Honeybee visitation to shared flowers increases Vairimorpha ceranae prevalence in bumblebees

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Abstract

Vairimorpha (=Nosema) ceranae is a widespread pollinator parasite that commonly infects honeybees and wild pollinators, including bumblebees. Honeybees are highly competent V. ceranae hosts and previous work in experimental flight cages suggests V. ceranae can be transmitted during visitation to shared flowers. However, the relationship between floral visitation in the natural environment and the prevalence of V. ceranae among multiple bee species has not been explored. Here, we analyzed the number and duration of pollinator visits to particular components of squash flowers—including the petals, stamen, and nectary—at six farms in southeastern Michigan, USA. We also determined the prevalence of V. ceranae in honeybees and bumblebees at each site. Our results showed that more honeybee flower contacts and longer duration of contacts with pollen and nectar was linked with greater V. ceranae prevalence in bumblebees. Honeybee visitation patterns appear to have a disproportionately large impact on V. ceranae prevalence in bumblebees even though honeybees are not the most frequent flower visitors. Floral visitation by other pollinators was not linked with V. ceranae prevalence in bumblebees. Further, V. ceranae prevalence in honeybees was unaffected by floral visitation behaviors by any pollinator species. These results suggest that honeybee visitation behaviors on shared floral resources may be an important contributor to increased V. ceranae spillover to bumblebees in the field. Understanding how V. ceranae infection risk is influenced by pollinator behavior in the shared floral landscape is critical for reducing parasite spillover into declining native bee populations.

INTRODUCTION

Recent declines in native and managed bee populations threaten the stability of pollination services that are vital for maintaining natural and agricultural ecosystems (Beismeijer et al. 2006, Potts et al. 2010). Several factors contribute to these declines, including the spread of multi-host pathogens, habitat loss, and climate change (Ricketts et al. 2008, Burkle et al. 2013, Furst et al. 2014). Losses in pollinator community biodiversity and abundance lead to changes in flower visitation patterns (Beismeijer et al. 2006, Albrecht et al. 2012, Burkle et al. 2013), as well as changes in the risk of infectious disease within reduced pollinator communities (Figueroa et al. 2020, Graystock et al. 2020, Fearon and Tibbetts 2021). Yet, it remains unclear how differences in floral visitation behaviors within pollinator communities affected by these declines may in turn affect the spread of pathogens.

Many pollinator pathogens and parasites (hereafter, 'parasites') are transmitted within and among species by visitation to flowers that were previously visited by infected bees (Durrer and Schmid-Hempel 1994, Graystock et al. 2015, Müller et al. 2019, Purkiss and Lach 2019). The likelihood of parasite deposition and subsequent transmission on flowers depends on multiple factors, including flower traits, flower morphology, pollinator behavior, and the environment (Durrer and Schmid-Hempel 1994, Alger et al. 2019, Figueroa et al. 2019, Russell et al. 2019). Depending on the parasite, different plant components, including the floral tissue, pollen, and nectar, are implicated in transmission among pollinators (reviewed by McArt et al. 2014). In particular, differences in the rates of parasite deposition and acquisition of microorganisms on various flower parts may depend on how bees interact with the flowers during foraging visits. For example, bees foraging for

pollen had greater rates of microbe deposition and acquisition on flowers than did bees foraging for nectar (Russell et al. 2019). However, pollinator visitation behaviors have been shown to have a complex relationship with the prevalence of bee parasites on flowers. In a study on pollinator viruses, flowers receiving longer visits were more likely to host viruses, but those with high visitation rates were less likely to host viruses (Alger et al. 2019). In a different study, *Crithidia bombii* survived longer when deposited inside the corolla rather than on the bract, but infection occurring from an encounter with the bract resulted in more intense infection (Figueroa et al. 2019). Therefore, the ways in which infected bees interact with specific flower features and the duration and frequency of their visits will alter the likelihood of parasite deposition on floral surfaces and influence the probability of infection for later visitors. However, most studies on this topic have been conducted in the laboratory and have not fully considered the potential for parasite transmission via shared floral resources in natural settings.

Agricultural fields and the surrounding hedgerows may represent potential 'hot spots' for parasite transmission within and among bee species on shared floral resources. Managed honeybees (*Apis mellifera)* are frequently brought to agricultural fields to provide pollination services, where they have ample opportunity to interact with wild pollinators that are also attracted to plentiful crop flowers or nearby hedgerows with wildflowers (Goulson and Hughes 2015). The worldwide dispersal of *A. mellifera* (hereafter honeybees) and its many parasites has consequently led to spillover (i.e., parasite transmission from reservoir populations to sympatric wildlife) to many naïve wild pollinators (Daszak et al. 2000, Keesing et al. 2006, Goulson and Hughes 2015, Purkiss and Lach 2019). Since honeybee colonies tend to send generalist foragers to a few flower patches at a time (Visscher and Seeley 1982), it is possible that an infected colony may create localized floral hot-spots where wild bees may acquire parasites. Increasingly, parasites previously thought to only infect honeybees are found in diverse populations of wild pollinators and seem to be contributing to their decline (Furst et al. 2014, Arbulo et al. 2015, Goulson and Hughes 2015, Porrini et al. 2017, Müller et al. 2019, Purkiss and Lach 2019).

One parasite of particular concern is the widely-dispersed microsporidian parasite *Vairimorpha* (= *Nosema*)*ceranae* (Tokarev et al. 2020), which has been rapidly infecting honeybees and spilling over into wild bee populations over the past three decades (Paxton et al. 2007, Chen et al. 2008, Fries 2010). Although *V. ceranae* is transmitted within honeybee hives through contaminated feces and pollen stores, transmission may also occur when bees encounter spores on contaminated flowers (Higes et al. 2008b, 2010). Graystock et al. (2015) demonstrated that multiple pollinator parasites, including *V. ceranae* , can be effectively dispersed onto flowers by competent hosts and then vectored from flowers back to colonies by other pollinator species. Additionally, *V. ceranae* spores have been detected on the flowers of at least 14 plant genera in the field (Graystock et al. 2020). Therefore, contamination of shared flower resources is a likely mode of transmission for *V. ceranae* between different pollinator species, with dispersal potentially occurring through defecation on floral surfaces or through the rubbing off of spores that were attached to the bee cuticle (Graystock et al. 2015, Bodden et al. 2019, Piot et al. 2020). Furthermore, Graystock et al. (2015) found that *V. ceranae*transmission was very rapid in small experimental flight cages, but they recognized that whether parasite dispersal is similar in nature will depend on the characteristics of pollinator communities and environmental conditions. Despite clear experimental evidence for *V. ceranae* transmission on flowers, the relationship between specific pollinator visitation patterns and *V. ceranae* prevalence across managed and wild pollinator species in the field has remained understudied.

Here, we examine whether the prevalence of *V. ceranae* in managed and wild bee populations is influenced by the floral visitation behaviors of bees in the natural environment. We conducted an observational study of *V. ceranae* in honeybee (*A. mellifera*) and bumblebee (*Bombus* spp.) populations among different pollinator communities to understand how floral visitation patterns differ among pollinator species and whether the visitation patterns are linked with *V. ceranae* prevalence in both host species. Specifically, we investigated how *V. ceranae* prevalence is linked with the number of honeybee, bumblebee, and other pollinator visits to flowers and the time each bee species spent interacting with different parts of the flowers during each visit. We hypothesized that higher numbers of visits and longer visits by potentially infected bees would increase the likelihood of *V. ceranae* transmission and correlate with higher *V. ceranae* prevalence. These findings

will be important for determining the pollinator visitation behaviors that contribute the most to *V. ceranae* exposure and subsequent infection in honeybees and bumblebees as well as helping to establish whether *V. ceranae* transmission on flowers occurs under field conditions.

METHODS

Study System

V. ceranae is a microsporidian parasite with a nearly global distribution. It was initially discovered in *Apis ceranae* and later spilled over into *A. mellifera* honeybees, where it appears to be more virulent than closely related parasites such as *V. apis* (Paxton et al. 2007). Recent studies have shown that wild native bees are also infected with *V. ceranae* , including many wild bumblebees (*Bombus* spp.), stingless bees (*Tetragonula hockingsi* , *Tetragonisca* spp., *Scaptotrigona* spp.,*Melipona* spp.), and solitary bees (*Osmia bicornis*) (Plischuk et al. 2009, Graystock et al. 2013, Furst et al. 2014, Müller et al. 2019, Purkiss and Lach 2019, Salvarrey et al. 2021, Cilia et al. 2022). Transmission of *V. ceranae* between individuals is primarily fecal–oral or oral–oral, as it is spread through ingestion of contaminated food or contact with the feces of diseased hosts (Chen et al. 2008, Smith 2012). *V. ceranae* germinates in the midgut of the bee, where the spore count can reach over 30 million, and it is then excreted as feces (Paxton et al. 2007, Chen et al. 2008, Higes et al. 2008a), potentially contributing very large numbers of spores to the environment (e.g., on floral surfaces). Symptoms of infection in honeybees include digestive disorders, shortened life spans, atypical breeding behavior, reduced sucrose sensitivity, and diminished honey production; however, colony infection is often asymptomatic until sharp depopulation occurs, often in autumn and winter (Chen et al. 2008, Higes et al. 2008a, 2010, Graystock et al. 2013). Symptoms are generally assumed to be the same for wild bees, but data on this is limited aside from a few reports that *V. ceranae* may cause reduced survival, learning impairment, sucrose sensitivity, and cellular immunosuppression in bumblebees or stingless bees (Graystock et al. 2013, Piiroinen and Goulson 2016, Macías-Macías et al. 2020). Furthermore, *V. ceranae*infections suppress the pollinator immune response, which can lead to coinfection with other pathogens or parasites and an increased likelihood of mortality (Antúnez et al. 2009). The drastic effects of *V. ceranae* on pollinator health have been linked to the sudden collapse of honeybee colonies (Higes et al. 2008a) and may be an important factor in the recent declines of some wild bees (Graystock et al. 2013, Furst et al. 2014, Goulson and Hughes 2015).

Sampling pollinators in the field

A. mellifera and *Bombus* spp. (hereafter honeybee and bumblebee, respectively) samples were collected from six winter squash farms in southeastern Michigan, USA (Appendix S1: Table S1) during two visits to each site between 26 July and 30 August 2016 during the peak squash bloom. The pollinator sampling described here includes a subset of the sites that were previously sampled in Fearon and Tibbetts (2021) and Fearon et al. (2022). In this study, we focus on *V. ceranae*infection in honeybees and bumblebees, while the prior studies examined links between the pollinator community composition and bee viral prevalence. Sites were at least 10 km apart to ensure that the pollinator communities were isolated from each other (Greenleaf et al. 2007). We only sampled on sunny days with windspeeds less than 2 m/s. To collect the bees, four 50 m transects were randomly placed at each field site. Three transects were placed in the field along the crop rows, while the fourth transect was placed along a field edge to sample bees foraging near native flowers and invasive weeds. All honeybees and bumblebees observed along the transect lines were collected using handheld nets or pan traps. Details on the trapping methods are included in Appendix S2 and Fearon and Tibbetts (2021).

All pollinator samples were stored on dry ice in the field, and later placed in a -80 @C freezer to maintain the integrity of the DNA for detection of *V. ceranae* infection. All bees were identified to species using the Discover Life key (Ascher and Pickering 2013). The collected bumblebee species were primarily *Bombus impatiens* , but also included *Bombus auricomus* , *Bombus bimaculatus* ,*Bombus griseocollis* , *Bombus fervidus, Bombus pensylvanicus* , *Bombus sandersoni* , and *Bombus vagans* at very low densities (< 8 individuals total). *A. mellifera* and *B. impatiens* were common at all six field sites.

Collecting Flower Visitation Data

During each visit to the six farms, we took 30-min video recordings of pollinators visiting eight randomly selected male squash flowers per site (N = 112, mean video length: 30.87 min [sd: 3.75 min]). Each video was recorded between 07:30 AM and 12:00 PM on sunny, non-windy days. Video recordings were watched to record data on the identity and frequency of pollinator visitors to the flowers. Pollinators captured on video were identified to genus where possible (e.g., *Apis* ,*Bombus* , *Eucera*), or to morphospecies for species that require close inspection and/or a key for accurate identification (Appendix S1: Table S2). Honeybees and bumblebees were easy to identify in the video recordings due to their relatively large body size and distinctive coloration. The behaviors of all other pollinators observed, including small green and olive halictids (e.g., *Augochlora* ,*Augochlorella* , *Augochloropsis* , *Halictus* , and*Lasioglossum* genera), *Melissodes* spp., *Eucera* spp.,*Triepeolus* spp., *Vespula* wasp spp., and hover flies, were grouped together into an 'other pollinators' category to compare to honeybee and bumblebee behaviors in later analyses (see Statistics section).

During each individual pollinator's visit to the observed flower, we recorded the duration (seconds) of each visitor's interactions with specific flower parts, including petals (petal-only), nectar (nectar-only), pollen (pollen-only), and both pollen and nectar simultaneously (pollen+nectar). Typically, large-bodied bees, including honeybees and bumblebees, could not avoid contacting the stamen while drinking nectar (pollen+nectar) and led to relatively few observations of nectar-only interactions with flowers (Appendix 1: Table S3). For this reason, the nectar-only interactions were not considered as a substantial interaction type and were not included as a response variable in our main analyses. For each flower observed, the total duration of all types of interactions were summed for each pollinator group (honeybees, bumblebees, or all other pollinators) and then divided by the number of flower visits for the respective pollinator group to generate the duration spent per visit by each pollinator group to each flower. Finally, to test how each pollinator group's visitation behavior impacted *V. ceranae* prevalence, we averaged the calculated visitation metrics for all flowers observed during the same site visit for each pollinator group. We followed the same process to calculate the average duration per visit of time spent on petal-only, pollen-only, and pollen+nectar interactions for each pollinator group. The number of visits for each pollinator group was the raw count of each type of pollinator that visited each observed flower within the 30-min observation period, which was then averaged for each of the two visits to each site.

Evaluating the average duration bees spent per floral visit ensured that the duration metrics accurately reflected the time bees spent interacting with flowers without being skewed by the number of bee visitors. Each additional bee visitor inherently increased the total duration of time bees spent on flowers ($r = 0.76$, $t = 11.52$, $df = 95$, $p < 0.001$) but did not necessarily increase the duration per visit time ($r = 0.02$, $t = 0.21$, $df = 95$, $p = 0.84$). We predicted that bees that spent a greater amount of time per visit interacting with flowers would have a greater likelihood of either depositing or picking up *V. ceranae* spores on flowers and would be correlated with higher *V. ceranae* prevalence.

Detecting V. ceranae Infection Presence

Approximately eight honeybee and eight bumblebee individuals per visit to each field site were randomly selected to test for the presence or absence of *V. ceranae* infection (target N per species per site = 16; total honeybee, n = 75; bumblebee, n = 86; Appendix S1: Table S4). Only sites with a minimum of eight bees total were included in the analysis. When less than eight individuals of each species were collected during one of the two visits to a site, infection was tested in all individuals collected (Appendix S1: Table S4). The selected bumblebees were predominantly *Bombus impatiens* , but also included single individuals from *Bombus fervidus, Bombus bimaculatus,* and*Bombus pensylvanicus* species that were all collected from a single field site visit that had relatively low *Bombus impatiens*abundance (Site E, Visit 1). Ultimately, we modeled the binary presence or absence of *V. ceranae* in individual honeybees and bumblebees and all sites had a minimum of eight samples tested for *V. ceranae* presence per host species.

Abdominal contents were dissected from each sample using sterilized forceps and immediately placed on dry ice. Half of the abdomen was placed in a microcentrifuge tube for DNA analysis, and the other half

was stored for reference. DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Germantown, MD, USA) following the manufacturer's instructions for tissue samples. Following extraction, DNA purity and concentration were quantified using Nanodrop 2000 software (Thermo Fisher Scientific, Waltham, MA, USA). One sample with a nucleic acid concentration less than 10 ng/μL was removed from the study due to insufficient DNA extraction (a honeybee from Site E, Visit 1).

To ensure adequate extraction of bee DNA, polymerase chain reactions (PCR) were conducted on all samples using *A. mellifera* 18S rRNA gene primers, which produced bands at 784 bp (Cardinal et al. 2010). Sequences for these bands were confirmed via Sanger sequencing. To determine presence or absence of *V. ceranae* infection in each sample, PCR was conducted with *V. ceranae* -positive and H_2O negative controls using the primers Nosema-F (5'-CGGATAAAAGAGTCCGTTACC-3') and Nosema-R (5'-TGAGCAGGGTTCTAGGGAT-3') for the *V. ceranae* large subunit ribosomal RNA gene (GenBank Accession No: DQ486027; Chen et al. 2008). Details on the PCR procedure can be found in Appendix S2. A subset of samples was selected for Sanger sequencing to confirm the identification of *V. ceranae* (GenBank Accession Numbers: bee 18S rRNA, OQ545564–OQ545565 and *V. ceranae* large subunit rRNA, OQ550096–OQ550100).

Statistical Analysis

Analyses were performed using the statistical program R (version 4.2.1; R Core Team 2020). First, we evaluated how pollinator visitation behavior varied among honeybees, bumblebees, and other pollinators with a separate model for the following response variables: 1) number of visits to flowers per 30 min video observation period, 2) total duration per visit of pollinator visits to flowers (seconds/visit), 3) duration pollinators spent on petals only per visit (seconds/visit), 4) duration pollinators spent on pollen only per visit (seconds/visit), and 5) duration pollinators spent simultaneously on pollen + nectar per visit (seconds/visit; model output in Appendix S1: Table S5). Each model was a zero-inflated generalized linear mixed effects model (GLMM) using a negative binomial distribution with a log link function and pollinator group (honeybees, bumblebees, and other pollinators) as the main predictor for both the zero-inflated and GLMM portions of the model (*glmmTMB* package; Brooks et al. 2017). The pollinator visitation data was aggregated by each flower observed for each pollinator group; therefore, a nested random effect of Flower ID (8 flowers/visit/site) within visit to a site (2 visits/site) within site (6 sites) was used in each model. To model the duration per visit, we used the duration of behaviors in seconds as the response variable with an offset of the log of the number of pollinator visits + 1 to correct for flowers with zero visits. We removed one outlier point from the number of visits per 30 min data, where other pollinators visited a single flower over twice as many times as the next most visited flower in our study. Each model was checked for overdispersion, zero-inflation, and spatial autocorrelation; none of these tests were significant (*DHARMa* package; Hartig 2020). Then we followed up each model with a post-hoc test to evaluate significant differences among honeybees, bumblebees, and other pollinators' visitation behaviors (Appendix S1: Table S6; *emmeans*package; Lenth et al. 2020).

To evaluate *V. ceranae* prevalence in honeybees and bumblebees, we initially calculated the total apparent *V. ceranae* prevalence in each host species (*epiR* package; Stevenson et al. 2021) and used a Chi-squared test of two proportions to determine if there was a significant difference among the two host species. Then we ran two sets of models: the first to evaluate how the number of visits to flowers (per 30 min) at each site influenced *V. ceranae*prevalence in honeybees and bumblebees, and the second to test how the duration per visit of specific behaviors on the flowers was correlated with *V. ceranae* prevalence in each host species. All models were generalized linear mixed effects models with *V. ceranae* prevalence in either honeybees or bumblebees as the response variable, a binomial distribution, and a logit link function (package *lme4* ; Bates et al. 2015). For all models, we used a random effect of each visit to a site nested within site to account for both the variation between the two different dates on which each site was visited and the variation among different sites, though the random effects were singular for many models (model outputs in Appendix S1: Table S7 and S8). In the first set, models included the average number of honeybee visits, bumblebee visits, and all other pollinator species visits to flowers during the 30-min observation period as main effects. In

the second set, we ran a series of models to evaluate the average total duration per visit (seconds/visit) and the durations per visit of petal-only, pollen-only, and pollen+nectar interactions of honeybees, bumblebees, and all other pollinators that visited the flowers. To deal with zeros in the data, all main effects had '1' added to the value before log transforming the variable, and then they were scaled and centered to generate standardized estimates from the models. The variance inflation factor (VIF) for all main effects in all models was < 2.2, indicating that there was no multicollinearity in our models. Additionally, none of the models were over-dispersed. There was no evidence of spatial autocorrelation in the model residuals, indicating that *V. ceranae* prevalence was not correlated among sites based on their spatial proximity (*DHARMa* package; Hartig 2020). Finally, we used a Bonferroni Correction of four comparisons to adjust our alpha significance threshold from 0.05 to 0.0125 to account for four separate analyses, one for each of four different pollinator duration behavioral parameters (total duration per visit, duration on petals per visit, duration on pollen per visit, duration on pollen + nectar per visit). The number of visits model was not included in the Bonferroni Correction and was evaluated with the usual 0.05 alpha threshold.

RESULTS

The number of pollinator visits by the three pollinator groups (honeybee, bumblebee, and other pollinators) varied considerably among observed flowers (ranges: honeybees = 0–7, bumblebees = 0–38, other = 0–54; $\chi^2 = 16.12$, df = 2, p = 0.0003). Honeybees had fewer visits compared to bumblebees (p = 0.0003) but did not differ from combined other pollinator visits (p = 0.15; Figure 1a; Appendix S1: Table S3, S5, and S6). On the other hand, the time bees spent on flowers per visit (seconds) did not differ among honeybees, bumblebees, and other pollinators (Figure 1b; $\chi^2 = 4.95$, df = 2, p = 0.08), despite substantial variation in total duration per visit among flowers observed (ranges: honeybees = 0–219.25, bumblebees = 0–222.75, other = 0–484 seconds). We further explored how bee species may differ in how much time per visit they spend interacting with different aspects of the flower, including the petals, pollen, and simultaneously contacting the pollen and nectar (pollen+nectar). Bumblebees spent less time per visit on petals compared to honeybees ($p < 0.0001$) or other pollinators ($p < 0.0001$; Figure 1c). Other pollinators spent more time per visit on pollen-only visits relative to honeybees (p = 0.0025) and bumblebees ($p < 0.0001$; Appendix S1: Fig. S1). On average, all three pollinator groups spent similar amounts of time per visit in contact with pollen+nectar (Figure 1d; $\chi^2 = 3.60$, df = 2, p = 0.17), though there was a wide range of visit times for pollen+nectar (ranges: honeybees = 0–197.75, bumblebees = 0–218.75, other = 0–295 seconds). Overall, each pollinator group differed in the number of visits and duration of time spent per visit interacting with different aspects of the flowers, which could contribute to variation in the likelihood of bees depositing or picking up parasite spores during floral visits.

V. ceranae was highly prevalent in both honeybees and bumblebees at all six field sites. In total, 68.0% (95% CI: 56.7–77.9%) of honeybees and 64.0% (95% CI: 52.9–73.6%) of bumblebees had *V. ceranae* detected in their midguts. *V. ceranae* prevalence did not significantly differ between host species ($\chi^2 = 0.14$, df = 1, p = 0.71). Among different sites, *V. ceranae*prevalence ranged from 57.1% to 81.3% in honeybees and from 40.0% to 93.8% in bumblebees (Appendix S1: Fig. S2, Table S9).

To determine whether floral visitation behaviors were linked with *V. ceranae* prevalence, we explored how the number of pollinator visits and the duration of time per visit spent interacting with certain parts of the flower correlated with *V. ceranae* prevalence in honeybees and bumblebees. Despite a lower number of honeybee visits compared to bumblebee visits (Figure 1a), the number of honeybee visits was the only factor that had a significant impact on *V. ceranae*prevalence. *V. ceranae* prevalence in bumblebees was positively linked with honeybee flower visits (p = 0.005; Table 1, Figure 2a) but not bumblebee flower visits (p = 0.98, Figure 3a). In contrast, *V. ceranae* prevalence in honeybees was not linked with honeybee flower visitation (p = 0.57, Table 1, Figure 2a) or bumblebee flower visitation (p = 0.47, Figure 3a). *V. ceranae* prevalence in honeybees and bumblebees was also not linked with flower visitation by other bee genera (both $p > 0.54$, Figure 3b; Table 1, Appendix S1: Table S7 and S8).

We also expected that greater amounts of time bees spent per visit on flowers and interacting with different aspects of the flower (e.g., petals, pollen, and pollen+nectar) would increase the *V. ceranae*prevalence by

increasing the chances of parasite transmission. For the duration per visit models, we used a Bonferroni-corrected significance threshold of 0.0125 because the durations per pollinator visit to each part of the flower were analyzed separately. *V. ceranae* in honeybees and bumblebees was not associated with the total duration per visit of honeybees, bumblebees, or other pollinators (Table 1). However, *V. ceranae* prevalence in bumblebees was marginally, but not significantly, higher the longer that honeybees spent interacting with flowers per visit ($p = 0.014$, Figure 2b). We further explored this result by breaking down the total floral visit duration by the duration of time that bees spent interacting with different flower parts, including petals, pollen, and pollen+nectar to determine which specific behaviors contributed most to *V. ceranae* prevalence in each host species (Table 1; Appendix S1: Table S7 and S8). *V. ceranae*prevalence in bumblebees was higher the longer honeybees interacted with pollen+nectar ($p = 0.009$; Figure 2c), despite no overall differences in time spent on pollen+nectar per visit among honeybees, bumblebees, and other pollinators (Figure 1d). *V. ceranae* in both host species was not impacted by bumblebee or other pollinator duration spent per visit on pollen+nectar (Figure 3c,d; Table 1). *V. ceranae* in honeybees and bumblebees was not correlated with the time per visit that bees spent on petals or pollen, regardless of bee species (Table 1).

DISCUSSION

We observed that honeybees, bumblebees, and other pollinators differed in the number of visits to flowers and the duration per visit to petals and pollen but did not vary in the total length of time they spent on flowers. Honeybees had fewer flower visits than bumblebees, and all visiting pollinators spent similar amounts of time per visit interacting with the pollen and nectar simultaneously (Figure 1). Yet, the sites with more and longer honeybee visits to shared flowers had higher *V. ceranae* prevalence in bumblebees. Therefore, honeybee visitation to flowers appears to have a disproportionate impact on *V. ceranae* prevalence in local bumblebee populations. Visitation by bumblebees or other pollinators, in terms of the number of visits or time spent on flowers, was not associated with *V. ceranae*prevalence in either host species. These findings suggest honeybees may play an important role in the spread of *V. ceranae* to bumblebees through indirect contact via shared flowers in the natural environment. Such pathogen spillover from honeybees to bumblebees is likely to have negative consequences for bumblebee populations (Colla et al. 2006, Furst et al. 2014).

The spillover from honeybees to bumblebees may occur differently compared to transmission among honeybees. *V. ceranae* is easily transmitted within honeybee hives when bees clean up fecal material, eat contaminated food, or perform trophallaxis (Chen et al. 2008, Higes et al. 2010). Further, drifting of honeybees among hives is known to occur and is thought to play a role in the transmission of parasites, including *V. ceranae* (Higes et al. 2010, Eberl and Muhammad 2022). As *V. ceranae* is a well-established concern for managed honeybee populations (Higes et al. 2013) and is thought to spill over from managed honeybee populations to native bumblebee populations (Furst et al. 2014, Goulson and Hughes 2015, Alger et al. 2018), high *V. ceranae* prevalence in honeybees may be driven by intraspecific transmission occurring among and within honeybee hives. In contrast, *V. ceranae* prevalence in bumblebees may be driven in part by parasite spillover from shared flowers with honeybees. Thus, spillover from honeybees to bumblebees could explain why honeybee visitation behavior was strongly correlated with *V. ceranae* prevalence in bumblebees, but not with prevalence in honeybees.

Our results are consistent with prior small-scale lab experiments which demonstrated that pollinator parasites, including *V. ceranae* , are transmitted via contact with flowers (Durrer and Schmid-Hempel 1994, Graystock et al. 2015, Purkiss and Lach 2019). Several recent studies have further shown that pollinator parasites are commonly found on flowers in the field, but their abundance varies based on flower morphology, the environment, and pollinator visitation patterns (Alger et al. 2019, Figueroa et al. 2019, Russell et al. 2019, Graystock et al. 2020). Furthermore, Graystock et al. (2015) experimentally showed that 23% of uninfected bumblebees that foraged on flowers recently visited by infected honeybees became infected with *V. ceranae* . This suggests that flowers can become hotspots for parasite dispersal once contaminated (Graystock et al. 2015). However, few studies have examined how differences in the pollinator community's floral visitation behaviors may impact parasite prevalence across multiple host species in nature (but see Graystock et al. 2020), and *V. ceranae* in particular has been neglected. As pathogens are a key driver of pollinator

population decline (Potts et al. 2010), it is crucial to understand patterns of their transmission within and among pollinator species in the natural environment. Our findings corroborate prior experimental work and add that honeybee visitation to shared flowers—especially in areas with generally high *V. ceranae*infection levels in honeybees—facilitates greater *V. ceranae*spillover from managed honeybees to wild bumblebees in the natural environment.

While *V. ceranae* spillover via contaminated flowers seems likely, little is known about how pollinator interactions with different parts of inflorescences may affect the likelihood of *V. ceranae*transmission. We examined the association between *V. ceranae*prevalence and the duration per visit by honeybees, bumblebees, and other pollinators to flower petals, nectaries, and pollen to explore which parts of inflorescences may have the greatest impact on *V. ceranae* spread. We found that higher *V. ceranae* prevalence in bumblebees was associated with longer durations of honeybee interactions per visit spent simultaneously contacting both the pollen and nectar of inflorescences (pollen+nectar). These visits were characterized by active foraging behavior for nectar and/or pollen while deeply embedded within the corolla of the large squash flowers. Additionally, since there was no difference among honeybees, bumblebees, and other pollinators in time spent per visit on the pollen+nectar (Figure 1d), our results suggest that time spent by honeybees on flowers disproportionately increases the likelihood of parasite spillover to bumblebees relative to time spend on flowers by bumblebees or other pollinators.

The length of time that infected honeybees spend closely interacting with both pollen and nectar—food resources that are consumed by many pollinator species—likely contributes to *V. ceranae* spore deposition on flower surfaces, which may be picked up and consumed by subsequent floral visitors. *V. ceranae* is a fecal-orally transmitted parasite (Chen et al. 2008, Smith 2012) and bees commonly defecate on floral surfaces while foraging, with longer visits increasing the likelihood of defecation (Bodden et al. 2019). *V. ceranae* has been detected in honeybee salivary glands (Chen et al. 2009) and viable and infectious *V. ceranae* spores have been found in the corbicular pollen of honeybees (Higes et al. 2008b), suggesting that pollen can become contaminated during pollen collection. Therefore, it is possible that the pollen on the stamen may be a key hot spot for the deposition of *V. ceranae* by infected bees and the acquisition of this contaminated pollen by susceptible bees. In contrast, nectar may be a poor location for pathogen transmission because high sugar concentrations can inhibit microbial growth and pathogen survival (Adler et al. 2021). We observed that honeybees and bumblebees seemed to spend more time on pollen+nectar interactions compared to pollen-only or nectar-only interactions (Appendix S1: Table S3), likely owing to their large size making it difficult to only contact one food source at a time. Therefore, the long visits with high floral contact during which honeybees and bumblebees foraged for pollen and nectar may have increased the chances for transmission to occur.

We did not observe any relationships between *V. ceranae*prevalence and the length of time pollinators spent interacting with only the petals or pollen. Though many bees spent time on the petals, bees were typically observed either resting or crawling on the petals for very short periods of time. Though other pollinator parasites are transmitted via floral petals (Figueroa et al. 2019), in our study petal-only interactions were not linked with *V. ceranae*prevalence. Pathogenic spores can often survive well on floral surfaces (McArt et al. 2014), but their survival likely varies among different plant species, flower parts, and the centrality of the plant in the plant–pollinator network (Palmer-Young et al. 2016, Naughton et al. 2017, Adler et al. 2018, Figueroa et al. 2019, Piot et al. 2020). Since this study only considered a single plant species and did not consider the plant–pollinator network, future studies are needed to empirically test how floral traits among different plant species affect pollinator–flower interactions, explore the distribution of spores on different floral surfaces in the natural environment, and determine the consequences for *V. ceranae* parasite dispersal via different parts of the inflorescences.

In contrast to *V. ceranae* prevalence in bumblebees, we consistently found that *V. ceranae* prevalence in honeybees was not correlated with flower visitation by any species. *V. ceranae*prevalence in honeybees was high at all sites (57.1% to 81.3%; Appendix S1 Figure S1, Table S9), indicating that honeybees experience consistently high *V. ceranae* infection levels across the landscape. The spillover of *V. ceranae* from managed

honeybee hosts to wild bumblebee populations would suggest that honeybees are a highly competent host for *V. ceranae* that could be facilitating transmission to other native bee species in pollinator communities through indirect interactions on shared flowers.

Conclusions

We found that *V. ceranae* prevalence in bumblebees was strongly associated with the floral visitation behaviors of honeybees. More honeybee visits and time spent interacting with both the pollen and nectar contributed to higher *V. ceranae* prevalence in bumblebees, despite honeybees visiting flowers less than bumblebees. These results suggest that even a few visits by honeybees to shared crop flowers may be having a disproportionately large effect on *V. ceranae*spillover from managed honeybee populations to wild bumblebee populations in the agricultural landscapes. Our study provides a first look at how specific pollinator visitation behaviors on flowers impact the likelihood of parasite spillover among native pollinators in nature. Understanding how the risk of *V. ceranae* infection for different bee species changes with regard to their shared floral landscape with honeybees is critical for reducing parasite spillover into declining native bee populations. This knowledge may be particularly important in agricultural settings where managed honeybees and wild pollinators from the surrounding environment may frequently interact on crop flowers and nearby hedgerows, creating potential hotspots for parasite transmission on flowers.

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Author contributions : M. Zbrozek and M. Fearon should be considered joint first author. MZ and MLF developed manuscript ideas, MLF carried out field data collection, and MZ and CW conducted the molecular data collection. MLF and MZ performed all analyses with advice from EAT. MZ and MLF wrote the first draft of the manuscript, and all authors contributed substantially to manuscript revisions.

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Competing Interests: The authors declare that they have no conflict of interest.

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TABLES

Table 1. Scale standardized model estimates for the effects of the number of visits, total duration per visit, duration per visit of interactions with petals only, pollen only, and pollen and nectary simultaneously by honeybees, bumblebees, and all other pollinators on *V. ceranae* prevalence in honeybees and bumblebees. Standardized estimates with a larger magnitude indicate a stronger relationship with *V. ceranae* prevalence. Full model output in Appendix S1: Table S7 and S8.

Response variable	Visitation by	Visit Number Estimates	Total Duration Per Visit Es
V. ceranae **prevalence in honeybees**	*Apis*	0.171	0.260
	Bombus	0.262	-0.312
	Other	0.190	-0.109
V. ceranae **prevalence in bumblebees**	*Apis*	**1.144****	0.825*
	Bombus	-0.008	-0.016
	Other	-0.118	-0.306

Significant estimates are bolded.

+ Bonferroni-corrected alpha threshold of 0.0125 for four comparisons applied to models with duration per visit main factors.

Significant: ** $p < 0.01$; trending: * $p < 0.05$

FIGURE CAPTIONS

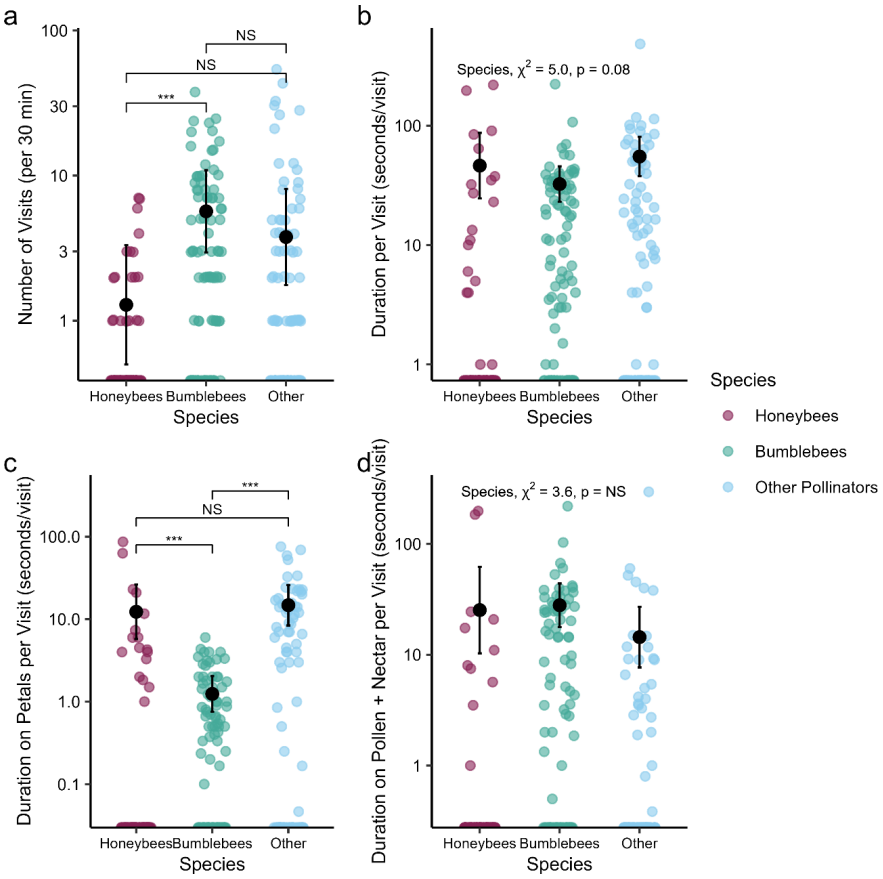
Figure 1. Honeybees had fewer visits to flowers compared to bumblebees and other pollinators, and honeybees and other pollinators spent more time on petals than bumblebees. Total duration per visit and duration on pollen + nectar per visit did not differ among pollinator species. (a) Number of visits observed per 30 min by pollinator species, (b) total duration per visit (seconds/visit) by pollinator species, (c) duration on petals per visit (seconds/visit), (d) duration on pollen + nectar per visit (seconds/visit). Y-axes are on a log scale, where zero values are on the x-axis. Colored points are the raw data per flower observed, and the black points are the model-predicted marginal means with 95% confidence intervals. Significant differences are indicated by the number of stars for each pair (Appendix S1: Table S6).

Figure 2. (a) The average number of honeybee visits to flowers was correlated with greater *V. ceranae* prevalence in bumblebees ($p = 0.005$), but not in honeybees ($p = 0.58$). (b) The average total duration of time honeybees spent on flowers per visit did not correlate with *V. ceranae* prevalence in honeybees ($p =$

0.40) or bumblebees (p = 0.014). (c) The average duration of time honeybees spent per visit interacting with pollen and nectar simultaneously correlated with greater *V. ceranae* prevalence in bumblebees (p = 0.009), but not *V. ceranae* prevalence in honeybees (p = 0.12). The number and duration of visits by honeybees were converted to their original scales for figure clarity. Significant slopes are indicated by solid lines, while insignificant slopes are indicated by dotted lines. Honeybee visits (per 30 min) were evaluated based on a 0.05 alpha threshold, while the visit duration (seconds/visit) were evaluated according to the Bonferroni-corrected alpha threshold of 0.0125.

Figure 3. Neither the number of bumblebee and other pollinator visits per 30 min nor the duration per visit to pollen + nectar impacted *V. ceranae* prevalence in honeybees or bumblebees. There was no change in *V. ceranae* prevalence in honeybees or bumblebees based on (a) average number of bumblebee visits (per 30 min), (b) average number of other pollinator visits (per 30 min), (c) average duration on pollen + nectar per bumblebee visit (second/visit), and (d) average duration on pollen + nectar per other pollinator visit (second/visit). Significant slopes are indicated by solid lines, while insignificant slopes are indicated by dotted lines.

FIGURES



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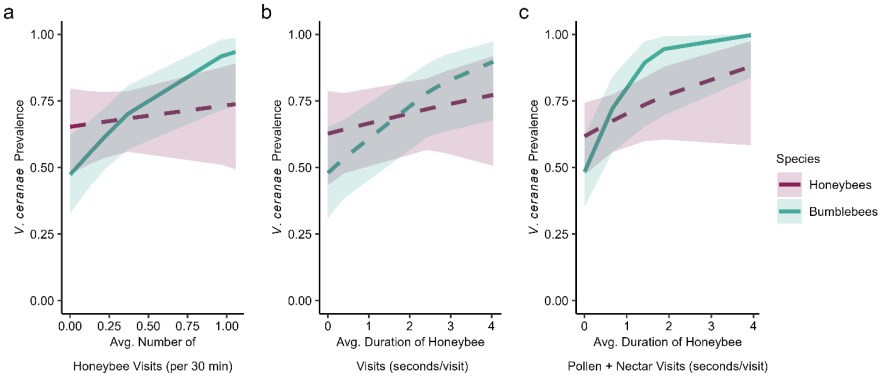

Figure 2. (a) The average number of honeybee visits to flowers was correlated with greater *V. ceranae* prevalence in bumblebees (p = 0.005), but not in honeybees (p = 0.58). (b) The average total duration of time honeybees spent on flowers per visit did not correlate with *V. ceranae* prevalence in honeybees (p = 0.40) or bumblebees (p = 0.014). (c) The average duration of time honeybees spent per visit interacting with pollen and nectar simultaneously correlated with greater *V. ceranae* prevalence in bumblebees (p = 0.009), but not *V. ceranae* prevalence in honeybees (p = 0.12). The number and duration of visits by honeybees were converted to their original scales for figure clarity. Significant slopes are indicated by solid lines, while insignificant slopes are indicated by dotted lines. Honeybee visits (per 30 min) were evaluated based on a 0.05 alpha threshold, while the visit duration (seconds/visit) were evaluated according to the Bonferroni-corrected alpha threshold of 0.0125.

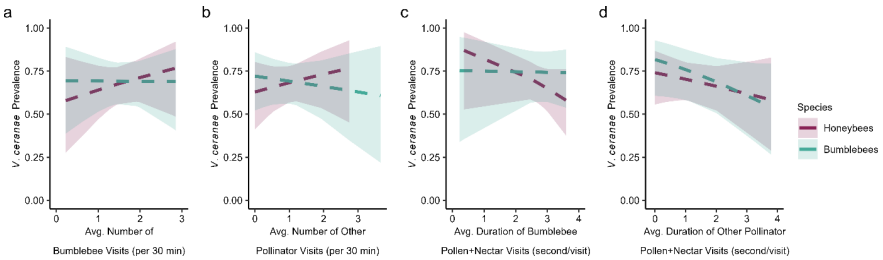

Figure 3. Neither the number of bumblebee and other pollinator visits per 30 min nor the duration per visit to pollen + nectar impacted *V. ceranae* prevalence in honeybees or bumblebees. There was no change in *V. ceranae* prevalence in honeybees or bumblebees based on (a) average number of bumblebee visits (per 30 min), (b) average number of other pollinator visits (per 30 min), (c) average duration on pollen + nectar per bumblebee visit (second/visit), and (d) average duration on pollen + nectar per other pollinator visit (second/visit). Significant slopes are indicated by solid lines, while insignificant slopes are indicated by dotted lines.

APPENDIX S1

Table S1. Field site abbreviation, farm name, dates of each visit to the field site (mm/dd/yyyy), zone, and Easting and Northing coordinates in the UTM GPS system. All field sites are located in the southeastern region of the Lower Peninsula of Michigan, USA. Permission from landowners was granted for all pollinator collection.

Site Code	Farm Name	First Visit	Second visit	Zone	Easting	Northing
BP	Brimley's Pumpkin Patch	8/10/2016	8/26/2016	16T	714474	4716740
K	Kapnick Orchards	8/21/2016	8/28/2016	17T	257729	4648607
PR	Peacock Road Farms	7/26/2016	8/23/2016	16T	714244	4746884
GT	Green Things Farm	8/17/2016	8/24/2016	17T	276741	4689607
E	Erwin Orchards	7/27/2016	8/22/2016	17T	280997	4708908
PL	Plymouth Orchards	8/11/2016	8/30/2016	17T	289557	4690343

Table S2. Morphospecies classifications used to identify individuals visiting squash flowers in the visitation videos. For analyses, visitations by all other pollinator groups excluding*Apis mellifera* and *Bombus* spp. were combined into the 'Other' category.

Code	**Possible included species**	**Total Num. Visits**	**Characteristics**
APIS	*Apis mellifera*	50	
AUGO	*Augochlora*, *Augochlorella*, *Augochloropsis*	232	Small green halictid
BOMB	*Bombus*	477	
HALI	*Halictus*, *Lasioglossum*	128	Small non-green halictid
HFLY	Hover fly	13	
MELI	*Melissodes*	2	
PEPO	*Eucera (=Peponapis)*	180	
TRIE	*Triepeolus*	1	Cuckoo bee, parasitizes *Eucera* spp.
VESP	*Vespula* (wasp)	1	

Table S3. Means and ranges of each visitation variable at each site. Durations per visit are calculated as the number of seconds that a bee species visited the flower per visit (i.e., number of seconds honeybees spent doing a given behavior divided by the number of honeybee visits to the flower).

	SITE	**Honeybees**	**Bumblebees**	**Other Pollinators**
VISIT NUMBER PER 30 MIN	**BP**	1.75 (0, 7)	9.25 (0, 25)	1.63 (0, 7)
	E	0 (0,0)	1.63 (0, 8)	7.31 (0, 43)
	GT	0.24 (0, 3)	4.47 (0, 15)	1.41 (0, 8)
	K	0 (0,0)	1.31 (0, 6)	1.31 (0, 4)
	PL	1 (0, 7)	4.44 (0, 15)	3.25 (0, 12)
	PR	0.13(0, 2)	8.44 (0, 38)	19 (0, 136)
TOTAL DURATION PER VISIT	**BP**	34.33 (0, 219.3)	19.57 (0, 70)	12.94 (0, 58.6)
	E	0 (0,0)	20.39 (0, 222.8)	19.01 (0, 113.7)
	GT	5.39 (0, 90.7)	18.90 (0, 42.3)	13.26 (0, 85)
	K	0 (0,0)	15.41 (0, 65)	31.08 (0, 94)
	PL	8.78 (0. 64.3)	20.63 (0, 107)	14.44 (0, 117.3)
	PR	5.28 (0, 84.5)	11.99 (0, 40.3)	63.71 (0, 484)
PETAL-ONLY DURATION PER VISIT	**BP**	4.03 (0, 23)	0.83 (0, 3.1)	6.35 (0, 33.7)
	E	0 (0,0)	0.29 (0, 4)	1.00 (0, 12)
	GT	5.10 (0, 86.7)	0.98 (0, 6)	5.54 (0, 32.8)
	K	0 (0,0)	0.85 (0, 4.3)	10.13 (0, 69.3)
	PL	2.31 (0, 11.7)	1.60 (0, 4)	5.70 (0, 23.4)
	PR	3.94 (0, 63)	0.55 (0, 4)	17.29 (0, 75.7)
NECTAR-ONLY DURATION PER VISIT	**BP**	0.29 (0, 2.5)	0.03 (0, 0.55)	1.40 (0, 14.3)
	E	0 (0,0)	0 (0,0)	0.02 (0, 0.4)

	SITE	Honeybees	Bumblebees	Other Pollinators
	GT	0 (0,0)	0 (0,0)	5.12 (0, 46.4)
	K	0 (0,0)	0.03 (0, 0.5)	18.15 (0, 80)
	PL	4.66 (0, 52.7)	0.67 (0, 8.4)	6.23 (0, 99.8)
	PR	0.66 (0, 10.5)	0 (0,0)	15.17 (0, 116)
POLLEN-ONLY DURATION PER VISIT	**BP**	2.00 (0, 11)	2.50 (0, 6.4)	1 (0, 6.3)
	E	0 (0,0)	1.30 (0, 7)	5.63 (0, 50.7)
	GT	0.29 (0, 4)	2.63 (0, 9)	1.49 (0, 12)
	K	0 (0,0)	0.80 (0, 3)	2.65 (0, 14)
	PL	0.22 (0, 3)	2.10 (0, 14.3)	1.21 (0, 9.1)
	PR	0.22 (0, 3.5)	1.26 (0, 4.6)	8.69 (0, 26.3)
POLLEN+NECTAR DURATION PER VISIT	**BP**	28.01 (0, 197.8)	16.17 (0, 67)	4.20 (0, 45.4)
	E	0 (0,0)	17.84 (0, 218.8)	12.36 (0, 60)
	GT	0 (0,0)	15.29 (0, 39.7)	1.10 (0, 14.8)
	K	0 (0,0)	13.66 (0, 60.5)	0.13 (0, 2)
	PL	1.59 (0, 17.4)	14.20 (0, 103)	1.29 (0, 9)
	PR	0.47 (0, 7.5)	9.93 (0, 37.8)	22.56 (0, 295)

Table S4. Numbers of honeybee (*Apis mellifera*) and bumblebee (*Bombus* spp.) individuals tested and positive for *V. ceranae* presence across six sites and two visits per site. We aimed to sample eight individuals per species per visit to each site where possible (target N = 16 per species per site), but there was some variation in the abundance of honeybees and bumblebees across visits to each field site.

Species	Species	honeybees	honeybees	Bumblebees	Bumblebees	Combined	Combined
Site	**VISIT**	**Tested**	**Positive**	**Tested**	**Positive**	**Tested**	**Positive**
BP	1	8	*4*	8	7	16	11
BP	2	8	*7*	8	8	16	15
E	1	8	*6*	7	2	15	8
E	2	8	*4*	8	4	16	8
GT	1	7	*3*	8	4	15	7
GT	2	5	*5*	7	4	12	9
K	1	3	*2*	7	4	10	6
K	2	5	*3*	8	4	13	7
PL	1	8	*5*	7	5	15	10
PL	2	8	*8*	8	8	16	16
PR	1	0	*0*	2	1	2	1
PR	2	7	*4*	8	4	15	8
TOTAL	*TOTAL*	**75**	51	**86**	55	**161**	106

Table S5. Full model output for each zero-inflated hurdle GLMM for pollinator species (honeybees, bumblebees, or other pollinators) effects on the number of visits per 30 min and total, petal, pollen, and pollen+nectar durations per visit. The table includes the model estimate, standard error, z value, and p-value for each main effect in the conditional and zero-inflated portions of the models, as well as the variance and standard deviation for the nested random effects of each observed flower within site visit (i.e., first or second visit to each site) within site. All models used a negative binomial distribution with a log link function. Some models have singular random effects, indicating no variation in all or some of the nested random effects. Significant p-values are bolded.

Response variable	Main Effect	Estimate	Std Error	z value	P-value	Random E
Number of visits per 30 min	**Conditional model:**					
	Intercept	0.25	0.48	0.52	0.602	FlowerID:
	Genus-Bumblebees	1.48	0.38	3.91	**<0.0001**	Visit:Site
	Genus-Other Pollinators	1.08	0.57	1.87	0.061	Site
	Zero-inflation model:					
	Intercept	0.58	0.43	1.34	0.179	
	Genus-Bumblebees	-2.06	0.54	-3.79	**0.00015**	
	Genus-Other Pollinators	-1.67	0.68	-2.47	**0.014**	
Total duration per visit	**Conditional model:**					
	Intercept	3.25	0.32	10.07	**<0.0001**	FlowerID:
	Genus-Bumblebees	-0.36	0.34	-1.04	0.3	Visit:Site
	Genus-Other Pollinators	0.18	0.36	0.49	0.624	Site
	Zero-inflation model:					
	Intercept	1.30	0.27	4.88	**<0.0001**	
	Genus-Bumblebees	-2.47	0.42	-5.90	**<0.0001**	
	Genus-Other Pollinators	-1.71	0.35	-4.88	**<0.0001**	
Duration on Petals per visit	**Conditional model:**					
	Intercept	1.92	0.39	5.0	**<0.0001**	FlowerID:
	Genus-Bumblebees	-2.29	0.36	-6.4	**<0.0001**	Visit:Site
	Genus-Other Pollinators	0.18	0.37	0.5	0.618	Site
	Zero-inflation model:					
	Intercept	1.22	0.30	4.07	**<0.0001**	
	Genus-Bumblebees	-20.12	3615.1	-0.01	0.996	
	Genus-Other Pollinators	-1.69	0.40	-4.20	**<0.0001**	
Duration on Pollen per visit	**Conditional model:**					
	Intercept	0.26	0.42	0.64	0.525	FlowerID:
	Genus-Bumblebees	0.19	0.41	0.46	0.643	Visit:Site
	Genus-Other Pollinators	1.45	0.44	3.33	**0.0009**	Site
	Zero-inflation model:					
	Intercept	1.12	0.41	2.71	**0.007**	
	Genus-Bumblebees	-18.04	4156.2	0.00	0.997	
	Genus-Other Pollinators	-1.12	0.48	-2.36	**0.018**	
Duration on Pollen + Nectar per visit	**Conditional model:**					
	Intercept	2.64	0.46	5.76	**<0.0001**	FlowerID:
	Genus-Bumblebees	0.10	0.44	0.23	0.821	Visit:Site
	Genus-Other Pollinators	-0.56	0.49	-1.15	0.25	Site
	Zero-inflation model:					
	Intercept	1.94	0.33	5.90	**<0.0001**	
	Genus-Bumblebees	-2.47	0.41	-6.08	**<0.0001**	
	Genus-Other Pollinators	-1.27	0.41	-3.14	0.0017	

Table S6. Pairwise contrasts between each pollinator species group (honeybees, bumblebees, and other pollinators) for each pollinator visitation behavior metric, including number of visits per 30 min, total duration per visit, and duration per visit on petals, pollen, and pollen+nectar. Odds ratios are shown for the pairwise difference between the two host species compared in each row and are calculated on the log scale. P values are Tukey adjusted for comparing a family of three, and significant p-values are bolded. Data for these tests are shown in Figure 1 and Figure S1.

Response variable	**Contrast**	**Ratio**	**Std Error**	**DF**	**z value**	**P-value**
Number of visits per 30 min	Honeybees / Bumblebees	0.227	0.086	Inf	-3.911	**0.0003**
	Honeybees / Other Pollinators	0.341	0.196	Inf	-1.873	0.1467
	Bumblebees / Other Pollinators	1.504	0.653	Inf	0.94	0.6152
Total duration per visit	Honeybees / Bumblebees	1.427	0.489	Inf	1.036	0.5539
	Honeybees / Other Pollinators	0.839	0.301	Inf	-0.49	0.876
	Bumblebees / Other Pollinators	0.588	0.143	Inf	-2.184	0.0738
Duration on Petals per visit	Honeybees / Bumblebees	9.902	3.5253	Inf	6.44	**<0.000**
	Honeybees / Other Pollinators	0.8326	0.3063	Inf	-0.498	0.8722
	Bumblebees / Other Pollinators	0.0841	0.0214	Inf	-9.739	**<0.000**
Duration on Pollen per visit	Honeybees / Bumblebees	0.827	0.3382	Inf	-0.464	0.8882
	Honeybees / Other Pollinators	0.235	0.1024	Inf	-3.326	**0.0025**
	Bumblebees / Other Pollinators	0.284	0.0653	Inf	-5.477	**<0.000**
Duration on Pollen + Nectar per visit	Honeybees / Bumblebees	0.904	0.402	Inf	-0.226	0.9722
	Honeybees / Other Pollinators	1.754	0.856	Inf	1.152	0.4824
	Bumblebees / Other Pollinators	1.94	0.685	Inf	1.876	0.1458

Table S7. Full model output for each GLMM for *V. ceranae*prevalence in honeybees, including the model estimate, standard error, z value, and p-value for each main effect in the models, as well as the variance and standard deviation for the nested random effects of each site visit (i.e., first or second visit to each site) within site. There are separate models for each pollinator behavior variable. Some models have singular random effects, indicating no variation in all or some of the nested random effects. Significant p-values are bolded, and all duration per visit models use the Bonferroni-corrected alpha threshold of 0.0125 instead of 0.05. Note that data on the nectar-only interactions are only included in the Appendix and were not part of the analyses in the main text and are not included as a group in the Bonferroni correction for four comparisons (Table 1).

Response variable	Main Effect	Estimate	Std Error	z value	P
V. ceranae in honeybees	Intercept	0.783	0.273	2.865	**0**
	Number of honeybee visits per 30 min	0.171	0.307	0.558	0
	Number of bumblebee visits per 30 min	0.262	0.365	0.717	0
	Number of other visits per 30 min	0.190	0.311	0.610	0
V. ceranae in honeybees	Intercept	0.783	0.276	2.842	**0**
	Total duration per visit of honeybee visits	0.260	0.310	0.840	0
	Total duration per visit of bumblebee visits	-0.312	0.355	-0.878	0
	Total duration per visit of other visits	-0.109	0.324	-0.336	0
V. ceranae in honeybees	Intercept	0.818	0.263	3.109	**0**
	Duration per visit of honeybee petal-only interactions	0.092	0.290	0.316	0
	Duration per visit of bumblebee petal-only interactions	0.714	0.348	2.051	0
	Duration per visit of other petal-only interactions	-0.504	0.341	-1.479	0
V. ceranae in honeybees	Intercept	2.025	1.191	1.700	0
	Duration per visit of honeybee nectar-only interactions	0.270	0.776	0.347	0
	Duration per visit of bumblebee nectar-only interactions	4.006	3.119	1.284	0
	Duration per visit of other nectar-only interactions	-0.392	0.302	-1.300	0
V. ceranae in honeybees	Intercept	0.783	0.286	2.742	**0**
	Duration per visit of honeybee pollen-only interactions	0.043	0.300	0.143	0
	Duration per visit of bumblebee pollen-only interactions	0.044	0.398	0.109	0
	Duration per visit of other pollen-only interactions	0.150	0.387	0.388	0
V. ceranae in honeybees	Intercept	0.798	0.258	3.087	**0**
	Duration per visit of honeybee pollen+nectar interactions	0.490	0.311	1.574	0

Response variable	Main Effect	Estimate	Std Error	z value	P
	Duration per visit of bumblebee pollen+nectar interactions	-0.408	0.315	-1.297	0
	Duration per visit of other pollen+nectar interactions	-0.230	0.294	-0.784	0

Table S8. Full model output for each GLMM for *V. ceranae*prevalence in *Bombus* spp. (bumblebees), including the model estimate, standard error, z value, and p-value for each main effect in the models, as well as the variance and standard deviation for the nested random effects of each site visit within site. Some models have singular random effects, indicating no variation in all or some of the nested random effects. Significant p-values are bolded, and all duration per visit models use the Bonferroni-corrected alpha threshold of 0.0125 instead of 0.05. Note that data on the nectar-only interactions are only included in the Appendix and were not part of the analyses in the main text and are not included as a group in the Bonferroni correction for four comparisons (Table 1).

Response variable	Main Effect	Estimate	Std Error	z value
V. ceranae in bumblebees	Intercept	0.807	0.288	2.803
	Number of honeybee visits per 30 min	1.144	0.410	2.789
	Number of bumblebee visits per 30 min	-0.008	0.333	-0.023
	Number of other visits per 30 min	-0.118	0.267	-0.443
V. ceranae in bumblebees	Intercept	0.683	0.284	2.407
	Total duration per visit of honeybee visits	0.825	0.335	2.465
	Total duration per visit of bumblebee visits	-0.016	0.342	-0.046
	Total duration per visit of other visits	-0.306	0.352	-0.868
V. ceranae in bumblebees	Intercept	0.648	0.271	2.387
	Duration per visit of honeybee petal-only interactions	0.573	0.274	2.088
	Duration per visit of bumblebee petal-only interactions	0.680	0.312	2.180
	Duration per visit of Other petal-only interactions	-0.389	0.305	-1.277
V. ceranae in bumblebees	Intercept	1.873	1.096	1.708
	Duration per visit of honeybee nectar-only interactions	0.616	0.762	0.808
	Duration per visit of bumblebee nectar-only interactions	3.948	3.048	1.295
	Duration per visit of other nectar-only interactions	-0.360	0.300	-1.200
V. ceranae in bumblebees	Intercept	0.708	0.263	2.692
	Duration per visit of honeybee pollen-only interactions	0.790	0.352	2.246
	Duration per visit of bumblebee pollen-only interactions	-0.094	0.356	-0.265
	Duration per visit of other pollen-only interactions	-0.376	0.333	-1.130
V. ceranae in bumblebee	Intercept	1.069	0.401	2.666
	Duration per visit of honeybee pollen+nectar interactions	1.878	0.722	2.601
	Duration per visit of bumblebee pollen+nectar interactions	-0.017	0.261	-0.064
	Duration per visit of other pollen+nectar interactions	-0.426	0.270	-1.578

Table S9. The *V. ceranae* prevalence for honeybees and bumblebees at each site, including the standard error (SE), degrees of freedom (df), and the lower and upper asymptotic confidence intervals.

Genus	Site	Estimated Prevalence	SE	df	Lower CL	Upper CL
Honeybees	BP	0.688	0.116	146	0.431	0.865
Honeybees	E	0.625	0.121	146	0.375	0.822
Honeybees	GT	0.667	0.136	146	0.374	0.870
Honeybees	K	0.625	0.171	146	0.282	0.876
Honeybees	PL	0.813	0.098	146	0.550	0.939

Genus	Site	Estimated Prevalence	SE	df	Lower CL	Upper CL
Honeybees	PR	0.571	0.187	146	0.228	0.858
Bumblebees	BP	0.938	0.061	146	0.661	0.991
Bumblebees	E	0.400	0.127	146	0.190	0.654
Bumblebees	GT	0.533	0.129	146	0.291	0.761
Bumblebees	K	0.533	0.129	146	0.291	0.761
Bumblebees	PL	0.867	0.088	146	0.592	0.967
Bumblebees	PR	0.500	0.158	146	0.223	0.777

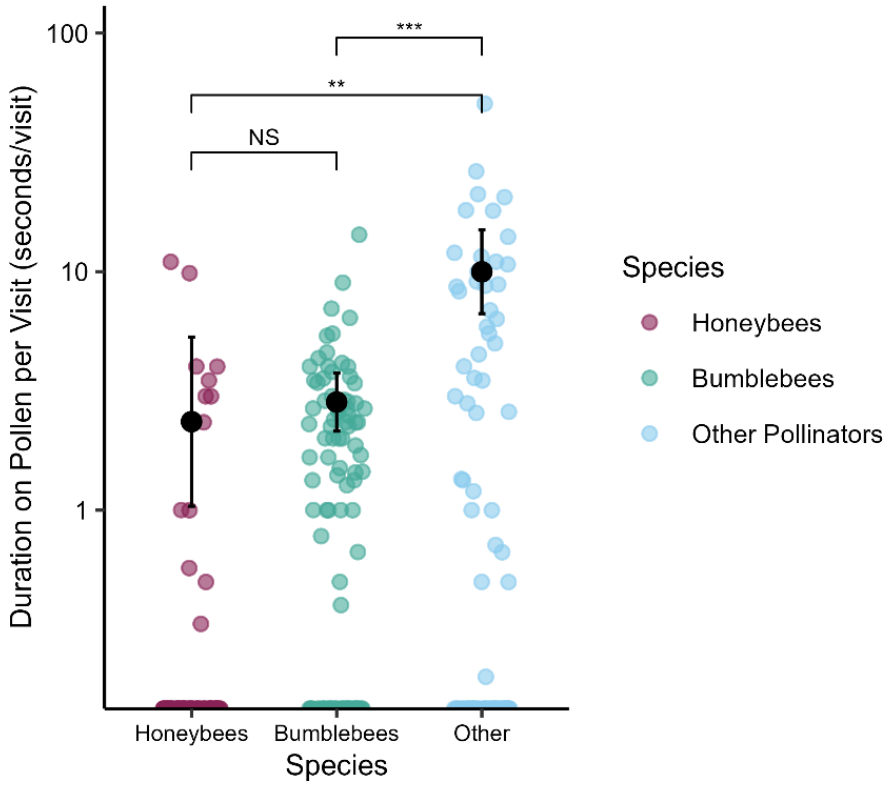

Figure S1. Other pollinators spent more time per visit (seconds/visit) on pollen than both honeybees and bumblebees. This finding may be driven by the time spent by *Eucera pruinosa* , a squash pollen specialist, that was included in the 'other pollinator' category. Significant differences are indicated by the number of stars for each pair (Appendix S1: Table S6). The y-axis is log scaled, and zero values are shown along the x-axis. Colored points are the raw data per flower observed, and the black points are the model predicted marginal means with 95% confidence intervals.

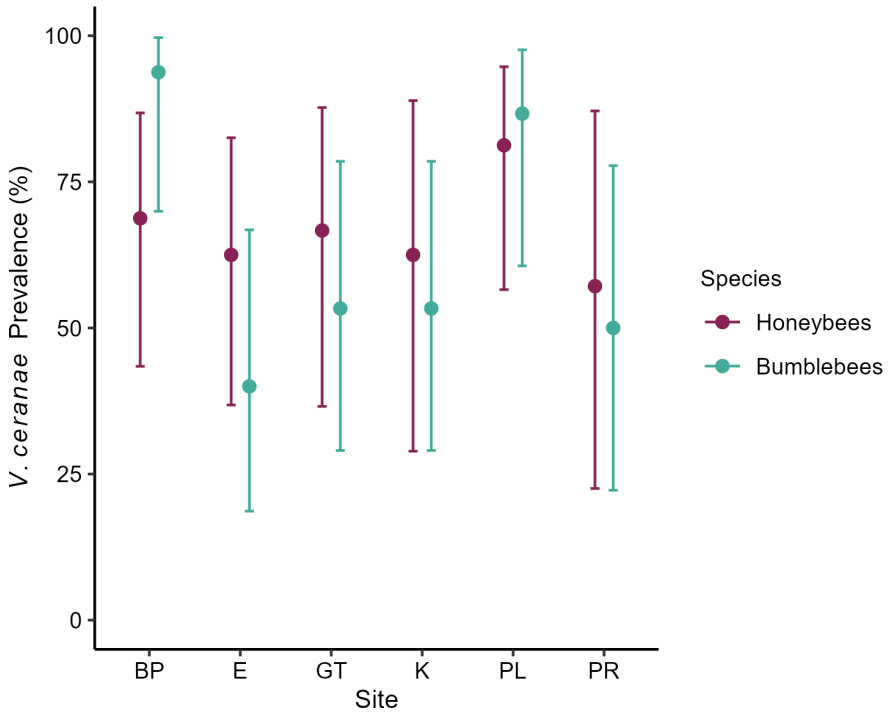

Figure S2. *V. ceranae* prevalence in honeybees and bumblebees at each of the six field sites. (Appendix: Tables S9). The error bars signify a 95% confidence interval.

APPENDIX S2

Trapping methodology

To catch bees using the netting method, each transect was walked once for 30 minutes at approximately 08:00, 10:00, 11:00, and 12:00 before the squash flowers closed around midday. Any observed honeybees and bumblebees that were visiting squash flowers within 1.5 m of the transect were captured. To allow for maximum sample sizes, pan traps were used alongside netting. Brightly colored pan traps attract bees, who are subsequently trapped by soapy liquid in the pans and drown (Roulston et al. 2007). In our study, fluorescent blue, yellow, and white pan traps were placed in an alternating color pattern 5 m apart along each transect between the crop rows. Each trap was filled with a mixture of water and clear dish soap. Traps were set up by 07:00 am, checked every three hours for captured pollinators, and collected after the squash flowers closed around midday, for a total average duration of six hours.

PCR procedure

The PCR master mix contained 12.5 µL dH_2O, 2 µL 10x buffer, 0.4 µL 10 mM dNTPs, 1 µL of each primer (10 mM), 2 µL 25 mM $MgCl_2$, and 0.1 µL 5 U/µL Taq polymerase (Invitrogen, Carlsbad, CA, USA) per reaction. Reactions were run with an initial denaturation step at 94 @C for 2 min, 40 cycles containing denaturation at 94 @C for 30 s, annealing at 61 @C for 45 s, and extension at 72 @C for 2 min, followed by a final extension at 72 @C for 7 min and a cooling period at 10 @C for 2 min.

The PCR product was visualized on a 2% agarose gel by observing a 250 bp band. We extracted the 250 bp band with a High Pure PCR Product Purification and Gel Extraction kit (Roche, Basel, Switzerland) to clean the product for sequencing.