DR. MICHELLE LYNDSEY FEARON (Orcid ID : 0000-0001-6849-4834)

Article type : Articles

**Journal: Ecology**

**Manuscript type: Article**

**Running Head:** Bee richness dilutes multiple viruses

**Title:** Pollinator community species richness dilutes prevalence of multiple viruses within multiple host species

**Author List:**

Michelle L. Fearon[1, 2] and Elizabeth A. Tibbetts[1]

[1]Department of Ecology & Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109

[2]Corresponding Author. E-mail: mlfearon@umich.edu

Manuscript received 21 September 2020; accepted 24 November 2020.

**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, contribute to pathogen spread for many host–pathogen systems. Evaluating relationships among multiple pathogens and hosts may clarify whether particular host or pathogen traits consistently drive links between community factors and pathogen prevalence. Pollinators are a good system to test how community composition influences pathogen spread because pollinator communities are extremely variable and contain several multi-host pathogens

transmitted on shared floral resources. We conducted a field survey of four pollinator species to test the prevalence of three RNA viruses (deformed wing virus, black queen cell virus, and sacbrood virus) among pollinator communities with variable species richness, abundance, and composition. All three viruses showed a similar pattern of prevalence among hosts. *Apis mellifera* and *Bombus impatiens* had significantly higher viral prevalence than *Lasioglossum* spp. and *Eucera pruinosa*. In each species, lower virus prevalence was most strongly linked with greater pollinator community species richness. In contrast, pollinator abundance, species-specific pollinator abundance, and community composition were not associated with virus prevalence. Our results support a consistent dilution effect for multiple viruses and host species. Pollinators in species-rich communities had lower viral prevalence than pollinators from species-poor communities, when accounting for differences in pollinator abundance. Species-rich communities likely had lower viral prevalence because species-rich communities contained more native bee species likely to be poor viral hosts than species-poor communities, and all communities contained the highly competent hosts *A. mellifera* and *B. impatiens*. Interestingly, the strength of the dilution effect was not consistent among hosts. Instead, host species with low viral prevalence exhibited weaker dilution effects compared to hosts with high viral prevalence. Therefore, host species susceptibility and competence for each virus may contribute to variation in the strength of dilution effects. This study expands biodiversity–disease studies to the pollinator–virus system, finding consistent evidence of the dilution effect among multiple similar pathogens that infect 'replicate' host communities.

**Key Words:** *Apis mellifera*, black queen cell virus, *Bombus*, community composition, deformed wing virus, dilution effect, biodiversity–disease, multi-host pathogens, native bees, sacbrood virus.

## 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. Heterogeneity among host species in their likelihood of encountering, becoming infected (i.e. susceptibility), and transmitting pathogens to other hosts (i.e. competency) contribute to variation in pathogen transmission and prevalence among

communities (Fenton et al. 2015). Therefore, the biodiversity, relative abundance, and identity of hosts present in a community may influence pathogen prevalence (LoGiudice et al. 2003, Keesing et al. 2006). For example, differences in bird community diversity, relative abundance, and composition predict differences in West Nile virus prevalence in birds and humans due to heterogeneity in bird host competence and transmission rates (Ezenwa et al. 2006, Kilpatrick et al. 2006).

Pathogen characteristics, such as host ranges and modes of transmission, also have strong effects on patterns of multi-host pathogen prevalence (Woolhouse and Gowtage-Sequeria 2005). 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 (hereafter, 'biodiversity–disease relationship')(Rohr et al. 2020). For example, Wood et al. found that pathogen characteristics were important for determining whether greater wildlife biodiversity could reduce, increase, or not affect prevalence of many human pathogens (Wood et al. 2014a). Thus far, few studies have evaluated variability among hosts and pathogens in how host community factors, 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 to influence disease dynamics: host species diversity, host abundance, and community composition (Keesing et al. 2010, Roche et al. 2012). 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 less competent hosts or reduce encounters, transmission, or density of the competent hosts (Ostfeld and Keesing 2000, Keesing et al. 2006). The dilution effect is supported by the tick-born Lyme disease system. High vertebrate biodiversity reduces *Borrelia burgdorferi* prevalence because ticks are more likely to feed on less competent hosts in diverse communities compared to species-poor communities dominated by highly competent white-footed mice (Ostfeld and Keesing 2000). Though there is growing evidence for the dilution effect in many multi-host–pathogen systems (Ezenwa et al. 2006, Clay et al. 2009, Johnson et al. 2013b, Venesky et al. 2014), other studies have found different biodiversity–disease relationships (Salkeld et al. 2013, Luis et al. 2018).

Biodiversity–disease relationships can also exhibit the 'amplification effect', where greater host species diversity increases pathogen prevalence (Keesing et al. 2006). The amplification effect is likely when highly competent hosts are found in species-rich rather than species-poor communities, or additional species facilitate greater pathogen transmission among hosts (Keesing et al. 2006, Luis et al. 2018). Additionally, some pathogens are not influenced by changes in community diversity, and therefore could have a neutral biodiversity–disease relationship (Wood et al. 2014a, Rohr et al. 2020). There is much interest in when different biodiversity–disease relationships are observed and their underlying mechanisms (Randolph and Dobson 2012, Wood and Lafferty 2013, 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 (Mihaljevic et al. 2014), therefore it is important to evaluate the relative contributions of host diversity and host abundance to observed biodiversity–disease relationships to elucidate their underlying mechanisms (Rudolf and Antonovics 2005). As biodiversity increases, the addition of less competent hosts can reduce the abundance of highly competent hosts to subsequently reduce pathogen transmission and prevalence, known as the 'susceptible host regulation' mechanism of the dilution effect (Keesing et al. 2006). For example, Mitchell et al. (2002) found reduced disease severity of several species-specific foliar fungal diseases in species-rich plant communities, but the pattern was driven by lower species-specific densities in the species-rich plots rather than biodiversity *per se*. Alternatively, diverse communities that contain multiple competent host species could result in 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 are shared among several abundant and susceptible host species in a community.

Host community composition, including both species identity and relative abundance, can also 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 (Ostfeld and LoGiudice 2003, Fenton et al. 2015). If highly competent hosts are common in species-poor communities and additional species in diverse communities are more likely to be less competent hosts, then a dilution effect pattern is more likely to occur. For example, Johnson et al. (2013b) 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. In this case, the dilution effect pattern is due to the presence of particular host species rather than host species richness alone. Previous studies have shown that the presence of highly competent or low competence "diluter" hosts can be important predictors of pathogen prevalence in diverse host–pathogen systems, including Lyme disease in vertebrates (LoGiudice et al. 2003), West Nile Virus in birds (Ezenwa et al. 2006), *Batrachochytrium dendrobatidis* in amphibians (Becker et al. 2014, Venesky et al. 2014), and *Metschnikowia* fungus in *Daphnia* (Strauss et al. 2018). Though many studies have tested the relative impacts of host community diversity, abundance, and composition on pathogen prevalence, few studies have compared the effects these 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. Similar traits among hosts or pathogens can lead to consistently negative biodiversity–disease relationships, where pathogen prevalence is diluted by increased host diversity or other community factors (Ezenwa et al. 2006, Johnson et al. 2013a, 2013b, Becker et al. 2014, Venesky et al. 2014). However, in some cases, biodiversity–disease outcomes may diverge from each other based on key differences in specific host traits or pathogen characteristics (Becker et al. 2014, Wood et al. 2014a, 2014b, Strauss et al. 2015, 2018). 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. 2014a, Strauss et al. 2015). Therefore, by simultaneously studying biodiversity–disease relationships for multiple similar pathogens each infecting multiple related host species, we can look for common patterns 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 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). 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). Pollinator species vary substantially in their flower preferences, sociality, and other life history traits (Williams et al. 2010), which could impact the likelihood of pathogen exposure and infection among different hosts and in different community contexts.

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). Growing evidence suggests that these viruses are bidirectionally transmitted among managed honey bees and native bees (Singh et al. 2010, Fürst et al. 2014, McMahon et al. 2015, Alger et al. 2019, Grozinger and Flenniken 2019). Though these viruses may be generalist pathogens capable of infecting a wide diversity of species, all three viruses are most commonly found in honey bees and less commonly detected in other native pollinator species (Singh et al. 2010, Manley et al. 2015, Dolezal et al. 2016). Viral infections in early life stages (e.g. larval or pupal) cause mortality in honey bees, while infected adults are typically asymptomatic but can still transmit the virus (Chen and Siede 2007, Grozinger and Flenniken 2019). Some native bees may experience reduced viral virulence compared to honey bees (Dolezal et al. 2016), but viral virulence in native bee species has received limited study. Viral transmission among conspecifics is likely food-borne or fecal-oral (Chen and Siede 2007) via contact on flowers (McArt et al. 2014). DWV and BQCV have been detected on whole flowers near apiaries and on pollen collected by bees, and honey bees can become infected after consuming virus-contaminated pollen (Singh et al. 2010, Mazzei et al. 2014, Alger et al. 2019).

Sampling pollinator communities

We collected pollinators from 14 winter squash farms in Michigan, USA, with permission granted by private landowners (Appendix S1: Table S1). All fields were adjacent to either corn or apple orchards, except for the GT and S sites, which had small plots of other specialty vegetables. Field sites were at least 10 km away from each other, so it is unlikely that bees observed at one site visited other field sites. We sampled the pollinator communities at each site twice during the peak squash flower bloom (July and August), and maintained even sampling effort 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 during the peak squash bloom period (18 July – 21 August 2015 and 26 July – 2 September 2016).

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. We hand-netted pollinators within 1.5-m of each transect line once for 30 minutes at 0800, 1000, 1100 and

1200. We did not collect in the afternoon because squash flowers close by midday. Fluorescent blue, yellow, and white pan traps were set along the transect between the crop rows 5-m apart in an alternating color pattern. 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 were frozen for later identification and viral analysis. Bee collection method (i.e. netting or pan traps) was not correlated with virus presence or absence (Appendix S1: Table S2).

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 total occurrences in our sample were identified to genus.

Detecting viral positive strand prevalence

We tested for BQCV, DWV, and SBV within four pollinator species: *Apis mellifera* (n = 237), *Bombus impatiens* (n = 252), *Eucera pruinosa* (n = 193), and *Lasioglossum* spp. (n = 255). These four species were the most consistently abundant species among all communities sampled (Appendix S1: Table S3). We tested up to 20 randomly selected individuals from each species per site, and tested all individuals available when less than 20 were collected at a site (Appendix S1: Table S4).

Tissue from half of each bee's abdomen was homogenized using a FastPrep-24 (MP Biomedicals, Santa Clara, CA, USA) for 1 minute at 4.0 M/sec. RNA was extracted using TRIzol reagent (Ambion, Austin, TX, USA) 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, Carlsbad, CA, USA). We found that RNA concentration did not impact the likelihood of detecting viral presence (Appendix S1: Table S2, Appendix S2: Section S1). 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, Madison, WI, USA) and 0.25 µM random hexamers (Invitrogen) according to manufacturer's instructions.

We tested for the presence or absence of BQCV, DWV, and SBV positive strand using PCR with established virus-specific primers (Appendix S1: Table S5). 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. To confirm adequate RNA extraction and reverse transcription of all bee samples, we ran PCR for

each sample with *A. mellifera* 18S rRNA gene primers (Cardinal et al. 2010) as a control. Further reaction details are provided in Appendix S2: Section S1. All PCR products were visualized with gel electrophoresis to determine virus presence or absence. We sequenced a subset of the PCR products to confirm identification of viral RNA and the 18S gene (GenBank Accession Numbers in Appendix S1: Table S6).

The BQCV, DWV, and SBV prevalence observed in this study are representative of current spillover among pollinator species. The primers we used were created from honey bee virus sequences (Singh et al. 2010), so they could slightly underestimate the virus prevalence in native bees. However, data from several studies indicate that native bees share the same virus strains with local honey 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). Further, the primers we use are well established for successfully testing for viral positive and negative strand presence in many bee, wasp, and non-Hymenopteran insect species (Singh et al. 2010, Levitt et al. 2013, Fürst et al. 2014, McMahon et al. 2015, Bailes et al. 2018).

Screening for the viral negative strand

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 host (Ongus et al. 2004, Yue and Genersch 2005). Up to 26 virus-positive bee samples from each of the focal bee species per virus were randomly selected from all sites to test for the presence 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 S1: Table S7). Negative-strand specific cDNA synthesis was carried out with 2.5 µl RNA template with M-MLV reverse transcriptase (Promega) and tagged negative-strand specific primers for BQCV, DWV, and SBV, followed by PCR with negative and virus-specific positive controls (primer details in Appendix S1: Table S5). All samples were visualized with gel electrophoresis, and a subset of samples were sequenced to confirm identification of the negative strand viral sequences (GenBank Accession Numbers in Appendix S1: Table S6). Additional reaction details are in Appendix S2: Section S2.

Statistical analysis

All analyses were performed in the program R v4.0.2 (R Core Team 2020). We used a global Generalized Linear Mixed effects model (GLMM) of virus prevalence including all three viruses within the four host species with a binomial distribution and logit link function (*lme4* package) (Bates et al. 2015). Here, we use 'virus prevalence' as the response variable in our global model based on the presence or absence of the viral positive strand for each individual bee. The 'infection prevalence', based on the presence of the viral negative strand, had insufficient sample size among hosts and sites to be used in the global model (see Appendix S2: Section S3.1 for further discussion). For random effects, we included visit number 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 BQCV, DWV, and SBV. All models included species richness, total pollinator abundance, virus type (BQCV, DWV, and SBV), and host species (*A. mellifera*, *B. impatiens*, *Lasioglossum* spp., and *E. 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- (Models 2a–f), three- (Models 3a–e), and four-way interactions (Model 4) in a model selection table ranked by lowest AICc score (*MuMIn* package; Table 1, Appendix S1: Table S8, top model selection details in Appendix S2: Section S3.2) (Barton 2020). Significant main effects do not differ between any of the top models, indicating that our key results are robust.

All top models included 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 (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 3, Appendix S1: Table S9) (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 S1: Table S10) (Dormann et al. 2013). Furthermore, we compared the results from the top 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 S1: Table S11). 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.

There was no evidence of significant spatial autocorrelation in the model residuals for any model, indicating that closely located communities did not have significantly similar virus prevalence (Moran's I test using packages *ape* and *DHARMa*; Appendix S1: Table S12) (Paradis and Schliep 2018, Hartig 2020). 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 divided by the number of virus-positive samples that were tested, which indicates active replication in the host (Ongus et al. 2004, Yue and Genersch 2005). We compared negative strand infection prevalence in each of the four host species within each virus using a Chi-squared test of two proportions. We used a Bonferroni correction for multiple comparisons to determine significant differences among host species ($\alpha^* = 0.05/6 = 0.0083$). The Chi-squared test approach achieved similar results when compared with the GLMM post-hoc analysis comparing differences in virus prevalence (positive strand) among the four host species (described above).

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. Community composition was assessed qualitatively through differences in the relative abundance of pollinator species and Non-metric Multi-Dimensional Scaling (NMDS) (described below). 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 S1: Fig. S1) (Oksanen et al. 2018). To determine if we captured the pollinator species richness within each community, we created individual-based rarefaction curves (*iNext* package) and compared the observed species richness to the estimated species richness at the asymptote of the rarefaction curve (Appendix S1: Fig. S2) (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 the ranking order of the communities (Appendix S1: Table S13). Additionally, we found that our results were robust regardless of method used to estimate species richness because models with two different methods of estimating species richness showed the same results as Model 2a (Appendix S1: Table S14 and S15, details in Appendix S2: Section S3.3). 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 all pollinator species identities and relative abundances collected at each site. Specifically, this analysis examines whether other community members beyond the four focal host species may be indicator species correlated with higher virus prevalence by evaluating the presence/absence and relative abundance of all pollinator species in the community. We predict that communities that include a key indicator species will show a consistent correlation with high virus prevalence, but we expect that rare and low-density pollinator species are unlikely to show significant correlation with virus prevalence. The NMDS ordination of the pollinator communities was created using a Bray-Curtis dissimilarity matrix (*vegan* package) (Oksanen et al. 2018). A two-dimensional solution for the NMDS ordination of pollinator community composition yielded a stress value of 0.1324, 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 BQCV, DWV, and SBV prevalence within each of the four host species and the ordination of pollinator communities using fitted smooth surfaces (i.e. contour lines) calculated using Generalized Additive Models (GAM) with thin-plate splines ('ordisurf' function; *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 (additional details in Appendix S2: Section S3.4).

## Results

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

*Virus and infection prevalence were highly variable among honey bees and native bees*

The BQCV, DWV, and SBV positive strands were detected in the four focal pollinator species: *Apis mellifera, Bombus impatiens*, *Lasioglossum* spp., and *Eucera pruinosa* (Fig. 1, Appendix S1: Table S16 and S17). 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 (Fig. 1, Table 1, Appendix S1: Table S18). BQCV and DWV had the same overall pattern of prevalence among the four host species tested, where *A. mellifera* had significantly higher prevalence than *B. impatiens*, which in turn was significantly higher than both *Lasioglossum* spp. and *E. pruinosa* (Fig. 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).

We also tested the prevalence of viral infection by testing for BQCV, DWV, and SBV negative strand in each host species (hereafter, 'infection prevalence'). The viral negative strand for all three viruses was present in all four host species, except for SBV in *Lasioglossum* spp. (Table 2). *Lasioglossum* spp. had very low SBV prevalence detected (0.2%, a single SBV-positive individual), so it is unsurprising that we found no evidence of the SBV negative 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, Appendix S1: Table S19). The infection prevalence presented here was an estimate since we only tested a subset of virus-positive specimen from each species, but the data clearly showed 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 varied in abundance, richness, and composition*

Across both sampling years, we collected 4,737 bees and wasps from 14 communities, including at least 126 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: 46 to 756 individuals) (Fig. 2). Furthermore, pollinator community composition varied qualitatively among sites, as the relative abundance of key pollinator species differed among communities (Fig. 2, Appendix S1: Fig. S3). 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 = 54.2°, p = 0.01; Appendix S1: Fig. S1). 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) ranged from 0.46 to 0.85 among the different communities (Appendix S1: Table S13).

*Virus prevalence was 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 (Model 2a; Table 3). Specifically, all four host species had significantly reduced DWV prevalence in communities with greater pollinator species richness (Fig. 3a). Additionally, *A. mellifera* and *B. impatiens* had significantly reduced BQCV and SBV prevalence in species-rich communities (Fig. 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 (Fig. 3b, Table 3, Appendix S1: Table S11).

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* SBV prevalence and *Lasioglossum* spp.

DWV prevalence (*A. mellifera* SBV: $F = 3.02$, $p = 0.03$, Adj $R^2 = 0.68$; *Lasioglossum* spp. DWV: $F = 2.15$, $p = 0.02$, Adj $R^2 = 0.60$; Appendix S1: Fig. S4a and S4c).

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

*Consistent relationships between virus prevalence and pollinator community species richness and abundance in hosts and pathogens*

All three viruses showed significantly reduced virus prevalence in species-rich communities within host species that had greater than 10% estimated virus prevalence (Fig. 3a). The strength of the negative relationships varied among host species based on their relative viral prevalence. BQCV and SBV showed 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* were rarely infected with BQCV or SBV, and showed no strong relationship between virus prevalence and species richness (Fig. 1, Fig. 3a). None of the host–virus pairs had greater virus prevalence in species-rich communities.

When comparing across either hosts or viruses, virus prevalence was largely unlinked with community composition. In two of the twelve host–virus pairs, there were significant relationships between viral prevalence and community composition, but the direction of the relationships varied (Appendix S1: Fig. S4).

There were no significant relationships between virus prevalence and pollinator community total abundance among all host species and viruses tested (Table 3, Fig. 3b, Appendix S1: Table S11).

## Discussion

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 for pollinator viruses for the first time. For multiple viruses within multiple bee host species, communities with greater pollinator species richness had lower viral prevalence than species-poor communities, but the strength of the relationships appear to vary based on each species' 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 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 (Fig. 3). 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 community-wide pathogen prevalence (Ezenwa et al. 2006, Johnson et al. 2013a, 2013b, Becker et al. 2014, Venesky et al. 2014, Strauss et al. 2018).

The pollinator–virus system has many characteristics that typically facilitate the dilution effect in other host–pathogen systems. The dilution effect is likely to occur when the most competent host dominates species-poor communities, and more disease resistant host species are common in species-rich communities (LoGiudice et al. 2003, Keesing et al. 2006, Johnson et al. 2013b). 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 S1: Fig. S1). Species poor communities are dominated by the four focal hosts in our study, two of which (*A. mellifera* and *B. impatiens*) are competent hosts with high prevalence for all three viruses (Fig. 1). Species-rich communities include many 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).

Our results suggest that the encounter reduction mechanism of the dilution effect may operate in the pollinator–virus system. 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 competence hosts in species-rich communities (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 and to improve future predictions of disease risk.

Species abundance

Community factors other than biodiversity were not strongly associated with virus prevalence, including total pollinator abundance and the species-specific abundances of the four focal host species (Fig. 3b, Table 3, Appendix S1: Table S11). Changes in community diversity often correspond with changes in the total host abundance and/or relative abundance of specific host species, which can lead to the 'susceptible host regulation' mechanism of the dilution effect (Rudolf and Antonovics 2005, Randolph and Dobson 2012, Mihaljevic et al. 2014). Susceptible host regulation could operate in the pollinator–virus system if additional low competence hosts compete with susceptible hosts to constrain their abundance and reduce pathogen spread (Keesing et al. 2006). Most of the other pollinator species in these communities were rare (less than 5 individuals observed per site) and are unlikely to explain community-level differences in virus prevalence (see Appendix S1: Fig. S4 for an analysis that considers additional pollinator species). Further, we found no relationship between pollinator host abundance and virus prevalence over all, so susceptible host regulation is unlikely to mediate the dilution effect.

The lack of relationship between host abundance and viral prevalence suggests that BQCV, DWV, and SBV may have frequency-dependent transmission rather than density-dependent transmission. The three viruses are likely transmitted within and among host species through interactions on flowers and through contaminated pollen (Singh et al. 2010, McArt et al. 2014, McMahon et al. 2015). 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. 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–virus 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, Becker et al. 2014). 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, which could lead to either dilution or amplification effects depending on the host species' traits (Loreau M. and Hector. A. 2001). However, virus prevalence among all four pollinator species was generally unrelated to community composition.

Community composition may also influence virus prevalence if the presence of particular pollinator species influences the likelihood of viral encounter or transmission by altering interactions among host species on shared flowers. Though our study does not evaluate the "complementarity effect" mechanism from BEF literature, where pathogen transmission is reduced through less habitat sharing among host species in diverse communities, it could occur in pollinator pathogen systems (Loreau M. and Hector. A. 2001, Becker et al. 2014). 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.

Consistent evidence of dilution among pathogens and hosts

We found similar, negative biodiversity–disease relationships among multiple viruses and multiple hosts, but the strength of the dilution effect varied among hosts. Variation in the strength of relationships between biodiversity and pathogen prevalence is likely due to variation in relative viral competence among different host species. *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. The four host species differ in their social behavior and whether they are floral specialists or generalists. Both factors may influence variation viral exposure and prevalence, and result in variable strength in the observed dilution effects among hosts.

Perhaps we found similar biodiversity–disease relationships among pathogens because the three viruses are quite similar. 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, McMahon et al. 2018). Similarly, Johnson et al. also found consistently reduced infection success with greater host diversity for five out of seven trematode parasites that share many pathogen characteristics (Johnson et al. 2013a). Previous studies and meta-analyses have compared biodiversity–disease relationships among highly divergent pathogens, generally finding that pathogen ecology, transmission mode, infectivity, or degree of host specialization influence these relationships (Randolph and Dobson 2012, Salkeld et al. 2013, Wood et al. 2014a, 2014b, Rohr et al. 2020). Utilizing a comparative approach for multiple pathogens within 'replicate' host communities will clarify 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 BQCV, DWV, and SBV are shared among many pollinator species (Singh et al. 2010, Fürst et al. 2014, McMahon et al. 2015, Dolezal et al. 2016). However, our study design more accurately assesses differences in BQCV, DWV, and SBV prevalence by using larger sample sizes per species. The results show that *A. mellifera* are highly susceptible and competent hosts for all three viruses. *B. impatiens*, a close relative of *A. mellifera*, was also a relatively competent host for all three viruses, but had lower DWV and BQCV prevalence. The more distantly related *E. pruinosa* and *Lasioglossum* spp. have lower viral and infection prevalence, suggesting that both are likely poor hosts, less susceptible, and/or less likely to encounter infective viruses.

BQCV, DWV, and SBV appear to vary in their host ranges from generalist to relatively specialist pathogens that primarily infect very closely related hosts. DWV appears to be the broadest generalist pathogen of the three, causing active infections in a wide range of Hymenoptera (Singh et al. 2010, Manley et al. 2015, Dolezal et al. 2016). Meanwhile, SBV has the most restrictive host range limited primarily to honey bees and bumblebees (Bombus spp.), and BQCV is intermediate between the two (Manley et al. 2015). Despite some differences in host range, all three viruses showed very similar biodiversity–disease relationships.

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) (Burkle et al. 2013), and in their specific foraging and nesting habitat requirements (Williams et al. 2010), which result in highly dynamic pollinator communities through time and space. Repeated temporal sampling of a few sites showed that pollinator community diversity declined throughout the growing season, but *Nosema* spp. and *Crithidia* spp. parasite prevalence increased with greater *A. mellifera* and *Bombus* spp. dominance in the communities (Graystock et al. 2020). Our study provides an initial investigation of biodiversity–disease relationships for pollinator viruses toward the end of the growing season and across many similar sites with variable surroundings. Future studies that examine these relationships over different spatial scales and with repeated temporal sampling will be critical for understanding the context-dependence of biodiversity–disease relationships in pollinator–pathogens (Johnson et al. 2015, Graystock et al. 2020, Rohr et al. 2020).

Conclusions

Overall, prevalence of three viruses in pollinator communities was most strongly linked with species richness, while host abundance and community composition were rarely associated with virus prevalence. Notably, virus prevalence was consistently negatively associated with greater species richness—providing evidence of the dilution effect in multiple viruses infecting multiple pollinator host species. However, we found that the strength of the biodiversity–disease

relationships varied based on relative viral prevalence in each host. Host species with high virus prevalence exhibited dilution effects, while hosts with very low virus prevalence did not show 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.

**Acknowledgments**

All molecular work for this study was conducted in the Biodiversity Laboratory of the Department of Ecology and Evolutionary Biology (EEB), and Sanger sequencing was carried out by the Advanced Genomics Core, University of Michigan (UM). We thank R. Marchan Rivadeneira for her help with developing the molecular methods and J. Graham, M. Scales, K. Lam, and Y. Oden-Plants for their assistance collecting pollinators. We also thank numerous undergraduate students that aided with pollinator identification and molecular work: M. Zbrozek, M. Frank, C. Weise, H. Zonnevylle, C. Fleming, G. Flint, and E. Collins-Carey. Funding for this work was provided by NSF IOS-1557564, the North American Pollinator Protection Campaign, Pollinator Partnership, The Garden Club of America, the UM Rackham Graduate School and Department of EEB. We thank the reviewers for their helpful comments that contributed to improving this manuscript. MLF and EAT developed manuscript ideas, MLF carried out data collection and analysis with advice from EAT, and MLF and EAT wrote the manuscript.

**Supporting Information**

Additional supporting information may be found online at: [link to be added in production]

**Data Availability**

All data and code used for the analyses and figures contained in this manuscript are available on Dryad (Fearson and Tibbetts 2021): https://doi.org/10.5061/dryad.zpc866t7g.

## 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.

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.

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. 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.

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.

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,

669 D. Zurell, and S. Lautenbach. 2013. Collinearity: a review of methods to deal with it and a
670 simulation study evaluating their performance. Ecography 36:27–46.
671 Estrada-Peña, A., R. S. Ostfeld, A. T. Peterson, R. Poulin, and J. de la Fuente. 2014. Effects of
672 environmental change on zoonotic disease risk: An ecological primer. Trends in
673 Parasitology 30:205–214.
674 Ezenwa, V. O., M. S. Godsey, R. J. King, and S. C. Guptill. 2006. Avian diversity and West Nile
675 virus: Testing associations between biodiversity and infectious disease risk. Proceedings of
676 the Royal Society B: Biological Sciences 273:109–117.
677 Fearon, M. and E. Tibbetts. 2021. Data from: Pollinator community species richness dilutes
678 prevalence of multiple viruses within multiple host species. Dryad, data set.
679 https://doi.org/10.5061/dryad.zpc866t7g
680 Fenton, A., D. G. Streicker, O. L. Petchey, and A. B. Pedersen. 2015. Are All Hosts Created
681 Equal? Partitioning Host Species Contributions to Parasite Persistence in Multihost
682 Communities. The American Naturalist 186:610–622.
683 Fox, J., and S. Weisberg. 2019. An {R} Companion to Applied Regression, Third Edition. Sage,
684 Thousand Oaks, CA. https://socialsciences.mcmaster.ca/jfox/Books/Companion/.
685 Fürst, M. A., D. P. McMahon, J. L. Osborne, R. J. Paxton, and M. J. F. Brown. 2014. Disease
686 associations between honeybees and bumblebees as a threat to wild pollinators. Nature
687 506:364–366.
688 Genersch, E., C. Yue, I. Fries, and J. R. De Miranda. 2011. Detection of Deformed wing virus, a
689 honey bee viral pathogen, in bumble bees (Bombus terrestris and Bombus pascuorum) with
690 wing deformities. Journal of Invertebrate Pathology 91:61–63.
691 Gotelli, N. J., and R. K. Colwell. 2001. Quantifying biodiversity: Procedures and pitfalls in the
692 measurement and comparison of species richness. Ecology Letters 4:379–391.
693 Graystock, P., W. H. Ng, K. Parks, A. D. Tripodi, P. A. Muñiz, A. A. Fersch, C. R. Myers, Q. S.
694 McFrederick, and S. H. McArt. 2020. Dominant bee species and floral abundance drive
695 parasite temporal dynamics in plant-pollinator communities. Nature Ecology & Evolution
696 4:1358–1367.
697 Grozinger, C. M., and M. L. Flenniken. 2019. Bee Viruses: Ecology, Pathogenicity, and Impacts.
698 Annual Review of Entomology 64:205–226.
699 Hartig, F. 2020. DHARMa: Residual Diagnostics for Hierarchical (Multi-Level/Mixed)

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

731 LoGiudice, K., R. S. Ostfeld, K. A. Schmidt, and F. Keesing. 2003. The ecology of infectious
732 disease: effects of host diversity and community composition on Lyme disease risk.
733 Proceedings of the National Academy of Sciences 100:567–71.
734 Loreau M., and Hector. A. 2001. Partitioning selection and complementarity in biodiversity
735 experiments. Nature 412:72–76.
736 Luis, A. D., A. J. Kuenzi, and J. N. Mills. 2018. Species diversity concurrently dilutes and
737 amplifies transmission in a zoonotic host–pathogen system through competing mechanisms.
738 Proceedings of the National Academy of Sciences 115:7979–7984.
739 Manley, R., M. Boots, and L. Wilfert. 2015. Emerging viral disease risk to pollinating insects:
740 ecological, evolutionary and anthropogenic factors. Journal of Applied Ecology 10:1–10.
741 Mazzei, M., M. L. Carrozza, E. Luisi, M. Forzan, M. Giusti, S. Sagona, F. Tolari, and A.
742 Felicioli. 2014. Infectivity of DWV associated to flower pollen: experimental evidence of a
743 horizontal transmission route. PloS ONE 9:e113448.
744 McArt, S. H., H. Koch, R. E. Irwin, and L. S. Adler. 2014. Arranging the bouquet of disease:
745 Floral traits and the transmission of plant and animal pathogens. Ecology Letters 17:624–
746 636.
747 McMahon, D. P., M. A. Fürst, J. Caspar, P. Theodorou, M. J. F. Brown, and R. J. Paxton. 2015.
748 A sting in the spit: widespread cross-infection of multiple RNA viruses across wild and
749 managed bees. Journal of Animal Ecology 84:615–624.
750 McMahon, D. P., L. Wilfert, R. J. Paxton, and M. J. F. Brown. 2018. Emerging Viruses in Bees:
751 From Molecules to Ecology. Pages 251–291 *in* C. M. Malmstrom, editor. Advances in
752 Virus Research: Environmental Virology and Virus Ecology. First edition. Elsevier Inc.,
753 London, UK.
754 Mihaljevic, J. R., M. B. Joseph, S. A. Orlofske, and S. H. Paull. 2014. The scaling of host density
755 with richness affects the direction, shape, and detectability of diversity-disease
756 relationships. PLoS ONE 9:e97812.
757 Mitchell, C. E., D. Tilman, and J. V. Groth. 2002. Effects of Grassland Plant Species Diversity,
758 Abundance, and Composition on Foliar Fungal Disease. Ecology 83:1713–1726.
759 Novotný, V., and Y. Basset. 2000. Rare species in communities of tropical insect herbivores:
760 Pondering the mystery of singletons. Oikos 89:564–572.
761 Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R.

762 B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, E. Szoecs, and H. Wagner. 2018.
763 vegan: Community Ecology Package. https://cran.r-project.org/package=vegan.
764 Ongus, J. R., D. Peters, J. M. Bonmatin, E. Bengsch, J. M. Vlak, and M. M. van Oers. 2004.
765 Complete sequence of a picorna-like virus of the genus Iflavirus replicating in the mite
766 Varroa destructor. Journal of General Virology 85:3747–3755.
767 Ostfeld, R. S., and F. Keesing. 2000. Biodiversity and Disease Risk: The Case of Lyme Disease.
768 Conservation Biology 14:722–728.
769 Ostfeld, R. S., and K. LoGiudice. 2003. Community dissassembly, biodiversity loss, and the
770 erosion of an ecosystem service. Ecology 84:1421–1427.
771 Paradis, E., and K. Schliep. 2018. ape 5.0: an environment for modern phylogenetics and
772 evolutionary analyses in R. Bioinformatics 35:526–528.
773 R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for
774 Statistical Computing, Vienna, Austria.
775 Rader, R., I. Bartomeus, J. M. Tylianakis, and E. Laliberté. 2014. The winners and losers of land
776 use intensification: Pollinator community disassembly is non-random and alters functional
777 diversity. Diversity and Distributions 20:908–917.
778 Radzevičiūtė, R., P. Theodorou, M. Husemann, G. Japoshvili, G. Kirkitadze, A. Zhusupbaeva,
779 and R. J. Paxton. 2017. Replication of honey bee-associated RNA viruses across multiple
780 bee species in apple orchards of Georgia, Germany and Kyrgyzstan. Journal of Invertebrate
781 Pathology 146:14–23.
782 Randolph, S., and A. Dobson. 2012. Pangloss revisited: a critique of the dilution effect and the
783 biodiversity-buffers-disease paradigm. Parasitology 139:847–63.
784 Roche, B., A. P. Dobson, J. F. Guégan, and P. Rohani. 2012. Linking community and disease
785 ecology: The impact of biodiversity on pathogen transmission. Philosophical Transactions
786 of the Royal Society B: Biological Sciences 367:2807–2813.
787 Rohr, J. R., D. J. Civitello, F. W. Halliday, P. J. Hudson, K. D. Lafferty, C. L. Wood, and E. A.
788 Mordecai. 2020. Towards common ground in the biodiversity–disease debate. Nature
789 Ecology and Evolution 4:24–33.
790 Rudolf, V. H. W., and J. Antonovics. 2005. Species Coexistence and Pathogens with Frequency-
791 Dependent Transmission. The American Naturalist 166:112–118.
792 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.

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.

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.

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. 2014a. Does biodiversity protect humans against infectious disease? Ecology 95:817–832.

Wood, C. L., S. A. Sandin, B. Zgliczynski, A. S. Guerra, and F. Micheli. 2014b. Fishing drives declines in fish parasite diversity and has variable effects on parasite abundance. Ecology 95:1929–1946.

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 1:** 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 S1: Table S8, and model results for Model 2a and Model 3a in Table 3 and Appendix S1: Table S9, 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:** DWV, BQCV, and SBV infection prevalence for *Apis mellifera*, *Bombus impatiens*, *Lasioglossum* spp., and *Eucera pruinosa* determined by the percent of virus-positive samples that had the viral negative strand present, 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 in Appendix S1: Table S7, and p-values for differences in infection prevalence are in Appendix S1: Table S19.

| 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 3:** Analysis of deviance table for 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.

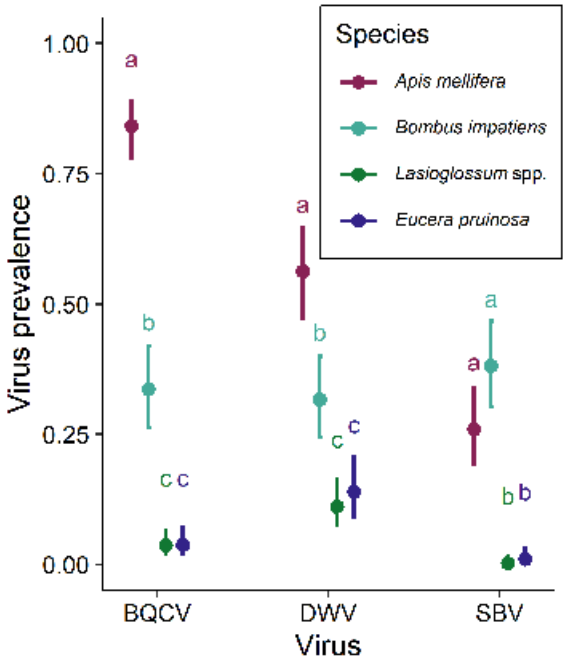
| **Main Factors** | $\chi^2$ | **df** | **P value** |
|---|---|---|---|
| Total Abundance | 1.71 | 1 | 0.1907 |
| Species Richness | 12.79 | 1 | **0.0003** |
| Virus Type | 34.63 | 2 | **< 0.0001** |
| Host Species | 165.25 | 3 | **< 0.0001** |
| Virus Type × Genus | 131.18 | 6 | **< 0.0001** |

**Figure legends**

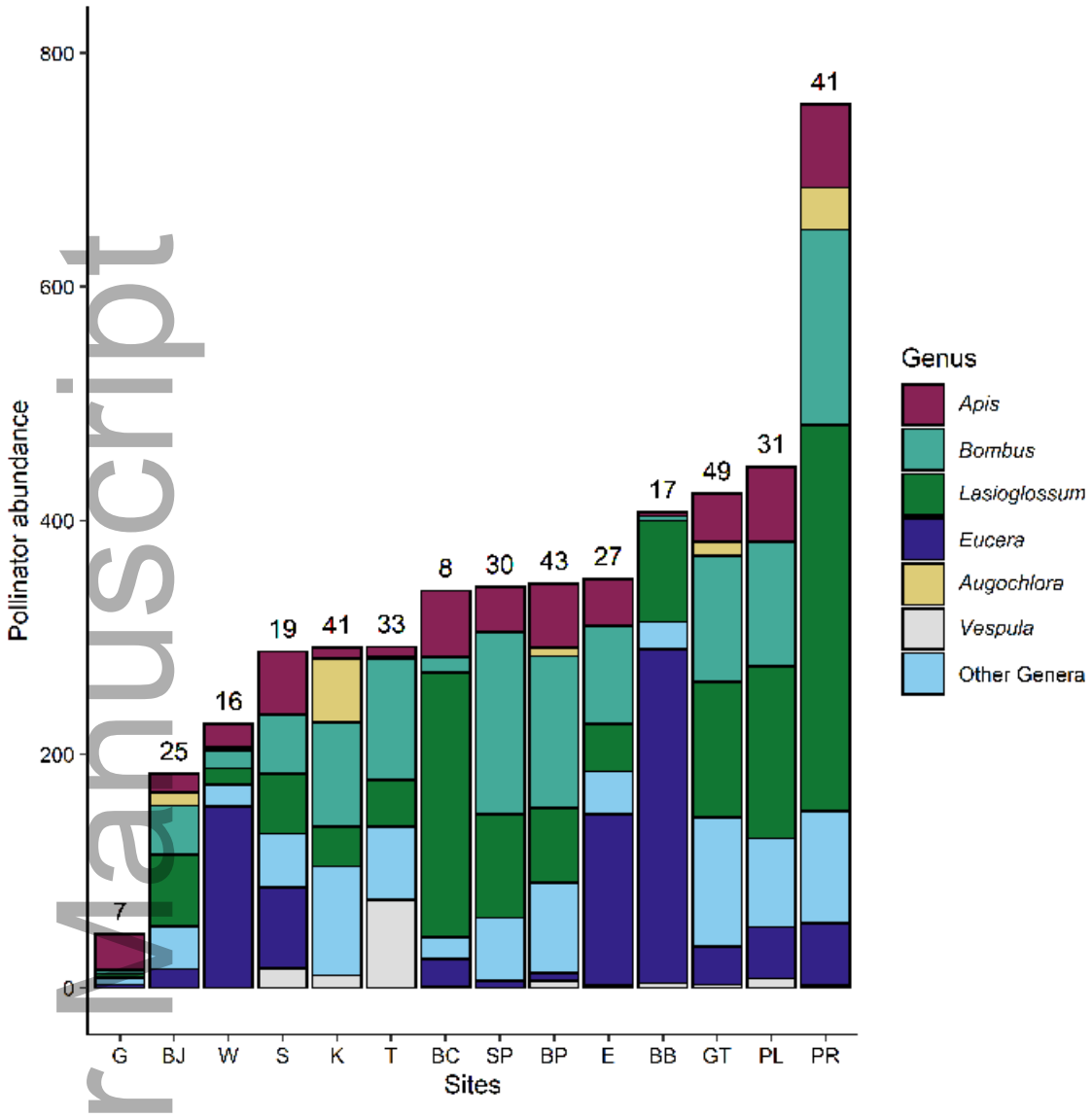
**Fig. 1:** Virus prevalence varied significantly among different host species. BQCV, DWV, and SBV prevalence with the 95% CI among *Apis mellifera*, *Bombus impatiens*, *Lasioglossum* spp., and *Eucera pruinosa* (Appendix S1: Table S17). Different letters indicate significant differences in virus prevalence among host species and within each virus type. The data shown correspond to the significant virus type × genus interaction ($p < 0.0001$) from the Model 2a analysis (Table 3), and post-hoc pairwise comparison with a Tukey p-value adjustment for multiple comparisons (Appendix S1: Table S18). Sample sizes per host species: *A. mellifera*, n = 237; *B. impatiens*, n = 252; *Lasioglossum* spp., n = 255; and *E. pruinosa*, n = 193 (Appendix S1: Table S16).

**Fig. 2:** Pollinator species richness, abundance, and community composition vary qualitatively 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 observed species richness at each site is shown at the top of each bar. Site abbreviation codes can be found in Appendix S1: Table S1.

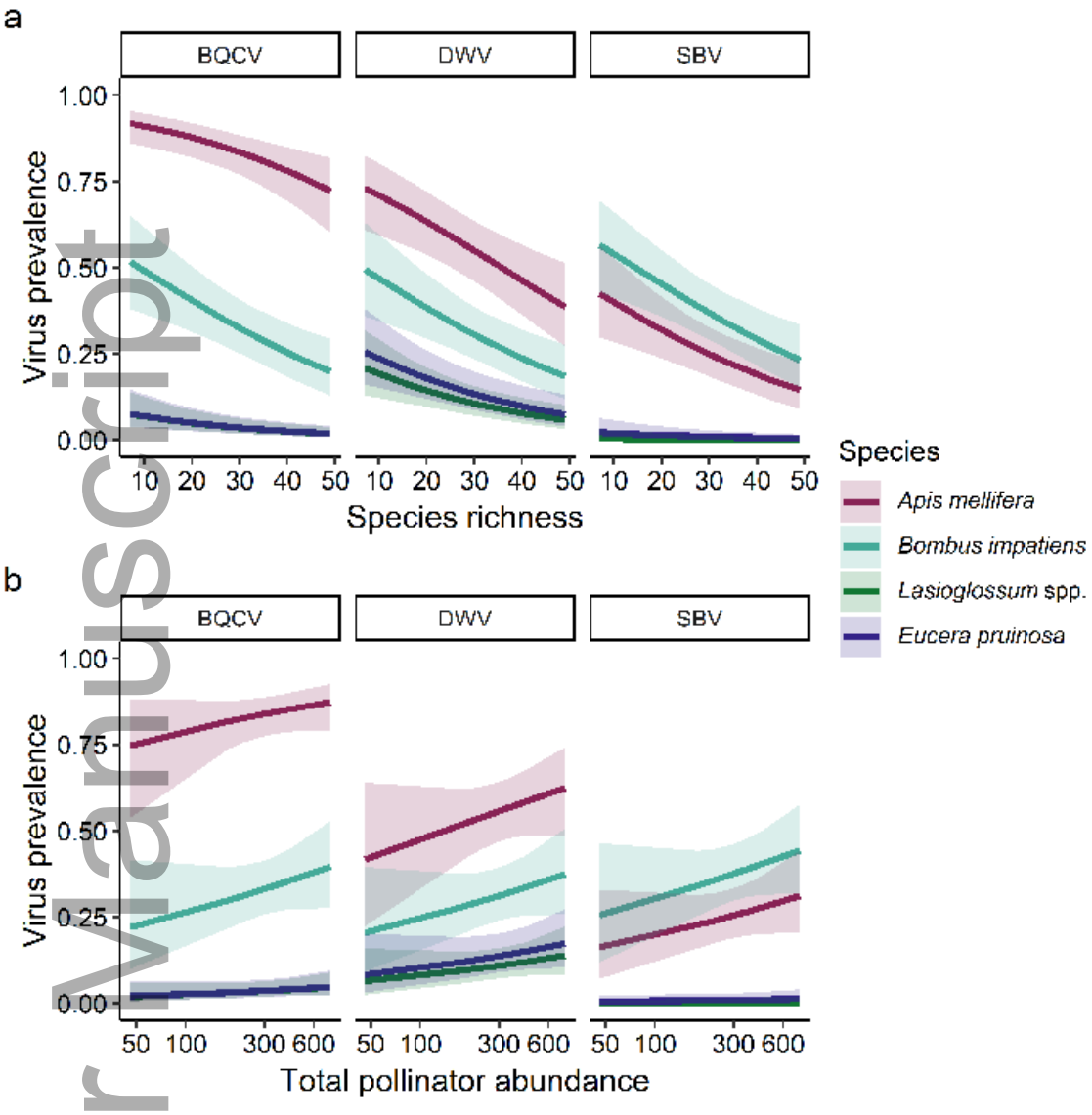
**Fig. 3:** a) Species-rich communities are significantly correlated with lower predicted virus prevalence in *Apis mellifera*, *Bombus impatiens*, *Lasioglossum*, and *Eucera pruinosa* ($p = 0.0003$). The strength of the negative slope varies among host–virus pairs depending on the host's relative virus prevalence (Fig. 1). b) Total pollinator abundance was not significantly correlated with pollinator virus prevalence ($p = 0.19$). Total pollinator abundance is on a log scale. The data shown correspond to Table 3.




ecy_3305_f1.tiff




ecy_3305_f2.tiff




ecy_3305_f3.tiff