Virus Prevalence in Pollinator Communities: The Role of Communities, Environments, and Host Interactions on Multi-Host–Multi-Pathogen Dynamics

by

Michelle L. Fearon

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Ecology and Evolutionary Biology)
in the University of Michigan
2020

Doctoral Committee:

Professor Elizabeth A. Tibbetts, Chair
Professor Meghan A. Duffy
Professor Mark D. Hunter
Associate Professor Adam S. Lauring
Assistant Professor Chelsea Wood, University of Washington

Michelle L. Fearon

mlfearon@umich.edu

ORCID iD: 0000-0001-6849-4834

Dedication

To Mike and Scott. You both are my greatest joy every day, and inspire me to always be curious about the world around us. Never stop exploring and discovering.

All my love,

–Michelle

Acknowledgements

It has been a privilege to be able to pursue my passion in science research, teaching, and mentoring over the last six years. I would like to thank my committee for their thoughtful comments on my thesis work throughout my PhD. Your feedback and ideas have challenged me to become a better scientist and have improved the quality of this work immensely. I look up to each of you as mentors and model scientists that I hope to be able to emulate one day. I am particularly grateful to my committee chair and primary advisor, Liz Tibbetts. Thank you for your insightful comments and hours of discussion about my work, even when it was outside of your main areas of research. Your advice and critique have always been incredibly helpful. Your instruction in developing research questions, overcoming challenges, analyzing data, and writing has taught me to be a rigorous and thoughtful scientist. Thank you to Meghan Duffy and her lab for providing a second "lab home" for me to gain experience with disease ecology and get feedback on my work. I have learned everything I know about disease ecology from my time with your lab. I look forward to working with you as a post-doc and diving even deeper into multi-host–multi-parasite dynamics. Thank you to Mark Hunter for challenging me to always consider how the bees, flowers, and viruses I study are connected and a part of the broader ecosystem. I have also really enjoyed the opportunity to teach with you and benefit from your experience and long career in science. Thank you to Adam Lauring for providing your expertise on viruses and for supporting my research. Thank you to Chelsea Wood for being an inspiration in your research program and for helping me to discover the specific areas of disease ecology that I find the most fascinating. Your advice always challenges me to think deeper about my

work and its role in the field of disease ecology. I always come away from our conversations excited about community and disease ecology, and I look forward to many years of future collaboration.

I would like to thank the many invaluable people that assisted with various aspects of my thesis work—it wouldn't have been possible without all the help and work done by the entire team. Jeremy Graham, Mike Scales, Kelly Lam, Yorick Oden-Plants, and Josh Peters for their help collecting pollinators in the field and recording videos of bees visiting flowers. Maryellen Zbrozek, Cara Fleming, Madeiline Frank, Gabrielle Flint, Leah Davis, and Eva Collins-Carey for their hours of identifying thousands of pollinators to genus and species. Raquel Marchan Rivadeneira taught me everything that I know about molecular laboratory work and provided advice to help trouble shoot and optimize the molecular methods (repeatedly). I don't know what I would have done without her! Maryellen Zbrozek, Madeleine Frank, Gabrielle Flint, Chloe Weise, and Hannah Zonnevylle for their assistance detecting the presence of the viruses in bees and on flowers. Maryellen Zbrozek, Cara Fleming, Kelly Lam, and Yorick Oden-Plants for their help quantifying landscape cover around each field site. Maryellen Zbrozek, Eva Collins-Carey, Madeleine Frank, Gabrielle Flint, Leah Davis, and Justice Martin-Johnston for watching hours of video and coding the details of individual bee interactions on flowers. Additionally, it was a pleasure and honor to get to mentor Maryellen Zbrozek and Chloe Weise in completing honors theses. The opportunity to mentor and teach so many talented and inspiring students has been one of the major highlights of my PhD!

Thank you for the unending love and support from my family as I have pursued my PhD and science career. My husband, Mike, has supported me in this goal since the beginning of our relationship. He has been there for me through the long, hard days of field and lab work (even

helping with my field work and providing advice on molecular methods) and has celebrated every exciting result and accomplishment with me. He has listened to and commented on numerous talks, reviewed my writing, and provided food, cookies, and coffee during some of the toughest writing days. There are not enough words to express my thanks—completing my PhD would not have been possible without you, Mike. The addition of my son, Scott, to our family has brought me joy every day and reminds me to be curious and explore the world around me as I see the world through his eyes. I can't wait to teach him all that I know about the natural world! Thank you to my parents, they made my education and career in science not only possible, but a reality. My parents and sister inspire me to work hard and be the best version of myself that I can be. Especially, I want to thank my mom for always understanding my drive to be a scientist (she knew that I would be a scientist *long* before I did) and the various challenges that I have faced throughout the graduate program. Mom, you were the first successful woman scientist that I looked up to and helped me believe that I could also become a scientist and get my PhD. I am so thankful for the love and support that I receive from Mike's and my families and our dear friends. Finally, I am incredibly thankful for the friends and scientific community that I have gained in our EEB department. I am honored to be a part of such an incredible group of scientists, and I have valued their friendship and scientific expertise immensely over the years. In particular, thank you to my good friend Jacqueline Popma. You have been an integral part of my graduate experience, from walking to and from lab in our first year, working in coffee shops, suffering through prelims, reviewing endless writing, supporting each other through the joys and challenges of being moms and scientists, to finally defending our dissertations. I have been so lucky to have a best friend by my side over the last six years!

Thank you to the variety of institutions and non-profits that provided funding to make my research projects a reality. Funding support came from the National Science Foundation IOS-1557564, the North American Pollinator Protection Campaign, the Pollinator Partnership, The Garden Club of America, the University of Michigan Rackham Graduate School, the UM Department of Ecology and Evolutionary Biology, UM Graham Sustainability Institute, Sigma Xi, and Phi Kappa Phi, University of Michigan Chapter 39. I would also like to thank all the landowners that allowed me to use their land to collect pollinators and flowers from their fields: Blast Corn Maze (Nixon Farms), Bird's Big Punk'ns, BJ Farm, Brimley's Pumpkin Patch, Erwin Orchards, Gust Brother's Pumpkin Farm, Green Things Farm, Kapnick Orchards, Plymouth Orchards, Peacock Road Farms, Spicer Orchards, Stone Coop Farm, Tantré Farm, and Wasem Fruit Farm. It was a joy to work on these farms and to visit to many corners of Michigan that I wouldn't have seen otherwise.

I acknowledge that the University of Michigan and land where my research was conducted resides on the traditional Territories of the Three Fire Peoples - the Ojibwe, Odawa and Bodewadmi, and the Wyandot. I further acknowledge that these aforementioned lands were obtained, generally in unconscionable ways, from indigenous peoples. In addition, our research on the ecology and evolutionary biology of Michigan has benefited and continues to benefit from access to land originally gained through the exploitation of others. Knowing where we live and work does not change the past, but a thorough understanding of the ongoing consequences of this past can empower us in our research, teaching, and outreach to create a future that supports human flourishing and justice for all individuals. As we continue to work, live and play on these territories we must keep in mind the ongoing effects of colonization, communities struggle for self-determination, colonial state violence and the recognition of Indigenous sovereignty.

Table of Contents

List of Tables

List of Figures

Abstract

How do host communities and their environment impact patterns of pathogen prevalence? This question is becoming increasingly important as disease ecologists shift their focus from single host–single pathogen systems to the more realistic and complex dynamics of multiple hosts infected by multiple pathogens. Although widespread environmental change and biodiversity loss are associated with increased infectious disease in human and wildlife populations, we still lack a detailed understanding of how community and environmental factors influence pathogen prevalence.

The central question of my dissertation asks, why does pathogen prevalence vary among communities? To address this question, I explore how host communities, the environment, and species interactions influence patterns of pathogen prevalence for three multi-host pathogens.

Specifically, I investigate variation in pathogen prevalence among pollinator communities for three widespread viruses: deformed wing virus, black queen cell virus, and sacbrood virus. These pathogens infect multiple pollinator species, including *Apis mellifera*, *Bombus impatiens, Lasioglossum* spp., and *Eucera pruinosa*. I conducted a field study to examine how pollinator community characteristics, local and landscape habitat factors, and patterns of pollinator visitation to flowers alter pathogen prevalence in multiple hosts.

My dissertation has three main conclusions: First, greater pollinator community species richness is consistently linked with lower virus prevalence for all three viruses in all competent host species. Total and species-specific host abundance and community composition are not strongly associated with virus prevalence. These findings fit with the ‘dilution effect’ hypothesis,

where biodiversity reduces pathogen prevalence, and is among the first studies to show consistent dilution in pathogen prevalence for multiple pathogens infecting a community of hosts.

Second, 'high-quality' habitat characteristics associated with improved pollinator nutrition are directly linked with differences in virus prevalence. Interestingly, this association either increased or decreased virus prevalence depending on the specific habitat characteristic. Habitat diversity and abundance characteristics that improve pollinator nutrition are also indirectly linked with reduced virus prevalence through habitat-mediated increases in pollinator species richness. In sum, the net effect of greater local and landscape habitat characteristics among all direct and indirect pathways predicted a strong reduction in viral prevalence for all three viruses. These findings support a new pattern that I termed the 'habitat–disease relationship', where habitat characteristics directly mediate patterns of pathogen prevalence, independent of concurrent links between biodiversity and pathogen prevalence. Future investigation is warranted to untangle the mechanistic links between habitat characteristics, host community diversity, and pathogen prevalence in multi-host–multi-pathogen systems.

Finally, 'high-quality' environmental characteristics, like greater natural area and landcover diversity, are associated with greater species richness and abundance of pollinator visitors to flowers. Greater visitation species richness is also correlated with reduced virus prevalence. The pollinator visitation data are consistent with prior results, and reveal that host interactions on flowers may be key to explaining community-level patterns in virus prevalence.

Overall, my dissertation shows that pathogen prevalence is critically linked with host community species richness and 'high-quality' environmental characteristics. My results demonstrate that both habitat–disease and biodiversity–disease relationships operate

synchronously for multiple pathogens infecting multiple host species, but also shows variation in individual links between habitat, hosts communities, and pathogen prevalence among hosts and pathogens. This work illustrates that multi-host–multi-pathogen dynamics are complex, and investigating patterns among multiple pathogens or multiple hosts can reveal consistent and biologically relevant relationships between communities, environment, and host interactions.

Chapter 1 : Introduction

The importance of multi-host–multi-pathogen systems

Traditionally, disease ecology has concentrated on understanding pairwise interactions between a single host and its pathogen. However, in nature many pathogens are capable of infecting multiple host species, and hosts are influenced by their environmental context (Woolhouse et al. 2001). A major challenge in disease ecology is to incorporate both community ecology and the environment into our understanding of the complex interactions between multiple hosts and multiple pathogens in natural systems (Rigaud et al. 2010, Budria and Candolin 2014, Johnson et al. 2015b). There is a growing interest in the interaction between host communities, environmental change, and higher pathogen prevalence as an increasing number of new emerging infectious diseases (EIDs) are linked with widespread biodiversity loss, habitat fragmentation and degradation, and climate change (Daszak et al. 2000, 2001, Parmesan 2006, Keesing et al. 2006, Begon 2008, Tylianakis et al. 2008). Therefore, understanding the underlying factors that allow EIDs to spill over into new hosts or radically increase in geographic range or incidence is of paramount importance.

EIDs are an important threat to both human public health and the maintenance of wildlife populations, and are becoming more common with increasing human-caused environmental change. In the last 20 years, many pandemics have been caused by pathogens that have spilled over from reservoir wildlife populations into human populations. These pandemics include Severe acute respiratory syndrome (SARS) coronavirus, Influenza A virus subtype H1N1, Middle East respiratory syndrome coronavirus (MERS), Ebola virus, Zika virus, and the current

2019 SARS coronavirus-2 pandemic (i.e. COVID-19), among many others (Li et al. 2006, Parrish et al. 2008, Taubenberger and Kash 2010, Gire et al. 2014, Milne-Price et al. 2014, Gutiérrez-Bugallo et al. 2019, Contini et al. 2020). Many new EIDs are caused by multi-host viruses, which are particularly predisposed to host shifts because they can rapidly adapt to new host environments (Domingo and Holland 1997, Woolhouse et al. 2005). Increasing human populations, conversion of natural habitat into agricultural and urban spaces, and rapid climate change all can result in greater contact between human populations and wildlife reservoirs, increasing the potential for viruses to spill over into new hosts (Daszak et al. 2000, 2001). Similarly, EIDs are also rising in wildlife populations and causing serious population declines and biodiversity loss in many taxa, including amphibians (Stuart et al. 2005, Wake and Vredenburg 2008, Fisher et al. 2009), marine invertebrates (Lessios 1988, Harvell et al. 1999, Kim and Harvell 2004), mammals (Holdo et al. 2009, Dobson et al. 2011) and pollinators (Potts et al. 2010, Cameron et al. 2011, Wilfert et al. 2016). Both human and wildlife EIDs are linked with human-caused environmental changes in wildlife biodiversity, habitat fragmentation and degradation, and climate change, which has provoked great interest in understanding how these factors contribute to patterns of disease risk from multi-host pathogens (Daszak et al. 2000, 2001, Altizer et al. 2013).

Patterns of pathogen prevalence can be altered by changing host communities and interactions among hosts, loss and degradation of natural habitats, or a combination of multiple factors (Daszak et al. 2000, 2001, Parmesan 2006, Keesing et al. 2006, Begon 2008, Tylianakis et al. 2008). Though we know that these factors impact pathogen prevalence and disease risk for some host–pathogen systems, we still lack a clear understanding of how these specific factors vary in relative importance and may operate concurrently among different communities of hosts

and environments. This is especially true for pathogens that infect many host species and may be at greater risk for becoming an EID. Fundamentally, this boils down to the question: Why are some host communities healthier than others? In this dissertation, I will address this question by exploring how differences in host communities and their environments are associated with patterns of pathogen prevalence in multiple host species, and how those patterns vary among multiple pathogens.

Intersection between community ecology and disease ecology

The importance of community diversity in reducing the spread of infectious disease has long been recognized qualitatively in agriculture through intercropping and crop rotation, where increasing crop diversity tends to reduce disease prevalence (Curl 1963, Vandermeer 1989). Furthermore, Charles S. Elton, a pioneer ecologist, noted that 'outbreaks [of infectious diseases] most often happen on cultivated or planted land …that is, in habitats and communities very much simplified by man' (Elton 1958, p147). These observations describe a single host–pathogen system, where greater host diversity can constrain transmission of infectious disease by reducing the density of the single host species (Mitchell et al. 2002, Dobson 2004, Begon 2008). However, predicting how community diversity will impact systems with multiple hosts and multiple pathogens becomes much more complex and intriguing because hosts can vary in quality for pathogens (i.e. host competence) and pathogens can infect different subsets of host species (Johnson et al. 2015a). Therefore, the diversity and abundance of hosts interacting in a community could alter a host's likelihood of encountering pathogenic agents and becoming infected. This disease risk will also differ among diverse pathogens. Consequently, a host's disease risk will vary with its community context but predicting specifically how different community-level factors influence multi-host pathogens remains complex.

Over the past few decades, theoretical and empirical work in many systems has explored community-level factors that influence disease, showing that factors like host community diversity, community composition, and abundance influence pathogen prevalence and transmission (Ostfeld and Keesing 2000a, Holt et al. 2003, Keesing et al. 2006, Johnson and Thieltges 2010, Johnson et al. 2013a, 2015b, Levi et al. 2016, Luis et al. 2018, Strauss et al. 2018). There is particularly strong interest in the influence of community diversity on pathogens, with some striking evidence that members of species-rich communities have reduced disease risk compared to members of species-poor communities. If the additional species in diverse host communities are less competent hosts that tend to reduce the density of highly competent hosts or reduce the contact rate among competent hosts, then the likelihood of pathogen transmission may be lower in species-rich host communities compared to species-poor communities (Ostfeld and Keesing 2000b, Keesing et al. 2006). This phenomenon is known as the "dilution effect" in the disease ecology literature (Ostfeld and Keesing 2000b, Schmidt and Ostfeld 2001, Ostfeld and LoGiudice 2003, Keesing et al. 2006). This negative biodiversity–disease relationship represents a "win-win" scenario for conserving biodiversity while also improving public and wildlife health (Kilpatrick et al. 2017a). The dilution effect theory was initially developed in vector-borne pathogens, such as Lyme disease in mammalian hosts (Ostfeld and Keesing 2000b, Schmidt and Ostfeld 2001) and West Nile Virus in birds (Ezenwa et al. 2006, Allan et al. 2009). More recent research has expanded recognition of the dilution effect to diverse host–pathogen systems, including directly-transmitted hantavirus in rodents (Clay et al. 2009a, Dizney and Ruedas 2009), parasites with complex life cycles like *Ribeiroia ondatrae* in amphibians (Johnson et al. 2013b), and environmentally-transmitted fungal *Metschnikowia bicuspidata* in *Daphnia*

(Strauss et al. 2018) and *Batrachochytrium dendrobatidis* in amphibians (Searle et al. 2011, Becker et al. 2014, Venesky et al. 2014).

Although the dilution effect has received support in several disease systems, it remains controversial. Many argue that there is a publication bias in favor of the dilution effect, and that it only occurs under specific conditions rather than as a general phenomenon in many host–pathogen systems (Begon 2008, Randolph and Dobson 2012, Lafferty and Wood 2013, Ostfeld 2013, Salkeld et al. 2013, Wood et al. 2014, 2017, Rohr et al. 2020). A central challenge in empirical biodiversity–disease studies revolves around disentangling the effects of host diversity from changes in host abundance and host identity (i.e. community composition). Host abundance often scales with species richness in most natural communities (Begon 2008, Mihaljevic et al. 2014), therefore it is important evaluate the relative contributions of host diversity and host density to observed biodiversity–disease relationships to elucidate their underlying mechanisms (Dobson 2004, Rudolf and Antonovics 2005). The relationship between host communities and pathogen prevalence is not simple, but understanding how different community-level factors interact and whether they have consistent effects on pathogen transmission is a critical next step in biodiversity–disease research (LoGiudice et al. 2003, Keesing et al. 2010, Roche et al. 2012, Randolph and Dobson 2012, Johnson et al. 2015a, Huang et al. 2016).

Three host community-level variables are thought to play a key role in disease dynamics: species diversity, abundance, and community composition. As previously discussed, the dilution effect predicts that diverse communities will have reduced pathogen transmission compared to species-poor communities, especially if a highly competent host (i.e. high potential to support and transmit pathogens) is common in the species-poor communities (Ostfeld and Keesing 2000b, Keesing et al. 2006). For example, communities with high vertebrate biodiversity tend to

have more host species that are less competent or non-competent hosts for the Lyme bacterium (*Borrelia burgdorferi*), which lowers the density of the most competent Lyme disease host, the white-footed mouse (*Peromyscus leucopus*), in the community. As a result, the nymphal ticks (*Ixodes* spp.) that vector Lyme disease feed more frequently on less competent hosts, which reduces Lyme disease prevalence in nymphal ticks and reduces transmission rates in diverse communities (Ostfeld and Keesing 2000b, Schmidt and Ostfeld 2001, LoGiudice et al. 2003). There are many theorized mechanisms to support the dilution effect hypothesis (e.g. encounter reduction, transmission reduction, susceptible host regulation, infected host mortality, and recovery augmentation (Keesing et al. 2006)), but most have not been thoroughly tested empirically in natural host–pathogen systems, especially in the context of multiple hosts and multiple pathogens. However, host diversity can also increase pathogen prevalence via the 'amplification effect'; this pattern tends to occur when the highly competent host is more likely to be found in diverse host communities and increase pathogen transmission among hosts (Keesing et al. 2006, 2010, Wood et al. 2014). As a result, the biodiversity–disease literature has been hotly debated to further refine the context-dependent conditions that different biodiversity–disease relationships are observed and their underlying mechanisms (Lafferty and Wood 2013, Salkeld et al. 2013, Wood and Lafferty 2013, Johnson et al. 2015a, Rohr et al. 2020). Expanding biodiversity–disease studies to additional multi-host–pathogen systems and comparing patterns among multiple pathogens infecting host communities are important frontiers to further understand the conditions at the community-level that lead to dilution, amplification, or neutral effects.

The abundance of hosts in a community is another important factor that can influence variation in pathogen prevalence among different communities. The positive relationship

between higher host density and increased density-dependent pathogen transmission has long been studied, particularly in systems with a single host and single pathogen (May and Anderson 1979, Anderson and May 1981, 1991). However, the impact of host density becomes more complex when considering communities of hosts, where host species vary in susceptibility and competence for a pathogen, and multiple pathogens have differing host ranges. Pathogens that are shared among multiple abundant and competent host species in a community are predicted to produce higher pathogen prevalence than pathogens with a single host or low-density host species (Holt et al. 2003). Furthermore, high host abundance is often correlated with high species diversity such that the 'susceptible host regulation' mechanism of the dilution effect predicts that the addition of non-hosts or less competent hosts could reduce the abundance of susceptible and highly competent hosts, and consequently lead to reduced pathogen transmission and prevalence (Dobson 2004, Rudolf and Antonovics 2005, Keesing et al. 2006, Randolph and Dobson 2012, Mihaljevic et al. 2014). For example, Mitchell et al. (2002) found reduced disease severity of several host species-specific foliar fungal diseases in species-rich plant communities, but that the observed pattern was driven by lower species-specific densities in the species-rich plots. Therefore, it is critical to not only control for host density, but also to evaluate the relative contribution of host diversity and density to biodiversity–disease relationships, especially for multi-host pathogens that may be shared among multiple highly abundant and susceptible host species in a community.

Finally, the third key community factor that influences pathogen prevalence is the composition of multi-host communities, which includes both identities and relative abundance of species present in the community. Host species vary in many factors that will influence their risk of infection, including their susceptibility to a pathogen, infectiousness (i.e. their ability to

transmit a pathogen), and behaviors that may facilitate contact with other infected hosts (Johnson et al. 2013a, Fenton et al. 2015, Huang et al. 2016). Communities are unlikely to have identical community composition; therefore, the specific composition of host species could have important impacts on the patterns of pathogen transmission and pathogen prevalence in the community. Furthermore, communities are usually non-randomly structured such that there are consistent patterns in which species are lost as community biodiversity declines (Ricklefs 1987). Therefore, the presence or absence of particular host species can alter patterns of pathogen prevalence. Specifically, if highly competent hosts are robust to biodiversity losses and tend to be common in species-poor communities, then a dilution effect is likely as additional species in diverse communities are more likely to be less competent hosts. For example, Johnson and colleagues showed that species-poor amphibian communities were dominated by highly competent hosts for the pathogen *Ribeiroia ondatrae*, while species-rich communities contained more resistant hosts, which resulted in a reduction in transmission in the diverse communities (Johnson et al. 2013b). However, understanding whether there are consistent patterns in how community composition is linked with pathogen prevalence is still an active area of research. Though many studies have accounted for the relative impact of host community diversity, abundance, and composition in recent biodiversity–disease studies, few studies have compared the effects these community-level factors on prevalence of several pathogens that infect the same sets of hosts (but see Johnson et al. 2013a).

Furthermore, differences in community diversity, relative abundance, and community composition will inherently alter patterns of interactions among host species, and consequently change exposure and transmission of pathogens among hosts. Yet we have a limited understanding of how changing host interactions—particularly those that may lead to pathogen

transmission—may scale up to drive changes in pathogen prevalence at the community level in natural systems (Kilpatrick et al. 2006, Clay et al. 2009b). Host interactions are likely representative of community-level diversity and relative abundance, but differences in host behavior may drive more frequent interactions among some hosts compared to others. For instance, a small proportion of deer mice (*Peromyscus maniculatus*) with bold behaviors were more likely to be infected with Sin Nombre Virus, and engaged more frequently with other deer mice—interactions that are more likely to lead to transmission events (Dizney and Dearing 2013, 2016). This result suggests that a few bold individuals in the population could be responsible for most of the SNV transmission in the community (i.e. superspreaders) (Dizney and Dearing 2013, 2016). Most studies broadly examine patterns of community diversity and pathogen prevalence, which is an important first step towards understanding multi-host pathogen dynamics. However, subsequent research needs to trace how individual interactions among hosts may be specifically contributing to patterns of pathogen prevalence in natural systems (McCann 2007). Furthermore, exploring variation in interactions and pathogen transmission among hosts in different communities will be important for understanding why pathogen prevalence varies among hosts in different communities.

Systems with multiple hosts and multiple pathogens provide a powerful model to test which community-level factors influence pathogen transmission and prevalence because we can tease apart commonalities among similar hosts or shared pathogens. Biodiversity–disease relationships may be consistent among multiple hosts infected with the same pathogen, such that prevalence of a particular pathogen is similarly influenced by the same community-level factors across multiple host species (Johnson et al. 2008). Several multi-host pathogen studies have shown consistent focal host-specific or community-wide reductions in pathogen prevalence with

increased host diversity (Ezenwa et al. 2006, Allan et al. 2009, Johnson et al. 2013b, Becker et al. 2014, Venesky et al. 2014), but in some cases variation in specific host traits can result in different biodiversity–disease outcomes (Becker et al. 2014, Strauss et al. 2015, 2018). Alternatively, community-level factors may influence pathogen prevalence in a similar way among several different pathogens that infect the same host species. For example, amphibian host species richness reduced infection in five of seven pathogen species tested, showing consistent negative biodiversity–disease relationships among multiple pathogens (Johnson et al. 2013a). Or biodiversity–disease relationships may be idiosyncratic and context-dependent on the specific combinations of host and pathogen traits (Salkeld et al. 2013, Wood et al. 2014, Strauss et al. 2015).

Surprisingly few empirical studies have examined biodiversity–disease relationships in multiple pathogens that infect the same communities of hosts (but see Johnson et al. 2013a), and most meta-analyses of biodiversity–disease studies compare diverse pathogens that infect very different groups of organisms (Salkeld et al. 2013, Wood et al. 2014, Civitello et al. 2015). Differences in either host or pathogen ecology may impact comparisons among disparate host–pathogen systems (Salkeld et al. 2013), so it is essential to choose hosts and pathogens that share common traits (e.g. pathogen type, genetic relatedness, transmission mode, virulence, host range, etc.). By simultaneously studying biodiversity–disease relationships for multiple similar pathogens each infecting multiple related host species, we can look for common patterns between community factors and pathogen prevalence among many host–pathogen pairs and identify potential host or pathogen traits that lead to different outcomes.

The role of the environment in community and disease dynamics

Recent environmental changes, including habitat fragmentation, habitat degradation, and climate change, are key drivers of widespread biodiversity loss that cause changes in community structure and interactions among species (Fahrig 2003, Foley et al. 2005, Parmesan 2006, Tylianakis et al. 2008, Kerr et al. 2015). Furthermore, both habitat and biodiversity loss are increasingly linked with greater infectious disease prevalence for many host–pathogen systems (Harvell et al. 1999, Daszak et al. 2000, 2001, McKenzie 2007, Jones et al. 2008, Altizer et al. 2013, Wilkinson et al. 2018). As a result, there is a growing appreciation for the role that the environment plays in mediating host–pathogen dynamics, whether indirectly through changing host communities or directly by altering host susceptibility and response to infection (**Figure 1.1**).

Environmental change as a driver of biodiversity–disease relationships

Environmental variability is often a key driver of differences in community diversity and abundance, and as I have reviewed above, communities can have important impacts on host-pathogen dynamics. Community assembly is determined first by environmental factors, and followed by the many biotic interactions among community members (Liebold 1997, Chesson 2000, HilleRisLambers et al. 2012, Kraft et al. 2015). Most of what we know about the effects of species interactions on community structure come from theory, careful experiments, or detailed empirical studies of a few specific species interactions (Gause 1932, Neill 1974, Liebold 1997, MacArthur 2009, De León et al. 2014). However, quantifying how environmental characteristics change interactions within and among species that subsequently affect community structure in natural systems remains challenging to study (McCann 2007, Tylianakis et al. 2008). Therefore, the environment could be an important driver of biodiversity–disease relationships by indirectly

altering host community diversity and abundance, but there is still much that remains unknown about the complex interactions between the environment, host communities, and pathogens.

Habitat fragmentation and degradation can reduce host biodiversity and/or alter host densities, both of which have important consequences on species interactions and likelihood of pathogen transmission. Despite the widespread implications of changing biodiversity and host densities on pathogen spread, we still have much to understand about the role that the environment plays in driving biodiversity–disease relationships (Estrada-Peña 2009, Estrada-Peña et al. 2014, Huang et al. 2016). For instance, in the classic dilution effect example, the Lyme disease system, forest fragmentation correlated with reduced mammal species diversity and increased the densities of the white-footed mice (*Peromyscus leucopus*), a key high-quality disease reservoir for the Lyme bacterium (*Borrelia burgdorferi*) (Ostfeld and Keesing 2000b). As a result, nymphal ticks that feed on the mice had higher densities and higher infection prevalence in the fragmented habitats, which corresponds to greater Lyme disease risk for humans (Allan et al. 2003). Therefore, forest fragmentation correlated with increased Lyme disease prevalence by increasing the contact rate between nymphal ticks and highly competent disease reservoirs (e.g. the white-footed mouse), while many other vertebrate species with lower transmission efficiencies were extirpated from those fragmented communities (Ostfeld and Keesing 2000b). Other host–pathogen systems, including hantaviruses in rodents and fungal *Metschnikowia bicuspidata* in *Daphnia* hosts, have also shown similar patterns of habitat degradation or fragmentation correlating with greater pathogen prevalence (Langlois et al. 2001, Suzán et al. 2008, Dearing and Dizney 2010, Penczykowski et al. 2014, Strauss et al. 2016).

Additionally, the effects of natural climate cycles and ongoing climate change can alter host densities and communities, thus impacting pathogen prevalence and disease risk. For

example, the increased precipitation and warmer winter temperatures associated with El Niño Southern Oscillation drives increased food resources (seeds and arthropods) for rodents, and results in substantially increased rodent population densities in the years following the El Niño event. Consequently, the number of human cases of Sin Nombre Virus (SNV) increased in years after the El Niño event, likely due to higher density of rodents infected with SNV (Yates et al. 2002, Dearing and Dizney 2010). These patterns suggest that global climate change could also have key impacts on host densities that underlie host–pathogen interactions and rates of pathogen spillover among hosts, but the effects of global climate change on biodiversity–disease relationships remains understudied (Tylianakis et al. 2008, Dearing and Dizney 2010, Estrada-Peña et al. 2014).

Overall, the effects of the environment often have correlated impacts on host species richness and densities, which makes it challenging to fully tease apart the effects of each on patterns of pathogen prevalence. Furthermore, environmental effects may vary across different geographic locations or spatial scales (Estrada-Peña 2009). Regardless, how different aspects of the environment (e.g. habitat quality, habitat area, climate, etc.) influence communities of hosts and their pathogens is a central challenge for developing a dynamic understanding of the interactions between multiple hosts and multiple pathogens.

Interactions between environment, nutrition, immunity, and infectious disease

The resources available to hosts in the environment can also influence host–pathogen dynamics through changes in host nutrition and immunity. Here, we use the term "habitat quality" throughout this dissertation to indicate habitats that provide hosts with good nutrition, which likely have important impacts on host immune function and response to infectious diseases. The impacts of nutrition on immunity and infectious disease have been well studied,

broadly showing that better nutrition tends to improve host immune function and decrease disease burdens (Ponton et al. 2013). However, the relationships between nutrition, immunity, and pathogens can sometimes have complex outcomes, where pathogens do better in hosts with better nutritional status by co-opting host resources or depressing host immune responses (Ponton et al. 2013). It is important to understand how various habitat quality characteristics are mechanistically associated with higher or lower pathogen fitness, transmission, and prevalence.

Much of the basis for our understanding of the interactions between nutrition, immunity, and disease burdens in wildlife comes from studies on gastrointestinal parasites in ruminants, but these ideas have more recently been expanded to many other host–pathogen systems (Coop and Kyrizakis 1999, Ponton et al. 2013). Successful immune function often results in increased immune gene expression and production of proteins to help fight off an infection; therefore, hosts in a nutritionally poor state may not be able to sustain the high protein demand during an infection, and may reduce their immune response and sustain a higher pathogen load as a result (Klasing 2007). For example, *Spodoptera littoralis* caterpillars given a diet with low protein to carbohydrate ratios (P:C) had greater susceptibility to nucleopolyhedrovirus (NPV) and lower constitutive immune function compared to caterpillars on high P:C diets (Lee et al. 2006). Findings in other host–pathogen systems have shown similar links between reduced nutrition, decreased immune function, and increased susceptibility to pathogens or parasites (Coop and Kyrizakis 1999, Ezenwa 2004, Suorsa et al. 2004, Alaux et al. 2010, Brunner et al. 2014, Santicchia et al. 2015); however, in natural populations the environmental context can also alter established links between nutrition and infectious disease. For example, Ezenwa (2004) found that during drought conditions, wild bovids with low-quality diets had greater susceptibility to gastrointestinal parasites compared to those with high-quality diets, but there was little difference

in susceptibility between the diets in non-drought conditions. Furthermore, individuals with low dietary crude protein had significantly higher gastrointestinal parasite burden than individuals with high crude protein levels. This work shows how environmental conditions, nutrition, and pathogen loads are critically linked in natural wildlife populations.

Hosts can also access exogenous sources of immunity (i.e. medicines) from resources in their environment to better deal with infections. Many plants and algae produce secondary metabolites with anti-bacterial or anti-fungal properties, which some herbivorous taxa can use as a medicine to gain protection from their pathogens (i.e. self-medication; Roode et al. 2013). For example, protozoan infected monarch butterflies lay their eggs on more toxic milkweed plants to reduce infection in their offspring that consume the milkweed (Lefèvre et al. 2010). Additionally, *Daphnia* that consumed toxic phytoplankton were protected from infection by a fungal parasite compared to diets with greater nutritional quality (Sánchez et al. 2019). Furthermore, secondary metabolites in a host's diet can interact with the host's immune response to infection and alter pathogen loads. For instance, bees that consumed secondary metabolites at doses typically occurring in nectar and pollen increased the expression of immune antimicrobial peptide genes and had reduced deformed wing virus loads (Palmer-Young et al. 2017). Therefore, the availability and diversity of resources in the environment may also directly mediate host immune function and severity of infections, which could have important consequences for patterns of pathogen prevalence at the community-level.

To date, relatively few studies have examined how the environment, particularly habitat degradation and alteration, influences pathogen prevalence and disease burdens (Becker et al. 2015). Yet, habitat degradation has led to depleted food resources and increased physiological stress for many wildlife species (Fahrig 2003, Suorsa et al. 2004, Wilkin et al. 2009, Potts et al.

2010, Thomason et al. 2013). For species that forage in the environment for food, host nutrition is inexorably linked with the quality and abundance of resources in their environment. For instance, Eurasian red squirrels (*Sciurus vulgaris*) in fragmented habitats had higher abundance of the dominant gastrointestinal helminth compared to those in continuous forest habitats, and parasite burdens were higher in years with low food availability regardless of habitat type (Santicchia et al. 2015). Yet careful examination is needed to confirm that habitat characteristics are linked with better nutrition and/or improved immunity for specific host–pathogen systems (Becker et al. 2015). For example, roe deer (*Capreolus capreolus*) helminth burden did not change with a higher degree of habitat fragmentation, and the authors suggest that the surrounding agricultural landscape could have provided deer with access to high-quality food and compensated for the potential negative effects of habitat fragmentation (Navarro-Gonzalez et al. 2011). Furthermore, the specific habitat characteristics critical for host nutrition, immune function, and disease resistance are likely specific to the ecology of each host–pathogen system. Though environmental changes to habitat quality and resource availability likely influence the prevalence and severity of pathogens on host populations, few studies have explored how these environmental factors impact multi-host–pathogen interactions.

Introducing the 'habitat–disease relationship' as an alternative and complementary explanation for observed biodiversity–disease relationships

Importantly, the same habitat characteristics that are linked with host nutrition and immune responses to pathogens can simultaneously alter host community diversity and interactions among hosts. I propose that there are two non-exclusive pathways that may contribute to previously observed biodiversity–disease relationships. First, environmental changes in habitat characteristics may directly alter pathogen prevalence through a new pattern

that I termed the 'habitat–disease relationship' (**Figure 1.1, pathway 1**) or indirectly change host community diversity and structure which subsequently alters pathogen prevalence through the well-studied biodiversity–disease relationship (**Figure 1.1, pathway 2**). The habitat–disease relationship differs from the biodiversity–disease relationship because it works directly via habitat effects on host susceptibility and resistance to infection rather than indirectly via habitat impacts on altering community diversity to change patterns of pathogen spread. However, just as biodiversity–disease relationships can both amplify or dilute pathogen prevalence with greater species richness, habitat–disease relationships may also increase or decrease pathogen prevalence with greater habitat quality characteristics. Though the link between community biodiversity and pathogen prevalence has been tested in many host–pathogen systems, the patterns remain variable and idiosyncratic among different host–pathogen systems (Salkeld et al. 2013, Wood et al. 2014, Kilpatrick et al. 2017b). Therefore, habitat–disease relationships represent a previously unexplored avenue that could contribute to and partially explain variable patterns of pathogen prevalence among communities and space.

Habitat–disease relationships are an alternative, but non-mutually exclusive, explanation for previously observed biodiversity–disease relationships because both pathways can operate concurrently or independently to influence pathogen prevalence. Furthermore, coexisting habitat–disease and biodiversity–disease links could either complement or oppose each other to result in reduced or increased pathogen prevalence depending on the direction and relative strength of each effect. It is critical to determine the relative impact of each pathway to pathogen prevalence among different communities. Studies that only investigate biodiversity–disease relationships may overly attribute the observed patterns of dilution or amplification to

biodiversity alone, when in fact the pattern is the net effect of both habitat–disease and biodiversity–disease pathways.

Habitat–disease relationships can be initially be tested by finding direct correlations between pathogen prevalence and habitat characteristics that are likely to mediate host health, while accounting for habitat driven changes in host species richness. Follow-up tests should then compare the relative strength and direction of those direct habitat–disease links to indirect links where the same habitat factors alter biodiversity to result in a biodiversity–disease relationship. These findings would indicate a habitat–disease relationship where habitat quality can directly impact host health and mediate pathogen prevalence among different host communities. Future work will need to investigate the underlying mechanism(s) that link specific habitat factors to pathogen prevalence by comparing host nutrition, host immune function, and host's relative susceptibility to an infection challenge along a gradient for each habitat characteristic.

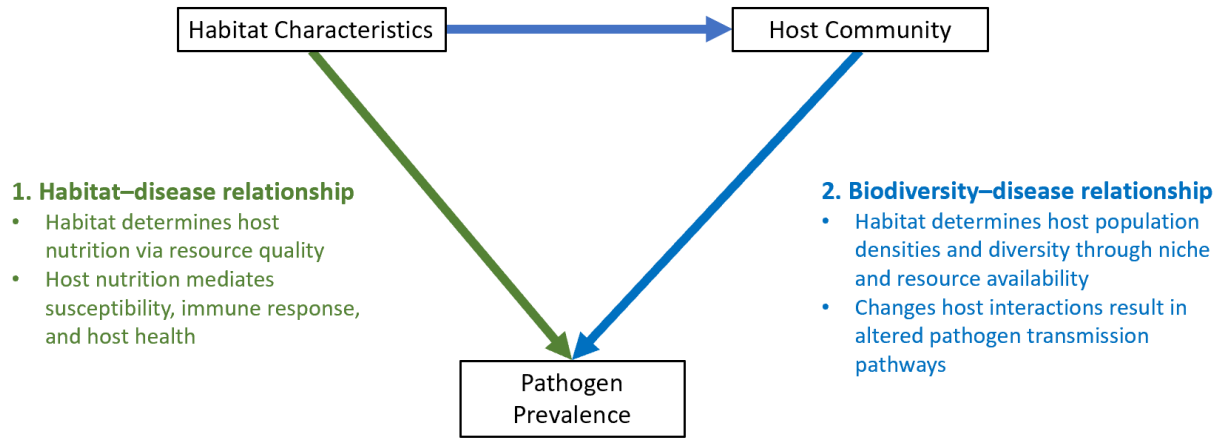


Figure 1.1: A conceptual diagram of the two proposed, non-mutually exclusive pathways that habitat characteristics are linked with host communities and pathogen prevalence: 1) Habitat–disease relationship and 2) Biodiversity–disease relationship.

Study system: Pollinators and their viruses

Pollinator communities and their pathogens are an ideal multi-host–multi-pathogen system to investigate how patterns of pathogen prevalence vary among hosts, pathogens, communities, and environments for several key reasons. First, several viruses are known to spill over between many diverse bee species through interactions on shared flowers (Singh et al. 2010, Mazzei et al. 2014, McArt et al. 2014, Manley et al. 2015). However, different bee species vary in exposure, susceptibility, and host quality for the pathogens, resulting in variable virus prevalence among hosts. Second, the composition of pollinator communities is highly variable depending on their environmental context (Potts et al. 2003, 2010, Kennedy et al. 2013, Koh et al. 2016), which has important implications for pollinator interactions on shared flowers and pathogen transmission (McArt et al. 2014, Alger et al. 2019, Figueroa et al. 2019, Truitt et al. 2019). Throughout my dissertation, I use the term 'pollinator community' to refer primarily to Hymenopteran bee and wasp pollinators that commonly share flowers and pathogens within a local area (Singh et al. 2010, Evison et al. 2012, Levitt et al. 2013, Manley et al. 2015), and exclude other pollinating taxa, such as flies and birds that are less susceptible to bee pathogens (but see Bailes et al. 2018). Finally, pollinator nutrition, body condition, immune function, and susceptibility to infectious disease are highly linked and depend on the quality of the surrounding environment (Alaux et al. 2010, DeGrandi-Hoffman et al. 2010, Di Pasquale et al. 2013, Donkersley et al. 2014, DeGrandi-Hoffman and Chen 2015). Therefore, this pollinator study system will elucidate the important interactions among pollinator communities, the environment, and pathogen prevalence within several related host species and multiple pathogens.

Three widespread multi-host viruses that infect bees

Bee pollinators, especially honey bees (*Apis mellifera*), are infected with a variety of pathogens and parasites that are contributing to current population declines worldwide, including at least 24 known viruses (Potts et al. 2010, Evison et al. 2012, Vanbergen 2013, Fürst et al. 2014, McMenamin and Genersch 2015, Meeus et al. 2018). In particular, three RNA viruses, deformed wing virus (DWV), black queen cell virus (BQCV), and sacbrood virus (SBV), are globally widespread due to the transportation of managed honey bees (Manley et al. 2015, Wilfert et al. 2016). These pathogens are shared among honey bees and many native bee species, and are considered recent emerging infectious diseases (Potts et al. 2010, Manley et al. 2015, McMenamin and Genersch 2015, Wilfert et al. 2016). These viruses have been well studied in honey bees, and all three can infect all stages of development in honey bees (Chen and Siede 2007). DWV infections cause crumpled and deformed wings, body discoloration, and early death in newly emerged adult honey bees that were infected as larva (**Figure 1.2A**). BQCV typically only cause symptoms in honey bee queen larvae and pupae, turning them black and killing them quickly (**Figure 1.2B**). Honey bee workers can become infected with BQCV, but are usually asymptomatic. SBV infected larva become discolored and filled with fluid, which kills the larva and gives it a sac-like appearance (**Figure 1.2C**). These viruses typically only show symptoms and cause mortality in early developmental stages (e.g. larvae or pupae) in honey bees (Chen et al. 2006, Chen and Siede 2007) (**Figure 1.2**). However, for all three viruses, adult bees can become infected as well, but they often do *not* show any obvious symptoms besides a slightly reduced lifespan (Chen and Siede 2007). Therefore, infected adult bees can still fly and forage on flowers, further spreading the viruses to other bees. However, the mortality and fitness effects of DWV, BQCV, and SBV have been less well-studied among most native bees. It is unclear if all

bee species respond identically, but initial evidence suggests that the viruses may have variable virulence among native bee species (Genersch et al. 2011, Yang et al. 2013, Fürst et al. 2014, Dolezal et al. 2016, Graystock et al. 2016).

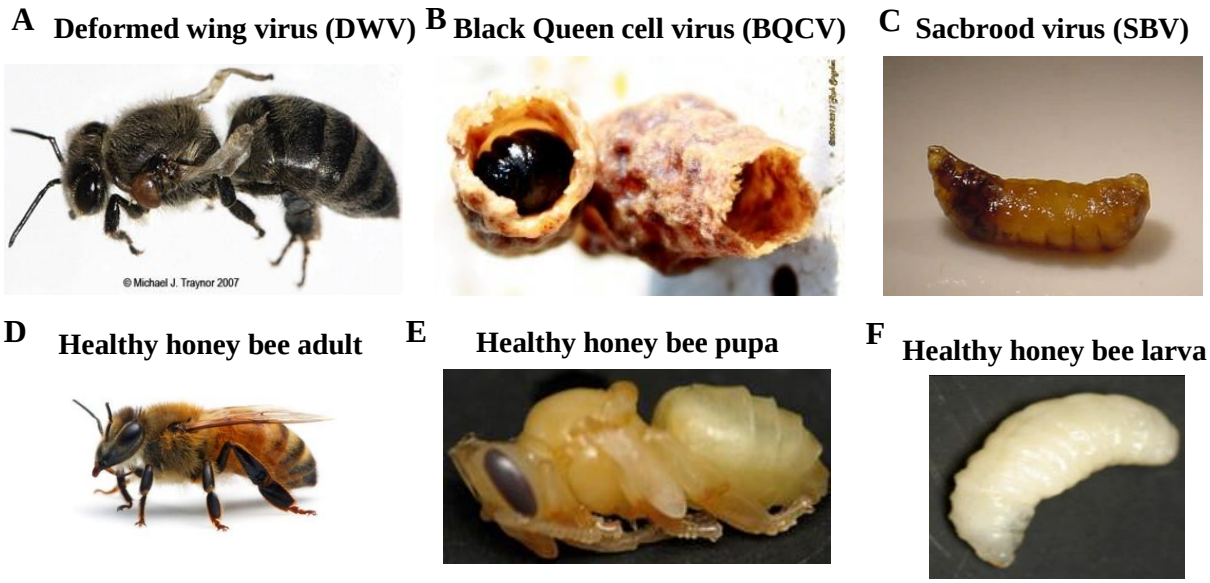


Figure 1.2: A) A young adult honey bee killed by deformed wing virus (DWV) infection and showing the characteristic deformed wings. **B)** A honey bee queen pupa that has turned black and died in her specialized queen cell due to black queen cell virus (BQCV). **C)** A honey bee larva infected with sacbrood virus (SBV) has become a brown and semi-transparent bag of water. For comparison, healthy honey bee **D)** adult, **E)** pupa, and **F)** larva. Note that honey bee queen pupae develop in a specialized cell as shown in B). Image sources: A) © Michael J. Traynor B) © Rob Snyder C) University of Georgia Honey Bee Program, D) ©Alex Wild, E) and F) photographs by Jason Graham and Ashley N. Mortensen, University of Florida.

Pollinator pathogens are a convenient study system because large sample sizes can be collected from the field, and simple molecular methods can be used to determine whether pollinator hosts have been exposed and are actively infected with these positive-strand RNA viruses. Bees that have been exposed to the virus will have the viral positive-strand RNA present in their gut. Viral presence is detected through reverse-transcription polymerase chain reaction (RT-PCR) with virus-specific primers, which amplifies a segment of the viral genome that can subsequently be sequenced to confirm viral identity (Benjeddou et al. 2001, Singh et al. 2010). It is important to distinguish that while virus presence is not necessarily indicative of an active

viral infection in bee hosts, it does indicate that viral exposure has occurred recently and sufficient viral load to be detectable by molecular methods (Ongus et al. 2004, Yue and Genersch 2005). In actively infected bees, the virus enters cells in the gut, and the positive-strand of the virus is copied to create the complementary negative-strand. The negative-strand is then used as a template to produce new positive-strand viral sequences for the viral progeny (Ongus et al. 2004, Yue and Genersch 2005). Therefore, presence of the negative-strand indicates that the virus is actively replicating and infecting the host (Ongus et al. 2004, Yue and Genersch 2005). Similar detection methods are used for the viral negative-strand with additional negative-strand specific primers (Yue and Genersch 2005, Peng et al. 2011, Gong et al. 2016). Throughout my dissertation, I will use the term "virus prevalence" to refer to tests of the presence of the virus positive-strand in pollinator hosts, and "infection prevalence" to refer to tests of active viral infections indicated by the presence of the negative-strand of the virus.

Virus prevalence varies among pollinator species

DWV, BQCV, and SBV are present and can actively infect many diverse species of bees and wasps around the world, likely spread by the global movement of honey bee colonies (Singh et al. 2010, Levitt et al. 2013, Fürst et al. 2014, Manley et al. 2015, McMahon et al. 2015, Dolezal et al. 2016, Wilfert et al. 2016, Alger et al. 2019). RNA viruses are particularly capable of shifting host species; high mutation rates and short replication times make RNA viruses highly adaptable to new host environments (Domingo and Holland 1997, Woolhouse et al. 2005). However, evidence from phylogenetic studies shows that viral sequences from different host species are almost identical, indicating that the viruses can move quickly among host species with little mutation required to shift to new host species (Singh et al. 2010, Genersch et al. 2011, Yang et al. 2013, Levitt et al. 2013, Fürst et al. 2014, McMahon et al. 2015, Radzevičiūtė et al.

2017, Bailes et al. 2018). Many native pollinator communities have only recently been exposed to DWV, BQCV, and SBV, and therefore have not adapted to these pathogens (Manley et al. 2015). This potentially leaves native bees at high risk for severe infections and widespread prevalence. However, the viral host ranges are not well understood because many native bees have not been tested, and few studies have critically evaluated how virus prevalence differs among a wide diversity of host species. Further, it is poorly understood how the pollinator community or environmental context may influence levels of virus prevalence among host species.

Though current data on prevalence for DWV, BQCV, and SBV among hosts is limited, we do know that virus prevalence varies among different pollinator groups and among the three viruses: DWV, BQCV, and SBV (**Figure 1.3**) (Manley et al. 2015, Dolezal et al. 2016). Variation in virus prevalence among hosts likely represents differences in host quality from the pathogen's perspective (i.e. host competence), due to differences in host exposure, susceptibility, and tolerance to infection. Honey bees (*Apis mellifera*) are commonly infected and have very high DWV, BQCV, and SBV prevalence (Chen et al. 2005, Singh et al. 2010, Fürst et al. 2014, McMahon et al. 2015, Dolezal et al. 2016). Closely related bumblebee species (*Bombus* spp.) also tend to have relatively high virus prevalence, but usually lower than honey bee virus prevalence (Evison et al. 2012, Fürst et al. 2014, Dolezal et al. 2016). Current data suggest that many other native bee species tend to have lower prevalence compared to honey bees and bumblebees (Singh et al. 2010, Evison et al. 2012, Dolezal et al. 2016) (**Figure 1.3A**). However, in most studies to date, native species are grouped together by family or broader categories based on ecological traits (e.g. solitary species), or have a limited number of individuals per species tested for virus prevalence. These metrics do not provide an accurate measure of virus

prevalence to compare among different bee species. Furthermore, most native bee species have never been tested for the viruses at all (e.g. *Eucera pruinosa*, squash bee). Therefore, based on current data, it appears that virus prevalence among different bee species can be quite variable, but few native bee species have been rigorously tested to get accurate measures of virus prevalence. The variation in virus prevalence among different host species suggests that hosts differ in host competence for the pathogens, and pollinator communities composed of different species will likely vary in patterns of virus prevalence within those communities. Yet, comparisons of how virus prevalence differs among different pollinator communities have never been tested.

Variation in the patterns of DWV, BQCV, and SBV prevalence within the same sets of host species indicates that the three viruses may have different host ranges (i.e. the number and relatedness of host species that each virus can infect). Though honey bees are the primary host for all three viruses (Chen and Siede 2007), the extent to which each virus is capable of switching among hosts may be different. Based on current evidence, DWV appears to be a broad generalist pathogen that can infect honey bees, bumblebees, native bees in non-honey bee families, social wasps, hoverflies, and other insects associated with apiaries with high exposure to DWV (Singh et al. 2010, Levitt et al. 2013, Manley et al. 2015, Dolezal et al. 2016). Furthermore, DWV prevalence consistently higher among all host species tested (Dolezal et al. 2016) (**Figure 1.3**). BQCV and SBV appear to have host ranges restricted primarily to honey bees and bumblebees, but can sometimes be found in hover flies and other social wasps (Singh et al. 2010, Levitt et al. 2013, Manley et al. 2015, Dolezal et al. 2016, Bailes et al. 2018). BQCV and SBV prevalence tends to be high in their primary hosts (e.g. honey bees and bumblebees), but appears to drop off rapidly for other host species when detected at all (Dolezal et al. 2016)

(**Figure 1.3**). These general patterns are intriguing and suggest that we may expect different patterns in virus prevalence among communities for the three different viruses. However, a general lack of thorough testing of virus prevalence in individual native bee species (see groupings by family in **Figure 1.3**) leaves some uncertainty of the actual host ranges for each of these viruses based on low resolution data.

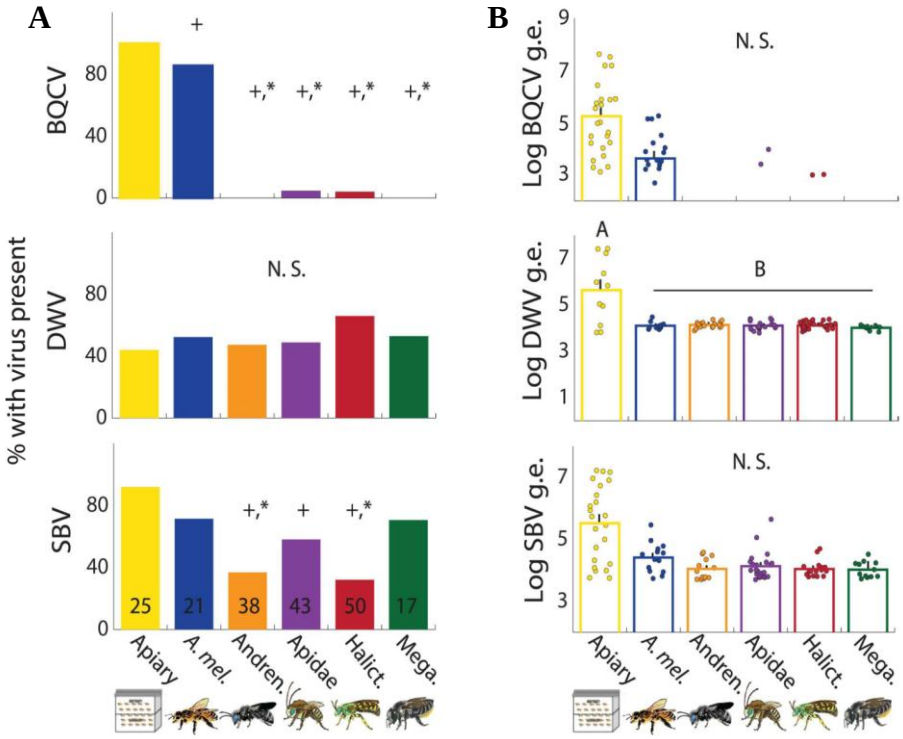


Figure 1.3: A) Black queen cell virus (BQCV), deformed wing virus (DWV), and sacbrood virus (SBV) prevalence and **B)** viral loads in *Apis mellifera* collected from apiaries (yellow) and foraging on flowers (blue), and in bees from the Andrenidae (orange), Apidae (purple), Halictidae (red), and Megachilidae (green) families. The * indicates significant difference from field collected honey bees (blue), and $^{+}$ denotes significant difference from the apiary collected honey bees (yellow). Prevalence is determined by the percent of samples that are virus positive. Viral load is the log of the estimated number of viral copies per sample determined by quantitative reverse-transcription polymerase chain reaction (qRT-PCR) and averaged among samples. Note that these findings are from a single study and may not represent prevalence and viral loads in these species in other regions. Figure modified from Dolezal et al. 2016.

Viral transmission via pollen on flowers

Flowers are believed to serve as a key site for viral transmission within pollinator communities. This is supported by the presence of DWV, BQCV, and SBV in pollen, bee

intestines, and feces (Chen et al. 2006, Singh et al. 2010, Mazzei et al. 2014, Alger et al. 2019). Further, when these viruses are transmitted horizontally between individuals, it primarily occurs through direct contact (e.g. saliva) and food-borne transmission (Chen et al. 2006, Singh et al. 2010). Therefore, horizontal transmission within and among species may be possible through interactions with infected bees or contaminated pollen on flowers (Singh et al. 2010, McArt et al. 2014). Nonetheless, intraspecific transmission is still likely much higher within the colonies of social bee species compared to the rates of interspecific transmission on flowers. Viruses have been detected on pollen from natural flowers as well as pollen collected from foraging bees (Singh et al. 2010, Mazzei et al. 2014, Alger et al. 2019). Furthermore, honey bees fed virus contaminated pollen became infected (Singh et al. 2010, Mazzei et al. 2014). However, there are still many unanswered questions due to the overall lack of experimental testing for the factors that could influence DWV, BQCV, and SBV horizontal transmission on flowers. We still do not know what proportion of bees become infected from visiting a contaminated flower, nor how bee visitation behavior may play a role in facilitating transmission. It is also unknown whether an infectious viral dose can accumulate on flowers or pollen. Experimental evidence for other bee parasites, *Crithidia bombi* and *Nosema ceranae*, have shown that infected bees transmit parasites to flowers, and subsequent healthy floral visitors can pick up parasites from the contaminated flowers (Ruiz-Gonzalez et al. 2012, Graystock et al. 2015, Figueroa et al. 2019). Furthermore, differences in bee foraging behaviors alter how microbes, and likely other pathogens, are dispersed within and among flowers (Russell et al. 2019). Therefore, the specific bee species visiting and pollinating flowers, and their likelihood of depositing viral particles onto the flower will be important factors to explore to better understand how interactions between pollinators on flowers may impact patterns of virus transmission and prevalence.

Environmental change at local and landscape scales alters pollinator communities and species interactions through non-random loss of species

Changing environments, particularly habitat loss, habitat degradation, and climate change, are important drivers of pollinator population declines and non-random patterns of species extirpation (Biesmeijer et al. 2006, Potts et al. 2010, Williams et al. 2010, Burkle et al. 2013, Kerr et al. 2015). Consequently, many studies have examined how local and landscape habitat factors influence pollinator communities and pollination services to agriculture. In general, greater availability of habitats that provide access to abundant and diverse flowers at local and landscape scales have important positive effects that increase pollinator community diversity and abundance, and pollinator visitation to flowers (Kremen et al. 2002, Klein et al. 2007, Ricketts et al. 2008, Lonsdorf et al. 2009, Garibaldi et al. 2011, Kennedy et al. 2013, Pardee and Philpott 2014, Rader et al. 2014). Specifically, at the landscape scale, a greater proportion of natural area (e.g. forest, grasslands, and wetlands) surrounding agricultural sites tends to increase pollinator community diversity and abundance (Ricketts et al. 2008, Lonsdorf et al. 2009, Kennedy et al. 2013, Shackelford et al. 2013). Additionally, Ricketts et al. (2008) found significant declines in pollinator species richness and pollination services provided to crops as the distance from natural habitat increased. At the local scale, areas with greater floral diversity of native plants correlated with increased wild bee species richness and increased density of honey bees and wild bees visiting wildflower patches (Blaauw and Isaacs 2014). Recent environmental changes, including conversion of natural areas into agricultural landscape, tend to reduce total natural area, diversity of land cover types, and local floral diversity (Kennedy et al. 2013). These changes diminish overall habitat quality for pollinators and pose

significant challenges to native pollinators that require access to specific floral resources only found in some habitat types (e.g. natural grasslands) (Kremen et al. 2007).

Furthermore, climate change also dramatically impacts pollinator species home ranges and patterns of visitation to flowers. The spatial distributions of bumblebee (*Bombus* spp.) home ranges in North America and Europe are contracting rapidly as global climate increases, resulting in the loss of species from the southern parts of their ranges (Kerr et al. 2015). Changing climate can also alter the phenology (i.e. timing) of plant and pollinator emergence, causing potential mis-matches between plants and their key pollinators in time and/or space (Burkle et al. 2013). Consequently, climate associated environmental features can result in significant changes in interaction networks of between bees on shared flowers, with significant potential changes to patterns of pathogen transmission.

Importantly, all these environmental changes result in non-random patterns of species loss from pollinator communities, as some bee species are more sensitive to environmental change than others. Specifically, pollinator species with a narrow diet breadth, above-ground nests, large body size, or solitary behavior are the first to be lost from pollinator communities in response to increasing agricultural intensity (Williams et al. 2010, Rader et al. 2014). Furthermore, rare pollinator species are extirpated from highly disturbed communities, while abundant species are capable of persisting despite the disturbance (Winfree et al. 2014). These findings suggest that there is non-random loss of pollinator species from the community in response to anthropogenic environmental change, and native bee species are disproportionately affected compared to honey bees. Additionally, honey bees are used to pollinate crop fields where pollination services from native pollinators have been lost or become unpredictable (Kremen et al. 2002). Therefore, highly disturbed communities are likely to be species poor and

dominated by honey bees (*A. mellifera*), while less disturbed pollinator communities are likely to have higher diversity and be composed of both abundant and rare native species, in addition to honey bees. Environmentally driven non-random patterns of species loss are important factors underlying changing pollinator communities and interactions among bees on shared flowers. Thus, these altered communities will likely have different patterns of pathogen prevalence and transmission, making pollinator communities an ideal model system to test how variation in community and environmental factors influence pathogen prevalence in multiple hosts.

The environment plays an important role in mediating pollinator health through nutrition and immune function

In addition to altering community structure, the surrounding environment can also have important effects on pollinator nutrition based on the diversity and abundance of flowers available. Pollinators depend on floral nectar and pollen as their primary sources of nutrition, and access to these resources at both local and landscape scales is important for pollinators to maintain adequate nourishment (Michener 2007, Vaudo et al. 2015). In particular, high-quality pollinator habitats include abundant and diverse flowers at the local scale, and greater natural area (i.e. forest, grassland, and wetlands) and greater diversity of land cover types at the landscape scale (Ebeling et al. 2008, Ricketts et al. 2008, Jha and Kremen 2013, Shackelford et al. 2013, Vaudo et al. 2016). Throughout the dissertation, I use 'habitat quality' to refer to habitat characteristics listed above that particularly benefit pollinator nutrition. Land-use changes, including conversion of natural areas to agriculturally intense landscapes (i.e. monoculture), generally decreases floral abundance and species richness and consequently decreases the nutrition quality of resources for bees (Biesmeijer et al. 2006, Winfree et al. 2011, Donkersley et al. 2014, Goulson et al. 2015). Though some agricultural landscapes can provide an abundance

of flowers, they may present only a single source of pollen or nectar, or only be present for a limited amount of time, both of which are insufficient to maintain pollinator health (Vaudo et al. 2015).

Furthermore, bees with access to high-quality resources will maintain better nutrition and body condition, which will allow them to resist or tolerate infections better (Dolezal and Toth 2018). Honey bees (*A. mellifera*) and bumblebees (*Bombus* spp.) with access to high-quality and diverse floral resources had increased colony growth, nutrition, and immunocompetence (DeGrandi-Hoffman et al. 2010, Alaux et al. 2011, Di Pasquale et al. 2013, Brunner et al. 2014, DeGrandi-Hoffman and Chen 2015, Vaudo et al. 2016). Additionally, honey bees experimentally fed diverse pollen diets had improved immune responses, and bees fed high protein diets had lower DWV loads compared to bees with poor-quality diets (Alaux et al. 2010, DeGrandi-Hoffman et al. 2010). Therefore, bees foraging on diverse flower sources may have improved nutrition and immune function, and lower pathogen loads.

Additionally, it is important to note that pollinators can also obtain plant secondary chemicals from plants, which can sometimes be used as medicines to improve immune function and/or reduce infection (Gherman et al. 2014, Gowler et al. 2015, Richardson et al. 2015, Palmer-Young et al. 2017, Koch et al. 2019). Understanding how bees may change their foraging behavior to prevent infection or self-medicate after infection could be critical to linking habitat characteristics with patterns of pathogen prevalence among different hosts. All together, these findings suggest that pollinator hosts in high-quality habitats may be able to maintain better body condition and immune function to resist becoming infected or tolerate infection better by reducing pathogen loads. Thus, high-quality habitat characteristics could be directly linked with pathogen prevalence via the habitat–disease relationship (**Figure 1.1, pathway 1**, described in

detail above). However, the direct link between habitat factors and virus prevalence indicative of the habitat–disease relationship remains untested in natural systems (Goulson et al. 2012, 2015, McArt et al. 2017), particularly in systems with multiple hosts and multiple pathogens. In my dissertation I show significant direct correlations between virus prevalence in pollinators and several habitat quality characteristics at the local and landscape scales indicative of the habitat–disease relationship, but I was unable to test for the mechanistic links of habitat characteristics on bee nutrition, immune function, and pathogen severity.

Importance of using a pollinator–pathogen system

Overall, pollinator health is influenced by a complex interplay between community structure, environmental characteristics, and species interactions (**Figure 1.1**). In this dissertation, I address the central challenge of identifying how these key factors interact and contribute to different patterns of pathogen prevalence for three viruses and among multiple pollinator hosts. Additionally, I expand upon existing theory for biodiversity–disease relationships by exploring how habitat characteristics may be directly linked with pollinator health (habitat–disease relationships) or indirectly linked with pathogen prevalence through altered pollinator communities (biodiversity–disease relationships). Furthermore, pollinator pathogen systems are an ideal multi-host–multi-pathogen system for exploring how differences in host or pathogen characteristics may alter the relationships among communities, environments, host interactions, and pathogen prevalence. These novel comparisons will further explore why pathogen prevalence varies among different host communities and among pathogens. In summary, my dissertation will improve our understanding of the important factors underlying differences in pathogen prevalence based on variable pollinator community and environmental contexts.

Dissertation Synopsis

Although widespread environmental change and biodiversity loss are linked with increased infectious disease in human and wildlife populations, we still lack a detailed understanding of how community and environmental factors influence pathogen prevalence. The central question of my dissertation asks, why does pathogen prevalence vary among communities? To address this question, I explore how host communities, the environment, and species interactions may influence patterns of pathogen prevalence among different communities of pollinator hosts for three multi-host viruses. Specifically, I conducted a broad field study to examine how pollinator communities, local and landscape scale habitat characteristics, and patterns of pollinator visitation to flowers affect the prevalence of multiple pathogens among multiple hosts.

Chapter 2 focuses on how different community factors, including host species richness, abundance, and community composition, impact DWV, BQCV, and SBV prevalence in four pollinator host species (*Apis mellifera*, *Bombus impatiens*, *Lasioglossum* spp. and *Eucera pruinosa*). First, I investigate how the prevalence of DWV, BQCV, and SBV differ among four pollinator host species to show that different hosts commonly share the three viruses, but vary substantially in prevalence among hosts. Second, I test how pathogen prevalence varies among pollinator communities that differ in species richness, relative abundance, and composition to determine which community factors are associated with differences in virus prevalence. Finally, I examine whether relationships between pathogen prevalence and community-level factors are similar among multiple hosts or multiple pathogens. This study represents the first examination of differences in pollinator pathogen prevalence among different pollinator communities, and demonstrates the remarkably consistent evidence of the dilution effect among multiple hosts and

pathogens. More broadly, it is one of the first studies to compare biodiversity–disease relationships in multiple similar pathogens that each infect multiple related host species to understand commonalities in how different community factors may correlate with pathogen prevalence among different hosts and pathogens. This chapter was initially submitted to *Ecology*, and given thorough and helpful reviews. Based on these reviews, I revised this chapter and will be resubmitting the manuscript to *Ecology*.

In Chapter 3, I build on the previous chapter by exploring whether local and landscape habitat characteristics associated with high-quality pollinator nutrition could be linked with differences in pollinator community diversity, pollinator abundance, and pathogen prevalence. Here, we present a new 'habitat–disease relationship', a non-mutually exclusive and complementary pattern to biodiversity–disease relationships, which predicts that habitat characteristics may directly mediate pathogen prevalence possibly though access to better nutritional resources and/or improve the immune response for hosts. I use a structural equation model to parse apart the relative importance of direct links between pathogen prevalence and habitat characteristics (habitat–disease relationship) to indirect links via changes in host diversity (biodiversity–disease relationship) while controlling for abundance. This study shows that both habitat–disease and biodiversity–disease relationships operate concurrently to mediate patterns of virus prevalence in pollinator communities, but the specific direct links between local and landscape scale habitat characteristics and virus prevalence varied among the three viruses. Habitat characteristics may be an important player in the complex interactions between hosts and pathogens, as a key driver of both changing species interactions in communities and mediating host susceptibility and response to infection.

In Chapter 4, I focus on how differences in host species interactions may drive pathogen prevalence patterns in different host communities and environments. Specifically, I quantify frequency and diversity of pollinator species visits to flowers among different communities, and test how the environment (habitat and climatic factors) influences pollinator interactions on flowers. I then explore the consequences of different pollinator visitation patterns on virus prevalence in two key bee hosts: *A. mellifera* (honey bees) and *B. impatiens* (bumblebees). Finally, I determine whether virus prevalence on flowers varies based on differences in the species richness of bee visits, honeybee and bumblebee visitation rates, and/or honey bee and bumblebee virus prevalence. This work begins to explore how small-scale interactions are affected by habitat and climatic environmental factors, and may scale up to influence broader patterns of pathogen prevalence in multiple host species.

Finally, in Chapter 5, I summarize the major conclusions of my dissertation and further discuss the implications of how pollinator communities, local and landscape habitat characteristics, and pollinator interactions on flowers are associated with patterns of pathogen prevalence. I conclude by briefly discussing potential future directions of this work to develop an understanding of the underlying mechanisms driving the intriguing patterns discovered in my dissertation.

Literature Cited

Alaux, C., F. Ducloz, D. Crauser, and Y. Le Conte. 2010. Diet effects on honeybee immunocompetence. Biology Letters 6:562–565.

Alaux, C., M. Folschweiller, C. McDonnell, D. Beslay, M. Cousin, C. Dussaubat, J.-L. Brunet, and Y. Le Conte. 2011. Pathological effects of the microsporidium Nosema ceranae on honey bee queen physiology (Apis mellifera). Journal of Invertebrate Pathology 106:380–5.

Alger, S. A., P. A. Burnham, H. F. Boncristiani, and A. K. Brody. 2019. RNA virus spillover from managed honeybees (Apis mellifera) to wild bumblebees (Bombus spp.). PLoS ONE 14:e0217822.

Allan, B. F., F. Keesing, R. S. Ostfeld, B. F. Allan, F. Keesing, and R. S. Ostfeldt. 2003. Effect of Forest Fragmentation on Lyme Disease Risk. Conservation Biology 17:267–272.

Allan, B. F., R. B. Langerhans, W. A. Ryberg, W. J. Landesman, N. W. Griffin, R. S. Katz, B. J. Oberle, M. R. Schutzenhofer, K. N. Smyth, A. D. S. Maurice, L. Clark, K. R. Crooks, D. E. Hernandez, R. G. Mclean, R. S. Ostfeld, and J. M. Chase. 2009. Ecological Correlates of Risk and Incidence of West Nile Virus in the United States. Oecologia 158:699–708.

Altizer, S., R. S. Ostfeld, P. T. J. Johnson, S. Kutz, and C. D. Harvell. 2013. Climate change and infectious diseases: from evidence to a predictive framework. Science 341:514–9.

Anderson, R. M., and R. M. May. 1981. The population dynamics of microparasites and their invetrebrate hosts. Philosophical Transactions of the Royal Society B: Biological Sciences 291:451–524.

Anderson, R. M., and R. M. May. 1991. Infectious Diseases of Humans. Oxford University Press, Oxford, UK.

Bailes, E. J., K. R. Deutsch, J. Bagi, L. Rondissone, M. J. F. Brown, and O. T. Lewis. 2018. First

detection of bee viruses in hoverfly (syrphid) pollinators. Biology Letters 14:20180001.

Becker, C. G., D. Rodriguez, L. F. Toledo, A. V. Longo, C. Lambertini, D. T. Correa, D. S. Leite, C. F. B. Haddad, and K. R. Zamudio. 2014. Partitioning the net effect of host diversity on an emerging amphibian pathogen. Proceedings of the Royal Society B: Biological Sciences 281:20141796.

Becker, D. J., D. G. Streicker, and S. Altizer. 2015. Linking anthropogenic resources to wildlife-pathogen dynamics: A review and meta-analysis. Ecology Letters 18:483–495.

Begon, M. 2008. Effects of host diversity on disease dynamics. Pages 12–29 *in* R. S. Ostfeld, F. Keesing, and V. T. Eviner, editors. Infectious Disease Ecology: Effects of Ecosystems on Disease and of Disease on Ecosystems. Princeton University Press, Princeton, NJ.

Benjeddou, M., N. Leat, M. Allsopp, and S. Davison. 2001. Detection of Acute Bee Paralysis Virus and Black Queen Cell Virus from Honeybees by Reverse Transcriptase PCR. Applied and Environmental Microbiology 67:2384–2387.

Biesmeijer, J. C., S. P. M. Roberts, M. Reemer, R. Ohlemuller, M. Edwards, T. Peeters, A. Schaffers, S. G. Potts, R. Kleukers, C. Thomas, J. Settele, and W. E. Kunin. 2006. Parallel Declines in Pollinators and Insect-Pollinated Plants in Britain and the Netherlands. Science 313:351–354.

Blaauw, B. R., and R. Isaacs. 2014. Larger patches of diverse floral resources increase insect pollinator density, diversity, and their pollination of native wildflowers. Basic and Applied Ecology 15:701–711.

Brunner, F. S., P. Schmid-Hempel, and S. M. Barribeau. 2014. Protein-poor diet reduces host-specific immune gene expression in Bombus terrestris. Proceedings of the Royal Society B: Biological Sciences 281:20140128.

Budria, A., and U. Candolin. 2014. How does human-induced environmental change influence host-parasite interactions? Parasitology 141:462–74.

Burkle, L. A., J. C. Marlin, and T. M. Knight. 2013. Plant-Pollinator Interactions over 120 Years: Loss of Species, Co-Occurrence, and Function. Science 339:1611–1615.

Cameron, S. A., J. D. Lozier, J. P. Strange, J. B. Koch, N. Cordes, L. F. Solter, T. L. Griswold, and G. E. Robinson. 2011. Patterns of widespread decline in North American bumbe bees. Proceedings of the National Academy of Sciences 108:662–667.

Chen, Y., J. Evans, and M. Feldlaufer. 2006. Horizontal and vertical transmission of viruses in the honey bee, Apis mellifera. Journal of Invertebrate Pathology 92:152–9.

Chen, Y. P., and R. Siede. 2007. Honey bee viruses. Pages 33–80 *in* K. Maramorosch, A. J. Shatkin, and F. A. Murphy, editors. Advances in Virus Research. First edition. Academic Press, San Diego, CA.

Chen, Y., J. S. Pettis, and M. F. Feldlaufer. 2005. Detection of multiple viruses in queens of the honey bee Apis mellifera L. Journal of Invertebrate Pathology 90:118–21.

Chesson, P. 2000. Mechanisms of maintenance of species diversity. Annual Review of Ecology and Systematics 31:343–366.

Civitello, D. J., J. Cohen, H. Fatima, N. T. Halstead, J. Liriano, T. A. McMahon, C. N. Ortega, E. L. Sauer, T. Sehgal, S. Young, and J. R. Rohr. 2015. Biodiversity inhibits parasites: Broad evidence for the dilution effect. Proceedings of the National Academy of Sciences 112:8667–8671.

Clay, C. A., E. M. Lehmer, S. St. Jeor, and M. D. Dearing. 2009a. Sin Nombre virus and rodent species diversity: A test of the dilution and amplification hypotheses. PLoS ONE 4:e6467.

Clay, C. A., E. M. Lehmer, A. Previtali, S. St Jeor, and M. D. Dearing. 2009b. Contact

heterogeneity in deer mice: Implications for Sin Nombre virus transmission. Proceedings of the Royal Society B: Biological Sciences 276:1305–1312.

Contini, C., M. Di Nuzzo, N. Barp, A. Bonazza, R. De Giorgio, M. Tognon, and S. Rubino. 2020. The novel zoonotic COVID-19 pandemic: An expected global health concern. The Journal of Infection in Developing Countries 14:254–264.

Coop, R. L., and I. Kyrizakis. 1999. Nutrition and Parasite Interaction. Veterinary Parasitology 84:187–204.

Curl, E. A. 1963. Control of plant diseases by crop rotations. Botanical Review 29:413–479.

Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2000. Emerging infectious diseases of wildlife - threats to biodiversity and human health. Science 287:443–449.

Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2001. Anthropogenic environmental change and the emergence of infectious diseases in wildlife. Acta Tropica 78:103–116.

Dearing, M. D., and L. Dizney. 2010. Ecology of hantavirus in a changing world. Annals of the New York Academy of Sciences 1195:99–112.

DeGrandi-Hoffman, G., and Y. Chen. 2015. Nutrition, immunity and viral infections in honey bees. Current Opinion in Insect Science 10:170–176.

DeGrandi-Hoffman, G., Y. Chen, E. Huang, and M. H. Huang. 2010. The effect of diet on protein concentration, hypopharyngeal gland development and virus load in worker honey bees (Apis mellifera L.). Journal of Insect Physiology 56:1184–1191.

Dizney, L., and M. D. Dearing. 2013. The role of behavioural heterogeneity on infection patterns: Implications for pathogen transmission. Animal Behaviour 86:911–916.

Dizney, L., and M. D. Dearing. 2016. Behavioural differences: A link between biodiversity and pathogen transmission. Animal Behaviour 111:341–347.

Dizney, L. J., and L. A. Ruedas. 2009. Increased host species diversity and decreased prevalence of sin nombre virus. Emerging Infectious Diseases 15:1012–1018.

Dobson, A. 2004. Population dynamics of pathogens with multiple host species. The American Naturalist 164 Suppl:S64–S78.

Dobson, A. P., R. M. Holdo, and R. D. Holt. 2011. Rinderpest. Page *in* D. Simberloff and M. Rejmanek, editors. Encyclopedia of biological invasions. University of California Press, Berkeley, CA.

Dolezal, A. G., S. D. Hendrix, N. A. Scavo, J. Carrillo-Tripp, M. A. Harris, M. J. Wheelock, M. E. O'Neal, and A. L. Toth. 2016. Honey Bee Viruses in Wild Bees: Viral Prevalence, Loads, and Experimental Inoculation. PloS ONE 11:e0166190.

Dolezal, A. G., and A. L. Toth. 2018. Feedbacks between nutrition and disease in honey bee health. Current Opinion in Insect Science 26:114–119.

Domingo, E., and J. J. Holland. 1997. RNA virus mutations and fitness for survival. Annual Review of Microbiology 51:151–178.

Donkersley, P., G. Rhodes, R. W. Pickup, K. C. Jones, and K. Wilson. 2014. Honeybee nutrition is linked to landscape composition. Ecology and Evolution 4:4195–206.

Ebeling, A., A. M. Klein, J. Schumacher, W. W. Weisser, and T. Tscharntke. 2008. How does plant richness affect pollinator richness and temporal stability of flower visits? Oikos 117:1808–1815.

Elton, C. S. 1958. The Ecology of Invasions by Animals and Plants. University of Chicago Press, Chicago, IL.

Estrada-Peña, A. 2009. Diluting the dilution effect: A spatial Lyme model provides evidence for the importance of habitat fragmentation with regard to the risk of infection. Geospatial

Health 3:143–155.

Estrada-Peña, A., R. S. Ostfeld, A. T. Peterson, R. Poulin, and J. de la Fuente. 2014. Effects of environmental change on zoonotic disease risk: An ecological primer. Trends in Parasitology 30:205–214.

Evison, S. E. F., K. E. Roberts, L. Laurenson, S. Pietravalle, J. Hui, J. C. Biesmeijer, J. E. Smith, G. Budge, and W. O. H. Hughes. 2012. Pervasiveness of Parasites in Pollinators. PLoS ONE 7:e30641.

Ezenwa, V. O. 2004. Interactions among host diet, nutritional status and gastrointestinal parasite infection in wild bovids. International Journal for Parasitology 34:535–542.

Ezenwa, V. O., M. S. Godsey, R. J. King, and S. C. Guptill. 2006. Avian diversity and West Nile virus: Testing associations between biodiversity and infectious disease risk. Proceedings of the Royal Society B: Biological Sciences 273:109–117.

Fahrig, L. 2003. Effects of Habitat Fragmentation on Biodiversity. Annual Review of Ecology, Evolution, and Systematics 34:487–515.

Fenton, A., D. G. Streicker, O. L. Petchey, and A. B. Pedersen. 2015. Are All Hosts Created Equal? Partitioning Host Species Contributions to Parasite Persistence in Multihost Communities. The American Naturalist 186:610–622.

Figueroa, L. L., M. Blinder, C. Grincavitch, A. Jelinek, E. K. Mann, L. A. Merva, L. E. Metz, A. Y. Zhao, R. E. Irwin, S. H. McArt, and L. S. Adler. 2019. Bee pathogen transmission dynamics: Deposition, persistence and acquisition on flowers. Proceedings of the Royal Society B: Biological Sciences 286.

Fisher, M. C., T. W. J. Garner, and S. F. Walker. 2009. Global Emergence of *Batrachochytrium dendrobatidis* and Amphibian Chytridiomycosis in Space, Time, and Host. Annual Review

of Microbiology 63:291–310.

Foley, J. A., R. DeFries, G. P. Asner, C. Barford, G. Bonan, S. R. Carpenter, F. S. Chapin, M. T. Coe, G. C. Daily, H. K. Gibbs, J. H. Helkowski, T. Holloway, E. A. Howard, C. J. Kucharik, C. Monfreda, J. A. Patz, I. C. Prentice, N. Ramankutty, and P. K. Snyder. 2005. Global consequences of land use. Science 309:570–574.

Fürst, M. A., D. P. McMahon, J. L. Osborne, R. J. Paxton, and M. J. F. Brown. 2014. Disease associations between honeybees and bumblebees as a threat to wild pollinators. Nature 506:364–366.

Garibaldi, L. A., I. Steffan-Dewenter, C. Kremen, J. M. Morales, R. Bommarco, S. A. Cunningham, L. G. Carvalheiro, N. P. Chacoff, J. H. Dudenhöffer, S. S. Greenleaf, A. Holzschuh, R. Isaacs, K. Krewenka, Y. Mandelik, M. M. Mayfield, L. A. Morandin, S. G. Potts, T. H. Ricketts, H. Szentgyörgyi, B. F. Viana, C. Westphal, R. Winfree, and A. M. Klein. 2011. Stability of pollination services decreases with isolation from natural areas despite honey bee visits. Ecology Letters 14:1062–1072.

Gause, G. F. 1932. Experimental Studies on the Struggle for Existence: I. Mixed Population of Two Species of Yeast. Journal of Experimental Biology 9:389–402.

Genersch, E., C. Yue, I. Fries, and J. R. De Miranda. 2011. Detection of Deformed wing virus, a honey bee viral pathogen, in bumble bees (Bombus terrestris and Bombus pascuorum) with wing deformities. Journal of Invertebrate Pathology 91:61–63.

Gherman, B. I., A. Denner, O. Bobiş, D. S. Dezmirean, L. a. Mărghitaş, H. Schlüns, R. F. a. Moritz, and S. Erler. 2014. Pathogen-associated self-medication behavior in the honeybee Apis mellifera. Behavioral Ecology and Sociobiology 68:1777–1784.

Gire, S. K., A. Goba, K. G. Andersen, R. S. G. Sealfon, D. J. Park, L. Kanneh, S. Jalloh, M.

Momoh, M. Fullah, G. Dudas, S. Wohl, L. M. Moses, N. L. Yozwiak, S. Winnicki, C. B. Matranga, C. M. Malboeuf, J. Qu, A. D. Gladden, S. F. Schaffner, X. Yang, P. Jiang, M. Nekoui, A. Colubri, M. R. Coomber, M. Fonnie, A. Moigboi, M. Gbakie, F. K. Kamara, V. Tucker, E. Konuwa, S. Saffa, J. Sellu, A. A. Jalloh, A. Kovoma, J. Koninga, I. Mustapha, K. Kargbo, M. Foday, M. Yillah, F. Kanneh, W. Robert, J. L. B. Massally, S. B. Chapman, J. Bochicchio, C. Murphy, C. Nusbaum, S. Young, B. W. Birren, D. S. Grant, J. S. Scheiffelin, and E. S. Lander. 2014. Genomic surveillance elucidates Ebola virus origin and transmission during the 2014 outbreak. Science 345:1369–1372.

Gong, H. R., X. X. Chen, Y. P. Chen, F. L. Hu, J. L. Zhang, Z. G. Lin, J. W. Yu, and H. Q. Zheng. 2016. Evidence of *Apis cerana* Sacbrood virus infection in *Apis mellifera*. Applied and Environmental Microbiology 82:2256–2262.

Goulson, D., E. Nicholls, C. Botías, and E. L. Rotheray. 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science 347:1255957.

Goulson, D., P. Whitehorn, and M. Fowley. 2012. Influence of urbanisation on the prevalence of protozoan parasites of bumblebees. Ecological Entomology 37:83–89.

Gowler, C. D., K. E. Leon, M. D. Hunter, and J. C. de Roode. 2015. Secondary Defense Chemicals in Milkweed Reduce Parasite Infection in Monarch Butterflies, Danaus plexippus. Journal of Chemical Ecology 41:520–523.

Graystock, P., D. Goulson, and W. O. H. Hughes. 2015. Parasites in bloom: flowers aid dispersal and transmission of pollinator parasites within and between bee species. Proceedings of the Royal Society Biological Sciences 282:20151371.

Graystock, P., I. Meeus, G. Smagghe, D. Goulson, and W. O. H. Hughes. 2016. The effects of single and mixed infections of Apicystis bombi and deformed wing virus in Bombus

terrestris. Parasitology 143:358–365.

Gutiérrez-Bugallo, G., L. A. Piedra, M. Rodriguez, J. A. Bisset, R. Lourenço-de-Oliveira, S. C. Weaver, N. Vasilakis, and A. Vega-Rúa. 2019. Vector-borne transmission and evolution of Zika virus. Nature Ecology and Evolution 3:561–569.

Harvell, C. D., K. Kim, J. M. Burkholder, R. R. Colwell, P. R. Epstein, D. J. Grimes, E. E. Hofmann, E. K. Lipp, A. D. M. E. Osterhaus, R. M. Overstreet, J. W. Porter, G. W. Smith, and G. R. Vasta. 1999. Emerging Marine Disease--Climate Links and Anthropogenic Factors. Science 285:1505–1510.

HilleRisLambers, J., P. B. Adler, W. S. Harpole, J. M. Levine, and M. M. Mayfield. 2012. Rethinking Community Assembly through the Lens of Coexistence Theory. Annual Review of Ecology, Evolution, and Systematics 43:227–248.

Holdo, R. M., A. R. E. Sinclair, A. P. Dobson, K. L. Metzger, B. M. Bolker, M. E. Ritchie, and R. D. Holt. 2009. A disease-mediated trophic cascade in the Serengeti and its implications for ecosystem C. PLoS Biology 7:e1000210.

Holt, R. D., A. P. Dobson, M. Begon, R. G. Bowers, and E. M. Schauber. 2003. Parasite establishment in host communities. Ecology Letters 6:837–842.

Huang, Z. Y. X., F. van Langevelde, A. Estrada-Peña, G. Suzán, and W. F. de Boer. 2016. The diversity–disease relationship: evidence for and criticisms of the dilution effect. Parasitology 143:1075–1086.

Jha, S., and C. Kremen. 2013. Resource diversity and landscape-level homogeneity drive native bee foraging. Proceedings of the National Academy of Sciences 110:555–8.

Johnson, P. T. J., R. B. Hartson, D. J. Larson, and D. R. Sutherland. 2008. Diversity and disease: community structure drives parasite transmission and host fitness. Ecology Letters

11:1017–1026.

Johnson, P. T. J., R. S. Ostfeld, and F. Keesing. 2015a. Frontiers in research on biodiversity and disease. Ecology Letters 18:1119–1133.

Johnson, P. T. J., D. L. Preston, J. T. Hoverman, and B. E. Lafonte. 2013a. Host and parasite diversity jointly control disease risk in complex communities. Proceedings of the National Academy of Sciences 110:16916–16921.

Johnson, P. T. J., D. L. Preston, J. T. Hoverman, and K. L. D. Richgels. 2013b. Biodiversity decreases disease through predictable changes in host community competence. Nature 494:230–3.

Johnson, P. T. J., J. C. de Roode, and A. Fenton. 2015b. Why infectious disease research needs community ecology. Science 349:1259504-1–9.

Johnson, P. T. J., and D. W. Thieltges. 2010. Diversity, decoys and the dilution effect: how ecological communities affect disease risk. The Journal of Experimental Biology 213:961–970.

Jones, K., N. Patel, M. Levy, A. Storeygard, D. Balk, J. Gittleman, and P. Daszak. 2008. Global trends in emerging infectious diseases. Nature 451:990–993.

Keesing, F., L. K. Belden, P. Daszak, A. Dobson, C. D. Harvell, R. D. Holt, P. Hudson, A. Jolles, K. E. Jones, C. E. Mitchell, S. S. Myers, T. Bogich, and R. S. Ostfeld. 2010. Impacts of biodiversity on the emergence and transmission of infectious diseases. Nature 468:647–52.

Keesing, F., R. D. Holt, and R. S. Ostfeld. 2006. Effects of species diversity on disease risk. Ecology Letters 9:485–98.

Kennedy, C. M., E. Lonsdorf, M. C. Neel, N. M. Williams, T. H. Ricketts, R. Winfree, R.

Bommarco, C. Brittain, A. L. Burley, D. Cariveau, L. G. Carvalheiro, N. P. Chacoff, S. A. Cunningham, B. N. Danforth, J. H. Dudenhöffer, E. Elle, H. R. Gaines, L. A. Garibaldi, C. Gratton, A. Holzschuh, R. Isaacs, S. K. Javorek, S. Jha, A. M. Klein, K. Krewenka, Y. Mandelik, M. M. Mayfield, L. Morandin, L. A. Neame, M. Otieno, M. Park, S. G. Potts, M. Rundlöf, A. Saez, I. Steffan-Dewenter, H. Taki, B. F. Viana, C. Westphal, J. K. Wilson, S. S. Greenleaf, and C. Kremen. 2013. A global quantitative synthesis of local and landscape effects on wild bee pollinators in agroecosystems. Ecology Letters 16:584–599.

Kerr, J. T., A. Pindar, P. Galpern, L. Packer, S. G. Potts, S. M. Roberts, P. Rasmont, O. Schweiger, S. R. Colla, L. L. Richardson, D. L. Wagner, and L. F. Gall. 2015. Climate change impacts on bumblebees converge across continents. Science 349:177–180.

Kilpatrick, A. M., P. Daszak, M. J. Jones, P. P. Marra, and L. D. Kramer. 2006. Host heterogeneity dominates West Nile virus transmission. Proceedings of the Royal Society B: Biological Sciences 273:2327–2333.

Kilpatrick, A. M., D. J. Salkeld, G. Titcomb, and M. B. Hahn. 2017a. Conservation of biodiversity as a strategy for improving human health and well-being. Philosophical Transactions of the Royal Society B 372:20160131.

Kilpatrick, M. A., A. D. M. Dobson, T. Levi, D. J. Salkeld, A. Swei, H. S. Ginsberg, A. Kjemtrup, K. Padgett, P. Jensen, D. Fish, and M. Diuk-Wasser. 2017b. Lyme disease ecology: concensus, uncertainty, and critical gaps for improving control. Phil Trans R Soc Lond B 372:20160117.

Kim, K., and C. D. Harvell. 2004. The Rise and Fall of a Six-Year Coral-Fungal Epizootic. The American Naturalist 164:S52–S63.

Klasing, K. C. 2007. Nutrition and the immune system. British Poultry Science 48:525–537.

Klein, A.-M., B. E. Vaissière, J. H. Cane, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, and T. Tscharntke. 2007. Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society Biological Sciences 274:303–313.

Koch, H., J. Woodward, M. K. Langat, M. J. F. Brown, and P. C. Stevenson. 2019. Flagellum Removal by a Nectar Metabolite Inhibits Infectivity of a Bumblebee Parasite. Current Biology 29:3494-3500.e5.

Koh, I., E. V Lonsdorf, N. M. Williams, C. Brittain, R. Isaacs, J. Gibbs, and T. H. Ricketts. 2016. Modeling the status, trends, and impacts of wild bee abundance in the United States. Proceedings of the National Academy of Sciences 113:140–145.

Kraft, N. J. B., P. B. Adler, O. Godoy, E. C. James, S. Fuller, and J. M. Levine. 2015. Community assembly, coexistence and the environmental filtering metaphor. Functional Ecology 29:592–599.

Kremen, C., N. M. Williams, M. A. Aizen, B. Gemmill-Herren, G. LeBuhn, R. Minckley, L. Packer, S. G. Potts, T. Roulston, I. Steffan-Dewenter, D. P. Vázquez, R. Winfree, L. Adams, E. E. Crone, S. S. Greenleaf, T. H. Keitt, A. M. Klein, J. Regetz, and T. H. Ricketts. 2007. Pollination and other ecosystem services produced by mobile organisms: A conceptual framework for the effects of land-use change. Ecology Letters 10:299–314.

Kremen, C., N. M. Williams, and R. W. Thorp. 2002. Crop pollination from native bees at risk from agricultural intensification. Proceedings of the National Academy of Sciences 99:16812–16816.

Lafferty, K. D., and C. L. Wood. 2013. It's a myth that protection against disease is a strong and general service of biodiversity conservation: Response to Ostfeld and Keesing. Trends in Ecology and Evolution 28:503–504.

Langlois, J. P., L. Fahrig, G. Merriam, and H. Artsob. 2001. Landscape structure influences continental distribution of hantavirus in deer mice. Landscape Ecology 16:255–266.

Lee, K. P., J. S. Cory, K. Wilson, D. Raubenheimer, and S. J. Simpson. 2006. Flexible diet choice offsets protein costs of pathogen resistance in a caterpillar. Proceedings of the Royal Society B: Biological Sciences 273:823–829.

Lefèvre, T., L. Oliver, M. D. Hunter, and J. C. De Roode. 2010. Evidence for trans-generational medication in nature. Ecology Letters 13:1485–1493.

De León, L. F., J. Podos, T. Gardezi, A. Herrel, and A. P. Hendry. 2014. Darwin's finches and their diet niches: The sympatric coexistence of imperfect generalists. Journal of Evolutionary Biology 27:1093–1104.

Lessios, H. A. 1988. Mass Mortality of Diadema Antillarum in the Caribbean: What Have We Learned? Annual Review of Ecology and Systematics 19:371–393.

Levi, T., F. Keesing, R. D. Holt, M. Barfield, and R. S. Ostfeld. 2016. Quantifying dilution and amplification in a community of hosts for tick-borne pathogens. Ecological Applications 26:484–498.

Levitt, A. L., R. Singh, D. L. Cox-Foster, E. Rajotte, K. Hoover, N. Ostiguy, and E. C. Holmes. 2013. Cross-species transmission of honey bee viruses in associated arthropods. Virus Research 176:232–240.

Li, W., S.-K. Wong, F. Li, J. H. Kuhn, I.-C. Huang, H. Choe, and M. Farzan. 2006. Animal Origins of the Severe Acute Respiratory Syndrome Coronavirus: Insight from ACE2-S-Protein Interactions. Journal of Virology 80:4211–4219.

Liebold, M. A. 1997. Similarity and local co-existence of species in regional biotas. Evolutionary Ecology 29:95–110.

LoGiudice, K., R. S. Ostfeld, K. A. Schmidt, and F. Keesing. 2003. The ecology of infectious disease: effects of host diversity and community composition on Lyme disease risk. Proceedings of the National Academy of Sciences 100:567–71.

Lonsdorf, E., C. Kremen, T. Ricketts, R. Winfree, N. Williams, and S. Greenleaf. 2009. Modelling pollination services across agricultural landscapes. Annals of Botany 103:1589–1600.

Luis, A. D., A. J. Kuenzi, and J. N. Mills. 2018. Species diversity concurrently dilutes and amplifies transmission in a zoonotic host–pathogen system through competing mechanisms. Proceedings of the National Academy of Sciences 115:7979–7984.

MacArthur, R. H. 2009. Population Ecology of Some Warblers of Northeastern Coniferous Forests. Population Ecology 39:599–619.

Manley, R., M. Boots, and L. Wilfert. 2015. Emerging viral disease risk to pollinating insects: ecological, evolutionary and anthropogenic factors. Journal of Applied Ecology 10:1–10.

May, R. M., and R. M. Anderson. 1979. Population biology of infectious diseases: Part II. Nature 280:455–461.

Mazzei, M., M. L. Carrozza, E. Luisi, M. Forzan, M. Giusti, S. Sagona, F. Tolari, and A. Felicioli. 2014. Infectivity of DWV associated to flower pollen: experimental evidence of a horizontal transmission route. PloS ONE 9:e113448.

McArt, S. H., H. Koch, R. E. Irwin, and L. S. Adler. 2014. Arranging the bouquet of disease: Floral traits and the transmission of plant and animal pathogens. Ecology Letters 17:624–636.

McArt, S. H., C. Urbanowicz, S. McCoshum, R. E. Irwin, and L. S. Adler. 2017. Landscape predictors of pathogen prevalence and range contractions in US bumblebees. Proc. R. Soc.

B 284:20172181.

McCann, K. 2007. Protecting biostructure. Nature 446:29.

McKenzie, V. J. 2007. Human land use and patterns of parasitism in tropical amphibian hosts. Biological Conservation 137:102–116.

McMahon, D. P., M. A. Fürst, J. Caspar, P. Theodorou, M. J. F. Brown, and R. J. Paxton. 2015. A sting in the spit: widespread cross-infection of multiple RNA viruses across wild and managed bees. Journal of Animal Ecology 84:615–624.

McMenamin, A. J., and E. Genersch. 2015. Honey bee colony losses and associated viruses. Current Opinion in Insect Science 8:121–129.

Meeus, I., M. Pisman, G. Smagghe, and N. Piot. 2018. Interaction effects of different drivers of wild bee decline and their influence on host–pathogen dynamics. Current Opinion in Insect Science 26:136–141.

Michener, C. D. 2007. The Bees of the World. 2nd edition. John Hopkins University Press.

Mihaljevic, J. R., M. B. Joseph, S. A. Orlofske, and S. H. Paull. 2014. The scaling of host density with richness affects the direction, shape, and detectability of diversity-disease relationships. PLoS ONE 9:e97812.

Milne-Price, S., K. L. Miazgowicz, and V. J. Munster. 2014. The emergence of the Middle East Respiratory Syndrome coronavirus. Pathogens and Disease 71:121–136.

Mitchell, C. E., D. Tilman, and J. V. Groth. 2002. Effects of Grassland Plant Species Diversity, Abundance, and Composition on Foliar Fungal Disease. Ecology 83:1713–1726.

Navarro-Gonzalez, N., H. Verheyden, H. Hoste, B. Cargnelutti, B. Lourtet, J. Merlet, T. Daufresne, S. Lavín, A. J. M. Hewison, S. Morand, and E. Serrano. 2011. Diet quality and immunocompetence influence parasite load of roe deer in a fragmented landscape.

European Journal of Wildlife Research 57:639–645.

Neill, W. E. 1974. The Community Matrix and Interdependence of the Competition Coefficients. The American Naturalist 108:399–408.

Ongus, J. R., D. Peters, J. M. Bonmatin, E. Bengsch, J. M. Vlak, and M. M. van Oers. 2004. Complete sequence of a picorna-like virus of the genus Iflavirus replicating in the mite Varroa destructor. Journal of General Virology 85:3747–3755.

Ostfeld, R. S. 2013. A Candide response to Panglossian accusations by Randolph and Dobson: biodiversity buffers disease. Parasitology 140:1196–1198.

Ostfeld, R. S., and F. Keesing. 2000a. Biodiversity series: The function of biodiversity in the ecology of vector-borne zoonotic diseases. Canadian Journal of Zoology 78:2061–2078.

Ostfeld, R. S., and F. Keesing. 2000b. Biodiversity and Disease Risk: The Case of Lyme Disease. Conservation Biology 14:722–728.

Ostfeld, R. S., and K. LoGiudice. 2003. Community dissassembly, biodiversity loss, and the erosion of an ecosystem service. Ecology 84:1421–1427.

Palmer-Young, E. C., C. O. Tozkar, R. S. Schwarz, Y. Chen, R. E. Irwin, L. S. Adler, and J. D. Evans. 2017. Nectar and Pollen Phytochemicals Stimulate Honey Bee (Hymenoptera: Apidae) Immunity to Viral Infection. Journal of Economic Entomology 110:1959–1972.

Pardee, G. L., and S. M. Philpott. 2014. Native plants are the bee's knees: local and landscape predictors of bee richness and abundance in backyard gardens. Urban Ecosystems 17:641–659.

Parmesan, C. 2006. Ecological and Evolutionary Responses to Recent Climate Change. Annual Review of Ecology, Evolution, and Systematics 37:637–669.

Parrish, C. R., E. C. Holmes, D. M. Morens, E.-C. Park, D. S. Burke, C. H. Calisher, C. A.

Laughlin, L. J. Saif, and P. Daszak. 2008. Cross-Species Virus Transmission and the Emergence of New Epidemic Diseases. Microbiology and Molecular Biology Reviews 72:457–470.

Di Pasquale, G., M. Salignon, Y. Le Conte, L. P. Belzunces, A. Decourtye, A. Kretzschmar, S. Suchail, J. L. Brunet, and C. Alaux. 2013. Influence of Pollen Nutrition on Honey Bee Health: Do Pollen Quality and Diversity Matter? PLoS ONE 8:1–13.

Penczykowski, R. M., S. R. Hall, D. J. Civitello, and M. A. Duffy. 2014. Habitat structure and ecological drivers of disease. Limnology and Oceanography 59:340–348.

Peng, W., J. Li, H. Boncristiani, J. P. Strange, M. Hamilton, and Y. Chen. 2011. Host range expansion of honey bee Black Queen Cell Virus in the bumble bee, Bombus huntii. Apidologie 42:650–658.

Ponton, F., K. Wilson, A. J. Holmes, S. C. Cotter, D. Raubenheimer, and S. J. Simpson. 2013. Integrating nutrition and immunology: A new frontier. Journal of Insect Physiology 59:130–137.

Potts, S. G., J. C. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger, and W. E. Kunin. 2010. Global pollinator declines: Trends, impacts and drivers. Trends in Ecology and Evolution 25:345–353.

Potts, S. G., B. Vulliamy, A. Dafni, G. Ne, and P. Willmer. 2003. Linking Bees and Flowers: How Do Floral Communities Structure Pollinator Communities ? Ecology 84:2628–2642.

Rader, R., I. Bartomeus, J. M. Tylianakis, and E. Laliberté. 2014. The winners and losers of land use intensification: Pollinator community disassembly is non-random and alters functional diversity. Diversity and Distributions 20:908–917.

Radzevičiūtė, R., P. Theodorou, M. Husemann, G. Japoshvili, G. Kirkitadze, A. Zhusupbaeva,

and R. J. Paxton. 2017. Replication of honey bee-associated RNA viruses across multiple bee species in apple orchards of Georgia, Germany and Kyrgyzstan. Journal of Invertebrate Pathology 146:14–23.

Randolph, S., and A. Dobson. 2012. Pangloss revisited: a critique of the dilution effect and the biodiversity-buffers-disease paradigm. Parasitology 139:847–63.

Richardson, L. L., L. S. Adler, A. S. Leonard, K. Henry, W. Anthony, J. S. Manson, and R. E. Irwin. 2015. Secondary metabolites in floral nectar reduce parasite infections in bumble bees. Proceedings of the Royal Society Biological Sciences 282:20142471.

Ricketts, T. H., J. Regetz, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, A. Bogdanski, B. Gemmill-Herren, S. S. Greenleaf, A. M. Klein, M. M. Mayfield, L. A. Morandin, A. Ochieng, and B. F. Viana. 2008. Landscape effects on crop pollination services: Are there general patterns? Ecology Letters 11:499–515.

Ricklefs, R. E. 1987. Community Diversity: Relative Roles of Local and Regional Processes. Science 235:167–171.

Rigaud, T., M.-J. Perrot-Minnot, and M. J. F. Brown. 2010. Parasite and host assemblages: embracing the reality will improve our knowledge of parasite transmission and virulence. Proceedings of the Royal Society Biological Sciences 277:3693–702.

Roche, B., A. P. Dobson, J. F. Guégan, and P. Rohani. 2012. Linking community and disease ecology: The impact of biodiversity on pathogen transmission. Philosophical Transactions of the Royal Society B: Biological Sciences 367:2807–2813.

Rohr, J. R., D. J. Civitello, F. W. Halliday, P. J. Hudson, K. D. Lafferty, C. L. Wood, and E. A. Mordecai. 2020. Towards common ground in the biodiversity–disease debate. Nature Ecology and Evolution 4:24–33.

Roode, J. C. De, T. Lefèvre, and M. D. Hunter. 2013. Self-Medication in Animals. Science 340:150–151.

Rudolf, V. H. W., and J. Antonovics. 2005. Species Coexistence and Pathogens with Frequency-Dependent Transmission. The American Naturalist 166:112–118.

Ruiz-Gonzalez, M. X., J. Bryden, Y. Moret, C. Reber-funk, P. Schmid-hempel, and M. J. F. Brown. 2012. Dynamic Transmission, Host Quality, and Population Structure in a Multihost Parasite of Bumblebees. Evolution 66:3053–3066.

Russell, A. L., M. Rebolleda-Gómez, T. M. Shaible, and T. L. Ashman. 2019. Movers and shakers: Bumble bee foraging behavior shapes the dispersal of microbes among and within flowers. Ecosphere 10:e02714.

Salkeld, D. J., K. A. Padgett, and J. H. Jones. 2013. A meta-analysis suggesting that the relationship between biodiversity and risk of zoonotic pathogen transmission is idiosyncratic. Ecology Letters 16:679–686.

Sánchez, K. F., N. Huntley, M. A. Duffy, and M. D. Hunter. 2019. Toxins or medicines? Phytoplankton diets mediate host and parasite fitness in a freshwater system. Proceedings of the Royal Society B: Biological Sciences 286.

Santicchia, F., C. Romeo, A. Martinoli, P. Lanfranchi, L. A. Wauters, and N. Ferrari. 2015. Effects of habitat quality on parasite abundance: Do forest fragmentation and food availability affect helminth infection in the Eurasian red squirrel? Journal of Zoology 296:38–44.

Schmidt, K. A., and R. S. Ostfeld. 2001. Biodiversity and the Dilution Effect in Disease Ecology. Ecology 82:609–619.

Searle, C. L., L. M. Biga, J. W. Spatafora, and A. R. Blaustein. 2011. A dilution effect in the

emerging amphibian pathogen Batrachochytrium dendrobatidis. Proceedings of the National Academy of Sciences of the United States of America 108:16322–16326.

Shackelford, G., P. R. Steward, T. G. Benton, W. E. Kunin, S. G. Potts, J. C. Biesmeijer, and S. M. Sait. 2013. Comparison of pollinators and natural enemies: A meta-analysis of landscape and local effects on abundance and richness in crops. Biological Reviews 88:1002–1021.

Singh, R., A. L. Levitt, E. G. Rajotte, E. C. Holmes, N. Ostiguy, D. Vanengelsdorp, W. I. Lipkin, C. W. Depamphilis, A. L. Toth, and D. L. Cox-Foster. 2010. RNA viruses in hymenopteran pollinators: evidence of inter-taxa virus transmission via pollen and potential impact on non-Apis hymenopteran species. PLoS ONE 5:e14357.

Strauss, A. T., A. M. Bowling, M. A. Duffy, C. E. Cáceres, and S. R. Hall. 2018. Linking host traits, interactions with competitors and disease: Mechanistic foundations for disease dilution. Functional Ecology 32:1271–1279.

Strauss, A. T., D. J. Civitello, C. E. Cáceres, and S. R. Hall. 2015. Success, failure and ambiguity of the dilution effect among competitors. Ecology Letters 18:916–926.

Strauss, A. T., M. S. Shocket, D. J. Civitello, J. L. Hite, M. Penczykowski, M. A. Duffy, C. E. Cáceres, and S. R. Hall. 2016. Habitat, predators, and hosts regulate disease in Daphnia through direct and indirect pathways. Ecological Monographs 86:393–411.

Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodrigues, D. Fischman, and R. W. Waller. 2005. Status and Trends of Amphibian Declines and Extinctions Worldwide. Science 306:1783–1786.

Suorsa, P., H. Helle, V. Koivunen, E. Huhta, A. Nikula, and H. Hakkarainen. 2004. Effects of forest patch size on physiological stress and immunocompetence in an area-sensitive passerine, the Eurasian treecreeper (Certhia familiaris): An experiment. Proceedings of the

Royal Society B: Biological Sciences 271:435–440.

Suzán, G., A. Armién, J. N. Mills, E. Marcé, G. Ceballos, M. Ávila, J. Salazar-bravo, L. Ruedas, B. Armién, and T. L. Yates. 2008. Epidemiological Considerations of Rodent Community Composition in Fragmented Landscapes in Panama. Journal of Mammalogy 3:684–690.

Taubenberger, J. K., and J. C. Kash. 2010. Influenza virus evolution, host adaptation, and pandemic formation. Cell Host and Microbe 7:440–451.

Thomason, C. A., T. L. Hedrick-Hopper, and T. L. Derting. 2013. Social and nutritional stressors: Agents for altered immune function in white-footed mice (Peromyscus leucopus). Canadian Journal of Zoology 91:313–320.

Truitt, L. L., S. H. McArt, A. H. Vaughn, and S. P. Ellner. 2019. Trait-based modeling of multihost pathogen transmission: Plant-pollinator networks. American Naturalist 193:E149–E167.

Tylianakis, J. M., R. K. Didham, J. Bascompte, and D. A. Wardle. 2008. Global change and species interactions in terrestrial ecosystems. Ecology Letters 11:1351–1363.

Vanbergen, A. J. 2013. Threats to an ecosystem service: Pressures on pollinators. Frontiers in Ecology and the Environment 11:251–259.

Vandermeer, J. 1989. The Ecology of Intercropping. Cambridge University Press, Cambridge.

Vaudo, A. D., H. M. Patch, D. A. Mortensen, J. F. Tooker, and C. M. Grozinger. 2016. Macronutrient ratios in pollen shape bumble bee (Bombus impatiens) foraging strategies and floral preferences. Proceedings of the National Academy of Sciences 113:E4035–E4042.

Vaudo, A. D., J. F. Tooker, C. M. Grozinger, and H. M. Patch. 2015. Bee nutrition and floral resource restoration. Current Opinion in Insect Science 10:133–141.

Venesky, M. D., X. Liu, E. L. Sauer, and J. R. Rohr. 2014. Linking manipulative experiments to field data to test the dilution effect. Journal of Animal Ecology 83:557–565.

Wake, D. B., and V. T. Vredenburg. 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. Proceedings of the National Academy of Sciences 105:11466–11473.

Wilfert, L., G. Long, H. C. Leggett, S. J. M. Martin, P. Schmid-Hempel, R. K. Butlin, and M. Boots. 2016. Deformed Wing Virus is a Recent Global Epidemic in Honeybees driven by Varroa Mites. Science 351:594–597.

Wilkin, T. A., L. E. King, and B. C. Sheldon. 2009. Habitat quality, nestling diet, and provisioning behaviour in great tits Parus major. Journal of Avian Biology 40:135–145.

Wilkinson, D. A., J. C. Marshall, N. P. French, and D. T. S. Hayman. 2018. Habitat fragmentation, biodiversity loss and the risk of novel infectious disease emergence. Journal of Royal Society Interface 15:20180403.

Williams, N. M., E. E. Crone, T. H. Roulston, R. L. Minckley, L. Packer, and S. G. Potts. 2010. Ecological and life-history traits predict bee species responses to environmental disturbances. Biological Conservation 143:2280–2291.

Winfree, R., I. Bartomeus, and D. P. Cariveau. 2011. Native pollinators in anthropogenic habitats. Annual Review of Ecology, Evolution, and Systematics 42:1–22.

Winfree, R., N. M. Williams, J. Dushoff, and C. Kremen. 2014. Species abundance, not diet breadth, drives the persistence of the most linked pollinators as plant-pollinator networks disassemble. The American Naturalist 183:600–11.

Wood, C. L., and K. D. Lafferty. 2013. Biodiversity and disease: A synthesis of ecological perspectives on Lyme disease transmission. Trends in Ecology and Evolution 28:239–247.

Wood, C. L., K. D. Lafferty, G. DeLeo, H. S. Young, P. J. Hudson, and A. M. Kuris. 2014. Does biodiversity protect humans against infectious disease? Ecology 95:817–832.

Wood, C. L., A. McInterff, H. S. Young, D. Kim, and K. D. Lafferty. 2017. Human infectious diease burdens decrease with urbanization but not with biodiversity. Philosophical Transactions of the Royal Society B: Biological Sciences 372.

Woolhouse, M. E. J., D. T. Haydon, and R. Antia. 2005. Emerging pathogens: the epidemiology and evolution of species jumps. Trends in Ecology and Evolution 20:238–244.

Woolhouse, M. E., L. H. Taylor, and D. T. Haydon. 2001. Population biology of multihost pathogens. Science 292:1109–1112.

Yang, B., G. Peng, T. Li, and T. Kadowaki. 2013. Molecular and phylogenetic characterization of honey bee viruses, Nosema microsporidia, protozoan parasites, and parasitic mites in China. Ecology and Evolution 3:298–311.

Yates, T. L., J. N. Mills, C. A. Parmenter, T. G. Ksiazek, R. R. Parmenter, J. R. Vande Castle, C. H. Calisher, S. T. Nichol, K. D. Abbott, J. C. Young, M. L. Morrison, B. J. Beaty, J. L. Dunnum, R. J. Baker, J. Salazar-Bravo, and C. J. Peters. 2002. The Ecology and Evolutionary History of an Emergent Disease: Hantavirus Pulmonary Syndrome. BioScience 52:989–998.

Yue, C., and E. Genersch. 2005. RT-PCR analysis of Deformed wing virus in honeybees (Apis mellifera) and mites (Varroa destructor). Journal of General Virology 86:3419–3424.

Chapter 2 : Pollinator Community Species Richness Dilutes Prevalence of Multiple Viruses Within Multiple Host Species

Abstract

Most pathogens are embedded in complex communities composed of multiple interacting hosts, but we are still learning how community-level factors, such as host diversity, abundance, and composition, may influence pathogen spread for many host–pathogen systems. In particular, we can better understand the key host and pathogen traits that consistently drive links between community diversity and pathogen prevalence by evaluating parallel relationships among multiple pathogens and multiple hosts. Pollinator communities are a good system to test how community-level factors influence pathogens because several multi-host pathogens can be tracked among hosts that share flowers and exist in variable communities. We conducted a field survey of four pollinators to test for presence and infection prevalence of three RNA viruses (deformed wing virus, black queen cell virus, and sacbrood virus) among variable pollinator communities. First, all three viruses showed a similar pattern of variation in prevalence among hosts: *Apis mellifera* and *Bombus impatiens* had significantly higher viral prevalence than *Lasioglossum* spp. and *Eucera pruinosa*. However, BQCV showed the widest range in prevalence (3.7% – 84%), followed by DWV (11% – 56%) and SBV prevalence (0% – 38%). Second, virus prevalence was most strongly linked with pollinator community species richness, while pollinator abundance, species-specific pollinator abundance, and community composition were not associated with virus prevalence. Specifically, our results support the dilution effect, as pollinators in species-rich communities had lower viral prevalence than pollinators from species-poor communities for multiple host species and all three viruses, when accounting for differences

in pollinator abundance. Pollinator communities were nested such that all communities had highly competent *A. mellifera* and *B. impatiens*, while species-rich communities contained more native bee species likely to be poor viral hosts. Third, relationships between pathogen prevalence and community-level factors were remarkably similar among different pathogens and host species that were infected with the three viruses. Our study suggests that variation in relative competence among different host species for each virus contributed to variation in biodiversity–disease relationships among the three viruses. Therefore, investigating multiple similar pathogens that infect 'replicate' host communities is a useful approach to elucidate why patterns of dilution vary among different host–pathogen systems.

Introduction

Host–pathogen interactions occur within complex ecological communities composed of multiple host species and multiple pathogens, which can influence patterns of transmission and disease outcomes. First, heterogeneity among host species contribute to variation in pathogen transmission and prevalence among communities. For multi-host pathogens, host species differ in their likelihood of encountering pathogens, becoming infected (susceptibility), and transmitting the pathogen to other hosts (competency). Therefore, the biodiversity, relative abundance, and identity of hosts present in a community may influence pathogen prevalence (Haydon et al. 2002, LoGiudice et al. 2003, Keesing et al. 2006, Fenton et al. 2015). For example, heterogeneity in host competency for West Nile virus and contact rates with WNV-infectious mosquito vectors among bird species contribute to extreme variability in pathogen transmission; American robins are WNV superspreaders, while crows and jays had negligible WNV transmission (Kilpatrick et al. 2006). Correspondingly, multiple community-level factors, including bird community diversity, relative abundance, and identity of key hosts predict

differences in WNV prevalence in birds and humans (Ezenwa et al. 2006, Allan et al. 2009). Second, multi-host pathogens vary in their host ranges, modes of transmission, and infection severity, which are known to affect patterns of prevalence among hosts (Woolhouse and Gowtage-Sequeria 2005, Rigaud et al. 2010). Multiple pathogens often circulate among the same communities of hosts, but pathogens with different traits are likely to show different relationships between biodiversity and infectious disease prevalence. For example, Wood et al. found that greater wildlife biodiversity would reduce, increase, or not affect prevalence of many human pathogens. In particular, pathogens with complex life cycles, frequency-dependent transmission, and broad host ranges are more likely to be linked with biodiversity and exhibit positive or negative biodiversity–disease relationships (Wood et al. 2014). Thus far, few studies have evaluated variability among multiple hosts and multiple pathogens in how host community metrics, such as host diversity, abundance, and composition, impact biodiversity–disease relationships.

Although the relationships between host communities and pathogen prevalence are not simple, three community-level variables are thought play a key role in mediating disease dynamics: host species diversity, host abundance, and community composition (Keesing et al. 2010, Roche et al. 2012, Johnson et al. 2013a). Greater host biodiversity is hypothesized to reduce pathogen prevalence through the 'dilution effect' (Keesing et al. 2006). The dilution effect is predicted to occur when species-poor communities are dominated by highly competent hosts, and additional species in diverse communities are competent hosts or reduce encounters, transmission, or density of the competent hosts (Ostfeld and Keesing 2000, Keesing et al. 2006). Evidence for the dilution effect is supported by the tick-born Lyme disease system, where high vertebrate biodiversity reduces *Borrelia burgdorferi* prevalence in ticks. Ticks are more likely to

feed on less competent hosts in diverse communities compared to the species-poor communities dominated by the highly competent white-footed mouse (*Peromyscus leucopus*), reducing Lyme disease transmission (Ostfeld and Keesing 2000, Schmidt and Ostfeld 2001, LoGiudice et al. 2003). Though there is growing evidence for the dilution effect in many multi-host–pathogen systems (Ostfeld and LoGiudice 2003, Ezenwa et al. 2006, Johnson et al. 2008, Allan et al. 2009, Clay et al. 2009, Searle et al. 2011, Johnson et al. 2013b, Becker et al. 2014, Venesky et al. 2014), other studies have found different biodiversity–disease relationships (Salkeld et al. 2013, Strauss et al. 2015, Halliday et al. 2017, Luis et al. 2018).

Biodiversity–disease relationships can also exhibit the 'amplification effect', where greater host species diversity can increase pathogen prevalence (Keesing et al. 2006, 2010). The amplification effect is likely when highly competent hosts are more likely to be found in diverse rather than species-poor communities, or additional species facilitate greater pathogen transmission among hosts (Keesing et al. 2006, Wood et al. 2014). Additionally, not all pathogens are likely to be influenced by changes in community diversity, and therefore could have a neutral biodiversity–disease relationship (Wood et al. 2014, Rohr et al. 2020). As a result, there is much interest in when different biodiversity–disease relationships are observed and the underlying mechanisms (Lafferty and Wood 2013, Salkeld et al. 2013, Wood and Lafferty 2013, Johnson et al. 2015, Rohr et al. 2020). Expanding biodiversity–disease studies to additional multi-host–pathogen systems is an important frontier to further understand the conditions at the community-level that lead to dilution, amplification, or neutral effects.

A central challenge in empirical biodiversity–disease studies revolves around disentangling the effects of host diversity, host abundance, and host identity (i.e. community composition) on pathogen prevalence to understand the mechanisms that drive biodiversity–

disease relationships. Host abundance scales with species richness in most natural communities (Begon 2008, Mihaljevic et al. 2014), therefore it is important evaluate the relative contributions of host diversity and host abundance to observed biodiversity–disease relationships to elucidate their underlying mechanisms (Dobson 2004, Rudolf and Antonovics 2005). As biodiversity increases, the addition of less competent hosts can regulate and reduce the abundance of highly competent hosts, and consequently lead to reduced pathogen transmission and prevalence (Keesing et al. 2006). This pattern describes the 'susceptible host regulation' mechanism of the dilution effect, and demonstrates how host abundance and diversity may covary to drive biodiversity–disease relationships. For example, Mitchell et al. (2002) found reduced disease severity of several host species-specific foliar fungal diseases in species-rich plant communities, but that the observed pattern was driven by lower species-specific densities in the species-rich plots rather than biodiversity *per se*. However, if additional hosts in species-rich communities are also highly susceptible to a shared pathogen, then diverse communities with multiple competent host species could have a greater abundance of susceptible hosts and maintain higher levels of pathogen prevalence (i.e. amplification) (Holt et al. 2003). Therefore, it is critical to control for host density in biodiversity–disease studies, especially for multi-host pathogens that may be shared among multiple highly abundant and susceptible host species in a community.

Host community composition, including both species identity and relative abundance, can have a strong effects on the relationship between host diversity and pathogen prevalence (Randolph and Dobson 2012, Mihaljevic et al. 2014). Host species differ in many factors (e.g. susceptibility, infectiousness, behavior, and competence), so the presence or absence of particular host species can alter patterns of pathogen prevalence (Ricklefs 1987, Ostfeld and LoGiudice 2003, Johnson et al. 2013a, Fenton et al. 2015, Huang et al. 2016). If highly

competent hosts are common in species-poor communities, additional species in diverse communities are more likely to be less competent hosts and potentially lead to a dilution effect pattern. For example, Johnson et al. found that species-poor communities dominated by the highly-competent amphibian host *Pseudacris regilla* tended to have higher infection prevalence for the trematode parasite *Ribeiroia ondatrae* compared to more diverse communities composed of more pathogen-resistant species (Johnson et al. 2013b). Therefore, the dilution effect pattern is due to the presence or absence of a particular host species rather than host species richness alone. Previous studies have shown that the presence of highly competent or "diluter" hosts can be important predictors of pathogen prevalence in diverse host–pathogen systems, including Lyme disease in vertebrates (LoGiudice et al. 2003, 2008, Levi et al. 2016), West Nile Virus in birds (Ezenwa et al. 2006, Kilpatrick et al. 2006), *Batrachochytrium dendrobatidis* in amphibians (Becker et al. 2014, Venesky et al. 2014), and *Metschnikowia* fungus in *Daphnia* (Hall et al. 2009, Strauss et al. 2018). Though many studies have accounted for the relative impacts of host community diversity, abundance, and composition in recent biodiversity–disease studies, few studies have compared the effects these community-level factors on prevalence of several pathogens that infect the same sets of hosts (but see Johnson et al. 2013a).

Systems with multiple hosts and multiple pathogens provide a powerful model to test which community-level factors influence pathogen transmission and prevalence because we can tease apart commonalities among similar hosts or shared pathogens. Biodiversity–disease relationships may be consistent among multiple hosts infected with the same pathogen, such that prevalence of a particular pathogen is influenced by the same community-level factors across multiple host species (Johnson et al. 2008). Several multi-host pathogen studies have shown consistent focal host-specific or community-wide reductions in pathogen prevalence with

increased host diversity (Ezenwa et al. 2006, Allan et al. 2009, Johnson et al. 2013b, Becker et al. 2014, Venesky et al. 2014). In some cases, variation in specific host traits can result in different biodiversity–disease outcomes (Becker et al. 2014, Strauss et al. 2015, 2018). Alternatively, community-level factors may influence pathogen prevalence in a similar way among several different pathogens that infect the same host species. For example, amphibian host species richness reduced infection in five of seven pathogen species tested, showing consistent negative biodiversity–disease relationships among multiple pathogens (Johnson et al. 2013a). Finally, biodiversity–disease relationships may be idiosyncratic and context-dependent on the specific combinations of host and pathogen traits (Salkeld et al. 2013, Wood et al. 2014, Strauss et al. 2015).

Surprisingly few empirical studies have examined biodiversity–disease relationships in multiple pathogens that infect the same communities of hosts (but see Johnson et al. 2013a), and most meta-analyses of biodiversity–disease studies compare diverse pathogens that infect very different groups of organisms (Salkeld et al. 2013, Wood et al. 2014, Civitello et al. 2015). Differences in either host or pathogen ecology may impact comparisons among disparate host–pathogen systems (Salkeld et al. 2013). Therefore, by simultaneously studying biodiversity–disease relationships for multiple similar pathogens each infecting multiple related host species, we can look for common patterns between community factors and pathogen prevalence among many host–pathogen pairs and identify potential host or pathogen traits that lead to different outcomes.

Pollinator communities are a good system to study biodiversity–disease relationships because many pollinator species are infected by several multi-host pathogens that may be affected by the community-level factors in different ways. Three related viruses, deformed wing

virus (DWV), black queen cell virus (BQCV), and sacbrood virus (SBV), have long been observed in honey bees (*Apis mellifera*). The same viral strains that infect honey bees also spill-over into other native bee species, but initial evidence suggests that native bees are less commonly infected compared to honey bees and may be less competent hosts (Singh et al. 2010, Fürst et al. 2014, Manley et al. 2015, McMahon et al. 2015, Alger et al. 2019). The viruses have spread worldwide through movement of managed honey bees, and are well-known contributors to global declines in honey bees and native pollinators, causing mortality in bee offspring (Chen and Siede 2007, Potts et al. 2010, Goulson et al. 2015, Manley et al. 2015, Wilfert et al. 2016, McMahon et al. 2018). Current evidence suggests that the viruses may be transmitted through contact with flowers shared among pollinators, particularly though contaminated pollen (Singh et al. 2010, McArt et al. 2014, Alger et al. 2019). However, pollinator species vary substantially in their flower preferences, sociality, and other life history traits, which could impact the likelihood of pathogen exposure and infection among different hosts. Thus far, biodiversity–disease relationships have not been assessed for pollinator pathogens.

We measured viral prevalence in pollinator communities to address: 1) How does pathogen prevalence differ among host species and pathogens?, 2) How does pathogen prevalence vary among communities that differ in host species richness, relative abundance, and composition?, and 3) Are relationships between pathogen prevalence and pollinator community-level factors similar among hosts or pathogens? First, we expected that all three viruses would be present in all host species tested, but that managed honey bees, as the main reservoir host, would have higher viral prevalence for all three viruses compared to other native bee species. Second, if pollinator host species vary in virus prevalence, then we predicted that community-level factors, such as pollinator community species richness, abundance, and community composition, would

all vary with virus prevalence among different communities. Specifically, we thought that greater species richness would be likely to reduce virus prevalence, while greater pollinator abundance would increase virus prevalence, and communities with similar host compositions would exhibit similar virus prevalence compared to disparate communities. Third, we expected that relationships between virus prevalence and the three community-level factors would show consistent patterns among the three related viruses and four common pollinator hosts.

Methods

Study system

Three picorna-like RNA viruses, black queen cell virus (BQCV) in the *Dicistroviridae* family, and deformed wing virus (DWV) and sacbrood virus (SBV) in the *Iflaviridae* family, commonly infect European honey bees (*Apis mellifera*) (Chen and Siede 2007, de Miranda and Genersch 2010). There is growing evidence that these viruses are also transmitted among managed honey bees and native bees (Singh et al. 2010, Levitt et al. 2013, McArt et al. 2014, Manley et al. 2015, McMahon et al. 2015, Dolezal et al. 2016, Alger et al. 2019). The same DWV, BQCV, and SBV strains are present in both phylogenetically close and distantly related species, including honey bees, bumblebees, halictid bees, vespoid wasps, and several other non-Hymenopteran insect taxa, suggesting little host specialization by these viruses (Singh et al. 2010, Levitt et al. 2013, Manley et al. 2015, Dolezal et al. 2016, Bailes et al. 2018). Though these viruses may be generalist pathogens capable of infecting a wide diversity of species, all three viruses are most commonly found in high prevalence in honey bees and less commonly detected or at lower prevalence in other native pollinator species (Singh et al. 2010, Manley et al. 2015, Dolezal et al. 2016). For all three viruses, infections in early life stages (e.g. larval or pupal) cause mortality in honey bees, while infected adults are asymptomatic but can still

transmit the virus (Chen and Siede 2007, Grozinger and Flenniken 2019). Viral transmission among conspecifics is food-borne or fecal-oral (Chen et al. 2006, Chen and Siede 2007), but the mechanism of viral transmission among different pollinator species has not been studied in detail. Other bee parasites, including *Nosema ceranae* and *Crithidia bombi* are frequently transmitted through contact with flowers shared among pollinators (Durrer and Schmid-Hempel 1994, McArt et al. 2014, Graystock et al. 2015). Viruses may also be transmitted on flowers, as infected bees forage actively and carry viruses in their saliva and guts (Chen et al. 2006, Chen and Siede 2007). Additionally, DWV and BQCV have been detected on whole flowers near apiaries, and pollen collected by bees can be contaminated with viruses (Chen and Siede 2007, Singh et al. 2010, Mazzei et al. 2014, Alger et al. 2019). Furthermore, honey bees can become infected after consuming pollen contaminated with viruses (Singh et al. 2010, Mazzei et al. 2014, McArt et al. 2014).

Sampling pollinator communities

Bees were collected from 14 winter squash farms in Michigan, USA, with permission granted by private landowners (**Appendix, Table S2.1**). All squash fields were grown adjacent to either corn or apple orchards, except for the GT and S sites, which had squash grown next to small plots of other specialty vegetables. Each field site was at least 10 km away from other sites. Most bee species' home ranges are less than 10 km (Greenleaf et al. 2007), so it is unlikely that bees observed at one site visited other field sites. We visited each field site twice during the peak squash flower bloom (July and August) to sample the pollinator communities, and maintained even sampling effort among each pollinator community in terms of both total time and area sampled per site. We sampled on sunny days with little cloud cover and wind speeds less than 2 m/s. Six sites were sampled between 18 July – 21 August 2015, and eight sites were

sampled between 26 July – 2 September 2016. The 2016 season had a later peak bloom than 2015 because it was cooler and dryer than 2015.

Bees were sampled via hand-netting and pan traps in four 50-m transects. Three transects were randomly placed within the field in line with the crop rows, and one transect was placed along the field edge. Edges typically contained a mixture of native flowers and weeds. Each transect was walked for 30 minutes at 0800, 1000, 1100 and 1200. We did not collect in the afternoon because squash flowers close by midday. Pollinators within 1.5-m of the transect line were collected. Fluorescent blue, yellow, and white pan traps were set along the transect between the crop rows 5-m apart in an alternating color pattern. All pan traps were filled with water mixed with a natural, clear dish soap to reduce the surface tension. Pan traps were set prior to 0700 and collected at 1200, after squash flowers close. Pan traps were checked every 3 hours. All insects collected from hand-netting and pan traps were placed in individual microcentrifuge tubes, freeze killed, stored on dry ice in the field, and transferred to a -80^{o}C freezer in the lab.

Each specimen was identified using the Discover Life key (http://www.discoverlife.org). Most specimens were identified to species. *Lasioglossum* and *Halictus* were identified to genus because they are very difficult to key out to species. Additionally, rare wasp genera with less than five occurrences total in our sample were identified to genus.

Detecting virus prevalence

We tested four pollinator species (*Apis mellifera*, *Bombus impatiens*, *Eucera pruinosa*, and *Lasioglossum* spp.) from each site for deformed wing virus (DWV), black queen cell virus (BQCV), and sacbrood virus (SBV). These four species were chosen because they were the most consistently abundant among all communities sampled (**Appendix, Table S2.2)**. We tested up to twenty randomly selected individuals from each species per site (**Appendix, Table S2.3**). In

total, we sampled *A. mellifera* (n = 237), *B. impatiens* (n = 252), *E. pruinosa* (n = 193), and *Lasioglossum* spp. (n = 255). When less than 20 individuals from a species were collected at a site, we tested all individuals collected.

Half of each bee's abdomen was used for RNA extraction, while the other half was archived at -80^{o}C. The tissue was homogenized using a FastPrep-24 (MP Biomedicals) for 1 minute at 4.0 M/sec. RNA was extracted using TRIzol reagent (Ambion) according to manufacturer's instructions, eluted in 30 µl DNAse/RNAse free H_2O, and RNA concentration was quantified using Qubit 3.0 Fluorometer (Invitrogen). Samples with eluted RNA concentrations <1 ng/µl were excluded from the study because samples with poor RNA extraction are less likely to provide accurate information about virus presence or absence (excluded samples: n_{Apis}= 8, n_{Bombus} = 3, n_{Eucera} = 2, $n_{Lasioglossum}$ = 27). *Lasioglossum* spp. have substantially smaller body size compared to the other bees tested (*Lasioglossum* spp.: 2-8 mm, *A. mellifera*: 12-16 mm) (Michener 2007), and therefore have less body tissue to use for RNA extraction, which could have contributed to the lower RNA concentrations for some individuals. However, most of the samples (~96%) produced large enough RNA concentrations for accurate detection of virus presence or absence based on the adequate amplification of our quality control bee 18S rRNA gene (details below), which suggested that 1ng/µl of RNA was sufficient for reliable viral assessment. Positive-strand complimentary DNA (cDNA) synthesis reactions were performed with 2 µl of RNA template in a 20 µl reaction using M-MLV reverse-transcriptase (Promega) and 0.25 µM random hexamers (Invitrogen) according to manufacturer's instructions.

We tested for the presence or absence of DWV, BQCV, and SBV using PCR with primers for DWV (Singh et al. 2010), SBV (Singh et al. 2010), and BQCV (Benjeddou et al. 2001) (**Appendix, Table S2.4**). The DWV primer did not differentiate between DWV-A, -B, or -

C variants, therefore reported DWV prevalence includes all three variants. All reactions included negative (H_2O) and virus-specific positive controls. Additionally, we ran PCR for each sample with *A. mellifera* 18S rRNA gene primers (Cardinal et al. 2010) as a control to confirm adequate RNA extraction and reverse transcription of all bee samples. Further reaction details are provided in **Appendix S1**. All PCR products were visualized under UV light on a 2% agarose gel to determine the presence or absence of the virus, along with reaction negative and virus positive controls. We excluded samples that failed to produce a band for the 18S rRNA gene because failure to amplify this highly expressed gene in bee tissues indicates poor RNA quality or quantity, which is unlikely to accurately amplify viral RNA (n_{Apis}= 0, n_{Bombus} = 0, n_{Eucera} = 1, $n_{Lasioglossum}$ = 2). A subset of the PCR products was sequenced to confirm identification of viral RNA and 18S gene (GenBank Accession Numbers in **Appendix Table S2.5**).

Current data from several studies indicate that native bees share the same virus strains with local honey bees. At least eight studies have compared viral sequences of DWV, BQCV, and/or SBV in honey bees, native bees, and other arthropods, and six found almost identical sequences among the different host species and no evidence of phylogenetic clustering by species (Singh et al. 2010, Genersch et al. 2011, Yang et al. 2013, Levitt et al. 2013, Fürst et al. 2014, McMahon et al. 2015, Radzevičiūtė et al. 2017, Bailes et al. 2018). Two studies found weak phylogenetic clustering of viral sequences by species, but the results were confounded by either stronger temporal or spatial clustering of sample collection (Singh et al. 2010, McMahon et al. 2015). Though our results may slightly underestimate the virus prevalence due to sequence variation found only in other native bees by using primers created from honey bee virus sequences, the evidence described above suggests there is relatively little sequence variation among host species. Furthermore, these primers have been previously used to successfully test

for virus presence and negative-strand presence in other bees, wasps, and non-Hymenopteran insects (Singh et al. 2010, Levitt et al. 2013, Fürst et al. 2014, McMahon et al. 2015, Bailes et al. 2018). Therefore, the DWV, BQCV, and SBV prevalence observed in this study are representative of current spillover among pollinator species.

Negative-Strand Detection

We determined the infection status of a subset of virus-positive samples with additional negative-strand specific RT-PCR. Identifying the negative-strand provides strong evidence of viral replication and an active infection within the sample (Ongus et al. 2004, Yue and Genersch 2005). Up to 26 virus-positive bee samples from each bee species (*A. mellifera*, *B. impatiens*, *E. pruinosa*, and *Lasioglossum* spp.) per virus were randomly selected from all the sampled sites to test for the presence or absence of the negative-strand. If fewer than 20 virus-positive bee samples for a species were available, then all virus-positive samples were used (**Appendix, Table S2.6**). Negative-strand specific cDNA synthesis was carried out with 2.5 ul RNA template with M-MLV reverse transcriptase (Promega) and tagged negative-strand specific primers for DWV (Fürst et al. 2014), BQCV (Yue and Genersch 2005, Peng et al. 2011), and SBV (Gong et al. 2016), followed by PCR with negative and virus-specific positive controls (**Appendix, Table S2.4**). All samples were visualized with UV light on 2% agarose gels, and a subset of samples were sequenced to confirm positive identification of the negative-strand viral sequences (see **Appendix Table S2.5** for GenBank Accession Numbers). Additional negative strand detection methods are included in the **Appendix S1**.

Statistical Analysis

All analyses were performed in the program R (R Core Team 2020) . We used a global model of virus prevalence with all three viruses and four host species using a Generalized Linear

Mixed effects model (GLMM) with a binomial distribution and logit link function ('glmer' function in *lme4* package) (Bates et al. 2015). For random effects, we included visit number to a site nested within site to account for bees collected from sites on different days and each bee's unique ID to account for testing each bee for presence or absence of BQCV, DWV, and SBV. The initial model included species richness, total pollinator abundance, virus (i.e. BQCV, DWV, and SBV), and host species (*A. mellifera*, *B. impatiens*, *Lasioglossum* spp., and *Eucera pruinosa*) as main effects. Total pollinator abundance was log transformed, and all continuous variables were z standardized. We evaluated the model without interactions (Model 1) and each combination of two- (Model 2a-f), three- (Model 3a-e), and four-way interactions (Model 4) in a model selection table ranked by lowest AICc score (*MuMIn* package) (**Appendix, Table S2.7**) (Barton 2020). Model 3a and Model 2a were the top two models with very similar AICc and the greatest overall weight among all the models tested. Both top models share a significant interaction between virus type and host species. We selected the simpler Model 2a as the main model to avoid overfitting. Model 2a and 3a have similar AICc values, but 2a includes a two-way interaction, while Model 3a includes a three-way interaction and three two-way interactions. Main effects do not differ between any of the top models, indicating that our key results are robust.

Model 2a includes a significant interaction between virus type and host species. Interaction effects in non-linear GLMMs are complicated and cannot simply be evaluated by the coefficient or significance of the interaction term (Ai and Norton 2003). Instead, we investigated the asymptotic variance of the interaction using a post-hoc pairwise comparison of predicted virus prevalence among each host species for each virus with a Tukey method for adjusting the p-value for multiple comparisons and effect (package *emmeans*) (Lenth 2020). We also

conducted a Type II Wald Chi-square test to construct an Analysis of Deviance table for the main factors in Model 2a and Model 3a (package 'car'; **Table 2.4** and **Appendix Table S2.8**, respectively) (Fox and Weisberg 2019). All factors in the top model had Variance Inflation Tests (VIF) < 6, below the standard threshold of 10 for collinearity issues (**Appendix Table S2.9**) (Dormann et al. 2013). Furthermore, we compared the results from the top GLMM Model 2a to a model that included *A. mellifera*, *B. impatiens*, *Lasioglossum* spp., and *E. pruinosa* specific abundances (log transformed) instead of total abundance, and found similar results to Model 2a (**Appendix Table S2.10**). Viral prevalence was not associated with any of the four focal host's species-specific abundances. However, we did not have the power to adequately test the effect of the abundance of all potential host species on virus prevalence because rarer species were not consistently found at all sites.

We tested each model's residuals for spatial autocorrelation based on each site's location using the Moran's I test (packages *ape* and *DHARMa*) (Paradis and Schliep 2018, Hartig 2020). There was no evidence of significant spatial autocorrelation in the model residuals, indicating that closely located communities did not have significantly similar virus prevalence (**Appendix, Table S2.11**). Therefore, we considered virus prevalence among different pollinator communities as independent of each other.

To calculate apparent infection prevalence (based on the presence of viral negative-strand) within each host species, we used the 'epi.prev' function in the *epiR* package (Stevenson et al. 2020). The negative-strand infection prevalence is determined by the number of samples with the viral negative-strand present and the subset of virus-positive samples that were tested and found to have the negative-strand present, which indicates active replication in the host (Ongus et al. 2004, Yue and Genersch 2005)(see Negative-stand detection methods above). We

compared negative-strand infection prevalence in each of the four host species within each virus using a Chi-squared test of two proportions. For example, to test for differences in DWV infection prevalence among host species, we compared the DWV infection prevalence for each pair of host species with Chi-squared tests of two proportions, for a total of six comparisons. We used a Bonferroni correction for multiple comparisons to determine significant differences among host species ($\alpha^* = 0.05/6 = 0.0083$). We completed the same process for BQCV, and SBV infection prevalence, using the same number of comparisons.

Species richness, Simpson's diversity index (1-D), and species-specific and total abundance for each pollinator community were determined from the collection data for each site. Differences in community composition were assessed qualitatively through Non-metric Multi-Dimensional Scaling (NMDS) (as described below) and observed differences in the relative abundance of pollinator species. We tested the nested temperature of the pollinator communities sampled compared to simulated null model communities following Johnson et al. 2013b (method: "r00", function 'oecosimu', package *vegan*; **Appendix, Figure S2.1**) (Oksanen et al. 2018). To determine if we captured the pollinator species richness within each community, we created individual-based rarefaction curves using the *iNext* package and compared the observed species richness to the estimated species richness at the asymptote of the rarefaction curve (**Appendix, Figure S2.2**) (Hsieh et al. 2016). For invertebrate communities, it is rare that the observed species richness ever reaches an asymptote (Novotný and Basset 2000, Gotelli and Colwell 2001). Although observed and estimated species richness differed, there was strong consistency in community ranking order. For example, the GT site had the highest observed and estimated species richness, while site G had the lowest diversity by both measures (**Appendix, Table S2.12**). We tested whether our results are robust regardless of method used to estimate

species richness. We ran a similar GLMM as described above for Model 2a with a single two-way interaction between virus type and host species, but substituted our observed species richness for the rarefaction estimated asymptotic species richness or the estimated species richness for 46 randomly selected individuals (the number of individuals detected at the least abundant site), as recommended by (Gotelli and Colwell 2001). We found that both models with estimated species richness showed the same results as Model 2a and were robust to the two different methods of estimating species richness (**Appendix Table S2.13 and S2.14**). Therefore, the observed species richness seemed to sufficiently describe differences among the pollinator communities based on our even sampling effort in both time spent sampling and area covered by transects at each site.

To examine how community composition influenced virus prevalence in different host species, we used Non-metric Multidimensional Scaling (NMDS) ordination of the pollinator species collected at each site. Prior to ordination, the pollinator community was reduced to 15 species by removing rare species that were less than 0.5% of the total count of pollinators (i.e. species with fewer than 23 individuals collected across all sites) from the pollinator community matrix. The NMDS ordination of the pollinator communities was created with the 'metaMDS' function in the *vegan* package using a Bray-Curtis dissimilarity matrix (Oksanen et al. 2018). A two-dimensional solution for the NMDS ordination of pollinator community composition yielded a stress value of 0.1149, which showed that the 2D fit corresponded well with the actual multivariate distance among communities and was well below the 0.2 stress threshold. We separately evaluated the correlation between DWV, BQCV, and SBV prevalence within each host species at each site and the ordination of pollinator communities using fitted smooth surfaces (i.e. contour lines) on the ordination, which were calculated using Generalized Additive

Models (GAM) with thin-plate splines (based on the 'ordisurf' function in the *vegan* package). The correlation between host–virus prevalence and pollinator community composition were evaluated with GAM fitted vectors that indicate the strongest linear gradient along the fitted contour lines of virus prevalence in the ordination (adjusted R^2). By comparing patterns of virus prevalence and directionality of the fitted vectors overlaid on the NMDS plots of pollinator community composition for each host–virus pair, we can determine whether communities with similar compositions tend to share patterns of virus prevalence. There were no *E. pruinosa* collected at site K, therefore we could not calculate DWV, BQCV, or SBV prevalence for that host species at that site. Consequently, we modified *E. pruinosa* DWV, BQCV, and SBV prevalence to zero at site K only to allow the GAM models to run properly, and not change the pollinator community ordination used in all the other models by removing site K.

Results

1) How does pathogen prevalence differ among host species and pathogens?

Virus and infection prevalence are highly variable among honey bees and native bees

DWV, BQCV, and SBV were detected in the four focal pollinator species (*Apis mellifera, Bombus impatiens, Lasioglossum* spp., and *Eucera pruinosa*) (**Figure 2.1, Appendix Table S2.15**). Furthermore, virus prevalence varied significantly among the three viruses and different host species, as all the top generalized linear mixed models (GLMM) from model selection included a significant interaction between virus type and host species. (**Figure 2.1, Table 2.2, Appendix Table S2.16**). DWV and BQCV had the same overall pattern of prevalence among the four host species tested, with *A. mellifera* showing significantly higher prevalence than *B. impatiens*, which in turn was significantly higher than both *Lasioglossum* spp. and *E. pruinosa* (**Figure 2.1**).SBV prevalence showed a different pattern among the four host species. *A.*

mellifera and *B. impatiens* had similar SBV prevalence, but SBV was extremely rare in *Lasioglossum* spp. and *E. pruinosa* (estimated 0.2% and 1.1% prevalence by Model 2a, respectively). All three viruses were found in at least one individual from each of the four bee species tested, but there was high variability in the virus prevalence observed among honey bees (*A. mellifera*) and native bee species.

We also tested viral infection by testing for DWV, BQCV, and SBV negative-strand in each host species. Presence of the negative strand indicates viral infection because the virus is actively replicating within the host (Ongus et al. 2004, Yue and Genersch 2005). We use 'virus prevalence' Maybe say how it differs from viral prevalence specifically. We found the presence of the viral negative-strand for all three viruses within all four host species, except for SBV in *Lasioglossum* spp. (**Table 2.3**). *Lasioglossum* spp. had very low SBV prevalence detected (<0.4%, a single SBV positive individual), so it is not surprising that we found no evidence of the negative SBV viral strand. Therefore, we can conclude that a subset of the native bee species sampled were actively infected.

'Infection prevalence' is based the proportion of individuals that had the viral negative-strand for each virus and host species, which differs from 'virus prevalence' described above which is based on the viral positive-strand. The patterns of infection prevalence varied among the pollinator hosts and viruses. In general, virus-positive *A. mellifera* and *B. impatiens* had higher infection prevalence compared to *Lasioglossum* spp. and *E. pruinosa* (**Table 2.3, Appendix Table S2.17**). The infection prevalence presented here may be an underestimate since we only tested a subset of virus-positive specimen from each species, but the data clearly show that there was variation in the likelihood of infection among host species for all three viruses.

2) How does pathogen prevalence vary among communities that differ in host species richness, relative abundance, and composition?

Pollinator communities vary in abundance, richness, and composition

Across both sampling years, we collected 4,737 bees and wasps from 14 communities, including at least 127 species and 78 genera from five bee families (Andrenidae, Apidae, Colletidae, Halictidae, and Megachilidae) and nine wasp families (Aulacidae, Crabonidae, Gasteruptiidae, Ichneumonidae, Pompilidae, Sphecidae, Thynnidae, Tiphiidae, and Vespidae). The most common genera were *Lasioglossum* (n = 1305), *Bombus* (n = 1071), *Eucera* (n = 843), *Apis* (n = 508), *Vespula* (n = 129), *Augochlora* (n = 127), and *Halictus* (n = 105). The pollinator communities varied in species richness (range: 7 to 49 species) and total pollinator abundance (range: 49 to 756 individuals) (**Figure 2.2)**. Furthermore, the pollinator community composition varied qualitatively among sites, as the relative abundance of key pollinator species differed among sampled pollinator communities (**Figure 2.2, Appendix Figure S2.3**). The pollinator communities were significantly nested compared to simulated null community matrices, such that species poor communities were composed of a subset of the species rich communities (observed nested temperature = 20.7°; average null model temperature = 53.3°, p = 0.01; **Appendix Figure S2.1**). All communities included *A. mellifera*, *B. impatiens*, *Lasioglossum* spp., and *E. pruinosa*, except *E. pruinosa* was absent from K site. Simpson's index of diversity (1-D), which incorporates both species richness and evenness of species relative abundances, ranged from 0.46 to 0.85 among the different communities (**Appendix, Table S2.12**).

Virus prevalence is linked with pollinator species richness, but not pollinator abundance nor community composition

Virus prevalence was more strongly associated with pollinator species richness than with other community characteristics, like total host abundance, or species-specific abundances. Pollinator community species richness was a significant main effect in the top GLMM (**Table 2.4**). Specifically, all four host species had significantly reduced DWV prevalence in communities with greater pollinator species richness (**Figure 2.3A**). Additionally, *A. mellifera* and *B. impatiens* had significantly reduced BQCV and SBV prevalence in species-rich communities (**Figure 2.3A**). *Lasioglossum* spp. and *E. pruinosa* had relatively low BQCV and SBV prevalence among all communities tested, and therefore did not show as much variation in viral prevalence. On the other hand, total pollinator abundance and the species-specific abundances of *A. mellifera*, *B. impatiens*, *Lasioglossum* spp. and *E. pruinosa* were not significant predictors of virus prevalence in any of the top models (**Figure 2.3B, Table 2.3, 2.4, Appendix Table S2.10**).

Pollinator community composition generally did not predict virus prevalence in most host species. The NMDS ordination was only significantly correlated with viral prevalence in two of the twelve host–virus pairs, specifically *A. mellifera* BQCV and SBV prevalence (*A. mellifera* BQCV: $F = 1.04$, $p = 0.019$, Adj $R^2 = 0.42$; SBV: $F = 5.78$, $p = 0.0016$, Adj $R^2 = 0.8$; **Appendix Figure S2.4A**). Virus prevalence increased from the lower right towards the upper left of the ordination, indicating that communities with overall compositions similar to sites BB, T, S, and BC correlated with higher virus prevalence in *A. mellifera*. Virus prevalence was not significantly correlated with the pollinator community ordination for any other host–virus pair (**Appendix, Figure S2.4**).

3) Are relationships between pathogen prevalence and community-level factors similar among hosts or pathogens?

Overall, the relationships between virus prevalence and pollinator community species richness and abundance were very consistent among the three viruses and four host species. All three viruses showed significantly reduced virus prevalence in species-rich communities within all host species that had greater than 10% virus prevalence (**Figure 2.3A**). The strength of the negative relationships varied among host species based on their relative prevalence for each virus. BQCV and SBV prevalence show clear negative slopes between virus prevalence and species richness in *A. mellifera* and *B. impatiens*, hosts with high BQCV and SBV prevalence. Meanwhile, *Lasioglossum* spp. and *E. pruinosa* are rarely infected with BQCV or SBV, and show no strong relationship between virus prevalence and species richness (**Figure 2.1 and 2.3A**). All four hosts exhibited reduced DWV prevalence with greater species richness, but *A. mellifera* and *B. impatiens* had a steeper slope compared to *Lasioglossum* spp. and *E. pruinosa* (**Figure 2.3A**). None of the host–virus pairs showed evidence of greater virus prevalence in species-rich communities.

In general, there was a consistent lack of any significant pattern between virus prevalence and pollinator community composition among either hosts or viruses (**Appendix Figure S2.4**). Only *A. mellifera* BQCV and SBV prevalence showed a similar significant correlation between virus prevalence and community composition among two pathogens infecting the same host species (**Appendix Figure S2.4A**).

The relationship between virus prevalence and pollinator community total abundance was not significant among all host species and viruses tested, indicating little variation in the relationship among either hosts or pathogens (**Table 2.4, Figure 2.3**).

Discussion

Understanding the patterns and mechanisms that underlie how host communities influence multi-host infectious disease has become increasingly important in the wake of rapid biodiversity loss and emerging infectious diseases. Key frontiers in biodiversity–disease research include investigating novel host–pathogen systems for biodiversity–disease relationships and exploring the differences in these patterns among multiple pathogens and multiple hosts. Our results show that species richness is the most important community factor associated with reduced pathogen prevalence across multiple hosts and multiple pathogens. In contrast, host abundance and community composition are not consistently associated with pathogen prevalence. This work illustrates the dilution effect pattern in a pollinator–virus system for the first time. In multiple viruses and multiple bee host species, communities with greater pollinator species richness had lower viral prevalence than species-poor communities, but the strength of the relationships vary based on host competence for each virus.

Species richness

Increasingly biodiversity–disease studies have begun to focus on multi-host–pathogen systems to evaluate how disease risk within different host species or communities respond to changes in host communities. However, investigations that simultaneously compare biodiversity–disease relationships in multiple pathogens that infect similar communities of hosts have been much rarer (but see Johnson et al. 2013a). Here, we find that pollinator communities with greater species richness exhibit consistently lower virus prevalence for three multi-host viruses within four focal bee species, while controlling for total host abundance. Broadly, our findings corroborate other multi-host pathogen studies that have found consistent patterns of dilution in pathogen prevalence among multiple co-occurring hosts or in community-wide

pathogen prevalence (Ezenwa et al. 2006, Allan et al. 2009, Johnson et al. 2013a, 2013b, Becker et al. 2014, Venesky et al. 2014, Strauss et al. 2018).

The pollinator–virus system has many of characteristics that have been shown to facilitate the dilution effect in other host–pathogen systems. The dilution effect is more likely to occur when the most competent host is common in species-poor communities and more disease resistant host species dominate species-rich communities (LoGiudice et al. 2003, Keesing et al. 2006, Begon 2008, Johnson et al. 2013b). In general, biodiversity is lost from pollinator communities in a non-random order, where solitary and specialist native bees tend to be extirpated first (Rader et al. 2014). Our results are consistent with this pattern, as pollinator communities in our study are nested (**Appendix Figure S2.1**). Species poor communities are dominated by the four focal hosts, *Apis mellifera*, *Bombus impatiens*, *Lasioglossum* spp., and *Eucera pruinosa*, two of which (*A. mellifera* and *B. impatiens*) are competent hosts with high prevalence for all three viruses (**Figure 2.1**). In contrast, species-rich communities include many other native bee species, which are likely to be less or non-competent viral hosts (Singh et al. 2010, Manley et al. 2015, Dolezal et al. 2016).

Beyond correlations between host biodiversity and pathogen prevalence, it is critical to understand the mechanisms that underlie these dilution effect patterns in pollinator viruses to improve future predictions of disease risk. Two key dilution effect mechanisms seem likely to operate in the pollinator–virus system: encounter reduction and susceptible host regulation. Specifically, species-rich host communities may have lower encounter rates between susceptible hosts and infectious viral particles or infected hosts due to a higher proportion of non-hosts or low competent hosts in species-rich communities (Keesing et al. 2006). Additional non-hosts and low competent hosts can also compete with susceptible hosts to constrain their abundance and

reduce pathogen spread through the susceptible host regulation mechanism (addressed in more detail in the following section) (Keesing et al. 2006). As highly competent hosts and floral generalists, *A. mellifera* and *B. impatiens* may disproportionally impact virus prevalence in species-poor communities by spreading viral particles to more flowers and increasing the likelihood of hosts encountering viral particles during visits to shared flowers (i.e. encounter reduction) (Keesing et al. 2006). Also, if native bee hosts in species-rich communities can act as decoys or "diluter hosts" that take up viral particles but do not become infected during visits to shared flowers, then susceptible hosts could have a reduced encounter rate with viral particles (Johnson and Thieltges 2010). Further investigation through paired experimental and natural studies is needed to elucidate the specific dilution effect mechanism(s) operating in pollinator pathogen systems.

By expanding biodiversity–disease studies to new study systems, such as pollinator pathogens, we can begin to examine patterns of dilution in more complex communities and compare patterns among multiple pathogens that infect pollinators. Pollinator communities provide an opportunity to examine communities with a wide range of species present (e.g. 7 to 49 species per community) and to test other multi-host bee pathogens for biodiversity–disease relationships, such as *Nosema ceranae* or *Crithidia bombi*. Future research in complex, natural communities with multiple pathogens and multiple hosts will be an important frontier to continue to test the conditions for the dilution effect versus the amplification effect beyond the well-established host–pathogen systems for biodiversity–disease studies.

Species abundance

Although biodiversity was strongly associated with virus prevalence, other community factors, including total pollinator abundance and the species-specific abundances of the four

most common host species, were not linked with virus prevalence (**Figure 2.3B, Table 2.4, Table S2.10**). Changes in community diversity often correspond to changes in the total host abundance and/or relative abundance of specific host species in the community, and susceptible host abundance is an important underlying mechanism of the dilution effect (Dobson 2004, Rudolf and Antonovics 2005, Randolph and Dobson 2012, Mihaljevic et al. 2014). However, the lack of relationship between host abundance and virus prevalence suggests that the 'susceptible host regulation' mechanism may not be a likely driver of the dilution effect in this pollinator pathogen system. Though we only were able to test total abundance and species-specific abundance for the four most common species in the pollinator communities, most other species were rare (less than 5 individuals observed per site) and unlikely to explain community-level differences in virus prevalence.

The lack of relationship between host abundance and viral prevalence suggests that DWV, BQCV, and SBV may have frequency-dependent transmission rather than density-dependent transmission. This means that the frequency of host contacts and contact with contaminated flowers has a greater impact on pathogen prevalence than host abundance. The three viruses are likely transmitted within and among host species through interactions on flowers and through contaminated pollen (Singh et al. 2010, Levitt et al. 2013, McMahon et al. 2015, Alger et al. 2019). As a result, viral transmission may depend on the frequency of pollinator visits to shared flowers rather than the abundance of pollinators in a community. In fact, pathogens with frequency-dependent transmission are more likely to exhibit decreased pathogen prevalence with greater community biodiversity (i.e. dilution effect) that is not influenced by the total number of hosts in the community (Rudolf and Antonovics 2005, Keesing et al. 2006). Future studies should explicitly examine the mode of transmission of pollinator

viruses and whether the frequency of bee contacts with flowers provide a better fit with patterns of pathogen prevalence among different pollinator communities than host abundance.

Community composition

Pollinator community composition was rarely found to influence virus prevalence among most host–pathogen pairs tested. This is interesting because community composition is an important driver of observed dilution effects in many host–pathogen systems (Roche et al. 2012, Johnson et al. 2013b, Salkeld et al. 2013, Han et al. 2015). Assuming that hosts species are not equally competent for a pathogen, the presence or absence of a particular species in a community can dramatically influence pathogen transmission dynamics. This process could be akin to the "selection effect" from the field of biodiversity–ecosystem function (BEF), where a particular species has a disproportionate impact on pathogen prevalence and/or transmission in species-rich communities (Loreau M. and Hector. A. 2001, Becker et al. 2014). For example, the identity of host grass species in a community influences foliar pathogen loads because some host species are more disease prone than others (Mitchell et al. 2002). Therefore, hosts in communities composed of several highly competent species may have higher pathogen prevalence compared to communities with different species composition. However, we found that virus prevalence among all four pollinator species was generally unrelated to community composition. These findings suggest that similarity in community composition, based on host identity and relative abundance, does not lead to consistent patterns of pathogen prevalence among communities.

Alternatively, virus prevalence may be altered through differences in likelihood of viral encounter or transmission if interactions among host species on shared flowers differ based on the presence of particular pollinator species. In that case, pathogen transmission is reduced through less habitat sharing among host species in diverse communities similar to the niche

partitioning mechanism of the "complementarity effect" from BEF literature (Loreau M. and Hector. A. 2001, Becker et al. 2014). Both selection and complementarity mechanisms have been shown in the amphibian *Batrachochytrium dendrobatidis* system, where one host species had lower infection loads while driving higher loads for other hosts present (selection) and greater host diversity led to reduced shared habitat use and likely lower pathogen transmission (complementarity; Becker et al. 2014). Though the selection mechanism seems unlikely to be driving patterns in the pollinator pathogen system based on our data, the complementarity mechanism could be important, but careful experimental studies would be needed to clearly display these patterns. Bees in diverse communities may reduce their shared flower use through greater specialization in foraging or utilize different parts of the flower (e.g. nectar vs. pollen), which could reduce the potential for viral encounter or transmission among species through a complementarity mechanism. Future work needs to investigate how specific pollinator interactions on flowers among different communities contribute to various dilution effect mechanisms, including encounter reduction, transmission reduction, and complementarity effects.

Consistent evidence of dilution among pathogens and hosts

Finally, we found remarkably similar negative biodiversity–disease relationships among multiple viruses and multiple hosts, but the strength of the dilution effect varied among hosts. Our study suggests that variation in relative competence among different host species for each virus likely led to variation observed in the relationships between biodiversity and pathogen prevalence. *A. mellifera* and *B. impatiens*, the two most highly competent hosts in our study displayed consistent dilution effects for all three viruses. Meanwhile *Lasioglossum* spp. and *E. pruinosa* are relatively less competent hosts for DWV, and have a weaker dilution effect

compared to *A. mellifera* and *B. impatiens*. For BQCV and SBV, *Lasioglossum* spp. and *E. pruinosa* are poor hosts with extremely low virus prevalence, and consequently there was little virus prevalence to dilute with greater community biodiversity. Furthermore, ecologically variable hosts species may have important differences in encounter rates with the viruses that may contribute to different virus prevalence among host species and further contribute to slight variation in the dilution effects observed. *A. mellifera* and *B. impatiens* are floral generalists and social species living in relatively large colonies, likely with greater exposure to the viruses on flowers or in their colonies. On the other hand, *E. pruinosa* are squash flower specialists, solitary, and ground nesting with limited contact with infected bees or viral particles except while visiting shared squash flowers (Hurd Jr et al. 1971, Michener 2007). Species from the *Lasioglossum* genus exhibit highly variable ecology; they range from floral specialists to generalists, solitary to highly social (but generally live in relatively small social or communal groups), and predominantly nest in the ground (Michener 2007, Gibbs et al. 2012). As a group, Lasioglossum spp. are likely intermediate in their exposure to the viruses, but showed very low prevalence for BQCV and SBV, which suggests that they may be incompetent hosts for those viruses. Incompetent hosts would be unable to become infected, and therefore would not be able to exhibit a biodiversity–disease relationship.

We expected similar biodiversity–disease relationships among pathogens because the three viruses are closely related (order *Picornavirales*, DWV and SBV from *Iflavirus* genus), predominantly infect Hymenopteran insects (bees and wasps), particularly honey bees (*A. mellifera*), and have similar modes of infection (i.e. fecal-oral and food-borne) (Chen and Siede 2007, de Miranda and Genersch 2010, McMahon et al. 2018). Johnson et al. similarly found reduced infection success with greater host diversity for five out of the seven trematode parasites

tested (Johnson et al. 2013a). However, to our knowledge, few other empirical studies have compared biodiversity–disease relationships among multiple co-infecting pathogens. Previous meta-analyses and reviews have compared biodiversity–disease relationships among highly divergent pathogens, generally finding context-dependent outcomes likely based on differences in pathogen ecology, such transmission mode, infectivity, and degree of host specialization (Randolph and Dobson 2012, Salkeld et al. 2013, Wood et al. 2014, but see Civitello et al. 2015). Further investigation utilizing a comparative approach for multiple pathogens within 'replicate' host communities is needed to better understand how differences in either host or pathogen ecology may dictate variation in biodiversity–disease relationships.

Virus prevalence in pollinators

Our virus prevalence results are consistent with other studies that found DWV, BQCV, and SBV are shared among many pollinator species (Singh et al. 2010, Evison et al. 2012, Levitt et al. 2013, Fürst et al. 2014, McMahon et al. 2015, Dolezal et al. 2016). However, our study design allows us to more accurately determine differences in DWV, BQCV, and SBV prevalence among pollinator species by using larger sample sizes per species. The results show that *A. mellifera* are highly susceptible and competent hosts for all three viruses. As a close relative of *A. mellifera*, *B. impatiens* had lower DWV and BQCV prevalence, but was also a relatively competent host for all three viruses. In contrast, the more distantly related *E. pruinosa* and *Lasioglossum* spp. have low viral and infection prevalence, suggesting that both are likely poor hosts, less susceptible, and/or unlikely to encounter infective viruses compared to *A. mellifera* and *B. impatiens*.

DWV, BQCV, and SBV appear to vary in their host ranges from generalist pathogens that broadly infect many host species to relatively specialist pathogens that primarily infect a

subset of very closely related hosts. DWV appears to be the broadest generalist pathogen of the three, causing active infections in a wide range of Hymenoptera (bees, wasps, and ants) from tests for the presence of the viral negative-strand or quantified viral loads (Singh et al. 2010, Levitt et al. 2013, Manley et al. 2015). Meanwhile, SBV has the most restrictive host range limited primarily to honey bees and bumblebees, and BQCV is intermediate between the DWV and SBV (Singh et al. 2010, Levitt et al. 2013, Manley et al. 2015, Dolezal et al. 2016, Gisder and Genersch 2017). By expanding our understanding of the host ranges and relative competence among hosts for these multi-host viruses, we can create better predictions for potential biodiversity–disease relationships and mechanisms at play within pollinator communities.

Limitations and future directions

Although our findings show intriguing patterns among pollinator communities and pathogen prevalence, they are inevitably limited in scale. Communities are rarely static through time and space as host species vary in phenology, behavior, home ranges, and migration patterns, which consequently can alter expected outcomes for biodiversity–disease relationships (Estrada-Peña et al. 2014, Rohr et al. 2020). In particular, pollinator species vary in their phenology from short (less than a month) to long (the full growing season) (Tuell and Isaacs 2010, Burkle et al. 2013), and in their specific foraging and nesting habitat requirements (Lonsdorf et al. 2009, Williams et al. 2010), which result in highly dynamic pollinator communities through time and space. Our study represents an initial investigation of biodiversity–disease relationships for pollinator pathogens over two seasons and an intermediate spatial scale. Future studies that examine these relationships over different spatial and temporal scales will be critical for understanding the context-dependence of biodiversity–disease relationships in pollinator-pathogens and their underlying mechanisms (Johnson et al. 2015, Rohr et al. 2020).

Conclusions

Overall, we found that species richness was consistently the most important community factor influencing prevalence of three viruses in pollinator communities, while host abundance and community composition rarely impacted virus prevalence. Furthermore, virus prevalence was negatively associated with greater species richness—the first evidence of the dilution effect in multiple viruses infecting pollinator communities. We found similar patterns in biodiversity–disease relationships among multiple related viruses infecting the same host species, but the strength of the relationships varied based on relative host competence. Host species with high virus prevalence exhibited dilution effects, while hosts with very low virus prevalence could not exhibit a clear biodiversity–disease relationship. Few empirical studies have compared biodiversity–disease relationships among multiple pathogens infecting multiple hosts. We show that this is a powerful approach to assess commonalities and differences in biodiversity–disease relationships within natural systems. Incorporating more realistic complexity of multi-host–multi-pathogen systems into community–disease ecology will improve our understanding of underlying mechanisms that drive differences in pathogen prevalence.

Literature Cited

Ai, C., and E. C. Norton. 2003. Interaction terms in logit and probit models. Economics Letters 80:123–129.

Alger, S. A., P. A. Burnham, H. F. Boncristiani, and A. K. Brody. 2019. RNA virus spillover from managed honeybees (Apis mellifera) to wild bumblebees (Bombus spp.). PLoS ONE 14:e0217822.

Allan, B. F., R. B. Langerhans, W. A. Ryberg, W. J. Landesman, N. W. Griffin, R. S. Katz, B. J. Oberle, M. R. Schutzenhofer, K. N. Smyth, A. D. S. Maurice, L. Clark, K. R. Crooks, D. E. Hernandez, R. G. Mclean, R. S. Ostfeld, and J. M. Chase. 2009. Ecological Correlates of Risk and Incidence of West Nile Virus in the United States. Oecologia 158:699–708.

Bailes, E. J., K. R. Deutsch, J. Bagi, L. Rondissone, M. J. F. Brown, and O. T. Lewis. 2018. First detection of bee viruses in hoverfly (syrphid) pollinators. Biology Letters 14:20180001.

Barton, K. 2020. MuMIn: Multi-Model Inference. https://cran.r-project.org/package=MuMIn.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software 67:1–48.

Becker, C. G., D. Rodriguez, L. F. Toledo, A. V. Longo, C. Lambertini, D. T. Correa, D. S. Leite, C. F. B. Haddad, and K. R. Zamudio. 2014. Partitioning the net effect of host diversity on an emerging amphibian pathogen. Proceedings of the Royal Society B: Biological Sciences 281:20141796.

Begon, M. 2008. Effects of host diversity on disease dynamics. Pages 12–29 *in* R. S. Ostfeld, F. Keesing, and V. T. Eviner, editors. Infectious Disease Ecology: Effects of Ecosystems on Disease and of Disease on Ecosystems. Princeton University Press, Princeton, NJ.

Benjeddou, M., N. Leat, M. Allsopp, and S. Davison. 2001. Detection of Acute Bee Paralysis

Virus and Black Queen Cell Virus from Honeybees by Reverse Transcriptase PCR. Applied and Environmental Microbiology 67:2384–2387.

Burkle, L. A., J. C. Marlin, and T. M. Knight. 2013. Plant-Pollinator Interactions over 120 Years: Loss of Species, Co-Occurrence, and Function. Science 339:1611–1615.

Cardinal, S., J. Straka, and B. N. Danforth. 2010. Comprehenisve phylogeny of apid bees reveals the evolutionary origins and antiquity of cleptparasitism. Proceedings of the National Academy of Sciences 107:16207–16211.

Chen, Y., J. Evans, and M. Feldlaufer. 2006. Horizontal and vertical transmission of viruses in the honey bee, Apis mellifera. Journal of Invertebrate Pathology 92:152–9.

Chen, Y. P., and R. Siede. 2007. Honey bee viruses. Pages 33–80 *in* K. Maramorosch, A. J. Shatkin, and F. A. Murphy, editors. Advances in Virus Research. First edition. Academic Press, San Diego, CA.

Civitello, D. J., J. Cohen, H. Fatima, N. T. Halstead, J. Liriano, T. A. McMahon, C. N. Ortega, E. L. Sauer, T. Sehgal, S. Young, and J. R. Rohr. 2015. Biodiversity inhibits parasites: Broad evidence for the dilution effect. Proceedings of the National Academy of Sciences 112:8667–8671.

Clay, C. A., E. M. Lehmer, S. St. Jeor, and M. D. Dearing. 2009. Testing mechanisms of the dilution effect: Deer mice encounter rates, sin nombre virus prevalence and species diversity. EcoHealth 6:250–259.

Dobson, A. 2004. Population dynamics of pathogens with multiple host species. The American Naturalist 164 Suppl:S64–S78.

Dolezal, A. G., S. D. Hendrix, N. A. Scavo, J. Carrillo-Tripp, M. A. Harris, M. J. Wheelock, M. E. O'Neal, and A. L. Toth. 2016. Honey Bee Viruses in Wild Bees: Viral Prevalence,

Loads, and Experimental Inoculation. PloS ONE 11:e0166190.

Dormann, C. F., J. Elith, S. Bacher, C. Buchmann, G. Carl, G. Carr, J. R. Garc, B. Gruber, B. Lafourcade, P. J. Leit, M. Tamara, C. Mcclean, P. E. Osborne, B. S. Der, A. K. Skidmore, D. Zurell, and S. Lautenbach. 2013. Collinearity : a review of methods to deal with it and a simulation study evaluating their performance. Ecography 36:27–46.

Durrer, S., and P. Schmid-Hempel. 1994. Shared Use of Flowers Leads to Horizontal Pathogen Transmission. Proceedings of the Royal Society Biological Sciences 258:299–302.

Estrada-Peña, A., R. S. Ostfeld, A. T. Peterson, R. Poulin, and J. de la Fuente. 2014. Effects of environmental change on zoonotic disease risk: An ecological primer. Trends in Parasitology 30:205–214.

Evison, S. E. F., K. E. Roberts, L. Laurenson, S. Pietravalle, J. Hui, J. C. Biesmeijer, J. E. Smith, G. Budge, and W. O. H. Hughes. 2012. Pervasiveness of Parasites in Pollinators. PLoS ONE 7:e30641.

Ezenwa, V. O., M. S. Godsey, R. J. King, and S. C. Guptill. 2006. Avian diversity and West Nile virus: Testing associations between biodiversity and infectious disease risk. Proceedings of the Royal Society B: Biological Sciences 273:109–117.

Fenton, A., D. G. Streicker, O. L. Petchey, and A. B. Pedersen. 2015. Are All Hosts Created Equal? Partitioning Host Species Contributions to Parasite Persistence in Multihost Communities. The American Naturalist 186:610–622.

Fox, J., and S. Weisberg. 2019. An {R} Companion to Applied Regression, Third Edition. Sage, Thousand Oaks, CA. https://socialsciences.mcmaster.ca/jfox/Books/Companion/.

Fürst, M. A., D. P. McMahon, J. L. Osborne, R. J. Paxton, and M. J. F. Brown. 2014. Disease associations between honeybees and bumblebees as a threat to wild pollinators. Nature

506:364–366.

Genersch, E., C. Yue, I. Fries, and J. R. De Miranda. 2011. Detection of Deformed wing virus, a honey bee viral pathogen, in bumble bees (Bombus terrestris and Bombus pascuorum) with wing deformities. Journal of Invertebrate Pathology 91:61–63.

Gibbs, J., S. G. Brady, K. Kanda, and B. N. Danforth. 2012. Phylogeny of halictine bees supports a shared origin of eusociality for Halictus and Lasioglossum (Apoidea: Anthophila: Halictidae). Molecular Phylogenetics and Evolution 65:926–939.

Gisder, S., and E. Genersch. 2017. Viruses of commercialized insect pollinators. Journal of Invertebrate Pathology 147:51–59.

Gong, H. R., X. X. Chen, Y. P. Chen, F. L. Hu, J. L. Zhang, Z. G. Lin, J. W. Yu, and H. Q. Zheng. 2016. Evidence of Apis cerana Sacbrood virus infection in Apis mellifera. Applied and Environmental Microbiology 82:2256–2262.

Gotelli, N. J., and R. K. Colwell. 2001. Quantifying biodiversity: Procedures and pitfalls in the measurement and comparison of species richness. Ecology Letters 4:379–391.

Goulson, D., E. Nicholls, C. Botías, and E. L. Rotheray. 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science 347:1255957.

Graystock, P., D. Goulson, and W. O. H. Hughes. 2015. Parasites in bloom: flowers aid dispersal and transmission of pollinator parasites within and between bee species. Proceedings of the Royal Society Biological Sciences 282:20151371.

Greenleaf, S. S., N. M. Williams, R. Winfree, and C. Kremen. 2007. Bee foraging ranges and their relationship to body size. Oecologia 153:589–596.

Grozinger, C. M., and M. L. Flenniken. 2019. Bee Viruses: Ecology, Pathogenicity, and Impacts. Annual Review of Entomology 64:205–226.

Hall, S. P. R., C. R. Becker, J. L. Simonis, M. A. Duffy, A. J. Tessier, and C. E. Caceres. 2009. Friendly competition: evidence for a dilution effect among competitors in a planktonic host – parasite system. Ecology 90:791–801.

Halliday, F. W., R. W. Heckman, P. A. Wilfahrt, and C. E. Mitchell. 2017. A multivariate test of disease risk reveals conditions leading to disease amplification. Proceedings of the Royal Society B: Biological Sciences 284.

Han, B. A., J. L. Kerby, C. L. Searle, A. Storfer, and A. R. Blaustein. 2015. Host species composition influences infection severity among amphibians in the absence of spillover transmission. Ecology and Evolution 5:1432–1439.

Hartig, F. 2020. DHARMa: Residual Diagnostics for Hierarchical (Multi-Level/Mixed) Regression Models. https://cran.r-project.org/package=DHARMa.

Haydon, D. T., S. Cleaveland, L. H. Taylor, and M. K. Laurenson. 2002. Identifying reservoirs of infection: A conceptual and practical challenge. Emerging Infectious Diseases 8:1468–1473.

Holt, R. D., A. P. Dobson, M. Begon, R. G. Bowers, and E. M. Schauber. 2003. Parasite establishment in host communities. Ecology Letters 6:837–842.

Hsieh, T. C., K. H. Ma, and A. Chao. 2016. iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). Methods in Ecology and Evolution 7:1451–1456.

Huang, Z. Y. X., F. van Langevelde, A. Estrada-Peña, G. Suzán, and W. F. de Boer. 2016. The diversity–disease relationship: evidence for and criticisms of the dilution effect. Parasitology 143:1075–1086.

Hurd Jr, P. D., E. G. Linsley, and T. W. Whitaker. 1971. Squash and Gourd Bees (Peponapis,

Xenoglossa) and the Origin of the Cultivated Cucurbita. Evolution 25:218–234.

Johnson, P. T. J., R. B. Hartson, D. J. Larson, and D. R. Sutherland. 2008. Diversity and disease: community structure drives parasite transmission and host fitness. Ecology Letters 11:1017–1026.

Johnson, P. T. J., R. S. Ostfeld, and F. Keesing. 2015. Frontiers in research on biodiversity and disease. Ecology Letters 18:1119–1133.

Johnson, P. T. J., D. L. Preston, J. T. Hoverman, and B. E. Lafonte. 2013a. Host and parasite diversity jointly control disease risk in complex communities. Proceedings of the National Academy of Sciences 110:16916–16921.

Johnson, P. T. J., D. L. Preston, J. T. Hoverman, and K. L. D. Richgels. 2013b. Biodiversity decreases disease through predictable changes in host community competence. Nature 494:230–3.

Johnson, P. T. J., and D. W. Thieltges. 2010. Diversity, decoys and the dilution effect: how ecological communities affect disease risk. The Journal of Experimental Biology 213:961–970.

Keesing, F., L. K. Belden, P. Daszak, A. Dobson, C. D. Harvell, R. D. Holt, P. Hudson, A. Jolles, K. E. Jones, C. E. Mitchell, S. S. Myers, T. Bogich, and R. S. Ostfeld. 2010. Impacts of biodiversity on the emergence and transmission of infectious diseases. Nature 468:647–52.

Keesing, F., R. D. Holt, and R. S. Ostfeld. 2006. Effects of species diversity on disease risk. Ecology Letters 9:485–98.

Kilpatrick, A. M., P. Daszak, M. J. Jones, P. P. Marra, and L. D. Kramer. 2006. Host heterogeneity dominates West Nile virus transmission. Proceedings of the Royal Society B:

Biological Sciences 273:2327–2333.

Lafferty, K. D., and C. L. Wood. 2013. It's a myth that protection against disease is a strong and general service of biodiversity conservation: Response to Ostfeld and Keesing. Trends in Ecology and Evolution 28:503–504.

Lenth, R. 2020. emmeans: Estimated Marginal Means, aka Least-Squares Means. https://cran.r-project.org/package=emmeans.

Levi, T., F. Keesing, R. D. Holt, M. Barfield, and R. S. Ostfeld. 2016. Quantifying dilution and amplification in a community of hosts for tick-borne pathogens. Ecological Applications 26:484–498.

Levitt, A. L., R. Singh, D. L. Cox-Foster, E. Rajotte, K. Hoover, N. Ostiguy, and E. C. Holmes. 2013. Cross-species transmission of honey bee viruses in associated arthropods. Virus Research 176:232–240.

LoGiudice, K., S. T. K. Duerr, M. J. Newhouse, A. Kenneth, M. E. Killilea, and R. S. Ostfeld. 2008. Impact of Host Community Composition on Lyme Disease Risk. Ecology 89:2841–2849.

LoGiudice, K., R. S. Ostfeld, K. A. Schmidt, and F. Keesing. 2003. The ecology of infectious disease: effects of host diversity and community composition on Lyme disease risk. Proceedings of the National Academy of Sciences 100:567–71.

Lonsdorf, E., C. Kremen, T. Ricketts, R. Winfree, N. Williams, and S. Greenleaf. 2009. Modelling pollination services across agricultural landscapes. Annals of Botany 103:1589–1600.

Loreau M., and Hector. A. 2001. Partitioning selection and complementarity in biodiversity experiments. Nature 412:72–76.

Luis, A. D., A. J. Kuenzi, and J. N. Mills. 2018. Species diversity concurrently dilutes and amplifies transmission in a zoonotic host–pathogen system through competing mechanisms. Proceedings of the National Academy of Sciences 115:7979–7984.

Manley, R., M. Boots, and L. Wilfert. 2015. Emerging viral disease risk to pollinating insects: ecological, evolutionary and anthropogenic factors. Journal of Applied Ecology 10:1–10.

Mazzei, M., M. L. Carrozza, E. Luisi, M. Forzan, M. Giusti, S. Sagona, F. Tolari, and A. Felicioli. 2014. Infectivity of DWV associated to flower pollen: experimental evidence of a horizontal transmission route. PloS ONE 9:e113448.

McArt, S. H., H. Koch, R. E. Irwin, and L. S. Adler. 2014. Arranging the bouquet of disease: Floral traits and the transmission of plant and animal pathogens. Ecology Letters 17:624–636.

McMahon, D. P., M. A. Fürst, J. Caspar, P. Theodorou, M. J. F. Brown, and R. J. Paxton. 2015. A sting in the spit: widespread cross-infection of multiple RNA viruses across wild and managed bees. Journal of Animal Ecology 84:615–624.

McMahon, D. P., L. Wilfert, R. J. Paxton, and M. J. F. Brown. 2018. Emerging Viruses in Bees: From Molecules to Ecology. Pages 251–291 *in* C. M. Malmstrom, editor. Advances in Virus Research: Environmental Virology and Virus Ecology. First edition. Elsevier Inc., London, UK.

Michener, C. D. 2007. The Bees of the World. 2nd edition. John Hopkins University Press.

Mihaljevic, J. R., M. B. Joseph, S. A. Orlofske, and S. H. Paull. 2014. The scaling of host density with richness affects the direction, shape, and detectability of diversity-disease relationships. PLoS ONE 9:e97812.

de Miranda, J. R., and E. Genersch. 2010. Deformed wing virus. Journal of Invertebrate

Pathology 103 Suppl:S48-61.

Mitchell, C. E., D. Tilman, and J. V. Groth. 2002. Effects of Grassland Plant Species Diversity, Abundance, and Composition on Foliar Fungal Disease. Ecology 83:1713–1726.

Novotný, V., and Y. Basset. 2000. Rare species in communities of tropical insect herbivores: Pondering the mystery of singletons. Oikos 89:564–572.

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, E. Szoecs, and H. Wagner. 2018. vegan: Community Ecology Package. https://cran.r-project.org/package=vegan.

Ongus, J. R., D. Peters, J. M. Bonmatin, E. Bengsch, J. M. Vlak, and M. M. van Oers. 2004. Complete sequence of a picorna-like virus of the genus Iflavirus replicating in the mite Varroa destructor. Journal of General Virology 85:3747–3755.

Ostfeld, R. S., and F. Keesing. 2000. Biodiversity and Disease Risk: The Case of Lyme Disease. Conservation Biology 14:722–728.

Ostfeld, R. S., and K. LoGiudice. 2003. Community dissassembly, biodiversity loss, and the erosion of an ecosystem service. Ecology 84:1421–1427.

Paradis, E., and K. Schliep. 2018. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35:526–528.

Peng, W., J. Li, H. Boncristiani, J. P. Strange, M. Hamilton, and Y. Chen. 2011. Host range expansion of honey bee Black Queen Cell Virus in the bumble bee, Bombus huntii. Apidologie 42:650–658.

Potts, S. G., J. C. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger, and W. E. Kunin. 2010. Global pollinator declines: Trends, impacts and drivers. Trends in Ecology and Evolution 25:345–353.

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rader, R., I. Bartomeus, J. M. Tylianakis, and E. Laliberté. 2014. The winners and losers of land use intensification: Pollinator community disassembly is non-random and alters functional diversity. Diversity and Distributions 20:908–917.

Radzevičiūtė, R., P. Theodorou, M. Husemann, G. Japoshvili, G. Kirkitadze, A. Zhusupbaeva, and R. J. Paxton. 2017. Replication of honey bee-associated RNA viruses across multiple bee species in apple orchards of Georgia, Germany and Kyrgyzstan. Journal of Invertebrate Pathology 146:14–23.

Randolph, S., and A. Dobson. 2012. Pangloss revisited: a critique of the dilution effect and the biodiversity-buffers-disease paradigm. Parasitology 139:847–63.

Ricklefs, R. E. 1987. Community Diversity: Relative Roles of Local and Regional Processes. Science 235:167–171.

Rigaud, T., M.-J. Perrot-Minnot, and M. J. F. Brown. 2010. Parasite and host assemblages: embracing the reality will improve our knowledge of parasite transmission and virulence. Proceedings of the Royal Society Biological Sciences 277:3693–702.

Roche, B., A. P. Dobson, J. F. Guégan, and P. Rohani. 2012. Linking community and disease ecology: The impact of biodiversity on pathogen transmission. Philosophical Transactions of the Royal Society B: Biological Sciences 367:2807–2813.

Rohr, J. R., D. J. Civitello, F. W. Halliday, P. J. Hudson, K. D. Lafferty, C. L. Wood, and E. A. Mordecai. 2020. Towards common ground in the biodiversity–disease debate. Nature Ecology and Evolution 4:24–33.

Rudolf, V. H. W., and J. Antonovics. 2005. Species Coexistence and Pathogens with Frequency-

Dependent Transmission. The American Naturalist 166:112–118.

Salkeld, D. J., K. A. Padgett, and J. H. Jones. 2013. A meta-analysis suggesting that the relationship between biodiversity and risk of zoonotic pathogen transmission is idiosyncratic. Ecology Letters 16:679–686.

Schmidt, K. A., and R. S. Ostfeld. 2001. Biodiversity and the Dilution Effect in Disease Ecology. Ecology 82:609–619.

Searle, C. L., L. M. Biga, J. W. Spatafora, and A. R. Blaustein. 2011. A dilution effect in the emerging amphibian pathogen Batrachochytrium dendrobatidis. Proceedings of the National Academy of Sciences of the United States of America 108:16322–16326.

Singh, R., A. L. Levitt, E. G. Rajotte, E. C. Holmes, N. Ostiguy, D. Vanengelsdorp, W. I. Lipkin, C. W. Depamphilis, A. L. Toth, and D. L. Cox-Foster. 2010. RNA viruses in hymenopteran pollinators: evidence of inter-taxa virus transmission via pollen and potential impact on non-Apis hymenopteran species. PLoS ONE 5:e14357.

Stevenson, M., T. Nunes, C. Heuer, J. Marshall, J. Sanchez, R. Thornton, J. Reiczigel, J. Robison-Cox, P. Sebastiani, P. Solymos, K. Yoshida, G. Jones, S. Pirikahu, S. Firestone, R. Kyle, J. Popp, M. Jay, and C. Reynard. 2020. epiR: Tools for the Analysis of Epidemiological Data.

Strauss, A. T., A. M. Bowling, M. A. Duffy, C. E. Cáceres, and S. R. Hall. 2018. Linking host traits, interactions with competitors and disease: Mechanistic foundations for disease dilution. Functional Ecology 32:1271–1279.

Strauss, A. T., D. J. Civitello, C. E. Cáceres, and S. R. Hall. 2015. Success, failure and ambiguity of the dilution effect among competitors. Ecology Letters 18:916–926.

Tuell, J. K., and R. Isaacs. 2010. Community and species-specific responses of wild bees to

insect pest control programs applied to a pollinator-dependent crop. Journal of Economic Entomology 103:668–675.

Venesky, M. D., X. Liu, E. L. Sauer, and J. R. Rohr. 2014. Linking manipulative experiments to field data to test the dilution effect. Journal of Animal Ecology 83:557–565.

Wilfert, L., G. Long, H. C. Leggett, S. J. M. Martin, P. Schmid-Hempel, R. K. Butlin, and M. Boots. 2016. Deformed Wing Virus is a Recent Global Epidemic in Honeybees driven by Varroa Mites. Science 351:594–597.

Williams, N. M., E. E. Crone, T. H. Roulston, R. L. Minckley, L. Packer, and S. G. Potts. 2010. Ecological and life-history traits predict bee species responses to environmental disturbances. Biological Conservation 143:2280–2291.

Wood, C. L., and K. D. Lafferty. 2013. Biodiversity and disease: A synthesis of ecological perspectives on Lyme disease transmission. Trends in Ecology and Evolution 28:239–247.

Wood, C. L., K. D. Lafferty, G. DeLeo, H. S. Young, P. J. Hudson, and A. M. Kuris. 2014. Does biodiversity protect humans against infectious disease? Ecology 95:817–832.

Woolhouse, M. E. J., and S. Gowtage-Sequeria. 2005. Host range and emerging and reemerging pathogens. Emerging Infectious Diseases 11:1842–7.

Yang, B., G. Peng, T. Li, and T. Kadowaki. 2013. Molecular and phylogenetic characterization of honey bee viruses, Nosema microsporidia, protozoan parasites, and parasitic mites in China. Ecology and Evolution 3:298–311.

Yue, C., and E. Genersch. 2005. RT-PCR analysis of Deformed wing virus in honeybees (Apis mellifera) and mites (Varroa destructor). Journal of General Virology 86:3419–3424.

Tables

Table 2.1: The number of individuals for each species that were virus positive for DWV, BQCV, and SBV. Virus presence was determined by RT-PCR, and virus prevalence for each host species is shown in **Figure 2.1**.

Species	**DWV**	**BQCV**	**SBV**	**Total Tested**
Apis mellifera	128	186	71	237
Bombus impatiens	85	89	98	252
Lasioglossum spp.	41	16	1	255
Eucera pruinosa	39	13	4	193

Table 2.2: Model selection table comparing top four models based on lowest AICc. The simpler Model 2a was selected (bolded) as the top model based on very close performance compared with Model 3a, but with only a single interaction term rather than a three-way interaction and three two-way interactions. The full model selection table can be found in **Appendix Table S2.7**, and model results for Model 2a and Model 3a in **Table 2.4** and **Appendix Table S2.8**, respectively.

Model	***Model details***	***K***	***logLik***	***AICc***	***delta***	***weight***
Model 3a	Abundance + Richness × Virus Type × Host Species	27	-1206.03	2466.61	0.00	0.420
Model 2a	**Abundance + Richness + Virus Type × Host Species**	**16**	**-1217.45**	**2467.10**	**0.49**	**0.328**
Model 3c	Abundance × Richness + Virus Type × Host Species	17	-1217.04	2468.31	1.69	0.180
Model 3b	Richness + Abundance × Virus Type × Host Species	27	-1207.79	2470.13	3.52	0.072

Table 2.3: DWV, BQCV, and SBV infection prevalence for *Apis mellifera*, *Bombus impatiens*, *Lasioglossum* spp., and *Eucera pruinosa* determined by a subset of virus-positive samples that were positive for the viral negative strand, indicating active viral infections. The 95% confidence intervals are in parentheses and data include samples randomly selected from all sites. Specific sample sizes for each host–virus pair are indicated in **Appendix Table S2.6**, and p-values for differences in infection prevalence are in **Appendix Table S2.11**.

Species	**DWV**	**BQCV**	**SBV**
Apis mellifera	26.9% (12.3, 46.5)	87.0% (68.0, 96.4)	96.0% (81.0, 99.8)
Bombus impatiens	68.2% (45.2, 85.5)	66.7% (44.9, 84.8)	88.0% (69.7, 96.7)
Lasioglossum spp.	15.0% (4.2, 36.9)	40.0% (18.6, 66.8)	0.0% (0.0, 95.0)
Eucera pruinosa	10.0% (1.8, 31.6)	7.7% (0.4, 33.7)	50.0% (9.8, 90.2)

Table 2.4: Analysis of deviance table for the main factors of the top Model 2a generalized linear mixed effects model (GLMM) output based on the Type II Wald Chi squared test. Factors with significant p-values are bolded.

Main Factors	χ^2	**d*f***	***P value***
Total *Abundance*	1.71	1	0.1907
Species *Richness*	12.79	1	**0.0003**
Virus Type	34.63	2	**< 0.0001**
Host Species	165.25	3	**< 0.0001**
Virus Type × *Genus*	131.18	6	**< 0.0001**

Figures

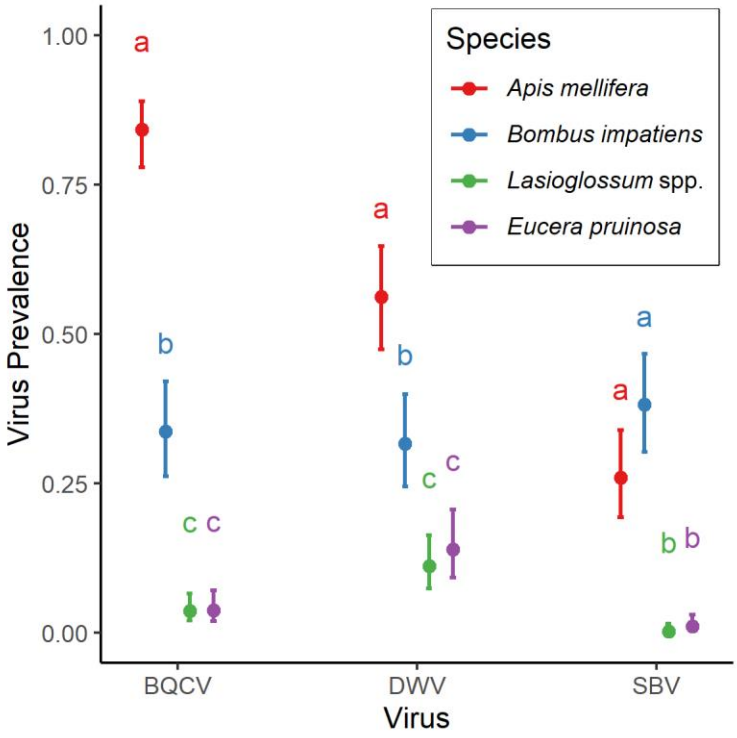


Figure 2.1: Virus prevalence varied significantly among different host species. DWV, BQCV, and SBV prevalence with the 95% CI among *Apis mellifera*, *Bombus impatiens*, *Lasioglossum* spp., and *Eucera pruinosa*. Different letters indicate significant differences in virus prevalence among host species and within each virus type. The data shown in this figure correspond to results from the Model 2a analysis, and post-hoc pairwise comparison with a Tukey p-value adjustment for multiple comparisons. Virus prevalence with 95% confidence intervals, and the pairwise comparisons tests with p-values can be found in the **Appendix, Table S2.15 and S2.16**. Sample sizes per host species: *A. mellifera*, n = 237; *B. impatiens*, n = 252; *Lasioglossum* spp., n = 255; and *E. pruinosa*, n = 193.

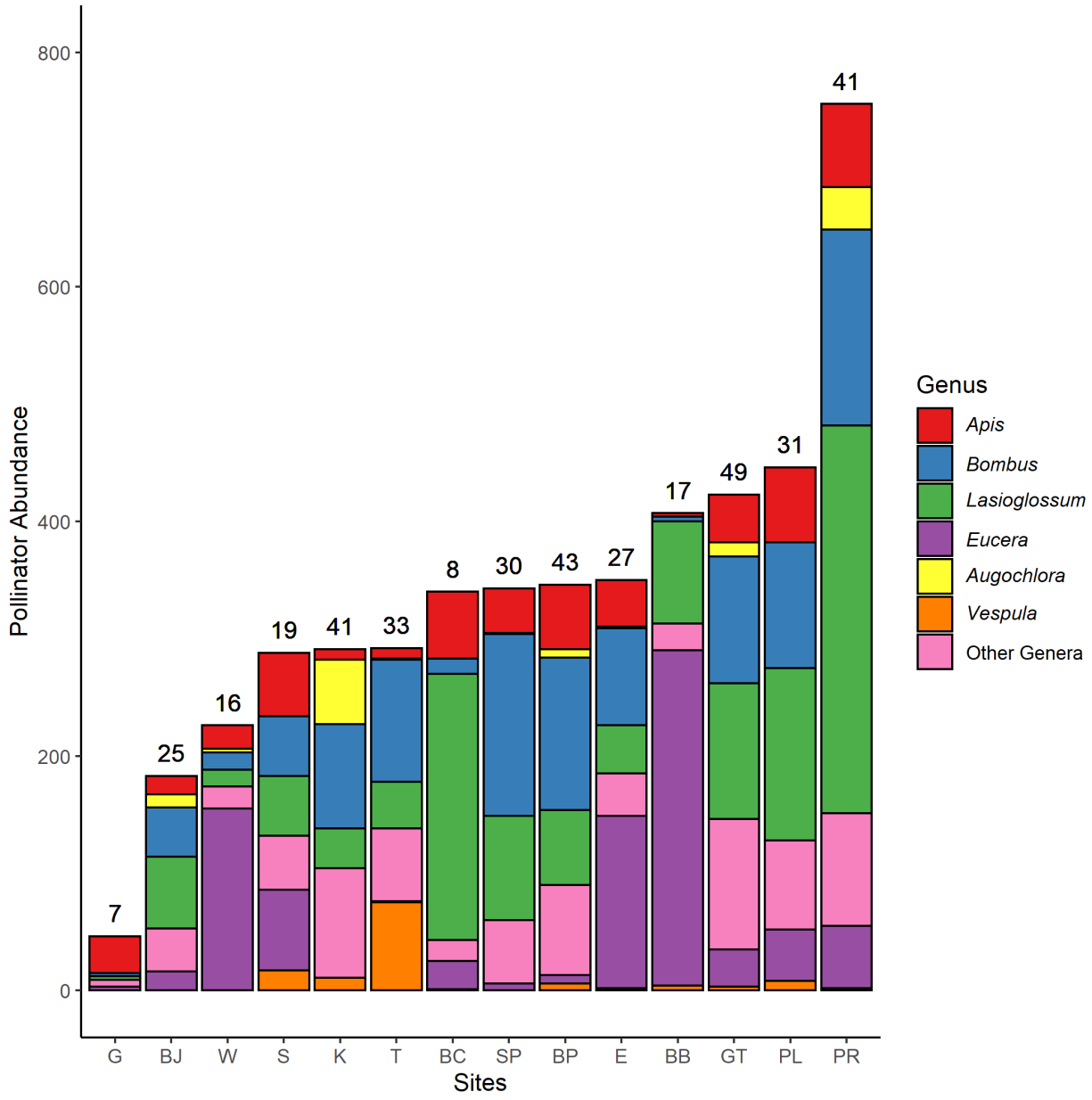


Figure 2.2: Pollinator species richness, abundance, and community composition vary among sites. Each bar depicts the relative abundance of the six most common genera and all other genera grouped together per site, with the total height of the bar representing the total pollinator abundance. The species richness found at each site is shown at the top of each bar. Site abbreviation codes can be found in **Appendix Table S2.1.**

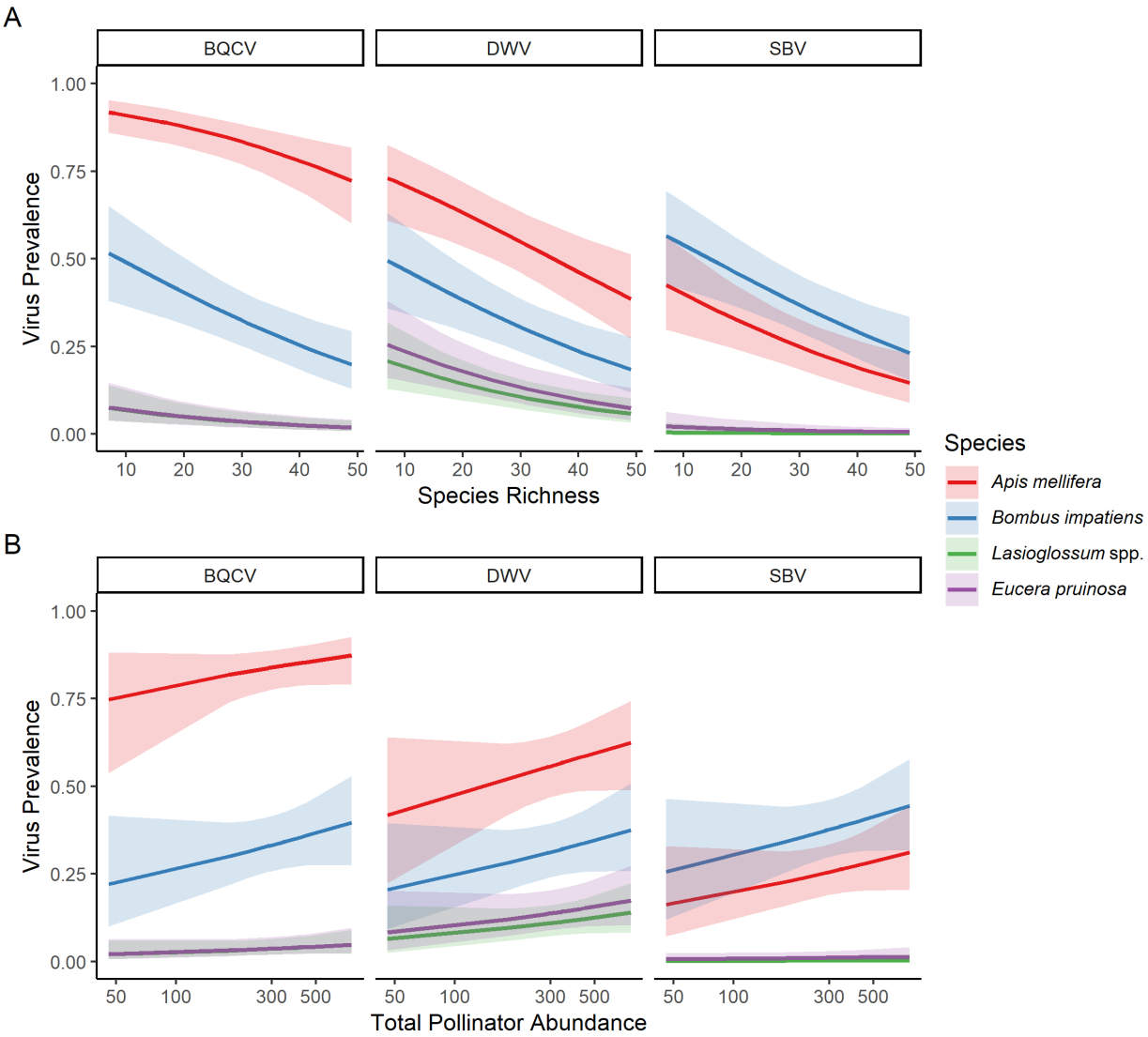


Figure 2.3: A) Species richness, Species-rich communities are significantly correlated with lower predicted virus prevalence in *Apis mellifera*, *Bombus impatiens*, *Lasioglossum*, and *Eucera pruinosa* (species richness, p = 0.0003). **B) Pollinator abundance,** Total pollinator abundance was not significantly correlated with pollinator virus prevalence (total pollinator abundance, p = 0.19). Total pollinator abundance is on a log scale. The data shown in this figure correspond to those used in the analysis presented in **Table 2.3**.

Appendix S1

Detection of positive-strand virus by RT-PCR

Positive-strand complimentary DNA (cDNA) synthesis reactions was performed with 2 µl of RNA template in a 20 µl reaction using M-MLV reverse-transcriptase (Promega). RNA template was denatured to remove any secondary structures at 70 ºC for 5 minutes with 0.25 µM random hexamers (Invitrogen), 0.5 mM dNTPs, and dH_2O. Then 0.25 U RNase inhibitor, M-MLV 0.4x buffer and 10 U M-MLV reverse transcriptase were added to the reaction. Samples were extended at 37 ºC for 60 minutes, followed by reverse transcriptase inactivation at 92 ºC for 10 minutes.

Presence or absence of three common bee viruses (Black Queen Cell Virus BQCV, Deformed Wing Virus, and Sacbrood Virus SBV) was determined by RT-PCR. All reactions had a total reaction volume of 20 µl, using 0.025 U of Platinum Taq DNA polymerase (Invitrogen), with 2 µl of template, 1x reaction buffer, 2.5 mM $MgCl_2$, 0.2 µM dNTPs, and 0.5 µM primers (each). For DWV, BQCV, and SBV reactions, samples were amplified at 94 °C for 8 minutes, followed by 35 cycles of 94 °C for 45 seconds, T_a for 1 minute, and 72 °C for 1 minute, and a final extension step of 72°C for 10 minutes (T_a and primer sequences are given in **Table S2.4**). For the 18S reactions, samples were amplified at 94 °C for 1 minute, followed by 35 cycles of 94 °C for 1 minute, T_a for 1 minute, and 72 °C for 1 minute, and a final extension step of 72°C for 7 minutes (T_a and primer sequences are given in **Table S2.4**). Positive and negative controls were included in each PCR run. PCR products were visualized under UV light on a 2% agarose gel stained with Gel Red stain (Phenix Research Products). Samples with unclear visualization

results (i.e. faint bands) were retested with RT-PCR to confirm either presence or absence. Select samples were sequenced to determine accurate identification of the positive-strand for each virus (see **Table S2.5** for GenBank Accession Numbers).

Detection of negative-strand virus by RT-PCR

Negative-strand specific cDNA synthesis was done with 2 µl of RNA template from virus-positive samples detected with positive strand RT-PCR in a 25 µl reaction with M-MLV reverse-transcriptase (Promega). RNA template was denatured to remove any secondary structures at 70 ºC for 5 minutes with 0.4 µM negative-strand specific primer (primer sequences are given in **Table S2.4**) and dH_2O. Followed by adding 0.2 U RNase inhibitor, 0.8 mM dNTPs, M-MLV 1x buffer and 8 U M-MLV reverse transcriptase. Samples were extended 50 ºC for 45 minutes, then the reverse transcriptase was inactivated at 95 ºC for 15 minutes.

Presence or absence of the negative-strand viral sequence for DWV, BQCV, and SBV was carried out with 2.5 µl of cDNA template in RT-PCR reactions. All reactions had a total reaction volume of 20 µl, using 0.025 U of Platinum Taq DNA polymerase (Invitrogen), with 2 µl of template, 1x reaction buffer, 2.5 mM $MgCl_2$, 0.2 µM dNTPs, and 0.5 µM primers (each). For DWV negative-strand reactions, samples were amplified at 94 °C for 5 minutes, followed by 30 cycles of 94 °C for 30 seconds, T_a for 30 seconds, and 72 °C for 2 minutes, and a final extension step of 72°C for 7 minutes. For BQCV and SBV negative-strand reactions, samples were amplified at 95 °C for 2 minutes, followed by 37 cycles of 95 °C for 30 seconds, T_a for 30 seconds, and 72 °C for 1 minute, and a final extension step of 72°C for 5 minutes (T_a and primer sequences are given in **Table S2.4**). Positive and negative controls were included in each PCR run. PCR products were visualized under UV light on a 2% agarose gel stained with Gel Red stain (Phenix Research Products). Samples with unclear visualization results (i.e. faint bands)

were retested with RT-PCR to confirm either presence or absence. Select samples were sequenced to determine accurate identification of the negative-strand for each virus (see **Table S2.5** for GenBank Accession Numbers).

Appendix S2

Table S2.1: Field site abbreviation, farm name, sampling year, dates of each visit to field site, and zone, easting and northing coordinates in the UTM GPS system. All field sites are in the Lower Peninsula of Michigan, USA.

Code	Farm Name	Year	First Visit	Second visit	Zone	Easting	Northing
W	Wasem Fruit Farm	2015	7/22/2015	7/30/2015	17T	282985	4668596
G	Gust Brother's Pumpkin Farm	2015	7/26/2015	8/5/2015	17T	267978	4625139
BC	The Blast Corn Maze (Nixon Farms)	2015	7/24/2015	8/2/2015	17T	264841	4692908
BB	Bird's Big Punk'ns	2015	7/18/2015	7/28/2015	17T	300395	4672764
BJ	BJ Farm	2015	7/23/2015	7/31/2015	17T	281032	4630188
S	Stone Coop Farm	2015	8/7/2015	8/21/2015	17T	271906	4704446
BP	Brimley's Pumpkin Patch	2016	8/10/2016	8/26/2016	16T	714474	4716740
T	Tantré Farm	2016	8/18/2016	9/2/2016	16T	738575	4681735
K	Kapnick Orchards	2016	8/21/2016	8/28/2016	17T	257729	4648607
SP	Spicer Orchards	2016	8/14/2016	9/1/2018	17T	274397	4729038
PR	Peacock Road Farms	2016	7/26/2016	8/23/2016	16T	714244	4746884
GT	Green Things Farm	2016	8/17/2016	8/24/2016	17T	276741	4689607
E	Erwin Orchards	2016	7/27/2016	8/22/2016	17T	280997	4708908
PL	Plymouth Orchards	2016	8/11/2016	8/30/2016	17T	289557	4690343

Table S2.2: The total number of individuals collected for each of the five most common species or genera at each field site. Totals of each species among all sites is indicated on the right.

Year	***2015***						***2016***								
Species Names	**BB**	**W**	**BJ**	**BC**	**G**	**S**	**PR**	**E**	**BP**	**PL**	**SP**	**GT**	**T**	**K**	**Species totals**
Apis mellifera	3	20	16	57	31	54	71	40	55	64	38	41	9	9	**508**
Bombus impatiens	4	13	42	13	3	50	167	76	126	105	153	105	103	89	**1049**
Lasioglossum spp.	87	14	61	227	3	51	331	41	64	147	89	116	40	34	**1305**
Eucera pruinosa	286	155	16	24	3	69	53	147	7	44	6	32	1	0	**843**
Halictus spp.	5	5	3	12	4	18	13	11	8	6	2	11	5	2	**105**

Table S2.3: The number of individuals tested for the presence of DWV, BQCV, and SBV. Where less than 20 individuals were collected, then all collected individuals were tested.

Year	***2015***						***2016***								
Species Names	**BB**	**W**	**BJ**	**BC**	**G**	**S**	**PR**	**E**	**BP**	**PL**	**SP**	**GT**	**T**	**K**	**Species totals**
Apis mellifera	3	20	16	21	20	20	20	20	20	20	20	20	8	9	**237**
Bombus impatiens	4	13	30	13	3	30	19	20	20	20	20	20	20	20	**252**
Lasioglossum spp.	22	12	19	22	2	19	20	19	20	20	20	20	20	20	**255**
Eucera pruinosa	20	21	16	20	3	20	20	20	6	20	6	20	1	0	**193**

Table S2.4: Primer pair sequences for positive- and negative-strand virus screens and RT-PCR annealing temperatures.

Virus/ Gene	Purpose	Primer name	Primer sequence	T_a	Product size	Reference
BQCV	RT-PCR screen	BQCV 3'UTR F	TGGTCAGCTCCCACTACCTTAAAC	57 ºC	700bp	(*1*)
		BQCV 3'UTR R	GCAACAAGAAGAAACGTAAACCAC			
DWV	RT-PCR screen	DWV VP1a F	CTCGTCATTTTGTCCCGACT	56 ºC	424bp	(*2*)
		DWV VP1a R	TGCAAAGATGCTGTCAAACC			
SBV	RT-PCR screen	SBV VP1b F	GCACGTTTAATTGGGGATCA	55 ºC	693bp	(*2*)
		SBV VP1b R	CAGGTTGTCCCTTACCTCCA			
18 S rRNA	RT-PCR screen (bee positive control)	18S H17F	AAATTACCCACTCCCGGCA	58 ºC	784bp	(*3*)
		18S H35R	TGGTGAGGTTTCCCGTGTT			
DWV	negative strand specific cDNA synthesis	Tag-F15	agcctgcgcaccgtggTCCATCAGGTTCTCCAATAACGGA	na	na	(*4*)
DWV	negative strand specific RT-PCR	B23	CCACCCAAATGCTAACTCTAAGCG	54.5 ºC	450bp	(*4*)
BQCV	negative strand specific cDNA synthesis	Tag-BQCV-sense	agcctgcgcaccgtggTCAGGTCGGAATAATCTCGA	na	na	(*5*)
general	negative strand specific RT-PCR	Tag	AGCCTGCGCACCGTGG	na	na	(*5*)
BQCV	negative strand specific RT-PCR	BQCV-antisense	GCAACAAGAAGAAACGTAAACCAC	55 ºC	420bp (with tag)	(*5*)
SBV	negative strand specific cDNA synthesis	Tag-SB7f	agcctgcgcaccgtggGGAGATGTTAGAAATACCAACCGATTCC	na	na	(*6*)
SBV	negative strand specific RT-PCR	SB8R	CCATTAAACAAATCGGTATAAGAGTCCACT	57 ºC	200bp	(*6*)

Table S2.5: GenBank Accession Numbers and associated information for sequenced samples.

GenBank Accession #	Gene or Virus/Gene	Target Strand	Isolate Species	Bee ID #
MN900314	18S ribosomal RNA gene		*Apis mellifera*	1496
MN900315	18S ribosomal RNA gene		*Apis mellifera*	2962
MN900316	18S ribosomal RNA gene		*Bombus impatiens*	3567
MN900317	18S ribosomal RNA gene		*Bombus impatiens*	2373
MN900318	18S ribosomal RNA gene		*Lasioglossum*	1353
MN900319	18S ribosomal RNA gene		*Lasioglossum*	4266
MN900320	18S ribosomal RNA gene		*Eucera pruinosa*	599
MN900321	18S ribosomal RNA gene		*Eucera pruinosa*	3375
MN902093	Black queen cell virus/capsid protein 4	Negative	*Bombus impatiens*	454
MN902094	Black queen cell virus/capsid protein 4	Negative	*Apis mellifera*	448
MN902095	Black queen cell virus/capsid protein 4	Negative	*Eucera pruinosa*	1302
MN902096	Black queen cell virus/capsid protein 4	Negative	*Apis mellifera*	519
MN902097	Black queen cell virus/capsid protein 4	Negative	*Bombus impatiens*	1698
MN902098	Black queen cell virus/capsid protein 4	Negative	*Apis mellifera*	1496
MN902099	Black queen cell virus/capsid protein 4	Negative	*Bombus impatiens*	4612
MN902100	Black queen cell virus/structural polyprotein	Positive	*Bombus impatiens*	454
MN902101	Black queen cell virus/structural polyprotein	Positive	*Apis mellifera*	448
MN902102	Black queen cell virus/structural polyprotein	Positive	*Apis mellifera*	1663
MN902103	Black queen cell virus/structural polyprotein	Positive	*Eucera pruinosa*	1302
MN902104	Black queen cell virus/structural polyprotein	Positive	*Apis mellifera*	519
MN902105	Black queen cell virus/structural polyprotein	Positive	*Bombus impatiens*	571
MN902106	Black queen cell virus/structural polyprotein	Positive	*Apis mellifera*	1294
MN902107	Black queen cell virus/structural polyprotein	Positive	*Apis mellifera*	1428
MN902108	Black queen cell virus/structural polyprotein	Positive	*Bombus impatiens*	3567
MN902109	Black queen cell virus/structural polyprotein	Positive	*Bombus impatiens*	2373
MN902110	Deformed wing virus/polyprotein	Negative	*Apis mellifera*	1319
MN902111	Deformed wing virus/polyprotein	Negative	*Bombus impatiens*	1631
MN902112	Deformed wing virus/polyprotein	Negative	*Bombus impatiens*	3567
MN902113	Deformed wing virus/polyprotein	Negative	*Apis mellifera*	4151

MN902114	Deformed wing virus/polyprotein	Negative	*Eucera pruinosa*	265
MN902115	Deformed wing virus/polyprotein	Negative	*Apis mellifera*	1663
MN902116	Deformed wing virus/polyprotein	Negative	*Apis mellifera*	1699
MN902117	Deformed wing virus/capsid protein	Positive	*Apis mellifera*	1663
MN902118	Deformed wing virus/capsid protein	Positive	*Apis mellifera*	1519
MN902119	Deformed wing virus/capsid protein	Positive	*Apis mellifera*	1699
MN902120	Deformed wing virus/capsid protein	Positive	*Bombus impatiens*	1311
MN902121	Deformed wing virus/capsid protein	Positive	*Lasioglossum*	563
MN902122	Deformed wing virus/capsid protein	Positive	*Lasioglossum*	930
MN902123	Deformed wing virus/capsid protein	Positive	*Eucera pruinosa*	436
MN902124	Deformed wing virus/capsid protein	Positive	*Apis mellifera*	465
MN902125	Deformed wing virus/capsid protein	Positive	*Apis mellifera*	1319
MN902126	Deformed wing virus/capsid protein	Positive	*Eucera pruinosa*	265
MN902127	Sacbrood virus/polyprotein	Negative	*Apis mellifera*	1450
MN902128	Sacbrood virus/polyprotein	Negative	*Apis mellifera*	1322
MN902129	Sacbrood virus/polyprotein	Negative	*Apis mellifera*	3462
MN902130	Sacbrood virus/polyprotein	Negative	*Apis mellifera*	2534
MN902131	Sacbrood virus/polyprotein	Negative	*Bombus impatiens*	2543
MN902132	Sacbrood virus/polyprotein	Negative	*Apis mellifera*	3734
MN902133	Sacbrood virus/capsid protein	Positive	*Bombus impatiens*	454
MN902134	Sacbrood virus/capsid protein	Positive	*Apis mellifera*	1450
MN902135	Sacbrood virus/capsid protein	Positive	*Apis mellifera*	1682
MN902136	Sacbrood virus/capsid protein	Positive	*Bombus impatiens*	2667
MN902137	Sacbrood virus/capsid protein	Positive	*Apis mellifera*	2239
MN902138	Sacbrood virus/capsid protein	Positive	*Bombus impatiens*	2379

Table S2.6: The number of randomly selected virus-positive individuals tested for the presence of the negative-strand sequence for DWV, BQCV, and SBV. All BQCV and SBV positive *Lasioglossum* spp. and *E. pruinosa* samples were included in tests for the negative-strand due to low positive-strand viral prevalence.

Species Names	**DWV**	**BQCV**	**SBV**
Apis mellifera	26	23	25
Bombus impatiens	22	21	25
Lasioglossum spp.	20	15	1
Eucera pruinosa	20	13	4
Total	**88**	**72**	**55**

Table S2.7: Model selection table for all model combinations tested ranked by lowest AICc. Model 2a (bolded) was selected as the main model presented in the main text as a simpler model with very similar AICc compared to Model 3a.

Model	**Model details**	**K**	**logLik**	**AICc**	**delta**	**weight**
Model 3a	Abundance + Richness × Virus Type × Host Species	27	-1206.03	2466.61	0.00	0.420
Model 2a	**Abundance + Richness + Virus Type × Host Species**	**16**	**-1217.45**	**2467.10**	**0.49**	**0.328**
Model 3c	Abundance × Richness + Virus Type × Host Species	17	-1217.04	2468.31	1.69	0.180
Model 3b	Richness + Abundance × Virus Type × Host Species	27	-1207.79	2470.13	3.52	0.072
Model 4	Abundance × Richness × Virus Type × Host Species	50	-1191.52	2484.88	18.27	0.000
Model 2b	Abundance + Richness × Virus Type + Host Species	12	-1291.31	2606.73	140.12	0.000
Model 2e	Abundance + Richness × Host Species + Virus Type	13	-1291.01	2608.16	141.54	0.000
Model 3d	Abundance x Richness × Virus Type + Host Species	17	-1288.84	2611.90	145.29	0.000
Model 3e	Abundance × Richness × Host Species + Virus Type	20	-1286.02	2612.34	145.73	0.000
Model 1	Abundance + Richness + Virus Type + Host Species	10	-1298.19	2616.47	149.85	0.000
Model 2c	Abundance × Richness + Virus Type + Host Species	11	-1297.77	2617.63	151.02	0.000
Model 2d	Richness + Abundance × Virus Type + Host Species	12	-1296.93	2617.97	151.36	0.000
Model 2f	Richness + Abundance × Host Species + Virus Type	13	-1296.18	2618.49	151.88	0.000

Table S2.8: Analysis of deviance table for Model 3a, for comparison to Model 2a (**Table 2.4**), the two top models from the model selection **Table S2.7**.

Main Factors	χ^2	**d*f***	**P value**
Total Abundance	1.27	1	0.26
Species Richness	11.23	1	**0.0008**
Virus Type	32.25	2	**< 0.0001**
Host Species	159.92	3	**< 0.0001**
Species Richness × Virus Type	2.89	2	0.24
Species Richness × Host Species	12.47	3	**0.0059**
Virus Type × Host Species	121.70	6	**< 0.0001**
Species Richness × Virus Type × Host Species	3.91	6	0.69

Table S2.9: The variance inflation factors (VIF) for each main factor in Model 2a.

Main Factor	**VIF**
Intercept	4.24
Total Abundance	1.43
Species Richness	1.44
Virus Type: DWV	5.41
Virus Type: SBV	4.57
Host Species: BOMB	4.43
Host Species: LASI	4.01
Host Species: PEPO	4.13
Virus Type DWV × Host Species BOMB	3.63
Virus Type SBV × Host Species BOMB	4.24
Virus Type DWV × Host Species LASI	3.77
Virus Type SBV × Host Species LASI	1.12
Virus Type DWV × Host Species PEPO	3.80
Virus Type SBV × Host Species PEPO	1.50

Table S2.10: Analysis of deviance table for a comparable model to Model 2a with species-specific abundance for the four most common species rather than total abundance. The species-specific abundance model shows similar results to **Table 2.4**.

Main Factors	χ^2	**d*f***	**P value**
Species Richness	5.05	1	**0.025**
A. mellifera Abundance	0.08	1	0.78
B. impatiens Abundance	0.01	1	0.94
Lasioglossum spp. Abundance	1.62	1	0.20
E. pruinosa Abundance	0.19	1	0.66
Virus Type	34.63	2	< **0.0001**
Host Species	161.24	3	< **0.0001**
Virus Type × Host Species	131.16	6	< **0.0001**

Table 2.11: Moran's I spatial autocorrelation test on the residuals for each generalized linear mixed model tested in **Table S2.7**. None of the models showed significant spatial autocorrelation.

Model	**Observed**	**Expected**	**SD**	**p value**
Model 1	-0.14889	-0.07692	0.053004	0.1746
Model 2a	-0.14837	-0.07692	0.052952	0.1772
Model 2b	-0.14744	-0.07692	0.052895	0.1825
Model 2c	-0.13868	-0.07692	0.052763	0.2418
Model 2d	-0.14959	-0.07692	0.052946	0.1699
Model 2e	-0.14376	-0.07692	0.053027	0.2075
Model 2f	-0.14831	-0.07692	0.053106	0.1789
Model 3a	-0.14314	-0.07692	0.053104	0.2124
Model 3b	-0.14962	-0.07692	0.053376	0.1732
Model 3c	-0.13892	-0.07692	0.052773	0.2401
Model 3d	-0.13838	-0.07692	0.052739	0.2439
Model 3e	-0.10368	-0.07692	0.052997	0.6136
Model 4	-0.09792	-0.07692	0.053123	0.6926

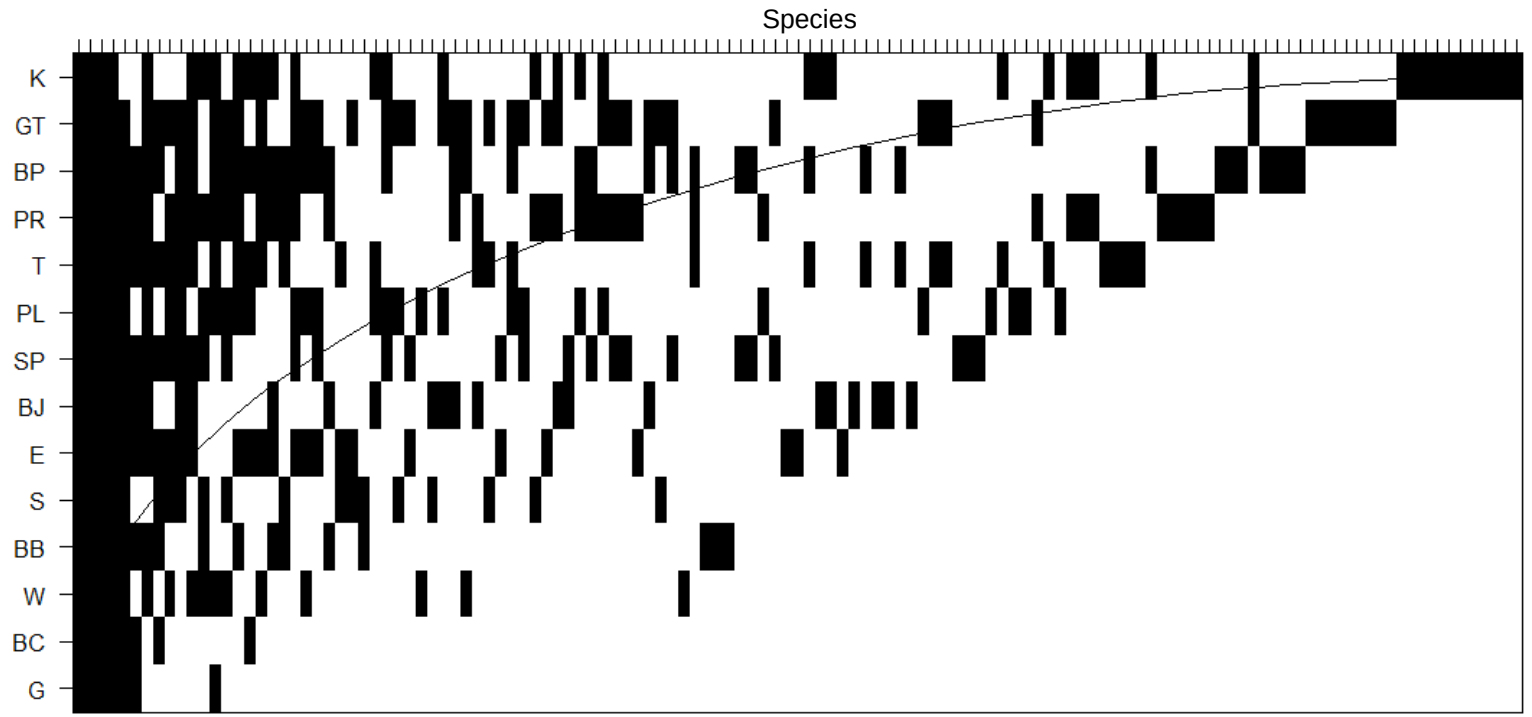


Figure S2.1: The pollinator communities sampled are significantly nested compared to simulated null community matrices, such that species poor communities are composed of a subset of the species rich communities (observed nested temperature = 20.7°; average null model temperature = 53.3°, p = 0.01). The incidence (black) of each species (columns) that occurs at each site (rows).

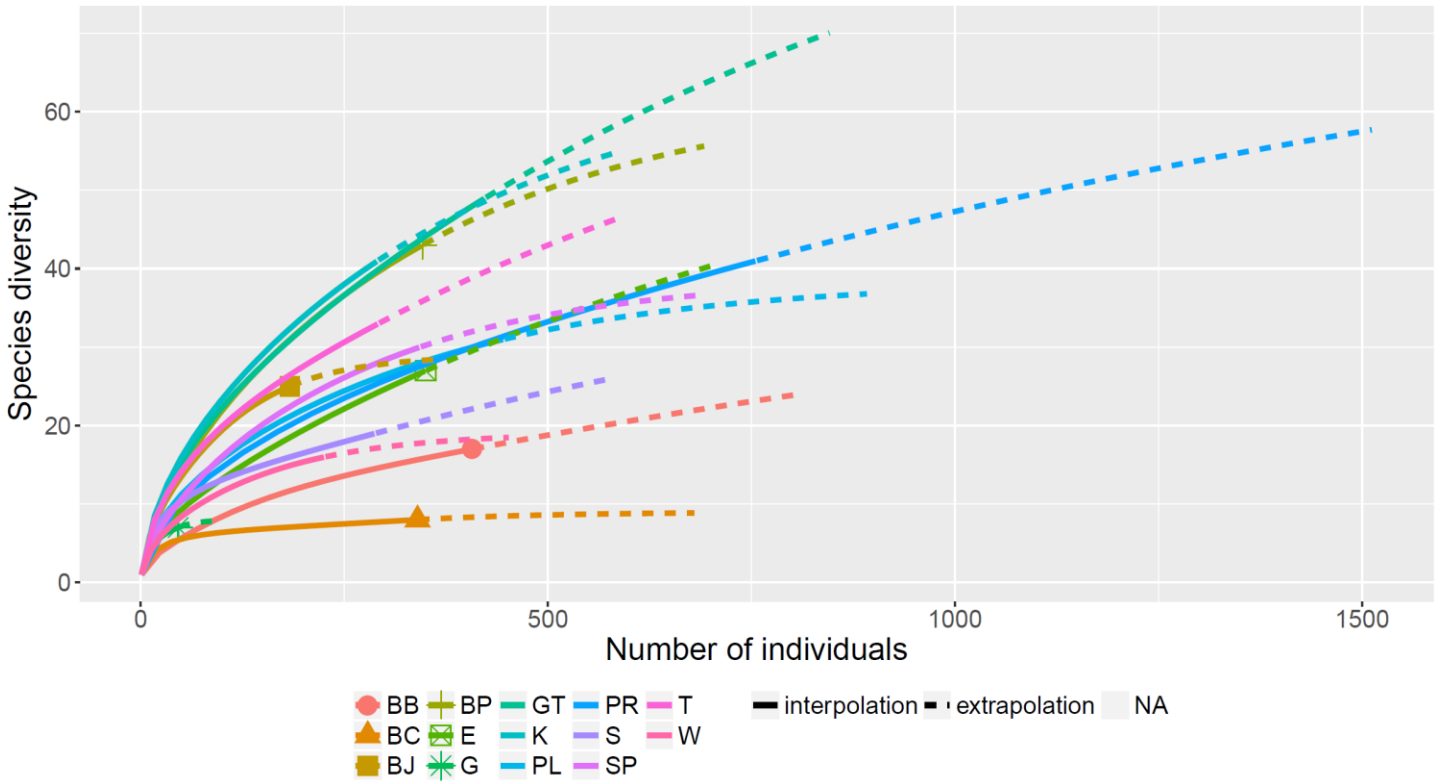


Figure S2.2: An individual-based rarefaction curve was produced for each field site sampled, combining the samples from the two visits at each site. The rarefaction curve based on the number of individuals sampled at a site is shown by the solid line, with the extrapolated species richness show by the dotted line up to double the number of individuals collected at each site.

Table S2.12: Summary table of observed pollinator community species richness, total pollinator abundance, Simpson's Index of diversity (1-D), and estimated richness at 46 individuals per site and at the asymptote of the rarefaction curve at each site, with standard error, and 95% confidence intervals.

Site	Observed			Estimated at 46 individuals			Estimated at Asymptote			
	Richness	Abundance	Simpson (1-D)	Estimated Richness	95% Lower	95% Upper	Estimated Richness	Standard error	95% Lower	95% Upper
BB	17	407	0.45984	5.4	4.5	6.3	48.9	39.5	21.8	228.1
BC	8	340	0.518218	5.4	4.9	6.0	9.0	2.2	8.1	21.8
BJ	25	183	0.814835	12.9	11.1	14.8	29.0	3.7	25.8	43.8
BP	43	346	0.803635	13.7	12.3	15.0	62.9	11.8	49.8	101.4
E	27	350	0.747902	9.0	7.6	10.3	69.5	33.1	38.0	191.1
G	7	46	0.524575	7.0	5.5	8.5	8.0	2.2	7.1	20.5
GT	49	423	0.842871	14.6	13.0	16.2	100.9	28.9	67.8	192.9
K	41	291	0.847486	15.2	13.3	17.2	65.9	15.1	49.3	115.4
PL	31	446	0.802449	10.8	9.9	11.7	38.5	5.9	32.9	60.5
PR	41	756	0.742441	10.0	9.1	10.9	85.0	28.3	54.9	180.5
S	19	288	0.838614	10.1	9.1	11.1	46.9	21.3	26.4	124.2
SP	30	343	0.719411	10.1	8.9	11.4	39.0	6.8	32.4	63.5
T	33	292	0.823701	13.6	12.2	15.0	75.5	33.1	44.0	197.0
W	16	226	0.513392	8.1	6.9	9.2	19.1	3.6	16.5	35.2

Table S2.13: Comparable model to Model 2a with estimated species richness at the asymptote of the rarefaction curve for each site (**Figure S2.2**) shows similar results to **Table 2.4**.

Main Factors	χ^2	**d*f***	**P value**
Estimated Species Richness at Asymptote	8.51	1	**0.0035**
Total Abundance	1.47	1	0.22
Virus Type	34.71	2	< **0.0001**
Host Species	164.10	3	< **0.0001**
Virus Type × Host Species	131.02	6	< **0.0001**

Table 2.14: Comparable model to Model 2a with estimated species richness at 46 individuals in the population based on the rarefaction curve for each site (**Figure S2.2**) shows similar results to **Table 2.4**. The site with the lowest abundance (G site) had only 46 pollinator individuals detected, therefore that was the threshold selected for estimating species richness at an even abundance at each site.

Main Factors	χ^2	**d*f***	**P value**
Estimated Species Richness at 46 individuals	7.69	1	**0.0055**
Total Abundance	0.04	1	0.84
Virus Type	34.612	2	< **0.0001**
Host Species	164.18	3	< **0.0001**
Virus Type × Host Species	131.11	6	< **0.0001**

Table S2.15: Model estimated virus prevalence, standard error (SE), and 95% asymptotic lower and upper confidence levels (CL) for each host and virus pair. The model estimated prevalence is shown as a proportion between 0 and 1, and is based on samples with the presence or absence of the viral positive-strand. Data visualized in **Figure 2.1**, and post-hoc pairwise comparison test shown in **Table S2.16**.

Virus	Host Species	Model estimated prevalence	SE	Lower CL	Upper CL
BQCV	APIS	0.842	0.028	0.78	0.89
	BOMB	0.336	0.041	0.26	0.42
	LASI	0.037	0.011	0.02	0.07
	PEPO	0.037	0.012	0.02	0.07
DWV	APIS	0.563	0.045	0.47	0.65
	BOMB	0.317	0.040	0.24	0.40
	LASI	0.111	0.022	0.07	0.16
	PEPO	0.140	0.029	0.09	0.21
SBV	APIS	0.259	0.037	0.19	0.34
	BOMB	0.381	0.042	0.30	0.47
	LASI	0.002	0.002	0.00	0.01
	PEPO	0.011	0.006	0.00	0.03

Table S2.16: Post-hoc pairwise comparison test of BQCV, DWV, and SBV prevalence with a Tukey p-value adjustment for multiple comparisons (family of four) for each host species, respectively. 95% confidence levels (CL) are shown as the asymptotic lower and upper confidence levels (CL). Significant comparisons are bolded.

Virus	Host Species Comparison	Odds Ratio	SE	df	Lower CL	Upper CL	Z ratio	p-value
BQCV	APIS / BOMB	10.49	2.74	Inf	5.36	20.51	9.00	**< 0.0001**
	APIS / LASI	139.12	52.22	Inf	53.04	364.89	13.15	**< 0.0001**
	APIS / PEPO	136.80	55.20	Inf	48.52	385.73	12.19	**< 0.0001**
	BOMB / LASI	13.27	4.47	Inf	5.58	31.51	7.68	**< 0.0001**
	BOMB / PEPO	13.05	4.80	Inf	5.07	33.59	6.98	**< 0.0001**
	LASI / PEPO	0.98	0.41	Inf	0.33	2.90	-0.04	1.000
DWV	APIS / BOMB	2.77	0.65	Inf	1.51	5.08	4.32	**< 0.0001**
	APIS / LASI	10.28	2.83	Inf	5.07	20.84	8.47	**< 0.0001**
	APIS / PEPO	7.92	2.28	Inf	3.78	16.57	7.20	**< 0.0001**
	BOMB / LASI	3.71	0.99	Inf	1.87	7.34	4.93	**< 0.0001**
	BOMB / PEPO	2.86	0.80	Inf	1.39	5.87	3.74	**0.001**
	LASI / PEPO	0.77	0.23	Inf	0.36	1.64	-0.89	0.811
SBV	APIS / BOMB	0.57	0.14	Inf	0.31	1.05	-2.36	**0.084**
	APIS / LASI	169.76	174.46	Inf	12.11	2379.19	5.00	**< 0.0001**
	APIS / PEPO	32.87	18.42	Inf	7.79	138.72	6.23	**< 0.0001**
	BOMB / LASI	299.02	307.21	Inf	21.35	4187.78	5.55	**< 0.0001**
	BOMB / PEPO	57.90	32.44	Inf	13.73	244.23	7.24	**< 0.0001**
	LASI / PEPO	0.19	0.22	Inf	0.01	3.58	-1.45	0.471

Table S2.17: P-values for DWV, BQCV, and SBV infection prevalence (based on the presence of the negative strand in virus positive samples) among four bee host species using a test of two proportions with six comparisons with a Bonferroni Correction, $\alpha^* = 0.05/6 = 0.0083$. Significant comparisons are bolded.

DWV	*Apis mellifera*	*Bombus impatiens*	*Lasioglossum* spp.	*Eucera pruinosa*
Apis mellifera	--			
Bombus impatiens	0.01	--		
Lasioglossum spp.	0.5	**0.0015**	--	
Eucera pruinosa	0.3	**0.0004**	0.99	--

BQCV	*Apis mellifera*	*Bombus impatiens*	*Lasioglossum* spp.	*Eucera pruinosa*
Apis mellifera	--			
Bombus impatiens	0.2	--		
Lasioglossum spp.	**0.0072**	0.2	--	
Eucera pruinosa	< **0.0001**	**0.0026**	0.12	--

SBV	*Apis mellifera*	*Bombus impatiens*	*Lasioglossum* spp.	*Eucera pruinosa*
Apis mellifera	--			
Bombus impatiens	0.6	--		
Lasioglossum spp.	0.1	0.3	--	
Eucera pruinosa	0.05	0.2	0.99	--

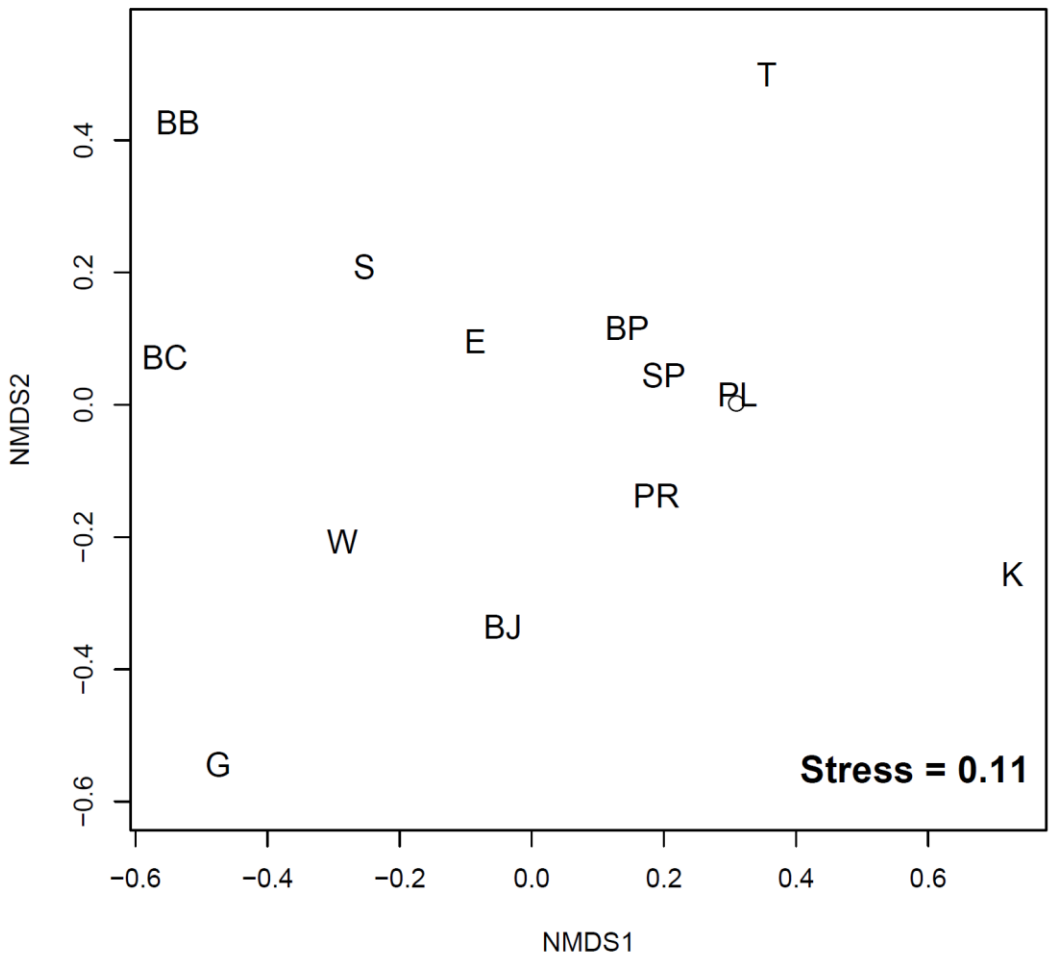
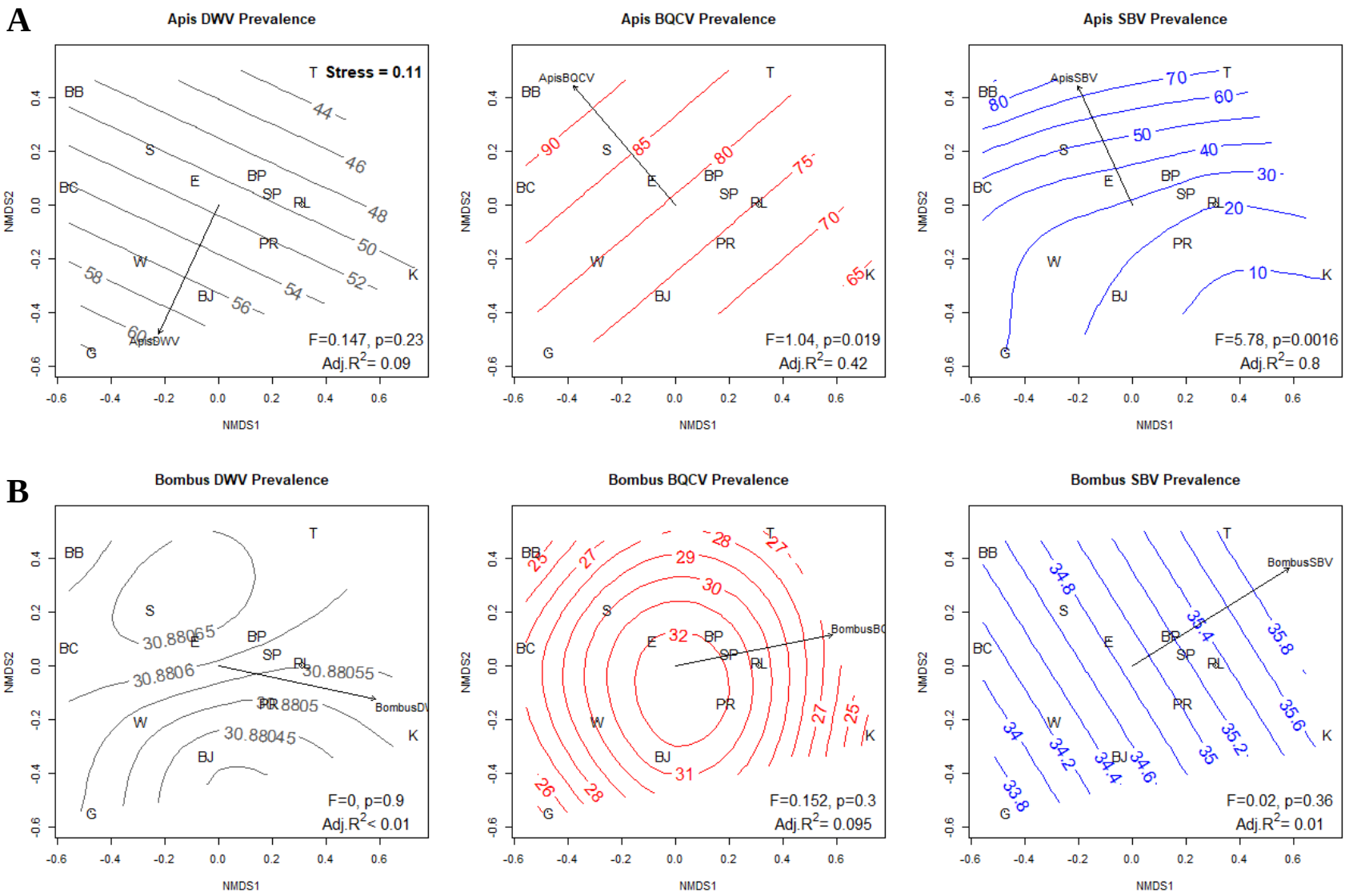
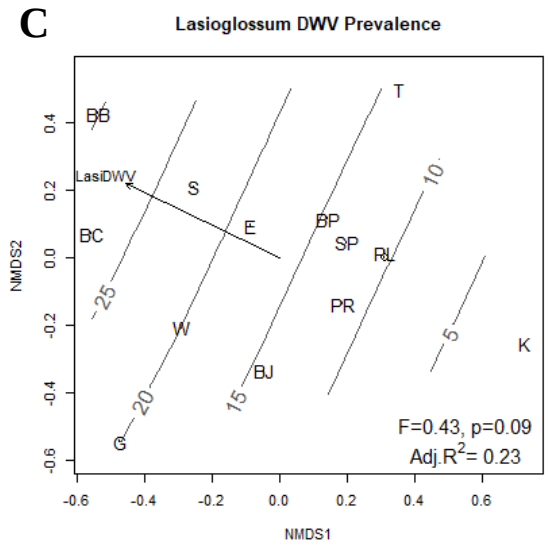
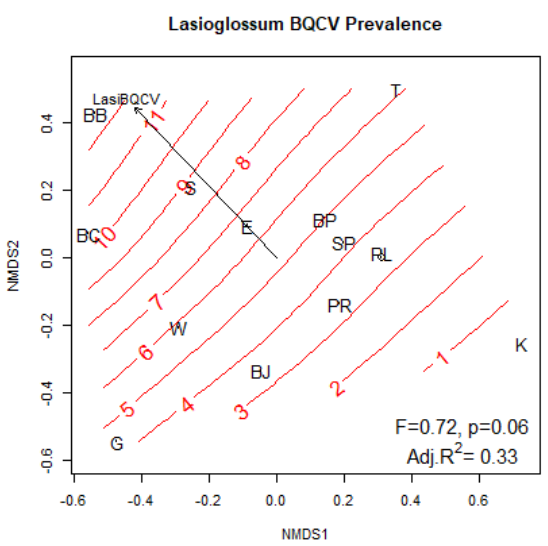


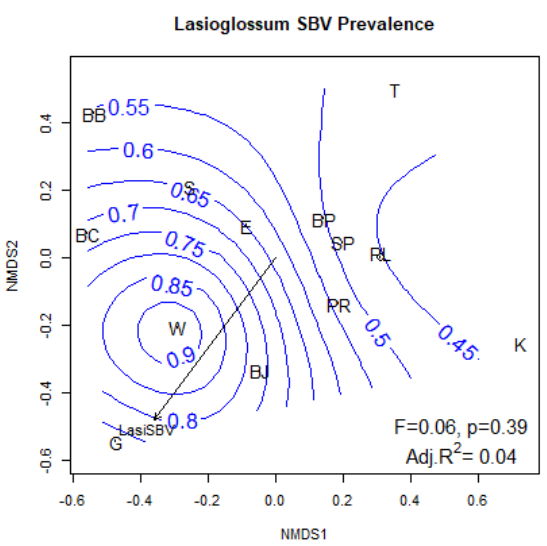
Figure S2.3: Non-metric Multidimensional Scaling (NMDS) ordination of pollinator communities shows that community composition varies among sites based on both the identity and relative abundance of pollinator species. Site abbreviation codes can be found in **Table S2.1** (the GT site is represented as a point because it overlaps with the PL site label).


A
Apis DWV Prevalence
T Stress = 0.11
F=0.147, p=0.23
Adj.R2= 0.09
Apis BQCV Prevalence
F=1.04, p=0.019
Adj.R2= 0.42
Apis SBV Prevalence
F=5.78, p=0.0016
Adj.R2= 0.8
B
Bombus DWV Prevalence
F=0, p=0.9
Adj.R2< 0.01
Bombus BQCV Prevalence
F=0.152, p=0.3
Adj.R2= 0.095
Bombus SBV Prevalence
F=0.02, p=0.36
Adj.R2= 0.01
NMDS1
NMDS2

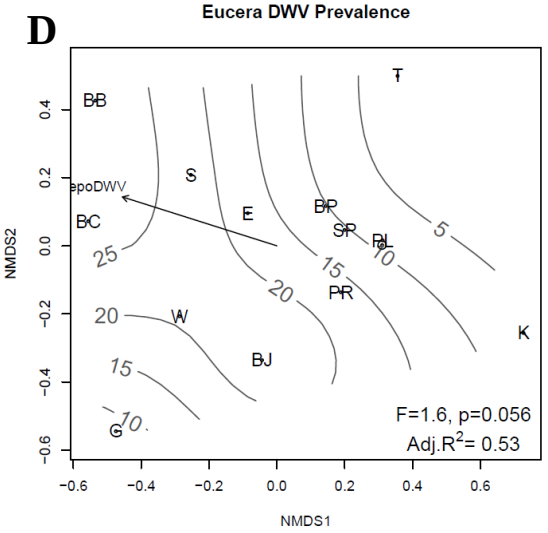
C

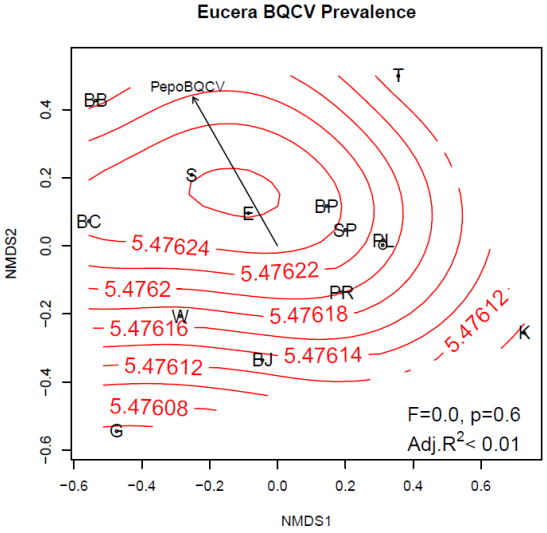

Lasioglossum DWV Prevalence
LasiDWV
F=0.43, p=0.09
Adj.R2= 0.23
NMDS1
NMDS2


Lasioglossum BQCV Prevalence
LasiBQCV
F=0.72, p=0.06
Adj.R2= 0.33
NMDS1
NMDS2


Lasioglossum SBV Prevalence
LasiSBV
F=0.06, p=0.39
Adj.R2= 0.04
NMDS1
NMDS2

D


Eucera DWV Prevalence
epoDWV
F=1.6, p=0.056
Adj.R2= 0.53
NMDS1
NMDS2


Eucera BQCV Prevalence
PepoBQCV
F=0.0, p=0.6
Adj.R2< 0.01
NMDS1
NMDS2

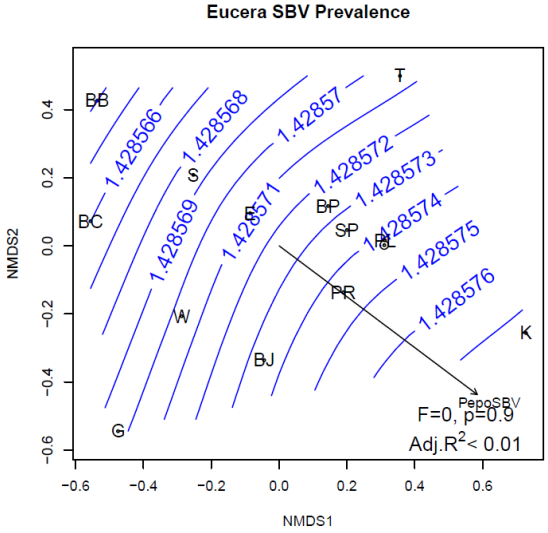

Eucera SBV Prevalence
PepoSBV
F=0, p=0.9
Adj.R2< 0.01
NMDS1
NMDS2

Figure S2.4: The patterns of A) *A. mellifera*, B) *B. impatiens*, C) *Lasioglossum spp*., and D) *E. pruinosa* DWV, BWCV, and SBV prevalence among pollinator communities in southeastern Michigan. The smooth contour lines represent the estimated DWV (gray), BQCV (red), and SBV (blue) prevalence in each host species by General Additive Model on the Non-metric Multidimensional Scaling ordination of pollinator communities. The vectors indicate the strongest linear gradient along the virus prevalence contour lines. Site abbreviation codes can be found in **Table S2.1**.

Literature Cited

1. M. Benjeddou, N. Leat, M. Allsopp, S. Davison, Detection of Acute Bee Paralysis Virus and Black Queen Cell Virus from Honeybees by Reverse Transcriptase PCR. *Appl. Environ. Microbiol.* **67**, 2384–2387 (2001).
2. R. Singh *et al.*, RNA viruses in hymenopteran pollinators: evidence of inter-taxa virus transmission via pollen and potential impact on non-Apis hymenopteran species. *PLoS One*. **5**, e14357 (2010).
3. S. Cardinal, J. Straka, B. N. Danforth, Comprehenisve phylogeny of apid bees reveals the evolutionary origins and antiquity of cleptparasitism. *Proc. Natl. Acad. Sci.* **107**, 16207–16211 (2010).
4. C. Yue, E. Genersch, RT-PCR analysis of Deformed wing virus in honeybees (Apis mellifera) and mites (Varroa destructor). *J. Gen. Virol.* **86**, 3419–3424 (2005).
5. W. Peng, J. Li, H. Boncristiani, J. P. Strange, M. Hamilton, Y. Chen, Host range expansion of honey bee Black Queen Cell Virus in the bumble bee, Bombus huntii. *Apidologie*. **42**, 650–658 (2011).
6. H. R. Gong, X. X. Chen, Y. P. Chen, F. L. Hu, J. L. Zhang, Z. G. Lin, J. W. Yu, H. Q. Zheng, Evidence of *Apis cerana* Sacbrood virus infection in *Apis mellifera*. *Appl. Environ. Microbiol.* **82**, 2256–2262 (2016).

Chapter 3 : Habitat Driven Dilution Effects: Comparing the Relative Effects of Biodiversity and Habitat on Pollinator Pathogen Prevalence

Abstract

Habitat characteristics influence host biodiversity, nutrition, and immune function, yet few studies have evaluated the how habitat may be linked with pathogen prevalence. Instead, most work has focused on how biodiversity reduces pathogen risk in many host-pathogen systems ('dilution effect hypothesis'). Here, we explore how habitat directly and indirectly influences disease. We propose 'habitat–disease relationship' as a non-mutually exclusive and complementary pattern to biodiversity–disease relationships. Habitat may directly influence pathogen prevalence by influencing host nutritional resources and immune response. Habitat characteristics may also indirectly influence pathogen prevalence by promoting host biodiversity, altering biodiversity–disease relationships and producing dilution or amplification effects. Here, we used a structural equation model to test the relative strength of direct links between pathogen prevalence and habitat characteristics (habitat–disease relationship) to indirect links via changes in host diversity (biodiversity–disease relationship) while controlling for abundance. We used a pollinator-virus system that exhibits the dilution effect to examine how prevalence of three bee viruses changed based on pollinator host species richness and abundance and local- and landscape-level habitat diversity and abundance characteristics. We selected specific habitat characteristics across associated with better nutrition and immune function in pollinators, including abundant and diverse floral resources at the local scale and larger natural areas and greater diversity of landcover (landscape richness) at the landscape scale. We found that landscape richness, natural area, floral richness, and floral density habitat characteristics were all

directly associated with changes in virus prevalence, but the direction of the effect varied among the three viruses. These results provide initial evidence for a direct habitat–disease relationship. Landscape richness, natural area, and floral richness were also indirectly linked with reduced virus prevalence, while floral density was indirectly linked with increased virus prevalence. All indirect pathways were mediated through habitat effects on pollinator species richness. Habitat had relatively strong effects on virus prevalence. In fact, the magnitude of the direct effects of habitat on virus prevalence were similar to the direct effects of pollinator species richness on virus prevalence. Our findings show that local and landscape habitat features are directly associated with pathogen prevalence, in addition to mediating changes in host biodiversity that influence pathogens via the dilution effect. Future work is needed to evaluate the underlying mechanisms for the habitat–disease relationships, and further evaluate the role of habitat characteristics in host–pathogen systems with established biodiversity–disease relationships.

Introduction

Habitat degradation is the primary driver of biodiversity loss (Fahrig 2003, Foley et al. 2005), and biodiversity loss is increasingly linked with greater emergence of infectious diseases (Daszak et al. 2000, Keesing et al. 2010). Relationships between host biodiversity and pathogen prevalence tend to show either a pattern of decreased pathogen prevalence in species-rich communities called the ‘dilution effect’, or increased pathogen prevalence with greater species richness called the ‘amplification effect’ (Keesing et al. 2006). The dilution effect represents a win-win scenario for conserving biodiversity and improving public or wildlife health (Kilpatrick et al. 2017), and has been shown in diverse host–pathogen systems, including Lyme disease in vertebrate hosts (Ostfeld and Keesing 2000, Schmidt and Ostfeld 2001), hantavirus in rodents (Clay et al. 2009, Dizney and Ruedas 2009), West Nile Virus in birds (Ezenwa et al. 2006,

Swaddle and Calos 2008, Allan et al. 2009), fungal *Metschnikowia* in *Daphnia* (Strauss et al. 2018), *Ribeiroia ondatrae* in amphibians (Johnson et al. 2013), *Batrachochytrium dendrobatidis* in amphibians (Searle et al. 2011, Becker et al. 2014, Venesky et al. 2014), and deformed wing virus (DWV), black queen cell virus (BQCV), and sacbrood virus (SBV) in pollinators (**Chapter 2**). Yet, in many of these host–pathogen systems, habitat loss or alteration are important drivers of changing community biodiversity. Therefore, changes in habitat characteristics can indirectly lead to the observed biodiversity–disease relationships, including the dilution effect (Faust et al. 2017).

However, habitat characteristics could also directly impact pathogen prevalence through access to better nutrition and altering immune responses to pathogens, a new pattern that we termed the 'habitat–disease relationship' (**Figure 3.1, pathway 1**). The habitat–disease relationship differs from the biodiversity–disease relationship because it works directly via habitat effects on host susceptibility and resistance to infection rather than indirectly via habitat impacts on altering community diversity to change patterns of pathogen spread. We argue that habitat–disease relationships are an alternative, but non-mutually exclusive, explanation for previously observed biodiversity–disease relationships. In fact, the same habitat characteristics that mediate host health may simultaneously promote greater host diversity and indirectly reduce pathogen prevalence via the dilution effect (**Figure 3.1, pathway 2**) (Faust et al. 2017). Therefore, habitat–disease relationships represent a previously unexplored avenue that could contribute to and explain variable patterns of pathogen prevalence among communities and space.

Current data suggest that habitat–disease relationships are plausible, but relatively few studies have examined the direct relationship between habitat factors and pathogen prevalence.

Host nutrition is a well-studied and important factor impacting immunity and infectious disease (Ponton et al. 2013), and host nutrition is inescapably linked with the quality of the resources in their environment. In general, poor nutrition tends to decrease host immune function and increase host susceptibility and/or disease burdens in many host–pathogen systems, though more complex interactions are possible (Coop and Kyrizakis 1999, Ezenwa 2004, Suorsa et al. 2004, Alaux et al. 2010, Ponton et al. 2013, Brunner et al. 2014, Santicchia et al. 2015). For instance, Eurasian red squirrels (*Sciurus vulgaris*) in fragmented habitats had higher abundance of the dominant gastrointestinal helminth compared to those in continuous forest habitats, and parasite burdens were higher in years with low food availability regardless of habitat type (Santicchia et al. 2015). We focused on evaluating habitat characteristics that provide good nutrition to hosts as the key 'high-quality' habitat characteristics that may be directly linked with pathogen prevalence; though the specific habitat characteristics critical for host nutrition, immune function, and disease resistance are likely to be specific to each host–pathogen system. Surprisingly, the link between specific habitat characteristics and infectious disease needs to be studied further.

Habitat–disease and biodiversity–disease relationships are likely to co-occur because the habitat characteristics that improve nutrition are often linked with high biodiversity (Franklin 1993, Thomas et al. 2001, Fahrig 2003, Ebeling et al. 2008, Prugh et al. 2008, Kennedy et al. 2013, Rader et al. 2014, Blaauw and Isaacs 2014, Vaudo et al. 2015). Hosts from both high-quality habitats and species-rich communities may be better able to resist pathogens than hosts from low-quality habitats and species-poor communities. However, it remains unclear whether habitat characteristics, host community biodiversity, or a combination of both drive the change in pathogen prevalence. Therefore, habitat factors can both directly and indirectly impact pathogen

prevalence and potentially underlie the pattern that is normal ascribed to the dilution effect alone. Thus, understanding the intricate links between habitat, host biodiversity, and pathogen prevalence are key to predicting how habitat characteristics may influence disease risk for hosts in many host–pathogen systems.

In this study, we explore whether habitat characteristics directly mediate host health in a host–pathogen system that has previously shown evidence of the dilution effect pattern. The habitat–disease relationship is not mutually exclusive with a biodiversity–disease relationship. Both could operate independently, complementarily, or in opposition each other. The net result may be reduced or increased pathogen prevalence depending on the direction and relative strength of habitat and biodiversity effects on pathogen prevalence. Therefore, determining the relative impact of habitat and biodiversity is key to explaining patterns of pathogen prevalence among different communities. Habitat–disease relationships can be initially be tested by finding direct links between pathogen prevalence and habitat characteristics that are likely to mediate host health, while accounting for habitat driven changes in host species richness. Follow-up tests should then compare the relative strength of those direct habitat–disease links to indirect links where the same habitat factors alter biodiversity to result in a biodiversity–disease relationship. These findings would indicate a habitat–disease relationships where habitat quality can directly impact host health and mediate pathogen prevalence among different host communities. Future work will need to investigate the underlying mechanism(s) that link specific habitat factors to pathogen prevalence by comparing host nutrition, host immune function, and host's relative susceptibility to an infection challenge along a gradient for each habitat characteristic.

Here, we conduct an initial test of how habitat influences pathogens in a pollinator pathogen system with multiple hosts and pathogens by testing whether pathogen prevalence is

directly linked with habitat characteristics important for pollinator nutrition. Pollinators are an ideal system to explore habitat–disease relationships because pollinators are experiencing severe declines due to wide-spread habitat loss and degradation and increased prevalence of multi-host viruses, including deformed wing virus (DWV), black queen cell virus (BQCV), and sacbrood virus (SBV) (Biesmeijer et al. 2006, Carvell et al. 2006, Potts et al. 2010, vanEngelsdorp and Meixner 2010, Goulson et al. 2015). A direct link between habitat characteristics and virus prevalence is probable because pollinators depend on flower pollen and nectar as their primary sources of nutrition, but DWV, BQCV, and SBV are also transmitted among bee species through contaminated pollen picked up on shared flowers (Michener 2007, Ebeling et al. 2008, Singh et al. 2010, Mazzei et al. 2014, McArt et al. 2014, Vaudo et al. 2015, Alger et al. 2019). Pollinator nutrition and health is tightly linked with access to abundant and diverse flowers at the local scale, and greater natural grassland and woodland area and diversity of flowers at the landscape spatial scale (Jha and Kremen 2013, Jha et al. 2013, Donkersley et al. 2014). Therefore, we used local floral richness and density, and landscape-level proportion of natural area and landscape richness (a proxy of floral diversity based on diversity of landcover types) as the key 'high-quality' habitat characteristics associated with pollinator nutrition to test for the habitat–disease relationship. Numerous experimental and field studies have shown that honey bees (*A. mellifera*) and bumblebees (*Bombus* spp.) with access to diverse floral resources had increased colony growth, nutrition, and immunocompetence, and one experiment showed that bees fed high protein diets had lower DWV loads compared to bees with poor-quality diets (DeGrandi-Hoffman et al. 2010, Alaux et al. 2011, Jha and Kremen 2013, Jha et al. 2013, Brunner et al. 2014, Donkersley et al. 2014, DeGrandi-Hoffman and Chen 2015, Vaudo et al. 2016). The effects of habitat characteristics on nutrition and immune function suggest that habitat–disease

relationship is plausible, but very few studies have directly linked these important habitat characteristics with patterns of infectious disease prevalence or burden.

In addition to mediating pollinator health, many of the same habitat characteristics can simultaneously influence pollinator community diversity and abundance. At the local scale, larger and more diverse floral patches were positively correlated with greater pollinator diversity and density (Kennedy et al. 2013, Blaauw and Isaacs 2014). At the landscape scale, access to diverse floral resources through greater landscape heterogeneity and greater proportion of natural area (i.e. forest, wetlands, and grasslands) surrounding agricultural fields had greater pollinator species richness and abundance (Ricketts et al. 2008, Lentini et al. 2012, Fabian et al. 2013, Kennedy et al. 2013, Shackelford et al. 2013). Therefore, these habitat features could also indirectly impact patterns of pathogen prevalence by altering host communities to facilitate a biodiversity–disease relationship. In fact, our previous research shows that three pollinator viruses exhibited diluted virus prevalence in species-rich pollinator communities compared to species-poor communities for multiple bee host species (**Chapter 2**). However, whether this pattern is driven by high-quality habitat characteristics, pollinator species richness, or a combination of both through direct and indirect pathways remains unclear. Investigating and disentangling the relative impact of direct habitat–disease relationships and the indirect effect of habitat on biodiversity–disease relationships is critical to fully understanding variation in pathogen prevalence among different communities (Rohr et al. 2020).

In this study, we conduct a survey of DWV, BQCV, and SBV prevalence in pollinator communities with variable local and landscape habitat characteristics and employ a structural equation model (SEM) to address three main questions: 1) How are habitat characteristics at the local and landscape scales associated with pollinator communities? and 2) Are local and

landscape-level habitat characteristics directly and/or indirectly associated with virus prevalence, and what is the relative magnitude and direction of these links? and 3) If so, are there consistent patterns among the three viruses? We use the SEM to evaluate the relative magnitude and directionality of all direct and indirect pathways between habitat characteristics and virus prevalence in the model, while accounting for the effects of all other significant pathways.

First, consistent with much previous work, we expect that increased pollinator species richness and abundance will be correlated with greater floral richness and density at the local scale, and greater natural area and landcover diversity at the landscape scale. Second, the SEM allows us to disentangle the relative effects of direct correlations between habitat factors and virus prevalence, and indirect pathways linked by either pollinator species richness or abundance. We predict that both local and landscape habitat characteristics will be directly linked with virus prevalence and indirectly linked via habitat effects on pollinator species richness. A combination of direct and indirect links supports both habitat–disease and biodiversity–disease relationships in the pollinator–pathogen system. Finally, we predict that the three viruses are likely to demonstrate similar habitat–disease and biodiversity–disease patterns based on previous work showing similar dilution effects for these viruses in multiple hosts (**Chapter 2**). This study investigates an important missing link in the biodiversity–disease literature, the role of habitat characteristics in mediating patterns of pathogen prevalence through the newly proposed habitat–disease relationship and the well-established biodiversity–disease relationship.

Methods

Pollinator community sampling

Pollinator communities were sampled at 13 winter squash farms located in southeastern Michigan, USA, with private landowner permission (**Appendix, Table S3.1**). The landscape surrounding the field sites varied from intense monoculture agriculture (6% natural area within 1000-m radius) to predominantly natural forests and wetlands (88% natural area within 1000-m radius). Field sites were situated 10-km or more away from each other, so it not likely that bees would be able to visit more than one field site because most bee species' home ranges are less than 10 km (Greenleaf et al. 2007). We visited five sites between 22 July – 21 August 2015, and eight sites between 26 July – 2 September 2016. We sampled the pollinator community and local habitat characteristics (additional details below) at each site twice during the peak squash flower bloom.

Pollinators were collected by hand-netting and pan traps along four 50-m transects; three transects were randomly placed in the field along crop rows, and one was placed along the nearby field edge. Edges generally contained a mixture of native and invasive flowering plants. Sampling effort of the pollinator communities was standardized in terms of both total time and area sampled: we netted all pollinators observed within 1.5-m of the each transect line during 30-minute time-interval. Each transect was sampled once between 0800 and 1200. Pan traps were set 5-m apart along each transect between the crop rows in an alternating color pattern (fluorescent blue, yellow, and white). Pan traps were left out for a total of six hours and checked for bees every three hours. All pollinator collection ceased by 1300 because squash flowers close around midday and pollinator activity declined precipitately in the afternoon. All collected insects were freeze killed, stored on dry ice, and placed in a -80^{o}C freezer in the lab.

Most pollinators sampled were identified to species using the Discover Life key (http://www.discoverlife.org). Some difficult and speciose groups (e.g. *Lasioglossum* spp.) or

very rare species in our collection (<5 total individuals) were only identified to genus. Then we determined the pollinator species richness (total number of species) and total abundance (total number of individual pollinators) at each site. We previously compared our species richness with the estimated species richness at the asymptote of rarefaction curves, and found that the overall ranking order of observed and estimated species richness were similar (**Chapter 2**). Additionally it is rare that the observed species richness ever reaches an asymptote for invertebrate communities (Novotný and Basset 2000, Gotelli and Colwell 2001).

Habitat quantification at local and landscape scales

We quantified habitat diversity and abundance characteristics through several measures at local and landscape spatial scales. We sampled local floral species richness and total floral density within 1-m^2 plots at 10-m intervals along the length of each of the four 50-m transects per site. At each plot, we recorded the number of flowers per plant species. Total floral density was obtained by summing the total number of flowers across all plots at a site and dividing by the total area sampled. Floral species richness was determined by the number of different herbaceous flowering plants recorded across all plots at a site.

We used landscape data obtained from the 2015 and 2016 USDA cropland data layers (https://nassgeodata.gmu.edu/CropScape/) that classifies each 30-m x 30-m grid cell of the USA for the dominant landcover type within that cell. We grouped forest, wetland, and grassland meadow landcover types into one "natural habitat" category because those landcover types provide native flowers for foraging and nesting resources for many native bee species (Williams et al. 2012, Jha et al. 2013, Williams and Kremen 2015, Koh et al. 2016). Geographic Information System (GIS) was used to quantify the proportion of natural habitat surrounding each site at 500-m, 1000-m, 1500-m, and 2000-m radii based on the landcover types given by the

cropland data layer. Additionally, we calculated 'landscape richness' by counting all of the different landcover types included within each radius. We found natural habitat area within a 1000-m radius had the strongest positive correlation with pollinator abundance and species richness (**Appendix, Figure S3.1**), corroborating with several other studies (Ricketts et al. 2008, Watson et al. 2011), and flight distances of many species (Greenleaf et al. 2007). Though landscape richness within a 2000-m radius had a higher Pearson's correlation than the 1000-m radius, there was still significant positive correlation at 1000-m and we wanted to maintain equivalent spatial scales for all landscape habitat factors in the model (**Appendix, Figure S3.1**). Most pollinator species foraging ranges are within a 1000-m radius due to species-specific ecological characteristics, including body size (e.g. *Lasioglossum* spp.) (Greenleaf et al. 2007) or limited dispersal from a specialist plant (e.g. *Eucera pruinosa*, squash specialist) (Hurd Jr et al. 1971), therefore the 1000-m radius is a relevant spatial scale for assessing the pollinator community.

Detecting virus prevalence and active replication

We tested for DWV, BQCV, and SBV prevalence and replication in up to 20 randomly selected *Apis mellifera* (n = 237), *Bombus impatiens* (n = 252), *Lasioglossum* spp. (n = 255), and *Eucera pruinosa* (n = 193) from each site (**Appendix, Table S3.2**). These four pollinator host species were the most common among all communities sampled, which allowed for adequate comparison of virus prevalence among hosts and different communities. Some sites had fewer than 20 individuals from a species, so we tested all individuals that we collected.

RNA was extracted from each bee's abdomen using TRIzol reagent (Ambion) according to manufacturer's instructions, and RNA concentration was quantified with a Qubit 3.0 Fluorometer (Invitrogen). We excluded samples with <1 ng/µl RNA concentrations because they

are unlikely to provide accurate information about virus presence or absence. Positive-strand complimentary DNA (cDNA) synthesis reactions were performed with 2 µl of RNA template in a 20 µl reaction using M-MLV reverse-transcriptase (Promega) and 0.25 µM random hexamers (Invitrogen).

We tested for the presence of DWV, BQCV, and SBV using PCR with virus-specific primers (Benjeddou et al. 2001, Singh et al. 2010). The DWV primer did not differentiate between DWV-A, -B, or -C variants, therefore reported DWV prevalence includes all three variants. As a positive control for RNA extraction and reverse transcription, we also tested each sample for the presence of the 18S rRNA gene (Cardinal et al. 2010). All reaction included positive and negative controls. All PCR products were visualized on a 2% agarose gel to determine the presence or absence of the virus. Samples that failed to produce the 18S rRNA gene band were excluded from the study due to poor RNA quality.

The presence of the viral negative-strand provides strong evidence of viral replication and an active infection within the host (Ongus et al. 2004, Yue and Genersch 2005). Therefore, we tested the infection status of a subset of randomly selected virus-positive samples from each species with additional negative-strand specific RT-PCR to confirm that all four hosts could produce active infections for all three viruses. If fewer than 20 virus-positive bee samples for a species were available, then all virus-positive samples were used (e.g. *Lasioglossum* spp. and E. *pruinosa* BQCV and SBV), and a maximum of 26 virus-positive samples per species were tested (**Appendix, Table S2.6**). Negative-strand specific cDNA synthesis was carried out with 2.5 ul RNA template with M-MLV reverse transcriptase and tagged negative-strand primers for DWV (Fürst et al. 2014), BQCV (Yue and Genersch 2005, Peng et al. 2011), and SBV (Gong et al. 2016), followed by PCR and visualization on 2% agarose gels (**Appendix, Table S2.4**).

Our previous work showed that there were significant differences in DWV, BQCV, and SBV prevalence of both the positive- and negative-strand among the four host species. In particular, *A. mellifera* had higher virus prevalence compared to *B. impatiens*, which was higher than both *Lasioglossum* spp. and *E. pruinosa* (**Chapter 2**). Additional details for virus positive- and negative-strand detection methods are included in the **Appendix S1**, and all primers are listed in **Table S2.4**. A subset of the PCR products was sequenced to confirm identification of viral positive and negative strand RNA (GenBank Accession Numbers: MN902093 – MN902138) and 18S rRNA gene (GenBank Accession Numbers: MN900314 – MN900321) (**Appendix, Table S2.5**).

Since the primers used in this study are based on viruses isolated from honey bees, our measures of DWV, BQCV, and SBV prevalence in native bees are likely underestimates because virus sequence variants that are only found in native bee species might be missed with this technique. However, these primers have been previously used in many studies to examine presence of both positive and negative strands of DWV, BQCV, and SBV in honey bees, native bee species, and non-Hymenopteran insects (Singh et al. 2010, Levitt et al. 2013, Fürst et al. 2014, McMahon et al. 2015, Bailes et al. 2018). Additionally, at least eight studies have tested for virus sequence clustering by host species, and six studies found that the viruses were commonly shared among honey bees and native bees (Singh et al. 2010, Genersch et al. 2011, Yang et al. 2013, Levitt et al. 2013, Fürst et al. 2014, McMahon et al. 2015, Radzevičiūtė et al. 2017, Bailes et al. 2018). Only two studies found weak evidence for virus sequence variation clustering by host species, but the sample collection time and location had a much larger effect on virus sequence variation (Singh et al. 2010, McMahon et al. 2015). Therefore, the variation in

DWV, BQCV, and SBV prevalence found at each site in this study are likely representative of levels of virus prevalence in each host species at the time of collection.

Statistical Analysis

All analyses were performed in R version 4.0.0 (R Core Team 2020) and models were fit and evaluated with *lme4* and *piecewiseSEM* packages (Bates et al. 2015, Lefcheck 2016). We used Structural Equation Modeling (SEM) to examine the relative impact of local- and landscape-level habitat characteristics and pollinator community features on DWV, BQCV, and SBV prevalence. The parameters in the model included landscape richness and proportion of natural area within 1000m radii of each site, floral richness, floral density (per m^2), pollinator community species richness and total abundance, and the presence or absence of DWV, BQCV, and SBV positive-strand within individually tested bees from four host species: *Apis mellifera*, *Bombus impatiens*, *Lasioglossum* spp., and *Eucera pruinosa* (**Appendix Table S3.3**). An insufficient number of bees were tested for the negative-strand of each virus to have an adequate sample size for the SEM analysis, so only presence or absence of the positive-strand was used to determine virus prevalence in the model. The proportion of natural habitat at 1000-m was arcsine square root transformed, species richness and total pollinator abundance were square root transformed, and all continuous main factors were z-standardized. Within our initial SEM, all local and landscape habitat factors could affect pollinator community species richness and abundance through linear regressions, and all variables could affect DWV, BQCV, and SBV prevalence through a generalized linear mixed model with a binomial distribution and logit link function (**Appendix Table S3.4**). Since our previous work indicated that there were significant differences in virus prevalence among these four host species tested for DWV, BQCV, and SBV (**Chapter 2**), we included host species as a categorical variable in the virus models within the

SEM. We compared the differences in virus prevalence based on host species with a post-hoc adjusted Tukey test using the *emmeans* package and reported estimated path coefficient (Lenth 2020) (**Appendix Table S3.5**).

We allowed the prevalence of the three viruses to covary in the SEM since the same hosts were tested for all three viruses and presence of one virus could impact the likelihood of infection with another virus. Additionally, we allowed pollinator species richness and abundance to covary to control for the positive correlation between the community factors. We evaluated all component models for their model assumptions, and conducted Variance Inflation Factors (VIF) tests. Though many of the factors covaried, all factors in the initial models had VIF < 3.5 and correlations < 0.7, indicating that there was acceptable levels of collinearity among factors in the model (Dormann et al. 2013) (**Appendix Table S3.6**). Furthermore, none of component models in the SEM showed evidence of spatial autocorrelation in the model residuals using the Moran's I test (packages *ape* and *DHARMa*), indicating that pollinator communities that were geographically closer to each other did not have significantly similar community composition or virus prevalence (**Appendix, Table S3.7**) (Paradis and Schliep 2018, Hartig 2020).

The initial SEM converged, but the model was highly saturated, therefore the tests of directed separation indicated a poor initial fit to the data (Fisher's $C = 14.45$, $df = 4$, $p = 0.006$). We updated the model by removing all non-significant paths with $p > 0.4$ from the model, and found that the updated model had an improved fit to the data (Fisher's $C = 22.58$, $df = 22$, $p = 0.4$). Additionally, an anova comparison of the two models indicated that the removed paths did not significantly change the model ($p = 0.97$). We then calculated the range standardized path coefficients to compare the relative impact of a full range shift of each factor on each response variable (i.e. virus prevalence or pollinator community richness or abundance). Finally, we

calculated the net indirect effect of habitat characteristics on virus prevalence by multiplying the coefficient of each component pathway.

Results

1. How are habitat characteristics at the local and landscape scales associated with pollinator communities?

The pollinator communities sampled along a landscape gradient were highly variable in species richness and abundance. In total, we collected 4,330 individuals from 124 species of bees and wasps from 13 sites with different local and landscape features. The most common genera collected were *Lasioglossum* (n = 1218), *Bombus* (n = 1067), *Eucera* (n = 557), *Apis* (n = 505), *Augochlora* (n = 127), *Vespula* (n = 125), and *Halictus* (n = 100). Additional details and analyses characterizing differences among the 14 pollinator communities were previously reported in **Chapter 2**.

The final SEM shows that all landscape and local habitat factors regardless of spatial scale were significant predictors of pollinator community characteristics, but landscape-level factors exhibited particularly strong, positive links with pollinator community species richness and abundance compared to the local habitat factors. The numbers in parentheses are the range standardized regression coefficients, which indicates the proportional shift in the response variable along its range given a full shift in the predictor variable (habitat characteristics) along its range, and allow for comparison of the relative effect of the predictor on the response, given the other variables in the model (Lefcheck 2019). Larger numbers indicate stronger effects (max = 1.0). Greater proportion of natural habitat at 1000-m was positively correlated with both greater pollinator species richness (range standardized coefficient = 0.89) and total pollinator abundance (0.66) (**Figure 3.2, Table 3.1**). Pollinator species richness and abundance were very

low at sites with low proportions of natural habitat (7 species, 46 bees, 6% natural area) and high at sites with high proportions of natural habitat (41 species, 756 bees, 88% natural area). Greater landscape richness at 1000-m was also an important predictor of higher pollinator species richness (0.49), but had a comparatively weaker association with greater pollinator abundance (0.06).

Local habitat factors exhibited less consistent directionality and lower magnitude associations with pollinator community characteristics compared to the landscape habitat characteristics. Greater floral richness was positively correlated with greater species richness (0.17), but negatively correlated with pollinator abundance (–0.15). In contrast, greater floral density was negatively associated with pollinator species richness (–0.23), and positively associated with pollinator abundance (0.17). Overall, both local and landscape habitat characteristics are directly linked with pollinator community characteristics, though local habitat factors appear to have opposite effects on pollinator communities.

2. Are local and landscape-level habitat characteristics directly and/or indirectly associated with virus prevalence, and what is the relative magnitude and direction of these links?

The final SEM shows that local and landscape habitat characteristics were directly associated with reduced or increased virus prevalence, and indirectly linked with reduced virus prevalence through habitat-mediated increases in pollinator species richness. Habitat characteristics did not indirectly mediate virus prevalence through changes in pollinator abundance (**Table 3.1, Figure 3.2 and 3.3**). Greater habitat characteristics had variable positive or negative direct effects on virus prevalence, but greater natural area, landscape richness, and floral richness all had an indirect net effect of reduced DWV and BQCV prevalence by increasing pollinator species richness (**Table 3.2**). The net indirect effect between habitat and

virus prevalence is a product of the coefficients of the component direct pathways between each habitat characteristic and pollinator species richness, and species richness and virus prevalence. Since most of the habitat characteristics had a positive association on the pollinator species richness (described above), and greater species richness was correlated with reduced DWV (–0.10) and BQCV prevalence (–0.16), therefore the net indirect effect of habitat factors on virus prevalence was also negative (**Figure 3.2, 3.3E, and 3.3F**). Overall, local and landscape habitat factors had weaker direct links with virus prevalence compared to direct links with pollinator community characteristics, and indirect links tended to be approximately equivalent or lower in magnitude compared to direct links.

Specifically, we assessed the direction and relative magnitude of the direct and indirect pathways between each habitat characteristic and virus prevalence. Greater landscape richness within 1000m was associated with less DWV prevalence (–0.10) and greater BQCV prevalence (0.08), but was not directly associated with SBV (**Figure 3.2 and 3.3A**). Landscape richness also had a net negative indirect link with DWV (–0.05) and BQCV prevalence (–0.08) because richer landscapes are linked with greater species richness (**Table 3.2**). Greater proportion of natural area within 1000m was only directly linked with greater BQCV prevalence (0.13), but greater natural area had a net negative correlation with DWV (–0.09) and BQCV prevalence (–0.14) through indirect changes in pollinator species richness (**Table 3.2, Figure 3.2 and 3.3B**). Greater local floral richness directly correlated with greater DWV (0.09) and BQCV prevalence (0.07), but had a very weak indirect link with reduced DWV (–0.02) and BQCV prevalence (–0.03) mediated by greater pollinator species richness (**Table 3.2, Figure 3.2 and 3.3C**). Local floral density exhibited an opposite trend compared to all other habitat characteristics. Greater floral density was only directly linked with reduced BQCV prevalence (–0.09), but very weakly

indirectly correlated with greater DWV (0.02) and BQCV prevalence (0.04) (**Table 3.2, Figure 3.2 and 3.3D**).

Host species was an important categorical factor that was associated with differences in DWV, BQCV, and SBV prevalence (**Table 3.1, Figure 3.2**). For DWV and BQCV, *Apis mellifera* had the highest virus prevalence, which was significantly higher than the virus prevalence in *Bombus impatiens* and both species had higher virus prevalence than both *Lasioglossum* spp. and *Eucera pruinosa* (**Appendix Table S3.5**). For SBV, *B. impatiens* had the highest virus prevalence, followed by *A. mellifera,* and *Lasioglossum* spp. and *E. pruinosa* rarely detected with SBV (**Appendix Table S3.5**). We previously investigated and discuss the details of differences in DWV, BQCV, and SBV prevalence among these four host species in **Chapter 2**.

3. If so, are there consistent patterns among the three viruses?

DWV and BQCV prevalence had many direct links with habitat and pollinator community characteristics, while SBV was not significantly associated with any of the habitat or pollinator community characteristics in the model. DWV and BQCV shared many of the same direct and indirect pathways with comparable magnitudes. When summing the coefficients for all direct and indirect pathways for each virus, we found that habitat and pollinator community characteristics have a negative net effect on all three viruses, but the effects are strongest in DWV and weakest in SBV (DWV: –0.23; BQCV: –0.17; SBV: –0.08; **Table 3.2**). Shared direct pathways among DWV and BQCV showed that greater floral richness correlated with greater virus prevalence, and greater pollinator species richness correlated with reduced virus prevalence. Both viruses also shared indirect pathways between all habitat factors through changes in species richness, with a net negative effect on virus prevalence (**Table 3.2**). Despite

the many similarities among viruses, all local and landscape habitat factors in the model were directly linked with BQCV prevalence, while only high landscape richness and low floral richness were associated with reduced DWV prevalence. Furthermore, both greater proportion of natural area and landscape richness were associated with an increase in BQCV prevalence, while high landscape richness correlated with reduced DWV prevalence.

Discussion

Our work expands our understanding of underlying drivers of biodiversity–disease relationships by showing that multiple habitat characteristics are both directly and indirectly linked with pathogen prevalence. These findings support a new pattern that we termed the 'habitat–disease relationship', where habitat characteristics directly mediate patterns of pathogen prevalence, even while accounting for the effects of concurrent biodiversity–disease relationships. Specifically, our work demonstrates that increased landscape richness and floral density are linked with reduced virus prevalence, while greater proportion of natural area, landscape richness, and floral richness are linked with increased virus prevalence. Only greater landscape richness exhibited both positive and negative effects on virus prevalence depending on the specific virus. This evidence suggests that these direct links between habitat characteristics and pathogen prevalence are an unexplored pathway that contribute to variation in pathogen prevalence among different communities. Furthermore, greater habitat quality characteristics at local and landscape scales are generally positively linked with pollinator species richness and abundance, but only greater species richness was linked with reduced virus prevalence. Pollinator abundance was not associated with patterns of virus prevalence. These findings show that habitat characteristics can indirectly mediate biodiversity–disease relationships to produce a dilution effect. Therefore, the net effect of habitat–disease relationships may complement,

oppose, or operate independently from biodiversity–disease relationships. Future work will need to investigate how the complex interactions between habitat factors and host communities influence host health. Furthermore, we need more studies that test the specific mechanisms that underlie habitat–disease relationships, such as altering host nutrition, susceptibility, and immune function of hosts.

Habitat–disease relationships

Habitat characteristics at the local and landscape scales are directly associated with virus prevalence, showing clear evidence of a habitat–disease relationship that is separate from the link between biodiversity and virus prevalence. These findings demonstrate that habitat–disease relationships could be an important, underexplored pathway that could contribute to variation in patterns of pathogen prevalence among different communities and spatial scales. We expected that greater landscape richness, natural area, floral richness, and floral density would be associated with reduced virus prevalence because these habitat characteristics are all linked with improved pollinator nutrition and increased immune function (Alaux et al. 2010, Donkersley et al. 2014, Vaudo et al. 2016). Instead, we observe both positive and negative direct links between habitat characteristics and virus prevalence. We show that increasing landscape richness and floral density are directly associated with decreased virus prevalence, while greater proportions of natural area, landscape richness, and floral richness directly correlate with increased virus prevalence. Interestingly, landscape richness is the only habitat characteristic to show both positive and negative correlations with virus prevalence; greater landscape richness is associated with greater BQCV prevalence and reduced DWV prevalence.

This variability in habitat–disease relationships suggest that different habitat characteristics may have complex interactions with host health through nutrition, susceptibility,

and immune function. Habitat characteristics linked with reduced virus prevalence may be closely associated with high-quality diets for bees that improve their health. Bees with diverse or high-protein pollen diets have better nutrition, greater immune gene expression, lower pathogen loads, and lower mortality rate from infection for multiple bee pathogens (DeGrandi-Hoffman et al. 2010, Di Pasquale et al. 2013). Furthermore, some flowers offer phytochemicals that can convey medicinal benefits to bees by reducing pathogen loads or increasing immune gene expression (Richardson et al. 2015, Palmer-Young et al. 2016, 2017). Therefore, diverse and abundant floral resources at the local and landscape scales may allow pollinators to optimally forage on diverse or preferred flowers with high-quality resources (e.g. protein-rich pollen and/or medicines) or avoid contaminated flowers (Fouks and Lattorff 2011, Jha et al. 2013, Gherman et al. 2014), which may convey health benefits such as reduced exposure or susceptibility, or better tolerance to infection (i.e. lower pathogen loads).

On the other hand, the mechanisms that underlie habitat–disease relationships that are associated with increased virus prevalence are unclear because we have limited experimental evidence that clearly untangles the complex interactions between resource quality from the environment and DWV, BQCV, and SBV prevalence in pollinator hosts. However, positive habitat–disease relationships may be possible in this pollinator pathogen system through two main avenues. First, high-quality habitat characteristics could be linked with higher virus prevalence if high-quality floral patches are more likely to be virus-contaminated and facilitate virus transmission among pollinators during visits to shared flowers. Although greater floral diversity and density provide better nutrition to pollinators (Donkersley et al. 2014, Vaudo et al. 2015), the possibility of disproportionately higher exposure to viral particles on flowers in high-quality floral patches could outweigh the benefits of nutrition. Second, the positive link between

habitat and virus prevalence may be due to complicated interactions occurring within a host through competition for limited resources between the host and one or more pathogen(s). Though diverse and abundant floral resources provide improved nutrition to hosts, evidence from other host–pathogen systems shows that some pathogens are able to do better in hosts with high-quality nutrition by exploiting the additional resources harbored by the host (Ponton et al. 2013, Lange et al. 2014). Future studies are needed to test the complex mechanisms by which different habitat characteristics may mediate higher or lower pathogen prevalence by investigating differences in host nutrition, immune function, and susceptibility to disease along gradients for each habitat characteristic.

Relative effects of habitat–disease and biodiversity–disease relationships

Critically, it is important to note that habitat–disease relationships are not mutually exclusive to biodiversity–disease relationships. Both habitat and biodiversity can concurrently influence pathogen prevalence, but both factors may have further complex effects on patterns of pathogen prevalence through direct and indirect pathways. Many of the same habitat characteristics that facilitate better pollinator nutrition also contribute to greater pollinator community diversity and abundance (Potts et al. 2003, Ebeling et al. 2008, Jha and Kremen 2013, Kennedy et al. 2013, Koh et al. 2016, Evans et al. 2018). Habitat driven changes in host community species richness could alter the rate of encounters with infected individuals on flowers (encounter reduction) or rate of transmission through consumption of virus-contaminated pollen (transmission reduction), two key mechanisms of the dilution effect (Keesing et al. 2006). Therefore, habitat factors can simultaneously affect pathogen prevalence through a habitat–disease relationship (**Figure 3.1, pathway 1**) and by altering community diversity and interactions among hosts and their pathogens (i.e. biodiversity–disease relationship; **Figure 3.1,**

pathway 2). However, most biodiversity–disease studies only examine the second pathway either as a direct link between host biodiversity and pathogen prevalence, or as an indirect link where habitat characteristics are used as a proxy for host community biodiversity effects on pathogen prevalence. By simultaneously evaluating direct habitat–disease links alongside concurrent links between habitat, host biodiversity, and pathogen prevalence, we can more critically investigate the relative contribution of each pathway to community-wide pathogen prevalence.

The relative magnitude of direct habitat–disease links and direct biodiversity–disease links was comparable, but greater species richness only correlated with lower virus prevalence, while high-quality habitat characteristics had mixed positive and negative associations with virus prevalence. Overall, the summed net effect of all direct and indirect links between habitat, biodiversity, and virus prevalence results in reduced prevalence for all three viruses. Therefore, despite some mixed results along individual pathways and between different viruses, sites with very high-quality habitat and high species richness are predicted to have significantly reduced virus prevalence by a maximum of 8 to 23% (depending on the virus) compared to sites with low-quality habitat and low species richness (**Table 3.2**). This result corroborates previous evidence of dilution effects for DWV, BQCV, and SBV in multiple pollinator host species (**Chapter 2**), but our structural equation model provides a more detailed view of the underlying contributing factors to the broadly observable patterns of pathogen prevalence. Investigation of whether habitat factors also directly and indirectly influence both host communities and pathogens in other host–pathogen systems with known biodiversity–disease relationships will be key to understanding the drivers of variable pathogen prevalence among different communities.

Consistencies among viruses

An ideal way to begin to understand how habitat characteristics will influence pathogen prevalence is to look for commonalities among pathogens within multi-host–multi-pathogen systems. We find that DWV and BQCV share many of the same links between habitat characteristics, pollinator species richness, and virus prevalence, while SBV was not significantly linked with any of habitat or pollinator characteristics. Most of the pathways that were shared among DWV and BQCV were similar in magnitude and direction, except greater landscape richness correlated with reduced DWV and increased BQCV. The majority of the shared links among the three viruses are likely due to the many common traits shared by the three viruses. All three viruses are positive-strand RNA viruses in the order *Picornavirales*, that primarily infect honey bees and other Hymenopteran pollinator species through food-borne transmission pathways (Chen and Siede 2007). Previous work has shown that differences in pathogen traits, particularly the mode of transmission, can lead to different biodiversity–disease relationships (Halliday et al. 2017), and could have similar divergent effects on habitat–disease relationships. The main differences observed in the number and type of direct links between habitat characteristics and prevalence of the three viruses may be due to more subtle interactions between each virus and host nutritional status and immune responses. Experimental work has shown that honey bees given a high-quality pollen diet have significantly reduced DWV load (number of viral copies) compared to bees given low-quality diets (DeGrandi-Hoffman et al. 2010), which could scale up to more broadly reduce virus prevalence at the landscape-level. However, there are few experimental studies that clearly link habitat characteristics, nutrition, or immune function to DWV, BQCV, or SBV prevalence, so we currently lack a clear mechanistic explanation for the differences in habitat–disease relationships among these viruses.

Potential for habitat–disease relationships in other host–pathogen systems

Habitat–disease relationships have the potential to be an important explanation for variability observed in the biodiversity–disease relationships in other host–pathogen systems. Several reviews and meta-analyses have found inconsistent evidence for the dilution and amplification effects, even when comparing the same host–pathogen system in different locations (Salkeld et al. 2013, Civitello et al. 2015a, Wood et al. 2017). Part of this variation may be explained, at least in part, by differences in habitat characteristics at different sites. The quality of resources in the surrounding environment is known to influence host nutrition, susceptibility, and immune function in many diverse taxa (Ezenwa 2004, Suorsa et al. 2004, Sorvari et al. 2008, Wilkin et al. 2009, Thomason et al. 2013, Santicchia et al. 2015), which may consequently alter host–pathogen interactions and broad patterns of pathogen prevalence. For example, evidence for dilution effect in *Daphnia* infected with a fungal pathogen *Metschnikowia* is highly dependent on the other hosts' competitive ability for phytoplankton resources (Strauss et al. 2015). However, the quality of phytoplankton resources also changes epidemic size and transmission potential by altering host foraging behavior and host size (Penczykowski et al. 2014, Civitello et al. 2015b), suggesting that habitat quality could play a key role in how these dilution effect dynamics play out. Furthermore, some phytoplankton have medicinal properties in the presence of the fungal pathogen, but can harm hosts in the absence of infection—adding further dimensions to how the quality of resources and habitat could affect patterns of pathogen prevalence (Sánchez et al. 2019). Clearly habitat factors can have multifaceted impacts on pathogen prevalence in many different host–pathogen systems through direct and indirect mechanisms that could explain the variable biodiversity–disease relationships we observe in natural systems. The specific habitat characteristics that constitute "high-quality habitat" and are

likely to influence patterns of pathogen prevalence will vary based on the ecology of specific hosts and pathogens, but need to be considered to improve predictions of biodiversity–disease relationships. Future work testing the predictions of the habitat–disease relationships in other host–pathogen systems will be important for determining how habitat characteristics may impact patterns of pathogen prevalence and biodiversity–disease relationships at broader spatial scales.

Conclusions

Here, we investigate the role of habitat quality characteristics on pollinator community characteristics and patterns of DWV, BQCV, and SBV prevalence that have previously been shown to exhibit a consistent dilution effect in multiple host species. We show that habitat characteristics are directly linked with virus prevalence and indirectly linked to virus prevalence through habitat effects on host species richness. Furthermore, we found that species richness correlated with reduced virus prevalence, while the direction of the individual habitat–disease pathways were more variable among different pathogens and specific habitat characteristics, but both pathways had similar magnitude. Overall, the combined net effect of greater habitat quality characteristics through all direct and indirect pathways resulted in a strong reduction in prevalence for all three viruses. These results support that habitat–disease relationships are important in mediating pathogen prevalence, and could contribute to variability in biodiversity–disease relationships observed in other host–pathogen systems. Habitat characteristics may be an important player in the complex interactions between hosts and pathogens, as a key driver of both changing species interactions in communities and mediating host susceptibility and immunity. Future studies should investigate potential mechanisms that link habitat characteristics to host nutrition, susceptibility, immunity, and pathogen prevalence and load, which will be critical for generating a predictive framework for habitat–disease relationships.

Literature Cited

Alaux, C., F. Ducloz, D. Crauser, and Y. Le Conte. 2010. Diet effects on honeybee immunocompetence. Biology Letters 6:562–565.

Alaux, C., M. Folschweiller, C. McDonnell, D. Beslay, M. Cousin, C. Dussaubat, J.-L. Brunet, and Y. Le Conte. 2011. Pathological effects of the microsporidium Nosema ceranae on honey bee queen physiology (Apis mellifera). Journal of Invertebrate Pathology 106:380–5.

Alger, S. A., P. A. Burnham, H. F. Boncristiani, and A. K. Brody. 2019. RNA virus spillover from managed honeybees (Apis mellifera) to wild bumblebees (Bombus spp.). PLoS ONE 14:e0217822.

Allan, B. F., R. B. Langerhans, W. A. Ryberg, W. J. Landesman, N. W. Griffin, R. S. Katz, B. J. Oberle, M. R. Schutzenhofer, K. N. Smyth, A. D. S. Maurice, L. Clark, K. R. Crooks, D. E. Hernandez, R. G. Mclean, R. S. Ostfeld, and J. M. Chase. 2009. Ecological Correlates of Risk and Incidence of West Nile Virus in the United States. Oecologia 158:699–708.

Bailes, E. J., K. R. Deutsch, J. Bagi, L. Rondissone, M. J. F. Brown, and O. T. Lewis. 2018. First detection of bee viruses in hoverfly (syrphid) pollinators. Biology Letters 14:20180001.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software 67:1–48.

Becker, C. G., D. Rodriguez, L. F. Toledo, A. V. Longo, C. Lambertini, D. T. Correa, D. S. Leite, C. F. B. Haddad, and K. R. Zamudio. 2014. Partitioning the net effect of host diversity on an emerging amphibian pathogen. Proceedings of the Royal Society B: Biological Sciences 281:20141796.

Benjeddou, M., N. Leat, M. Allsopp, and S. Davison. 2001. Detection of Acute Bee Paralysis Virus and Black Queen Cell Virus from Honeybees by Reverse Transcriptase PCR. Applied

and Environmental Microbiology 67:2384–2387.

Biesmeijer, J. C., S. P. M. Roberts, M. Reemer, R. Ohlemuller, M. Edwards, T. Peeters, A. Schaffers, S. G. Potts, R. Kleukers, C. Thomas, J. Settele, and W. E. Kunin. 2006. Parallel Declines in Pollinators and Insect-Pollinated Plants in Britain and the Netherlands. Science 313:351–354.

Blaauw, B. R., and R. Isaacs. 2014. Larger patches of diverse floral resources increase insect pollinator density, diversity, and their pollination of native wildflowers. Basic and Applied Ecology 15:701–711.

Brunner, F. S., P. Schmid-Hempel, and S. M. Barribeau. 2014. Protein-poor diet reduces host-specific immune gene expression in Bombus terrestris. Proceedings of the Royal Society B: Biological Sciences 281:20140128.

Cardinal, S., J. Straka, and B. N. Danforth. 2010. Comprehenisve phylogeny of apid bees reveals the evolutionary origins and antiquity of cleptparasitism. Proceedings of the National Academy of Sciences 107:16207–16211.

Carvell, C., D. B. Roy, S. M. Smart, R. F. Pywell, C. D. Preston, and D. Goulson. 2006. Declines in forage availability for bumblebees at a national scale. Biological Conservation 132:481–489.

Chen, Y. P., and R. Siede. 2007. Honey bee viruses. Pages 33–80 *in* K. Maramorosch, A. J. Shatkin, and F. A. Murphy, editors. Advances in Virus Research. First edition. Academic Press, San Diego, CA.

Civitello, D. J., J. Cohen, H. Fatima, N. T. Halstead, J. Liriano, T. A. McMahon, C. N. Ortega, E. L. Sauer, T. Sehgal, S. Young, and J. R. Rohr. 2015a. Biodiversity inhibits parasites: Broad evidence for the dilution effect. Proceedings of the National Academy of Sciences

112:8667–8671.

Civitello, D. J., R. M. Penczykowski, A. N. Smith, M. S. Shocket, M. A. Duffy, and S. R. Hall. 2015b. Resources, key traits and the size of fungal epidemics in Daphnia populations. Journal of Animal Ecology 84:1010–1017.

Clay, C. A., E. M. Lehmer, S. St. Jeor, and M. D. Dearing. 2009. Sin Nombre virus and rodent species diversity: A test of the dilution and amplification hypotheses. PLoS ONE 4:e6467.

Coop, R. L., and I. Kyrizakis. 1999. Nutrition and Parasite Interaction. Veterinary Parasitology 84:187–204.

Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2000. Emerging infectious diseases of wildlife - threats to biodiversity and human health. Science 287:443–449.

DeGrandi-Hoffman, G., and Y. Chen. 2015. Nutrition, immunity and viral infections in honey bees. Current Opinion in Insect Science 10:170–176.

DeGrandi-Hoffman, G., Y. Chen, E. Huang, and M. H. Huang. 2010. The effect of diet on protein concentration, hypopharyngeal gland development and virus load in worker honey bees (Apis mellifera L.). Journal of Insect Physiology 56:1184–1191.

Dizney, L. J., and L. A. Ruedas. 2009. Increased host species diversity and decreased prevalence of sin nombre virus. Emerging Infectious Diseases 15:1012–1018.

Donkersley, P., G. Rhodes, R. W. Pickup, K. C. Jones, and K. Wilson. 2014. Honeybee nutrition is linked to landscape composition. Ecology and Evolution 4:4195–206.

Dormann, C. F., J. Elith, S. Bacher, C. Buchmann, G. Carl, G. Carr, J. R. Garc, B. Gruber, B. Lafourcade, P. J. Leit, M. Tamara, C. Mcclean, P. E. Osborne, B. S. Der, A. K. Skidmore, D. Zurell, and S. Lautenbach. 2013. Collinearity : a review of methods to deal with it and a simulation study evaluating their performance. Ecography 36:27–46.

Ebeling, A., A. M. Klein, J. Schumacher, W. W. Weisser, and T. Tscharntke. 2008. How does plant richness affect pollinator richness and temporal stability of flower visits? Oikos 117:1808–1815.

Evans, E., M. Smart, D. Cariveau, and M. Spivak. 2018. Wild, native bees and managed honey bees benefit from similar agricultural land uses. Agriculture, Ecosystems and Environment 268:162–170.

Ezenwa, V. O. 2004. Interactions among host diet, nutritional status and gastrointestinal parasite infection in wild bovids. International Journal for Parasitology 34:535–542.

Ezenwa, V. O., M. S. Godsey, R. J. King, and S. C. Guptill. 2006. Avian diversity and West Nile virus: Testing associations between biodiversity and infectious disease risk. Proceedings of the Royal Society B: Biological Sciences 273:109–117.

Fabian, Y., N. Sandau, O. T. Bruggisser, A. Aebi, P. Kehrli, R. P. Rohr, R. E. Naisbit, and L. F. Bersier. 2013. The importance of landscape and spatial structure for hymenopteran-based food webs in an agro-ecosystem. Journal of Animal Ecology 82:1203–1214.

Fahrig, L. 2003. Effects of Habitat Fragmentation on Biodiversity. Annual Review of Ecology, Evolution, and Systematics 34:487–515.

Faust, C. L., A. P. Dobson, N. Gottdenker, L. S. P. Bloomfield, H. I. Mccallum, T. R. Gillespie, M. Diuk-Wasser, R. K. Plowright, G. TR, and P. RK. 2017. Null expectations for disease dynamics in shrinking habitat: dilution or amplification? Phil Trans R Soc B 372:20160173.

Foley, J. A., R. DeFries, G. P. Asner, C. Barford, G. Bonan, S. R. Carpenter, F. S. Chapin, M. T. Coe, G. C. Daily, H. K. Gibbs, J. H. Helkowski, T. Holloway, E. A. Howard, C. J. Kucharik, C. Monfreda, J. A. Patz, I. C. Prentice, N. Ramankutty, and P. K. Snyder. 2005. Global consequences of land use. Science 309:570–574.

Fouks, B., and H. M. G. Lattorff. 2011. Recognition and avoidance of contaminated flowers by foraging bumblebees (Bombus terrestris). PloS ONE 6:e26328.

Franklin, J. F. 1993. Preserving Biodiversity: Species, Ecosystems, or Landscapes? Ecological Applications 3:202–205.

Fürst, M. A., D. P. McMahon, J. L. Osborne, R. J. Paxton, and M. J. F. Brown. 2014. Disease associations between honeybees and bumblebees as a threat to wild pollinators. Nature 506:364–366.

Genersch, E., C. Yue, I. Fries, and J. R. De Miranda. 2011. Detection of Deformed wing virus, a honey bee viral pathogen, in bumble bees (Bombus terrestris and Bombus pascuorum) with wing deformities. Journal of Invertebrate Pathology 91:61–63.

Gherman, B. I., A. Denner, O. Bobiş, D. S. Dezmirean, L. a. Mărghitaş, H. Schlüns, R. F. a. Moritz, and S. Erler. 2014. Pathogen-associated self-medication behavior in the honeybee Apis mellifera. Behavioral Ecology and Sociobiology 68:1777–1784.

Gong, H. R., X. X. Chen, Y. P. Chen, F. L. Hu, J. L. Zhang, Z. G. Lin, J. W. Yu, and H. Q. Zheng. 2016. Evidence of Apis cerana Sacbrood virus infection in Apis mellifera. Applied and Environmental Microbiology 82:2256–2262.

Gotelli, N. J., and R. K. Colwell. 2001. Quantifying biodiversity: Procedures and pitfalls in the measurement and comparison of species richness. Ecology Letters 4:379–391.

Goulson, D., E. Nicholls, C. Botías, and E. L. Rotheray. 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science 347:1255957.

Greenleaf, S. S., N. M. Williams, R. Winfree, and C. Kremen. 2007. Bee foraging ranges and their relationship to body size. Oecologia 153:589–596.

Halliday, F. W., R. W. Heckman, P. A. Wilfahrt, and C. E. Mitchell. 2017. A multivariate test of

disease risk reveals conditions leading to disease amplification. Proceedings of the Royal Society B: Biological Sciences 284.

Hartig, F. 2020. DHARMa: Residual Diagnostics for Hierarchical (Multi-Level/Mixed) Regression Models. https://cran.r-project.org/package=DHARMa.

Hurd Jr, P. D., E. G. Linsley, and T. W. Whitaker. 1971. Squash and Gourd Bees (Peponapis, Xenoglossa) and the Origin of the Cultivated Cucurbita. Evolution 25:218–234.

Jha, S., and C. Kremen. 2013. Resource diversity and landscape-level homogeneity drive native bee foraging. Proceedings of the National Academy of Sciences 110:555–8.

Jha, S., L. Stefanovich, and C. Kremen. 2013. Bumble bee pollen use and preference across spatial scales in human-altered landscapes. Ecological Entomology 38:570–579.

Johnson, P. T. J., D. L. Preston, J. T. Hoverman, and K. L. D. Richgels. 2013. Biodiversity decreases disease through predictable changes in host community competence. Nature 494:230–3.

Keesing, F., L. K. Belden, P. Daszak, A. Dobson, C. D. Harvell, R. D. Holt, P. Hudson, A. Jolles, K. E. Jones, C. E. Mitchell, S. S. Myers, T. Bogich, and R. S. Ostfeld. 2010. Impacts of biodiversity on the emergence and transmission of infectious diseases. Nature 468:647–52.

Keesing, F., R. D. Holt, and R. S. Ostfeld. 2006. Effects of species diversity on disease risk. Ecology Letters 9:485–98.

Kennedy, C. M., E. Lonsdorf, M. C. Neel, N. M. Williams, T. H. Ricketts, R. Winfree, R. Bommarco, C. Brittain, A. L. Burley, D. Cariveau, L. G. Carvalheiro, N. P. Chacoff, S. A. Cunningham, B. N. Danforth, J. H. Dudenhöffer, E. Elle, H. R. Gaines, L. A. Garibaldi, C. Gratton, A. Holzschuh, R. Isaacs, S. K. Javorek, S. Jha, A. M. Klein, K. Krewenka, Y.

Mandelik, M. M. Mayfield, L. Morandin, L. A. Neame, M. Otieno, M. Park, S. G. Potts, M. Rundlöf, A. Saez, I. Steffan-Dewenter, H. Taki, B. F. Viana, C. Westphal, J. K. Wilson, S. S. Greenleaf, and C. Kremen. 2013. A global quantitative synthesis of local and landscape effects on wild bee pollinators in agroecosystems. Ecology Letters 16:584–599.

Kilpatrick, A. M., D. J. Salkeld, G. Titcomb, and M. B. Hahn. 2017. Conservation of biodiversity as a strategy for improving human health and well-being. Philosophical Transactions of the Royal Society B 372:20160131.

Koh, I., E. V Lonsdorf, N. M. Williams, C. Brittain, R. Isaacs, J. Gibbs, and T. H. Ricketts. 2016. Modeling the status, trends, and impacts of wild bee abundance in the United States. Proceedings of the National Academy of Sciences 113:140–145.

Lange, B., M. Reuter, D. Ebert, K. Muylaert, and E. Decaestecker. 2014. Diet quality determines interspecific parasite interactions in host populations. Ecology and Evolution 4:3093–3102.

Lefcheck, J. S. 2016. PIECEWISE SEM : Piecewise structural equation modelling in R for ecology , evolution , and systematics. Methods in Ecology and Evolution 7:573–579.

Lefcheck, J. S. 2019. Structural Equation Modeling in R for Ecology and Evolution. https://jslefche.github.io/sem_book/index.html.

Lenth, R. 2020. emmeans: Estimated Marginal Means, aka Least-Squares Means. https://cran.r-project.org/package=emmeans.

Lentini, P. E., T. G. Martin, P. Gibbons, J. Fischer, and S. A. Cunningham. 2012. Supporting wild pollinators in a temperate agricultural landscape: Maintaining mosaics of natural features and production. Biological Conservation 149:84–92.

Levitt, A. L., R. Singh, D. L. Cox-Foster, E. Rajotte, K. Hoover, N. Ostiguy, and E. C. Holmes. 2013. Cross-species transmission of honey bee viruses in associated arthropods. Virus

Research 176:232–240.

Mazzei, M., M. L. Carrozza, E. Luisi, M. Forzan, M. Giusti, S. Sagona, F. Tolari, and A. Felicioli. 2014. Infectivity of DWV associated to flower pollen: experimental evidence of a horizontal transmission route. PloS ONE 9:e113448.

McArt, S. H., H. Koch, R. E. Irwin, and L. S. Adler. 2014. Arranging the bouquet of disease: Floral traits and the transmission of plant and animal pathogens. Ecology Letters 17:624–636.

McMahon, D. P., M. A. Fürst, J. Caspar, P. Theodorou, M. J. F. Brown, and R. J. Paxton. 2015. A sting in the spit: widespread cross-infection of multiple RNA viruses across wild and managed bees. Journal of Animal Ecology 84:615–624.

Michener, C. D. 2007. The Bees of the World. 2nd edition. John Hopkins University Press.

Novotný, V., and Y. Basset. 2000. Rare species in communities of tropical insect herbivores: Pondering the mystery of singletons. Oikos 89:564–572.

Ongus, J. R., D. Peters, J. M. Bonmatin, E. Bengsch, J. M. Vlak, and M. M. van Oers. 2004. Complete sequence of a picorna-like virus of the genus Iflavirus replicating in the mite Varroa destructor. Journal of General Virology 85:3747–3755.

Ostfeld, R. S., and F. Keesing. 2000. Biodiversity and Disease Risk: The Case of Lyme Disease. Conservation Biology 14:722–728.

Palmer-Young, E. C., B. M. Sadd, P. C. Stevenson, R. E. Irwin, and L. S. Adler. 2016. Bumble bee parasite strains vary in resistance to phytochemicals. Scientific Reports 6:37087.

Palmer-Young, E. C., C. O. Tozkar, R. S. Schwarz, Y. Chen, R. E. Irwin, L. S. Adler, and J. D. Evans. 2017. Nectar and Pollen Phytochemicals Stimulate Honey Bee (Hymenoptera: Apidae) Immunity to Viral Infection. Journal of Economic Entomology 110:1959–1972.

Paradis, E., and K. Schliep. 2018. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35:526–528.

Di Pasquale, G., M. Salignon, Y. Le Conte, L. P. Belzunces, A. Decourtye, A. Kretzschmar, S. Suchail, J. L. Brunet, and C. Alaux. 2013. Influence of Pollen Nutrition on Honey Bee Health: Do Pollen Quality and Diversity Matter? PLoS ONE 8:1–13.

Penczykowski, R. M., B. C. P. Lemanski, R. D. Sieg, S. R. Hall, J. Housley Ochs, J. Kubanek, and M. A. Duffy. 2014. Poor resource quality lowers transmission potential by changing foraging behaviour. Functional Ecology 28:1245–1255.

Peng, W., J. Li, H. Boncristiani, J. P. Strange, M. Hamilton, and Y. Chen. 2011. Host range expansion of honey bee Black Queen Cell Virus in the bumble bee, Bombus huntii. Apidologie 42:650–658.

Ponton, F., K. Wilson, A. J. Holmes, S. C. Cotter, D. Raubenheimer, and S. J. Simpson. 2013. Integrating nutrition and immunology: A new frontier. Journal of Insect Physiology 59:130–137.

Potts, S. G., J. C. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger, and W. E. Kunin. 2010. Global pollinator declines: Trends, impacts and drivers. Trends in Ecology and Evolution 25:345–353.

Potts, S. G., B. Vulliamy, A. Dafni, G. Ne, and P. Willmer. 2003. Linking Bees and Flowers: How Do Floral Communities Structure Pollinator Communities? Ecology 84:2628–2642.

Prugh, L. R., K. E. Hodges, A. R. E. Sinclair, and J. S. Brashares. 2008. Effect of habitat area and isolation on fragmented animal populations. Proceedings of the National Academy of Sciences 105:20770–20775.

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for

Statistical Computing, Vienna, Austria.

Rader, R., I. Bartomeus, J. M. Tylianakis, and E. Laliberté. 2014. The winners and losers of land use intensification: Pollinator community disassembly is non-random and alters functional diversity. Diversity and Distributions 20:908–917.

Radzevičiūtė, R., P. Theodorou, M. Husemann, G. Japoshvili, G. Kirkitadze, A. Zhusupbaeva, and R. J. Paxton. 2017. Replication of honey bee-associated RNA viruses across multiple bee species in apple orchards of Georgia, Germany and Kyrgyzstan. Journal of Invertebrate Pathology 146:14–23.

Richardson, L. L., L. S. Adler, A. S. Leonard, K. Henry, W. Anthony, J. S. Manson, and R. E. Irwin. 2015. Secondary metabolites in floral nectar reduce parasite infections in bumble bees. Proceedings of the Royal Society Biological Sciences 282:20142471.

Ricketts, T. H., J. Regetz, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, A. Bogdanski, B. Gemmill-Herren, S. S. Greenleaf, A. M. Klein, M. M. Mayfield, L. A. Morandin, A. Ochieng, and B. F. Viana. 2008. Landscape effects on crop pollination services: Are there general patterns? Ecology Letters 11:499–515.

Rohr, J. R., D. J. Civitello, F. W. Halliday, P. J. Hudson, K. D. Lafferty, C. L. Wood, and E. A. Mordecai. 2020. Towards common ground in the biodiversity–disease debate. Nature Ecology and Evolution 4:24–33.

Salkeld, D. J., K. A. Padgett, and J. H. Jones. 2013. A meta-analysis suggesting that the relationship between biodiversity and risk of zoonotic pathogen transmission is idiosyncratic. Ecology Letters 16:679–686.

Sánchez, K. F., N. Huntley, M. A. Duffy, and M. D. Hunter. 2019. Toxins or medicines? Phytoplankton diets mediate host and parasite fitness in a freshwater system. Proceedings of

the Royal Society B: Biological Sciences 286.

Santicchia, F., C. Romeo, A. Martinoli, P. Lanfranchi, L. A. Wauters, and N. Ferrari. 2015. Effects of habitat quality on parasite abundance: Do forest fragmentation and food availability affect helminth infection in the Eurasian red squirrel? Journal of Zoology 296:38–44.

Schmidt, K. A., and R. S. Ostfeld. 2001. Biodiversity and the Dilution Effect in Disease Ecology. Ecology 82:609–619.

Searle, C. L., L. M. Biga, J. W. Spatafora, and A. R. Blaustein. 2011. A dilution effect in the emerging amphibian pathogen Batrachochytrium dendrobatidis. Proceedings of the National Academy of Sciences of the United States of America 108:16322–16326.

Shackelford, G., P. R. Steward, T. G. Benton, W. E. Kunin, S. G. Potts, J. C. Biesmeijer, and S. M. Sait. 2013. Comparison of pollinators and natural enemies: A meta-analysis of landscape and local effects on abundance and richness in crops. Biological Reviews 88:1002–1021.

Singh, R., A. L. Levitt, E. G. Rajotte, E. C. Holmes, N. Ostiguy, D. Vanengelsdorp, W. I. Lipkin, C. W. Depamphilis, A. L. Toth, and D. L. Cox-Foster. 2010. RNA viruses in hymenopteran pollinators: evidence of inter-taxa virus transmission via pollen and potential impact on non-Apis hymenopteran species. PLoS ONE 5:e14357.

Sorvari, J., H. Hakkarainen, and M. J. Rantala. 2008. Immune Defense of Ants Is Associated with Changes in Habitat Characteristics. Environmental Entomology 37:51–56.

Strauss, A. T., A. M. Bowling, M. A. Duffy, C. E. Cáceres, and S. R. Hall. 2018. Linking host traits, interactions with competitors and disease: Mechanistic foundations for disease dilution. Functional Ecology 32:1271–1279.

Strauss, A. T., D. J. Civitello, C. E. Cáceres, and S. R. Hall. 2015. Success, failure and ambiguity

of the dilution effect among competitors. Ecology Letters 18:916–926.

Suorsa, P., H. Helle, V. Koivunen, E. Huhta, A. Nikula, and H. Hakkarainen. 2004. Effects of forest patch size on physiological stress and immunocompetence in an area-sensitive passerine, the Eurasian treecreeper (Certhia familiaris): An experiment. Proceedings of the Royal Society B: Biological Sciences 271:435–440.

Swaddle, J. P., and S. E. Calos. 2008. Increased avian diversity is associated with lower incidence of human West Nile infection: Observation of the dilution effect. PLoS ONE 3.

Thomas, J. A., N. A. D. Bourn, R. T. Clarke, K. E. Stewart, D. J. Simcox, G. S. Pearman, R. Curtis, and B. Goodger. 2001. The quality and isolation of habitat patches both determine where butterflies persist in fragmented landscapes. Proceedings of the Royal Society B: Biological Sciences 268:1791–1796.

Thomason, C. A., T. L. Hedrick-Hopper, and T. L. Derting. 2013. Social and nutritional stressors: Agents for altered immune function in white-footed mice (Peromyscus leucopus). Canadian Journal of Zoology 91:313–320.

vanEngelsdorp, D., and M. D. Meixner. 2010. A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. Journal of Invertebrate Pathology 103 Suppl:S80-95.

Vaudo, A. D., H. M. Patch, D. A. Mortensen, J. F. Tooker, and C. M. Grozinger. 2016. Macronutrient ratios in pollen shape bumble bee (Bombus impatiens) foraging strategies and floral preferences. Proceedings of the National Academy of Sciences 113:E4035–E4042.

Vaudo, A. D., J. F. Tooker, C. M. Grozinger, and H. M. Patch. 2015. Bee nutrition and floral resource restoration. Current Opinion in Insect Science 10:133–141.

Venesky, M. D., X. Liu, E. L. Sauer, and J. R. Rohr. 2014. Linking manipulative experiments to field data to test the dilution effect. Journal of Animal Ecology 83:557–565.

Watson, J. C., A. T. Wolf, and J. S. Ascher. 2011. Forested Landscapes Promote Richness and Abundance of Native Bees (Hymenoptera: Apoidea: Anthophila) in Wisconsin Apple Orchards. Environmental Entomology 40:621–632.

Wilkin, T. A., L. E. King, and B. C. Sheldon. 2009. Habitat quality, nestling diet, and provisioning behaviour in great tits Parus major. Journal of Avian Biology 40:135–145.

Williams, N. M., and C. Kremen. 2015. Resource Distributions among Habitats Determine Solitary Bee Offspring Production in a Mosaic Landscape. Ecological Applications 17:910–921.

Williams, N. M., J. Regetz, and C. Kremen. 2012. Landscape-scale resources promote colony growth but not reproductive performance of bumble bees. Ecology 93:1049–1058.

Wood, C. L., A. McInterff, H. S. Young, D. Kim, and K. D. Lafferty. 2017. Human infectious disease burdens decrease with urbanization but not with biodiversity. Philosophical Transactions of the Royal Society B 372:20160122.

Yang, B., G. Peng, T. Li, and T. Kadowaki. 2013. Molecular and phylogenetic characterization of honey bee viruses, Nosema microsporidia, protozoan parasites, and parasitic mites in China. Ecology and Evolution 3:298–311.

Yue, C., and E. Genersch. 2005. RT-PCR analysis of Deformed wing virus in honeybees (Apis mellifera) and mites (Varroa destructor). Journal of General Virology 86:3419–3424.

Tables

Table 3.1: Final SEM unstandardized estimated pathway coefficients, standard error, degrees of freedom, critical value, p-value, range standardized estimated coefficient, and R^2 for each model pathway. The last four rows are correlated errors. P-values are bolded for significant pathways.

Response variable	Predictor variables	Estimate	Std Error	DF	Critical Value	P-value	Range Std Estimate	R^2
Species Richness	Prop. Natural Area 1000m	0.609	0.020	883	29.782	**<0.001**	0.892	0.65
	Landscape Richness 1000m	0.494	0.025	883	20.080	**<0.001**	0.493	
	Floral Richness	0.173	0.027	883	6.473	**<0.001**	0.165	
	Floral Density	-0.261	0.026	883	-10.117	**<0.001**	-0.226	
Abundance	Prop. Natural Area 1000m	0.677	0.023	883	29.980	**<0.001**	0.664	0.59
	Landscape Richness 1000m	0.087	0.027	883	3.216	**0.001**	0.058	
	Floral Richness	-0.235	0.030	883	-7.973	**<0.001**	-0.150	
	Floral Density	0.300	0.028	883	10.551	**<0.001**	0.174	
DWV	Landscape Richness 1000m	-0.346	0.102	881	-3.379	**0.001**	-0.098	0.23
	Floral Richness	0.316	0.093	881	3.396	**0.001**	0.085	
	Species Richness	-0.335	0.094	881	-3.557	**<0.001**	-0.095	
	Host Species	NA	NA	3	38.006	**<0.001**	NA	
	Host Species = *Apis*	0.174	0.136	Inf	1.279	0.201	NA	
	Host Species = *Bombus*	-0.660	0.139	Inf	-4.764	**<0.001**	NA	
	Host Species = *Lasioglossum*	-1.913	0.198	Inf	-9.683	**<0.001**	NA	
	Host Species = *Eucera*	-1.671	0.206	Inf	-8.113	**<0.001**	NA	
BQCV	Prop. Natural Area 1000m	0.390	0.138	879	2.822	**0.005**	0.132	0.50
	Landscape Richness 1000m	0.336	0.139	879	2.419	**0.016**	0.078	
	Floral Richness	0.303	0.131	879	2.319	**0.020**	0.067	
	Floral Density	-0.438	0.132	879	-3.326	**0.001**	-0.088	
	Species Richness	-0.674	0.161	879	-4.185	**<0.001**	-0.156	
	Host Species	NA	NA	3	122.212	**<0.001**	NA	
	Host Species = *Apis*	1.313	0.163	Inf	8.040	**<0.001**	NA	
	Host Species = *Bombus*	-0.599	0.137	Inf	-4.381	**<0.001**	NA	
	Host Species = *Lasioglossum*	-2.898	0.289	Inf	-10.023	**<0.001**	NA	
	Host Species = *Eucera*	-2.629	0.294	Inf	-8.936	**<0.001**	NA	

SBV	Species Richness	-0.168	0.094	883	-1.781	0.075	-0.035	0.33
	Host Species	NA	NA	3	69.495	**<0.001**	NA	
	Host Species = *Apis*	-0.923	0.146	Inf	-6.320	**<0.001**	NA	
	Host Species = *Bombus*	-0.407	0.131	Inf	-3.117	**0.002**	NA	
	Host Species = *Lasioglossum*	-5.441	1.002	Inf	-5.429	**<0.001**	NA	
	Host Species = *Eucera*	-3.782	0.507	Inf	-7.460	**<0.001**	NA	
DWV	BQCV	0.203	NA	888	6.167	**<0.001**	0.203	
BQCV	SBV	0.161	NA	888	4.853	**<0.001**	0.161	
SBV	DWV	0.174	NA	888	5.260	**<0.001**	0.174	
Abundance	Species Richness	0.175	NA	888	5.276	**<0.001**	0.175	

Table 3.2: The range standardized regression coefficients for the direct and indirect pathways between habitat characteristics and DWV, BQCV, and SBV prevalence. Indirect pathway coefficients are a product of the two direct pathway coefficients, with the specific pathway indicated by the → below. The net effect is the sum of all direct and indirect pathways connected to each virus. The range standardized coefficients and net effects are colored based on the magnitude (darker = larger) and direction (red = negative; blue = positive) of the proportional shift in virus prevalence from a full range shift in each predictor. Coefficients that are composed of all significant pathways are bolded.

Response variable	Pathway	Link Type	Std. Range Estimate	Net Effect
DWV	Landscape Richness 1000m	Direct	**-0.098**	**-0.234**
	Floral Richness	Direct	**0.085**	
	Species Richness	Direct	**-0.095**	
	Natural Area → Species Richness	Indirect	**-0.085**	
	Landscape Richness → Species Richness	Indirect	**-0.047**	
	Floral Richness → Species Richness	Indirect	**-0.016**	
	Floral Density → Species Richness	Indirect	**0.022**	
BQCV	Prop. Natural Area 1000m	Direct	**0.132**	**-0.174**
	Landscape Richness 1000m	Direct	**0.078**	
	Floral Richness	Direct	**0.067**	
	Floral Density	Direct	**-0.088**	
	Species Richness	Direct	**-0.156**	
	Natural Area → Species Richness	Indirect	**-0.139**	
	Landscape Richness → Species Richness	Indirect	**-0.077**	
	Floral Richness → Species Richness	Indirect	**-0.026**	
	Floral Density → Species Richness	Indirect	**0.035**	
SBV	Species Richness	Direct	-0.035	-0.082
	Natural Area → Species Richness	Indirect	-0.032	
	Landscape Richness → Species Richness	Indirect	-0.017	
	Floral Richness → Species Richness	Indirect	-0.006	
	Floral Density → Species Richness	Indirect	0.008	

Figures

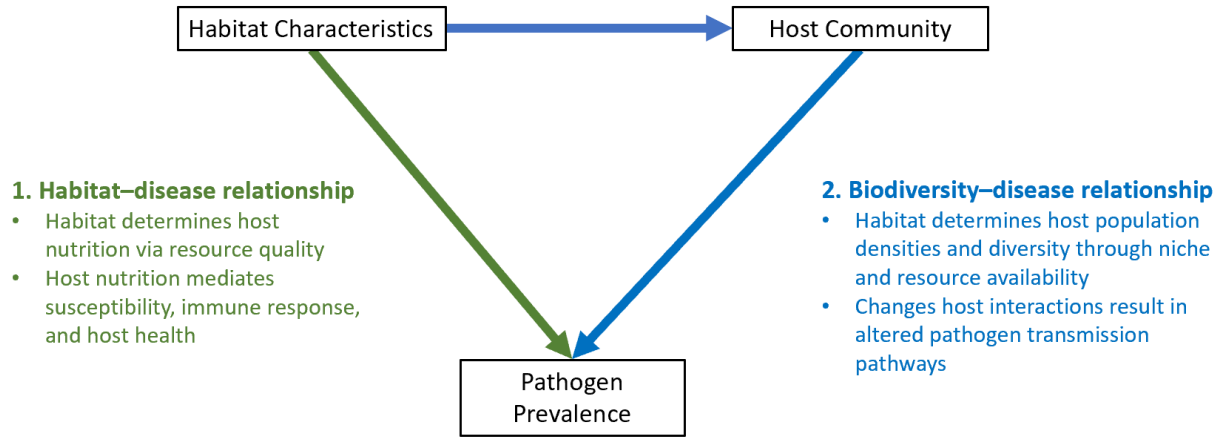


Figure 3.1: A conceptual diagram of the two non-mutually exclusive hypotheses, 1) Habitat–disease relationship and 2) Biodiversity–disease relationship, for how habitat characteristics, host communities, and pathogen prevalence may be linked together.

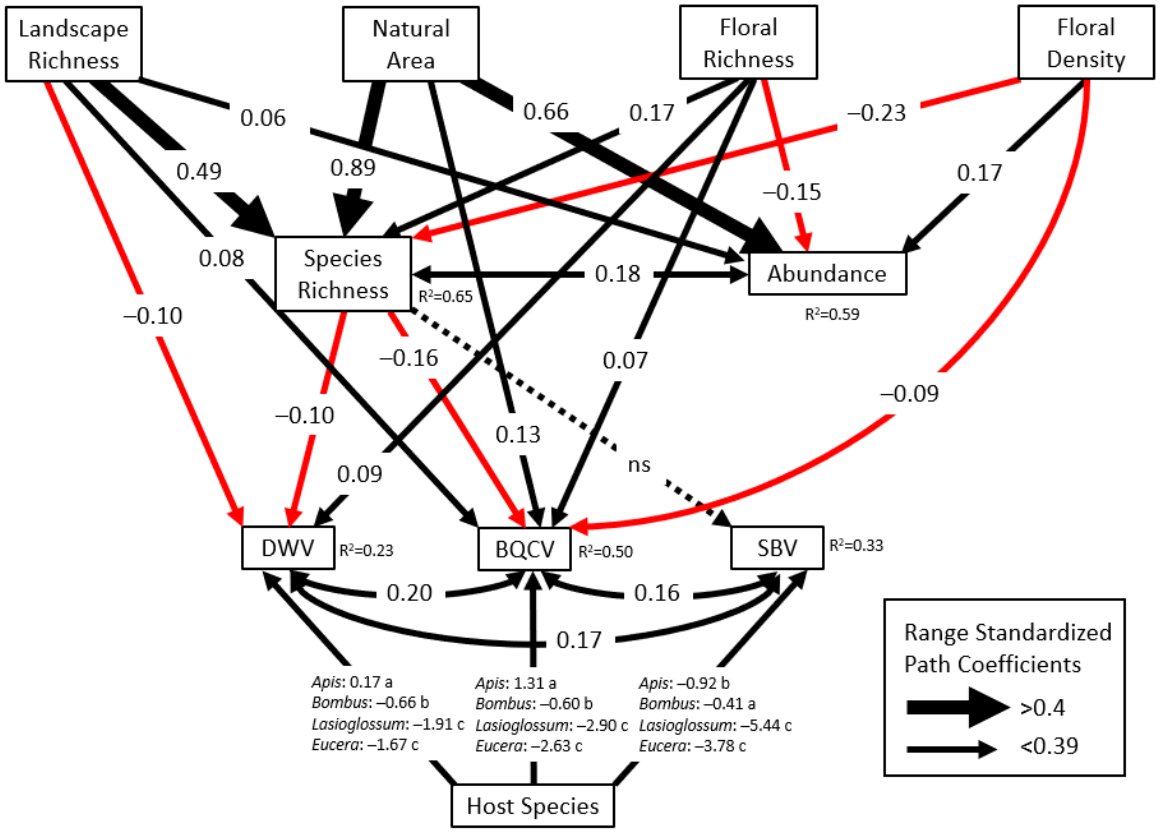


Figure 3.2: Structural Equation Model for the effect of local- and landscape-level habitat and pollinator community characteristics on BQCV, DWV, and SBV prevalence within four focal pollinator host species: *Apis mellifera*, *Bombus impatiens*, *Lasioglossum* spp., and *Eucera pruinosa*. Landscape-level habitat characteristics have a strong positive correlation with pollinator species richness and abundance, while links between habitat characteristics and viral prevalence tend to be comparatively weaker and vary in directionality. Though pollinator species richness tends to have a negative correlation with DWV BQCV. SBV is not significantly related with any of the habitat or pollinator community characteristics modeled. Solid red and black paths denote significant negative and positive associations between linked variables, respectively, and path thickness corresponds to the magnitude of the coefficient. Dotted lines indicate non-significant pathways, and double headed arrows indicate correlated errors included in the model. All path coefficients are range standardized regression coefficients, except the host species have unstandardized coefficients with different letters within each virus denoting significant differences based on post-hoc tests (**Appendix Table S3.5**). Full model statistics can be found in **Table 3.1**.

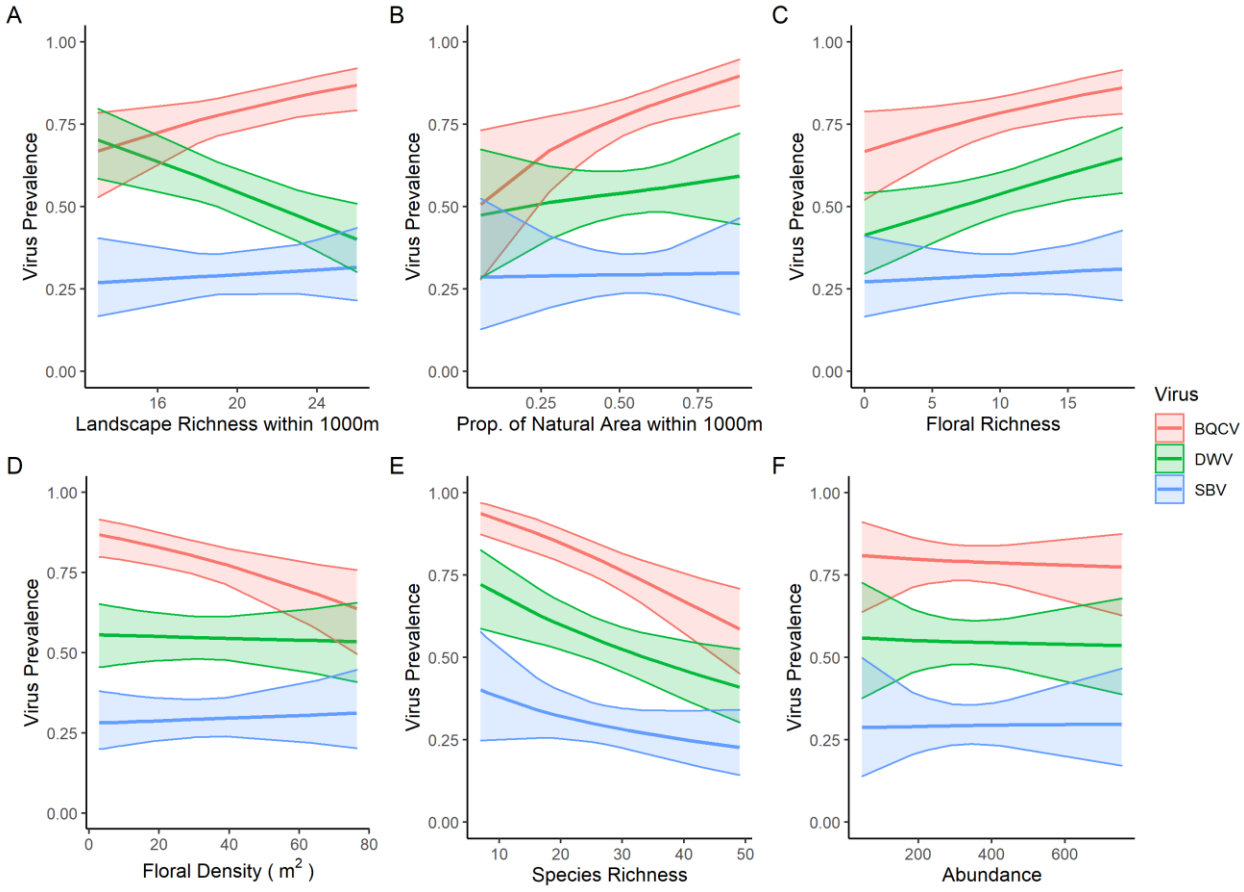


Figure 3.3: Model predicted BQCV, DWV, and SBV prevalence with 95% confidence intervals varies among sites with increasing A) landscape richness within 1000m, B) proportion natural area within 1000m, C) floral richness, D) floral density, E) pollinator community species richness, and F) total abundance. Based on component BQCV, DWV, and SBV binomial generalized linear models in the initial SEM (**Appendix Table S3.4**). Red = black queen cell virus (BQCV); Green = deformed wing virus (DWV); Blue = sacbrood virus (SBV).

Appendix S3

Table S3.1: Field site abbreviation, farm name, sampling year, dates of each visit to field site, and zone, easting and northing coordinates in the UTM GPS system. All field sites are in the Lower Peninsula of Michigan, USA.

Code	Farm Name	Year	First Visit	Second visit	Zone	Easting	Northing
W	Wasem Fruit Farm	2015	7/22/2015	7/30/2015	17T	282985	4668596
G	Gust Brother's Pumpkin Farm	2015	7/26/2015	8/5/2015	17T	267978	4625139
BC	The Blast Corn Maze (Nixon Farms)	2015	7/24/2015	8/2/2015	17T	264841	4692908
BJ	BJ Farm	2015	7/23/2015	7/31/2015	17T	281032	4630188
S	Stone Coop Farm	2015	8/7/2015	8/21/2015	17T	271906	4704446
BP	Brimley's Pumpkin Patch	2016	8/10/2016	8/26/2016	16T	714474	4716740
T	Tantré Farm	2016	8/18/2016	9/2/2016	16T	738575	4681735
K	Kapnick Orchards	2016	8/21/2016	8/28/2016	17T	257729	4648607
SP	Spicer Orchards	2016	8/14/2016	9/1/2018	17T	274397	4729038
PR	Peacock Road Farms	2016	7/26/2016	8/23/2016	16T	714244	4746884
GT	Green Things Farm	2016	8/17/2016	8/24/2016	17T	276741	4689607
E	Erwin Orchards	2016	7/27/2016	8/22/2016	17T	280997	4708908
PL	Plymouth Orchards	2016	8/11/2016	8/30/2016	17T	289557	4690343

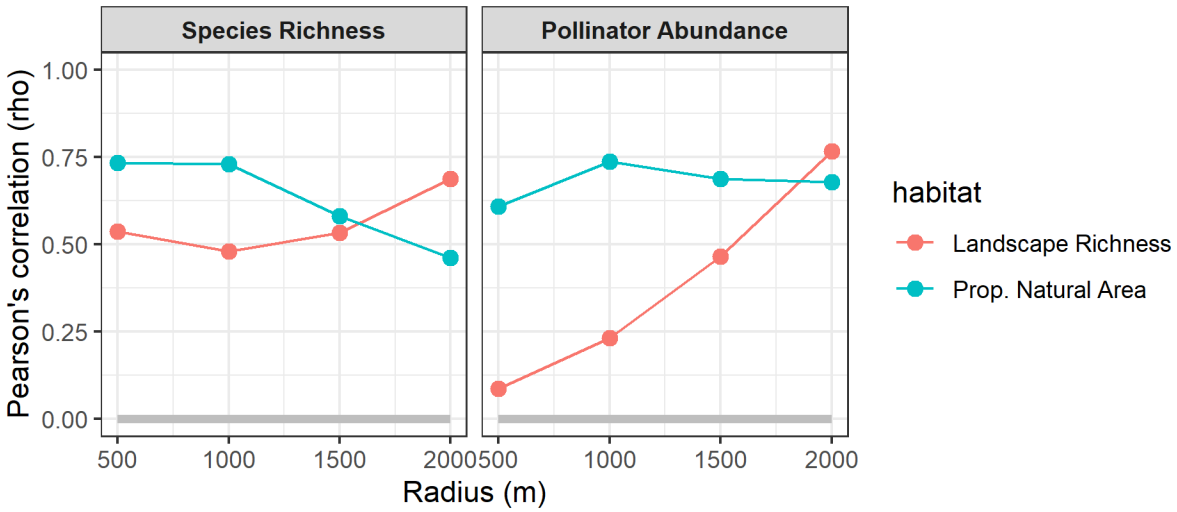


Figure S3.1: Pearson's correlations of landscape richness and the proportion of natural area at 500, 1000, 1500, and 2000 meter spatial scales for pollinator community species richness and total abundance.

Table S3.2: The number of individuals tested for the presence of DWV, BQCV, and SBV. Where less than 20 individuals for a species were collected, then all collected individuals for that species were tested for virus prevalence.

Year	***2015***					***2016***								
Species Names	**W**	**BJ**	**BC**	**G**	**S**	**PR**	**E**	**BP**	**PL**	**SP**	**GT**	**T**	**K**	**Species totals**
Apis mellifera	20	16	21	20	20	20	20	20	20	20	20	8	9	**234**
Bombus impatiens	13	30	13	3	30	19	20	20	20	20	20	20	20	**248**
Lasioglossum spp.	12	19	22	2	19	20	19	20	20	20	20	20	20	**233**
Eucera pruinosa	21	16	20	3	20	20	20	6	20	6	20	1	0	**173**

Table S3.3: Description of variables included in the structural equation model fitting process. Table contains the name, type, applied transformations, mean, standard deviation, and a brief description of each variable.

Variable Name	Type	Transformation	Mean	SD	Description
DWV	binomial	none	na	na	Presence (1) or absence (0) of DWV in individual bees.
BQCV	binomial	none	na	na	Presence (1) or absence (0) of BQCV in individual bees.
SBV	binomial	none	na	na	Presence (1) or absence (0) of SBV in individual bees.
Host Species	categorical	none	*Apis* is reference	na	The host species identity that was tested for each virus. Apis = *Apis mellifera*, Bombus = *Bombus impatiens*, Lasioglossum = *Lasioglossum* spp., Eucera = *Eucera pruinosa*
Species Richness	integer	square root	27.64	13.12	The number of pollinator host species detected per site.
Abundance	integer	square root	338.36	158.66	The total number of pollinator individuals detected per site
Landscape Richness	integer	none	19.64	3.84	The number of different landcover types that occur within a 1000m radius of each site.
Natural Area	continuous	arcsine square root	0.53	0.2	The proportion of area within a 1000m that was classified as 'Natural Area'. We classified forest, wetland, meadow landcover types as 'Natural Area'.
Floral Richness	integer	none	9.93	5.43	The number of different floral species detected at each site.
Floral Density (m^2)	continuous	none	30.51	23.87	The density of all flower per m^2 detected at each site.

Table S3.4: Final SEM unstandardized estimated pathway coefficients, standard error, degrees of freedom, critical value, p-value, range standardized estimated pathway coefficient, and R^2 estimate for each model pathway. The last four rows are correlated errors included in the final model. P-values are bolded for significant pathways.

Response variable	Predictor variable	Estimate	Std Error	DF	Critical Value	P-value	Range Std Estimate	R^2
Species Richness	Prop. Natural Area 1000m	0.609	0.020	883	29.782	<**0.001**	0.892	0.65
	Landscape Richness 1000m	0.494	0.025	883	20.080	<**0.001**	0.493	
	Floral Richness	0.173	0.027	883	6.473	<**0.001**	0.165	
	Floral Density	-0.261	0.026	883	-10.117	<**0.001**	-0.226	
Abundance	Prop. Natural Area 1000m	0.677	0.023	883	29.980	<**0.001**	0.664	0.59
	Landscape Richness 1000m	0.087	0.027	883	3.216	**0.001**	0.058	
	Floral Richness	-0.235	0.030	883	-7.973	<**0.001**	-0.150	
	Floral Density	0.300	0.028	883	10.551	<**0.001**	0.174	
DWV	Prop. Natural Area 1000m	0.065	0.131	878	0.493	0.622	0.027	0.23
	Landscape Richness 1000m	-0.318	0.118	878	-2.697	**0.007**	-0.090	
	Floral Richness	0.326	0.116	878	2.812	**0.005**	0.088	
	Floral Density	-0.036	0.122	878	-0.294	0.769	-0.009	
	Species Richness	-0.375	0.136	878	-2.760	**0.006**	-0.106	
	Abundance	-0.026	0.122	878	-0.215	0.829	-0.011	
	Host Species	NA	NA	3	37.730	<**0.001**	NA	
	Host Species = *Apis*	0.177	0.137	Inf	1.294	0.196	NA	
	Host Species = *Bombus*	-0.667	0.140	Inf	-4.771	<**0.001**	NA	
	Host Species = *Lasioglossum*	-1.915	0.198	Inf	-9.688	<**0.001**	NA	
	Host Species = *Eucera*	-1.668	0.207	Inf	-8.069	<**0.001**	NA	
BQCV	Prop. Natural Area 1000m	0.409	0.157	878	2.615	**0.009**	0.139	0.50
	Landscape Richness 1000m	0.335	0.139	878	2.414	**0.016**	0.078	
	Floral Richness	0.293	0.136	878	2.150	**0.032**	0.065	
	Floral Density	-0.424	0.143	878	-2.968	**0.003**	-0.085	
	Species Richness	-0.665	0.165	878	-4.041	<**0.001**	-0.154	
	Abundance	-0.038	0.142	878	-0.264	0.792	-0.013	
	Host Species	NA	NA	3	121.431	<**0.001**	NA	
	Host Species = *Apis*	1.314	0.163	Inf	8.045	<**0.001**	NA	
	Host Species = *Bombus*	-0.604	0.138	Inf	-4.378	<**0.001**	NA	
	Host Species = *Lasioglossum*	-2.896	0.289	Inf	-10.020	<**0.001**	NA	
	Host Species = *Eucera*	-2.624	0.295	Inf	-8.901	<**0.001**	NA	

SBV	Prop. Natural Area 1000m	0.003	0.161	878	0.017	0.987	0.001	0.34
	Landscape Richness 1000m	0.069	0.142	878	0.485	0.628	0.014	
	Floral Richness	0.055	0.135	878	0.404	0.686	0.011	
	Floral Density	0.044	0.141	878	0.316	0.752	0.008	
	Species Richness	-0.235	0.166	878	-1.418	0.156	-0.049	
	Abundance	0.012	0.148	878	0.082	0.935	0.004	
	Host Species	NA	NA	3	65.958	**<0.001**	NA	
	Host Species = *Apis*	-0.922	0.147	Inf	-6.263	**<0.001**	NA	
	Host Species = *Bombus*	-0.408	0.132	Inf	-3.080	**0.002**	NA	
	Host Species = *Lasioglossum*	-5.446	1.002	Inf	-5.433	**<0.001**	NA	
	Host Species = *Eucera*	-3.796	0.508	Inf	-7.476	**<0.001**	NA	
DWV	BQCV	0.203	NA	888	6.155	**<0.001**	0.203	
BQCV	SBV	0.161	NA	888	4.845	**<0.001**	0.161	
SBV	DWV	0.173	NA	888	5.238	**<0.001**	0.173	
Abundance	Richness	0.175	NA	888	5.276	**<0.001**	0.175	

Table S3.5: Pairwise Tukey adjusted post-hoc tests of DWV, BQCV, and SBV prevalence within each hosts species: *Apis mellifera*, *Bombus impatiens*, *Lasioglossum* spp., and *Eucera pruinosa*.

Model Response Variable	Contrast	Estimate	SE	Z Ratio	P-value
DWV	*Apis - Bombus*	0.835	0.195	4.273	**<0.001**
	Apis - Lasioglossum	2.087	0.241	8.677	**<0.001**
	Apis - Eucera	1.846	0.248	7.450	**<0.001**
	Bombus - Lasioglossum	1.253	0.241	5.201	**<0.001**
	Bombus - Eucera	1.011	0.247	4.084	**<0.001**
	Lasioglossum - Eucera	-0.242	0.282	-0.856	0.827
BQCV	*Apis - Bombus*	1.913	0.215	8.880	**<0.001**
	Apis - Lasioglossum	4.211	0.334	12.605	**<0.001**
	Apis - Eucera	3.942	0.339	11.636	**<0.001**
	Bombus - Lasioglossum	2.298	0.319	7.213	**<0.001**
	Bombus - Eucera	2.029	0.323	6.274	**<0.001**
	Lasioglossum - Eucera	-0.269	0.409	-0.657	0.913
SBV	*Apis - Bombus*	-0.516	0.197	-2.620	**0.044**
	Apis - Lasioglossum	4.519	1.013	4.462	**<0.001**
	Apis - Eucera	2.859	0.526	5.432	**<0.001**
	Bombus - Lasioglossum	5.034	1.011	4.981	**<0.001**
	Bombus - Eucera	3.375	0.524	6.441	**<0.001**
	Lasioglossum - Eucera	-1.659	1.123	-1.478	0.451

Table S3.6: Pearson's correlation between each continuous variable included in the SEM below the diagonal, with the corresponding p-values above the diagonal.

	Species Richness	Abundance	Landscape Richness	Natural Area	Floral Richness	Floral Density
Species Richness	--	<0.001	<0.001	<0.001	<0.001	0.123
Abundance	0.533	--	<0.001	<0.001	0.33	<0.001
Landscape Richness	0.496	0.203	--	<0.001	<0.001	<0.001
Natural Area	0.657	0.671	0.205	--	<0.001	<0.001
Floral Richness	0.281	0.033	0.333	0.266	--	<0.001
Floral Density	0.123	0.328	0.454	0.249	0.52	--

Table S3.7: Moran's I spatial autocorrelation model output for each of the component models in the SEM.

Model Response Variable	Observed	Expected	SD	P-value
Species Richness	-0.121	-0.083	0.061	0.54
Abundance	-0.045	-0.083	0.062	0.53
DWV	-0.077	-0.083	0.061	0.92
BQCV	-0.107	-0.083	0.059	0.69
SBV	-0.037	-0.083	0.057	0.41

Chapter 4 : Virus Prevalence in Bee Hosts and on Flowers is Linked with Bee Visitation to Flowers and the Environment

Abstract

Although we know that species interactions change based on differences in the surrounding community and environment, we have a limited understanding of how changes in interactions among host species—particularly those interactions that may lead to pathogen transmission—may scale up to drive patterns of pathogen prevalence at the community level. Environmental factors that alter the diversity or frequency of interactions among host species that vary in competence for a multi-host pathogen are likely to change patterns of pathogen transmission and pathogen prevalence among communities. In this study, we investigate how environmental characteristics are associated with patterns of individual interactions among bees on flowers, and subsequently test how species richness and abundance of bee visitors are linked with deformed wing virus (DWV), black queen cell virus (BQCV), and sacbrood virus (SBV) prevalence in bee hosts and on flowers. We use video surveillance of bee visitation to flowers at eight field sites with variable environmental characteristics, and test field collected honey bees, bumble bees, and flowers for DWV, BQCV, and SBV. Greater natural area and landscape richness are associated with greater bee visit richness and total bee visits to flowers, but the correlations between habitat and weather environmental characteristics are more variable for species-specific visitation rates to flowers. Additionally, lower virus prevalence in *Apis mellifera* (honey bees) and *Bombus impatiens* (Eastern bumblebee) is associated with higher species richness of bee visitors to flowers, while the number of bee visitors is not strongly linked with

virus prevalence in either host. These findings suggest that more diverse interactions among hosts, even at very small scales (i.e. on flowers), could contribute to patterns of the dilution effect at the community scale. Finally, DWV prevalence on flowers is not strongly correlated with bee visitation patterns, but DWV prevalence on flowers is lower at sites with high *A. mellifera* DWV prevalence. Overall, changes in the environment can be linked with variation in individual interactions among hosts at key sites of virus transmission in different communities, which could alter pathogen prevalence among communities. Investigating the dynamic complexity of individual interactions among hosts is increasingly important for understanding patterns of pathogen transmission among multiple host species in different community and environmental contexts.

Introduction

Incorporating multiple hosts into models of disease ecology is becoming increasingly important, particularly in natural systems, because many pathogens commonly infect multiple host species (Rigaud et al. 2010, Budria and Candolin 2014, Johnson et al. 2015b). Additionally, the diversity and relative abundance of host species can be correlated with patterns of pathogen prevalence among different communities (Randolph and Dobson 2012, Mihaljevic et al. 2014). For example, diverse host communities can reduce pathogen prevalence in a focal host by reducing the rate of encounters or transmission events with the focal host—a hypothesis known as the 'dilution effect' (Schmidt and Ostfeld 2001, LoGiudice et al. 2003, Keesing et al. 2006, Begon 2008, Ostfeld and Keesing 2012). Furthermore, the relative abundance of different host species in communities can dramatically alter predictions of pathogen prevalence through density-dependent processes on pathogen transmission or by correlating with community diversity (Anderson and May 1991, Holt et al. 2003, LoGiudice et al. 2003, Begon 2008,

Mihaljevic et al. 2014). For example, when a highly competent host is common in the community, pathogen prevalence will be maintained at higher levels. In contrast, when a highly competent host is a rare species in the community, pathogen prevalence will remain low (Holt et al. 2003, Keesing et al. 2006, Begon 2008, Randolph and Dobson 2012, Johnson et al. 2013). Understanding the effects of community factors on disease dynamics is critical to predicting patterns of pathogen prevalence among different communities.

The surrounding environment, including habitat and climatic factors, can influence host communities, and multi-host disease dynamics. As previously described in **Chapter 3**, the environment can be directly linked with host health through the habitat–disease relationship, or indirectly affect contacts and pathogen transmission among hosts by altering species richness and community composition (Ostfeld and LoGiudice 2003, Santicchia et al. 2015, Huang et al. 2016). Habitat factors that are connected with the abundance of critical resources in the environment can have important direct effects on host nutrition, susceptibility, and immune function, which can subsequently affect patterns of pathogen prevalence by mediating host health in different environments (**Chapter 3**) (Ezenwa 2004, DeGrandi-Hoffman and Chen 2015, Santicchia et al. 2015). For instance, the Eurasian red squirrel (*Sciurus vulgaris*) had increased gastrointestinal helminth (*Tyrpanoxyuris* (*Rodentoxyuris*) *sciuri*) infections in fragmented habitats, and were less able to reduce their parasite load due to poorer nutritional status (Santicchia et al. 2015). Additionally, recent anthropogenic environmental and climatic changes are important drivers of reduced host biodiversity and density, which can alter the rate of interactions within and among species, and modify patterns of pathogen transmission among hosts (Daszak et al. 2001, Tylianakis et al. 2008, Rader et al. 2014, Faust et al. 2017, Halliday et al. 2017). For example, the addition of backyard bird feeders (i.e. augmented food resources) has

led to greater contact rates within and among wild bird species at feeding stations, and resulted in substantial increases in mycoplasmal conjunctivitis prevalence among birds (Hartup et al. 1998, Adelman et al. 2015). However, little is known about how environmentally driven changes in host communities alter patterns of interactions and pathogen transmission.

Community assembly is determined first by environmental factors, followed by the many biotic interactions among community members (Liebold 1997, Chesson 2000, HilleRisLambers et al. 2012, Kraft et al. 2015). Most of what we know about the effects of species interactions on community structure comes from theory, careful experiments, or detailed studies of a few specific species interactions (Gause 1932, Neill 1974, Liebold 1997, MacArthur 2009, De León et al. 2014). However, quantifying how changes in interactions within and among species are linked with differences in community structure in natural systems remains challenging to study. To date, most studies focus on the effects of broader patterns of community diversity on community-level processes, instead of individual interactions (McCann 2007).

Although we know that species interactions are important, we have a limited understanding of how changes in interactions among host species—particularly those that may lead to pathogen transmission—may scale up to drive changes in pathogen prevalence at the community level. Specifically, the diversity and frequency of interactions among host species that vary in competence for a pathogen are likely alter patterns of pathogen transmission for multi-host pathogen systems, resulting in differences in pathogen prevalence among communities (Keesing et al. 2006, Begon 2008). Transmission is usually more frequent within host species than among species, therefore, we need to examine how species diversity and the abundance of interactions among hosts influences patterns of pathogen prevalence (Woolhouse et al. 2001, Holt et al. 2003, Randolph and Dobson 2012, Rohr et al. 2020). First, a greater

diversity of interspecific interactions could reduce the encounter rate among highly competent hosts and consequently reduce pathogen prevalence in highly competent hosts, a dilution effect mechanism known as 'encounter reduction' that could operate at the individual interaction scale (Keesing et al. 2006, Begon 2008). For example, the encounter rate between Lyme infected ticks and highly competent white-footed mice (*Peromyscus leucopus*) was reduced by the addition of chipmunks, a poor-quality host, to mammal communities (Schmidt and Ostfeld 1999, Ostfeld et al. 2006). Greater diversity of species interactions may cause a highly competent host to interact more frequently with less competent host species rather than with conspecifics, consequently reducing the likelihood of pathogen transmission and overall pathogen prevalence (Keesing et al. 2006). Alternatively, a higher abundance of interactions increases the probability of encountering highly competent hosts that are more likely to be infected, which could lead to increased pathogen transmission and higher pathogen prevalence at the community level. For example, communities with a greater proportion of intraspecific interactions among deer mice (*Peromyscus maniculatus*), the most highly competent host in the community, had higher Sin Nombre Virus prevalence independent of deer mouse density (Clay et al. 2009). Most studies broadly examine patterns of community diversity and pathogen prevalence, rather than tracking how individual interactions among hosts may be specifically contributing to patterns of pathogen prevalence in natural systems.

With that said, variable environmental factors contribute to complex patterns of species interactions and pathogen transmission for multi-host pathogens. Yet we have a limited understanding of how the environment may drive changes in the diversity and frequency of interactions among host species (Tylianakis et al. 2008), and whether those interactions lead to different patterns of pathogen transmission in natural systems (Roche et al. 2012, Dearing et al.

2015). Various aspects of the environment, such as habitat and weather, can impact hosts in a community differently, and are critical in altering host behavior that dictates which species interact regularly (Estrada-Peña et al. 2014). Habitat fragmentation can lead to unusually high concentrations of hosts (Dearing and Dizney 2010), while land-use conversion to cropland can increase the densities of highly competent hosts (Young et al. 2017). Both of these patterns of environmental change can dramatically change interactions among hosts in those communities. Additionally, weather and climate can be important factors that alter interactions among hosts by altering host geographic distributions (Parmesan 2006, Estrada-Peña et al. 2014), host or vector developmental and population growth rates (Delatte et al. 2009, Elderd and Reilly 2014), or host behavior (Elderd and Reilly 2014). Therefore, we need to develop a more thorough understanding of how environmental variables can dynamically alter the type and frequency of host interactions, and how patterns of pathogen transmission are subsequently changed as a result.

Here, we use a pollinator system to test how individual interactions among bees on flowers are associated with different environmental conditions, and how bee visitation patterns are correlated with pathogen prevalence among bee hosts and on shared flowers. We focus on the prevalence of three key viruses, deformed wing virus (DWV), black queen cell virus (BQCV), and sacbrood (SBV), which commonly infect honey bees (*Apis mellifera*), bumblebees (*Bombus* spp.), and to a lesser extent, several native bee species (Potts et al. 2010, Singh et al. 2010, Fürst et al. 2014, Manley et al. 2015, McMahon et al. 2015). Virus spillover among bee species likely occurs through consumption of contaminated pollen during visits to shared flowers (Singh et al. 2010, Mazzei et al. 2014, Alger et al. 2019). High DWV, BQCV, and SBV prevalence in honey bees and bumblebees compared to other native bee species (**Chapter 1**, Singh et al. 2010, Evison

et al. 2012, Manley et al. 2015, Dolezal et al. 2016) suggests that these species are more likely to carry the virus, deposit viral particles on flowers during visits, and increase transmission of these viruses through shared flowers with other bees in the community. Therefore, understanding the visitation patterns of different bee species to shared flowers is a critical component to determining the likelihood of virus deposition on to flowers and relative virus prevalence among host species.

Furthermore, patterns of bee visitation to flowers are never static—they vary substantially with changes in the environment, including seasonal, habitat, and climatic factors. Greater natural area and diversity of flowers tend to increase the abundance and diversity of bee visitors to flowers (Kremen et al. 2007, Ricketts et al. 2008, Rader et al. 2014, Blaauw and Isaacs 2014), while climatic conditions, such as temperature and wind speed, differentially affect some bee species' foraging patterns based on their size, coloration, and behaviors (Brittain et al. 2013, Scaven and Rafferty 2013). Exploring how environmental characteristics are correlated with the frequency and diversity of bee visitation to shared flowers will be important to better understand the potential impacts on pathogen transmission and patterns of pathogen prevalence among different communities.

In this study, we conducted video surveillance of bee visitation to flowers at eight agricultural field sites with variable environmental characteristics, and tested field collected honey bees, bumble bees, and flowers for DWV, BQCV, and SBV to ask 1) Are bee visitation patterns to flowers associated with environmental characteristics, 2) Is virus prevalence in bee hosts associated with bee visitation to flowers, and 3) Is virus prevalence detected on flowers linked with bee visitation patterns or with virus prevalence in bee hosts? First, we expected that environmental features such as more natural area and greater richness in land cover types in the

landscape, higher temperatures, and lower wind speeds would be correlated with increased bee species richness and visitation rates to flowers. Second, we expected that virus prevalence in honey bees and bumblebees would increase with higher bee visitation rates, especially with higher honey bee and bumblebee visitation rates. However, we also predicted that virus prevalence would potentially decrease with greater species richness of flower visitors because the likelihood of encounters between highly infected honey bees and/or bumblebees would be reduced on flowers. Third, we expected that higher honey bee and bumblebee visitation rates and higher virus prevalence in honey bees and bumblebees would increase the likelihood of virus deposition on flowers. We also expected that higher visit richness may reduce virus prevalence on flowers through additional, less competent species either reducing the likelihood of virus deposition on flowers or by consuming and removing virus contaminated pollen without becoming infected. Although we were unable to directly test if virus is removed from the flowers by visiting bees, we will test the association between higher visit richness and virus prevalence on flowers and in bee hosts.

Methods

Pollinator Collection

To assess deformed wing virus (DWV), black queen cell virus (BQCV), and sacbrood virus (SBV) prevalence, we collected pollinators from eight winter squash farms in Michigan, USA (**Appendix S4.1**). Permission was granted by private landowners for bee and flower collection, and videotaping bee visitation to squash flowers. All sites were greater than 10 km apart from each other, therefore it is unlikely that bees could visit two sites because most bee species' home ranges are less than 10 km (Greenleaf et al. 2007). We sampled each field site twice between 26 July – 2 September 2016 during the peak squash flower bloom at each site. All

sampling was conducted on primarily sunny days with low wind speeds. Bees were collected through a combination of hand nets and pan traps along four 50-m transects. Three transects were randomly placed in the fields along crop rows, and the fourth was placed in hedgerows with native and weedy plants adjacent to the fields. We walked each transect once for 30-min and collected all observed pollinators at 0800, 1000, 1100 and 1200. Pan traps were set 5-m apart along each transect in alternating fluorescent blue, yellow, and white; pan traps were filled with soapy water. We set out pan traps prior to dawn and collected all bees in the pan traps every three hours until 1300 when squash flowers closed, and bee activity diminished. All collected bees were placed in individual microcentrifuge tubes, frozen, stored on dry ice in the field, and transferred to a -80^{o}C freezer in the lab. We later identified all bees to species or genus level using the Discover Life key (http://www.discoverlife.org). We initially selected four bee species (*Apis mellifera, Bombus impatiens*, *Lasioglossum* spp. and *Eucera pruinosa*) that were consistently common among all sites to test for DWV, BQCV, and SBV prevalence (virus detection methods below). However, only *A. mellifera* and *B. impatiens* had sufficient virus prevalence to adequately test association with bee visitation patterns to flowers.

Pollinator Visitation to Flowers

To assess bee visitation patterns to squash flowers, we recorded videos of bee visitation to eight randomly selected squash flowers during each of two visits to each site. Selected flowers were covered prior to dawn to prevent any insect visitation to the flower. Flowers were uncovered immediately prior to video recording. Videos were approximately 30-min in length and videos recorded all pollinator visits to the flower during that period (mean: 31.1 min, sd: 3.58 min). Some videos were much shorter than 30-min due to battery failure in the field or because the view of the flower became obstructed during the video (n = 3). We only used

visitation data from portions of the video where the flower was clearly visible. The number of bee visits was used as the response variable with the total video time included as an offset in the model to account for differences in the total observed time. We also calculated visitation rates to normalize the visitation data to the total duration of the videos in minutes where visitation rate was used as a predictor variable in our analysis. All videos were conducted between 730 and 1200 while squash flowers were open and actively pollinated. At the start of each video, we recorded the temperature (degrees F), wind speed (meters/sec), and estimated percent cloud cover (0 to 10 scale, where 0 = completely clear sky, 10 = fully overcast). Immediately after the video recording was completed, we collected whole flowers in 50 mL conical tubes and the flower stamen in separate microcentrifuge tubes. We placed flower samples on dry ice in the field and stored them in a -80^{o}C freezer in the lab. The flowers were later tested for the presence of viruses (detailed methods below). Squash flowers are open for a single day at dawn and senesce shortly after midday, which make them ideal for testing if bee visits add virus to flowers.

We watched all videos and scored the duration, number, and type of pollinator visitors to squash flowers. We identified bees to morphospecies that could be accurately and consistently differentiated in the videos. Where possible, we identified bees to species or genus level, but other bee groups were identified to the tribe-level. All hoverflies were grouped together. Morphospecies groupings were as follows: *Apis mellifera* (honey bees), *Bombus* spp. (all bumblebees), non-green Halictini sweat bees (e.g. *Halictus* or *Lasioglossum* spp.), bright-green Augochlorini sweat bees (e.g. *Augochlora*, *Augochorella*, and *Augochloropsis* spp.), *Eucera* spp. (squash bees), *Vespula* spp. wasps, *Melissodes* spp. (long horned bees), *Triepeolus* spp. (cuckoo bee), and hoverflies. From the videos, we calculated the number of morphospecies that visited each flower as the visit richness per flower, the total visitation rate per minute (number of all bee

visits divided by the total video time in minutes), and morphospecies-specific visitation rates per minute (number of morphospecies visits divided by the total video time in minutes).

Landscape Habitat Quantification

We obtained data about the habitat surrounding each field site from the USDA cropland data layer (https://nassgeodata.gmu.edu/CropScape/), which classifies each 30-m x 30-m grid cell of the USA for the dominant landcover type within that cell. We grouped forest, wetland, and grassland meadow landcover types into one "natural habitat" category because those landcover types provide native flowers for foraging and nesting resources for many native bee species (Williams et al. 2012, Jha et al. 2013, Williams and Kremen 2015, Koh et al. 2016). Then we used Geographic Information System (GIS) to calculate the total proportion of natural habitat area surrounding each site within 500-m and 1000-m radii. We also calculated the 'landscape richness' by counting the total number of all different landcover types provided by the cropland data layer within each radius for each site (including all natural, agricultural and urban landcover types) to account for the variety of habitats available in the surrounding landscape. Many bee species have a flight range of about 1000-m (Greenleaf et al. 2007), therefore we used a 1000-m radius for total bee visitation rate and bee visitation richness models, and visitation rate models for larger, generalist species, such as *Apis mellifera* and *Bombus* spp. We used a smaller radius of 500-m for the other morphospecies-specific models because some native bees either have much smaller flight distances (< 100-m) based on their smaller body size (e.g. Halictini and Augochlorini species) (Greenleaf et al. 2007) or have limited dispersal based on the location of their specialist plant (e.g. *Eucera pruinosa*, squash specialist) (Hurd Jr et al. 1971).

Detection of viruses in bees and on flowers

We tested for the presence of DWV, BQCV, and SBV in up to 20 randomly selected individuals per host species at each site (**Appendix Table S4.2**). We used the two pollinator species that were the most common among all sites: *Apis mellifera* (n = 137) and *Bombus impatiens* (n = 159). RNA was extracted from each bee's abdomen with the TRIzol reagent (Ambion) using the manufacturer's protocol, and used a Qubit 3.0 Fluorometer (Invitrogen) to quantify each sample's RNA concentration. Samples with < 10 ng/ µl RNA concentrations were excluded from the study because they are unlikely to amplify viral RNA. Then we converted the RNA to complementary DNA (cDNA) through positive-strand cDNA synthesis reactions using 2 µl of RNA template in a 20 µl reaction using M-MLV reverse-transcriptase (Promega) and 0.25 µM random hexamers (Invitrogen).

We tested for the presence of DWV, BQCV, and SBV using PCR with virus-specific primers (Benjeddou et al. 2001, Singh et al. 2010). The DWV primer did not differentiate between DWV-A, -B, or -C variants, therefore reported DWV prevalence includes all three variants. As a positive control for RNA extraction and reverse transcription, we also tested each sample for the presence of the 18S rRNA gene (Cardinal et al. 2010). All PCR products were visualized on a 2% agarose gel to determine the presence or absence of the virus. Samples that failed to produce the 18S rRNA gene band were excluded from the study due to poor RNA quality.

Additional details for virus positive-strand detection methods are included in the **Appendix S1**, and all primers are listed in **Appendix Table S2.4**. We also tested for viral infections in each host using negative-strand specific RT-PCR, which is further explained in **Chapter 1** and **Appendix S1**. We sequenced a subset of the PCR products to confirm

identification of viral positive and negative strand RNA (GenBank Accession Numbers: MN902093 – MN902138) and 18S rRNA gene (GenBank Accession Numbers: MN900314 – MN900321) (**Appendix Table S2.5**).

To evaluate virus prevalence on squash flowers, we subsampled parts of the stamen and pollen from flowers that had been video tapped to record bee visitation (n = 129). Previous work shows that pollen collected from foraging bees and flowers near apiaries can contain viruses, and pollen contaminated with viruses can lead to infections in honeybees (*A. mellifera*) (Singh et al. 2010, Levitt et al. 2013, Mazzei et al. 2014, Alger et al. 2019). Therefore, the stamen and pollen are the most likely sites on flowers for viruses to be deposited and picked up by bees visiting flowers. We followed the same protocols for RNA extraction, and RT-PCR to test for presence of DWV, BQCV, and SBV on flowers. Unfortunately, we were only able to test for DWV prevalence on all flowers before the sample RNA became degraded beyond usable quality. Consequently, we were unable to complete tests on all flowers for BQCV and SBV, but did confirm that both BQCV and SBV were present on the stamen and/or pollen of some squash flowers. We only used DWV prevalence in the analyses of how bee visitation and bee virus prevalence is linked with virus prevalence on flowers.

Statistical Analysis

All statistical analyses were performed in the program R (R Core Team 2020). To analyze the association between environmental factors and bee visitation patterns, we used separate generalized linear mixed effects models with a Poisson distribution and a log link function for bee visit richness per flower (number of morphospecies visiting a flower), total number of bee visits, number of *A. mellifera* visits, number of *Bombus* spp. visits, number of Halictini visits, number of *Eucera pruinosa* visits, and number of Augochlorini visits with the

log of total duration of video observation included as an offset in the models (full model output in **Appendix Table S4.3**; glm function in lme4 package) (Bates et al. 2015). The total video duration offset accounts for differences in the video length of observed flowers, and effectively models the log of the bee visitation rate to flowers. However, we still refer to the response variables in the models and figures as visit number to maintain clarity. We also included visit nested in site, and start hour of the video as random effects in each model, except for the visit richness model which was singular for both random effects and run as a generalized linear model instead. Additionally, the total bee, *A. mellifera*, *Bombu*s spp., *and Eucera pruinosa* visit number models were initially overdispersed, so we included Flower ID as a random effect to reduce the overdispersion in those models.

Each model included the proportion of natural area, landscape richness (number of landcover types surrounding a site), temperature, wind speed, and cloud cover as main factors, which were all z transformed. Cloud cover was not significant in any of the models, so we removed that factor, and proceeded with all remaining factors. Since we generally recorded visitation videos on non-cloudy days, our data did not have enough variation in cloud cover to produce an effect on bee visitation patterns. We varied the spatial scale used for the proportion of natural area and landscape richness depending on the response variable tested. We used a smaller 500-m radius for bee species with small home ranges and body sizes (e.g. Halictini and Augochlorini) (Greenleaf et al. 2007) or for specialist species that do not travel far from their host plant (e.g. *Eucera pruinosa*, a squash specialist) (Hurd Jr et al. 1971). We used 1000-m radius for all other species based on their larger body sizes and home ranges (Greenleaf et al. 2007). Main factors had variance inflation factors of < 2, suggesting that collinearity among environmental factors in the model was low (**Appendix Table S4.4**). Additionally, Pearson's

product-moment correlation tests (function cor.test) showed that landscape richness and proportion of natural habitat were not significantly correlated at either spatial scale (500m: cor = -0.28, t = -0.71, df = 6, p = 0.5; 1000m: cor = -0.46, t = -1.28, df = 6, p = 0.25). We removed all visitation data for one flower from the dataset as an outlier due to an exceptionally high bee visitation rate that was an order of magnitude higher than all other visitation rates observed (PR site, visit 1, flower 4). We plotted the marginal effects from each model using the ggpredict function (package "ggeffects") to show how the predictors and response variable are associated in the model (Lüdecke 2018). The response variables were shown on a log scale to produce a linear fitted line, and we added one to the response variable to avoid flowers with zero visits producing infinite numbers. Finally, the residuals for each model was tested for spatial autocorrelation with a Moran's I test to ensure that sites were not spatially clustered in bee visitation patterns (packages "ape" and "DHARMa") (Paradis and Schliep 2018, Hartig 2020). None of the models showed significant spatial autocorrelation, which suggests that the pollinator communities are independent and not similar due to close geographic proximity (**Appendix Table S4.5**).

Additionally, we evaluated the correlations between visit richness and total bee visit number, and with the visit number for each morphospecies per flower using Pearson's product-moment correlation tests (function cor.test). All correlations were significantly positive, except for visit richness and *Eucera* visit number (**Appendix Table S4.6, Figure S4.1**). In particular, the total number of bee visits, *A. mellifera* visits, and *B. impatiens* visits increase as the number of bee species visiting flowers increases. But, all correlations are below the threshold of $r > 0.7$, so they are less likely to cause collinearity problems in our models of virus prevalence (detailed below) (Dormann et al. 2013).

We calculated apparent DWV, BQCV, and SBV prevalence based on the number of virus-positive bees in each host species at each site using the "epi.prev" function in the epiR package (Stevenson et al. 2020). Then to analyze the impact of bee visitation patterns on DWV, BQCV, and SBV prevalence in *Apis mellifera* and *Bombus impatiens*, we used General Linear Mixed effects Models (GLMM) with a binomial distribution and logit link function (full model output in **Appendix Table S4.7**; glmer function in lme4 package) (Bates et al. 2015). All models included virus presence or absence as the response variable, and main factors were average visitation species richness per flower, average *A. mellifera* visitation rate per minute (number of visits divided by total video duration in minutes), and average *Bombus* spp. visitation rate per minute to flowers for each site. All main factors were z standardized to improve model fit and allow for comparison of model estimates. The transect ID indicates the specific transect that a bee was collected from, and was initially included as the random effect in all models. In some models, transect ID was a singular term with a variance of zero. For those models, we removed the transect ID random effect, and ran a generalized linear model (GLM) instead. Due to the correlations between bee visitation factors found above, we tested all factors for collinearity with Variance Inflation Factor (VIF) Tests. We found that all factors had VIF below a threshold of 5 in all the models, indicating that there is some moderate collinearity, but it remains well below an acceptable threshold (**Appendix Table S4.8**) (Dormann et al. 2013). Additionally, we tested for spatial autocorrelation in the residuals of the virus prevalence models, as described above, and found no evidence of geographic clustering in virus prevalence among the sites tested (**Appendix Table S4.5**).

Finally, we calculated the apparent DWV prevalence on squash flowers based on the number of virus-positive flowers at each site using the 'epi.prev' function in the epiR package

(Stevenson et al. 2020). Then we modeled how DWV prevalence on flowers was associated with bee visitation and bee DWV prevalence using a generalized linear model (GLM) with a binomial distribution (glm function in lme4 package) (Bates et al. 2015). We include bee visit richness per flower, *A. mellifera* visitation rate per flower, *Bombus* spp. visitation rate per flower, *A. mellifera* DWV prevalence per site, and *B. impatiens* DWV prevalence per site as main factors in the model. All main factors had a VIF < 3 (Visit Richness: 1.53, *Apis* visitation rate: 1.58, *Bombus* visitation rate: 1.11, *Apis* DWV prevalence: 2.02, *Bombus* DWV prevalence: 2.06), showing low collinearity among factors in the model. Furthermore, there was no significant spatial autocorrelation in the residuals of the flower DWV prevalence model, indicating that virus prevalence detected on flowers was not similar based on sites that are closer together in geographic space (**Appendix Table S4.5**).

Results

1) Are bee visitation patterns to flowers associated with environmental characteristics?

Overall, visitation diversity and rates were quite variable among the eight squash farms (**Appendix Table S4.9**). We observed a total of nine morphospecies visiting squash flowers among all the sites, but the total visit species richness at a site ranged from eight to four morphospecies. The visit species richness per flower (*hereafter*, visit richness) ranged from zero visitors to a maximum of five morphospecies visiting the same flower. In total, our videos recorded 1,169 pollinator visits to squash flowers during 67.13 hours of video (n = 129 videos). *Bombus* spp. were the most common pollinators of the squash flowers (646 visits), followed by *E. pruinosa* (177 visits), Augochlorini (130 visits), Halictini (109 visits), *A. mellifera* (67 visits), hoverflies (11 visits), *Vespula* spp. wasps (11 visits), *Melissodes* spp. (2 visits), and *Triepeolus*

spp. cuckoo bees (1 visit). The average number of visits by each morphospecies to flowers at each site can be found in **Appendix Table S4.10**.

We tested how bee visit richness and visitation rates for all bees and each morphospecies were associated with environmental characteristics, such as proportion of natural area, number of landcover types (*hereafter*, landscape richness), temperature, and wind speed (**Table 4.1,** full model details in **Appendix Table S4.3**). Significantly more bee species visited each flower at sites with a greater proportion of natural area and greater landscape richness at a 1000-m radius (**Figure 4.1**). Additionally, higher temperatures correlated with higher visit species richness, but wind speed did not strongly affect visit richness (**Appendix Figure S4.2**).

In general, total bee and morphospecies-specific (*Apis mellifera*, *Bombus* spp., Halictini, *Eucera pruinosa*, and Augochlorini) number of visits to flowers showed similar directionality of relationships with environmental characteristics, but few relationships were consistently significant among species (**Table 4.1**). The number of total bee (including all species) and Augochlorini visits were higher at sites with more natural area at a 1000-m and 500-m radii, respectively, but none of the four other morphospecies visit numbers were associated with natural area (**Figure 4.2, Appendix Figure S4.3**). Landscape richness was significantly correlated with the number of bee visits in two of the six models (**Figure 4.3, Appendix Figure S4.4**). The number of total bee and *Apis mellifera* visits were higher at sites with greater landscape richness within 1000-m radius (**Figure 4.3**).

Higher temperatures during recording periods were significantly associated with higher visit numbers in two of the six models. Higher temperatures were associated with higher numbers of Halictini and Augochlorini visits, but did not affect the number of total bee, *A. mellifera*, *Bombus* spp., or *Eucera pruinosa* visits (**Appendix Figure S4.5**). On the other hand,

higher wind speeds were generally associated with reduced numbers of visits to flowers in four of the six models (**Table 4.1**). The number of total bee, *A. mellifera*, Halictini, and *Eucera pruinosa* visits to flowers were significantly lower with higher wind speeds, but the number of *Bombus* spp. and Augochlorini visits were unaffected by wind speed (**Appendix Figure S4.6**). Overall *Bombus* spp. visit number to flowers was not significantly associated with any of the habitat or weather environmental characteristics tested in this study, though *Bombus* spp. did have a slight trend of higher visit number in areas with greater natural area at 1000m radii ($p = 0.06$) (**Table 4.1, Figure 4.2C**).

2) Is virus prevalence in bee hosts associated with bee visitation to flowers?

Deformed wing virus (DWV), black queen cell virus (BQCV), and sacbrood virus (SBV) prevalence in *Apis mellifera* (honey bees) and, to a lesser degree, *Bombus impatiens* (Eastern bumblebee), were strongly associated with the number of bee species that visited flowers (*hereafter*, visit richness). High visit species richness was associated with reduced virus prevalence in three of the six models for each host–virus pair (**Table 4.2, Appendix Table S4.7**). Specifically, DWV prevalence in both host species, *A. mellifera* and *B. impatiens*, were significantly lower at sites with high visit richness (**Figure 4.4**). Additionally, lower *A. mellifera* SBV prevalence was associated with greater visit richness per flower. BQCV prevalence was not strongly associated with visit richness per flower in either host species.

Virus prevalence was less strongly associated with *A. mellifera* or *Bombus* spp. flower visitation rate compared to visit richness overall. Only two of the six models for each host–virus pair found that virus prevalence increased with higher *A. mellifera* visitation rates (**Table 4.2, Appendix Table S4.7**). Higher *A. mellifera* BQCV and *B. impatiens* DWV prevalence were associated with higher *A. mellifera* visitation rates (**Figure 4.5**). Similarly, virus prevalence in

both host species was not significantly correlated with *Bombus* spp. visitation rates. Only *A. mellifera* DWV prevalence was significantly higher at sites with high *Bombus* spp. visitation rates to flowers (**Figure 4.6**). Overall, higher morphospecies-specific visitation rates tended to be associated with higher virus prevalence, but were rarely significant for most host–virus pairs tested. Additionally, *B. impatiens* BQCV and SBV prevalence were not strongly impacted by differences in bee species visitation richness, nor *A. mellifera* or *Bombus* spp. visitation rates.

3) Is virus prevalence detected on flowers linked with bee visitation patterns or with virus prevalence in bee hosts?

Deformed wing virus (DWV) was readily detected on the pollen and stamens of squash flowers at all eight sites. The relative DWV prevalence at each site was quite variable and ranged from 6.25% to 43.75% (**Table 4.3**). DWV prevalence on flowers was primarily explained by DWV prevalence in bee hosts at a site, rather than by bee visitation patterns to flowers (**Table 4.4**). Intriguingly, higher DWV prevalence on squash flowers was negatively correlated with *A. mellifera* DWV prevalence, and showed a non-significant trend with higher *B. impatiens* DWV prevalence (**Figure 4.7**). DWV prevalence on flowers was not associated with bee visit richness, *A. mellifera* or *Bombus* spp. visitation rates to flowers.

Discussion

In this study, we investigate how environmental characteristics are associated with patterns of individual interactions among bees on flowers, and test how richness and abundance of bee visitors are linked with patterns of deformed wing virus (DWV), black queen cell virus (BQCV), and sacbrood virus (SBV) prevalence in bee hosts and on flowers. We show that environmental characteristics, including both habitat and weather variables, are correlated with bee visit richness and the number of morphospecies-specific visits to flowers, but relationships

with environmental characteristics vary among different pollinator species. Our data also suggest that differences in individual interactions on flowers among communities may contribute to altered virus transmission dynamics. Lower *Apis mellifera* (honey bees) and *Bombus impatiens* (Eastern bumblebee) virus prevalence is associated with higher species richness of bee visitors to flowers, while bee visitation rate is not strongly linked with virus prevalence in either host. These findings suggest that more diverse interactions among hosts, even at very small scales (i.e. on flowers), could contribute to patterns of the dilution effect at the larger community scale. Finally, DWV prevalence on flowers is not strongly correlated with bee visitation patterns, but DWV prevalence on flowers is lower at sites with high *A. mellifera* DWV prevalence. Overall, changes in the environment are linked with variation in individual interactions among hosts in different communities, and correlate with altered patterns of disease dynamics. Investigating the dynamic complexity of individual interactions and pathogen transmission will become increasingly important to improve our predictions of pathogen transmission among multiple host species.

Bee visitation patterns to flowers are species specific and vary with environmental characteristics

Patterns of bee visitation to flowers are significantly linked with differences in environmental characteristics among sites, but some bee species were more strongly associated with environmental factors than others. In general, features of the environment that correspond with greater habitat diversity and abundance in the surrounding landscape are linked with greater species richness and frequency of bee visitors to flowers, which corroborates much of the existing pollination biology literature (Kremen et al. 2002, Ricketts et al. 2008, Garibaldi et al. 2011, Klein et al. 2012, Kennedy et al. 2013). Specifically, we show that higher visit species richness per flower (visit richness) and total number of bee visits are associated with a greater

proportion of natural habitat and a greater number of landcover types (landscape richness) within 1000-m radii. More natural area and greater landscape richness at the landscape level provides abundant and diverse floral and nesting resources for bees, which supports higher densities and diverse assemblages of bees (Potts et al. 2003, Ebeling et al. 2008, Jha and Kremen 2013, Kennedy et al. 2013, Koh et al. 2016, Evans et al. 2018).

Additionally, climatic environmental characteristics, such as temperature, had variable associations with bee visitation patterns to flowers that tended to be species-specific. Higher temperatures correlated with increased the number of Halictini and Auguchlorini visits, and led to higher overall visit richness to flowers. Different bee species' physiological limits for flight are often linked with temperature (Harrison and Fewell 2002, Hamblin et al. 2018), and smaller bees (e.g. Halictini and Augochlorini) need to wait for warmer temperatures to begin foraging, while larger bee species (e.g. *Bombus* and *Eurcera* spp.) may be largely unaffected by temperature. High wind speed had the most consistent effect on reducing bee visitation to flowers among most bee species tested. High wind speeds tend to reduce overall bee visitation rates because flying and landing on flowers becomes more difficult, but some bee species (e.g. *Bombus* spp.) are less affected by wind than others due to larger body size. In particular, *A. mellifera* are sensitive to high wind speeds and stop visiting almond tree flowers, while some other native bees can maintain similar visitation rates regardless of wind speed (Brittain et al. 2013). Overall, it is unsurprising that bee species visitation patterns to flowers vary with a variety of environmental factors because bees are highly ecologically variable in terms of floral specialization and preferences, sociality, body size, and coloration, which will affect their ability to respond to different environmental conditions (Scaven and Rafferty 2013, Hamblin et al. 2018).

Importantly, these environmental characteristics clearly have significant impacts on the frequency and types of bees that are interacting with each other on shared flowers. Since several bee viruses are associated with pollen and transmitted through consuming infected pollen (Singh et al. 2010, Mazzei et al. 2014, Alger et al. 2019), these changes in individual interactions between bees on flowers could lead to critical differences in viral encounter or transmission rates. Furthermore, these differences in individual interactions could culminate to affect patterns of viral prevalence among bee host species and among different communities. Therefore, how the environment may alter the frequency and diversity of host species interactions is a critical gap in our understanding of multi-host–pathogen dynamics in many natural systems.

Visit richness is associated with diluted virus prevalence in multiple hosts, while species specific visitation rates are not strongly linked with virus prevalence

To understand if bee visitation to flowers is linked with virus prevalence, we tested two key bee host species, *A. mellifera* and *B. impatiens*, for DWV, BQCV, and SBV prevalence and compared the diversity and frequency of bee visits to flowers. Overall, bee visitation patterns were important factors linked with virus prevalence in *A. mellifera* and *B. impatiens*, but the number of bee species visiting flowers and the visitation rate for *A. mellifera* and *Bombus* spp. had opposing correlations with virus prevalence. First, higher visit richness is associated with lower virus prevalence in multiple viruses and hosts. DWV prevalence in *A. mellifera* and *B. impatiens* and SBV prevalence in *A. mellifera* were lower at sites with higher visit richness to flowers. These results show initial evidence that greater species richness of hosts visiting flowers could dilute virus prevalence in key host species at the community level. Furthermore, these findings corroborate and strengthen our previous evidence of a dilution effect in *A. mellifera* DWV and SBV prevalence (**Chapter 2**). Though *B. impatiens* DWV prevalence did not

previously show evidence of a dilution effect, our findings herein could suggest that for some host species dilution effects may only be observable at very small scales. We initially hypothesized that greater species richness of visitors to flowers could reduce the *A. mellifera* and *B. impatiens* encounter rates with viral particles on flowers by reducing the visitation rate of high-competent and highly infected hosts (*A. mellifera* and/or *B. impatiens*). However, our data did not support this hypothesis because we found that the number of visits by *A. mellifera* and *B. impatiens* increased with greater visit richness to flowers (**Appendix Table S4.6, Figure S4.1**). Therefore, it is unlikely that virus prevalence is diluted through direct encounters with highly competent hosts on flowers. Alternatively, our other hypothesis was that greater visit richness could reduce bee virus prevalence. This pattern could occur if additional low or non-competent host species could remove or consume viral particles associated with pollen during visits to flowers, consequently reducing the encounter rate for *A. mellifera* and *B. impatiens* with viral particles on pollen during visits to flowers (rather than with other infected bees themselves). We tested the association between visit richness and virus prevalence detected on flowers to begin to investigate this possible avenue for dilution of virus prevalence in the third question in this study (discussed in more detail below). Finally, the additional host species visiting flowers could alter *A. mellifera* and *B. impatiens* visitation behavior in more complex ways that changes the likelihood of susceptible and infected hosts sharing flowers (e.g. competition among host species could reduce the likelihood of sharing flowers). There are few empirical tests of encounter reduction in natural host–pathogen systems, and future work tracing contacts between bee hosts on shared flowers through network analyses may open the door to a more mechanistic understanding of the dynamics at play in this system.

Second, species-specific visitation rates are not an important factor associated with virus prevalence among the two host species and three viruses tested, but higher visitation rates are linked with higher virus prevalence in some host–virus pairs. *A. mellifera* DWV prevalence was higher with higher *Bombus* spp. visitation rates, and *B. impatiens* DWV prevalence was higher with higher *A. mellifera* visitation rates. These results may suggest that pathogen spillover between host species is occurring regularly for DWV, which is consistent with evidence from virus phylogenies which have shown that the same virus strains are shared among bee species, further suggesting that spillover is frequent (Singh et al. 2010, Genersch et al. 2011, Yang et al. 2013, Levitt et al. 2013, Fürst et al. 2014, McMahon et al. 2015, Radzevičiūtė et al. 2017, Bailes et al. 2018). However, the rate of interspecific virus transmission occurring on flowers is likely much lower than the rate of intraspecific virus transmission within colonies, particularly for social bee species (e.g. *A. mellifera* and *B. impatiens*). This is because the number of contacts between bees that occur within densely populated colonies is likely much greater than the number of interactions among bees on shared flowers. Future work is needed to determine the relative rates of intra- and interspecific virus transmission in this system, and how that may impact patterns of pathogen prevalence among different hosts in the community.

Although we found that higher visit richness is linked with lower pathogen prevalence and higher *A. mellifera* and *Bombus* spp. visitation rates can increase pathogen prevalence, these two processes are likely occurring simultaneously despite their opposing effects on virus prevalence at the community level. The resulting patterns of virus prevalence among host species could be dependent on the specific individual interactions occurring at a given site, and whether those interactions tend to more strongly dilute or amplify virus transmission. For example, a site with high visit richness and low to moderate *Bombus* spp. visitation rate may have relatively low

virus prevalence as a result. Meanwhile, a site with high visit richness and high *Bombus* spp. visitation rate may have moderate to high virus prevalence due to the greater exposure by the highly competent *Bombus* spp. hosts. Dilution on the flowers could occur in some scenarios, but may be context dependent on the visitation rate by highly competent and highly infected hosts sharing flowers with others, and consequently altering the force of infection and rate of encounter with infected bees. Unfortunately, the results of this work are unable to tease apart these specific mechanisms, but future studies that track individual host interactions on shared flowers though detailed network analyses may be able to untangle the specific contexts that we observe dilution on flowers versus increased viral transmission on flowers.

Virus prevalence on flowers is linked with virus prevalence in honey bee hosts, but not with bee visitation patterns

Contrary to our expectations, DWV prevalence on flowers decreased with higher *A. mellifera* DWV prevalence, and was not strongly associated with bee visit richness and *A. mellifera* and *Bombus* spp. visitation rates to flowers. These findings are surprising because we expected higher DWV prevalence in *A. mellifera* and *B. impatiens* would increase the likelihood of DWV deposition on flowers during *A. mellifera* and *B. impatiens* visits. However, several sites with low DWV prevalence on flowers and high *A. mellifera* DWV prevalence at the site also have low *A. mellifera* visitation to flowers, which could partially explain why DWV on flowers is lower at sites with high *A. mellifera* DWV prevalence. Overall, these results show that rates of deposition of virus on flowers may be more complex than we expected, and we need to investigate the complicated interactions between bee visitation rates and virus prevalence in bees and on flowers or other factors that we did not account for in our study that are impacting DWV prevalence on flowers.

Another interesting aspect of these findings include that bee visit richness was not an important predictor of DWV prevalence on flowers. We expected that greater species richness of bee visitors to flowers may be associated with reduced virus prevalence on flowers, especially if additional bee species visitors are more likely to remove virus contaminated pollen from the flowers. However, our data do not support the association between bee visit richness and DWV prevalence on flowers, suggesting that greater visit richness may not change the rate of bees encountering viral particles on flowers. More specific experimental tests of the capacity for different bee species to remove virus contaminated pollen from flower are needed to test this idea thoroughly. Furthermore, though we observe an association between greater bee visit richness and lower DWV prevalence in *A. mellifera* and *B. impatiens* that is in line with the dilution effect pattern, the mechanism that underlies this pattern remains unclear. Our data suggest that encounter reduction among highly competent hosts nor with viral particles on flowers are not the main mechanisms for the observed dilution effect in this pollinator pathogen system. Future work should investigate other potential dilution mechanisms, such as transmission reduction or susceptible host regulation, as potential alternative explanations to understand how host interactions on flowers may be correlated with pathogen prevalence.

Previous work has shown that other bee parasites can be transmitted through direct and indirect interactions on flowers (Durrer and Schmid-Hempel 1994, McArt et al. 2014, Figueroa et al. 2019). Viruses are associated with pollen on bee and flowers, and can infect bees that consume virus contaminated pollen (Singh et al. 2010, Mazzei et al. 2014, Alger et al. 2019), which is suggestive evidence that viruses can also be transmitted on flowers. One experiment with Israeli Acute Paralysis virus (IAPV), a related virus to BQCV, showed likely transmission of virus through only shared flowers (Singh et al. 2010). However, in general, there is very

limited research on bee virus transmission via flowers, and many potential factors might affect the likelihood of effective transmission (e.g. viability on flower due to UV radiation or microbes present, time between visits, flower shape and type) (McArt et al. 2014). In our study, we were limited to examining bee visitation patterns to a single type of flower (winter squash, *Cucurbita pepo*), and it is possible that virus transmission could be greater on other native flowers visited by a wider variety of bee species or flowers with different shapes or pollen structures. Furthermore, we still lack critical knowledge about how frequently viruses are deposited on flowers, how long they remain viable or infectious on flowers, how much virus accumulated on flowers is an infectious dose, and what is the likelihood of infection from visiting a virus contaminated flower (McArt et al. 2014, Alger et al. 2019). Virus transmission via flowers is a critical avenue for cross-species transmission in pollinators, and we need to understand the details of how this transmission pathway to better predict patterns of pathogen prevalence and spillover to other bee species. Future work needs to take a more mechanistic approach using networks of bee interactions on shared flowers to understand how viruses may be deposited on flowers and contribute to transmission among species.

The potential role of individual interactions in understanding biodiversity–disease relationships in other host–pathogen systems

The role of individual interactions in contributing to infectious disease transmission has been well studied in social networks and contact tracing for single species hosts (Hamede et al. 2009, Salathé et al. 2010, Adelman et al. 2015), but little work has investigated how interactions within and among species explain patterns of multi-host pathogen transmission. Furthermore, the host communities and the interactions among community members are usually dynamic and change over time and space, and in response to changing environmental conditions, which will

subsequently cause dynamic changes to pathogen transmission among host species (Estrada-Peña et al. 2014, Johnson et al. 2015a, Cohen et al. 2016). Understanding the factors that contribute to variable patterns of interactions in different community and environmental contexts will allow us to improve predictions of pathogen prevalence in multiple host species in different settings. Particularly for host–pathogen systems that have biodiversity–disease relationships (e.g. the dilution effect), studying these patterns at the individual interaction scale will help to elucidate the specific mechanisms and conditions that lead to pathogen dilution in some communities but not others.

Conclusions

In conclusion, our study shows that environmental characteristics are linked with differences in bee visit richness and number of bee visits to flowers for many species, and in turn, different visitation patterns are associated with changes in pathogen prevalence in multiple bee species. However, there is variation among different bee species in the strength of their correlations with different environmental characteristics. Therefore, environmental characteristics could have complex, but important effects on the rate of interactions within and among host species. Furthermore, we found that DWV prevalence in *A. mellifera* and *B. impatiens* are significantly lower at sites with a greater number of bee species visiting flowers, which suggests that virus prevalence may be diluted at the interaction level on flowers. Further study is needed to determine the specific dilution effect mechanisms at play, but there is potential for diverse bee interactions on flowers to facilitate encounter reduction with viral particles. On the other hand, virus prevalence is less correlated with the visitation rate of *A. mellifera* and *Bombus* spp. Finally, virus prevalence on flowers was not associated with bee visitation, but there was less virus on flowers at sites with high *A. mellifera* DWV prevalence. This result indicates that virus

deposition on flowers may have a more complex relationship with bee visitation patterns to flowers, and there may be other factors that need to be considered. Overall, the results demonstrate that environmental effects can contribute to differences in community and host–pathogen dynamics at the individual interaction level, which are important for incorporating greater reality into our understanding of multi-host–multi-pathogen systems. Future work is needed to understand mechanistically how bees that share flowers may alter patterns of virus deposition on flowers, and consequently lead to successful transmission to other bee species possibly through contact tracing and social network analyses.

Literature Cited

Adelman, J. S., S. C. Moyers, D. R. Farine, and D. M. Hawley. 2015. Feeder use predicts both acquisition and transmission of a contagious pathogen in a North American songbird. Proceedings of the Royal Society Biological Sciences 282:20151429-.

Alger, S. A., P. A. Burnham, H. F. Boncristiani, and A. K. Brody. 2019. RNA virus spillover from managed honeybees (Apis mellifera) to wild bumblebees (Bombus spp.). PLoS ONE 14:e0217822.

Anderson, R. M., and R. M. May. 1991. Infectious Diseases of Humans. Oxford University Press, Oxford, UK.

Bailes, E. J., K. R. Deutsch, J. Bagi, L. Rondissone, M. J. F. Brown, and O. T. Lewis. 2018. First detection of bee viruses in hoverfly (syrphid) pollinators. Biology Letters 14:20180001.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software 67:1–48.

Begon, M. 2008. Effects of host diversity on disease dynamics. Pages 12–29 *in* R. S. Ostfeld, F. Keesing, and V. T. Eviner, editors. Infectious Disease Ecology: Effects of Ecosystems on Disease and of Disease on Ecosystems. Princeton University Press, Princeton, NJ.

Benjeddou, M., N. Leat, M. Allsopp, and S. Davison. 2001. Detection of Acute Bee Paralysis Virus and Black Queen Cell Virus from Honeybees by Reverse Transcriptase PCR. Applied and Environmental Microbiology 67:2384–2387.

Blaauw, B. R., and R. Isaacs. 2014. Larger patches of diverse floral resources increase insect pollinator density, diversity, and their pollination of native wildflowers. Basic and Applied Ecology 15:701–711.

Brittain, C., C. Kremen, and A. M. Klein. 2013. Biodiversity buffers pollination from changes in

environmental conditions. Global Change Biology 19:540–547.

Budria, A., and U. Candolin. 2014. How does human-induced environmental change influence host-parasite interactions? Parasitology 141:462–74.

Cardinal, S., J. Straka, and B. N. Danforth. 2010. Comprehenisve phylogeny of apid bees reveals the evolutionary origins and antiquity of cleptparasitism. Proceedings of the National Academy of Sciences 107:16207–16211.

Chesson, P. 2000. Mechanisms of maintenance of species diversity. Annual Review of Ecology and Systematics 31:343–366.

Clay, C. A., E. M. Lehmer, S. St. Jeor, and M. D. Dearing. 2009. Testing mechanisms of the dilution effect: Deer mice encounter rates, sin nombre virus prevalence and species diversity. EcoHealth 6:250–259.

Cohen, J. M., D. J. Civitello, A. J. Brace, E. M. Feichtinger, C. N. Ortega, J. C. Richardson, E. L. Sauer, X. Lui, and J. R. Rohr. 2016. Spatial scale modulates the strength of ecological processes driving disease distributions. Proceedings of the National Academy of Sciences 113:3359–3364.

Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2001. Anthropogenic environmental change and the emergence of infectious diseases in wildlife. Acta Tropica 78:103–116.

Dearing, M. D., C. Clay, E. Lehmer, and L. Dizney. 2015. The roles of community diversity and contact rates on pathogen prevalence. Journal of Mammalogy 96:29–36.

Dearing, M. D., and L. Dizney. 2010. Ecology of hantavirus in a changing world. Annals of the New York Academy of Sciences 1195:99–112.

DeGrandi-Hoffman, G., and Y. Chen. 2015. Nutrition, immunity and viral infections in honey bees. Current Opinion in Insect Science 10:170–176.

Delatte, H., G. Gimonneau, A. Triboire, and D. Fontenille. 2009. Influence of Temperature on Immature Development, Survival, Longevity, Fecundity, and Gonotrophic Cycles of Aedes albopictus, Vector of Chikungunya and Dengue in the Indian Ocean. Journal of Medical Entomology 46:33–41.

Dolezal, A. G., S. D. Hendrix, N. A. Scavo, J. Carrillo-Tripp, M. A. Harris, M. J. Wheelock, M. E. O'Neal, and A. L. Toth. 2016. Honey Bee Viruses in Wild Bees: Viral Prevalence, Loads, and Experimental Inoculation. PloS ONE 11:e0166190.

Dormann, C. F., J. Elith, S. Bacher, C. Buchmann, G. Carl, G. Carr, J. R. Garc, B. Gruber, B. Lafourcade, P. J. Leit, M. Tamara, C. Mcclean, P. E. Osborne, B. S. Der, A. K. Skidmore, D. Zurell, and S. Lautenbach. 2013. Collinearity : a review of methods to deal with it and a simulation study evaluating their performance. Ecography 36:27–46.

Durrer, S., and P. Schmid-Hempel. 1994. Shared Use of Flowers Leads to Horizontal Pathogen Transmission. Proceedings of the Royal Society Biological Sciences 258:299–302.

Ebeling, A., A. M. Klein, J. Schumacher, W. W. Weisser, and T. Tscharntke. 2008. How does plant richness affect pollinator richness and temporal stability of flower visits? Oikos 117:1808–1815.

Elderd, B. D., and J. R. Reilly. 2014. Warmer temperatures increase disease transmission and outbreak intensity in a host – pathogen system. Journal of Animal Ecology 83:838–849.

Estrada-Peña, A., R. S. Ostfeld, A. T. Peterson, R. Poulin, and J. de la Fuente. 2014. Effects of environmental change on zoonotic disease risk: An ecological primer. Trends in Parasitology 30:205–214.

Evans, E., M. Smart, D. Cariveau, and M. Spivak. 2018. Wild, native bees and managed honey bees benefit from similar agricultural land uses. Agriculture, Ecosystems and Environment

268:162–170.

Evison, S. E. F., K. E. Roberts, L. Laurenson, S. Pietravalle, J. Hui, J. C. Biesmeijer, J. E. Smith, G. Budge, and W. O. H. Hughes. 2012. Pervasiveness of Parasites in Pollinators. PLoS ONE 7:e30641.

Ezenwa, V. O. 2004. Interactions among host diet, nutritional status and gastrointestinal parasite infection in wild bovids. International Journal for Parasitology 34:535–542.

Faust, C. L., A. P. Dobson, N. Gottdenker, L. S. P. Bloomfield, H. I. Mccallum, T. R. Gillespie, M. Diuk-Wasser, R. K. Plowright, G. TR, and P. RK. 2017. Null expectations for disease dynamics in shrinking habitat: dilution or amplification? Phil Trans R Soc B 372:20160173.

Figueroa, L. L., M. Blinder, C. Grincavitch, A. Jelinek, E. K. Mann, L. A. Merva, L. E. Metz, A. Y. Zhao, R. E. Irwin, S. H. McArt, and L. S. Adler. 2019. Bee pathogen transmission dynamics: Deposition, persistence and acquisition on flowers. Proceedings of the Royal Society B: Biological Sciences 286.

Fürst, M. A., D. P. McMahon, J. L. Osborne, R. J. Paxton, and M. J. F. Brown. 2014. Disease associations between honeybees and bumblebees as a threat to wild pollinators. Nature 506:364–366.

Garibaldi, L. A., I. Steffan-Dewenter, C. Kremen, J. M. Morales, R. Bommarco, S. A. Cunningham, L. G. Carvalheiro, N. P. Chacoff, J. H. Dudenhöffer, S. S. Greenleaf, A. Holzschuh, R. Isaacs, K. Krewenka, Y. Mandelik, M. M. Mayfield, L. A. Morandin, S. G. Potts, T. H. Ricketts, H. Szentgyörgyi, B. F. Viana, C. Westphal, R. Winfree, and A. M. Klein. 2011. Stability of pollination services decreases with isolation from natural areas despite honey bee visits. Ecology Letters 14:1062–1072.

Gause, G. F. 1932. Experimental Studies on the Struggle for Existence: I. Mixed Population of

Two Species of Yeast. Journal of Experimental Biology 9:389–402.

Genersch, E., C. Yue, I. Fries, and J. R. De Miranda. 2011. Detection of Deformed wing virus, a honey bee viral pathogen, in bumble bees (Bombus terrestris and Bombus pascuorum) with wing deformities. Journal of Invertebrate Pathology 91:61–63.

Greenleaf, S. S., N. M. Williams, R. Winfree, and C. Kremen. 2007. Bee foraging ranges and their relationship to body size. Oecologia 153:589–596.

Halliday, F. W., R. W. Heckman, P. A. Wilfahrt, and C. E. Mitchell. 2017. A multivariate test of disease risk reveals conditions leading to disease amplification. Proceedings of the Royal Society B: Biological Sciences 284.

Hamblin, A. L., E. Youngsteadt, and S. D. Frank. 2018. Wild bee abundance declines with urban warming, regardless of floral diversity. Urban Ecosystems 21:419–428.

Hamede, R. K., J. Bashford, H. McCallum, and M. Jones. 2009. Contact networks in a wild Tasmanian devil (Sarcophilus harrisii) population: using social network analysis to reveal seasonal variability in social behaviour and its implications for transmission of devil facial tumour disease. Ecology Letters 12:1147–1157.

Harrison, J. F., and J. H. Fewell. 2002. Environmental and genetic influences on flight metabolic rate in the honey bee, Apis mellifera. Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology 133:323–333.

Hartig, F. 2020. DHARMa: Residual Diagnostics for Hierarchical (Multi-Level/Mixed) Regression Models. https://cran.r-project.org/package=DHARMa.

Hartup, B. K., H. O. Mohammed, G. V Kollias, and A. A. Dhondt. 1998. Risk factors associatied with mycoplasmal conjunctivitis in house finches. Journal of Wildlife Diseases 34:281–288.

HilleRisLambers, J., P. B. Adler, W. S. Harpole, J. M. Levine, and M. M. Mayfield. 2012.

Rethinking Community Assembly through the Lens of Coexistence Theory. Annual Review of Ecology, Evolution, and Systematics 43:227–248.

Holt, R. D., A. P. Dobson, M. Begon, R. G. Bowers, and E. M. Schauber. 2003. Parasite establishment in host communities. Ecology Letters 6:837–842.

Huang, Z. Y. X., F. van Langevelde, A. Estrada-Peña, G. Suzán, and W. F. de Boer. 2016. The diversity–disease relationship: evidence for and criticisms of the dilution effect. Parasitology 143:1075–1086.

Hurd Jr, P. D., E. G. Linsley, and T. W. Whitaker. 1971. Squash and Gourd Bees (Peponapis, Xenoglossa) and the Origin of the Cultivated Cucurbita. Evolution 25:218–234.

Jha, S., and C. Kremen. 2013. Resource diversity and landscape-level homogeneity drive native bee foraging. Proceedings of the National Academy of Sciences 110:555–8.

Jha, S., L. Stefanovich, and C. Kremen. 2013. Bumble bee pollen use and preference across spatial scales in human-altered landscapes. Ecological Entomology 38:570–579.

Johnson, P. T. J., R. S. Ostfeld, and F. Keesing. 2015a. Frontiers in research on biodiversity and disease. Ecology Letters 18:1119–1133.

Johnson, P. T. J., D. L. Preston, J. T. Hoverman, and K. L. D. Richgels. 2013. Biodiversity decreases disease through predictable changes in host community competence. Nature 494:230–3.

Johnson, P. T. J., J. C. de Roode, and A. Fenton. 2015b. Why infectious disease research needs community ecology. Science 349:1259504-1–9.

Keesing, F., R. D. Holt, and R. S. Ostfeld. 2006. Effects of species diversity on disease risk. Ecology Letters 9:485–98.

Kennedy, C. M., E. Lonsdorf, M. C. Neel, N. M. Williams, T. H. Ricketts, R. Winfree, R.

Bommarco, C. Brittain, A. L. Burley, D. Cariveau, L. G. Carvalheiro, N. P. Chacoff, S. A. Cunningham, B. N. Danforth, J. H. Dudenhöffer, E. Elle, H. R. Gaines, L. A. Garibaldi, C. Gratton, A. Holzschuh, R. Isaacs, S. K. Javorek, S. Jha, A. M. Klein, K. Krewenka, Y. Mandelik, M. M. Mayfield, L. Morandin, L. A. Neame, M. Otieno, M. Park, S. G. Potts, M. Rundlöf, A. Saez, I. Steffan-Dewenter, H. Taki, B. F. Viana, C. Westphal, J. K. Wilson, S. S. Greenleaf, and C. Kremen. 2013. A global quantitative synthesis of local and landscape effects on wild bee pollinators in agroecosystems. Ecology Letters 16:584–599.

Klein, A. M., C. Brittain, S. D. Hendrix, R. Thorp, N. Williams, and C. Kremen. 2012. Wild pollination services to California almond rely on semi-natural habitat. Journal of Applied Ecology 49:723–732.

Koh, I., E. V Lonsdorf, N. M. Williams, C. Brittain, R. Isaacs, J. Gibbs, and T. H. Ricketts. 2016. Modeling the status, trends, and impacts of wild bee abundance in the United States. Proceedings of the National Academy of Sciences 113:140–145.

Kraft, N. J. B., P. B. Adler, O. Godoy, E. C. James, S. Fuller, and J. M. Levine. 2015. Community assembly, coexistence and the environmental filtering metaphor. Functional Ecology 29:592–599.

Kremen, C., N. M. Williams, M. A. Aizen, B. Gemmill-Herren, G. LeBuhn, R. Minckley, L. Packer, S. G. Potts, T. Roulston, I. Steffan-Dewenter, D. P. Vázquez, R. Winfree, L. Adams, E. E. Crone, S. S. Greenleaf, T. H. Keitt, A. M. Klein, J. Regetz, and T. H. Ricketts. 2007. Pollination and other ecosystem services produced by mobile organisms: A conceptual framework for the effects of land-use change. Ecology Letters 10:299–314.

Kremen, C., N. M. Williams, and R. W. Thorp. 2002. Crop pollination from native bees at risk from agricultural intensification. Proceedings of the National Academy of Sciences

99:16812–16816.

De León, L. F., J. Podos, T. Gardezi, A. Herrel, and A. P. Hendry. 2014. Darwin's finches and their diet niches: The sympatric coexistence of imperfect generalists. Journal of Evolutionary Biology 27:1093–1104.

Levitt, A. L., R. Singh, D. L. Cox-Foster, E. Rajotte, K. Hoover, N. Ostiguy, and E. C. Holmes. 2013. Cross-species transmission of honey bee viruses in associated arthropods. Virus Research 176:232–240.

Liebold, M. A. 1997. Similarity and local co-existence of species in regional biotas. Evolutionary Ecology 29:95–110.

LoGiudice, K., R. S. Ostfeld, K. A. Schmidt, and F. Keesing. 2003. The ecology of infectious disease: effects of host diversity and community composition on Lyme disease risk. Proceedings of the National Academy of Sciences 100:567–71.

Lüdecke, D. 2018. ggeffects : Tidy Data Frames of Marginal Effects from Regression Models. Journal of Open Source Software 3:772.

MacArthur, R. H. 2009. Population Ecology of Some Warblers of Northeastern Coniferous Forests. Population Ecology 39:599–619.

Manley, R., M. Boots, and L. Wilfert. 2015. Emerging viral disease risk to pollinating insects: ecological, evolutionary and anthropogenic factors. Journal of Applied Ecology 10:1–10.

Mazzei, M., M. L. Carrozza, E. Luisi, M. Forzan, M. Giusti, S. Sagona, F. Tolari, and A. Felicioli. 2014. Infectivity of DWV associated to flower pollen: experimental evidence of a horizontal transmission route. PloS ONE 9:e113448.

McArt, S. H., H. Koch, R. E. Irwin, and L. S. Adler. 2014. Arranging the bouquet of disease: Floral traits and the transmission of plant and animal pathogens. Ecology Letters 17:624–

636.

McCann, K. 2007. Protecting biostructure. Nature 446:29.

McMahon, D. P., M. A. Fürst, J. Caspar, P. Theodorou, M. J. F. Brown, and R. J. Paxton. 2015. A sting in the spit: widespread cross-infection of multiple RNA viruses across wild and managed bees. Journal of Animal Ecology 84:615–624.

Mihaljevic, J. R., M. B. Joseph, S. A. Orlofske, and S. H. Paull. 2014. The scaling of host density with richness affects the direction, shape, and detectability of diversity-disease relationships. PLoS ONE 9:e97812.

Neill, W. E. 1974. The Community Matrix and Interdependence of the Competition Coefficients. The American Naturalist 108:399–408.

Ostfeld, R. S., and F. Keesing. 2012. Effects of Host Diversity on Infectious Disease. Annual Review of Ecology, Evolution, and Systematics 43:157–182.

Ostfeld, R. S., F. Keesing, and K. LoGiudice. 2006. Community ecology meets epidemiology: the case of Lyme disease. Pages 28–40 *in* S. K. Collinge and C. Ray, editors. Disease Ecology: Community Structure and Pathogen Dynamics. 1st edition. Oxford University Press, Oxford.

Ostfeld, R. S., and K. LoGiudice. 2003. Community dissassembly, biodiversity loss, and the erosion of an ecosystem service. Ecology 84:1421–1427.

Paradis, E., and K. Schliep. 2018. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35:526–528.

Parmesan, C. 2006. Ecological and Evolutionary Responses to Recent Climate Change. Annual Review of Ecology, Evolution, and Systematics 37:637–669.

Potts, S. G., J. C. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger, and W. E. Kunin. 2010.

Global pollinator declines: Trends, impacts and drivers. Trends in Ecology and Evolution 25:345–353.

Potts, S. G., B. Vulliamy, A. Dafni, G. Ne, and P. Willmer. 2003. Linking Bees and Flowers: How Do Floral Communities Structure Pollinator Communities? Ecology 84:2628–2642.

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rader, R., I. Bartomeus, J. M. Tylianakis, and E. Laliberté. 2014. The winners and losers of land use intensification: Pollinator community disassembly is non-random and alters functional diversity. Diversity and Distributions 20:908–917.

Radzevičiūtė, R., P. Theodorou, M. Husemann, G. Japoshvili, G. Kirkitadze, A. Zhusupbaeva, and R. J. Paxton. 2017. Replication of honey bee-associated RNA viruses across multiple bee species in apple orchards of Georgia, Germany and Kyrgyzstan. Journal of Invertebrate Pathology 146:14–23.

Randolph, S., and A. Dobson. 2012. Pangloss revisited: a critique of the dilution effect and the biodiversity-buffers-disease paradigm. Parasitology 139:847–63.

Ricketts, T. H., J. Regetz, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, A. Bogdanski, B. Gemmill-Herren, S. S. Greenleaf, A. M. Klein, M. M. Mayfield, L. A. Morandin, A. Ochieng, and B. F. Viana. 2008. Landscape effects on crop pollination services: Are there general patterns? Ecology Letters 11:499–515.

Rigaud, T., M.-J. Perrot-Minnot, and M. J. F. Brown. 2010. Parasite and host assemblages: embracing the reality will improve our knowledge of parasite transmission and virulence. Proceedings of the Royal Society Biological Sciences 277:3693–702.

Roche, B., A. P. Dobson, J. F. Guégan, and P. Rohani. 2012. Linking community and disease

ecology: The impact of biodiversity on pathogen transmission. Philosophical Transactions of the Royal Society B: Biological Sciences 367:2807–2813.

Rohr, J. R., D. J. Civitello, F. W. Halliday, P. J. Hudson, K. D. Lafferty, C. L. Wood, and E. A. Mordecai. 2020. Towards common ground in the biodiversity–disease debate. Nature Ecology and Evolution 4:24–33.

Salathé, M., M. Kazandjieva, J. W. Lee, P. Levis, M. W. Feldman, and J. H. Jones. 2010. A high-resolution human contact network for infectious disease transmission. Proceedings of the National Academy of Sciences of the United States of America 107:22020–22025.

Santicchia, F., C. Romeo, A. Martinoli, P. Lanfranchi, L. A. Wauters, and N. Ferrari. 2015. Effects of habitat quality on parasite abundance: Do forest fragmentation and food availability affect helminth infection in the Eurasian red squirrel? Journal of Zoology 296:38–44.

Scaven, V. L., and N. E. Rafferty. 2013. Physiological effects of climate warming on flowering plants and insect pollinators and potential consequences for their interactions. Current Zoology 59:418–426.

Schmidt, K. A., and R. S. Ostfeld. 1999. Infestation of Peromyscus leucopus and Tamias striatus by Ixodes scapularis (Acari: Ixodidae) in Relation to the Abundance of Hosts and Parasites. Journal of Medical Entomology 36:749–757.

Schmidt, K. A., and R. S. Ostfeld. 2001. Biodiversity and the Dilution Effect in Disease Ecology. Ecology 82:609–619.

Singh, R., A. L. Levitt, E. G. Rajotte, E. C. Holmes, N. Ostiguy, D. Vanengelsdorp, W. I. Lipkin, C. W. Depamphilis, A. L. Toth, and D. L. Cox-Foster. 2010. RNA viruses in hymenopteran pollinators: evidence of inter-taxa virus transmission via pollen and potential impact on

non-Apis hymenopteran species. PLoS ONE 5:e14357.

Stevenson, M., T. Nunes, C. Heuer, J. Marshall, J. Sanchez, R. Thornton, J. Reiczigel, J. Robison-Cox, P. Sebastiani, P. Solymos, K. Yoshida, G. Jones, S. Pirikahu, S. Firestone, R. Kyle, J. Popp, M. Jay, and C. Reynard. 2020. epiR: Tools for the Analysis of Epidemiological Data.

Tylianakis, J. M., R. K. Didham, J. Bascompte, and D. A. Wardle. 2008. Global change and species interactions in terrestrial ecosystems. Ecology Letters 11:1351–1363.

Williams, N. M., and C. Kremen. 2015. Resource Distributions among Habitats Determine Solitary Bee Offspring Production in a Mosaic Landscape. Ecological Applications 17:910–921.

Williams, N. M., J. Regetz, and C. Kremen. 2012. Landscape-scale resources promote colony growth but not reproductive performance of bumble bees. Ecology 93:1049–1058.

Woolhouse, M. E., L. H. Taylor, and D. T. Haydon. 2001. Population biology of multihost pathogens. Science 292:1109–1112.

Yang, B., G. Peng, T. Li, and T. Kadowaki. 2013. Molecular and phylogenetic characterization of honey bee viruses, Nosema microsporidia, protozoan parasites, and parasitic mites in China. Ecology and Evolution 3:298–311.

Young, H., D. McCauley, R. Dirzo, C. Nunn, M. Campana, B. Agwanda, E. Otarola-Castillo, R. Pringle, K. Veblen, D. Salkeld, K. Stewardson, R. Fleischer, E. Lambin, T. Palmer, and K. Helgen. 2017. Interacting effects of land use and climate on rodent-borne pathogens in central Kenya. Philosophical Transactions of the Royal Society B: Biological Sciences 372:20160116.

Tables

Table 4.1: Estimated slope coefficients from the models of bee visitation to flowers with significant main factors bolded (* = P<0.05, ** =P<0.01, *** = P<0.001). Models were generalized linear mixed effects models with a Poisson distribution and log link function and with the total video duration (min) as an offset in the model. All models include start hour of the video and visit number to each site nested within site as random effects, except for the visit richness model which was singular. Flower ID was also included as a random effect to correct for overdispersion in the total bee, *Apis mellifera*, *Bombus* spp., and *Eucera pruinosa* visit number models. Full model results in **Appendix Table S4.5**.

Response variable	Natural Area 1000m	Landscape Richness 1000m	Temperature	Wind Speed
Visit Richness (per flower)	**0.262*** **	**0.175***	**0.251*** **	-0.026
Total Bee Visit Number	**0.554*** **	**0.334***	0.093	**-0.217***
***Apis mellifera* Visit Number**	0.647	**1.137** **	0.307	**-0.965***
***Bombus* spp. Visit Number**	0.483	0.407	-0.040	-0.033

Table 4.2: Estimated slope coefficient from the models of DWV, BQCV, and SBV prevalence in *Apis mellifera* and *Bombus impatiens* with significant main factors bolded (* = P<0.05, ** =P<0.01, *** = P<0.001). Models were binomial GLMM or GLM models. If the random effect, Transect ID, had no variance in the GLMM model, then model was run as a GLM without random effects. Full model results in **Appendix Table S4.7**.

Response variable				**Main Factors**		
Species	Virus	Model	Random effect	Visit Richness per Flower	*Apis mellifera* visitation rate (min^{-1})	*Bombus* spp. visitation rate (min^{-1})
Apis mellifera	DWV	GLM	--	**-0.855****	0.275	**0.709***
Apis mellifera	BQCV	GLM	--	-0.310	**0.610***	-0.100
Apis mellifera	SBV	GLMM	Transect ID	**-1.289****	0.403	0.531
Bombus impatiens	DWV	GLM	--	**-0.659***	**0.527***	0.137
Bombus impatiens	BQCV	GLMM	Transect ID	-0.420	0.244	0.553
Bombus impatiens	SBV	GLM	--	0.084	0.192	-0.152

Table 4.3: Summary of DWV prevalence (%) on squash flowers at each site including the 95% confidence interval (CI), number of DWV-positive flowers, and the total number of flowers tested.

Site	Flower DWV prevalence (%)	95% Lower CI	95% Upper CI	Number DWV-positive	Total Tested
BP	25	9.02	50.01	4	16
E	6.25	0.318	30.04	1	16
GT	29.4	12.37	54.43	5	17
K	12.5	2.26	36.83	2	16
PL	18.75	5.31	43.45	3	16
PR	6.25	0.32	30.04	1	16
SP	18.75	5.31	43.45	3	16
T	43.75	20.11	69.96	7	16

Table 4.4: Full GLM model output of DWV prevalence on squash flowers with a binomial distribution. Significant p-values are bolded. All main factors were z transformed.

Main Factors	Estimate	Std. Error	z value	p-value
Intercept	-1.51517	0.24767	-6.118	**<0.0001**
Visitation Richness	-0.04015	0.27801	-0.144	0.88518
***Apis mellifera* visitation rate (min^{-1})**	0.19796	0.24614	0.804	0.42123
***Bombus* spp. visitation rate (min^{-1})**	0.23879	0.22918	1.042	0.29744
***Apis mellifera* DWV**	-0.84869	0.31345	-2.708	**0.00678**
***Bombus impatiens* DWV**	0.68095	0.36285	1.877	0.06057

Figures

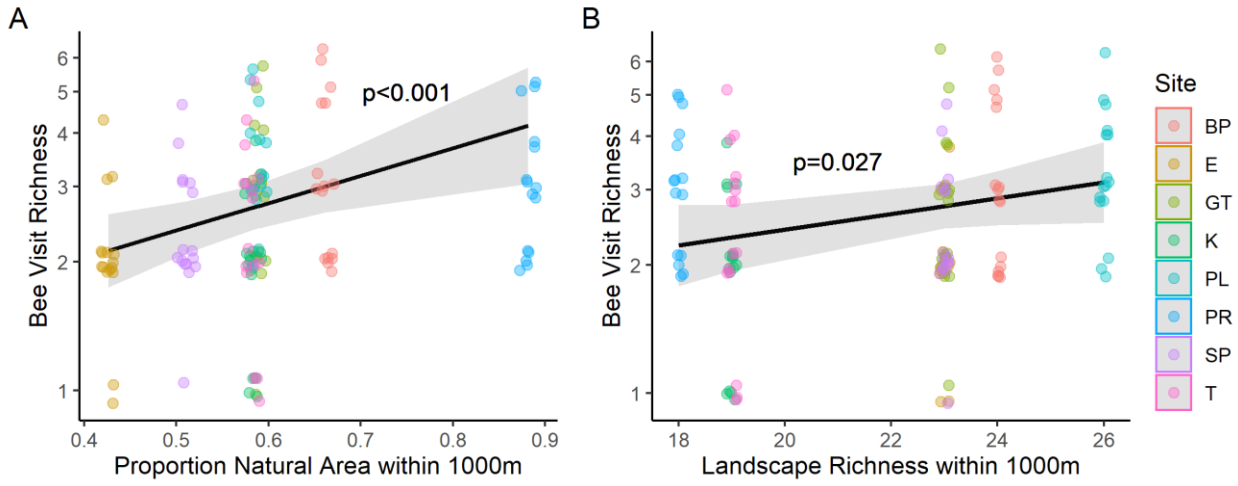


Figure 4.1: Higher bee visit richness is correlated with **A)** greater landscape richness at 1000m and **B)** greater proportion natural area at 1000m. Bee visit richness is plotted on a log + 1 scale. The number of bee species visiting flowers per approximately 30 min are plotted on a log + 1 scale. The fitted line is the predicted number of bee species + 1 from each corresponding model in **Table 4.1 and Appendix Table S4.3**, and accounts for the other fixed and random effects including the total time each flower was observed.

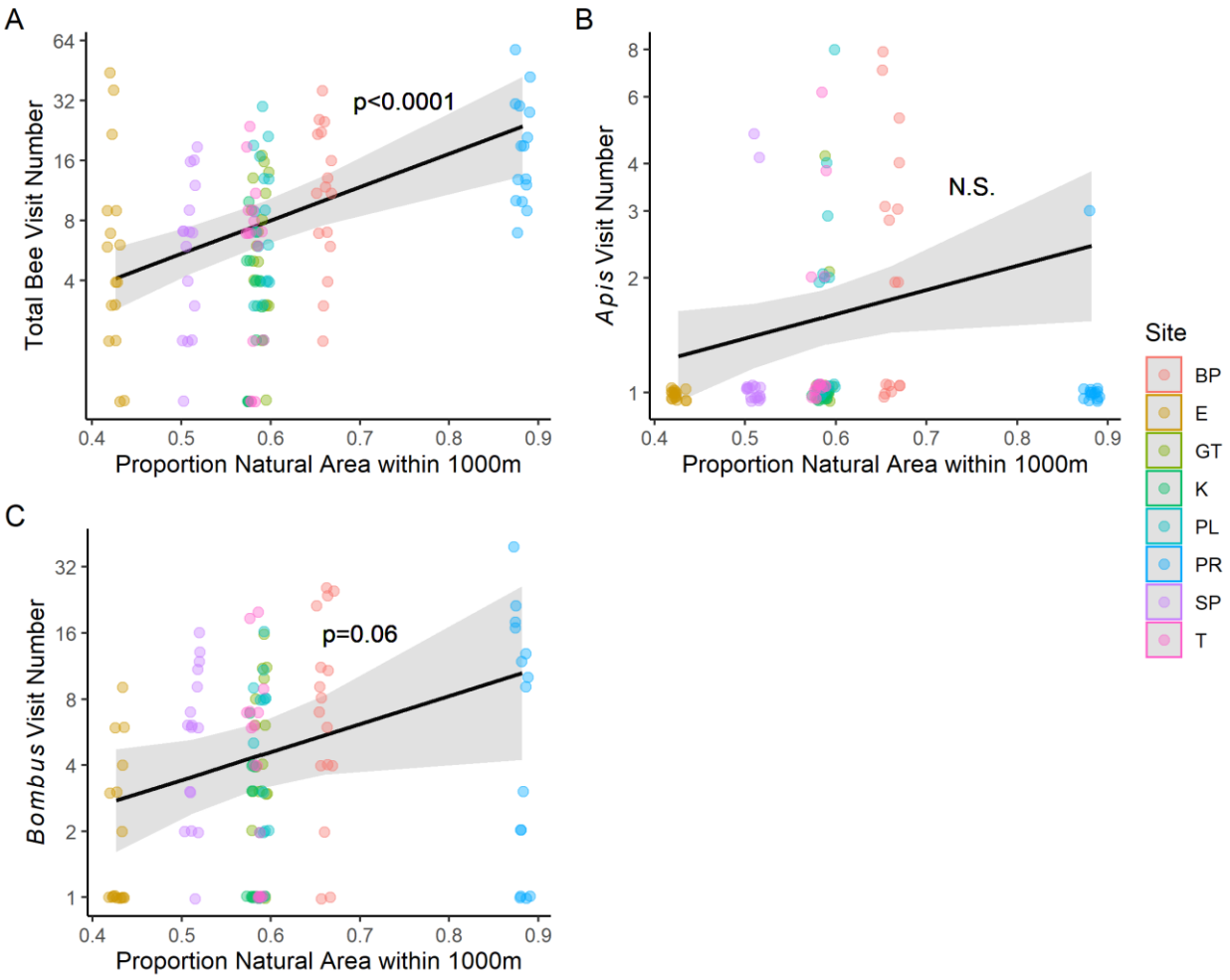


Figure 4.2: The association between the proportion of natural area at 1000-m and **A)** number of total bee, **B)** *Apis mellifera*, and **C)** *Bombus* spp. visits to flowers during approximately 30 min of observation. A greater number of total bee visits was linked with higher proportion of natural area, but not linked with the number *A. mellifera* or *Bombus* spp. visits. The number of bee visits per approximately 30 min are on a log + 1 scale. The fitted line is the predicted number of bee visits + 1 from each corresponding model in **Table 4.1 and Appendix Table S4.3**, and accounts for the other fixed and random effects, including the total time each flower was observed.

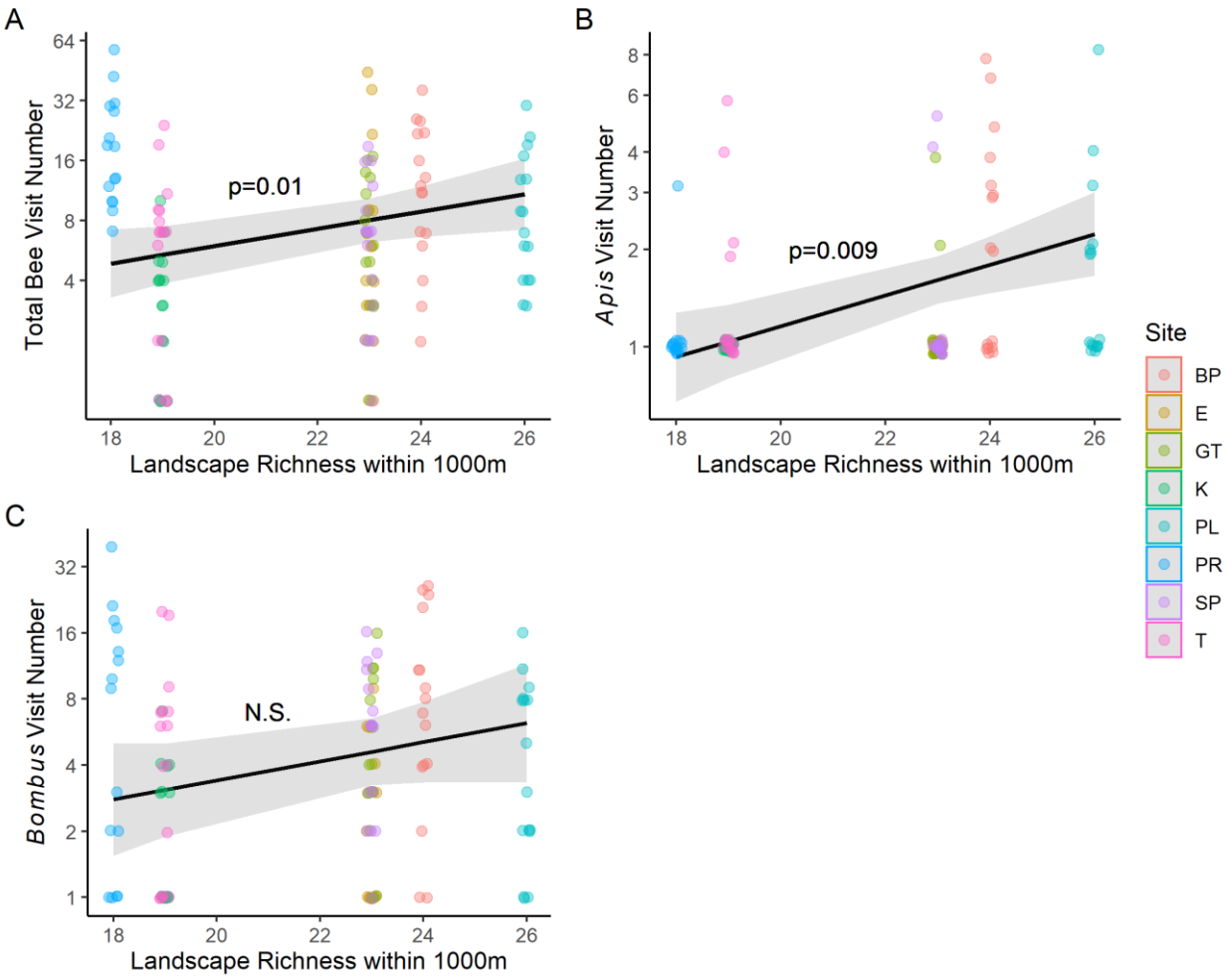


Figure 4.3: The association between landscape richness at 1000-m and **A)** total bee, **B)** *Apis mellifera*, and **C)** *Bombus* spp. visitation rates per minute to flowers. Higher total bee and *A. mellifera* visitation rates were significantly correlated with higher landscape richness. The number of bee visits per approximately 30 min are on a log + 1 scale. The fitted line is the predicted number of bee visits + 1 from each corresponding model in **Table 4.1 and Appendix Table S4.3**, and accounts for the other fixed and random effects, including the total time each flower was observed.

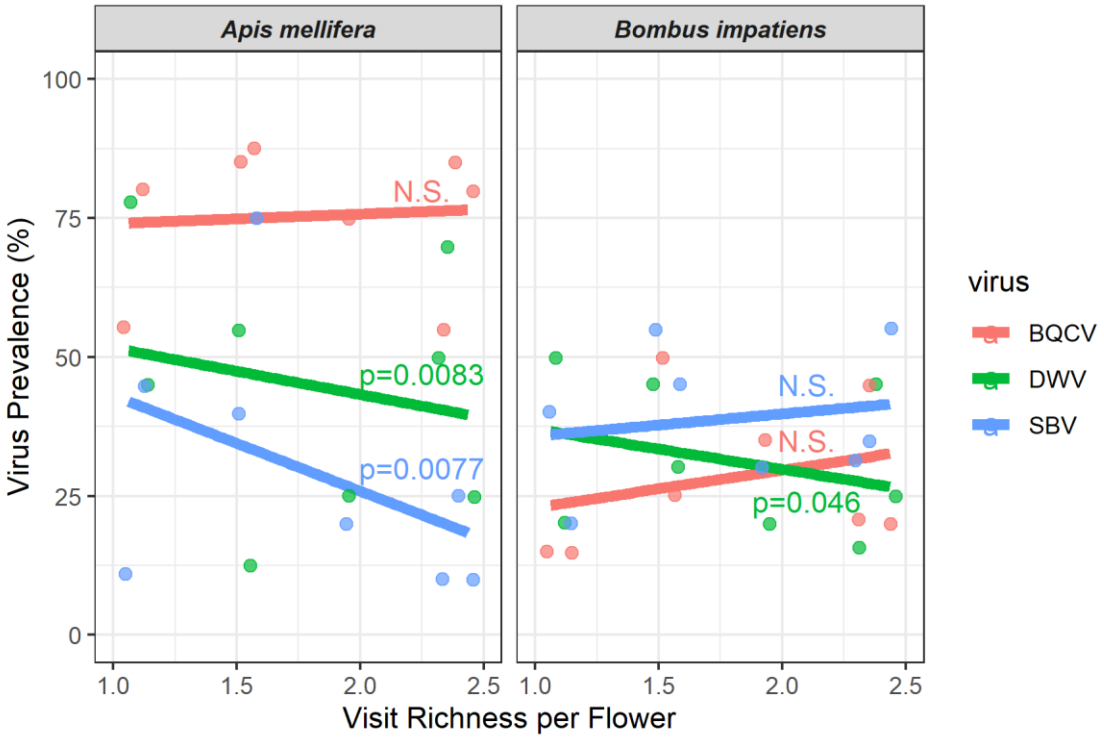


Figure 4.4: *Apis mellifera* DWV and SBV and *Bombus impatiens* DWV prevalence are lower at sites with higher average bee species richness to flowers. All model results are reported in **Table 4.2** and **Appendix Table S4.7**. P-values are reported for significant factors in the models. Green = deformed wing virus (DWV); Red = black queen cell virus (BQCV); Blue = sacbrood virus (SBV).

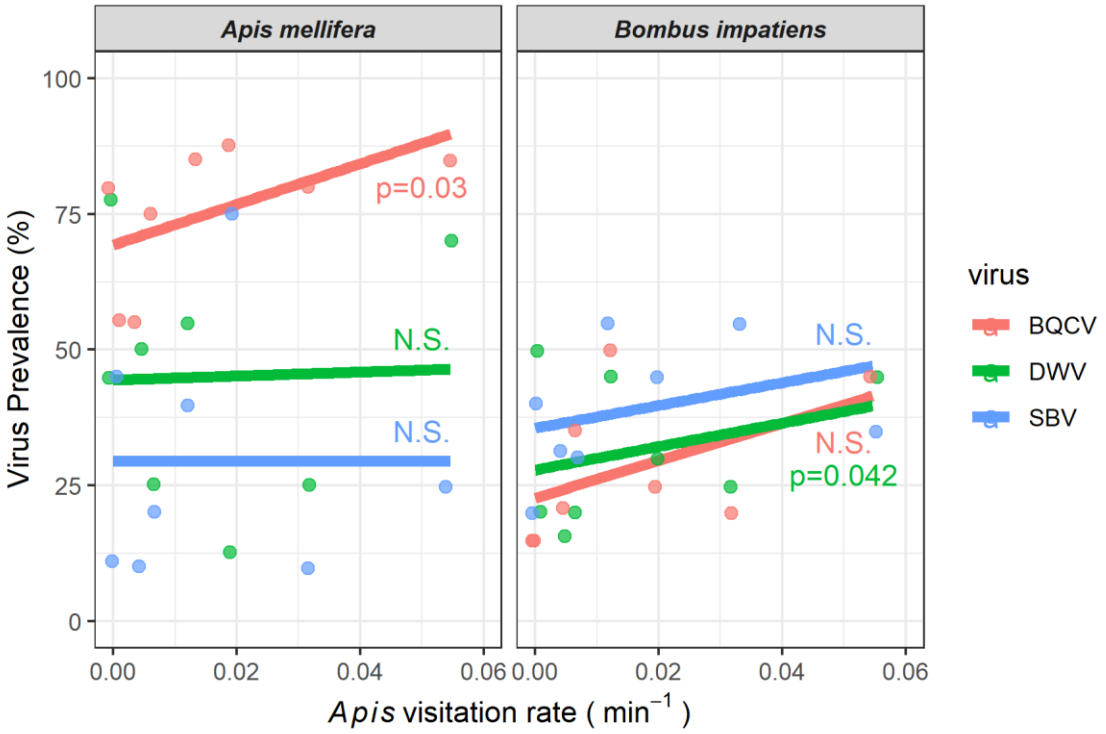


Figure 4.5: *Apis mellifera* BQCV and *Bombus impatiens* DWV prevalence increased with greater average *Apis mellifera* visitation rate to flowers per minute over the course of an approximately 30 min period. All model results are reported in **Table 4.2** and **Appendix Table S4.7**. P-values are reported for significant factors in the models. Green = deformed wing virus (DWV); Red = black queen cell virus (BQCV); Blue = sacbrood virus (SBV).

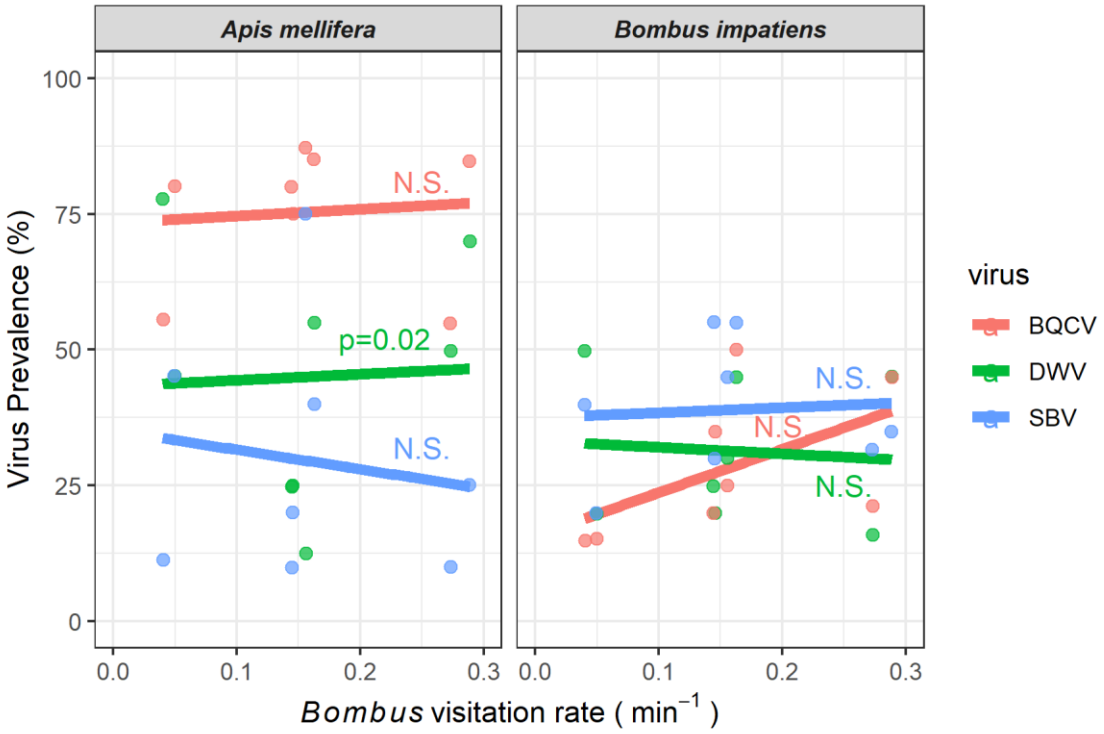


Figure 4.6: Only *Apis mellifera* DWV prevalence was positively associated with greater average *Bombus* spp. visitation rate to flowers per minute during the 30 min observed period. All model results are reported in **Table 4.2** and **Appendix Table S4.7**. P-values are reported for significant factors in the models. Green = deformed wing virus (DWV); Red = black queen cell virus (BQCV); Blue = sacbrood virus (SBV).

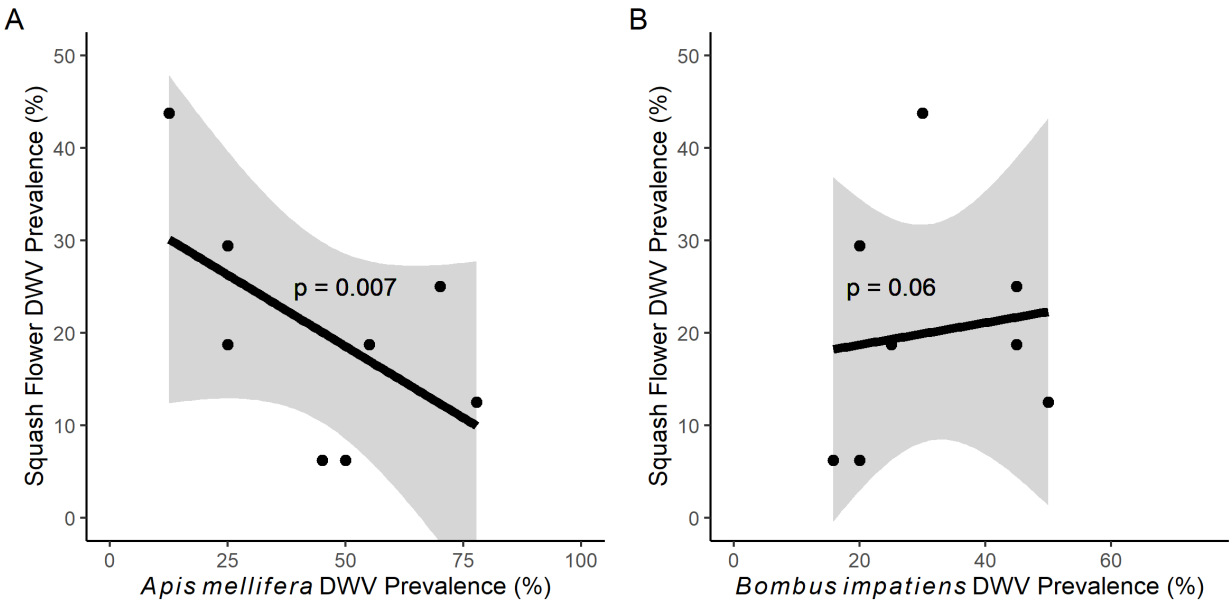


Figure 4.7: DWV prevalence on squash flowers decreases with **A)** *Apis mellifera* DWV prevalence, but not with **B)** *Bombus impatiens* DWV prevalence. Full model results are reported in **Table 4.4**. P-values are reported for significant factors in the model.

Appendix S4

Table S4.1: Field site abbreviation, farm name, sampling year, dates of each visit to field site, and zone, easting and northing coordinates in the UTM GPS system. All field sites are in the Lower Peninsula of Michigan, USA.

Code	Farm Name	Year	First Visit	Second visit	Zone	Easting	Northing
BP	Brimley's Pumpkin Patch	2016	8/10/2016	8/26/2016	16T	714474	4716740
E	Erwin Orchards	2016	7/27/2016	8/22/2016	17T	280997	4708908
GT	Green Things Farm	2016	8/17/2016	8/24/2016	17T	276741	4689607
K	Kapnick Orchards	2016	8/21/2016	8/28/2016	17T	257729	4648607
PL	Plymouth Orchards	2016	8/11/2016	8/30/2016	17T	289557	4690343
PR	Peacock Road Farms	2016	7/26/2016	8/23/2016	16T	714244	4746884
SP	Spicer Orchards	2016	8/14/2016	9/1/2018	17T	274397	4729038
T	Tantré Farm	2016	8/18/2016	9/2/2016	16T	738575	4681735

Table S4.2: The number of individuals tested for the presence of DWV, BQCV, and SBV. Where less than 20 individuals were collected, then all collected individuals were tested.

Year	***2016***								
Species Names	**PR**	**E**	**BP**	**PL**	**SP**	**GT**	**T**	**K**	**Species totals**
Apis mellifera	20	20	20	20	20	20	8	9	**137**
Bombus impatiens	19	20	20	20	20	20	20	20	**159**

Table S4.3: Generalized linear mixed models of bee visitation to flower for all weather and habitat variables, with a Poisson distribution and log link function and the total video duration (min) as an offset in the model. Start hour of the video and visit number to each site nested within site are included as random effects, except for the visit richness model which was singular and ran without random effects. Flower ID was also included as a random effect to correct for overdispersion in the total bee, *Apis mellifera, Bombus* spp., and *Eucera pruinosa* visit number models.

Response Variable	Main effect	Estimate	Std. Error	z value	p value
Visit Richness	Intercept	-2.94	0.07	-41.67	**<0.0001**
	Temperature	0.25	0.07	3.76	**0.000168**
	Wind Speed	-0.03	0.07	-0.40	0.691559
	Natural area 1000m	0.26	0.08	3.46	**0.000541**
	Landscape Richness 1000m	0.18	0.08	2.21	**0.027299**
Total Bee Visit Number	Intercept	-1.69	0.14	-11.98	**<0.0001**
	Temperature	0.09	0.11	0.84	0.3995
	Wind Speed	-0.22	0.10	-2.24	**0.025**
	Natural area 1000m	0.55	0.13	4.34	**<0.0001**
	Landscape Richness 1000m	0.33	0.13	2.50	**0.0124**
***Apis mellifera* Visit Number**	Intercept	-6.35	0.63	-10.05	**<0.0001**
	Temperature	0.31	0.34	0.90	0.36674
	Wind Speed	-0.97	0.45	-2.16	**0.03092**
	Natural area 1000m	0.65	0.42	1.53	0.12571
	Landscape Richness 1000m	1.14	0.43	2.62	**0.00885**
***Bombus* spp. Visit Number**	Intercept	-2.56	0.25	-10.24	**<0.0001**
	Temperature	-0.04	0.21	-0.19	0.8469
	Wind Speed	-0.03	0.12	-0.27	0.7865
	Natural area 1000m	0.48	0.26	1.87	0.0612
	Landscape Richness 1000m	0.41	0.26	1.57	0.1161
Halictini Visit Number	Intercept	-4.96	0.46	-10.88	**<0.0001**
	Temperature	0.59	0.21	2.82	**0.00479**
	Wind Speed	-0.37	0.16	-2.32	**0.02017**
	Natural area 500m	0.66	0.39	1.71	0.08747
	Landscape Richness 500m	0.20	0.37	0.53	0.59621
***Eucera pruinosa* Visit Number**	Intercept	-6.33	0.71	-8.88	**<0.0001**
	Temperature	0.10	0.37	0.26	0.7916
	Wind Speed	-0.72	0.36	-2.02	**0.0435**
	Natural area 500m	0.60	0.59	1.00	0.3163

	Landscape Richness 500m	0.62	0.56	1.12	0.265
Augochlorini Visit Number	Intercept	-6.06	0.60	-10.15	**<0.0001**
	Temperature	0.80	0.31	2.61	**0.00902**
	Wind Speed	-0.06	0.23	-0.26	0.79272
	Natural area 500m	1.13	0.37	3.02	**0.0025**
	Landscape Richness 500m	-0.55	0.34	-1.60	0.10886

Table S4.4: Variance Inflation Factors (VIFs) for each of the main factors in each model of bee visitation. Models referenced by their response variable from **Appendix Table S4.3**.

Response variable	Temperature	Wind Speed	Natural Area	Landscape Richness
Total Bee Visit Number	1.143464	1.095907	1.369642	1.436461
Bee Visit Richness	1.102975	1.122277	1.381791	1.525221
Apis visits	1.086294	1.098173	1.298936	1.380577
Bombus visits	1.160034	1.092305	1.331445	1.355072
Halictini visits	1.104429	1.106217	1.120006	1.143572
Eucera visits	1.163859	1.121758	1.156891	1.159777
Augochlorini visits	1.037132	1.18509	1.278017	1.453341

Table S4.5: Moran's I spatial autocorrelation model output for the residuals from each bee visitation and virus prevalence model listed by the main response variable.

Model Response Variable	Observed	Expected	SD	P-value
Total Bee Visit Number	-0.149097	-0.142857	0.091407	0.9456
Bee Visit Richness	-0.250653	-0.142857	0.096673	0.2648
Apis visits	-0.239321	-0.142857	0.093438	0.3019
Bombus visits	-0.108824	-0.108824	0.097701	0.7276
Halictini visits	-0.195343	-0.142857	0.090556	0.5622
Eucera visits	-0.050081	-0.142857	0.090821	0.307
Augochlorini visits	-0.08901	-0.142857	0.066907	0.4209
Apis DWV	-0.191196	-0.142857	0.087452	0.5804
Apis BQCV	-0.113035	-0.142857	0.094682	0.7528
Apis SBV	-0.141395	-0.142857	0.088945	0.9869
Bombus DWV	-0.246352	-0.142857	0.092258	0.2619
Bombus BQCV	-0.217362	-0.142857	0.089408	0.4047
Bombus SBV	-0.233867	-0.142857	0.096704	0.3466
Flower DWV prevalence	-0.200112	-0.142857	0.090849	0.5285

Table S4.6: Pearson's product moment correlation test output for each pair of bee visitation factors with 95% confidence intervals in parentheses. Correlations shown in **Appendix Figure S4.1**. Significant p-values are bolded.

Bee Visitation Factors		Correlation (95% CI)	t value	df	p value
Total Bee Visit Number	Visit Richness	0.489 (0.35, 0.61)	6.30	126	**<0.0001**
Apis visits	Visit Richness	0.509 (0.37, 0.63)	6.63	126	**<0.0001**
Bombus visits	Visit Richness	0.176 (0.003, 0.34)	2.01	126	**0.047**
Halictini visits	Visit Richness	0.443 (0.29, 0.57)	5.54	126	**<0.0001**
Eucera visits	Visit Richness	0.129 (-0.05, 0.30)	1.46	126	0.148
Augochlorini visits	Visit Richness	0.249 (0.08, 0.40)	2.89	126	**0.0046**
Apis visits	Total Bee Visit Number	0.274 (0.11, 0.43)	3.20	126	**0.0018**
Bombus visits	Total Bee Visit Number	0.547 (0.41, 0.66)	7.34	126	**<0.0001**
Halictini visits	Total Bee Visit Number	0.489 (0.34, 0.61)	6.29	126	**<0.0001**
Eucera visits	Total Bee Visit Number	0.479 (0.33, 0.60)	6.12	126	**<0.0001**
Augochlorini visits	Total Bee Visit Number	0.525 (0.39, 0.64)	6.92	126	**<0.0001**

Table S4.7: Full model results of visitation to flowers effects on *Apis mellifera* or *Bombus impatiens* DWV, BQCV, and SBV prevalence with significant main factors bolded. Models were binomial GLMM or GLM models. If the random effect, Transect ID, had no variance in the GLMM model, then model was run as a GLM without random effects. All main factors were z transformed to scale and center the data.

Species	Virus	Random effects	Main effect	Estimate	Std. Error	z value	p value
Apis mellifera	DWV	None	Intercept	-0.207	0.178	-1.161	0.245
			Visit Richness Per Flower	-0.855	0.324	-2.641	**0.0083**
			Apis mellifera visit rate	0.275	0.230	1.193	0.233
			Bombus spp. visit rate	0.709	0.304	2.334	**0.0196**
Apis mellifera	BQCV	None	Intercept	1.202	0.210	5.734	**9.81E-09**
			Visit Richness Per Flower	-0.310	0.370	-0.84	0.401
			Apis mellifera visit rate	0.610	0.281	2.174	**0.0297**
			Bombus spp. visit rate	-0.100	0.339	-0.295	0.768
Apis mellifera	SBV	Transect ID	Intercept	-1.159	0.254	-4.556	**5.22E-06**
			Visit Richness Per Flower	-1.289	0.484	-2.665	**0.0077**
			Apis mellifera visit rate	0.403	0.348	1.16	0.246
			Bombus spp. visit rate	0.531	0.482	1.102	0.270
Bombus impatiens	DWV	None	Intercept	-0.821	0.178	-4.617	**3.90E-06**
			Visit Richness Per Flower	-0.659	0.330	-1.996	**0.046**
			Apis mellifera visit rate	0.527	0.259	2.033	**0.042**
			Bombus spp. visit rate	0.137	0.324	0.423	0.672
Bombus impatiens	BQCV	Transect ID	Intercept	-1.025	0.226	-4.535	**5.76E-06**
			Visit Richness Per Flower	-0.420	0.392	-1.072	0.284
			Apis mellifera visit rate	0.244	0.276	0.887	0.375
			Bombus spp. visit rate	0.553	0.378	1.465	0.143
Bombus impatiens	SBV	None	Intercept	-0.451	0.163	-2.76	**0.0058**
			Visit Richness Per Flower	0.084	0.293	0.286	0.775
			Apis mellifera visit rate	0.192	0.222	0.864	0.388
			Bombus spp. visit rate	-0.152	0.281	-0.54	0.590

Table S4.8: Variance Inflation Factors (VIFs) for each of the main factors in each model of bee visitation. Models referenced by their response variable from **Appendix Table S4.7**.

Response Variable	Visit Richness	Apis visitation rate (min^{-1})	Bombus visitation rate (min^{-1})
Apis DWV	3.322727	1.671891	3.036729
Apis BQCV	3.135296	1.434553	2.638807
Apis SBV	4.37196	2.50213	4.725033
Bombus DWV	3.584425	2.397576	3.521059
Bombus BQCV	3.295381	1.82548	3.126632
Bombus SBV	3.222009	1.895142	2.929078

Table S4.9: Summary of average bee visitation to squash flowers at each site, including the number of all bee visits, bee visitation rate per minute, number of bee morphospecies visits to each flower, total number of morphospecies that visited flowers at a site, and sum of the duration of all bee visits to flowers at a site in seconds.

Site	Visit Number	Visitation Rate (min^{-1})	Visit Richness Per Flower	Total Visit Richness at Site	Visit Duration (sec)
BP	12.9375	0.404166	2.375	6	626.125
E	8.9375	0.278019	1.125	5	833.5
GT	6.470588	0.215015	1.941176	8	537.0588
K	2.5625	0.07819	1.0625	4	786.125
PL	9.5	0.311036	2.4375	6	355.0625
PR	27.8125	0.933944	2.3125	6	1050.938
SP	6.5	0.189078	1.5	6	631.625
T	6.5625	0.205419	1.5625	5	747.5625

Table S4.10: Summary of the average number of visits to squash flowers for each morphospecies observed at each site during the approximately 30-min videos.

Site	*Apis mellifera* visits	*Bombus* spp. visits	Halictini visits	Hover-fly visits	*Eucera pruinosa* visits	Augochlorini visits	*Melissodes* spp. visits	*Vespula* spp. visits	*Triepeolus* spp. visits
BP	1.75	9.25	0.875	0.125	0.6875	0	0.0625	0	0
E	0	1.625	0.125	0	6.9375	0.1875	0	0.0625	0
GT	0.24	4.47	0.823	0.176	0.235	0.235	0.059	0	0.059
K	0	1.3125	0.125	0	0.0625	1.125	0	0	0
PL	1	4.4375	0.75	0.1875	2.125	0.375	0	0	0
PR	0.125	8.4375	5.25	0.3125	1.1875	12.5625	0	0	0
SP	0.44	5.56	0.3125	0.0625	0.0625	0.0625	0	0	0
T	0.625	5	0.25	0	0	0.0625	0	0.625	0

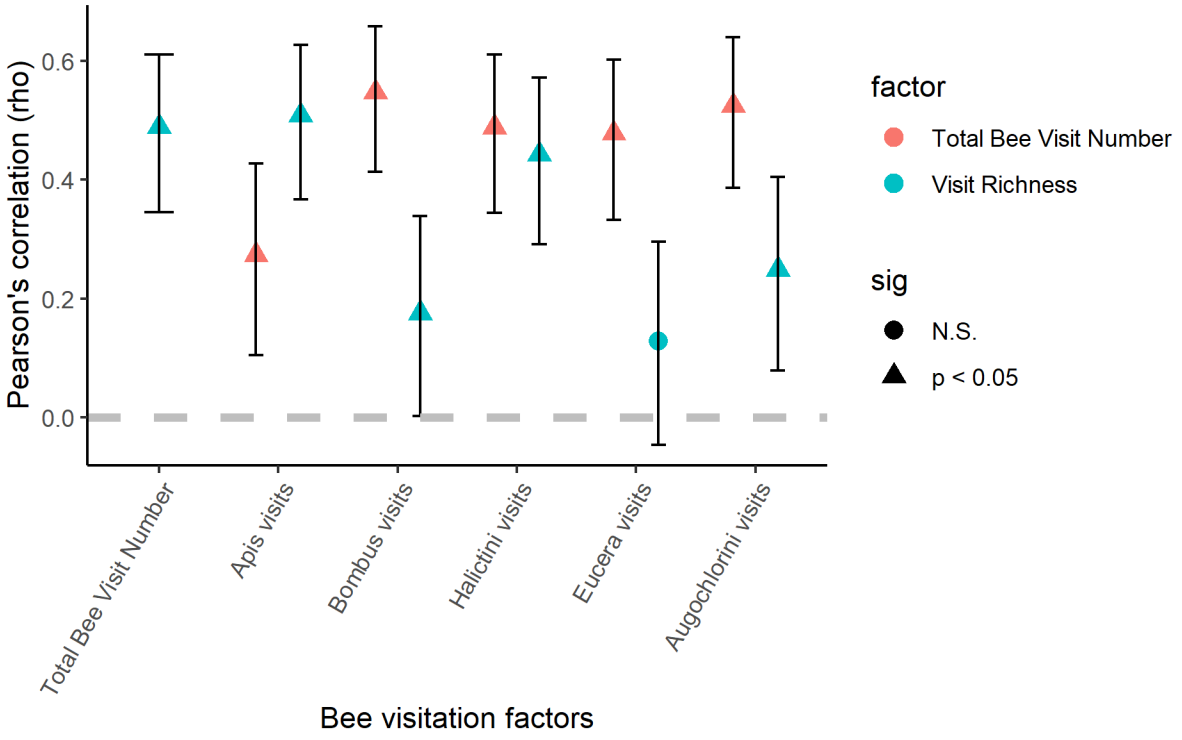


Figure S4.1: Visit richness and total visit number are positively correlated with all other morphospecies-specific bee visitation numbers. Pearson's product moment correlations were tested for each pair of bee visitation factors, with red points for factors tested with total bee visit number, and blue points for factors tested with visit richness (Appendix Table S4.6). The error bars are the 95% confidence intervals, and the gray dashed line shows whether the error bar crosses zero. Correlations where the error bar crosses zero are not significantly different from zero (circle), while those that do not cross zero are significant correlations (triangle).

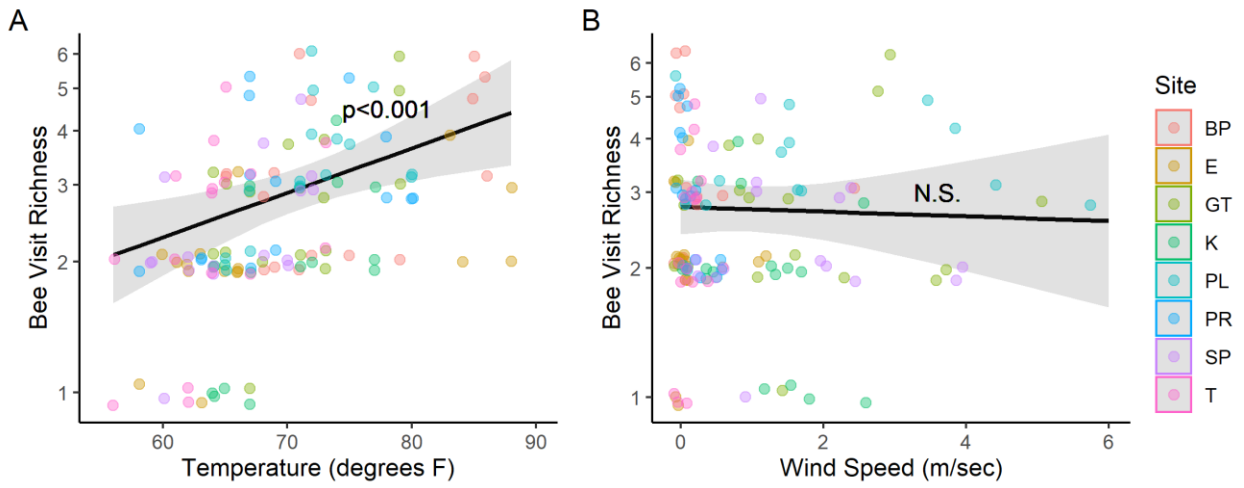


Figure S4.2: A) Higher temperatures (degrees F) were associated with greater bee visit richness. **B)** Wind speed (m/sec) was not associated with any differences in bee visit richness. The number of bee species visiting flowers per approximately 30 min are plotted on a log + 1 scale. The fitted line is the predicted number of bee species + 1 from each corresponding model in **Table 4.1 and Appendix Table S4.3**, and accounts for the other fixed and random effects including the total time each flower was observed.

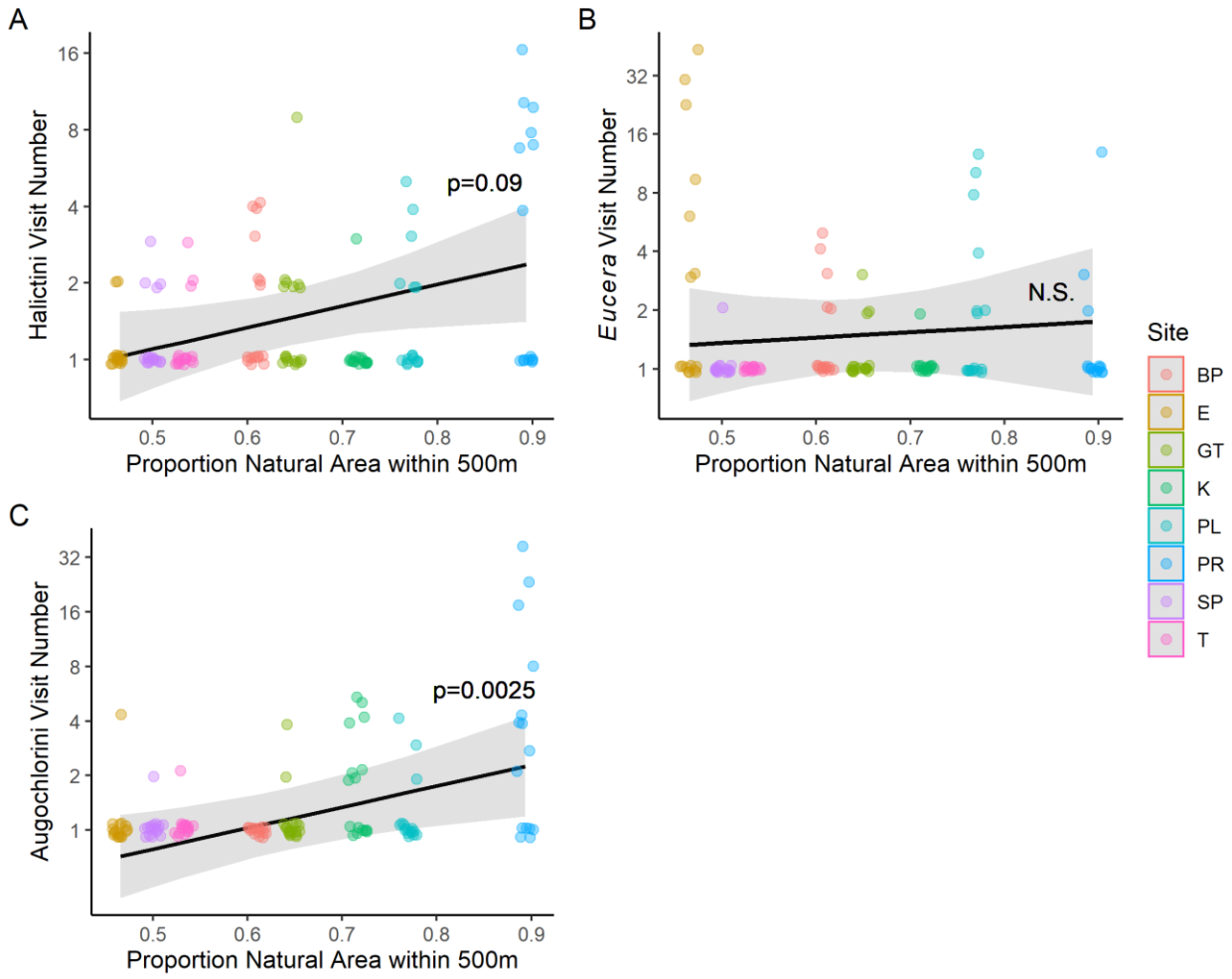


Figure S4.3: Greater proportion of natural area at 500-m was not correlated with the number of **A)** Halictini visits nor **B)** *Eucera pruinosa* visits, but was associated with a greater number of **C)** Augochlorini visits to flowers. The number of bee visits per approximately 30 min are on a log + 1 scale. The fitted line is the predicted number of bee visits + 1 from each corresponding model in **Table 4.1 and Appendix Table S4.3**, and accounts for the other fixed and random effects, including the total time each flower was observed.

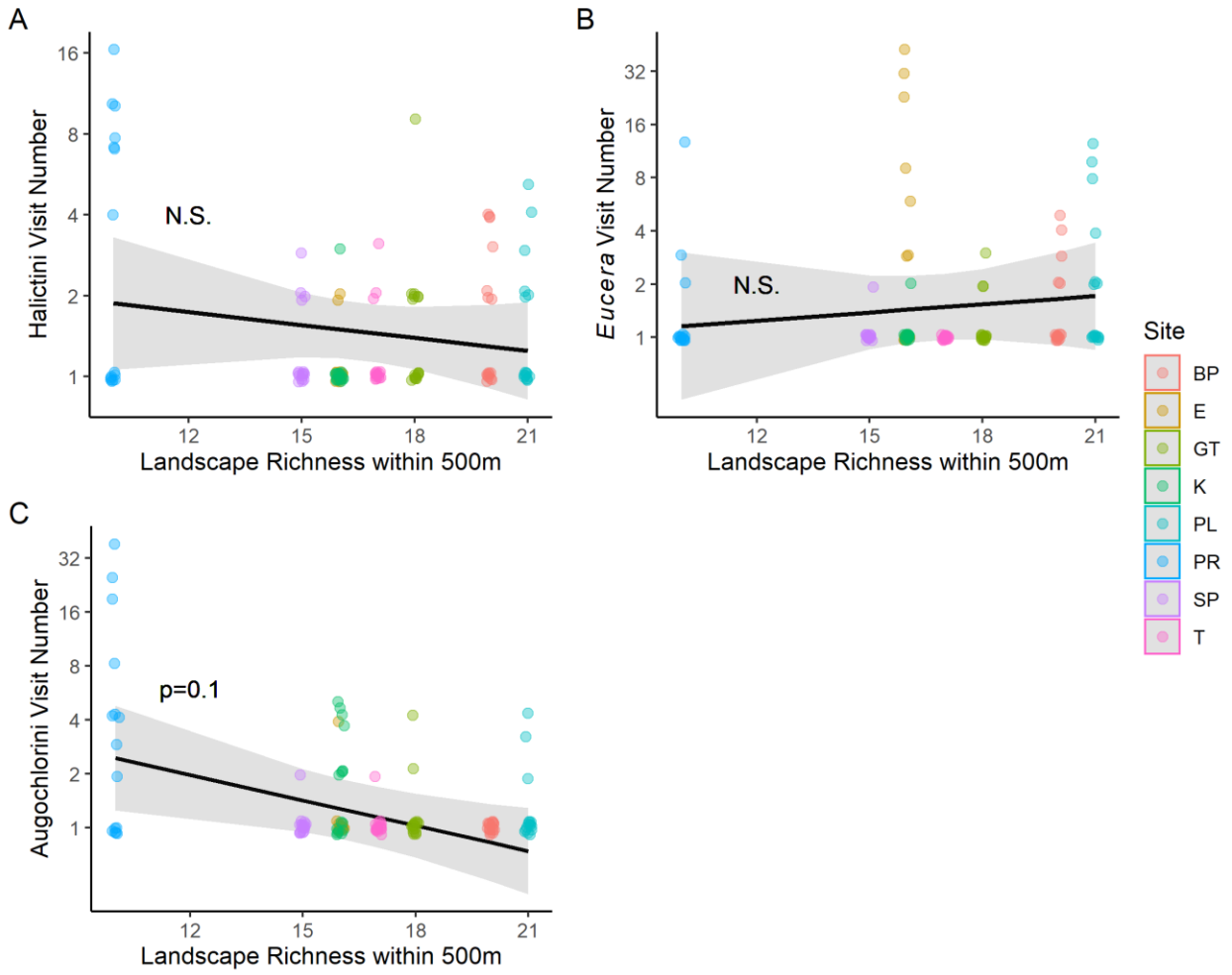


Figure S4.4: Higher landscape richness at 500-m was not correlated with the number of **A)** Halictini, **B)** *Eucera pruinosa*, or **C)** Augochlorini visits to flowers. The number of bee visits per approximately 30 min are on a log + 1 scale. The fitted line is the predicted number of bee visits + 1 from each corresponding model in **Table 4.1 and Appendix Table S4.3**, and accounts for the other fixed and random effects, including the total time each flower was observed.

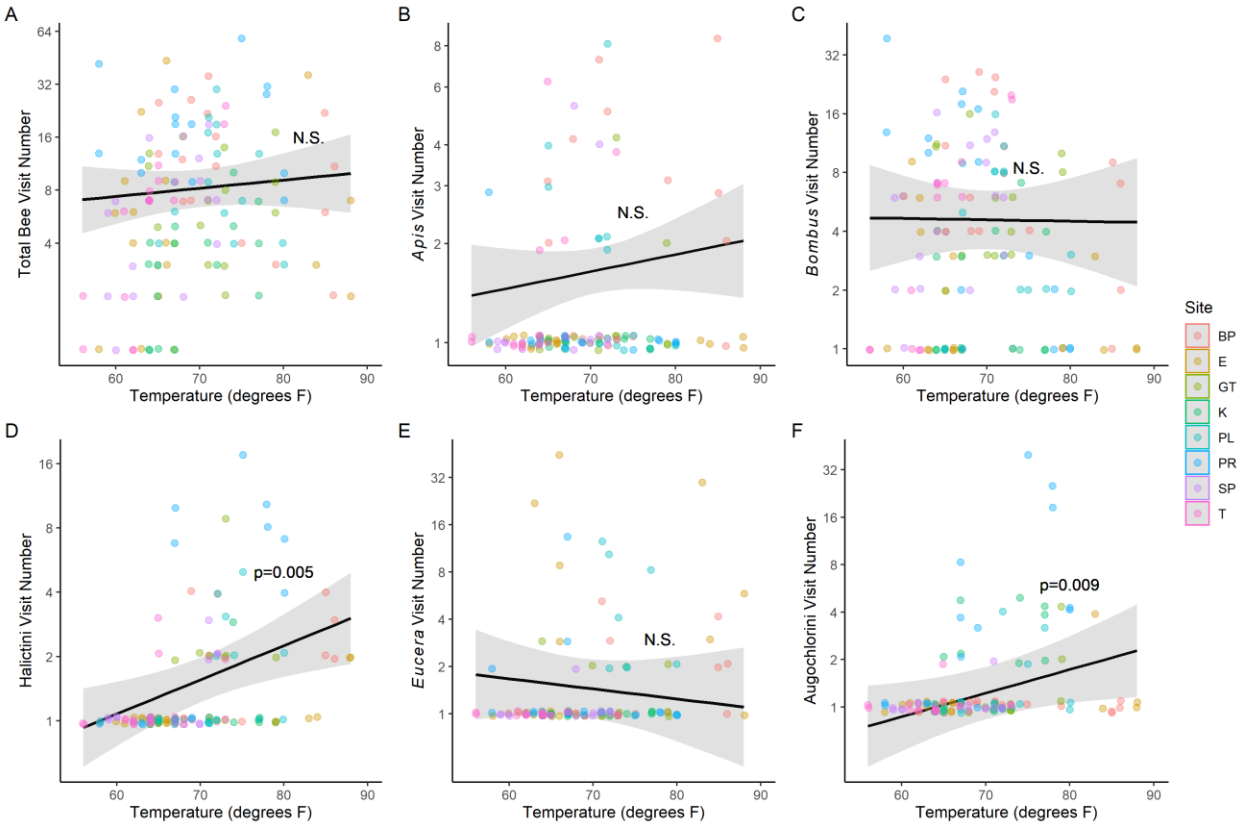


Figure S4.5: The associations between temperature (degrees F) and the number of visits by **A)** all bees, **B)** *Apis mellifera*, **C)** *Bombus* spp., **D)** Halictini, **E)** *Eucera pruinosa*, and **F)** Augochlorini to flowers. Higher temperature was only associated with higher number of Halictini and Augochlorini visits. Total bee and all other morphospecies-specific visits numbers to flowers were not strongly correlated with temperature. The number of bee visits per approximately 30 min are on a log + 1 scale. The fitted line is the predicted number of bee visits + 1 from each corresponding model in **Table 4.1 and Appendix Table S4.3**, and accounts for the other fixed and random effects, including the total time each flower was observed.

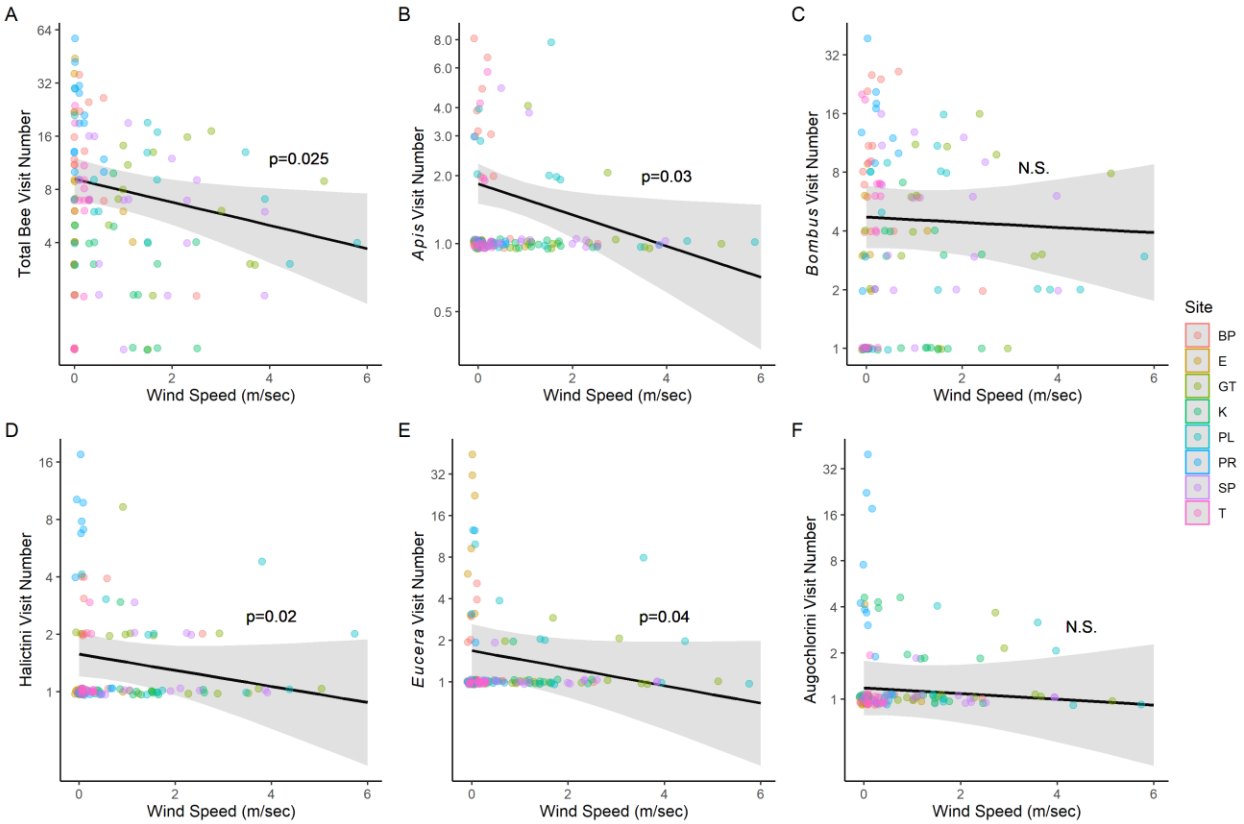


Figure S4.6: The associations between wind speed (m/sec) and the number of visits by **A)** all bees, **B)** *Apis mellifera*, **C)** *Bombus* spp., **D)** Halictini, **E)** *Eucera pruinosa*, and **F)** Augochlorini to flowers. Higher wind speeds were associated with lower visit numbers by total bees, *A. mellifera*, Halictini, and *E. pruinosa*. *Bombus* spp. and Augochlorini visitation rates were not correlated with wind speed. The number of bee visits per approximately 30 min are on a log + 1 scale. The fitted line is the predicted number of bee visits + 1 from each corresponding model in **Table 4.1 and Appendix Table S4.3**, and accounts for the other fixed and random effects, including the total time each flower was observed.

Chapter 5 : Conclusion

In my dissertation, I explored how communities, environment, and species interactions are linked with deformed wing virus (DWV), black queen cell virus (BQCV), and sacbrood virus (SBV) prevalence in four key pollinator host species. First, I used a two-year field study to examine how different community factors, including species richness, total abundance, and community composition correlated with virus prevalence in *Apis mellifera* (honey bees), *Bombus impatiens* (Eastern bumblebees), *Lasioglossum* spp. (sweat bees), and *Eucera pruinosa* (squash bees). Then I built upon on this study by assessing the effects of several local and landscape habitat characteristics that are important for pollinator nutrition on patterns of virus prevalence. We tested if habitat characteristics could be directly linked with virus prevalence, as well as whether habitat-mediated changes in pollinator community diversity indirectly impact virus prevalence. Finally, I took a closer look at bee visitation patterns on flowers to investigate potential mechanisms for the observed reduction in virus prevalence in diverse pollinator communities. Specifically, I wanted to understand how environmental factors correlate with pollinator visitation patterns, and whether the diversity and/or frequency of interactions on flowers was associated with the likelihood of DWV, BQCV, and SBV deposition on flowers and prevalence in *A. mellifera* and *B. impatiens* hosts. Overall, my results were very consistent among all chapters, showing that local and landscape scale habitat characteristics mediate greater pollinator community species richness, which is strongly linked with lower levels of virus prevalence among communities in multiple hosts and multiple viruses (i.e. biodiversity–disease relationship). Additionally, I found initial and novel evidence supporting a new ‘habitat–disease relationship’ that I termed, where virus prevalence is directly linked with habitat quality

characteristics associated with improved pollinator nutrition, separate from co-occurring biodiversity–disease relationships.

In Chapter 2, focusing specifically on the relationship between pollinator communities and virus prevalence, I provide evidence that species richness is the most important community factor associated with reduced virus prevalence in multiple hosts and multiple viruses. Most significantly, all three viruses had lower virus prevalence in species-rich communities compared to species-poor communities, while controlling for changes in pollinator abundance. In contrast, total and species-specific host abundance and community composition were not strongly associated with virus prevalence for most host–virus pairs. These findings support the 'dilution effect' hypothesis, where biodiversity reduces pathogen prevalence, which has never been previously shown for a pollinator–pathogen system. Interestingly, the strength of the dilution effect appears to vary based on host competence for each virus, where highly competent hosts exhibited greater negative slopes between virus prevalence and pollinator species richness. Low competent hosts, *Lasioglossum* spp. and *Eucera pruinosa*, were unable to show a correlation between biodiversity and virus prevalence because those hosts were rarely infected with BQCV and SBV. The mechanism(s) underlying this dilution effect is currently unknown, but may be explained by differences in the encounter or transmission rate between bees that share flowers in species-rich versus species-poor communities.

More and more biodiversity–disease studies are beginning to investigate how host community diversity impacts multi-host pathogen prevalence in multiple host species that vary in competence for the pathogen. Current evidences shows that multi-host pathogens can consistently dilute pathogen prevalence among multiple co-occurring hosts or in community-wide pathogen prevalence (Ezenwa et al. 2006a, Allan et al. 2009, Johnson et al. 2013a, 2013b,

Becker et al. 2014, Venesky et al. 2014, Strauss et al. 2018). These findings corroborate with my results that pollinator communities with greater species richness exhibit consistently lower virus prevalence for three multi-host viruses within multiple bee species, while controlling for total host abundance. However, variation in the strength of the dilution effect or lack of relationship between host diversity and virus prevalence is likely due to variation in pollinator hosts' exposure, susceptibility, or competence for the three viruses.

On the other hand, studies that compare biodiversity–disease relationships among multiple pathogens and multiple hosts have been rare. Johnson et al. similarly found reduced infection success with greater host diversity for five out of the seven trematode parasites that infect amphibian hosts (Johnson et al. 2013a). Meanwhile, Halliday et al. showed that pathogens with different modes of transmission tended to exhibit opposing biodiversity–disease relationships (i.e. dilution and amplification effects) (Halliday et al. 2017). My work contributes to expanding this multi-pathogen literature by adding that closely related pathogens that share many key traits can exhibit similar dilution effect patterns among the same host communities. These findings lend some support to the generality of the dilution effect in multiple hosts and pathogens, where conservation of biodiversity leads to a reduction in pathogen prevalence or disease risk—at least in some host–pathogen systems.

Current debates about biodiversity–disease relationships suggest that either the dilution effect is idiosyncratic or that amplification will be more common in most host–pathogen systems (Salkeld et al. 2013, Wood et al. 2014, 2017). Though amplification may be occurring in other pollinator host species or bee pathogens not tested here, overall, my results lend support to the idea that the dilution effect can generally occur among hosts and pathogens that share many traits. However, we observe the greatest differences in the dilution effect pattern among hosts

that vary in competence for the viruses. Future work that investigates biodiversity–disease relationships among a greater diversity of pathogens and hosts within the 'replicate' communities may further tease apart how differences in pathogen traits (e.g. generalist vs. specialist, transmission mode, etc.) and host traits (e.g. behavior, competence, etc.) contribute to variation in biodiversity–disease relationships.

In Chapter 3, I show that the habitat quality characteristics that are important for maintaining pollinator nutrition are linked with patterns of pathogen prevalence among different pollinator communities through two possible avenues. First, both local and landscape habitat features associated with high quality environments were directly linked with virus prevalence in multiple host species. However, the direction of the direct effects between habitat and virus prevalence varied among the specific habitat characteristics. These results provide the first evidence supporting a new pattern that I termed the 'habitat–disease relationship', where high-quality habitat is directly linked with pathogen prevalence as an independent, but not mutually exclusive pattern to habitat driven effects on biodiversity–disease relationships.

The habitat–disease relationship could function through habitat-mediated effects on host nutrition and immune function in high-quality environments to alter patterns of pathogen prevalence. In general, we predicted that habitat characteristics, such as greater natural area, landscape richness, floral richness and floral density, would benefit pollinator health through positive associations with nutrition and immune function, and consequently reduce virus prevalence in pollinator hosts. Instead, greater floral density tended to reduce virus prevalence, while greater natural area and floral richness correlated with increase virus prevalence, and greater landscape richness either increased or reduced virus prevalence depending on the specific virus. Though I was not able to test the specific mechanism(s) that might drive these variable

direct relationships, my data suggest that different habitat characteristics could have coinciding or differing impacts on patterns of pathogen prevalence depending on how habitat interacts with host nutrition, immune function, and pathogen severity. Bee nutrition and immunity are particularly linked with the quality of their environment (e.g. access to abundant and diverse flowers at local and landscape scales), given that recent declines in bees are associated with loss of these important resources (Biesmeijer et al. 2006, Potts et al. 2010, Di Pasquale et al. 2013, Donkersley et al. 2014, DeGrandi-Hoffman and Chen 2015, Vaudo et al. 2015). However, high quality habitat characteristics could also lead to increased pathogen prevalence if pathogens can co-opt host resources and benefit from hosts with greater nutritional resources (increasing pathogen severity and transmission rates), or if high-quality habitat features result in altered behavior that lead to higher exposure rates with the pathogen. Further study of the underlying mechanisms and interactions between habitat characteristics and host nutritional status, immunity, and susceptibility and/or tolerance to infection are needed to better predict how habitat factors will impact pathogen prevalence.

The second key avenue by which the high-quality habitat characteristics could alter patterns of pathogen prevalence is through changing community structure and host interactions, which consequently indirectly changes pathogen transmission patterns. I found that high-quality habitats with a greater proportion of natural area, landscape richness, and floral richness were associated with higher pollinator community species richness and abundance, but only greater pollinator species richness reduced virus prevalence. This demonstrates that habitat mediates changes in host diversity to result in a dilution effect, but the indirect link does not occur through changes in host density. Other biodiversity–disease studies have also shown that habitat fragmentation or degradation are important factors that alter community diversity and contribute

to dilution and amplification effects on pathogen prevalence (Langlois et al. 2001, Allan et al. 2003, Estrada-Peña 2009, Dearing and Dizney 2010, Huang et al. 2016, Faust et al. 2017). However, I found that the same sets of habitat characteristics simultaneously affect pathogen prevalence through the habitat–disease relationship, and by altering community diversity and interactions among hosts to produce a dilution effect. These results demonstrate that the role of habitat characteristics could be a missing link in our understanding of variability in pathogen prevalence among different communities and geographic locations.

Overall, the summed net effect of all direct and indirect links between habitat, pollinator species richness, and virus prevalence produced lower virus prevalence for all three viruses. Therefore, despite some mixed positive and negative effects on virus prevalence along individual pathways and among different viruses, in general, sites with very high-quality habitat and high species richness are predicted to have significantly reduced virus prevalence compared to sites with low-quality habitat and low species richness. These findings corroborate with previous evidence of dilution effects for DWV, BQCV, and SBV in multiple pollinator host species (**Chapter 2**), but our structural equation model provides a useful tool for generating hypotheses and testing the relative impact of different factors that contribute to observed patterns of pathogen prevalence in pollinator pathogens and other host–pathogen systems. Future direction of research should begin to untangle the complex links between the environment, hosts, and pathogens in multi-host–multi-pathogen systems.

Though some biodiversity–disease studies have shown the indirect effects of habitat characteristics such as habitat fragmentation, on dilution or amplification effects, few studies have examined the potential role of the habitat–disease relationship in studies that have shown a dilution effect pattern. Many experimental studies have found that host nutrition can mediate

immune function and pathogen loads, but lack connections to specific habitat characteristics and broad patterns of pathogen prevalence among different communities. Furthermore, it is often difficult to establish the causal connections necessary to elucidate the mechanisms that underlie habitat–disease and biodiversity–disease relationships in natural systems (Rohr et al. 2020). I recommend paired field and experimental studies to better to tease apart the intricate links between habitat quality and host nutrition, immunity, and infectious disease susceptibility and tolerance in pollinator and other host–pathogen systems (Venesky et al. 2014). First, initial field studies should examine differences in pathogen prevalence, host nutrition, and immune function along gradients of various habitat characteristics that are suspected to impact host health, and employ structural equation modeling to evaluate the relative impact of each factor on the outcomes of pathogen prevalence. Then, for host–pathogen systems that are amenable to experiments, a paired experiment should be used to further test the pathways established from the field study in a more thoroughly controlled design. This paired field and experimental approach will allow for the generation of host–pathogen system specific predictions based on their ecology, and can be followed with tests of the underlying mechanisms that drive these patterns.

Finally, in Chapter 4 I found that small scale individual interactions among bees on flowers were linked with patterns of virus prevalence among communities. Specifically, greater numbers of different pollinator species visiting flowers (visit richness) correlated with reduced DWV prevalence in *A. mellifera* and *B. impatiens*, showing that diversity in individual interactions can be associated with community-level dilution effect patterns in pathogen prevalence. Interestingly, the visitation rates of *A. mellifera* and *Bombus* spp. to flowers, two highly competent hosts that often have high virus prevalence, were not consistently associated

with virus prevalence among different communities. However, greater *A. mellifera* and *Bombus* spp. visitation rates was correlated with greater DWV prevalence in the other host species, possibly suggesting that higher visitation rates other heterospecifics may lead to greater cross-species transmission. All together, these results suggest that pollinator interactions on flowers may be the key to further explore potential dilution effect mechanisms, such as encounter or transmission reduction, in this pollinator–pathogen system. Further investigation of the factors that alter host interactions on shared flowers will improve our understanding of how different host species contribute to virus spread in pollinator communities.

Environment features—particularly those associated with high-quality habitats and weather—were also correlated with differences in pollinator visitation to flowers. The environment had strong but variable associations with bee visit richness and species-specific visitation rates to flowers. Therefore, changes in the environment could dramatically affect patterns of individual interactions among hosts in different communities, and could result in differing pathways of pathogen transmission and variable pathogen prevalence. Pollinator viruses are associated with pollen on flowers (Singh et al. 2010, Mazzei et al. 2014, Alger et al. 2019), suggesting that shared flowers are likely an important pathway of cross-species transmission in this system. Therefore, understanding how pollinator host species interactions on shared flowers vary among different community and environmental contexts is critical for determining differences in exposure, effective transmission, and the resulting prevalence of pollinator viruses.

Finally, DWV prevalence on flowers varied among different pollinator communities, which suggests that flowers may represent a key site for viral transmission within and among pollinator host species. Higher DWV prevalence on flowers was associated with lower DWV in

A. mellifera, but not with *A. mellifera* and *Bombus* spp. visitation rates or species richness of visitors to flowers. This confusing result indicates that patterns of virus deposition on flowers may be more complex than expected, and we need to use a more thorough and dynamic approach that can simultaneously assess differences in bee visitation rates, probability of a bee visitor being infected, and likelihood of depositing viral particles to flowers. Further study on how the three viruses are deposited on flowers and subsequently picked up by bees visiting shared flowers is key for understanding virus transmission among host species in this pollinator–pathogen system.

Future work to investigate how interactions among hosts may contribute to community-level patterns of pathogen prevalence could use social networks to trace direct and indirect interactions within and among bee species on flowers. These more dynamic approaches will be important for testing whether particular host species have disproportionately large impacts on pathogen transmission pathways (e.g. superspreader hosts) and could also shed light on the underlying mechanisms of the dilution effect (e.g. encounter or transmission reduction). Social networks have been used to understand the spread of many human infectious diseases, and other wildlife systems with a single host and pathogen (Hamede et al. 2009, Salathé et al. 2010, Kappeler et al. 2015, Fountain-Jones et al. 2017). However, applying these networks to model transmission for multi-host pathogens with differing rates transmission of within and among host species and varying host competence remains challenging. Initial studies utilizing this approach have shown dynamic patterns of transmission that need to be incorporated into our understanding of how multi-host pathogens move through communities of hosts (Rigaud et al. 2010, Ruiz-Gonzalez et al. 2012, Graystock et al. 2015, Antonovics 2017, Wilber et al. 2019), and may be a useful approach to understanding encounter reduction mechanisms of the dilution effect.

Dynamic patterns of transmission among host species also vary across temporal and spatial scales. Communities and environments are rarely static through time and space as host species vary in phenology, behavior, home ranges, and migration patterns based on cues from the environment, which consequently can alter expected outcomes for biodiversity–disease relationships (Duffy et al. 2010, Estrada-Peña et al. 2014, Rohr et al. 2020). Pollinator communities in the same location can vary substantially throughout the year because many pollinator species vary in phenology of their active periods from short (less than a month) to long (the full growing season) (Tuell and Isaacs 2010, Burkle et al. 2013). Though this dissertation only captured a 'snapshot' of the pollinator communities and pathogen prevalence at a couple of time points, this approach gives us an initial glimpse into the potential patterns of dilution occurring in this system. To date, few biodiversity–disease studies have examined how the relationship between community factors and pathogen prevalence change over time (Estrada-Peña et al. 2014, Johnson et al. 2015). Future work that explores how pollinator communities and their environment vary across time, and the impacts of those changes on patterns of pathogen prevalence will be critical for moving towards a more dynamic understanding of interactions among multiple hosts and multiple pathogens.

Furthermore, many have shown that biodiversity–disease relationships are scale dependent, and most studies that have observed dilution effects were done at relatively small spatial scales (Moore and Borer 2012, Lafferty and Wood 2013, Wood and Lafferty 2013, Johnson et al. 2015, Huang et al. 2016, Rohr et al. 2020). Therefore, will a biodiversity–disease relationship observed a local scale still hold true at a regional or global scale? The disease ecology literature remains unclear on this topic, with studies finding both dilution (Ezenwa et al. 2006b, Swaddle and Calos 2008, Allan et al. 2009) and amplification (Jones et al. 2008, Wood

and Lafferty 2013) at broad spatial scales. Host–pathogen dynamics can be influenced by factors at many scales, from local community composition and interactions, to landscape-level topography, climate, and species distributions (Duffy et al. 2010, Estrada-Peña et al. 2014). In pollinator systems, host traits vary substantially with their ecology, including foraging specialization, nesting requirements, degree of sociality, flight distances, and more (Michener 2007, Lonsdorf et al. 2009, Williams et al. 2010, Murray et al. 2012). Therefore, the likelihood of two pollinator species interacting on shared flowers will vary substantially across space and depending on the surrounding environment. Additionally, the impact of the environment may vary based on spatial scale, and effects at different spatial scales may interact in complex ways. For example, local habitats with larger sown wildflower plots in areas with few semi-natural landscape characteristics had higher pathogen prevalence in bumblebees, but this relationship was not found in landscapes with many semi-natural characteristics (Piot et al. 2019). An important frontier in biodiversity–disease relationships will be integrating these studies, including both environmental and community factors, across scales to explain why biodiversity–disease relationships may differ among local and regional scales.

Finally, one powerful aspect of my dissertation is that I examine patterns of pathogen prevalence for multiple pathogens that infect multiple hosts. These comparisons are key because we can further tease apart whether consistent patterns among communities, environments, and pathogen prevalence are driven by similarity in host traits or pathogen traits. Though many disease ecologists recognize that multi-host–multi-pathogen systems are relatively common in nature, few studies evaluate how multiple pathogens infecting multiple hosts may each respond to variable community and habitat factors. My dissertation allows for comparison of biodiversity–disease and habitat–disease relationships among three widespread viruses and four

focal pollinator host species to elucidate some general patterns. Overall, I found remarkably consistent dilution effect patterns among multiple viruses and multiple host species. Though there was some variation in the strength of the dilution effect or lack of biodiversity–disease relationship for some host–virus pairs based on relative host competence, I did not observe any evidence of an amplification effect across the three studies. Some specific habitat characteristics correlated with increased virus prevalence, in apparent opposition to the general pattern of dilution effects for the three viruses. However, when all direct and indirect pathways between habitat characteristics, pollinator community species richness, and virus prevalence were summed together, I found strong negative effects on virus prevalence for all three viruses. DWV had the strongest negative associations, followed by BQCV, and SBV had the weakest correlation. The variation in the strength of these links among the three viruses may be due to differences pathogen traits. In this system, host specificity of multi-host pathogens appears to play an important role; DWV is the most generalist pathogen, while BQCV and SBV are more specialized to infect just a few host species. Careful investigation into differences in specific pathogen traits of these viruses (e.g. host range, frequency of cross-species transmission, etc.) could further elucidate the variability among DWV, BQCV, and SBV in their responses to community and environmental factors.

Meanwhile, differences in host traits, such as life history, sociality, and other important behaviors, may be useful for explaining variation in pathogen prevalence and host competence among different host species. Host traits have been shown to have important impacts on community assembly and disease dynamics (Ruiz-Gonzalez et al. 2012, Strauss et al. 2018, Kirk et al. 2019, Truitt et al. 2019), but these relationships still need to be explored in many other host–pathogen systems. Future work that explicitly tests for the differences among host species

and among pathogens in how they respond to different community and environmental factors will be critical to explain why pathogen prevalence varies among hosts in different communities.

In conclusion, my dissertation work shows that both habitat–disease and biodiversity–disease relationships can operate simultaneously to produce reduced pathogen prevalence for multiple pathogens infecting multiple host species, despite some variation in the direction and relative effect of individual links between habitat characteristics, host communities, and pathogen prevalence. This work shows that multi-host–multi-pathogen dynamics are complex, and investigating patterns among similar hosts or similar pathogens can reveal consistent and biologically relevant relationships between communities, environment, and host interactions. Environmental and community factors have concurrent effects on patterns of pathogen prevalence, but whether they have opposing or synergistic effects on hosts could result in multifaceted outcomes for disease risk.

The findings presented in this dissertation suggest that generally improving habitat quality and increasing pollinator community diversity could have broad health benefits for honey bees and native bee species. However, careful study of the connections among environments, communities, and host interactions within specific host–pathogen systems will be needed to develop similar management strategies for other wildlife populations.

Literature Cited

Alger, S. A., P. A. Burnham, H. F. Boncristiani, and A. K. Brody. 2019. RNA virus spillover from managed honeybees (Apis mellifera) to wild bumblebees (Bombus spp.). PLoS ONE 14:e0217822.

Allan, B. F., F. Keesing, and R. S. Ostfeld. 2003. Effect of Forest Fragmentation on Lyme Disease Risk. Conservation Biology 17:267–272.

Allan, B. F., R. B. Langerhans, W. A. Ryberg, W. J. Landesman, N. W. Griffin, R. S. Katz, B. J. Oberle, M. R. Schutzenhofer, K. N. Smyth, A. D. S. Maurice, L. Clark, K. R. Crooks, D. E. Hernandez, R. G. Mclean, R. S. Ostfeld, and J. M. Chase. 2009. Ecological Correlates of Risk and Incidence of West Nile Virus in the United States. Oecologia 158:699–708.

Antonovics, J. 2017. Transmission dynamics: critical questions and challenges. Philosophical Transactions of the Royal Society B: Biological Sciences 372:20160087.

Becker, C. G., D. Rodriguez, L. F. Toledo, A. V. Longo, C. Lambertini, D. T. Correa, D. S. Leite, C. F. B. Haddad, and K. R. Zamudio. 2014. Partitioning the net effect of host diversity on an emerging amphibian pathogen. Proceedings of the Royal Society B: Biological Sciences 281:20141796.

Biesmeijer, J. C., S. P. M. Roberts, M. Reemer, R. Ohlemuller, M. Edwards, T. Peeters, A. Schaffers, S. G. Potts, R. Kleukers, C. Thomas, J. Settele, and W. E. Kunin. 2006. Parallel Declines in Pollinators and Insect-Pollinated Plants in Britain and the Netherlands. Science 313:351–354.

Burkle, L. A., J. C. Marlin, and T. M. Knight. 2013. Plant-Pollinator Interactions over 120 Years: Loss of Species, Co-Occurrence, and Function. Science 339:1611–1615.

Dearing, M. D., and L. Dizney. 2010. Ecology of hantavirus in a changing world. Annals of the

New York Academy of Sciences 1195:99–112.

DeGrandi-Hoffman, G., and Y. Chen. 2015. Nutrition, immunity and viral infections in honey bees. Current Opinion in Insect Science 10:170–176.

Donkersley, P., G. Rhodes, R. W. Pickup, K. C. Jones, and K. Wilson. 2014. Honeybee nutrition is linked to landscape composition. Ecology and Evolution 4:4195–206.

Duffy, M. A., C. E. Cáceres, S. R. Hall, A. J. Tessier, and A. R. Ives. 2010. Temporal, spatial, and between-host comparisons of patterns of parasitism in lake zooplankton. Ecology 91:3322–3331.

Estrada-Peña, A. 2009. Diluting the dilution effect: A spatial Lyme model provides evidence for the importance of habitat fragmentation with regard to the risk of infection. Geospatial Health 3:143–155.

Estrada-Peña, A., R. S. Ostfeld, A. T. Peterson, R. Poulin, and J. de la Fuente. 2014. Effects of environmental change on zoonotic disease risk: An ecological primer. Trends in Parasitology 30:205–214.

Ezenwa, V. O., M. S. Godsey, R. J. King, and S. C. Guptill. 2006a. Avian diversity and West Nile virus: Testing associations between biodiversity and infectious disease risk. Proceedings of the Royal Society B: Biological Sciences 273:109–117.

Ezenwa, V. O., M. S. Godsey, R. J. King, and S. C. Guptill. 2006b. Avian diversity and West Nile virus: Testing associations between biodiversity and infectious disease risk. Proceedings of the Royal Society B: Biological Sciences 273:109–117.

Faust, C. L., A. P. Dobson, N. Gottdenker, L. S. P. Bloomfield, H. I. Mccallum, T. R. Gillespie, M. Diuk-Wasser, R. K. Plowright, G. TR, and P. RK. 2017. Null expectations for disease dynamics in shrinking habitat: dilution or amplification? Phil Trans R Soc B 372:20160173.

Fountain-Jones, N. M., C. Packer, J. L. Troyer, K. VanderWaal, S. Robinson, M. Jacquot, and M. E. Craft. 2017. Linking social and spatial networks to viral community phylogenetics reveals subtype-specific transmission dynamics in African lions. Journal of Animal Ecology 86:1469–1482.

Graystock, P., D. Goulson, and W. O. H. Hughes. 2015. Parasites in bloom: flowers aid dispersal and transmission of pollinator parasites within and between bee species. Proceedings of the Royal Society Biological Sciences 282:20151371.

Halliday, F. W., R. W. Heckman, P. A. Wilfahrt, and C. E. Mitchell. 2017. A multivariate test of disease risk reveals conditions leading to disease amplification. Proceedings of the Royal Society B: Biological Sciences 284:20171340.

Hamede, R. K., J. Bashford, H. McCallum, and M. Jones. 2009. Contact networks in a wild Tasmanian devil (Sarcophilus harrisii) population: using social network analysis to reveal seasonal variability in social behaviour and its implications for transmission of devil facial tumour disease. Ecology Letters 12:1147–1157.

Huang, Z. Y. X., F. van Langevelde, A. Estrada-Peña, G. Suzán, and W. F. de Boer. 2016. The diversity–disease relationship: evidence for and criticisms of the dilution effect. Parasitology 143:1075–1086.

Johnson, P. T. J., R. S. Ostfeld, and F. Keesing. 2015. Frontiers in research on biodiversity and disease. Ecology Letters 18:1119–1133.

Johnson, P. T. J., D. L. Preston, J. T. Hoverman, and B. E. Lafonte. 2013a. Host and parasite diversity jointly control disease risk in complex communities. Proceedings of the National Academy of Sciences 110:16916–16921.

Johnson, P. T. J., D. L. Preston, J. T. Hoverman, and K. L. D. Richgels. 2013b. Biodiversity

decreases disease through predictable changes in host community competence. Nature 494:230–3.

Jones, K., N. Patel, M. Levy, A. Storeygard, D. Balk, J. Gittleman, and P. Daszak. 2008. Global trends in emerging infectious diseases. Nature 451:990–993.

Kappeler, P. M., S. Cremer, and C. L. Nunn. 2015. Sociality and health: Impacts of sociality on disease susceptibility and transmission in animal and human societies. Philosophical Transactions of the Royal Society B: Biological Sciences 370:20140116.

Kirk, D., D. Shea, and D. Start. 2019. Host traits and competitive ability jointly structure disease dynamics and community assembly. Journal of Animal Ecology 88:1379–1391.

Lafferty, K. D., and C. L. Wood. 2013. It's a myth that protection against disease is a strong and general service of biodiversity conservation: Response to Ostfeld and Keesing. Trends in Ecology and Evolution 28:503–504.

Langlois, J. P., L. Fahrig, G. Merriam, and H. Artsob. 2001. Landscape structure influences continental distribution of hantavirus in deer mice. Landscape Ecology 16:255–266.

Lonsdorf, E., C. Kremen, T. Ricketts, R. Winfree, N. Williams, and S. Greenleaf. 2009. Modelling pollination services across agricultural landscapes. Annals of Botany 103:1589–1600.

Mazzei, M., M. L. Carrozza, E. Luisi, M. Forzan, M. Giusti, S. Sagona, F. Tolari, and A. Felicioli. 2014. Infectivity of DWV associated to flower pollen: experimental evidence of a horizontal transmission route. PloS ONE 9:e113448.

Michener, C. D. 2007. The Bees of the World. 2nd edition. John Hopkins University Press.

Moore, S. M., and E. T. Borer. 2012. The influence of host diversity and composition on epidemiological patterns at multiple spatial scales. Ecology 93:1095–1105.

Murray, T. E., Ú. Fitzpatrick, A. Byrne, R. Fealy, M. J. F. Brown, and R. J. Paxton. 2012. Local-scale factors structure wild bee communities in protected areas. Journal of Applied Ecology 49:998–1008.

Di Pasquale, G., M. Salignon, Y. Le Conte, L. P. Belzunces, A. Decourtye, A. Kretzschmar, S. Suchail, J. L. Brunet, and C. Alaux. 2013. Influence of Pollen Nutrition on Honey Bee Health: Do Pollen Quality and Diversity Matter? PLoS ONE 8:1–13.

Piot, N., I. Meeus, D. Kleijn, J. Scheper, T. Linders, and G. Smagghe. 2019. Establishment of wildflower fields in poor quality landscapes enhances micro-parasite prevalence in wild bumble bees. Oecologia 189:149–158.

Potts, S. G., J. C. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger, and W. E. Kunin. 2010. Global pollinator declines: Trends, impacts and drivers. Trends in Ecology and Evolution 25:345–353.

Rigaud, T., M.-J. Perrot-Minnot, and M. J. F. Brown. 2010. Parasite and host assemblages: embracing the reality will improve our knowledge of parasite transmission and virulence. Proceedings of the Royal Society Biological Sciences 277:3693–702.

Rohr, J. R., D. J. Civitello, F. W. Halliday, P. J. Hudson, K. D. Lafferty, C. L. Wood, and E. A. Mordecai. 2020. Towards common ground in the biodiversity–disease debate. Nature Ecology and Evolution 4:24–33.

Ruiz-Gonzalez, M. X., J. Bryden, Y. Moret, C. Reber-funk, P. Schmid-hempel, and M. J. F. Brown. 2012. Dynamic Transmission, Host Quality, and Population Structure in a Multihost Parasite of Bumblebees. Evolution 66:3053–3066.

Salathé, M., M. Kazandjieva, J. W. Lee, P. Levis, M. W. Feldman, and J. H. Jones. 2010. A high-resolution human contact network for infectious disease transmission. Proceedings of

the National Academy of Sciences of the United States of America 107:22020–22025.

Salkeld, D. J., K. A. Padgett, and J. H. Jones. 2013. A meta-analysis suggesting that the relationship between biodiversity and risk of zoonotic pathogen transmission is idiosyncratic. Ecology Letters 16:679–686.

Singh, R., A. L. Levitt, E. G. Rajotte, E. C. Holmes, N. Ostiguy, D. Vanengelsdorp, W. I. Lipkin, C. W. Depamphilis, A. L. Toth, and D. L. Cox-Foster. 2010. RNA viruses in hymenopteran pollinators: evidence of inter-taxa virus transmission via pollen and potential impact on non-Apis hymenopteran species. PLoS ONE 5:e14357.

Strauss, A. T., A. M. Bowling, M. A. Duffy, C. E. Cáceres, and S. R. Hall. 2018. Linking host traits, interactions with competitors and disease: Mechanistic foundations for disease dilution. Functional Ecology 32:1271–1279.

Swaddle, J. P., and S. E. Calos. 2008. Increased avian diversity is associated with lower incidence of human West Nile infection: Observation of the dilution effect. PLoS ONE 3.

Truitt, L. L., S. H. McArt, A. H. Vaughn, and S. P. Ellner. 2019. Trait-based modeling of multihost pathogen transmission: Plant-pollinator networks. American Naturalist 193:E149–E167.

Tuell, J. K., and R. Isaacs. 2010. Community and species-specific responses of wild bees to insect pest control programs applied to a pollinator-dependent crop. Journal of Economic Entomology 103:668–675.

Vaudo, A. D., J. F. Tooker, C. M. Grozinger, and H. M. Patch. 2015. Bee nutrition and floral resource restoration. Current Opinion in Insect Science 10:133–141.

Venesky, M. D., X. Liu, E. L. Sauer, and J. R. Rohr. 2014. Linking manipulative experiments to field data to test the dilution effect. Journal of Animal Ecology 83:557–565.

Wilber, M. Q., K. M. Pepin, H. Campa III, S. E. Hygnstrom, M. J. Lavelle, T. Xifara, K. C. VerCauteren, and C. T. Webb. 2019. Modelling multi-species and multi-mode contact networks: Implications for persistence of bovine tuberculosis at the wildlife–livestock interface. Journal of Applied Ecology 56:1471–1481.

Williams, N. M., E. E. Crone, T. H. Roulston, R. L. Minckley, L. Packer, and S. G. Potts. 2010. Ecological and life-history traits predict bee species responses to environmental disturbances. Biological Conservation 143:2280–2291.

Wood, C. L., and K. D. Lafferty. 2013. Biodiversity and disease: A synthesis of ecological perspectives on Lyme disease transmission. Trends in Ecology and Evolution 28:239–247.

Wood, C. L., K. D. Lafferty, G. DeLeo, H. S. Young, P. J. Hudson, and A. M. Kuris. 2014. Does biodiversity protect humans against infectious disease? Ecology 95:817–832.

Wood, C. L., A. McInterff, H. S. Young, D. Kim, and K. D. Lafferty. 2017. Human infectious diease burdens decrease with urbanization but not with biodiversity. Philosophical Transactions of the Royal Society B: Biological Sciences 372:20160122.