspot vs dilution hypothesis) has been discussed at great length and no consensus has yet been reached (Schmidt and Ostfeld, 2001, Johnson et al., 2015). Using this disease ecology approach and a combination of survey work, lab experiments and computational modeling, I hope to show how spillover and biodiversity, traditionally studied in isolation, may together shed light on the complexity of disease dynamics and emerging infectious disease.

The added complexity inherent in studying a disease system in this manner necessitates an integrated experimental and computational approach. Lab and field experiments aimed at testing this proposed model would quickly become too large and unwieldly to have the required predictive power. Likewise, a modeling exercise without any empirical framework may prove to be misguided. Here I propose a series of surveys and experiments in the bumble bee-honeybee disease system with the goal of parametrizing an agent-based epidemiological model. The model will be capable of its own predictions that can be compared to empirical bumble bee survey data and shed light on important epidemiological questions. Modeling disease systems based on empirical parameters affords the scientific community with the opportunity to better understand disease dynamics without losing our understanding of the natural system. It increases our ability to predict how an outbreak will behave in future scenarios and allows the us to make generalizations about large-scale emergent properties in epidemiology that may inform future decisions in human and non-human disease outbreaks alike (Handel, 2017). In order to integrate empirical science and theoretical modeling, a natural disease system (like the bumble bee-honeybee system) is required that can be easily manipulated, measured and has analogs for all of the required areas of study. In addition to their agricultural value, the

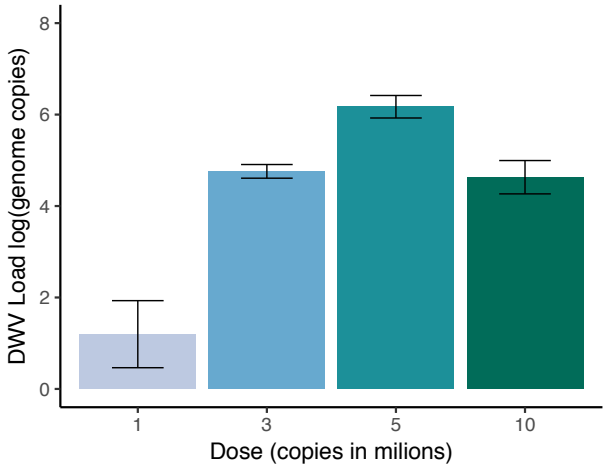

Figure 1 One mil. copies are sufficient to drive replicating infection. Viral load 3 days after inoculation increases as a function of the magnitude of infection dose.

bumble bee (*Bombus Spp.)* system can be easily measured in the field or taken into the lab. The system also exhibits a diverse array of pathogens and parasites and experiences spillover making it an ideal candidate for parameterizing mathematical models aimed at explaining the link between these epidemiological phenomena.

In the field of disease ecology, it is well understood that biotic factors like host density and parasite prevalence have an influence over the dynamics of a disease outbreak (Knolle, 1989). However, the effect that biodiversity has on the trajectory and outcome of a given disease is a relatively understudied area. Only recently has diversity come to the forefront as a potential factor in the disease literature. The idea, termed the "dilution hypothesis" suggests that host diversity may dampen or "dilute" the effectiveness of a pathogen or vector reducing the prevalence of the diseased state (Ostfeld & Keesing, 2012). Although past work on dilution has helped to improve our view of how ecological factors might influence epidemiological research, there is still much debate over this hypothesis. Evidence to support this hypothesis has been found, but the underlying mechanism is unknown (Strauss et al., 2015). To better understand diversity's influence over disease dynamics, I propose a study to examine a dilution hypothesis mechanism in the honeybee-bumble bee disease system.

Evidence is mounting that RNA viruses, likely originating from honeybees, have been spilling over into wild bee populations (Fürst et al., 2014; Alger et al., in review). Previous work in this system has shown that flower species can harbor viruses each with a unique prevalence. In addition, bumble bee species have different susceptibilities to these viruses (Alger et al., in review). Although transmission between bee species likely occurs through the use of shared flowers, only two published studies have directly examined this floral transmission route and no study has yet examined their role in RNA virus dissemination. In addition, mine will be the first study to model the route with the goals of characterizing its emergent properties and examining how floral diversity might influence overall disease dynamics. In this study I aim to experimentally test how flowers may be facilitating the spillover of RNA viruses from honey bees into bumble bee communities. To examine the dilution hypothesis, I will use a combination of experimental parametrization and mathematical modeling to examine how both floral and host diversity and density might influence the prevalence of RNA viruses in bumble bee communities.

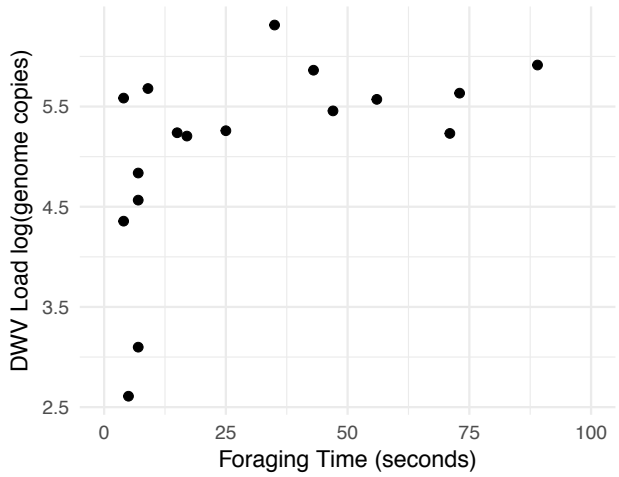

Figure 2 Bees pickup viruses on flowers. The log (base-10) of viral load in genome copies/bee after foraging on field-realistically inoculated artificial flowers (10^6 genome copies) by foraging time.

Methods

In the summer of 2018, I conducted a series of experiments to examine the transmission route of Deformed Wing Virus from honeybees to bumble bees through red clover, a flower commonly visited by both species and known to harbor DWV. I quantified the number of virus particles per flower to order of magnitude (10^4 to 10^6). Fifteen individuals from each of four commercial bumble bee colonies were tested for DWV using RT-qPCR and were found to be negative. Colonies were fed 30% sucrose solution and gamma-irradiated pollen to ensure no active DWV particles were introduced. Twelve micro-colonies were made from these four main colonies in four treatments (3 colonies/treatment) from 15 workers. Micro-colonies were pollen starved for three days and remaining bees were transferred to 8"x5"x4" boxes and exposed to infected red clover. In the random flowers treatment (RF), three colonies were exposed to a new set of three haphazardly selected red clover flowers from an infected honeybee apiary for each of three days. In the hand inoculated experiment (HI), three colonies were exposed to 3 sets of clover inoculated with 10^6 genome copies per flower with a new set presented for on each of three days. In the honey bee inoculation experiment (HBI), flowers were inoculated by infected in honeybee micro-colonies for three days before being presented to bumble bee colonies. To control for potential viral contamination, control groups were presented with sets of three artificial flowers inoculated with pure 30% sucrose throughout the course of the experiment and assayed for DWV using RT-qPCR along with the other treatment groups.

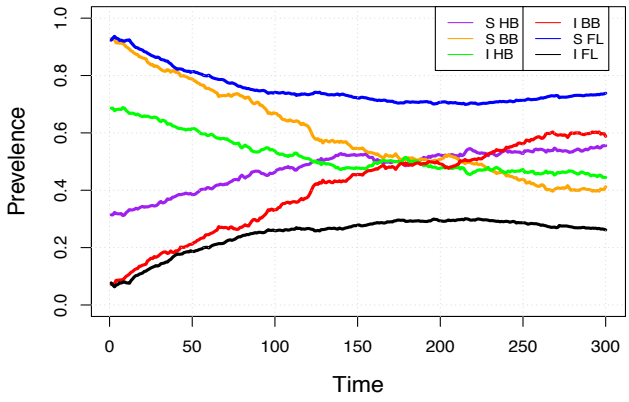

Figure 3 Preliminary CA model output shown as prevalence of infected (I) and susceptible (S) honeybees (HB), bumblebees (BB) and flowers (FL) through time. Infected BB prevalence (red) increases as a function of infected FL (black) driven by infected HB (green).

In addition to this demonstration of the route using real flowers, I conducted a series of three experiments with artificial flowers in order to estimate parameters for a cellular automata model of the system (Table 1). To determine the number of viral particles that can be acquired as a function of foraging time, I allowed 60 bees to forage on artificial flowers inoculated with 10^6 genome copies of DWV for between 1 and 120 seconds. Prevalence and load was analyzed as a function of foraging time in a regression design. The amount of virus required to contract a replicating infection, was determined by creating a dose curve by inoculating 50 bees with 1, 3, 5 and 10 million genome copies of DWV and allowing for three days of incubation time. To determine if the route might work in reverse (bumble bees to honeybees), the rate at which bumble bees deposit viruses on flowers was measured by allowing 30 infected bumble bees to forage on artificial flowers for 10 seconds. I then measured the number of viral particles in the bee and in the artificial nectary using RT-qPCR and analyzed with a regression model.

Remaining Work: RNA extractions have been completed for all samples, however RT-qPCR for the remaining experiments remains to be conducted. Data need to be analyzed in order to derive the parameter estimates required for the modeling component. I have constructed a preliminary

model, but the functionalities or floral constancy, floral diversity and host diversity have yet to be added. In addition, I will examine a wide range of parameter values (parameter sweep) in order to determine the emergent properties of different regions of the parameter space and their relevancy to the system and the dilution hypothesis.

Table 1 Model parameter description, preliminary value estimate and source. Parameters from this study as well as from previous studies will be used to parameterize a CA model of disease spillover. Ranges of flower viral prevalence and host susceptibilities will be used to understand the mechanism behind how biodiversity influences disease transmission (hotspot vs dilution hypothesis).

Parameter description	Value	Source
Probability of HB deposition	-	TBD from Chapter II
Proportion of infected flowers	0.1-0.5	(Alger et al., in review)
Proportion of infected *Bombus*	-	TBD from Chapter I
Probability or reverse route	-	TBD from Chapter II
Probability of infection	0.167	Determined from Chapter II
Required infection dose	10^6	Determined from Chapter II
Probability of virus Acquisition	-	TBD from Chapter II
Amount of virus on one flower	10^3-10^6	(Alger et al., in review)

Preliminary Findings: Preliminary results show for the first time that DWV can be picked up by bumble bees from hand inoculated artificial flowers. In addition, I demonstrated on artificial flowers and that forage time influences the amount acquired. A diminishing returns model of virus acquisition is implicated indicating that only a short amount of time is required for a bee to pick up a relatively large viral load (Figure 2). In addition, a dosage of 1 million genome copies leads to a replicating infection 16.7% of the time. This probability of replication increases as dosage increases (Figure 1). These new data coupled with the known prevalence of DWV in nature and the high abundance of infected flowers in honey bee apiaries indicates that the route is very probable as a primary mode of spillover. In addition, the preliminary CA model shows a sensitivity to the prevalence of infected honeybee colonies (Figure 3).

Conclusion & Broader Impacts:

The above work bridges the two import fields of conservation biology and disease ecology. The documented decline of important pollinators has garnered much attention and concern in recent years. Bumble bees in particular are important native pollinators whose decline has been understudied in light of managed honeybee losses (van Engelsdorp et al., 2008). Around 50% of bumble bee species have decreased in abundance since the 1960s (Colla et al., 2012). Spillover of honey bee diseases into naïve bumble bee populations has been implicated as one of the driving factors for these losses (Cox-Foster et al., 2007). The above proposed work will shed light on the mechanisms behind these losses and provide management recommendations to mitigate them. In addition to these conservation implications, this work proposes to use both experimental and modeling approaches in a single system to construct a unified principle of how spillover and host diversity can operate together to affect the trajectory and outcome of a disease outbreak. While these areas have been studied in the past, a reductionist view has often been imposed (Johnson et al., 2016). I aim to show that in combining experimental biology with computational epidemiology, the relationships between isolated fields in the literature may be incorporated into a larger holistic picture of disease ecology. The implications of this understanding may be used in future to inform recommendations for dealing with emerging infectious disease in both human and non-human systems.

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