BEE PATHOGEN TRANSMISSION IN PLANT-POLLINATOR NETWORKS

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Bees are important pollinators globally that have experienced population declines linked to pathogens. However, we have a limited understanding of how pathogens spread between bees through shared use of flowers and how this impacts entire bee communities. Here I developed a mechanistic understanding of bee pathogen transmission on flowers and assessed how these patterns scaled to the community level. In Chapter 1, using bumble bees as a model system, I experimentally evaluated bee pathogen deposition, persistence, and acquisition on flowers, finding differences among flower species, flower parts, and environmental conditions. I found that bumble bees frequently defecated on plants while foraging and did so more when infected with a gut pathogen. In Chapter 2 I combined empirical data with mathematical modeling to evaluate how pathogen prevalence in plant-pollinator networks was affected by landscape simplification. I found that landscape simplification indirectly shaped pathogen prevalence via the diet breadth of the dominant bee species. Moreover, I found that network connectance was a key parameter driving disease spread and prevalence at the community level. In Chapter 3 I evaluated whether bee functional traits explained the variance in prevalence found in the bee communities. I found that functional traits, namely nesting location and intraspecific body size for the dominant species, were linked to prevalence of certain pathogen groups but not others. Lastly, in Chapter 4 I experimentally assessed whether two solitary bee species could become

infected with a gut pathogen known to infect bumble bees, and evaluated whether diet played a role in infection dynamics and host survival. Both solitary bee species presented evidence of pathogen replication, regardless of whether they had access to pollen. These results expand the current understanding of bee epidemiology beyond honey bees and bumble bees, highlighting the need to evaluate the host range and impact of pathogens on wild bee communities. Overall, these findings expand our understanding of bee pathogen transmission dynamics. Furthermore, insights from this work can help inform the development of wildflower mixes that maximize forage while minimizing disease spread in pollinator communities.

BIOGRAPHICAL SKETCH

Laura grew up between Colombia and Oklahoma, always seeking ways of being in nature and nurturing her immense fondness for the outdoors. She has always cared deeply for the environment and from an early age has committed her life to doing what she can to help understand and conserve ecosystems around the world. As such, she has developed her curiosity and interest for the living world into a career as an ecologist and conservation advocate.

Before her time at Cornell, Laura completed her Bachelor of Science degree at the University of Oklahoma, graduating *Summa Cum Laude* with a Biology Major and a Minor in Interdisciplinary Perspectives on the Environment. She worked at the Oklahoma Biological Survey for four years, under the mentorship of Dr. Elizabeth Bergey, evaluating the spread of non-native terrestrial snails through the horticultural trade, as well as conducting the first standardized survey of bumble bees in Oklahoma. From her work with Dr. Bergey she co-authored three publications, including a first-author publication.

Throughout her PhD, Laura researched bee pathogen transmission in plant-pollinator networks. She worked to understand the mechanisms underpinning transmission on flowers, how this scaled to entire bee communities, and the effects of pathogens on historically under-evaluated bee species. She was very active in the Tropical Biology and Conservation Graduate Student Association, serving as the President in her final year as a graduate student, as well as being the Sustainability Fellow at the Big Red Barn. While at Cornell, she was actively involved in science outreach to numerous audiences, ranging from school children to policy makers.

I dedicate this thesis to my extraordinary parents and brother for their unwavering support, brilliant advice, and endless love.

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CHAPTER 1

BEE PATHOGEN TRANSMISSION DYNAMICS: DEPOSITION, PERSISTENCE AND ACQUISITION ON FLOWERS

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Abstract

Infectious diseases are a primary driver of bee decline worldwide, but limited understanding of how pathogens are transmitted hampers effective management. Flowers have been implicated as hubs of bee disease transmission, but we know little about how interspecific floral variation affects transmission dynamics. Using bumble bees (*Bombus impatiens*), a trypanosomatid pathogen (*Crithidia bombi*), and three plant species varying in floral morphology, we assessed how host infection and plant species affect pathogen deposition on flowers, and plant species and flower parts impact pathogen survival and acquisition at flowers. We found that host infection with *Crithidia* increased defecation rates on flowers, and that bees deposited feces onto bracts of *Lobelia siphilitica* and *Lythrum salicaria* more frequently than onto *Monarda didyma* bracts. Among flower parts, bracts were associated with the lowest pathogen survival but highest resulting infection intensity in bee hosts. Additionally, we found that *Crithidia* survival across flower parts was reduced with sun exposure. These results suggest that efficiency of pathogen transmission depends on where deposition occurs and the timing and place of acquisition, which varies among plant species and environmental conditions. This information could be utilized for development of wildflower mixes that maximize forage while minimizing disease spread.

Introduction

Infectious diseases are a global concern for both humans and wildlife, with examples ranging from the shifting ecology of Ebola virus (Jones *et al.*, 2008) to the rapid and devastating expansion of the chytrid fungus in amphibian populations (Lips *et al.*, 2006). Pathogens are one of the primary threats to pollinator health (Goulson *et al.*, 2015). However, how infectious diseases spread across pollinator communities is poorly understood, limiting effective conservation. Specifically, the mechanisms mediating bee pathogen transmission through shared use of flowers are largely unknown (Adler *et al.*, 2018; McArt *et al.*, 2014), despite flowers being linked to pathogen spillover and spread (Otterstatter & Thomson, 2008). Increasing dependence on bees for crop pollination heightens the urgency to understand disease transmission dynamics (Aizen *et al.*, 2008).

Effective bee disease transmission requires that pathogens be deposited onto a plant species and flower part where they can survive long enough to be encountered by, acquired, and infect new susceptible hosts. Recent findings that transmission rates vary across flower species and floral traits (Adler *et al.*, 2018; Durrer & Schmid-Hempel, 1994; Graystock *et al.*, 2015) show that infected foraging bees can transmit disease to susceptible bees that subsequently visit the same flowers (Durrer & Schmid-Hempel, 1994; Graystock *et al.*, 2015). Yet the mechanisms governing how pathogen transmission occurs on flowers, including deposition, survival, and acquisition of bee pathogens, are largely unknown. Such information could help us predict which plants are more likely than others to function as disease hubs, which is important given the

increasing role that wildflower plantings play in pollinator protection efforts (Williams *et al.*, 2015).

Infection can alter behavior and physiology in ways that facilitate or impede disease spread. For example, infection can induce changes in the social network of ant colonies in ways that suppress pathogen transmission (Stroeymeyt *et al.*, 2018). Conversely, honey bees infected with the fecal-orally transmitted microsporidian *Nosema apis* often present symptoms of dysentery, which facilitates spread within the colony (Bailey & Ball, 1991). Whether infection-induced changes could influence defecation rates on flowers is unknown. Bumble bees infected with *Crithidia bombi*, a fecal-orally transmitted trypanostomatid pathogen, are cognitively impaired (Gegear *et al.*, 2006) and less efficient foragers (Gegear *et al.*, 2005; Otterstatter *et al.*, 2005), spending more time learning floral information and consequently visiting each flower for more time. Either of these mechanisms, physiologically induced defecation or altered foraging patterns, could result in more feces deposited on flowers by infected bees. Whether infection affects bee defecation patterns on flowers represents a serious knowledge gap in bee disease transmission dynamics.

The ways bees interact with flowers vary greatly across floral morphologies and architectures, and depend on traits of the bees themselves, such as body size. Depending on the interaction between a bee and a flower, defecation patterns and pathogen deposition may be altered (McArt *et al.*, 2014). Moreover, bee size is highly variable across and within bee species, and may play an important role in pathogen deposition on flowers (Adler *et al.*, 2018). For example, small bodied bees may fit entirely within flowers with long tubular corollas, resulting

in higher likelihood of pathogen deposition inside the corolla tube than for larger bees that can only access the nectar at the end of the tubular corolla via their proboscis. Conversely, for flowers with short corollas, bee feces may be unlikely to be deposited inside the corolla regardless of bee size, but instead may fall onto the bract subtending the flower, or onto other flowers in the inflorescence. These deposition dynamics could have consequences for pathogen survival and transmission, but the role of floral morphology and architecture in mediating host-pathogen dynamics is largely unknown.

Once deposited, horizontally transmitted pathogens depend on environmental conditions to remain infectious before being encountered by a new host. For example, the bee microsporidian *Nosema apis* can remain infectious up to six years under optimal conditions, but loses infectivity within hours when exposed to ultra–violet (UV) radiation (Schmid-Hempel, 1998). Similarly, bumble bees develop a stronger infection when inoculated with freshly prepared *Crithidia bombi* compared to inoculum that has been stored for 45 minutes (Schmid-Hempel *et al.*, 1999). Depending on where pathogens are deposited on a plant, their exposure to UV radiation and phytochemicals may vary (e. g., inside a corolla tube compared to an exposed petal). Moreover, pollen and nectar phytochemicals can have growth-inhibitory effects on *C. bombi* (Palmer-Young *et al.*, 2016), and floral volatiles can kill certain plant pathogens (Huang *et al.*, 2012). Therefore, we predicted that pathogen survival and infectiousness would vary across floral parts within the same plant and across species and environmental conditions, and would be lowest for floral parts more exposed to the sun's UV radiation, such as outside the corolla and on flower bracts.

We evaluated multiple mechanisms hypothesized to contribute to bee disease transmission through shared use of flowers. Specifically, we investigated whether: (1) infection influences fecal deposition on flowers; (2) the frequency of feces deposited varies with plant species and flower part (inside the corolla, outside the corolla, flower bract, and leaves); (3) pathogen survival depends on pathogen deposition and environmental conditions (sun exposure) across flower parts; and (4) pathogen acquisition and subsequent infection of bees vary among different parts of the flower in different plant species. We asked these questions by conducting three experiments. In the first experiment (questions 1 and 2), we allowed experimentally infected and uninfected bees fed fluorescent diet to forage on three flower species, and determined how many times and where they defecated on the plants. We predicted that infected bees would defecate more on flowers than uninfected bees, and that defecation patterns would depend on how the bees interact with the morphology of each plant species. In the second experiment (question 3), we placed pathogen inoculum on three flower parts and determined survival for three hours across three plant species, either in sun exposed or shaded conditions. We predicted that the pathogen would survive longer inside the flower corolla and under shaded conditions, due to reduced exposure to UV radiation. In the third experiment (question 4), we allowed uninfected bees to forage on flowers upon which we had placed inoculum on a discrete flower part, and quantified the resulting infection loads one week after exposure. We predicted that resulting infections would be lowest when inoculum was encountered inside the flower corolla, due to increased presence of phytochemicals in pollen and nectar. This study lies at the intersection of bee foraging ecology and epidemiology, and aims to expand the current understanding of bee disease transmission.

Materials and methods

(a) Study system

All experiments were conducted using common eastern bumble bee (*Bombus impatiens*) workers and the trypanosome *Crithidia bombi*. Native to eastern North America, *Bombus impatiens* (Hymenoptera, Apidae) is an abundant generalist bee, frequently used for commercial pollination (Velthuis & van Doorn, 2006). The pathogen *Crithidia bombi* (Kinetoplastea; Trypanosomatida; hereafter *Crithidia*) is a horizontally transmitted gut pathogen known to reduce bumble bee foraging efficiency and increase mortality under stressful conditions, and is associated with reduced reproduction in wild bumble bee colonies (Brown *et al.*, 2000; Goulson *et al.*, 2018; Otterstatter *et al.*, 2005). All experiments were conducted using *Crithidia* from wild *B. impatiens* workers collected in Massachusetts, USA (GPS: 42°22'17.53"N 72°35'13.52"W) and maintained in laboratory bumble bee colonies (Biobest, Leamington, Ontario); infected colonies were only used as source of inoculum and not as source of bees in experimental trials. For the duration of the experiments, we conducted weekly pathogen screenings of 5 bees from each experimental colony to ensure colonies were *Crithidia*–free. *Crithidia bombi* species identity was verified by sequencing the 18S rRNA (Schmid-Hempel & Tognazzo, 2010).

This study compared three plant species that are visited by bumble bees in northeastern North America and vary in their floral morphology and architectures: *Monarda didyma* (Lamiaceae), *Lobelia siphilitica* (Campanulaceae), and *Lythrum salicaria* (Lythraceae), hereafter *Monarda*, *Lobelia*, and *Lythrum* (Figure 1). *Monarda* and *Lobelia* are native to eastern North America,

whereas *Lythrum* is a non-native species introduced from Europe that is highly abundant and attractive to pollinators (Flanagan *et al.*, 2010).

(b) Experimental protocol

(i) Experiment 1: Effect of plant species and infection status on bee defecation patterns across flower parts

To evaluate the role of infection on bee defecation across plant species, we infected bees with *Crithidia*. The *Crithidia* inoculum used in the trials was prepared fresh daily by dissecting the gut of infected bees maintained in the laboratory and combining with Ringer's solution (Sigma–Aldrich, St. Louis, MO) to create a solution with 1200 cells/µl, which was then mixed with equal amount of 50% sucrose solution to create an inoculum with 25% sucrose and 600 cells per µl (Richardson *et al.*, 2015). We used 25% sucrose in Ringer's solution without *Crithidia* for a control (sham) inoculum. We selected 18 bees from each of three experimental colonies. Half were infected, while the other half were sham-infected, for a total of 54 bees inoculated each date (13 days: July 10, 12, 16, 19, 21, 26, and 28, and August 1, 3, 9, 10, 17 and 21, 2017), by feeding 10 µl of inoculum or sucrose solution using a micropipette. Three similarly-sized bees of the same treatment and colony were maintained in microcolony containers with 30% sucrose and pollen provided *ad libitum* for 7 – 12 days prior to trial to allow infection to develop (Schmid-Hempel & Schmid-Hempel, 1993).

To determine defecation patterns, bees were given sucrose mixed with fluorescent dye (2.5 g of fluorescent powder (Dayglo Color, Cleveland, OH) dissolved in 500 mL of 30% sucrose) *ad libitum* 24 – 48h prior to field trials. Defecation trials were conducted during summer 2017 (*Monarda* July 10 – 19, *Lythrum* July 21 – August 3, *Lobelia* August 9 – 21). The day of the trial, bees were cooled at 4 °C and transported in a cooler to the field site in Massachusetts (42°28'45.5" N, 72°34'46.06"W). Each trial consisted of a single flight cage (45.7 cm x 71.0 cm x 55.6 cm) in which three clipped field-grown inflorescences were placed in tubes with water, held upright by tube racks. The number of flowers per inflorescence was held constant within species. The bottom of each cage was lined with newspaper, which was replaced before each trial to eliminate cross contamination across trials. Cooling bees prior to trials facilitated foraging. Due to mortality during the period in which infection was allowed to grow, not all trials included three bees; there was no difference in mortality between infected and uninfected bees ($\chi^2_1 = 0.11$, $p = 0.742$), nor did number of bees in a trial affect defecation patterns ($\chi^2_1 = 1.32$, $p = 0.250$ and $\chi^2_1 = 1.67$, $p = 0.200$ for presence/absence and number of fecal droplets, respectively). The number of bees and time when each bee was placed in a cage and started foraging were noted. If bees did not forage within 15 minutes, a flower was raised towards the bees to induce foraging (20% of bees were induced). If presentation of the flower did not induce foraging, that trial was excluded from the experiment. Cages were checked for bee feces three hours after foraging began; the cage was brought into a darkened barn and a handheld black light was used to count the number of fluorescent fecal droplets on each plant part (Escolite UV Flashlight Black Light, 51 LED 395 nM). The plant parts were divided into four categories: "inside" the flower (inside the corolla), "outside" the flower (surface of the corolla), on the bract (on the modified leaf subtending the inflorescence), or on a leaf (excluding the bract; Figure 1).

We also recorded feces elsewhere in the cage, to determine the proportion of feces deposited on plants for each plant species. Post-trial, bees were returned to the lab and maintained on 30% sucrose until the following day, when they were dissected to confirm infection status. We removed the right forewing and measured marginal cell length as a proxy for bee size (Harder, 1982).

Statistical analyses

Data analyses were conducted using R studio (R version 3.5.1) with the lme4 and lsmeans packages (Bates *et al.*, 2015; Lenth, 2016; R Development Core Team, 2008). We excluded trials for bees that were inoculated but did not develop infection (n = 3) and control trials in which bees developed infection (n = 3), for a resulting sample size of n = 163 trials (*Lobelia* n = 54, *Lythrum* n = 61, and *Monarda* n = 48). To evaluate the factors that predicted defecation, we constructed a generalized linear mixed model (GLMM) that evaluated feces on plant (presence/absence) as the response, predicted by bee infection status (infected/uninfected), plant species, average bee size, and number of bees in the trial. To determine whether bees were defecating differently across parts of the plant, we developed a GLMM that included number of fecal droplets as the response variable and evaluated part (inside of flower, outside of flower, bract, or leaf), infection status (infected/uninfected), plant species, average bee size, and number of bees in trial as explanatory variables. Both models included observation level (trial), experimental colony, and date as random effects, and fit a Poisson distribution, which is suitable for count data (Ellison & Gotelli, 2004). Experimental colony did not explain variance in either model and affected convergence, so was removed from subsequent analyses. No variable in the

model produced a Variance Inflation Factor (VIF) greater than two, indicating low co-linearity (O'Brien, 2007). To determine the role of each explanatory variable, we employed a likelihood ratio test to compare the full model to identical models that excluded the variable in question. Significance of interactions was determined by comparing the original model with and without interactions (flower part by either average bee size, plant species, or infection status); we removed non-significant interactions. Significant interactions were evaluated using the *lstrends* function (Lenth, 2016).

(ii) Experiment 2: *Crithidia* survival across plant species and flower parts

Pathogen survival was evaluated across plant species and parts on flowers. We made *Crithidia* inoculum based on realistic fecal volumes and sugar concentrations; we did not consider other nutrients or compounds that may be in feces. We used Ringer's solution, a saline solution often used to study insect physiology (Alghamdi *et al.*, 2008), as we expected it would be a more realistic proxy for bee feces than water. We determined realistic fecal volumes by placing 10 worker *Bombus impatiens* in individual vials for 2 – 4 hours and measuring fecal volume using microcapillary tubes (Sigma–Aldrich: 20 µl). The largest volume observed was 33 µl, so we used 35 µl of *Crithidia* inoculum in trials, representing the upper limit of realistic fecal quantity. Given *Crithidia*'s susceptibility to sugar (Cisarovsky & Schmid-Hempel, 2014), we evaluated the sugar concentration of bee feces using a refractometer. The values ranged from 0 – 1% sugar, and so, unlike Experiment 1 and 3, no sugar was added to inoculum.

Trials were conducted during summer 2017. Inoculum was made fresh each trial day, with at least 3,300 *Crithidia* cells per microliter of Ringer's solution (mean: 3,617, range 3,300 – 3,900); this high concentration was chosen for ease of visualization in the hemocytometer. We used the same three plant species from Experiment 1, each evaluated in one day: *Monarda* (July 12), *Lythrum* (July 21), and *Lobelia* (August 1). Because environmental conditions and inoculum strength varied between days, and flower species did not have co-occurring blooming periods, we are not able to compare viability across plant species. Flowers were bagged in the field two days prior to trial to avoid pathogen deposition from foraging bees. On the day of the trial, inflorescences were cut, individually marked, and placed in tubes with water. The experiment was conducted in large covered hexagonal tents (71 x 160.5 in). To evaluate the effect of the sun, one tent had a UV-protected cover while the other had a mesh cover that allowed UV exposure but prevented wild bees from entering. *Monarda* was only evaluated in shaded (UV-protected) conditions due to rainy and overcast weather. Within each tent, we measured the temperature, relative humidity (AcuRite, 01083 Pro Accuracy Indoor Temperature and Humidity Monitor), and ultraviolet radiation (Apogee instruments, MU–100).

We placed 35 µl of inoculum on two parts of each inflorescence (inside corolla and bract; exception was *Monarda* where we also evaluated outside the corolla). We evaluated pathogen survival for three hours, taking five inflorescences every 30 minutes into the laboratory, where the inoculum on each part was pipetted into a hemocytometer to count mobile *Crithidia*. We did not evaluate infectivity of *Crithidia*, using mobility instead as a proxy for survival, in part because infectiousness of *Crithidia* is highly variable, even within a single day (Otterstatter & Thomson, 2006). If the inoculum evaporated, we pipetted 10 µl of distilled water onto the part to

collect any *Crithidia* cells and checked for mobile *Crithidia*; we were successful in detecting mobile *Crithidia* in some instances when the inoculum had visibly evaporated. The sample size for the shaded samples were: *Lobelia* n = 58 parts (29 inflorescences), *Lythrum* n = 60 (30 inflorescences), and *Monarda* n = 88 (31 inflorescences). The sample sizes for sun-exposed plants were: *Lobelia* n = 58 (29 inflorescences) and *Lythrum* n = 60 (30 inflorescences).

Statistical analyses

We conducted survival analyses using Cox proportional hazards mixed–models via the coxme package in RStudio (R Development Core Team, 2008; Therneau & Therneau, 2015). The survival analysis evaluated *Crithidia* survival (count of moving cells per 0.02 µl) by time elapsed when the flower was inspected for each of the three plant species. The model included part on flower and shade treatment as explanatory variables, as well as individual plant as the random effect. To determine significance of the treatments (flower part and shade), we conducted a likelihood ratio test comparing the full model of each species with a model that included the same random effect structure but excluded either explanatory variable or included an additive relationship instead of an interaction. Differences in survival across flower parts were determined *post hoc* with Tukey's HSD using the *lsmeans* function (Lenth, 2016).

(iii) Experiment 3: Effects of plant species and flower part on pathogen acquisition and subsequent intensity of infection

We evaluated the effect of plant species and flower part on *Crithidia* transmission by placing pathogen inoculum on flowers, allowing uninfected bees to forage, and subsequently determining infection (presence/absence and intensity) in the bees. Trials were conducted in 2016 on *Monarda* (June 30 – July 15), *Lythrum* (July 18 – Aug 9), and *Lobelia* (Aug 18 – 26). Experimental bees and inoculum were transported to the field site in a cooler with insulated ice packs. We used bees from 4 experimental colonies for *Monarda*, 5 for *Lythrum*, and 6 for *Lobelia*; colonies mostly overlapped for the first two species and had approximately 50% overlap for the second and third species. We accounted for colony origin in the analyses (see Statistical analyses). For each trial, we collected an inflorescence of the target species at the field site and placed it in a tube filled with water. Each trial was randomly assigned to one of three treatments of inoculum placement: inside corolla, outside corolla, or bract. For all the treatments, we added four 10 µl drops of inoculum in 25% sucrose solution (see Experiment 1 for inoculum preparation) on the inflorescence in the specified treatment part using a micropipette (Figure 1); inoculated flowers were marked using a paint pen. Inflorescences were from field-grown plants that were bagged with mesh for at least two days prior to trials to prevent *Crithidia* deposition from wild foraging bees. We placed the prepared inflorescence in a small flight cage and released a single, chilled worker bee into the cage (see Experiment 1 for cage details). We allowed the bee to forage and recorded total time spent foraging (i.e., probing flowers, not including time moving between flowers), number of flowers probed, and number of drops probed. We also recorded the time of the trial so that we could calculate elapsed time between inoculum preparation and each trial for use as a covariate. When the bee stopped foraging (usually a clear change in behavior from probing flowers to flying around the cage), we

recaptured it in a vial. Bees were excluded if they did not probe any inoculum drops or foraged for less than thirty seconds.

Bees were collected and subsequently maintained individually for one week in the laboratory to allow infection to develop. We fed each bee daily 500 µl of 30% sucrose solution and a ~0.15 g pollen ball (30% sucrose and commercial mixed wildflower pollen (Koppert Biological Systems; Linden Apiaries, Walpole, NH, USA)). We maintained the bees in an incubator set at 27 °C in darkness. After seven days, we dissected each bee and placed the gut in 300 µl of Ringer's solution. The mixture was allowed to incubate for four hours before *Crithidia* was quantified using a hemocytometer (Richardson *et al.*, 2015). We removed the right forewing and measured marginal cell length as a proxy for size (Harder, 1982). Sample sizes for each species were n = 40 bees for *Monarda*, n = 67 for *Lythrum*, and n = 89 for *Lobelia*.

Statistical analyses

Data analyses were conducted using R studio with packages lme4, DHARMa, RVAidememo and lsmeans (Bates *et al.*, 2015; Hartig, 2017; Hervé, 2015; Lenth, 2016; R Development Core Team, 2008). To manage zero-inflated and overdispersed count data, we used manual two-step hurdle models (Zuur *et al.*, 2009). We first evaluated an "incidence" model (evaluating presence or absence of *Crithidia* infection), followed by an "intensity" model (*Crithidia* counts of the infected bees). In the first step, we modeled pathogen incidence using a binomial distribution (logit link), given the binary outcome of whether bees were infected or not. Next, we modeled *Crithidia* intensity when present (i.e., the non-zero outcomes) with a Poisson

distribution (log link). We evaluated overdispersion in the Poisson model using the *overdisp.glmer* function in the RVAideMemoire package (Hervé, 2015). To ensure our data were well-modeled by the specified distributions and to check model assumptions, we used the DHARMa package (Hartig, 2017). Our incidence model was evaluated using a GLMM, with presence or absence of infection as the response variable, predicted by flower part, plant species, their interaction, bee size, foraging time, and time since the inoculum was made (related to its infectiousness). The model included colony and date as random effects, thus accounting for overlap in colonies during trials. The intensity model had the same random effect structure as the incidence model, plus an observation-level random effect to correct for overdispersion (Harrison, 2014). To determine significance, we conducted a likelihood ratio test by comparing the full GLMM model to a model that excluded the factor of interest. Significant factors were determined *post hoc* with Tukey's HSD using the *lsmeans* function (Lenth, 2016).

Results

(i) Experiment 1: Effect of plant species and infection status on bee defecation patterns across flower parts

Overall, bees defecated on plants in 65% of trials. Infected bees were more likely to defecate on plants than uninfected bees ($\chi^2_1 = 4.26$, $p = 0.039$; Figure 2), although there was no relationship between infection status and the number of fecal droplets observed ($\chi^2_1 = 1.05$, $p = 0.306$) or where bees defecated ($\chi^2_3 = 3.78$, $p = 0.287$). Flower part significantly predicted the number of fecal droplets observed ($\chi^2_4 = 23.05$, $p < 0.001$). Moreover, we found a strong plant

species by part interaction ($\chi^2_6 = 166.74$, $p < 0.001$; Figure 3a and Table S1), such that the most deposition occurred on leaves and bracts for *Lobelia*, on bracts and inside the flower for *Lythrum*, and outside the flower for *Monarda*. We observed a bee size by flower part interaction for number of fecal droplets observed ($\chi^2_3 = 9.08$, $p = 0.028$; Figure 3b), whereby fewer droplets were detected inside flowers visited by larger bees (Tukey HSD: $z = -2.87$, $p = 0.004$). Plant species and average bee size did not predict presence or number of fecal droplets observed on flowers ($\chi^2_2 = 1.32$, $p = 0.517$ and $\chi^2_1 = 0$, $p = 0.991$ respectively for presence of feces; $\chi^2_2 = 0.978$, $p = 0.614$ and $\chi^2_1 = 0.50$, $p = 0.478$ respectively for number of fecal droplets). Bee size had no relationship with number of fecal droplets observed on the outside of the flower, on the bract, or on leaves ($z = 1.55$, $p = 0.122$, $z = 1.11$, $p = 0.268$ and $z = 1.34$ and $p = 0.180$, respectively). The proportion of total fecal droplets that landed on the plants (compared to elsewhere in the cage) varied across plant species ($\chi^2_2 = 28.65$, $p < 0.001$), being 0.55, 0.29 and 0.25 for *Lobelia*, *Lythrum*, and *Monarda* respectively.

(iii) Experiment 2: *Crithidia* survival across plant species and flower parts

Crithidia became non-motile within three hours of placement on flowers in 71% of trials. Furthermore, mortality varied by plant species ($\chi^2_1 = 0.001$, $p < 0.001$), at 90% for *Lobelia*, 90% for *Lythrum*, and 20% for *Monarda*. *Crithidia* survival was influenced by flower part on all plant species ($\chi^2_1 = 4.67$, $p = 0.031$, $\chi^2_1 = 5.49$, $p = 0.019$ and $\chi^2_2 = 6.30$, $p = 0.043$ for *Lobelia*, *Lythrum*, and *Monarda* respectively*;* Figure 4a, b). For *Lobelia* and *Lythrum*, *Crithidia* survived longer inside the corolla than on the bract (Tukey HSD test: $z = 2.09$, $p = 0.037$ and $z = 2.29$, $p = 0.022$ for *Lobelia* and *Lythrum* respectively). *Post hoc* evaluation of *Crithidia* survival across

parts on *Monarda* flowers did not yield significant pairwise comparisons (Table S2), likely due to low overall mortality in this species. *Crithidia* survival was also greater in shaded than sunny conditions ($\chi^2_1 = 6.87$, $p = 0.009$ and $\chi^2_1 = 4.53$, $p = 0.033$ for *Lobelia* and *Lythrum* respectively*;* Figure 4c, d). There was no flower part by sun exposure interaction in either species ($\chi^2_1 = 0.02$, $p = 0.892$ and $\chi^2_1 = 1.48$, $p = 0.223$, for *Lobelia* and *Lythrum*, respectively).

(ii) Experiment 3: Effects of plant species and flower part on *Crithidia* acquisition and subsequent intensity of infection

The probability of becoming infected did not depend on plant species, part where inoculum was placed, their interaction, or bee size ($\chi^2 < 4.68$, $p > 0.137$ for all). However, part on flower did predict *Crithidia* intensity for the infected bees ($\chi^2_2 = 13.66$, $p = 0.001$; Figure 5). Specifically, when bees picked up inoculum on the bract of a flower, they developed a more intense *Crithidia* infection than if they encountered the pathogen on the outside of the flower (Tukey HSD: $z = 3.77$, $p < 0.001$). Similarly, bees developed a marginally more intense *Crithidia* infection when encountered on the bract than the inside of the flower ($z = 2.29$, $p = 0.057$). There was no difference in infection intensity between the inside and outside of the flower ($z = 1.35$, $p = 0.370$). For infected bees, bee size did not explain *Crithidia* intensity ($\chi^2_1 = 0.83$, $p = 0.363$), nor did plant species ($\chi^2_2 = 1.01$, $p = 0.602$), or plant species by flower part interaction ($\chi^2_4 = 4.54$, $p = 0.338$).

Discussion

The intersection of bee foraging ecology and epidemiology is a novel area of research that can give rise to new understanding of pollinator disease spread and evidence-based conservation strategies. Here we show that foraging bumble bees often defecate on plants, and do so more when they are infected with *Crithidia* (Figure 2). There is not a universal part on plants where bees are more likely to defecate. That pattern depends on plant species, which may in turn be related to floral traits, such as shape or size. These deposition dynamics are also influenced by bee traits, with bigger bees defecating fewer times inside flowers (Figure 3b), possibly because they are too large to fit inside the flowers. Similarly, for pathogen survival on flowers, we found differences across flower parts for some species but not for others (Figure 4a). Moreover, the flower part where inoculum is encountered influenced the intensity of the resulting infection (Figure 5), further highlighting the complexity of bee pathogen transmission dynamics via flowers. Taken together, these data suggest variation in plant-pollinator interaction patterns, from encounter rates to trait matching, are expected to influence pathogen transmission and warrant further research.

Bees defecated on plants in 65% of trials, and did so significantly more when infected with *Crithidia* (Figure 2). Increased likelihood of defecation on plants could hasten the spread of multiple diseases, especially because bumble bees are often infected with several fecal-orally transmitted pathogens (Cordes *et al.*, 2012; Schmid-Hempel, 1998). Whether the increased defecation is a by-product of dysentery, as in honey bees infected with *Nosema apis* (Bailey & Ball, 1991) or due to increased time spent on each flower by infected bees (Gegear *et al.*, 2005; Otterstatter & Thomson, 2006), remains unknown.

We found a plant species by part interaction on the number of fecal droplets observed, such that each plant species had a different part where droplets were most likely to be found (Figure 3a). Differential handling of the flowers across plant species could have led to this pattern, especially given the diversity of floral morphologies and plant architectures (Figure 1). For *Monarda* (Figure 1c), the inside of the small floral tube is only accessible to the bee proboscis, likely explaining why we seldom observed feces there, compared to the outside of the corolla where the bees crawl to reach subsequent flowers. Similarly, *Lobelia* (Figure 1a) rarely had feces inside of the flower, despite an entirely different floral morphology. The floral tube of *Lobelia* is quite large, such that the entire head of the bees can fit inside, but usually the abdomen protrudes, enabling defecation onto leaves or bracts subtending the flower. However, the smallest bees in the trials fit entirely within the *Lobelia* flowers, likely contributing to the bee size by part interaction. *Lythrum* differed in that it often had feces on the inside of its flowers. This is likely because the tube of *Lythrum* is extremely short and narrow and surrounded by wide, flat petals (Figure 1b), so that bees will crawl over the entire flower after foraging to reach the next flower. These differential deposition dynamics across plant species are the first step towards horizontal transmission, which can result in transferring the pathogen to new colonies via foragers.

Horizontally transmitted pathogens must remain viable to be acquired by a new host. However, the decay rate of many pathogens outside of their host is unknown (Richardson & Gorochowski, 2015). *Crithidia* survived longer on the inside of the corolla than the bract of *Lythrum* and *Lobelia* flowers (Figure 4a, b). We had predicted that the inside would provide

more protection from desiccation, extending survival compared to more exposed parts. However, we did not observe that pattern for *Monarda*, which aligns with the lower overall *Crithidia* mortality on this species. Floral chemistry or other unknown mechanisms could mediate the lack of differences across floral parts for this species, as could more humid environmental conditions during the day of trial. In general, we found that within three hours of being placed on flowers, most *Crithidia* had died. Incorporating rate of decay between deposition by infected bees and acquisition by the incoming susceptible foragers could enhance disease spread models (Richardson & Gorochowski, 2015).

Once pathogens have been deposited on the plant, environmental factors could influence pathogen survival. *Crithidia* on sun–exposed flowers had shorter survival times than shaded plants (Figure 4c, d). This may be because of UV radiation, temperature, and/or increased desiccation, all of which were greater in the sun–exposed conditions. Pulsed UV radiation can decrease *Crithidia* viability (Naughton *et al.*, 2017). Otterstatter & Thompson experimentally varied the time and number of *Crithidia* cells placed on *Brassica rapa* nectaries encountered by susceptible foraging bumble bees. They found that most foraging bees became infected when exposed to *Crithidia* that had been placed on the flower for less than 10 minutes; by 85 minutes the probability of infection was under 15% (Otterstatter & Thomson, 2008). They determined the half-life of *Crithidia* to be 77 minutes, largely mirroring our results. Floral mechanisms that maximize exposure to direct sunlight, such as heliotropism, could reduce bee pathogen survival on flowers and warrant further investigation. Similarly, whether environmental gradients that affect exposure to UV radiation (e. g., along an altitudinal gradient or from the forest canopy to

the ground layer) influence bee pathogen transmission dynamics on flowers is entirely unknown and is an important area for future research.

For bees that developed an infection after foraging on inoculated plants (Experiment 3), those that encountered inoculum on the bract had more intense *Crithidia* infections than when they encountered it on the outside of the flower (Figure 5). This pattern may be due to fewer phytochemicals from nectar and pollen encountered on the bract (Palmer-Young *et al.,* 2019). For *Lobelia* and *Lythrum*, bumble bees defecated many times on the bract (Experiment 1), which was also the part associated with the most intense *Crithidia* infection (Experiment 3). However, in this part *Crithidia* survived shorter amounts of time, and so the ability to transmit *Crithidia* will depend on how quickly feces are encountered by a new host. *Lythrum* is very frequently visited by bees, especially *B. impatiens,* in its non-native North American range (Flanagan *et al.*, 2010), which could then minimize the impact of short pathogen survival time and facilitate pathogen spread in the community. Conversely, foraging bumble bees seldom defecated on *Monarda* bracts, the part that resulted in the greatest infection intensity. These results suggest *Lobelia* and *Lythrum* may be more effective disease transmission hubs than *Monarda*, but transmission will also depend on frequency of visitation. We hypothesize that floral morphologies that facilitate overlap in where pollinator feces are deposited and acquired (e.g. flat composites on which bees walk and forage for long periods of time) would result in higher rates of disease transmission compared to morphologies for which deposition and acquisition may be disjointed (e.g., Solanaceous plants that are visited for short period of times and do not have a landing platform).

In the face of increasing dependence on bees for ecosystem services (Aizen *et al.*, 2008), there is a pressing need to understand factors that shape pollinator health. Pathogen-induced stress and spillover from commercial bees via flowers are factors consistently linked to pollinator decline (Goulson *et al.*, 2015; Otterstatter & Thomson, 2008), yet the mechanisms governing how flowers serve as disease transmission venues have been largely unexplored. Flowers are multifunctional hubs, providing not only nutrition, microbial symbionts (McFrederick *et al.*, 2017), and pathogen-suppressing chemical compounds (Manson *et al.*, 2010; Richardson *et al.*, 2015), but also many of the pathogens themselves (Singh *et al.*, 2010). Infection-induced changes in foraging and/or physiology are predicted to affect probability of transmission (Koch *et al.*, 2017; Otterstatter & Thomson, 2006), but had yet to be empirically evaluated until now. Understanding how flowers contribute to bee pathogen transmission is a necessary component of promoting pollinator health. Given our results, we recommend assessing floral traits associated with pathogen transmission across a diversity of plant and pollinator species, in an effort to develop wildflower mixes that not only maximize forage but also minimize disease spread.

Figure 1: Flower parts where the common eastern bumble bee (*Bombus impatiens*) defecated or *Crithidia bombi* inoculum was placed on (a) *Lobelia siphilitica*, (b) *Lythrum salicaria* and (c) *Monarda didyma* (photo credit: N. Milano).

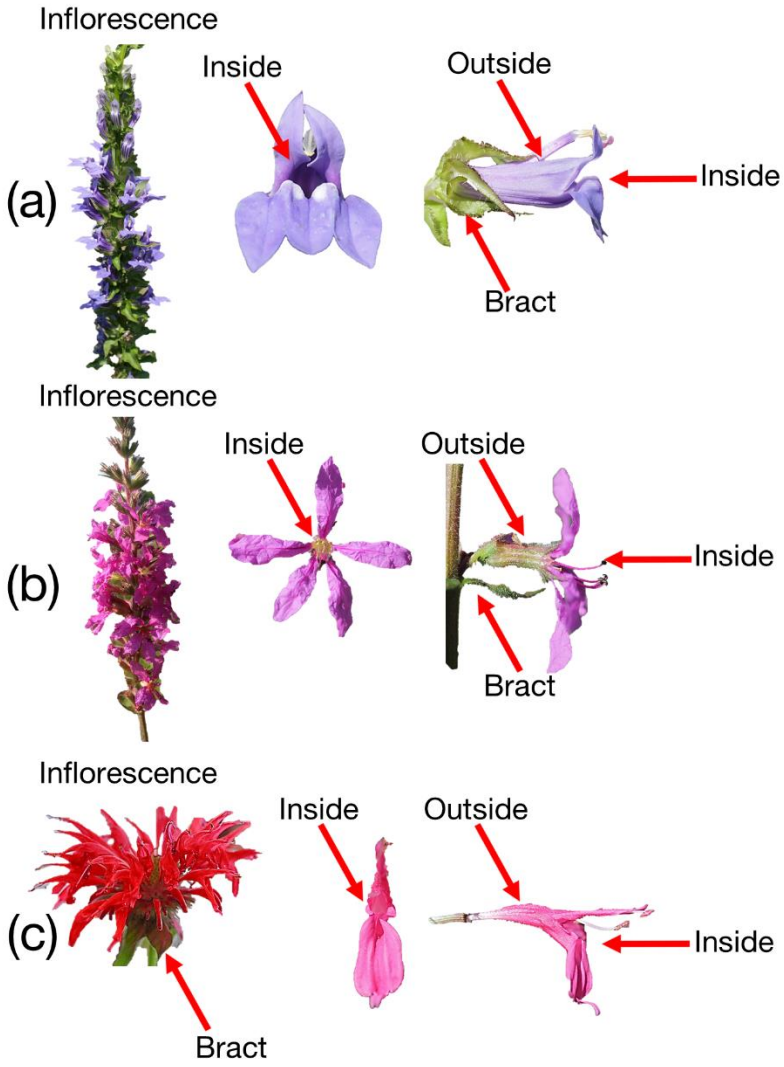

Figure 2. Experiment 1: Effect of *Crithidia* infection status on *Bombus impatiens* defecation rate on plants (mean ± s.e). Infected worker bees were more likely to defecate on plants than uninfected bees.

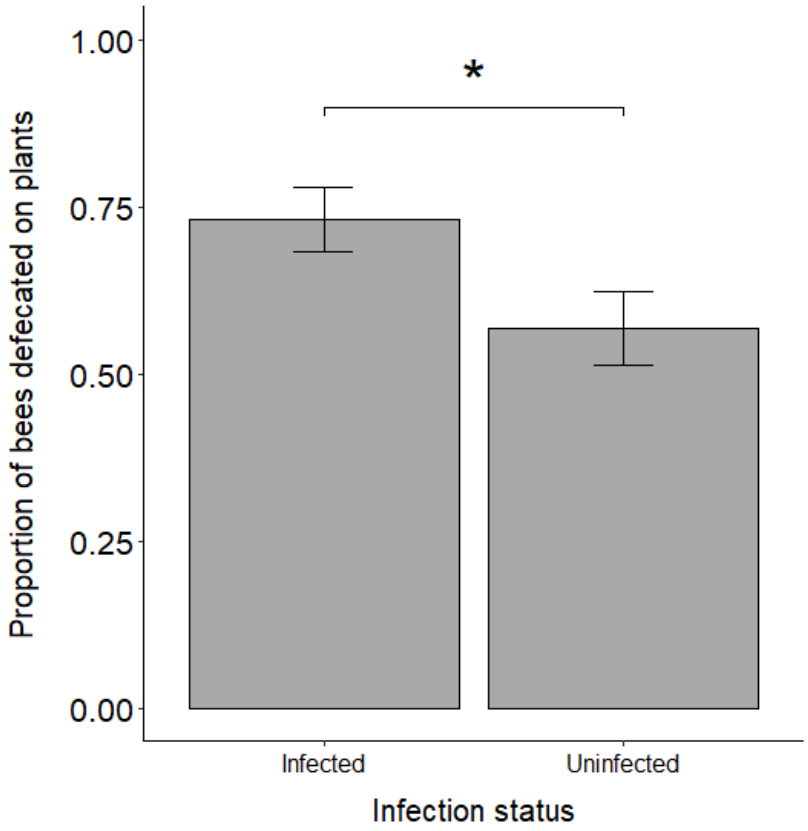

Figure 3. Experiment 1: (a) Effect of plant species and flower part on defecation by *B. impatiens* workers. Data are mean ± s.e. (for post-hoc comparisons see Table S1). (b) Effect of *B. impatiens* size on defecation among different flower parts. Solid lines indicate significance ($p < 0.05$) while dashed lines indicate no significant relationship.

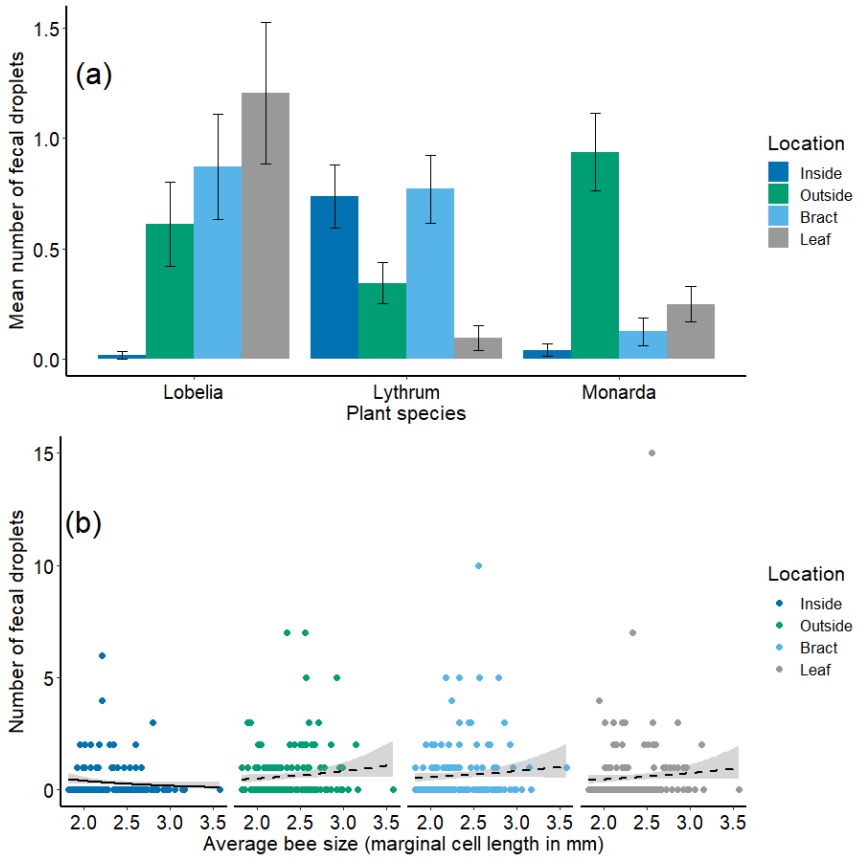

Figure 4. Experiment 2: *Crithidia* survival across plant species and flower parts. Survival differed across flower part and exposure to sun in *Lythrum* (a, c) and *Lobelia* (b, d). *Monarda* was only evaluated in shade conditions (see methods); we did not find significant differences among flower parts in *Monarda*, likely due to a high overall *Crithidia* survival (80%).

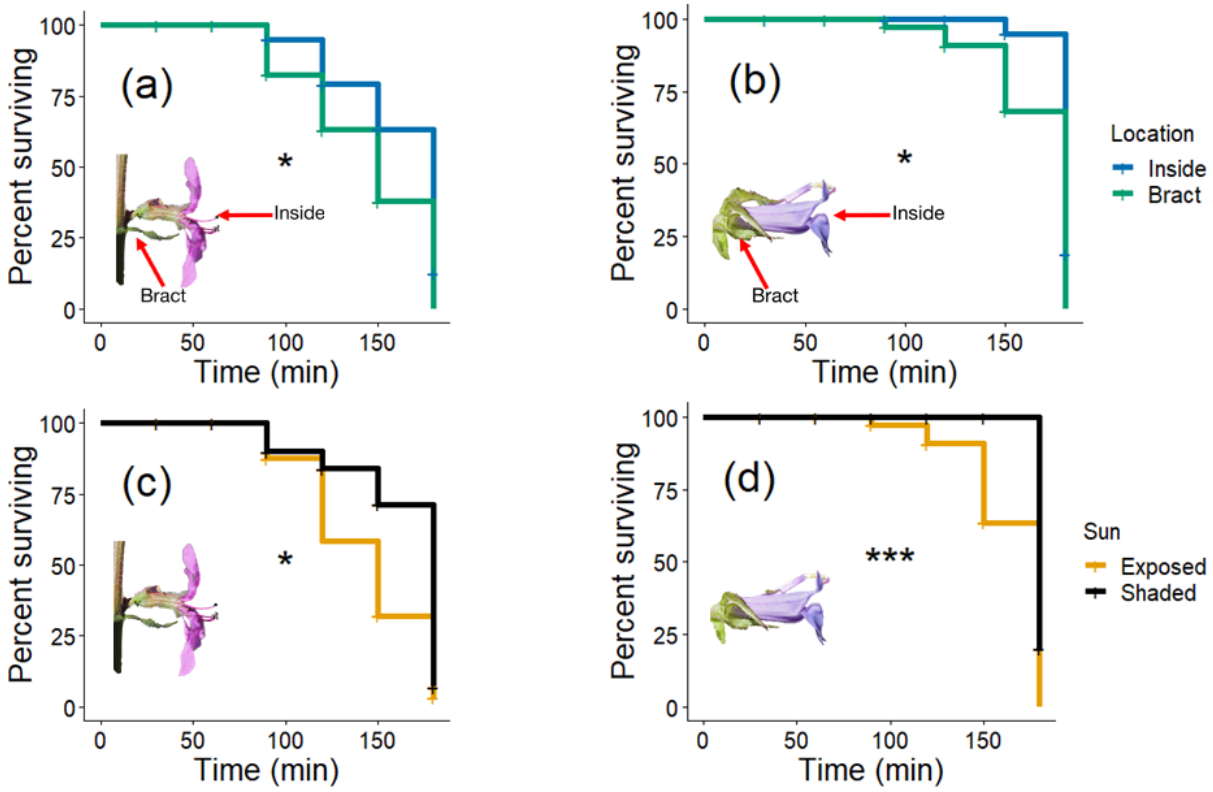

Figure 5. Experiment 3: Effects of plant species and flower part on *Crithidia* acquisition and subsequent intensity of infection (*Crithidia* cells per ul) in *B. impatiens* workers. Data are means ± s.e.

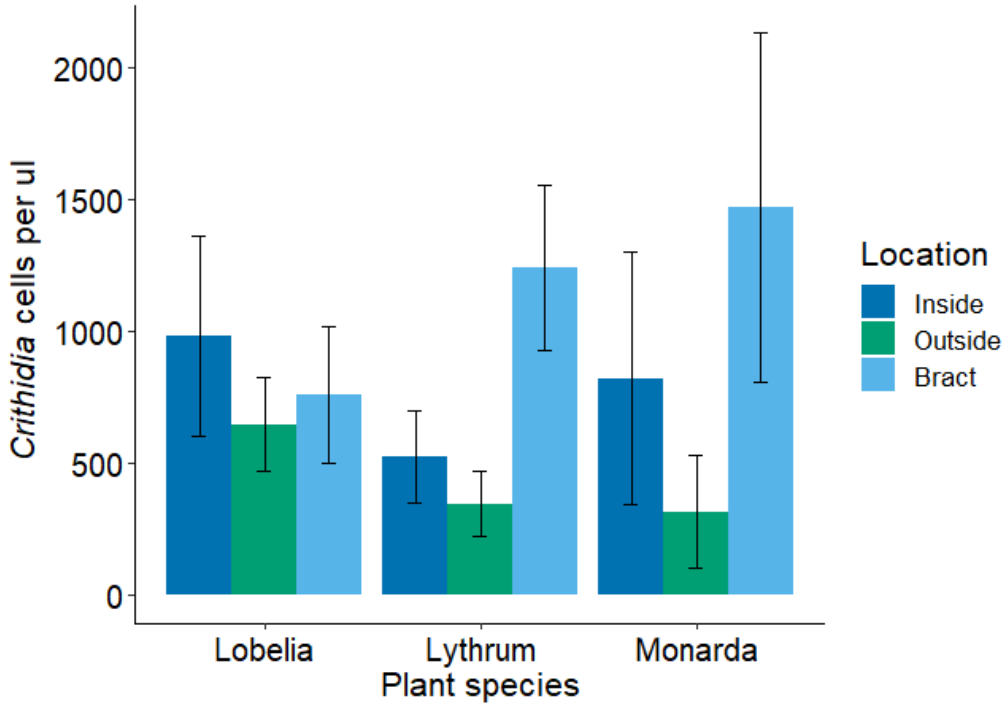

Table S1. Experiment 1: Post-hoc analysis **(**Tukey's HSD) for flower species by part interaction on number of times feces were observed.

Contrast	estimate	SE	z ratio	*p* value
Lythrum inside - Lythrum leaf	**2.01**	**0.43**	**4.64**	**<0.001**
Lythrum bract - Lythrum leaf	**1.99**	**0.44**	**4.58**	**<0.001**
Lythrum leaf - Monarda outside	**-2.29**	**0.52**	**-4.42**	**0.001**
Lobelia leaf - Lythrum leaf	**2.17**	**0.50**	**4.36**	**0.001**
Lobelia inside - Lobelia leaf	**-4.17**	**1.01**	**-4.14**	**0.002**
Monarda bract - Monarda outside	**-1.82**	**0.44**	**-4.13**	**0.002**
Lobelia inside - Monarda outside	**-4.29**	**1.05**	**-4.09**	**0.003**
Lobelia inside - Lythrum inside	**-4.02**	**1.04**	**-3.86**	**0.006**
Lythrum bract - Lobelia inside	**4.00**	**1.04**	**3.84**	**0.007**
Lobelia bract - Lobelia inside	**3.85**	**1.01**	**3.81**	**0.008**
Lobelia bract - Lythrum leaf	**1.84**	**0.50**	**3.66**	**0.013**
Lobelia inside - Lobelia outside	**-3.50**	**1.01**	**-3.45**	**0.028**
Monarda leaf - Monarda outside	**-1.13**	**0.33**	**-3.39**	**0.034**
Monarda bract - Lobelia leaf	**-1.70**	**0.51**	**-3.34**	**0.040**
Lobelia leaf - Lobelia outside	0.68	0.21	3.17	0.067
Lobelia inside - Lythrum outside	-3.26	1.05	-3.09	0.084
Monarda bract - Lythrum inside	-1.55	0.51	-3.02	0.103
Lythrum bract - Monarda bract	1.53	0.51	2.97	0.116
Lobelia inside - Monarda leaf	-3.17	1.08	-2.94	0.127
Lythrum leaf - Lobelia outside	-1.49	0.51	-2.91	0.138
Lythrum inside - Lythrum outside	0.76	0.26	2.88	0.147
Lythrum bract - Lythrum outside	0.74	0.27	2.79	0.185
Lythrum leaf - Lythrum outside	-1.25	0.46	-2.71	0.223
Lythrum outside - Monarda outside	-1.03	0.39	-2.68	0.238
Lobelia bract - Monarda bract	1.38	0.52	2.67	0.240
Lobelia leaf - Lythrum outside	0.91	0.36	2.55	0.309
Lobelia leaf - Monarda leaf	1.01	0.42	2.40	0.406
Monarda bract - Lobelia inside	2.47	1.12	2.22	0.536
Lobelia outside - Monarda outside	-0.80	0.37	-2.17	0.571
Lythrum leaf - Monarda leaf	-1.16	0.57	-2.04	0.669
Lythrum inside - Monarda leaf	0.86	0.42	2.02	0.680
Lythrum bract - Monarda leaf	0.83	0.42	1.96	0.718
Monarda bract - Lobelia outside	-1.02	0.52	-1.95	0.726
Lobelia inside - Lythrum leaf	-2.01	1.11	-1.81	0.813
Lobelia bract - Lobelia leaf	-0.32	0.19	-1.69	0.872
Lobelia bract - Lythrum outside	0.59	0.37	1.61	0.907
Lobelia bract - Monarda leaf	0.68	0.43	1.60	0.909
Lobelia bract - Lobelia outside	0.35	0.23	1.56	0.924
Lythrum inside - Lobelia outside	0.53	0.34	1.53	0.931
Lythrum bract - Lobelia outside	0.50	0.34	1.47	0.950
Monarda bract - Lythrum outside	-0.79	0.54	-1.47	0.950
Monarda bract - Monarda leaf	-0.69	0.50	-1.39	0.966
Lobelia bract - Monarda outside	-0.44	0.35	-1.25	0.985
Lythrum bract - Monarda outside	-0.29	0.35	-0.83	1.000
Lythrum inside - Monarda outside	-0.27	0.35	-0.77	1.000
Monarda leaf - Lobelia outside	-0.33	0.44	-0.75	1.000
Monarda bract - Lythrum leaf	0.47	0.64	0.73	1.000
Lobelia bract - Lythrum bract	-0.15	0.33	-0.46	1.000
Lobelia bract - Lythrum inside	-0.17	0.33	-0.52	1.000
Lobelia bract - Monarda inside	17.62	256.00	0.07	1.000
Lythrum bract - Lythrum inside	-0.02	0.21	-0.11	1.000
Lythrum bract - Monarda inside	17.77	256.00	0.07	1.000
Lythrum bract - Lobelia leaf	-0.17	0.32	-0.54	1.000
Monarda bract - Monarda inside	16.24	256.00	0.06	1.000
Lobelia inside - Monarda inside	13.77	256.00	0.05	1.000
Lythrum inside - Monarda inside	17.79	256.00	0.07	1.000
Lythrum inside - Lobelia leaf	-0.15	0.32	-0.47	1.000
Monarda inside - Lobelia leaf	-17.94	256.00	-0.07	1.000
Monarda inside - Lythrum leaf	-15.77	256.00	-0.06	1.000
Monarda inside - Monarda leaf	-16.93	256.00	-0.07	1.000
Monarda inside - Lobelia outside	-17.26	256.00	-0.07	1.000
Monarda inside - Lythrum outside	-17.03	256.00	-0.07	1.000
Monarda inside - Monarda outside	-18.06	256.00	-0.07	1.000
Lobelia leaf - Monarda outside	-0.12	0.35	-0.34	1.000
Monarda leaf - Lythrum outside	-0.09	0.45	-0.21	1.000
Lobelia outside - Lythrum outside	0.24	0.38	0.62	1.000

Table S2. Experiment 2: Post-hoc analyses (Tukey's HSD) table for differences in *Crithidia* survival across parts on the flower for each plant species.

Species	contrast	estimate	SE	z ratio	*p* value
Lythrum	Bract - Inside	0.67	0.29	2.29	**0.022**
Lobelia	Bract - Inside	0.86	0.41	2.09	**0.037**
Monarda	Bract - Inside	23.65	72798.34	0.00	1.000
Monarda	Bract - Outside	-0.21	0.77	-0.28	0.958
Monarda	Inside - Outside	-23.87	72798.34	0.00	1.000

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Supporting data and R code can be accessed at https://doi.org/10.5061/dryad.jc4hf80.

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CHAPTER 2

LANDSCAPE SIMPLIFICATION SHAPES PATHOGEN PREVALENCE IN PLANT-POLLINATOR NETWORKS

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Abstract

Species interaction networks, which play an important role in determining pathogen transmission and spread in ecological communities, can shift in response to agricultural landscape simplification. However, we know surprisingly little about how landscape simplification-driven changes in network structure impact epidemiological patterns. Here, we combine mathematical modeling and data from eleven bipartite plant-pollinator networks observed along a landscape simplification gradient to elucidate how changes in network structure shape disease dynamics. Our empirical data show that landscape simplification reduces pathogen prevalence in bee communities via increased diet breadth of the dominant species. Furthermore, our empirical data and theoretical model indicate that increased connectance reduces the likelihood of a disease outbreak and decreases variance in prevalence among bee species in the community, resulting in a dilution effect. Because infectious diseases are implicated in pollinator declines worldwide, a better understanding of how land use change impacts species interactions is therefore critical for conserving pollinator health.

Introduction

Landscape simplification driven by agricultural expansion is one of the most significant anthropogenic changes to the planet, influencing resource availability, biodiversity, and ecosystem functioning (Tscharntke *et al.*, 2005). An important but often overlooked consequence of landscape simplification is the deterioration of interaction patterns across trophic levels, including host-parasitoid and plant-pollinator networks (Fortuna & Bascompte, 2006; Spiesman & Inouye, 2013; Tylianakis *et al.*, 2007). These interactions between species are essential for maintaining ecosystem function and stability (Thébault & Fontaine, 2010), and they also shape how pathogens are transmitted and spread. Moreover, the predictive power of disease spread models is often improved when interaction patterns are considered (White *et al.*, 2017). Yet we currently do not understand how landscape simplification impacts disease dynamics via changes to interaction networks. This is an important knowledge gap given increasing global dependence on pollinators for ecosystem service provisioning (Aizen *et al.*, 2008) and the documented links between pathogens and bee declines worldwide (Goulson *et al.*, 2015).

The impacts of landscape simplification on network structure have been generally attributed to species turnover and reduced specialization (Grass *et al.*, 2013). Plant-pollinator networks are characterized by diverse bee species assemblages with frequently overlapping visitation patterns (Petanidou *et al.*, 2008; Pocock *et al.*, 2012). Given that generalist floral visitors are typically less susceptible to habitat loss (Fortuna & Bascompte, 2006), pollinator communities in simplified landscapes could be dominated by generalist species. In such a scenario, we would expect interaction networks with high proportions of realized links between bee and flower species

(high connectance), in which interactions occur evenly throughout (low modularity), and with few specialists present to interact with generalists (low nestedness), which has been found in some (Spiesman & Inouye, 2013; Vanbergen *et al.*, 2014), but not all systems (Redhead *et al.*, 2018; Traveset *et al.*, 2018). Moreover, all of these metrics have been highlighted as potential key mediators of disease transmission (*i.e.* connectance/nestedness (Wei *et al.*, 2015), modularity (Sah *et al.*, 2017), and abundance/diversity (Johnson *et al.*, 2015)). Ultimately, how changes to network structure influence pathogen prevalence in plant-pollinator communities is largely unknown, limiting our understanding of how agricultural expansion impacts the health of bee communities.

Dominant species often disproportionately influence ecosystem functions, including disease transmission dynamics (Keesing *et al.*, 2010), due to their high abundance, broad diet breadth, and/or dispersal abilities. Focusing on dominant species can provide an initial assessment of the impact that landscape simplification has on network stability (Hagen *et al.*, 2012). These dominant species could influence encounter probabilities, subsequent exposure to pathogens, and ultimately, disease spread dynamics at the community level (Keesing *et al.*, 2010). Thus, the way in which dominant species respond to landscape simplification and influence plant-pollinator network structure could have significant implications for pathogen transmission and prevalence at the community level.

Pathogen transmission can occur among multiple bee species when susceptible individuals forage on contaminated flowers (Durrer & Schmid-Hempel, 1994). Furthermore, bees can simultaneously vector multiple pathogens onto flowers, even without developing active infections (Graystock *et al.*, 2015). Recently, pathogens known to infect honey bees and bumble bees have

been detected in a broad array of bee species, including social and solitary taxa (Cordes *et al.*, 2012; Evison *et al.*, 2012; Ravoet *et al.*, 2014), highlighting the existing knowledge gap in the host range of these pathogens and more generally in bee inter-species transmission dynamics. Moreover, differences in pathogen prevalence in bee communities across land use gradients (Piot *et al.*, 2019; Theodorou *et al.*, 2016) suggest that changes in host density and interaction patterns may be key mediators in how communities respond to landscape simplification.

Here, we characterized plant-pollinator networks and bee pathogen prevalence in 11 replicated wildflower plots established across a landscape simplification gradient in order to address three major questions. First, does landscape simplification shape pathogen prevalence in bee communities mediated by changes in network structure? Specifically, we used structural equation modeling to quantify the role of network connectance, modularity, and nestedness, as well as bee abundance, species richness, and diet breadth of the dominant bee species. Second, does the role of a bee species in the network predict its likelihood of harboring pathogens? To address this question we computed species-level network descriptors such as centrality and diet breadth, as well as pairwise interactions between bee species mediated by shared floral resources. Finally, what mechanisms underpin the empirical relationship between network structure and pathogen prevalence in bipartite plant-pollinator networks? To shed light on this question, we developed mathematical models using empirically derived parameters from our data and additional literature to determine drivers of pathogen prevalence and spread.

Material and methods

Field sites, sample collection, and network assessment

We evaluated the plant-pollinator networks of 11 replicated wildflower plots in upstate New York from June 10 to September 26, 2015 (Fig. S1). Each 10 × 15 meter planting was established with native perennial wildflower species in 2012 following regional guidelines for species that encompass a broad phenological range and are known foraging resources for wild bees (Grab *et al.*, 2018; Tuell *et al.*, 2008). Not all plant species established equally well at each of the sites, and the parentheses indicate the number of sites that included each species: *Silphium perfoliatum* (11), *Solidago canadensis* (11), *Penstemon digitalis* (11), *Veronicastrum virginicum* (10), *Lobelia siphilitica* (10), *Coreopsis lanceolata* (5), and *Agastache nepetoides* (4). Sites were also invaded by weedy species: *Erigeron annuus* (9), *Trifolium repens* (9), *Medicago sativa* (7), *Trifolium pratens* (7), *Daucus carota* (5), and *Cirsium arvense* (3). Plant species richness was assessed within the planted wildflower plots at each site.

We characterized each network by conducting weekly ten-minute visitation surveys at each site, where all bee-flower interactions occurring within the site were recorded to the finest taxonomic resolution possible. Each site was surveyed 8 to 12 times throughout the summer from 08:00 to 17:00 hrs on sunny and low wind days, with temperatures ranging from 16 to 33 °C, and visited in rotating sequence. During the visitation surveys we recorded 28 bee morphogroups via visual assessments, 17 of which were identifiable at the species level, 10 to genus only, and a few unidentified bees. After each visitation assessment, we collected bees actively foraging on the flowers for 1.5 ± 0.5 person hours, depending on foraging activity levels. Bees were collected by hand from flowers into sterile vials and a sample of each observed flower species was also

collected using sterilized 50 ml falcon tubes. Samples were immediately placed on dry ice in the field, transported to the laboratory and stored at –80 °C until processing. Collected bees were used for subsequent pathogen screening and species determination using reference materials located in the Cornell University Insect Collection, published keys, and barcoding methods (Appendix 2).

Overall, 91% of bee samples were identified to species (46 species). Rarefaction analyses indicated sufficient sampling of the pollinator communities (Fig. S2). We compared analyses with networks constructed at the taxonomic resolution possible in the field ("unresolved") and those that were later fully resolved to species in the laboratory ("resolved"), finding no significant differences for any of our analyses (Table S1); here we present unresolved data to minimize assumptions. We screened 12 of the 13 flower species found in the wildflower sites for bee pathogens (Appendix 3).

DNA extraction and pathogen screening

We surface sterilized each bee to ensure only pathogens inside of the bees were detected (Lacey, 1997). The pathogens screened (trypanosomes, neogregarines, *Nosema ceranae*, and *Nosema bombi*) display broad tissue tropism (Schmid-Hempel, 1998), therefore we extracted gut, fat body, and Malpighian tubules in each sample. To maximize the amount of nucleic acid collected, we also pipetted 20 µl UltraPure™ water (ThermoFisher, Grand Island, NY, USA) into the abdomen during dissection. The dissected bee organs and water mixture were placed in sterile vials containing two sterilized 2.4 mm steel beads, 100 µl of sterilized 0.1 mm zirconia beads, and 800 µl of TRIsure™ reagent (BIOLINE, Boston, MA, USA). Samples were homogenized for 30

seconds at 6.5 m/s using a bead mill homogenizer (Omni International, Kennesaw, GA, USA), then immediately placed on ice. The solution was transferred to a new sterile vial, taking care to not pipette beads or large tissue fragments. DNA extraction from the TRIsure™ reagent then proceeded using the manufacturer's protocol. Each extraction batch included a negative control. Pathogen DNA on 100 mg of floral tissue per species per site was similarly extracted (Appendix 3) using established PCR primers (Appendix 3, Table S3 - S4).

Landscape characterization

The 11 replicated wildflower sites were established along a landscape simplification gradient in the fall of 2012 (Fig. S1). We used ArcGIS v10 (Environmental Systems Research Institute, Redlands, CA, USA) and the 2015 Cropland Data Layer (30 m resolution, USDA NASS) to evaluate the landscape composition within a 500, 750, and 1,250 meter radius of each site, encompassing the typical foraging range of most wild bees (Greenleaf *et al.*, 2007). In North American farmlands, pasture management frequently includes mowing and herbicide use (Singh *et al.*, 2006), both of which can affect the distribution of flowering plants available for bees at the landscape scale. Thus, we evaluated the proportion of agricultural cover (defined as corn, soy, barley, wheat, vegetable crops, alfalfa, orchards, hay fields, and grass/pastures) at each of the 11 sites. Agricultural cover was positively correlated with cropland (defined as corn, soy, barley, wheat, vegetable crops, alfalfa, and orchards: $F_{1,9} = 22.39$, $P = 0.001$; $F_{1,9} = 51.12$, $P < 0.001$; and $F_{1,9} = 55.67$, $P < 0.001$, for the 500, 750, and 1,250 meter scales, respectively) and negatively correlated with natural area (defined as deciduous, evergreen and mixed forest, woody and herbaceous wetlands, old fields, and shrub lands, as well as open and low intensity developed

lands, such as roadside edges: $F_{1,9} = 10.2$, $P = 0.011$; $F_{1,9} = 3.39$, $P = 0.100$; and $F_{1,9} = 6.33$, $P = 0.033$, for the 500, 750, and 1,250 meter scales, respectively). As such, we analyzed the effect of agricultural cover on network metrics and pathogen prevalence in our system.

Network characterization and statistical analyses

Network characterization and statistical analyses were conducted in R version 3.5.1 using the bipartite, vegan, epiR, lme4, ape and piecewiseSEM packages (Bates *et al.*, 2015; Dormann *et al.*, 2008; Lefcheck, 2016; Oksanen *et al.*, 2007; Paradis *et al.*, 2004; R Development Core Team, 2008), unless otherwise stated. Qualitative and quantitative network indices were calculated using the *networklevel* function in bipartite: connectance, nestedness (weighted NODF), and bee species richness (Dormann *et al.*, 2008). Modularity (Q) was estimated using the QuaBiMo algorithm (*computeModules* function; (Dormann & Strauss, 2014)). Module membership, indicating similarity in visitation patterns, was categorically determined for each bee morphogroup at each site, hereafter referred to as species (Table S5). In modular networks, pathogen transmission is much more frequent within modules than in the overall community (Sah *et al.*, 2017). As such, we hypothesized that module membership would predict bee pathogen prevalence given the higher likelihood of indirect pathogen exposure via flowers for bee species in the same module as those with pathogens. Taxon-specific network indices (betweenness centrality and degree) were calculated for bee species at each site using the *specieslevel* function in bipartite. Betweenness centrality, by measuring the frequency of shortest paths that pass through a species, can indicate likely "hotspots" of pathogen transmission. We expected bee species that most closely connected the greatest number of other bee species to have the highest likelihood of harboring a pathogen.

Bee abundance was calculated by summing the total number of interactions observed at each site throughout the summer (Fort *et al.*, 2016; Vázquez *et al.*, 2007; Vázquez *et al.*, 2005).

We evaluated relationships in bee community composition via non-metric multidimensional scaling (NMDS with the Bray-Curtis dissimilarity measure on the species-abundance matrix following Wisconsin double standardization (Oksanen, 2015)). We investigated how the proportion of agricultural cover related to the ordination by fitting the agricultural landscape variable to the ordination axes (*envfit* function with 999 permutations to determine significance).

Prior to analyses, degree, betweenness centrality, nestedness (weighted NODF), and modularity (Q) were standardized based on comparisons to null models (Dormann, 2011; Dormann *et al.*, 2009). We selected the *vaznull* null model because by maintaining the number of links and marginal totals in the null model equal to those of the real world network it can more closely mirror real ecological and/or evolutionary processes, including the existence of trait mismatching that impedes interactions from occurring (Dormann *et al.*, 2009). We computed 1000 null models for each network index at each site using the *vaznull* method, which first creates a binary matrix with randomized interaction probabilities proportional to each species' relative abundance, constrained by the connectance of the original network. Once the matrix was created with the same number of filled cells as the original network, the remaining interactions were distributed among the filled cells, thus constraining connectance while including interaction frequency (Vázquez *et al.*, 2007). We then calculated the z-score for the network indices observed at each site by comparing to the mean and standard deviation of the computed null models. The resulting z-scores were the values

employed in subsequent statistical analyses. The z-score of connectance was not calculated because *vaznull* constrains connectance.

We employed structural equation models to evaluate the hypothesized indirect effects of landscape simplification on pathogen prevalence via network metrics (Appendix 5). Our models evaluated whether landscape simplification (proportion agricultural cover at either 500, 750, or 1250 meters) predicted network-level metrics (connectance, modularity, nestedness, bee species richness, and scaled bee abundance), as well as the diet breadth (degree) of the dominant species in our system, *Bombus impatiens*, which is known to vector pathogens on flowers and is linked to pathogen spillover from commercial colonies to wild bees (Otterstatter & Thomson 2008). All network metrics (including *B. impatiens* diet breadth) were modeled to co-vary *a priori* because directionality was unclear. The landscape and network metrics were based on linear regression of values for each of the sites (n = 11); we evaluated model assumptions using the olsrr package (Hebbali 2017). The role of landscape simplification and each network metric on pathogen prevalence (trypanosomes, neogregarines, *Nosema bombi*, and/or *Nosema ceranae*) was evaluated using a Generalized Linear Mixed Model (GLMM), which included pathogen presence in individual bees as the binary response, all network metrics and proportion of agricultural cover as predictor variables, as well as bee species and site as random effects (n = 575). We then simplified our model by removing non-significant terms (Appendix 5). Our simplified model, presented in the main text, included proportion of agricultural cover at the 500-meter scale, *B. impatiens* diet breadth (degree), network connectance, overall bee abundance, and presence of pathogens in individual bees (Table S1). Results from the full model and the three spatial scales are presented in Table S6.

To determine whether each bee species' position in the network predicted its likelihood of harboring pathogens, we conducted a GLMM that included three taxon-specific indices: module membership, diet breadth (degree), and betweenness centrality. We calculated the variance inflation factor for a model including all three indices and found that module membership greatly increased estimate variances; therefore, the impact of module membership was analyzed separately from the other two factors. The taxon-specific network metrics were evaluated as the explanatory variable for presence of pathogens in bees using a binomial GLMM that included site and bee species as random effects. We conducted a likelihood ratio test to determine the significance of coefficients by comparing against a null model that only included the random effects. The taxon-specific analyses were conducted only on bee species for which we had both pathogen and visitation data at a site (14 species). Furthermore, we tested the hypothesis that similarity in foraging patterns between bee species in a given network predicted similarity in pathogen prevalence using Mantel tests, but found that number of shared floral partners was a poor predictor (Appendix 6).

Modelling pathogen transmission in bipartite networks with varying connectance

Existing theoretical models have generally suggested that increasing network connectance results in higher rates of disease transmission (Moslonka-Lefebvre *et al.*, 2009; Shirley & Rushton, 2005; Strona *et al.*, 2018), contrasting with our empirical findings (see *Results*). However, previous models generally assume that a host's contact rate scales with its network degree, corresponding to the niche breadth. This is unlikely to be true for plant-pollinator networks, unless floral resources

are so scarce that foraging rates become search-time limited, and hence warrants a re-evaluation of existing results under a different set of assumptions. As such, we developed a new theoretical model parameterized with empirically derived values to understand how connectance could impact a pathogen's basic reproductive number (R_0), linearized prevalence growth rate (λ), and steady state prevalence based on realistic assumptions for the plant-pollinator system.

We considered a simple deterministic susceptible – infected – susceptible (SIS) model with demography, assuming no latent period for exposed bees, and that bees and flowers become susceptible again after clearing infection. We assumed constant population sizes (equal birth and death rates), and that bees emerged uninfected as adults. Here, "infected" bees included host and non-host vectors that carry pathogens. Transmission of pathogens was assumed to occur only from fecal deposition of infected bees on uncontaminated ("susceptible") flowers, and from susceptible bees ingesting pathogens while foraging on contaminated ("infected") flowers (Figueroa *et al.*, 2019). Within-hive transmission and demographic processes were ignored (*i.e.*, we assumed that the population of each bee and flower species remained constant).

With the above assumptions, the SIS model was described by the system of equations (definitions provided in Table 1):

$$\dot{y}_q = \sum_{k=1}^{K} \beta_{q,k} \cdot s_q \cdot c_k - \left(\gamma_q + \mu_q\right) \cdot y_q, \qquad s_q = 1 - y_q,$$

$$\dot{c}_k = \sum_{q=1}^{Q} \frac{\alpha_{q,k} \cdot M_q}{N_k} \cdot y_q \cdot u_k - \zeta_k \cdot c_k, \qquad u_k = 1 - c_k,$$

We generated 100,000 random plant-pollinator networks as follows: for each network, we sampled the degree of each bee or flower species from a zero-truncated binomial distribution, with one binomial proportion used for bees and a different one for flowers. The zero-truncated binomial distribution was chosen for its simplicity and because it fit the observed degree distributions well, albeit being slightly over-dispersed. The Gale-Ryser criterion (Krause, 1996) was used to check whether a bipartite network could be constructed using the two sampled degree sets; if not, the flower degree set was re-sampled until the criterion was satisfied. Edges from bee and flower species were then joined at random while ensuring that no parallel edges were created. Changes to the two binomial proportions used in the zero-truncated binomial altered the expected degree of each bee or flower species, hence allowing connectance to vary. The bee binomial proportions fit from the empirically observed degree distributions ranged from 0.13 to 0.31, so we allowed the bee proportion in each network to vary between 0.05 and 0.4. The corresponding flower proportion was chosen such that the expected value for the sum of flower degrees equaled that of the bee degrees. We fixed the number of bee and flower species at $Q = 13$ and $K = 7$, based on the mean from our empirical networks. Missing edges in each network hence indicated that transmission parameters $\alpha_{q,k}$ and $\beta_{q,k}$ were zero. The two transmission parameters $\alpha_{q,k}$ and $\beta_{q,k}$ in the SIS model were combinations of other parameters defined in Table 1; importantly, both contain a multiplicative factor $\eta_{q,k}$, where $\sum_{k=1}^{K} \eta_{q,k} = 1$, to account for the division of total foraging efforts among multiple flower species, a departure from existing theoretical models on the effects of connectance.

The SIS model depended on four combination of parameters $\alpha_{q,k} \cdot M_q / N_k$, $\beta_{q,k}$, $\gamma_q + \mu_q$, and ζ_k. Therefore, to account for heterogeneity among bee and flower species, for example

between bee species that are non-host vectors versus those that can develop active infection, we first calculated reference values of these combinations (Table 1), except without $\eta_{q,k}$ for the first two combinations. Next, we allowed the values of these combinations for each q and k to fluctuate about the reference values by a multiplicative factor of $\sqrt{10}$ in either direction, so that the range of values spanned one order of magnitude. Finally, to complete the first two combinations, we randomly generated $\eta_{q,k}$ for each bee species q, with the requirements that $\sum_{k=1}^{K} \eta_{q,k} = 1$ and that they were zero if the corresponding edges between q and k were missing in the network. This process was repeated for each random network.

To quantify disease transmission, we defined two matrices $\boldsymbol{T}$ and $\boldsymbol{\Sigma}$ of dimensions $(Q + K) \times (Q + K)$ with matrix elements

$$T_{i,j} = \begin{cases} \beta_{q,k}, & i = q \\ & j = Q + k \\ \dfrac{\alpha_{q,k} \cdot M_q}{N_k}, & i = Q + k \\ & j = q \\ 0, & \text{otherwise} \end{cases}, \qquad \Sigma_{i,j} = \begin{cases} -\gamma_q, & i = j = q \\ -\zeta_k, & i = j = Q + k \\ 0, & \text{otherwise} \end{cases},$$

For each random network, we then calculated three metrics:

- Reproduction number R_0, given by the dominant eigenvalue of the next-generation matrix $-\boldsymbol{T}\boldsymbol{\Sigma}^{-1}$ (Diekmann *et al.*, 2009).
- Linearized prevalence growth rate λ, given by the dominant eigenvalue of the time evolution matrix $\boldsymbol{T} + \boldsymbol{\Sigma}$.
- Distribution of steady-state pathogen prevalence among the bee species.

Note that while we have used an SIS model, many pollinator pathogens are believed to cause chronic infections. Nonetheless, both SIS and SI models can give similar results once we include population dynamics (mortality and reproduction) in the SI model, so that reproduction in the SI model plays the same role as recovery in the SIS model in terms of recruiting new susceptibles. Therefore, for species that can develop active infection, mortality rates replace recovery rates in our model (assuming constant population to balance reproduction rates).

All computations were performed in R using the packages extraDistr (Wolodzko, 2018) for zero-truncated binomial distributions and rootSolve (Soetaert & Herman, 2009) for numerical evaluation of the steady-state prevalence.

Results

We recorded 2,936 bee visits across 143 plant-pollinator interaction pairs and screened 575 bees (46 species) and 81 flowers (12 species), finding that 65% of bee species (39% of bees; 95% Confidence Interval (CI), 35 – 44%) and 75% of flower species (31% of flowers; 95% CI, 20 – 43%) harbored at least one of the three pathogen groups (trypanosomes, neogregarines, *Nosema bombi*, and/or *Nosema ceranae;* Fig. 1, Fig. S3 & S4).

We employed structural equation models to evaluate the role of landscape simplification on network metrics, diet breadth of the dominant bee species, and, subsequently, on pathogen prevalence at the community level. In more simplified agricultural landscapes, there were fewer foraging bees and the dominant bee species, *Bombus impatiens*, visited more plant species (higher

degree: broader diet breadth) (Table S1). Bee species richness did not vary along the landscape gradient (Table S6), nor did species composition (NMDS stress = 0.62, R^2 = 0.07, P = 0.79), indicating that landscape simplification affected interaction patterns rather than interaction partners *per se* (Tylianakis *et al.*, 2007). Consequently, we infer that species loss was not the mechanism by which landscape simplification altered interaction patterns.

At sites where *B. impatiens* visited more plant species, pathogen prevalence in the community was lower (Fig. 2 bottom; Table S1). Conversely, bee abundance did not explain pathogen prevalence in the bee community (Fig. 2 center; Table S1). We found that in more connected networks, bees were less likely to harbor pathogens (Fig. 2 top; Table S1), a pattern not driven by landscape simplification. There was no direct relationship between agricultural cover and pathogen prevalence in the bee communities (Fig. 2 & Fig. S5). Instead, the relationship between landscape simplification and pathogen prevalence was mediated by *B. impatiens* diet breadth (Fig. 2).

We found that modules differed in pathogen prevalence, suggesting that groups of bee species with similar interaction patterns were more likely to share pathogens ($\chi^2_{29} = 48.1$, $P = 0.01$; Fig. S6). However, bee species' centrality (degree and betweenness centrality) did not explain likelihood of harboring pathogens ($\chi^2_2 = 0.98$, $P = 0.61$), nor did similarity of pairwise species interaction partners explain similarity in prevalence (number of shared interaction partners: $\bar{r} = -0.31, P = 0.76$, Jaccard index: $\bar{r} = 0.30, P = 0.77$, and weighted Jaccard index: $\bar{r} = 0.35, P = 0.73$). Furthermore, network modularity did not predict pathogen prevalence at the community level (Table S6).

From our theoretical model with empirically derived parameter values, we found that the rate of pathogen spread in the bee community, as measured both by pathogen reproduction number (R_0) and linearized prevalence growth rate (λ), decreased with connectance in bipartite networks (Fig. 3). In the initial pathogen spread phase, before steady-state prevalence had been reached, lower R_0 and λ in more connected networks suggest reduced prevalence. These results are in agreement with our empirical data, which found a negative relationship between connectance and pathogen prevalence in the community (Fig. 2). We also examined the steady-state pathogen prevalence of each bee species in the networks, finding that the outcome was highly dependent on changes to model parameter values within realistic ranges. Pathogen prevalence of the most infected species was consistently lower in well-connected networks, while prevalence of the least infected species was consistently higher (Fig. 4).

Discussion

In this study, we found widespread pathogen prevalence in plant-pollinator networks along an experimentally established agricultural landscape gradient. We found that landscape simplification can impact pathogen prevalence by altering the visitation patterns of the dominant bee species, *Bombus impatiens*. Empirical and theoretical model results indicate that more diverse plant-pollinator interactions dilute pathogen prevalence. In the field, networks that were more connected and had dominant species with broader diet-breath had lower pathogen prevalence in the bee communities. Similarly, in our newly developed and realistically-parameterized SIS

models, more connected networks had lower rates of pathogen spread and reduced variance in steady-state prevalence within the communities.

The dominant species in our system, *Bombus impatiens*, visited more plant species in simplified landscapes. Others have found that along experimental gradients of floral abundance, increased resource availability decreased *B. terrestris* diet breadth (Fontaine *et al.*, 2008). Thus, along our landscape simplification gradient, the dominant bumble bee species may have foraged more generally due to reduced abundance of floral resources at the landscape scale (Tscharntke *et al.*, 2005) or within the wildflower plantings (see Appendix 5 for further discussion). Both possibilities have the potential to alter disease dynamics. For example, a recent study found that increases in floral abundance in old-field communities reduce prevalence of bee pathogens on flowers, thus decreasing the potential for transmission (Graystock *et al.*, 2020b). Given that landscape context can differentially influence diet breath across bee species (Cusser *et al.*, 2019) and that life histories, including diet breadth, dispersal ability, and body size, shape how species respond to habitat fragmentation (Hagen *et al.*, 2012), evaluating whether and how these functional traits in diverse bee communities mediate the effect of landscape simplification on pathogen transmission and spread is highly warranted.

When *Bombus impatiens* visited more plant species, we found lower pathogen prevalence in the community. Others have estimated that infected *B. impatiens* deposit pathogens on only 1% of flowers visited (Otterstatter & Thomson, 2008), and that bumble bee species differ in their transmission potential on flowers (Ruiz-González *et al.*, 2012). The low deposition rate may therefore underlie the negative relationship between *B. impatiens* diet breadth and pathogen

prevalence. Determining which bee traits, such as deposition rate and host competence, contribute to differential dynamics among species is an important future direction in pollinator epidemiology. Furthermore, this pattern supports the importance of dilution, as a known host visiting a broader array of plants and not concentrating exclusively on one hub of disease transmission may have reduced the overall encounter likelihood for the next incoming foraging bee.

Land use change is often associated with a reduction in specialists, which are disproportionately lost along habitat fragmentation gradients (Hagen *et al.*, 2012). On this basis we had expected an increase in network connectance in simplified habitats. However, we did not see a change in bee species richness or composition along our landscape gradient, nor in network connectance. Our results are in agreement with recent studies showing that network connectance does not always respond to landscape simplification (Redhead *et al.*, 2018). Evaluating the importance of land use histories, ecoregions, and gradients of landscape simplification are important future directions in the field of plant-pollinator network ecology.

Our model found that greater connectance decreased both the rate of pathogen spread as well as variance in the prevalence distribution across species (Fig. 3 – 4). Given that steady-state prevalence is sensitive to the non-linear structure of the model, rates of pathogen spread are likely better indicators of observed prevalence. Thus, our theoretical prediction that increased connectance leads to slower pathogen spread is in agreement with the negative relation between connectance and prevalence in our empirical results (Fig. 2). Both theoretical results can be explained as follows. First, in a highly connected network, an infected bee would visit and hence contaminate more plant species; however, assuming a constant contact rate, the contamination

would be distributed among more flowers, thus decreasing the probability that a susceptible bee will encounter a contaminated flower ("dilution"; Fig. 5). This slows disease spread among the competent host species that usually drive disease growth rate. We note that others (Moslonka-Lefebvre *et al.*, 2009; Strona *et al.*, 2018) have predicted opposite patterns, where increased connectance enhances transmission. However, existing models assume that a host's contact rate scales with its degree, hence undoing any dilution. Given that we expect the foraging rate of an individual pollinator to remain roughly constant regardless of diet breadth (assuming similar search and handling times), our model is more appropriate for plant-pollinator networks. Second, high connectance facilitates pathogen transmission from competent hosts to other species within the network by increasing indirect interactions via flowers ("amplification"; Fig. 5); this raises the prevalence in other species toward that of the competent hosts, thus reducing the variance across species (Fig. 4).

Modules often form in ecological networks, within which interactions occur more frequently than in the overall community. Highly modular networks have the potential to structurally suppress pathogen spread (Gilarranz *et al.*, 2017). While we found that modules differed in pathogen prevalence (Fig. S6), network modularity did not predict overall pathogen prevalence nor did bee species' centrality predict likelihood of harboring pathogens. Furthermore, pairwise similarity between bees' interaction partners did not explain similarity in prevalence. Our data and models suggest that an understanding of how all species interact (*e.g.*, network connectance) is more informative than the interaction patterns of any given pair. Disentangling the role of foraging behavior, phylogeny, and susceptibility to infection in diverse bee communities is an important future direction for pollinator epidemiology.

The development of cost-effective pollinator conservation strategies across landscapes is of pressing global concern given documented bee declines and increasing dependence on crop pollinators (Aizen *et al.*, 2008; Goulson *et al.*, 2015). Our data and model illustrate that while pathogens can spread more slowly in highly connected plant-pollinator networks due to a "dilution" effect, resulting pathogen prevalence varies among bee species. These results suggest that management for species of conservation concern may differ from community-wide approaches and those targeting dominant species. Currently, one of the predominant strategies for promoting pollinators is the establishment of wildflower plantings (Williams *et al.*, 2015). Given our results showing that connectance is a key mediator of pathogen spread in bee communities, we recommend evaluating this metric in bee communities vulnerable to disease outbreaks, for example those in wildflower strips near apiaries or commercial bumble bee operations (Furst *et al.*, 2014; Otterstatter & Thomson, 2008), and adjusting seed mixes accordingly. Thus, wildflower plantings promoting pollinator health could be designed to maximize food resources while minimizing disease spread.

Fig. 1. Pathogen prevalence is widespread in plant-pollinator networks. Composite quantitative bipartite network of interactions (links) between bees (top bars) and flowers (bottom bars). Width of bars reflects relative abundance of bees and flowers, and width of links indicates interaction frequency. Color intensity of bars indicates pathogen prevalence in bees and on flowers (links colored according to prevalence in bees). Gray bars indicate species that were not screened. At least one screened pathogen (trypanosomes, neogregarines, *Nosema ceranae*, and *Nosema bombi*) was present in 65% of bee species and 75% of flower species. There were 42 bee species in the resolved network and 28 bee morphogroups in the unresolved network. Fig. S3 provides pathogen-specific depictions of prevalence across networks.

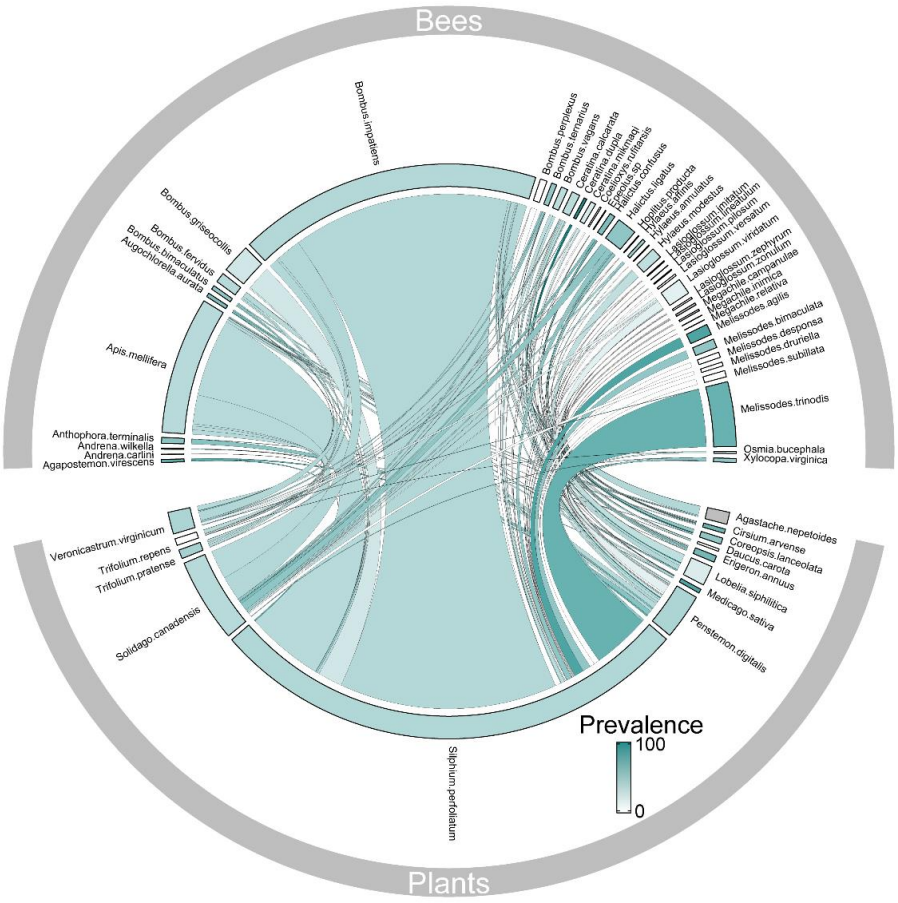


Fig. 2. Landscape simplification indirectly influences pathogen prevalence in bee communities by altering the diet breadth of the dominant species, *Bombus impatiens*. Unstandardized coefficients are shown for each path due to differences in sample sizes (n =11 for site-level comparisons compared to n = 575 for pathogen prevalence calculations). Solid lines indicate significant relationships ($P < 0.05$), dashed lines indicate a non-significant relationship ($P > 0.05$), black indicates positive relationships, and red indicates negative relationships. Ultimately, 76% of overall bee abundance, 40% of *Bombus impatiens* diet breadth (degree), 12% of overall pathogen prevalence, and 6% of network connectance variance was explained by the model. * $P < 0.05$, ** $P < 0.01$, * ** $P < 0.001$.

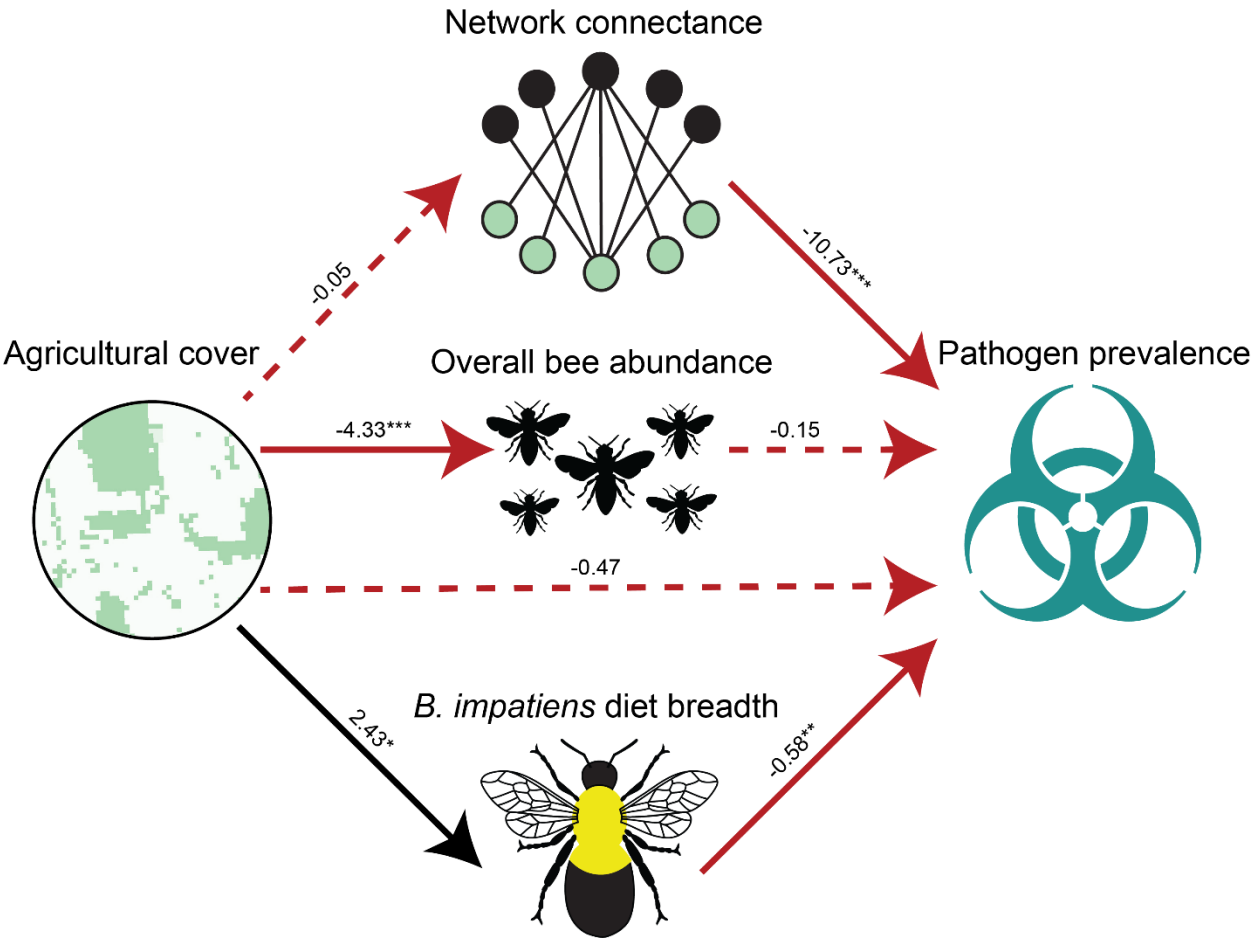


Fig. 3. (a) Connectance reduces pathogen reproductive number (R_0). Based on results from a SIS model run on 100,000 random networks of varying connectance. **(b) Connectance reduces pathogen linearized prevalence growth rate (λ).** Results from a SIS model run on 100,000 random networks of varying connectance show that λ decreases with increasing connectance. Lower R_0 and λ imply lower pathogen prevalence during the early stages of an outbreak. The solid line is a smoothing curve fitted through median values of R_0 and λ for each connectance bin.

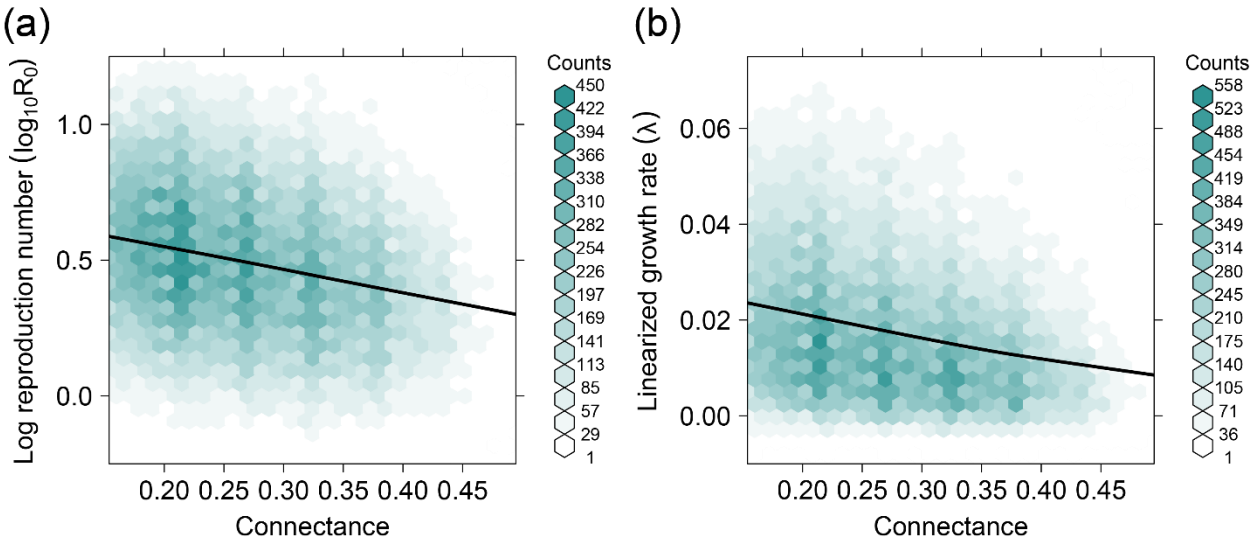


Fig. 4. Connectance reduces variance in the distribution of steady-state pathogen prevalence within a network. Steady-state pathogen prevalence of all bee species from a sample of 5,000 networks are shown, with the more infected species in each network shown as points in purple (top) and the less infected species in yellow (bottom). Points are slightly jittered in the horizontal direction to reduce overlap. For each bee species, a smoothing curve of the same color is fitted through the median prevalence values for each connectance bin. Connectance decreases the prevalence level of the more infected species while increasing that of the less infected ones.

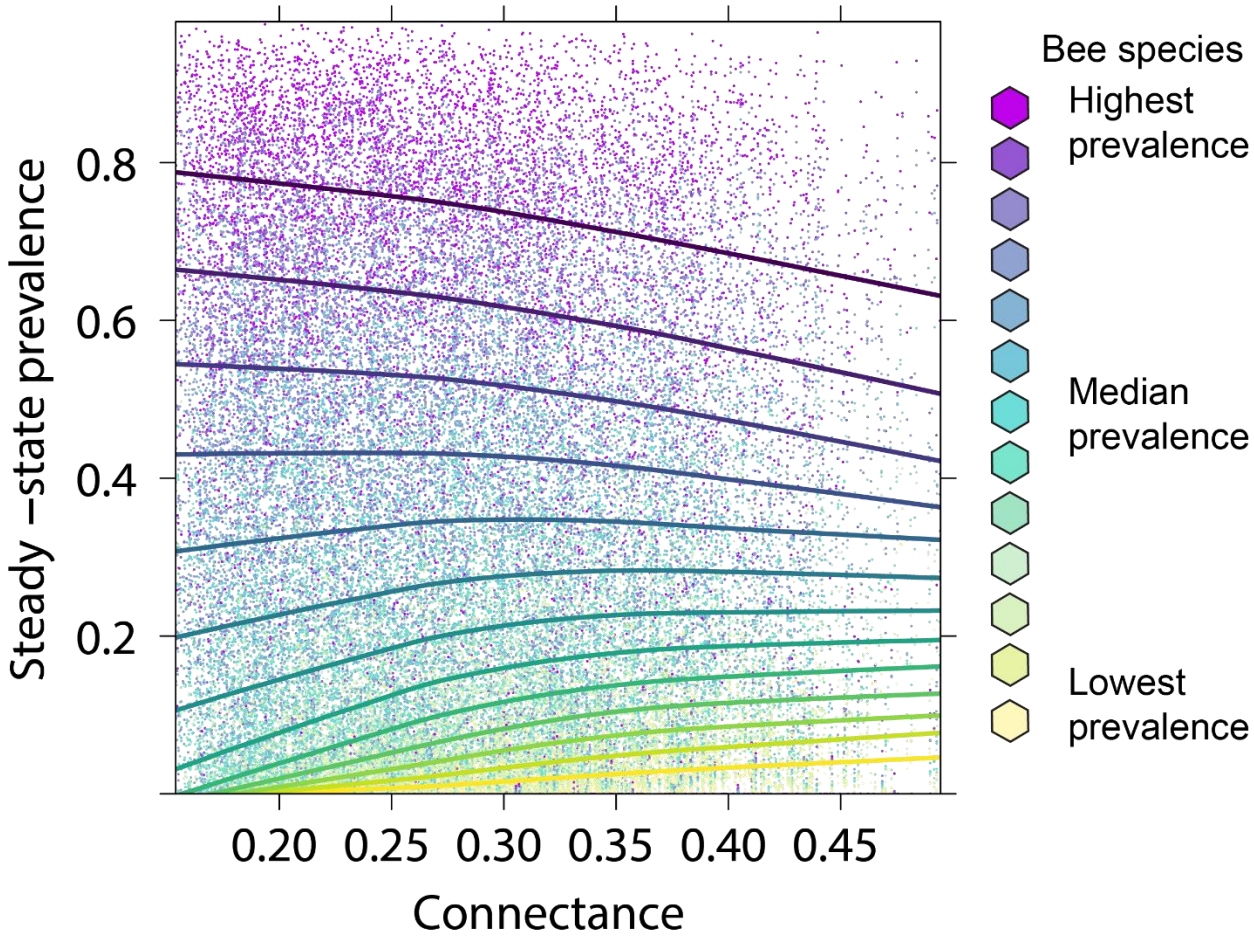


Fig. 5. Conceptual diagram illustrating how connectance can influence bee pathogen transmission in bipartite plant-pollinator networks. Low connectance networks (top row) are represented by two realized links between bees and plants, while high connectance networks (bottom row) have four realized links. Bees of the same species always forage on the same set of plant species (left column), while bees of different species can have either overlapping (center column) or non-overlapping (top right column) visitation patterns. Here, the bee vectoring pathogens will "infect" two flowers before clearing the infection. Under dilution scenarios (left and center columns), connectance reduces the probability that an incoming susceptible bee will encounter an infected flower because the total number of possible flowers increases. Conversely, under the amplification scenario, connectance increases diet overlap between infected and uninfected bees, resulting in greater encounter probability (right column).

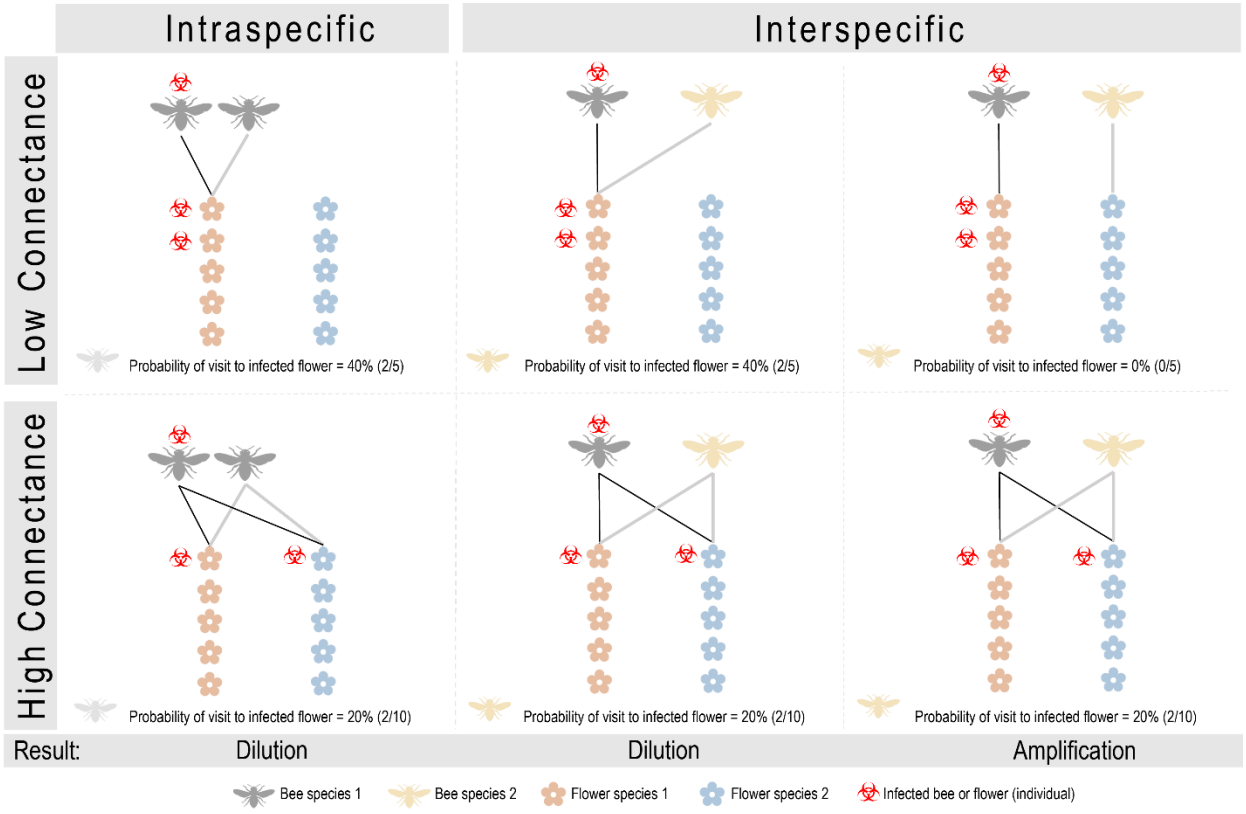


Table 1. Variables, parameters, and their definitions in the SIS system of equations. Parameter values are realistic for plant-pollinator networks. The only exception is N_k, which was adjusted to reproduce realistic values of R_0, λ, and mean steady-state prevalence in the models. The value of N_k is much higher than the observed floral abundance in the wildflower plots, due to the fact that the SIS model assumes a closed system, whereas the bees observed did not forage exclusively inside the plot. Therefore, the correct N_k should be one where the plot area has been sufficiently extended to support the population of bees.

Variables and parameters	Short description	Central value, comments, and reference
y_q and c_k	Fractions of infected/contaminated individuals of bee species q and flower species k	
s_q and u_k	Fractions of susceptible/uncontaminated individuals of bee species q and flower species k	
Q and K	Bee and flower species richness	Mean from empirical data
q and k	Labels for bee and flower species	
M_q and N_k	Abundance of bee species q and flower species k	Bee abundance 50, order-of-magnitude estimate based on observations at the wildflower plots. Flower abundance 2000 (see caption).
$1 / (\gamma_q + \mu_q)$	Reciprocal of the sum of bee recovery and mortality rates	3 days. Central value between species that are non-host vectors (recovery time < 1 day) and species that can develop active infections (if chronic, life expectancy of ~1 month; however, not all exposed individuals become infected, so we chose an intermediate value of 10 days).
$1 / \zeta_k$	Flower decontamination time	3 hours (Figueroa *et al.*, 2019)
$\alpha_{q,k}$	Rate at which uncontaminated flowers of species k become contaminated when foraged on by an infected bee of species q	Combination of other parameters $\alpha_{q,k} = \eta_{q,k} \cdot f_q \cdot r_q \cdot \phi_{q,k}$.
$\beta_{q,k}$	Rate at which a susceptible bee of species q becomes infected when foraging on contaminated flowers of species k	Combination of other parameters $\beta_{q,k} = \eta_{q,k} \cdot f_q \cdot r_q \cdot \psi_{q,k}$.
$\eta_{q,k}$	Fraction of foraging time a bee of species q spends on flowers of species k	We require that $\sum_{k=1}^{K} \eta_{q,k} = 1$.
f_q	Fraction of day bee species q spends foraging	2 hours / day (Otterstatter & Thomson, 2008)
r_q	Foraging rate for a bee of species q	5 flowers / minute, order-of-magnitude estimate based on observations
$\phi_{q,k}$	Bee-to-flower transmission: probability of an infected bee of species q contaminating a single flower of species k when foraging on it	0.01 (Otterstatter & Thomson, 2008)
$\psi_{q,k}$	Flower-to-bee transmission: probability of a bee of species q becoming infected when foraging on a contaminated flower of species k	0.01 (Truitt *et al.*, 2019)

Fig. S1. Map of sites, landscape simplification gradient, and corresponding plant-pollinator networks among the 11 sites. Black circles indicate locations of the sites; three example landscapes are shown on the right at the 750 m scale. The plant-pollinator networks corresponding to each site are on the right. The mean distance between sites was 38.85 km, ranging from 0.21 km to 70.92 km. While the shortest distance is within the maximum flight distance for the larger bodied bees, including bumble bees (Greenleaf et al. 2007; McArt et al. 2017), the average flight range for many solitary bee species ranges from 100 – 300 m (Zurbuchen et al. 2010). Pathogen prevalence was not spatially-autocorrelated (Moran's I = – 0.20, $P = 0.71$, Moran's I = 0.14, $P = 0.37$, Moran's I = – 0.02, $P = 0.74$, and Moran's I = – 0.27, $P = 0.42$ for overall, trypanosome, *Nosema ceranae*, and neogregarine prevalence, respectively), indicating the sites were sufficiently independent to test the hypotheses outlined in the manuscript. Sites were in New York State, USA.

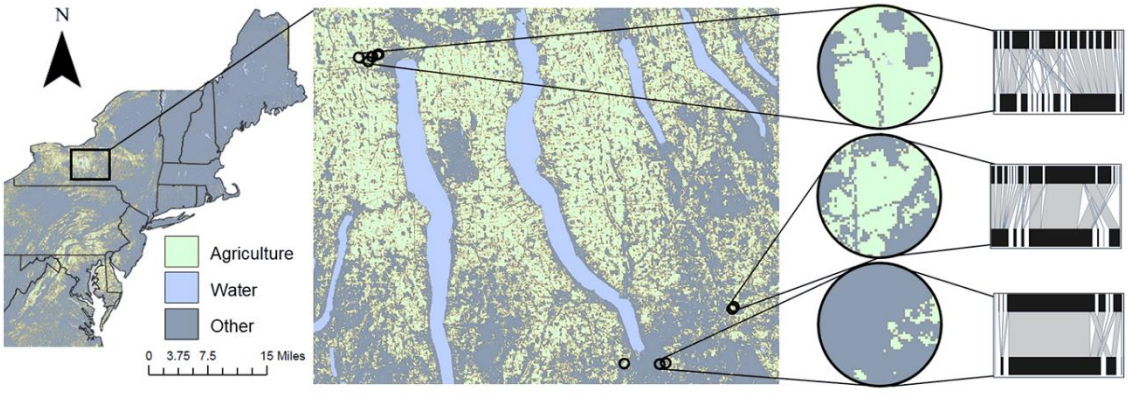
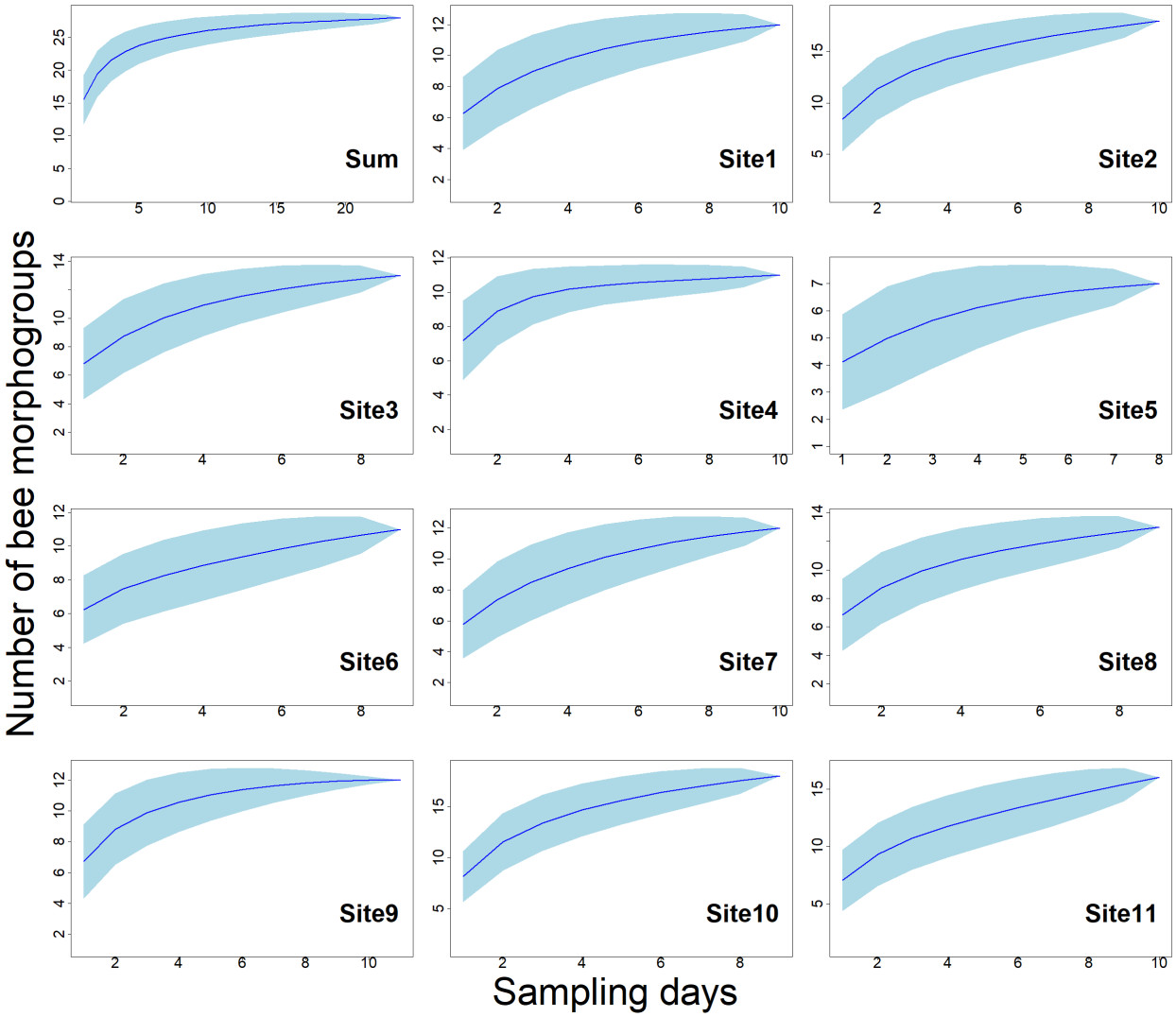


Fig. S2. Rarefaction curves for each bee community. Rarefaction curves are shown for the aggregate pollinator community (summed across the 11 sites), as well as for each individual site, indicating sufficient sampling of the bee communities at the taxonomic resolution possible in the field.



Appendix 2: Taxonomic resolution of the plant-pollinator network

Bee species identification: keys used and barcoding protocol (Sanger sequencing)

Most collected bees were identified to species post-dissection (see *DNA extraction and pathogen screening* in *Materials and Methods*), though some specimens lost key identifiable features. We used numerous published keys for identifying the bees (Gibbs, 2011; Gibbs *et al.*, 2013; LaBerge, 1986; Mitchell, 1960; Mitchell, 1962; Rehan & Sheffield, 2011).

For bees in the genus *Lasioglossum*, which are notoriously difficult to identify morphologically, we amplified and sequenced a 900 bp region of the protein coding gene elongation factor 1-alpha, which includes two introns and is commonly used in bee phylogenetic studies (Danforth *et al.*, 1999). This marker provides a reliable barcode for species determinations while avoiding the problem of co-amplifying endosymbiont (*Wolbachia*) DNA that commonly occurs when using traditional CO1 barcode primers (Gerth *et al.*, 2011; Magnacca & Brown, 2012; Smith *et al.*, 2012).

Taxonomic resolution of networks

To assess how taxonomic resolution of our communities influenced results, species identities of collected individuals were used to resolve morphogroups in the visitation network by allocating visits to each species based on their proportional representation in collections specific to each date, flower species, and site ("resolved network" compared to "unresolved network" that

maintained taxonomic resolution possible in the field). We compared analyses from the resolved and unresolved network, finding no significant differences (Table S1); in the main text we present analyses with the unresolved data to minimize assumptions. There was broad coverage of the network for pathogen screening, as only two very rare taxa that were observed during visitation assessments had no specimens collected for pathogen screening (*Andrena carlini* and *Epeolus* sp.).

Table S1: Results from the simplified structural equations model. Analyses were conducted on networks constructed with bee taxonomic resolution possible in the field (*i.e.,* some bees only identified to genus: "unresolved network") and with all bees identified to species based on proportional representation in collections specific to each date, flower species, and site ("resolved network"). Bold signifies $P < 0.05$. Connectance was not correlated with bee species richness (resolved: $F_{1,9} = 2.71$, $P = 0.134$ and unresolved: $F_{1,9} = 3.16$, $P = 0.109$) or abundance at our sites (resolved: $F_{1,9} = 0.08$, $P = 0.780$ and unresolved: $F_{1,9} = 0.06$, $P = 0.813$).

Response	Predictor	Resolved network					Unresolved network				
		Scaled estimate	Unscaled estimate	SE	DF	*P* value	Scaled estimate	Unscaled estimate	SE	DF	*P* value
***B. impatiens* diet breadth**	**Agricultural cover (500 m)**	**0.70**	**2.76**	**1.02**	**9**	**0.025**	**0.65**	**2.43**	**0.98**	**9**	**0.035**
Overall bee abundance	**Agricultural cover (500 m)**	**-0.87**	**-4.33**	**0.81**	**9**	**0.001**	**-0.87**	**-4.33**	**0.81**	**9**	**0.001**
Connectance	Agricultural cover (500 m)	-0.32	-0.08	0.07	9	0.297	-0.21	-0.05	0.07	9	0.486
Pathogen prevalence	Agricultural cover (500 m)	NA	-1.46	1.20	575	0.221	NA	-0.47	1.15	575	0.684
Pathogen prevalence	Overall bee abundance	NA	-0.32	0.23	575	0.168	NA	-0.15	0.21	575	0.483
Pathogen prevalence	**Connectance**	**NA**	**-10.36**	**2.47**	**575**	**0.000**	**NA**	**-10.73**	**2.42**	**575**	**0.000**
Pathogen prevalence	***B. impatiens* diet breadth**	**NA**	**-0.52**	**0.17**	**575**	**0.003**	**NA**	**-0.58**	**0.18**	**575**	**0.001**
~~Connectance	~~*B. impatiens* diet breadth	-0.54	-0.54	NA	11	0.052	-0.51	-0.51	NA	11	0.066
~~Connectance	~~Overall bee abundance	-0.45	-0.45	NA	11	0.096	-0.26	-0.26	NA	11	0.232
~~*B. impatiens* diet breadth	~~Overall bee abundance	0.40	0.40	NA	11	0.126	0.32	0.32	NA	11	0.186

Table S2: Results from the simplified structural equations model excluding honey bee pathogen prevalence. The results of the path analyses are nearly equivalent when honey bee pathogen prevalence is included (Table S1). Bold signifies $P < 0.05$.

Response	Predictor	Resolved network					Unresolved network				
		Scaled estimate	Unscaled estimate	SE	DF	*P* value	Scaled estimate	Unscaled estimate	SE	DF	*P* value
***B. impatiens* diet breadth**	**Agricultural cover (500 m)**	**0.70**	**2.76**	**1.02**	**9**	**0.025**	**0.68**	**2.56**	**1.06**	**9**	**0.039**
Overall bee abundance	**Agricultural cover (500 m)**	**-0.87**	**-4.33**	**0.81**	**9**	**0.001**	**-0.90**	**-4.50**	**0.80**	**9**	**0.000**
Connectance	Agricultural cover (500 m)	-0.32	-0.08	0.07	9	0.297	-0.24	-0.06	0.07	9	0.444
Pathogen prevalence	Agricultural cover (500 m)	NA	-1.19	1.22	542	0.328	NA	-0.18	1.17	542	0.879
Pathogen prevalence	Overall bee abundance	NA	-0.29	0.23	542	0.215	NA	-0.12	0.22	542	0.574
Pathogen prevalence	**Connectance**	**NA**	**-10.33**	**2.55**	**542**	**0.000**	**NA**	**-10.93**	**2.49**	**542**	**0.000**
Pathogen prevalence	***B. impatiens* diet breadth**	**NA**	**-0.55**	**0.18**	**542**	**0.003**	**NA**	**-0.62**	**0.19**	**542**	**0.001**
~~Connectance	~~*B. impatiens* diet breadth	-0.54	-0.54	NA	11	0.052	-0.50	-0.50	NA	11	0.069
~~Connectance	~~Overall bee abundance	-0.45	-0.45	NA	11	0.096	-0.34	-0.34	NA	11	0.170
~~*B. impatiens* diet breadth	~~Overall bee abundance	0.40	0.40	NA	11	0.126	0.44	0.44	NA	11	0.103

Appendix 3: Molecular screening of pathogens

Additional information of DNA extraction from flowers

The number of samples of each species screened for pathogens are as follows: *Silphium perfoliatum* (screened from 11 sites), *Solidago canadensis* (from 9 sites), *Penstemon digitalis* (from 8 sites), *Veronicastrum virginicum* (from 8 sites), *Erigeron annuus* (from 8 sites), *Trifolium repens* (from 8 sites), *Lobelia siphilitica* (from 6 sites), *Coreopsis lanceolata* (from 6 sites), *Medicago sativa* (from 5 sites), *Trifolium pratens* (from 5 sites), *Daucus carota* (from 4 sites), *Cirsium arvense* (from 3 sites), and *Agastache nepetoides* (not screened due to low abundance).

Flower samples were standardized by weight before DNA extractions: 100 mg of floral tissue was placed in a new vial with 1 ml of TRIsure™ (BIOLINE, Boston, MA, USA) reagent. Pathogens on the flower surface were isolated by first shaking the petals and TRIsure in the tissue lyser without any beads. The samples were then vortexed for 30 sec before being placed in the centrifuge at 12,000 g for 10 minutes at 4 °C. The solution was transferred (leaving petal tissue behind) to a new vial that included two sterilized 2.4 mm steel beads and 100 µl of sterilized 0.1 mm zirconia beads, and homogenized as before (30 seconds at 6.5 m/s). The vials were centrifuged at 12,000 g for 10 minutes at 4 °C and transferred to sterile tubes for DNA extraction following the manufacturer's guidelines (BIOLINE, Boston, MA, USA). Each batch included a negative control.

PCR protocol

We screened for presence of trypanosomes, neogregarines, *Nosema apis, Nosema bombi,* and *Nosema ceranae* using established PCR primers (Table S3; (Gisder & Genersch, 2013; Klee *et al.*, 2006; Meeus *et al.*, 2010)). The PCR was performed using OneTaq® 2X Master Mix with Standard Buffer (New England BioLabs, Ipswich, MA, USA) following the manufacturer's recommendations. Positive and negative controls were included for each set and PCR plate. The elongation temperature was reduced slightly to comply with the enzyme used in OneTaq (68°C) and the annealing temperature was raised slightly to increase oligonucleotide annealing specificity (*Nosema ceranae/apis*: 60°C and trypanosome/Host: 61°C). PCR products were run on a gel (1% agarose on ethidium bromide), then visualized under UV light. Sanger sequence data from a subset of pathogen positives confirmed that the PCR product successfully amplified the pathogens of interest (see below), except for *Nosema apis*, which was thereafter removed from analyses.

Verification that host control primer was not biased phylogenetically

Although the host control primer we used was developed for bees in the Apidae family (Meeus *et al.*, 2010), we found that it amplified DNA (18S rDNA) from bees in all five families, all 19 genera and 46 species in the dataset. A Chi-square test indicated no significant difference in likelihood of successfully amplifying host DNA among the bee families ($\chi^2_4 = 1.29$, $P = 0.86$). The number of bees with a successful extraction, as determined by either a host control or pathogen amplification, was 575 out of the 745 collected. Thus, we focused only on the 575-bee dataset for subsequent analyses.

Pathogen Sanger sequencing

Pathogen PCR results were verified by sequencing a random subset from each of the pathogen positives in the forward direction. Pathogen sequences were obtained by following Danforth et al. (2011) EXO-SAP, RTL sequencing, and edge biosynthesis 96-well dye terminator removal protocols (Danforth *et al.*, 2011). The edge plates were submitted to the Cornell Biotechnology Resource Center (BRC) for Sanger sequencing. Sequence data was manually edited in Sequencher (version 5.4.6, Gene Codes Corporation, Ann Arbor, MI, USA) and then compared to the NIH Standard Nucleotide Database using the BLAST® blastn algorithm search engine. At least a 98% identity match to known pathogens was required to confirm identity of the sequences (Table S4). Neogregarine and trypanosome positives could not be unequivocally identified to species due to shared sequence similarity to several different taxa, but all neogregarines had very high similarity to *Apicystis bombi* (5/5 samples, collected from *Bombus impatiens*, *Calliopsis nebraskensis*, and *Halictus ligatus*). Most trypanosome positives had very high similarity to *Crithidia bombi* (6/9 samples, present in *Bombus impatiens*, *Bombus bimaculatus*, and *Halictus ligatus*). We also had trypanosome positives with high similarity to *Crithidia mellificae* (in *Apis mellifera*) and *Herpetomonas pessoai* (in *Hylaeus affinis*), a pathogen known from flies (Borghesan *et al.*, 2013). Based on a comparison of our sequencing results, trypanosomes and neogregarines present in *Halictus ligatus* (Halictidae) were indistinguishable from those present in *Bombus impatiens* (Apidae) (NCBI Query cover 100%, E value 1 < 10-102 and Identity 99%). While we were unable to amplify *Nosema apis* from the PCR product (thus removed from the analyses), we found that *Nosema bombi* correctly amplified (from *Bombus impatiens* and *Bombus*

vagans), as did *Nosema ceranae* (from *Hylaeus affinis*). Infection with the trypanosome *Crithidia bombi* is known to affect bumble bee foraging behavior, cognitive function (Gegear *et al.*, 2005; Gegear *et al.*, 2006), and reproduction (Goulson *et al.*, 2018). Similarly, the neogregarine *Apicystis bombi* and microsporidians *Nosema bombi*, and *Nosema ceranae* can increase mortality in bees (Graystock *et al.*, 2016b; Graystock *et al.*, 2013a; Otti & Schmid-Hempel, 2007).

Table S3. Molecular conditions used for pathogen screening (PCR).

Primers and source	Thermal Cycling			Amplicon size (bp)
	Denaturing Min | Temp	Replication Sec | Temp	Elongation Min | Temp	
Apidae[A] (host) and Trypanosomes[T] (Meeus et al., 2010)	2 | 94	35x 30 | 94 30 | 61 45 | 68	5 | 68	130[A] 420[T]
ApidaeF(5-3): AGATGGGGGCATTCGTATTG				
ApidaeR(5-3): ATCTGATCGCCTTCGAACCT				
SEF(5-3): CTTTTGGTCGGTGGAGTGAT				
SER(5-3): GGACGTAATCGGCACAGTTT				
Neogregarines (Meeus et al., 2010)	2 | 94	35x 30 | 94 30 | 60.7 45 | 68	3 | 68	260
NeoF (5-3): CCAGCATGGAATAACATGTAAGG				
NeoR(5-3): GACAGCTTCCAATCTCTAGTCG				
Nosema apis[A], N ceranae[Nc] (Gisder & Genersch, 2013)	4 | 95	35x 60 | 95 60 | 60 60 | 68	5 | 68	297[Na] 662[Nc]
NosaRNAPol-F2 (5-3): AGCAAGAGACGTTTCTGGTACCTCA				
NosaRNAPol-R2 (5-3): CCTTCACGACCACCCATGGCA				
NoscRNAPol-F2 (5-3): TGGGTTCCCTAAACCTGGTGGTTT				
NoscRNAPol-R2 (5-3): TCACATGACCTGGTGCTCCTTCT				
Nosema bombi (Klee et al., 2006)	4 | 95	35x 60 | 95 60 | 50 60 | 68	4 | 68	323
Nbombi-SSU-Jf1 (5-3): CCA TGC ATG TTT TTG AAG ATT ATT AT				
Nbombi-SSU-Jr1 (5-3): CAT ATA TTT TTA AAA TAT GAA ACA ATA A				

Table S4. Pathogen sequencing results. Sequence data are available in the GenBank under accession numbers (MK128972 – MK128988; MK138674).

Species	Primer target	Closest match	Max score	Query cover	E value	Identity
Calliopsis nebraskensis	Neogregarines	*Apicystis bombi*	340	100%	8E-90	99%
Halictus ligatus	Neogregarines	*Apicystis bombi*	340	100%	8E-90	99%
Bombus impatiens	Neogregarines	*Apicystis bombi*	352	100%	1E-93	99%
Bombus impatiens	Neogregarines	*Apicystis bombi*	336	100%	1E-88	99%
Bombus impatiens	Neogregarines	*Apicystis bombi*	352	99%	1E-93	99%
Bombus vagans	*Nosema bombi*	*Nosema bombi*	496	99%	2E-136	100%
Bombus impatiens	*Nosema bombi*	*Nosema bombi*	496	99%	2E-136	100%
Bombus impatiens	*Nosema bombi*	*Nosema bombi*	496	99%	2E-136	100%
Hylaeus affinis	*Nosema ceranae*	*Nosema ceranae*	699	100%	0E+00	99%
Bombus impatiens	Trypanosomes	*Crithidia bombi*	621	99%	3E-174	99%
Bombus impatiens	Trypanosomes	*Crithidia bombi*	619	99%	1E-173	99%
Bombus impatiens	Trypanosomes	*Crithidia bombi*	554	99%	4E-154	99%
Bombus bimaculatus	Trypanosomes	*Crithidia bombi*	579	100%	3E-161	100%
Halictus ligatus	Trypanosomes	*Crithidia bombi*	573	100%	1E-159	99%
Bombus impatiens	Trypanosomes	*Crithidia bombi*	569	100%	2E-158	99%
Apis mellifera	Trypanosomes	*Crithidia mellificae*	579	100%	3E-161	100%
Hylaeus affinis	Trypanosomes	*Herpetomonas pessoai*	483	100%	2E-132	100%
Solidago juncea	Trypanosomes	Trypanostomatidae sp.	619	99%	1E-173	98%

Appendix 4. Module membership and additional network metrics evaluated (H2 and d').

Evaluation of H2 and d' as network level and species-specific network metrics

Quantitative network metrics, especially H2' and d', have been highlighted as valuable proxies for generalization and diet breadth of interacting species at network and species levels, respectively (Blüthgen *et al.*, 2008; Blüthgen & Menzel, 2006; Blüthgen *et al.*, 2007). We calculated the z score for the H2 of each plant-pollinator network and d' for each bee species at each site for which we had both pathogen and visitation data (see *Materials and Methods*). We found that the d' z-score was 0 for all of the bees in 10/11 sites (over 90 % of the bees processed), indicating that the values were no different than those expected from abundance alone (Dormann, 2011), and was thus not analyzed via GLMM as were degree, betweenness centrality, and module membership. We analyzed the null-standardized z-score of H2 in the path analyses, finding that it was neither affected by landscape (path analysis estimate (estimate) = – 0.45, P = 0.833 and estimate = 0.92, P = 0.798 for the effect of agricultural cover at the 500 m scale on the resolved and unresolved networks, respectively), nor predicted pathogen prevalence (resolved: estimate = – 0.11, P = 0.311 and unresolved: estimate = – 0.09, P = 0.174). Moreover, we found that H2 was correlated with weighted NODF (quantitative nestedness index), which is currently presented in the results summary table (resolved: $F_{1,9}$ = 5.11, P = 0.050 and unresolved: $F_{1,9}$ = 5.14, P = 0.050; Table S6).

Table S5: Constituent bee species of modules across sites. The number indicates samples of each species at each site that were screened.

Site	Site1	Site1	Site1	Site1	Site2	Site2	Site2	Site2	Site2	Site2	Site2	Site2	Site3	Site3	Site3
Module group	1	2	3	4	1	2	3	4	5	6	7	8	1	2	3
Overall pathogen prevalence	0.30	0.07	0.25	0.29	0.50	1.00	0.20	0.50	0.67	0.50	0.42	0.21	0.54	0.68	0.80
Bee species															
Agapostemon virescens								2							
Andrena sp															
Andrena wilkella				1											
Anthophora terminalis							4							8	
Apis mellifera			4							8			5		
Augochlorella aurata											1				
Bombus bimaculatus	2										8				
Bombus fervidus															1
Bombus griseocollis	1											4	1		
Bombus impatiens		4						36					4		
Bombus perplexus															
Bombus ternarius											3				
Bombus vagans															1
Calliopsis andreniformis															1
Ceratina calcarata							2						2		
Ceratina dupla						1									
Ceratina mikmaqi															1
Ceratina sp															
Coelioxys rufitarsis													1		
Halictus confusus										4					3
Halictus ligatus		4								8				10	
Halictus rubicundis															
Heriades carinata															
Hoplitis producta															
Hoplitis sp												1			
Hylaeus affinis				1	2										
Hylaeus annulatus												1			
Hylaeus modestus		2													
Hylaeus sp												3			
Lasioglossum coriaceum															
Lasioglossum cressonii															
Lasioglossum hitchensi												1			
Lasioglossum imitatum															
Lasioglossum lineatulum				1			2								
Lasioglossum perpunctatum															
Lasioglossum pilosum															
Lasioglossum sp				1								5			
Lasioglossum versatum															
Lasioglossum viridatum		4										8		1	
Lasioglossum weemsi												1			
Lasioglossum zephyrum															
Megachile sp												1			
Melissodes agilis									1						1
Melissodes bimaculata	1														
Melissodes desponsa													2		
Melissodes druriella												1			
Melissodes sp				3								2			
Melissodes subillata	1														
Melissodes trinodis	5												13		
Osmia atriventris															1
Osmia bucephala							2								
Peponapis pruinosa															1
Unknown															
Xylocopa virginica									2						

Table S5 continued:

Site	Site4	Site4	Site4	Site4	Site4	Site5	Site6	Site6	Site6	Site6	Site6	Site7	Site7	Site7	Site7	Site7
Module group	1	2	3	4	5	1	1	2	3	4	5	1	2	3	4	5
Overall pathogen prevalence	0.18	0.58	0.71	0.25	0.40	0.54	0.08	0.00	0.13	0.54	0.35	0.15	0.25	0.00	0.00	0.36
Bee species																
Agapostemon virescens																3
Andrena sp																
Andrena wilkella																
Anthophora terminalis					1											1
Apis mellifera										3				1		
Augochlorella aurata			5								1					1
Bombus bimaculatus				2							5					1
Bombus fervidus															1	
Bombus griseocollis	2															
Bombus impatiens				2		16	12						3			
Bombus perplexus																
Bombus ternarius																
Bombus vagans					3	1						3				
Calliopsis andreniformis																
Ceratina calcarata																
Ceratina dupla																
Ceratina mikmaqi												14				
Ceratina sp																
Coelioxys rufitarsis																
Halictus confusus		3														1
Halictus ligatus		6								3				1		
Halictus rubicundis																
Heriades carinata																
Hoplitis producta																
Hoplitis sp																
Hylaeus affinis		3														
Hylaeus annulatus																
Hylaeus modestus																
Hylaeus sp																
Lasioglossum coriaceum																
Lasioglossum cressonii																
Lasioglossum hitchensi																
Lasioglossum imitatum																
Lasioglossum lineatulum																
Lasioglossum perpunctatum																
Lasioglossum pilosum			1								2					1
Lasioglossum sp					1						8					2
Lasioglossum versatum									2							
Lasioglossum viridatum			1						6			3				
Lasioglossum weemsi																
Lasioglossum zephyrum											1					
Megachile sp					1											
Melissodes agilis	1									1						
Melissodes bimaculata	1															
Melissodes desponsa	3															
Melissodes druriella								1								1
Melissodes sp					4											
Melissodes subillata													1			
Melissodes trinodis	4					7				6						
Osmia atriventris																
Osmia bucephala																
Peponapis pruinosa																
Unknown																
Xylocopa virginica																

Table S5 continued:

Site	Site8	Site8	Site8	Site8	Site8	Site9	Site9	Site9	Site9	Site9	Site10	Site10	Site10	Site10	Site11	Site11	Site11	Site11	Site11
Module group	1	2	3	4	5	1	2	3	4	5	1	2	3	4	1	2	3	4	5
Overall pathogen prevalence	0.00	1.00	0.36	0.53	0.75	0.17	0.15	0.25	0.45	0.37	0.36	0.40	0.73	0.56	0.40	0.25	0.17	0.64	0.38
Bee species																			
Agapostemon virescens										2								4	
Andrena sp					1														
Andrena wilkella																1			
Anthophora terminalis								3						4		1			
Apis mellifera				10														2	
Augochlorella aurata					3			1			7				3				
Bombus bimaculatus					2					1									1
Bombus fervidus																		1	
Bombus griseocollis			1																
Bombus impatiens			26						4				3				7		
Bombus perplexus			1																
Bombus ternarius				1															
Bombus vagans										1			1						
Calliopsis andreniformis																			
Ceratina calcarata				1			7				1								
Ceratina dupla		1																	
Ceratina mikmaqi	1																		1
Ceratina sp					1					2									
Coelioxys rufitarsis																			
Halictus confusus						5						2							1
Halictus ligatus										3			2		2				
Halictus rubicundis														1					
Heriades carinata														1					
Hoplitis producta														1					
Hoplitis sp																			
Hylaeus affinis				4						1									
Hylaeus annulatus																			1
Hylaeus modestus				1			6				3					2			
Hylaeus sp																			
Lasioglossum coriaceum										1				1					1
Lasioglossum cressonii														1					
Lasioglossum hitchensi																			
Lasioglossum imitatum										2									
Lasioglossum lineatulum																			
Lasioglossum perpunctatum														1					
Lasioglossum pilosum						6													
Lasioglossum sp					1					1				3					1
Lasioglossum versatum										1									
Lasioglossum viridatum		1						4				3					5		
Lasioglossum weemsi																			
Lasioglossum zephyrum						1							2						
Megachile sp		2											1						
Melissodes agilis									1										
Melissodes bimaculata									2										
Melissodes desponsa																		1	
Melissodes druriella																			
Melissodes sp										3									2
Melissodes subillata									1										
Melissodes trinodis			5						3				2					6	
Osmia atriventris																			
Osmia bucephala										1				1					
Peponapis pruinosa																			
Unknown														2					
Xylocopa virginica																			

Appendix 5. Pathogen prevalence among bee species and landscapes

Structural equation modeling

After removing non-significant terms in the structural equation models, we used the d-separation test to evaluate whether the non-hypothesized independent paths were significant and whether the models could be improved with their inclusion (Shipley, 2009). The d-separation test indicated that the overall model fit would be improved by explicitly modeling the direct relationships between agricultural cover and pathogen prevalence as well as the path between bee abundance and pathogen prevalence, and were thus included in the simplified model, although these paths were generally not found to be significant in explaining pathogen prevalence (Table S1).

Role of floral abundance in wildflower plantings

The abundance of and rewards provided by specific flower species influence pollinator visitation patterns, with clear implications for the structure and stability of the plant-pollinator network (Carvalheiro *et al.*, 2014). Moreover, pairwise species interaction patterns in plant-pollinator networks depend on floral composition, defined as the abundance and identity of flowering plant species, more so than on overall floral abundance (Kaiser-Bunbury *et al.*, 2014). In the context of wildflower plantings, overall floral abundance and richness is not always predictive of pollinator abundance or richness, with landscape context being a much stronger predictor (Grass *et al.*, 2016). While we standardized the wildflower sites as much as was possible

by planting the same species in equal initial densities at each site, we did not measure floral abundance for each species at each site throughout the sampling period as it was not logistically feasible given other data collection constraints. Future evaluations of the interdependent dynamics between landscape context, wildflower strip establishment and blooming patterns, plant-pollinator network dynamics, and ultimately disease spread, is important for optimizing wildflower mixtures to promote pollinator health.

Pathogen prevalence spatial auto-correlation

Overall pathogen prevalence at the site was defined as the proportion of samples that tested positive for any of the following pathogens: trypanosomes, neogregarines, *Nosema bombi*, and *Nosema ceranae*. We computed the true pathogen prevalence with a 95% confidence interval correcting for varying sample sizes at each site. The test sensitivity was set to 95% using the Blaker method for two-sided confidence intervals using the *epi.prev* function of the epiR package in R (Stevenson *et al.*, 2012). We evaluated overall pathogen prevalence instead of specific taxa because the two most prevalent pathogens were highly correlated (trypanosomes and *Nosema ceranae*: $\chi^2_1 = 10.31$, $P = 0.001$), while the other pathogens had very low prevalence ($\leq 1\%$; Fig. S3). We assessed spatial auto-correlation of site-level pathogen prevalence across our sites by calculating the inverse distance matrix and using the *Moran.I* function of the ape package (Paradis *et al.*, 2004). Temporal patterns were not a significant predictor of pathogen prevalence in our communities (Appendix 7) and were therefore excluded from all models.

The effect of honey bee abundance in the overall bee community

Despite being recorded in visitation assessments, honey bees (*Apis mellifera*) were not collected for pathogen sampling during the first two months of sampling (June and July), although they were collected in August and September as their abundance increased (194 vs. 373 visits observed, respectively). Path analyses yielded similar results when honey bees were included (Table S1) and excluded (Table S2) from pathogen prevalence calculations. We evaluated the relationship between *A. mellifera* abundance and non-honey bee abundance, connectance, modularity, nestedness, *B. impatiens* degree, and overall pathogen prevalence and found no significant results for either the resolved or unresolved networks ($F_{1,9} < 3.08$ and $P > 0.113$ for all).

Fig. S3. Pathogen prevalence in composite plant-pollinator networks by pathogen group: (a) trypanosomes, (b) *Nosema ceranae*, (c) neogregarines, and (d) *Nosema bombi*. Darker colors indicate higher pathogen prevalence in bees (top bars) and on flowers (bottom bars). Species identity same as Fig. 1. The four pathogens screened varied in prevalence: 24% (95% CI, 20 – 28), 12% (95% CI, 9 – 15), 1% (95% CI, 0 – 3%), and virtually absent (95% CI, 0 – 0) for trypanosomes, *Nosema ceranae*, neogregarines, and *Nosema bombi*, respectively.

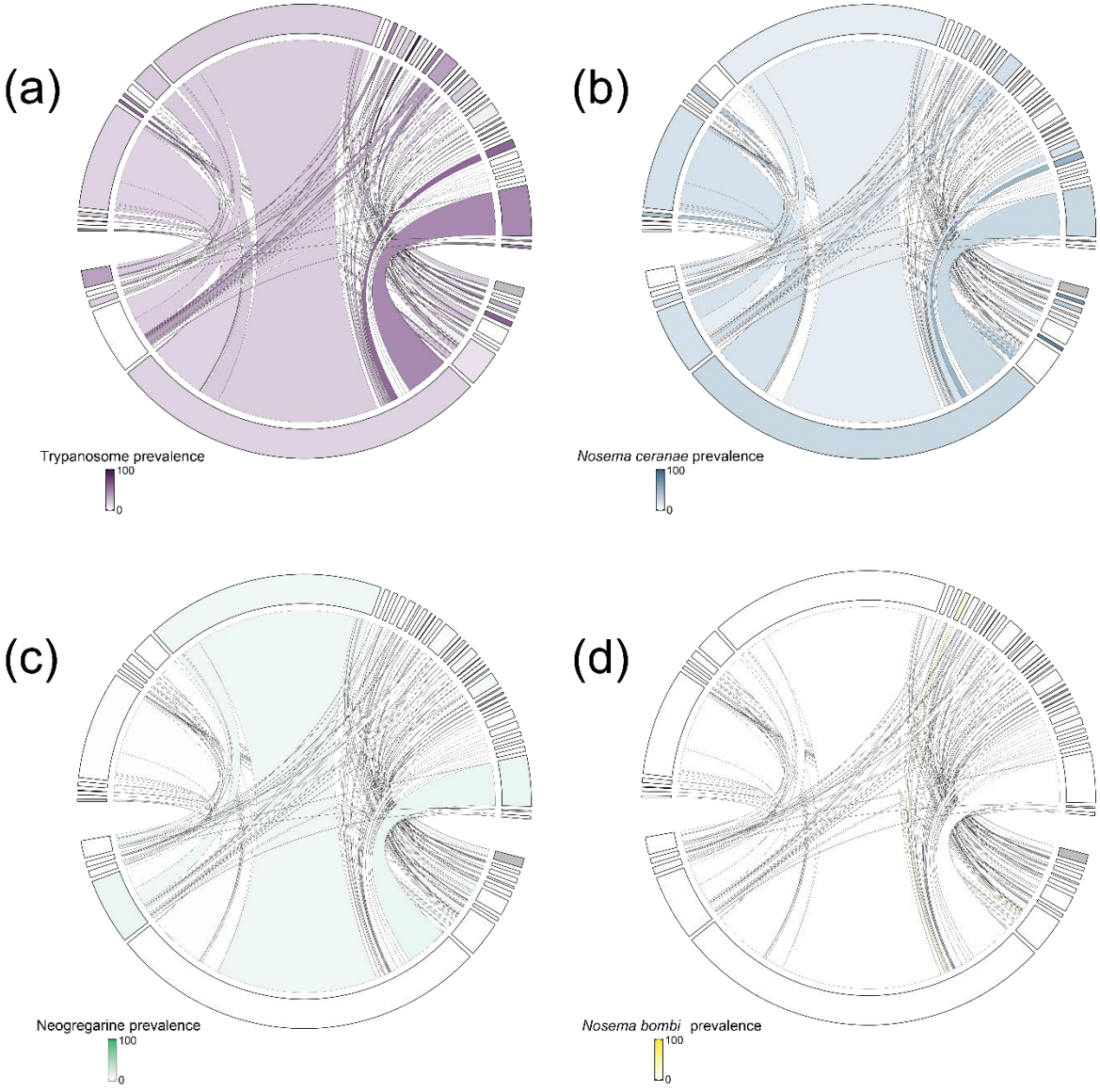


Fig. S4. Variation in presence of pathogens in bee genera and on flower species. (a) number of pathogen groups found in individual bees from each genus and (b) number of pathogen groups found in individual flowers from each plant species.

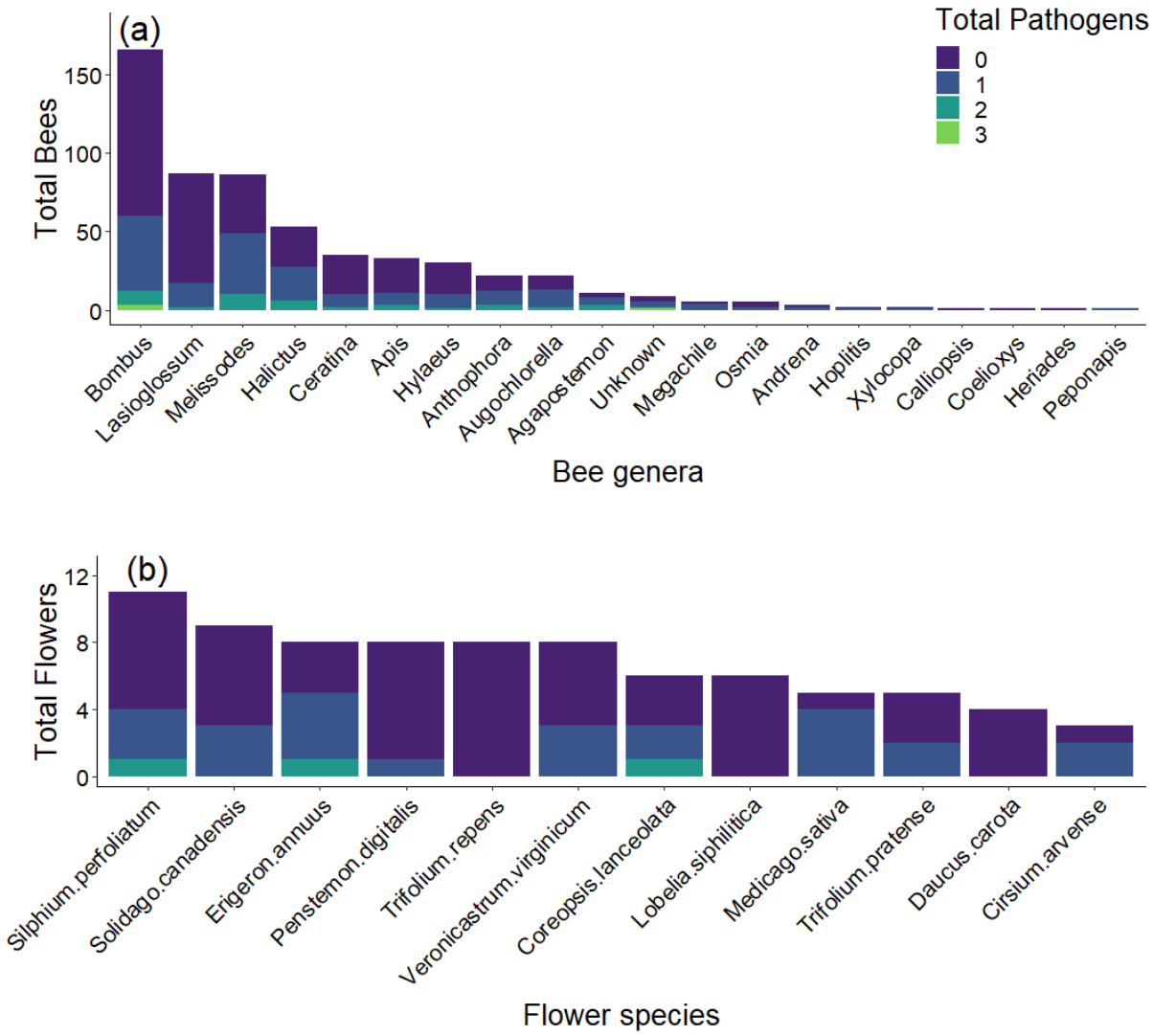


Fig. S5. Direct relationship between agricultural cover and pathogen prevalence across the 11 pollinator communities. There was not a significant direct relationship between landscape simplification and pathogen prevalence ($P > 0.05$); instead the effect was mediated by an increase in *Bombus impatiens* diet breadth (Fig. 2 and Table S1).

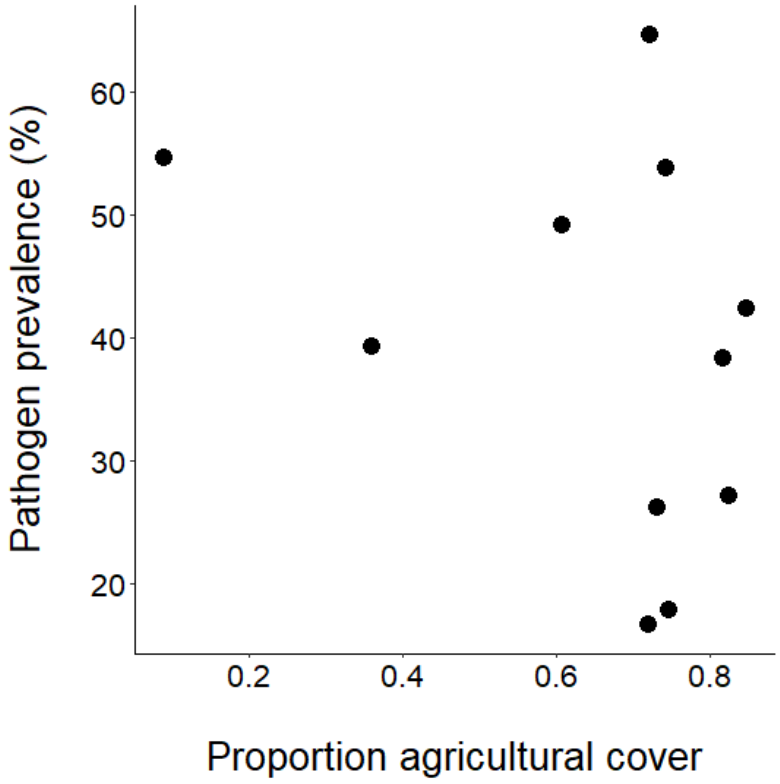


Fig. S6. Pathogen prevalence in bees by module group. Bars represent proportion of bees in each module with pathogens. Shown for (a) overall, (b) trypanosome, (c) *Nosema ceranae*, and (d) neogregarine prevalence. *Nosema bombi* results not shown because virtually absent from the community.

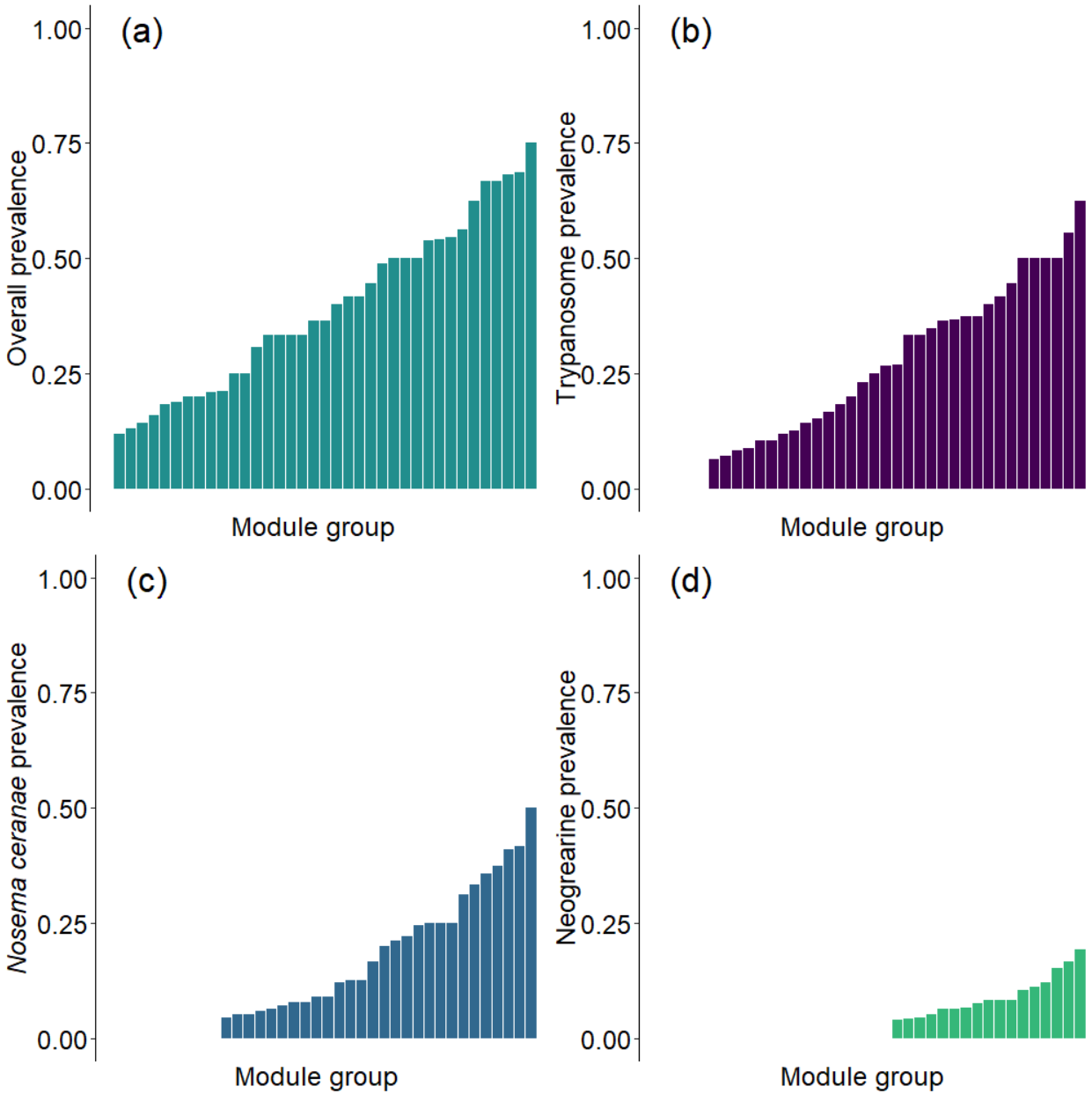


Table S6: Full structural equation model output for three landscape scales. Landscape variables that significantly predicted network metrics are in bold ($P < 0.05$). (a) Agricultural cover at 500 m; (b) Agricultural cover at 750 m; and (c) Agricultural cover at 1250 m.

(a)

Response	Predictor	Resolved network					Unresolved network				
		Scaled estimate	Unscaled estimate	SE	DF	*P* value	Scaled estimate	Unscaled estimate	SE	DF	*P* value
Connectance	Agricultural cover (500 m)	-0.32	-0.08	0.07	9	0.297	-0.21	-0.05	0.07	9	0.486
***B. impatiens* diet breadth**	**Agricultural cover (500 m)**	**0.70**	**2.76**	**1.02**	**9**	**0.025**	**0.65**	**2.43**	**0.98**	**9**	**0.035**
Modularity	Agricultural cover (500 m)	-0.03	-0.25	2.50	9	0.924	-0.42	-4.94	3.48	9	0.190
Nestedness	Agricultural cover (500 m)	-0.27	-1.05	1.19	9	0.400	-0.35	-1.38	1.25	9	0.298
Overall bee abundance	**Agricultural cover (500 m)**	**-0.87**	**-4.33**	**0.81**	**9**	**0.001**	**-0.87**	**-4.33**	**0.81**	**9**	**0.001**
Bee species richness	Agricultural cover (500 m)	0.50	6.85	4.17	9	0.135	0.23	3.53	4.55	9	0.458
Pathogen prevalence	**Agricultural cover (500 m)**	**NA**	**-6.52**	**2.52**	**575**	**0.010**	NA	-1.87	2.46	575	0.448
Pathogen prevalence	**Connectance**	**NA**	**-18.67**	**5.99**	**575**	**0.002**	**NA**	**-13.73**	**3.45**	**575**	**0.000**
Pathogen prevalence	***B. impatiens* diet breadth**	**NA**	**-0.60**	**0.19**	**575**	**0.002**	**NA**	**-0.49**	**0.21**	**575**	**0.019**
Pathogen prevalence	Modularity	NA	-0.19	0.12	575	0.111	NA	0.05	0.06	575	0.383
Pathogen prevalence	**Nestedness**	**NA**	**-0.82**	**0.37**	**575**	**0.025**	NA	-0.10	0.24	575	0.672
Pathogen prevalence	**Overall bee abundance**	**NA**	**-1.21**	**0.47**	**575**	**0.011**	NA	-0.43	0.41	575	0.298
Pathogen prevalence	Bee species richness	NA	-0.08	0.07	575	0.242	NA	-0.04	0.05	575	0.483
~~Connectance	~~*B. impatiens* diet breadth	-0.54	-0.54	NA	11	0.052	-0.51	-0.51	NA	11	0.066
~~Connectance	~~Modularity	0.40	0.40	NA	11	0.126	**0.62**	**0.62**	**NA**	**11**	**0.029**
~~Connectance	~~Nestedness	-0.28	-0.28	NA	11	0.215	0.03	0.03	NA	11	0.469
~~Connectance	~~Overall bee abundance	-0.45	-0.45	NA	11	0.096	-0.26	-0.26	NA	11	0.232
~~Connectance	~~Bee species richness	-0.38	-0.38	NA	11	0.138	-0.48	-0.48	NA	11	0.081
~~*B. impatiens* diet breadth	~~Modularity	-0.08	-0.08	NA	11	0.414	-0.40	-0.40	NA	11	0.128
~~*B. impatiens* diet breadth	~~Nestedness	-0.08	-0.08	NA	11	0.410	0.05	0.05	NA	11	0.443
~~*B. impatiens* diet breadth	Overall bee abundance	0.40	0.40	NA	11	0.126	0.32	0.32	NA	11	0.186
~~*B. impatiens* diet breadth	~~Bee species richness	0.13	0.13	NA	11	0.355	0.23	0.23	NA	11	0.266
~~Modularity	**~~Nestedness**	**-0.67**	**-0.67**	**NA**	**11**	**0.018**	-0.37	-0.37	NA	11	0.145
~~Modularity	Overall bee abundance	-0.08	-0.08	NA	11	0.412	0.09	0.09	NA	11	0.400
~~Modularity	~~Bee species richness	0.43	0.43	NA	11	0.107	-0.36	-0.36	NA	11	0.150
~~Nestedness	**Overall bee abundance**	-0.48	-0.48	NA	11	0.078	**-0.67**	**-0.67**	**NA**	**11**	**0.018**
~~Nestedness	~~Bee species richness	-0.18	-0.18	NA	11	0.312	-0.03	-0.03	NA	11	0.469
~~Overall bee abundance	~~Bee species richness	-0.04	-0.04	NA	11	0.452	-0.28	-0.28	NA	11	0.218

(b)

Response	Predictor	Resolved network					Unresolved network				
		Scaled estimate	Unscaled estimate	SE	DF	*P* value	Scaled estimate	Unscaled estimate	SE	DF	*P* value
Connectance	Agricultural cover (750 m)	0.04	0.01	0.10	9	0.911	0.11	0.03	0.09	9	0.727
B. impatiens diet breadth	Agricultural cover (750 m)	0.48	2.39	1.57	9	0.162	0.45	2.12	1.46	9	0.181
Modularity	Agricultural cover (750 m)	0.21	2.29	3.10	9	0.479	-0.25	-3.77	4.76	9	0.449
Nestedness	Agricultural cover (750 m)	-0.40	-1.96	1.44	9	0.207	-0.32	-1.59	1.62	9	0.352
Overall bee abundance	**Agricultural cover (750 m)**	**-0.90**	**-5.63**	**0.98**	**9**	**0.000**	**-0.90**	**-5.63**	**0.98**	**9**	**0.000**
Bee species richness	Agricultural cover (750 m)	0.49	8.43	5.39	9	0.152	0.25	4.69	5.80	9	0.440
Pathogen prevalence	**Agricultural cover (750 m)**	**NA**	**-5.84**	**2.60**	**575**	**0.025**	NA	-4.08	3.40	575	0.230
Pathogen prevalence	Connectance	NA	-4.18	4.50	575	0.353	NA	-6.06	6.60	575	0.359
Pathogen prevalence	***B. impatiens* diet breadth**	**NA**	**-0.55**	**0.19**	**575**	**0.004**	**NA**	**-0.46**	**0.19**	**575**	**0.015**
Pathogen prevalence	Modularity	NA	-0.18	0.12	575	0.126	NA	0.02	0.07	575	0.827
Pathogen prevalence	Nestedness	NA	-0.55	0.31	575	0.080	NA	-0.25	0.28	575	0.380
Pathogen prevalence	**Overall bee abundance**	**NA**	**-0.76**	**0.35**	**575**	**0.030**	NA	-0.58	0.40	575	0.141
Pathogen prevalence	Bee species richness	NA	0.05	0.06	575	0.382	NA	0.01	0.04	575	0.770
~~Connectance	**~~*B. impatiens* diet breadth**	**-0.71**	**-0.71**	**NA**	**11**	**0.011**	**-0.65**	**-0.65**	**NA**	**11**	**0.020**
~~Connectance	**~~Modularity**	0.39	0.39	NA	11	0.134	**0.70**	**0.70**	**NA**	**11**	**0.012**
~~Connectance	~~Nestedness	-0.15	-0.15	NA	11	0.335	0.15	0.15	NA	11	0.337
~~Connectance	~~Overall bee abundance	0.28	0.28	NA	11	0.216	0.41	0.41	NA	11	0.122
~~Connectance	**~~Bee species richness**	**-0.56**	**-0.56**	**NA**	**11**	**0.045**	**-0.56**	**-0.56**	**NA**	**11**	**0.045**
~~*B. impatiens* diet breadth	~~Modularity	-0.22	-0.22	NA	11	0.272	-0.50	-0.50	NA	11	0.070
~~*B. impatiens* diet breadth	~~Nestedness	-0.08	-0.08	NA	11	0.418	-0.05	-0.05	NA	11	0.440
~~*B. impatiens* diet breadth	Overall bee abundance	-0.09	-0.09	NA	11	0.404	-0.12	-0.12	NA	11	0.371
~~*B. impatiens* diet breadth	~~Bee species richness	0.25	0.25	NA	11	0.241	0.25	0.25	NA	11	0.246
~~Modularity	**~~Nestedness**	**-0.60**	**-0.60**	**NA**	**11**	**0.033**	-0.27	-0.27	NA	11	0.226
~~Modularity	Overall bee abundance	0.45	0.45	NA	11	0.097	0.42	0.42	NA	11	0.114
~~Modularity	~~Bee species richness	0.29	0.29	NA	11	0.207	-0.39	-0.39	NA	11	0.136
~~Nestedness	**Overall bee abundance**	**-0.83**	**-0.83**	**NA**	**11**	**0.002**	**-0.64**	**-0.64**	**NA**	**11**	**0.023**
~~Nestedness	~~Bee species richness	-0.12	-0.12	NA	11	0.374	-0.03	-0.03	NA	11	0.463
~~Overall bee abundance	~~Bee species richness	-0.07	-0.07	NA	11	0.426	-0.27	-0.27	NA	11	0.228

(c)

Response	Predictor	Resolved network					Unresolved network				
		Scaled estimate	Unscaled estimate	SE	DF	*P* value	Scaled estimate	Unscaled estimate	SE	DF	*P* value
Connectance	Agricultural cover (1250 m)	-0.20	-0.06	0.09	9	0.521	-0.10	-0.03	0.08	9	0.734
B. impatiens diet breadth	Agricultural cover (1250 m)	0.52	2.32	1.36	9	0.122	0.46	1.97	1.28	9	0.159
Modularity	Agricultural cover (1250 m)	0.12	1.17	2.81	9	0.686	-0.31	-4.20	4.14	9	0.337
Nestedness	Agricultural cover (1250 m)	-0.34	-1.50	1.32	9	0.285	-0.48	-2.13	1.34	9	0.146
Overall bee abundance	**Agricultural cover (1250 m)**	**-0.84**	**-4.70**	**1.04**	**9**	**0.001**	**-0.84**	**-4.70**	**1.04**	**9**	**0.001**
Bee species richness	Agricultural cover (1250 m)	0.58	9.05	4.48	9	0.074	0.32	5.50	5.01	9	0.301
Pathogen prevalence	Agricultural cover (1250 m)	NA	-0.86	1.30	575	0.506	NA	-0.45	1.76	575	0.797
Pathogen prevalence	**Connectance**	**NA**	**-8.53**	**4.31**	**575**	**0.048**	**NA**	**-12.76**	**3.41**	**575**	**0.000**
Pathogen prevalence	***B. impatiens* diet breadth**	**NA**	**-0.59**	**0.20**	**575**	**0.003**	**NA**	**-0.59**	**0.16**	**575**	**0.000**
Pathogen prevalence	Modularity	NA	-0.10	0.11	575	0.394	NA	0.06	0.06	575	0.366
Pathogen prevalence	Nestedness	NA	-0.09	0.24	575	0.709	NA	0.00	0.24	575	0.997
Pathogen prevalence	Overall bee abundance	NA	-0.16	0.23	575	0.499	NA	-0.19	0.26	575	0.470
Pathogen prevalence	Bee species richness	NA	0.02	0.06	575	0.784	NA	-0.01	0.03	575	0.882
~~Connectance	**~~*B. impatiens* diet breadth**	**-0.59**	**-0.59**	**NA**	**11**	**0.035**	-0.54	-0.54	NA	11	0.053
~~Connectance	**~~Modularity**	0.43	0.43	NA	11	0.107	**0.64**	**0.64**	**NA**	**11**	**0.023**
~~Connectance	~~Nestedness	-0.26	-0.26	NA	11	0.238	0.06	0.06	NA	11	0.435
~~Connectance	~~Overall bee abundance	-0.16	-0.16	NA	11	0.330	-0.03	-0.03	NA	11	0.468
~~Connectance	~~Bee species richness	-0.44	-0.44	NA	11	0.099	-0.50	-0.50	NA	11	0.069
~~*B. impatiens* diet breadth	~~Modularity	-0.17	-0.17	NA	11	0.316	-0.48	-0.48	NA	11	0.082
~~*B. impatiens* diet breadth	~~Nestedness	-0.09	-0.09	NA	11	0.403	0.04	0.04	NA	11	0.457
~~*B. impatiens* diet breadth	Overall bee abundance	-0.05	-0.05	NA	11	0.443	-0.11	-0.11	NA	11	0.377
~~*B. impatiens* diet breadth	~~Bee species richness	0.18	0.18	NA	11	0.306	0.20	0.20	NA	11	0.285
~~Modularity	**~~Nestedness**	**-0.63**	**-0.63**	**NA**	**11**	**0.026**	-0.38	-0.38	NA	11	0.139
~~Modularity	Overall bee abundance	0.19	0.19	NA	11	0.299	0.28	0.28	NA	11	0.217
~~Modularity	~~Bee species richness	0.35	0.35	NA	11	0.163	-0.36	-0.36	NA	11	0.157
~~Nestedness	**Overall bee abundance**	-0.54	-0.54	NA	11	0.055	**-0.81**	**-0.81**	**NA**	**11**	**0.002**
~~Nestedness	~~Bee species richness	-0.11	-0.11	NA	11	0.379	0.06	0.06	NA	11	0.436
~~Overall bee abundance	~~Bee species richness	0.06	0.06	NA	11	0.433	-0.12	-0.12	NA	11	0.368

Appendix 6. Mantel tests comparing similarity of foraging patterns and pathogen prevalence.

We tested the hypothesis that similarity in floral visitation patterns is associated with similarity in bee pathogen prevalence. For pathogen k and each site s, we calculated $\Delta_{ij}^{ks} = |p_i - p_j|$, the absolute value of the difference in mean pathogen prevalence p observed in bee species i and j. We only included bee species at a site in this analysis if there were at least two individuals of a species assayed for pathogens at that site. We aimed to compare this pairwise measure of "pathogen prevalence similarity" with a suitably defined pairwise measure of "floral visitation similarity".

For each pair of bee species present at a given site, we defined a variety of (dis)similarity measures for that pair based upon properties of the observed visitation network. At each site, we constructed a weighted bipartite network for which nodes are bee and flower species observed at that site, with edges connecting a bee and flower species if there was some observed visitation. We defined two related edge weights: one based on the total number of observed visits V_{ij} between bees of species i and flowers of species j, and one based on the relative visitation frequency $\phi_{ij} = V_{ij} / \sum_j V_{ij}$ (number of visits of bees of species i to flowers of species j, divided by the total number of observed visits for bees i). Various pairwise measures between species of bees can be defined, some making use just of the topology of the networks, and others making use of both the topology and of the edge weights.

We considered the following three network measures M_{ij} between all pairs of bee species observed at each site: number of shared floral partners (n_shared), the Jaccard distance between the sets of floral partners visited by each bee species (Jaccard), and the weighted Jaccard distance between the sets of floral partners visited by each bee species, with weights given by the relative frequencies ϕ_{ij} (weighted Jaccard). It should be noted that n_shared is a similarity measure between pairs of bees (bees sharing more floral partners have more similar visitation patterns), while the Jaccard and weighted Jaccard distances are dissimilarity measures. If similarity in floral visitation pattern is associated with similarity in bee pathogen prevalence, we would expect a positive correlation between the difference in bee pathogen prevalence Δ_{ij}^{ks} and the two Jaccard measures, and a negative correlation in the case of n_shared.

To assess the correlation between the matrices that encode the prevalence differences Δ_{ij}^{ks} and each of the three network measures M_{ij}, we ran Mantel tests for each site s separately. This was done using the scikit-bio package, calculating the Pearson correlation and assessing significance by computing a *P* value using a two-sided test based on 10000 random permutations. To determine an average correlation $\bar{r}$ for each network measure over all sites, we combined correlations by first transforming them to z scores using the Fisher Z transformation, then averaging those z scores, and finally reversed transforming the average z score to arrive at an estimate of $\bar{r}$ (Alexander, 1990). To assess the significance of this average correlation, we computed a two-sided *P* value associated with the average z score from a Student's t distribution with n degrees of freedom, where n is the number of sites for which we were able to compute a correlation via the Mantel test (n = 7).

Similarity of interaction patterns between bee species pairs in a community did not explain similarity in prevalence ($\bar{r}$ = -0.31, P = 0.76; $\bar{r}$ = 0.30, P = 0.77; $\bar{r}$ = 0.35, P = 0.73, for number of shared interaction partners, Jaccard index, and weighted Jaccard index, respectively).

Appendix 7: Temporal patterns of pathogen prevalence

We tested for differences in pathogen prevalence across months using generalized linear mixed effects models (GLMM), with pathogen status (proportion of pathogen positive bees out of the bees screened at each site) as a binomial response, month as the explanatory variable, and site as a random effect. We conducted a likelihood ratio test to determine the significance of coefficients by comparing against a null model that only included the random effect (site). We found that month did not significantly predict pathogen prevalence in our bee communities (June – September) ($\chi^2_3 = 0.64$, $P = 0.89$, $\chi^2_3 = 0.13$, $P = 0.98$, $\chi^2_3 = 0.30$, $P = 0.96$, $\chi^2_3 = 0.92$, $P = 0.82$ for overall, trypanosome, *Nosema ceranae*, and neogregarine prevalence, respectively). These results suggest there was not substantial variation in pathogen prevalence over time in our dataset.

Furthermore, our plant-pollinator networks were not characterized by rapid turnover of flower species, as most of the flower species were chosen for inclusion in the wildflower plots based on traits such as long blooming periods (Grab *et al.*, 2018; Tuell *et al.*, 2008). One flower species, *Silphium perfoliatum*, received 68% of all recorded visits. This species was abundant at all sites and had a blooming period of up to eight weeks (July 16 to September 11), largely encapsulating the entirety of the season. *Silphium* began blooming in 10/11 sites within a 2-week period and was blooming at all sites throughout August.

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CHAPTER 3

FUNCTIONAL TRAITS LINKED TO PATHOGEN PREVALENCE IN WILD BEE COMMUNITIES

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Abstract

Recent reports of pollinator decline have prompted efforts to understand contributing factors and protect vulnerable species. While pathogens are among the major factors linked to honey bee and bumble bee declines, variation in pathogen prevalence in diverse bee communities are far less understood. Functional traits have been proposed as a framework to predict susceptibility to stressors, including pathogens, in species-rich communities. Here we evaluated the relationship between functional traits and the prevalence of trypanosomes, neogregarines, and the microsporidian *Nosema ceranae* in diverse wild bee communities. We further evaluated the impact of intra-specific size variation on pathogen prevalence of the most common bee species in our system (*Bombus impatiens*). We found that ground-nesting bees were more likely to harbor trypanosomes and neogregarines than were above-ground nesting bees. Moreover, this pattern was not driven by bumble bees (all of which are ground-nesting in our community), as the relationship for trypanosomes persisted even when bumble bees were excluded from the analysis. While functional traits did not explain *Nosema ceranae* prevalence at the community level, larger *B. impatiens* workers were less likely to harbor *N. ceranae*. These results indicate that functional traits can provide a useful groundwork for understanding disease prevalence patterns in species-rich communities where baseline pathogen data are often not available. Furthermore, the results highlight that ground-nesting bees are more likely to harbor pathogens and indicate a clear need to evaluate vulnerability and disease spreading potential in this group.

Introduction

The functional trait framework has been proposed as a way of increasing synthesis, generalizability, and predictability of ecological systems (Wong *et al.*, 2019), critical elements in a world characterized by accelerating global change and biodiversity loss. This framework allows for non-taxonomic grouping of species based on any measurable property of an organism that strongly influences performance (McGill *et al.*, 2006) and has been effective at predicting plant community assembly, functioning, and structure (Wong *et al.*, 2019). For ecologically important taxa, such as pollinators, numerous studies have found that traits can predict susceptibility to stressors (Forrest *et al.*, 2015; Williams *et al.*, 2010) and their effect on ecosystem service provisioning (Gagic *et al.*, 2015; Woodcock *et al.*, 2019), though not always consistently (Bartomeus *et al.*, 2018). Given the recognized role of pathogens in pollinator declines worldwide (Goulson *et al.*, 2015), their widespread prevalence in surveyed plant-pollinator communities (Evison *et al.*, 2012; Figueroa *et al.*, 2020; Ravoet *et al.*, 2014), and the absence of baseline data for the majority of the world's bee species, developing generalizable ways of predicting infections could strengthen management practices to benefit pollinators more broadly. Moreover, trait-based evaluations have greatly enhanced the ability to predict disease risk in numerous systems, especially vertebrates (Han *et al.*, 2015; Paull *et al.*, 2012; Perkins *et al.*, 2003). However, the empirical relationship between pollinator functional traits and pathogen prevalence in diverse bee communities has not been previously tested, limiting our ability to evaluate potential vulnerability.

Functional traits mediate how individual bees interact with their environment, potentially influencing the probability of exposure to pathogens and well as likelihood of transmitting the pathogen to other bees. Increased screening efforts have found widespread pathogen prevalence in pollinator communities (Dolezal *et al.*, 2016; Evison *et al.*, 2012; Figueroa *et al.*, 2020; Levitt *et al.*, 2013; Ngor *et al.*, 2020; Ravoet *et al.*, 2014), highlighting the need to understand disease dynamics in these systems. Determining predictive traits can inform future hypotheses regarding factors associated with vulnerability to pathogen infection and more effectively predict disease risk.

Numerous functional traits could influence pathogen prevalence, such as body size, nesting location, sociality, diet breadth, and voltinism. Moreover, these are traits that are also frequently affected by habitat loss, and thus may be indicators of general susceptibility to stressors (Forrest *et al.*, 2015; Williams *et al.*, 2010). Body size is correlated with foraging distance in bees (Greenleaf *et al.*, 2007), and could therefore influence access to floral resources across larger spatial scales. Moreover, bee body size can vary substantially within species and can influence interactions with specific floral morphologies (Figueroa *et al.*, 2019). The common eastern bumble bee, *Bombus impatiens*, can vary greatly both in size and in pathogen prevalence estimates even within the worker caste, and size can be a predictor of bee immunity in this species (Otterstatter & Thomson, 2006). As such, *B. impatiens* can serve as an important model organism for addressing questions of intra-specific body size and pathogen prevalence.

Nesting location could influence pathogen survival and subsequently transmission and prevalence. For multiple bee pathogens, temperature, moisture, and ultra-violet radiation can

reduce survival (Schmid-Hempel, 1998). For example, the trypanosomatid bee pathogen *Crithidia bombi* has been shown to survive an average of 3 h on plants when exposed to the sun (Figueroa *et al.*, 2019). We hypothesized that the nest of ground-nesting bees would experience more stable moisture and temperature conditions and thus be more likely to facilitate the persistence and subsequent transmission of pathogens, either from mother to offspring (both for social and solitary bees) or between nest mates (social bees). As such, we predicted that ground-nesting bees would be more likely to harbor pathogens than above-ground nesting bees.

Sociality may be a key driver of pathogen transmission and subsequent prevalence in bees. We hypothesized that social bees would be more likely to harbor pathogens due to increased exposure and facilitated transmission within colonies; for example, social feeding (trophallaxis) and higher density of individuals are all factors that could promote disease transmission (Schmid-Hempel, 1998; Williams *et al.*, 2010). Even certain hygienic behaviors in honey bee colonies can actually facilitate disease spread in the hive (Schmid-Hempel, 1998).

Diet specialization is predicted to influence pathogen prevalence, though the directionality is unclear. There is a growing understanding that plant species vary in their transmission potential to incoming bees (Adler *et al.*, 2020; Adler *et al.*, 2018; Durrer & Schmid-Hempel, 1994; Graystock *et al.*, 2015), suggesting that all else being equal having access to more plant species may raise the likelihood of coming into contact with a high-transmission plant species. However, bumble bees may be able to discriminate against flowers contaminated with pathogens (Fouks & Lattorff, 2011). As such, having more choices could result in lower likelihood of encounter with contaminated flowers. Whether increasing the diet breadth will

result in dilution of the pathogen (*i.e.* lower prevalence) or amplification (*i.e.* greater prevalence) depends on interaction patterns at the community level (Figueroa *et al.*, 2020).

The number of reproductive broods each year (voltinism) that a bee has could also influence pathogen prevalence. For pathogen without transovarial transmission (*i.e.* from mother to offspring via the egg), such as *C. bombi*, new generations of hosts must reacquire the pathogen, often through contaminated flowers (social and solitary bees) or nest mates (social bees). Consequently, bees that have multiple generations per year may thus be able to "clear" the pathogen more effectively than those that only have one generation, all else being equal. For other pathogens, such as microsporidian *Nosema* spp., for which sexual and transovarial transmission may be possible (Roberts *et al.*, 2015; Rutrecht & Brown, 2008), voltinism may not be a strong predictor of prevalence or could even result in greater pathogen prevalence for multivoltine species if infections could accumulate over successive generation. As such, despite numerous bee pathogens being fecal-orally transmitted via flowers (Graystock *et al.*, 2015; Schmid-Hempel, 1998; Singh *et al.*, 2010), differences in other routes of transmission and persistence under varying environmental conditions among pathogen groups could differentially drive links between bee functional traits and pathogen prevalence.

Here, we characterize species' traits, including body size, nesting location, sociality, diet breadth, and voltinism, and evaluated associations with pathogen prevalence in wild bee communities. We were able to obtain functional trait information for 470 bees, representing 42 species, to associate with prevalence of three common fecal-orally transmitted bee pathogens

(trypanosomes, neogregarines, and *Nosema ceranae*). We thus were able to evaluate the relationship between bee functional traits and pathogen prevalence in wild bee communities.

Methods

Field Sample Collection

We collected bees foraging in 11 established wildflower plantings in central New York, USA, from June 17 to September 11, 2015 (Figueroa *et al.*, 2020). The 10 m x 15 m plots were established in 2012 with native perennial wildflower species and were subsequently invaded by weedy species. We collected bees for 1.5 ± 0.5 person hours from 8:00 to 17:00 hrs on sunny and low wind days, with 8 to 12 collection days per site throughout the season. Bees were collected while foraging on flowers and immediately placed on dry ice in the field and stored at – 80°C until processing. In addition to the bees collected directly on the flowers growing in the wildflower strip (Figueroa *et al.*, 2020), we also collected bees foraging on flowers adjacent to the wildflower strip.

Pathogen screening

Infection with trypanosomes, such as *Crithidia bombi*, can alter bumble bee foraging behavior, cognitive function (Gegear *et al.*, 2005; Gegear *et al.*, 2006), and reproduction (Goulson *et al.*, 2018). Similarly, the neogregarine *Apicystis bombi* and microsporidians *Nosema bombi* and *N. ceranae* can reduce bee survival (Graystock *et al.*, 2016b; Graystock *et al.*, 2013a;

Otti & Schmid-Hempel, 2007). We screened for presence of *Nosema ceranae*, trypanosomes, and neogregarines in the bee guts using established Polymerase Chain Reaction (PCR) primers (Figueroa *et al.*, 2020). While the primers for trypanosomes and neogregarines were developed to be taxonomically inclusive, upon Sanger sequencing a subset of the samples from numerous bee species we found that 66% of the trypanosome positive samples had very high sequence similarity with *C. bombi* (others present were *C. mellificae*, *Herpetomonas pessoai*, and Trypanostomatidae sp.) and that 100% of the neogregarive positive samples had very high sequence similarity with *A. bombi* (Figueroa *et al.*, 2020). We surface sterilized the bees before dissecting and evaluating pathogen presence in the bee gut tissues. To reduce risk of DNA degradation and cross-contamination, we identified the bee species post-dissection. As such, not all specimens retained the characters necessary for identification. Bees were predominantly identified using reference materials located in the Cornell University Insect Collection and published keys (Gibbs, 2011; Gibbs *et al.*, 2013; LaBerge, 1986; Mitchell, 1960; Mitchell, 1962; Rehan & Sheffield, 2011). Bees in the genus *Lasioglossum*, which are notoriously difficult to identify morphologically, were identified using barcoding methods (we amplified and sequenced a 900 bp region of the protein-coding gene elongation factor 1-alpha, details in (Figueroa *et al.*, 2020)). Ultimately, we were able to identify 86% of all bees to species, 13% of the bees to genus only, and unable to identify 1% of the bees. Unidentified bees were removed from the analyses. To be conservative, we conducted analyses only for bees that amplified positive control (bee DNA) or pathogen DNA (separate because multiplex primers developed to favor pathogen amplification).

Functional trait assessment

We selected five functional traits that we hypothesized would be important for pathogen prevalence or transmission: bee size (empirically determined), nesting (above/below ground), sociality (social/solitary), floral specialization (oligolectic/polylectic), and voltinism (univoltine/multivoltine). Moreover, these traits are among the most commonly evaluated traits in the bee literature (Bartomeus *et al.*, 2013; Bartomeus *et al.*, 2018; Coutinho *et al.*, 2018) and we were able to obtain information for most of the species in our study (Table S1). We determined body size by measuring the intertegular distance (ITD), defined as the distance between the wing bases (Cane, 1987; Greenleaf et al., 2007) using an Olympus SZX10 microscope and CellSens Standard software (Olympus Corporation of the Americas, Scientific Solutions Group, Waltham, Massachusetts, USA). Due to risk of cross-contamination and DNA-degradation, we measured ITD post-dissection. As such, some specimens were not in suitable condition for adequately measuring ITD (77/621). In order to include the specimens that were not able to be measured in the statistical analyses, we first calculated the average size (ITD) for each species at each site based on the bees we had measurements for, and then used this number for all bees of that species at that site (including those not directly measured). To avoid using nesting-trait categories that would have been represented by few species, we summarized stem and wood as above-ground to compare with ground nesters, as described in (Williams *et al.*, 2010). We also consolidated sociality according to (Forrest *et al.*, 2015), in which social bees include communal and facultative social species, as compared to obligate solitary species. Voltinism, defined as the number of reproductive generations per year, is separated into univoltine compared to multivoltine species. For diet breath, bees were determined to be pollen generalists (polylectic) or pollen specialists (oligolectic). The full list of species and traits is in

Table S1. Honey bees were collected in the latter half of the sampling period (Figueroa *et al.*, 2020), so to evaluate whether their inclusion altered significance patterns, we re-ran the statistical analyses excluding honey bees, finding that the results were unchanged (SI).

Statistical analyses

Statistical analyses were conducted in RStudio (R Core group, version 3.4.0) using the lme4 and epiR packages (Bates *et al.*, 2015; Stevenson *et al.*, 2015). We computed the true pathogen prevalence for each of the three pathogen groups (trypanosomes, neogregarines, and *Nosema ceranae* for all of the bees screened; $n = 580$) with the *epi.prev* function of the epiR package in R; the test sensitivity was set to 95% using the Blaker method for two-sided confidence intervals (Stevenson *et al.*, 2012). This method was likewise used to evaluate pathogen prevalence for the most abundant species in the system *Bombus impatiens* ($n = 106$).To determine whether bee genera differed in pathogen prevalence, we constructed a Generalized Linear Mixed Model (GLMM) that included pathogen presence/absence in individual bees as the binary response, predicted by bee genus in a model that included species nested within site as a random effect. To determine significance, we conducted a likelihood ratio test that compared that model to one that only included the random effects. This analysis was only conducted for bee genera for which we had at least 10 individual female bees, which resulted in a comparison of 10 genera: *Agapostemon*, *Anthophora*, *Apis*, *Augochlorella*, *Bombus*, *Ceratina*, *Halictus*, *Hylaeus*, *Lasioglossum*, and *Melissodes* (Table S1). Given potential sex differences, this analysis was conducted exclusively for bees identified as female ($n = 362$). We only had two genera for which we screened more than 10 individual male bees, therefore we did not analyze differences among

genera for males. This analysis was conducted separately for trypanosomes, neogregarines, and *Nosema ceranae*.

We evaluated the relationship between bee pathogen prevalence and bee functional traits using a GLMM that included pathogen presence/absence in individual bees as the binomial response predicted by each of the five traits (average bee size for each species at each site, nesting location, voltinism, sociality, and diet breadth), including species nested within site as the random effects. While we successfully screened 621 bees, we analyzed all traits simultaneously in a single GLMM and hence only used species without missing trait data (n = 470 individuals). We conducted this analysis separately for each pathogen group (trypanosomes, neogregarines, and *Nosema ceranae*). The variance inflation factor of the five factors was equal to or less than two, indicating that factors were not highly collinear and hence could be analyzed simultaneously (Rhodes *et al.*, 2009). To determine significance, we conducted likelihood ratio tests that compared the full model, which included all the functional traits, to models that selectively excluded each trait. We evaluated whether patterns were driven by the dominant genus in our system (*Bombus*) by re-analyzing significant models with the exclusion of all bumble bees from the dataset. Given that female and male bees can vary in size and behavior (Teder & Tammaru, 2005), we re-analyzed the data for female bees (n = 324; we did not have sufficient male bees to analyze separately: n = 45; the full dataset includes bees that were unable to be sexed). We also evaluated whether functional traits or taxonomic groupings resulted in greater model fit using Akaike Information Criterion (AIC) scores. To do this we compared GLMMs that included pathogen presence as the response (trypanosome or neogregarine; see *Results*) predicted by either nesting location (see *Results*) or genus; both models included species

nested within site as the random effect and were evaluated on the dataset used in the functional trait analyses (n = 470).

Lastly, we evaluated whether individual body size predicted presence of each of the pathogens in the dominant species of the system (*Bombus impatiens*), for which we had a large sample (n = 106 females). This was the only species for which we had sufficient numbers to evaluate intra-specific trait variation (*i.e.* >= 50 individuals; (Wong *et al.*, 2019)). To address this question, we conducted a GLMM that included pathogen presence as the binary response, predicted by individual bee size, and site as the random effect; this was done separately for each of the three pathogen groups. Significance was determined by comparing to null models that only included the random effect.

Results

Pathogen prevalence was widespread in the bees screened, ranging from 24% for trypanosomes (95% Confidence Interval (CI), 20 – 28%), to 12% for *Nosema ceranae* (CI, 9% – 15%), and 1% for neogregarines (CI, 0 – 3%). Pathogen prevalence varied among the most abundant bee genera for trypanosomes ($\chi^2_9 = 29.69$, $p < 0.001$) and *Nosema ceranae* ($\chi^2_9 = 17.69$, $p = 0.039$), but not neogregarines ($\chi^2_9 = 13.17$, $p = 0.155$; Figure 1).

We found that nesting location was an important predictor of pathogen prevalence, with ground-nesting bees being more likely to harbor trypanosomes and neogregarines than above ground-nesting bees ($\chi^2_1 = 9.66$, $p = 0.002$ and $\chi^2_1 = 4.52$, $p = 0.033$, respectively; Figure 2).

Specifically, the mean trypanosome prevalence for ground-nesting bees was 28% compared to 21% in above ground-nesting bees. For trypanosomes, this pattern was not driven by bumble bees, as excluding all individuals from this genus did not alter the significance ($\chi^2_1 = 12.06$, $p = 0.001$), though it did for neogregarines ($\chi^2_1 = 0.41$, $p = 0.522$). Similarly, we found that sociality was a marginally significant predictor of pathogen presence, with social bees having lower neogregarine prevalence than solitary bees ($\chi^2_1 = 3.81$, $p = 0.051$), though this pattern was no longer present when bumble bees were excluded from the analysis ($\chi^2_1 = 0.28$, $p = 0.595$). Bee size, diet breadth, voltinism, and sociality did not explain trypanosome prevalence ($\chi^2_1 \leq 2.79$, $p \geq 0.095$), nor did bee size, diet breadth, and voltinism explain neogregarine prevalence ($\chi^2_1 \leq 1.21$, $p \geq 0.271$). None of the functional traits evaluated significantly explained *Nosema ceranae* prevalence at the community level ($\chi^2_1 \leq 0.46$, $p \geq 0.496$). All significance patterns remained unchanged for the female-only analysis (SI). Nesting location as a predictor variable resulted in a greater model fit than the taxonomic model based on lower AIC scores for both trypanosome and neogregarine prevalence (ΔAIC = 6.25 and ΔAIC = 2.03, respectively).

For the dominant species in our system, we evaluated the role of intra-specific size variation in relation to pathogen presence. Pathogen prevalence in *Bombus impatiens* ranged from 19% for trypanosomes (95% Confidence Interval (CI), 10 – 28%), to 11% for *Nosema ceranae* (CI, 5% – 20%), and 5% for neogregarines (CI, 0 – 13%). Larger *B. impatiens* females were less likely to harbor *N. ceranae* ($\chi^2_1 = 4.13$, $p = 0.042$; Figure 3). However, neither trypanosome nor neogregarine presence was associated with size in *B. impatiens* females ($\chi^2_1 = 0.18$, $p = 0.671$ and $\chi^2_1 = 0.36$, $p = 0.546$, respectively).

Discussion

Pathogen prevalence was widespread in the bee communities surveyed and varied among bee genera for trypanosomes and *Nosema ceranae*. We found that bee functional traits predicted pathogen prevalence, though patterns differed by trait and pathogen group and whether evaluations were conducted at the community or intraspecific level. Overall, our work highlights that specific functional traits can explain pathogen prevalence patterns in diverse pollinator communities and pinpoints future areas for research in bee epidemiology.

We had predicted that nests in the ground would have greater stability of temperature and moisture, which could facilitate persistence and transmission of pathogens, and therefore result in greater prevalence. We found that nesting location predicted pathogen prevalence in bee communities. Specifically, ground-nesting bees were more likely to harbor trypanosomes and neogregarines than above-ground nesting bees. Given that trypanosomes include the well-known bumble bee pathogen *Crithidia bombi* and that bumble bees are ground nesting bees, we evaluated whether this pattern was driven by this abundant taxonomic group. However, the pattern remained significant after excluding bumble bees from the analysis for trypanosomes, suggesting that nesting location more than simple taxonomic identity was at play. When bumble bees were excluded the pattern was no longer significant for neogregarines, though this could be a product of insufficient power due to very low prevalence (1% compared to 24% for trypanosomes). Moreover, ground-nesting bees may be more tolerant of environmental stressors in general (Forrest *et al.*, 2015; Williams *et al.*, 2010), though to date relationship with pathogen prevalence had not been evaluated at a community level. Empirically testing the persistence and

transmission potential of pathogens across different nesting substrates is therefore a future direction highlighted by this work.

Interestingly, the microsporidian pathogen evaluated, *N. ceranae*, was not linked to functional traits at the community level, indicating that pathogen-specific factors influence relationships between pathogen prevalence and bee traits. For example, while *Nosema* spp. shares the fecal-oral transmission mode with trypanosomes and neogregarines, it can also be transmitted via trophallaxis, sex, and possibly transovarially (Roberts *et al.*, 2015; Rutrecht & Brown, 2008; Schmid-Hempel, 1998). Whether these additional modes of transmission for *Nosema ceranae* compared to trypanosomes and neogregarines dilute the importance of nesting substrate remains to be tested.

There is an increasing understanding of the contribution of intra-specific variability, which can be comparable to or even greater than effects of species differences for species coexistence, ecosystem functioning, and response to global change (Wong *et al.*, 2019). Individual body size influenced *Nosema ceranae* incidence in the dominant bee species in our study, the common eastern bumble bee (*Bombus impatiens*). The finding that larger bees were less likely to harbor the microsporidian pathogen is consistent with patterns found for *B. impatiens* and *C. bombi* (Manson *et al.*, 2010; Palmer-Young *et al.*, 2018). Whether this pattern is driven by a more robust immune system in larger bees (Otterstatter & Thomson, 2006), pathogen dilution due to broader diet breadth (Figueroa *et al.*, 2020), or some other mechanism, is unknown. Given that bees, including bumble bees, are often smaller in more simplified agricultural landscapes (Bommarco *et al.*, 2010; Grab *et al.*, 2019; Persson & Smith, 2011;

Renauld *et al.*, 2016), where numerous interacting stressors are co-occurring (*e.g.* decreased floral diversity, increased pesticide exposure, and competition with honey bees (Goulson & Sparrow, 2009)), future research should determine whether bees that are smaller in simplified agricultural landscapes are also more susceptible to infection.

The differences in pathogen prevalence among bee genera within communities clearly indicate a need to evaluate the potential impacts of pathogens in historically under-evaluated bee species. Given that pathogen prevalence was determined using molecular methods, we cannot distinguish between innocuous pathogen DNA and active infections. Beyond our limited understanding of the host range of these pathogens, we do not understand the fitness consequences or the context-dependency of their virulence beyond honey bees and bumble bees (Figueroa et al., *submitted*). This work highlights the need for experimental assessments of the host range and negative health consequences of infections beyond honey bees and bumble bees, in addition to determining which bee species play key roles in pathogen transmission (Figueroa *et al.*, 2020; Ruiz-González *et al.*, 2012).

Incorporating functional traits in community ecology promises synthesis, generality and prediction that is independent of geography or taxonomic identity (Wong *et al.*, 2019). We found that two of the five functional traits evaluated predicted pathogen prevalence in bee communities, though they did so differently depending on the pathogen group and on whether patterns were evaluated across or within bee species. For the common eastern bumble bee, *B. impatiens*, body size predicted *N. ceranae* prevalence. At the community level, we found that the trait based model using nesting location had a lower AIC value than the comparable taxonomic

based model. Similarly, trait-based models evaluating the pathogen transmission potential of flowers to bees have lower AIC values than those based on plant taxonomy (Adler *et al.*, 2018). Trait-based models, by requiring many fewer parameters, can therefore have greater predictive power, especially in high diversity communities. Our work illustrates that specific traits, especially those well described in the community, can increase our understanding of pathogen prevalence in diverse pollinator communities.

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Figure 1: Prevalence of (A) Trypanosomes, (B) *Nosema ceranae*, and (C) Neogregarines among abundant bee genera. Differences were significant ($p < 0.05$) for trypanosomes and *Nosema ceranae*.

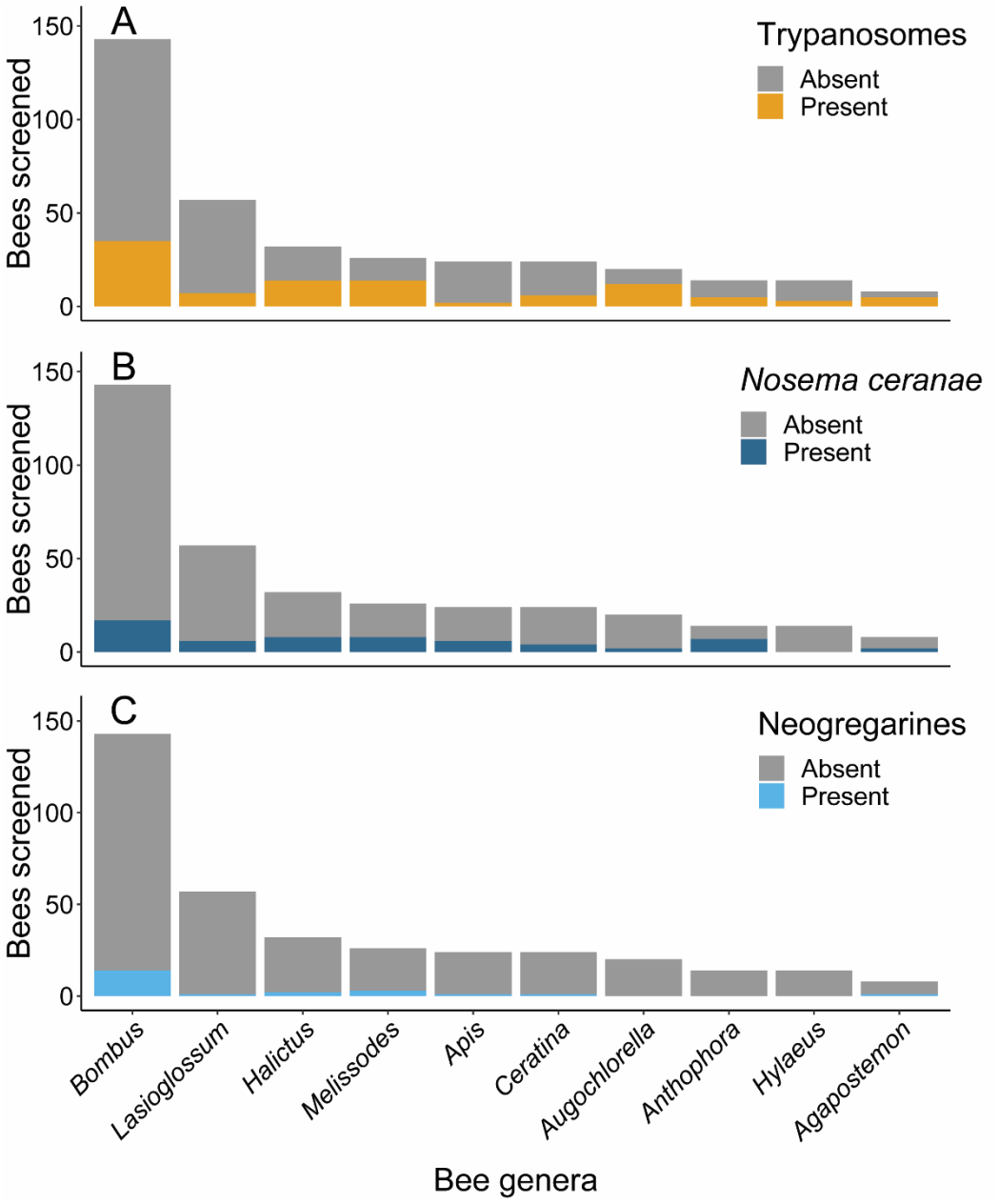

Figure 2: Effect of nesting location on (A) trypanosome prevalence in overall community (mean + s.e); (B) trypanosome prevalence, excluding bumble bees (mean + s.e); (C) neogregarine prevalence in overall community (mean + s.e); and (D) neogregarine prevalence, excluding (mean + s.e). Below-ground nesting bees were more likely to harbor trypanosomes and neogregarines than above-ground nesting bees. (* $p < 0.05$; n.s. $p > 0.05$).

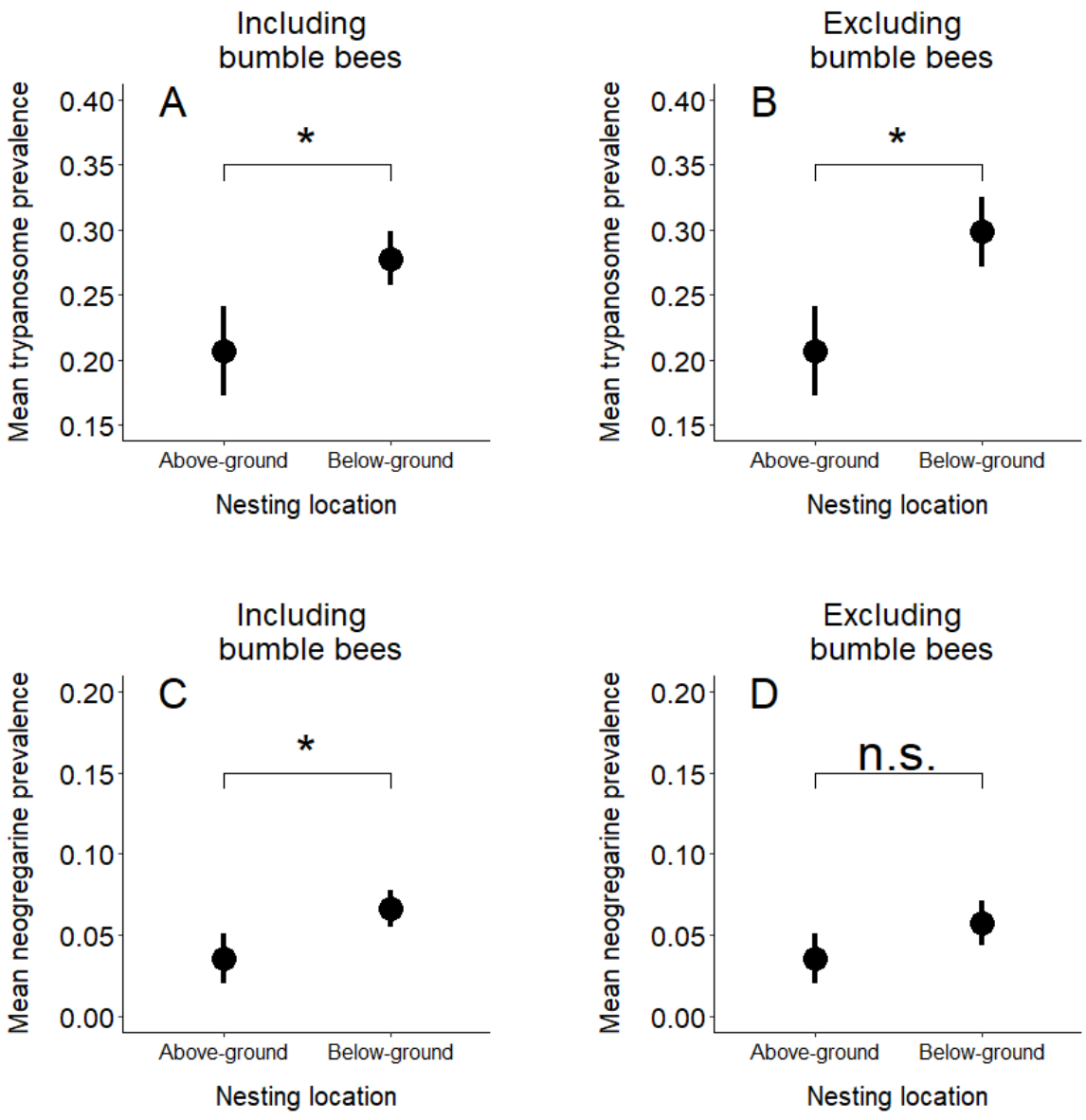

Figure 3. Relationship between individual bee size (intertegular distance) for *Bombus impatiens* and likelihood of *Nosema ceranae* presence. Larger *B. impatiens* were less likely to harbor the microsporidian in their gut. Shaded confidence interval corresponds to standard error.

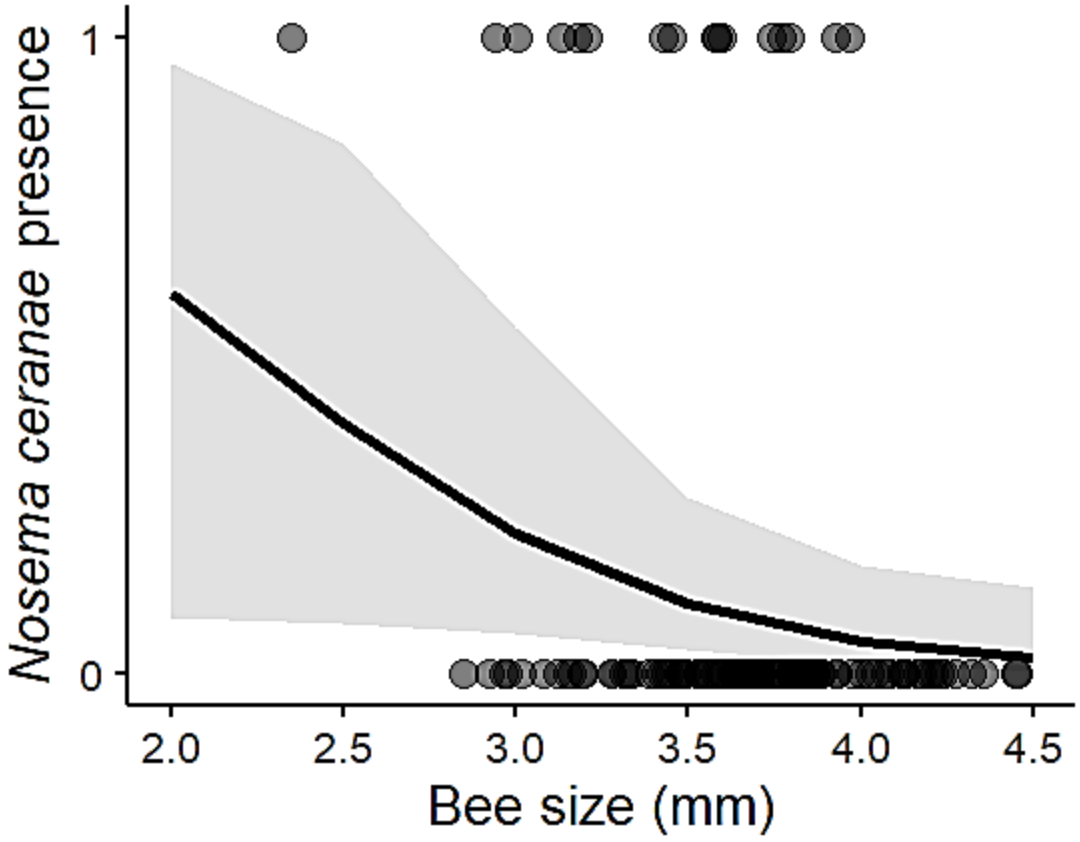

Table 1: Predicted and observed relationships between functional traits and pathogen prevalence in bee communities. Arrows indicate direction of relationship (↓ = negative relationship; ↑ = positive relationship; n.s. = non-significant relationship).

Trait	Hypotheses	Results
Body size	↓↑	↓ *N. cerana* (within *B. impatiens*)
Nesting location	Ground nesting ↑	Ground nesting ↑ trypanosomes and neogregarines
Sociality	Social bees ↑	Social bees ↓ neogregarines
Diet breadth	↓↑	n.s.
Voltinism	Multivoltine ↓	n.s.

Table S1: Summary of bee species and functional traits. Statistical analyses conducted on species for which we had all functional trait data (n = 470).

Family	Species	Size (mm)	Diet	Nesting location	Sociality	Voltinism	*n*
Halictidae	*Agapostemon virescens*	2.261	Polylectic	Below-ground	Social	Multivoltine	11
Andrenidae	*Andrena simplex*	2.002	Oligolectic	Below-ground	Solitary	Multivoltine	1
Andrenidae	*Andrena sp*	1.821		Below-ground	Solitary	Univoltine	
Andrenidae	*Andrena wilkella*	2.354	Polylectic	Below-ground	Solitary	Univoltine	2
Apidae	*Anthophora terminalis*	3.084	Polylectic	Above-ground	Solitary	Multivoltine	23
Apidae	*Apis mellifera*	3.064	Polylectic	Above-ground	Social	Multivoltine	33
Halictidae	*Augochlora pura*	1.744	Polylectic	Above-ground	Solitary	Multivoltine	2
Halictidae	*Augochlorella aurata*	1.438	Polylectic	Below-ground	Social	Multivoltine	24
Apidae	*Bombus bimaculatus*	3.827	Polylectic	Below-ground	Social	Univoltine	21
Apidae	*Bombus fervidus*	3.842	Polylectic	Below-ground	Social	Univoltine	3
Apidae	*Bombus griseocollis*	4.479	Polylectic	Below-ground	Social	Univoltine	8
Apidae	*Bombus impatiens*	3.656	Polylectic	Below-ground	Social	Univoltine	122
Apidae	*Bombus perplexus*	3.846	Polylectic	Below-ground	Social	Univoltine	1
Apidae	*Bombus ternarius*	3.221	Polylectic	Below-ground	Social	Univoltine	5
Apidae	*Bombus vagans*	3.391	Polylectic	Below-ground	Social	Univoltine	10
Andrenidae	*Calliopsis andreniformis*	1.219	Polylectic	Below-ground	Solitary	Multivoltine	1
Andrenidae	*Calliopsis nebraskensis*	1.124	Oligolectic	Below-ground	Solitary	Multivoltine	1
Apidae	*Ceratina calcarata*	1.393	Polylectic	Above-ground	Social	Univoltine	15
Apidae	*Ceratina dupla*	1.170	Polylectic	Above-ground	Social	Multivoltine	2
Apidae	*Ceratina mikmaqi*	1.513	Polylectic	Above-ground	Solitary	Univoltine	18
Apidae	*Ceratina sp*	1.478	Polylectic	Above-ground		Univoltine	
Megachilidae	*Coelioxys rufitarsis*	2.631		Below-ground		Multivoltine	
Colletidae	*Colletes kincaidii*	2.573	Polylectic	Below-ground	Social	Univoltine	1
Halictidae	*Halictus confusus*	1.370	Polylectic	Below-ground	Social	Multivoltine	17
Halictidae	*Halictus ligatus*	1.635	Polylectic	Below-ground	Social	Univoltine	40
Halictidae	*Halictus rubicundis*	1.887	Polylectic	Below-ground	Social	Multivoltine	1
Megachilidae	*Heriades carinata*	1.525	Polylectic	Above-ground	Solitary	Univoltine	1
Megachilidae	*Hoplitis producta*	1.656	Polylectic	Above-ground	Solitary	Univoltine	1
Megachilidae	*Hoplitis sp*	1.324	Polylectic	Above-ground	Solitary	Univoltine	1
Colletidae	*Hylaeus affinis*	1.178	Polylectic	Above-ground	Solitary	Multivoltine	11
Colletidae	*Hylaeus annulatus*	1.049	Polylectic	Above-ground	Solitary	Multivoltine	3
Colletidae	*Hylaeus modestus*	1.154	Polylectic	Above-ground	Solitary	Multivoltine	14
Colletidae	*Hylaeus sp*	1.085	Polylectic	Above-ground	Solitary	Multivoltine	3
Halictidae	*Lasioglossum coriaceum*	2.006	Polylectic	Below-ground	Social	Multivoltine	3
Halictidae	*Lasioglossum hitchensi*	1.003	Polylectic	Below-ground	Social		
Halictidae	*Lasioglossum imitatum*	0.853	Polylectic	Below-ground	Social	Multivoltine	2
Halictidae	*Lasioglossum inconditum*	1.251	Polylectic	Below-ground	Solitary		
Halictidae	*Lasioglossum lineatulum*	1.227	Polylectic	Below-ground	Social		
Halictidae	*Lasioglossum perpunctatum*	1.308	Polylectic	Below-ground	Social		
Halictidae	*Lasioglossum pilosum*	1.256	Polylectic	Below-ground	Social	Multivoltine	11
Halictidae	*Lasioglossum sp*	1.386	Polylectic	Below-ground			
Halictidae	*Lasioglossum versatum*	1.163	Polylectic	Below-ground	Social	Multivoltine	3
Halictidae	*Lasioglossum viridatum*	1.129	Polylectic	Below-ground	Social		
Halictidae	*Lasioglossum weemsi*	1.079	Polylectic	Below-ground	Social		
Halictidae	*Lasioglossum zephyrum*	0.973	Polylectic	Below-ground	Social	Multivoltine	7
Megachilidae	*Megachile sp*	3.697	Polylectic		Solitary		
Apidae	*Melissodes agilis*	2.445	Oligolectic	Below-ground	Solitary	Univoltine	4
Apidae	*Melissodes bimaculata*	2.808	Polylectic	Below-ground	Solitary	Univoltine	2
Apidae	*Melissodes desponsa*	2.835	Oligolectic	Below-ground	Solitary	Univoltine	6
Apidae	*Melissodes druriella*	2.530	Oligolectic	Below-ground	Solitary	Univoltine	2
Apidae	*Melissodes sp*	2.625		Below-ground	Solitary	Univoltine	
Apidae	*Melissodes subillata*	2.142	Oligolectic	Below-ground	Solitary	Univoltine	2
Apidae	*Melissodes trinodis*	2.763	Polylectic	Below-ground	Solitary	Univoltine	26
Megachilidae	*Osmia atriventris*	2.226	Polylectic	Above-ground	Solitary	Univoltine	1
Megachilidae	*Osmia bucephala*	3.784	Polylectic	Above-ground	Solitary	Univoltine	4
Apidae	*Peponapis pruinosa*	2.971	Oligolectic	Below-ground	Solitary	Univoltine	1
Apidae	*Xylocopa virginica*		Polylectic	Above-ground	Social	Univoltine	

SI:

Female-only analysis results

We found that nesting location was a predictive factor, with ground nesting bees being more likely to harbor trypanosomes ($\chi^2_1 = 9.46$, $p = 0.002$) than above ground-nesting bees. Moreover, this pattern was not driven by bumble bees, as excluding all individuals from this genus did not alter the significance ($\chi^2_1 = 12.28$, $p < 0.001$). Bee size, voltinism, and sociality did not explain trypanosome prevalence in female bees ($\chi^2_1 \leq 2.56$, $p \geq 0.110$), and none of the five functional traits evaluated significantly explained *Nosema ceranae* or neogregarine prevalence in female bees ($\chi^2_1 \leq 2.27$, $p \geq 0.132$). For female bees, diet breadth was a significant explanatory variable, with specialists having lower trypanosome prevalence ($\chi^2_1 = 5.93$, $p = 0.015$), though these results should be interpreted with caution due to very low sample sizes for specialist bees.

Trait analyses excluding honey bees

When honey bees were excluded from the trait analyses, we found comparable results to when they were included. Specifically, we found that nesting location remained an important predictor, with ground-nesting bees being more likely to harbor trypanosomes and neogregarines ($\chi^2_1 = 6.06$, $p = 0.014$ and $\chi^2_1 = 4.5$, $p = 0.034$, respectively; Figure 2). For trypanosomes, this pattern was not driven by bumble bees, as excluding all individuals from this genus did not alter the significance ($\chi^2_1 = 5.23$, $p = 0.022$), though it did for neogregarines ($\chi^2_1 = 0$, $p = 1$). We found that sociality was a significant predictor of pathogen presence, with social bees having lower neogregarine prevalence than solitary bees ($\chi^2_1 = 4.01$, $p = 0.045$), though this pattern was no longer significant when bumble bees were excluded from the analysis ($\chi^2_1 = 0$, $p = 1$). Bee size, diet breadth, voltinism, and sociality did not explain trypanosome prevalence ($\chi^2_1 \leq 2.49$, $p \geq 0.155$), nor did bee size, diet breadth, and voltinism explain neogregarine prevalence ($\chi^2_1 \leq 0.942$, $p \geq 0.332$). None of the functional traits evaluated significantly explained *Nosema ceranae* prevalence at the community level ($\chi^2_1 \leq 0.351$, $p \geq 0.554$).

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CHAPTER 4

CRITHIDIA BOMBI CAN INFECT TWO SOLITARY BEE SPECIES WHILE HOST SURVIVORSHIP DEPENDS ON DIET

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Abstract

Pathogens and lack of floral resources interactively impair global pollinator health. However, epidemiological and nutritional studies aimed at understanding bee declines have historically focused on social species, with limited evaluations of solitary bees. Here, we asked whether *Crithidia bombi*, a trypanosomatid gut pathogen known to infect bumble bees, could infect the solitary bees *Osmia lignaria* (females) and *Megachile rotundata* (males), and whether nutritional stress influenced infection patterns and bee survival. We found that *C. bombi* was able to infect both solitary bee species, with 59% of *O. lignaria* and 29% of *M. rotundata* bees experiencing pathogen replication 5–11 days following inoculation. Moreover, access to pollen resulted in *O. lignaria* living longer, although it did not influence *M. rotundata* survival. Access to pollen did not affect infection probability or resulting pathogen load in either species. Similarly, inoculating with the pathogen did not drive survival patterns in either species during the 5–11 day laboratory assays. Our results demonstrate that solitary bees can be hosts of a known bumble bee pathogen, and that diet is an important contributing factor for survival, thus expanding our understanding of factors contributing to solitary bee health.

Introduction

Pathogen pressure, inadequate nutrition, and the interaction between these stressors can drastically impair pollinator health (Goulson *et al.*, 2015). The relationship between nutrition and bee disease dynamics is complex, as diet quality and diversity can support immunocompetence and reduce infections in some instances, while increasing disease burdens in other cases (Dolezal & Toth, 2018; Koch *et al.*, 2017). A bee's diet is made up of nectar and pollen, both of which have primary metabolites such as sugars, amino acids, and lipids, as well as secondary compounds, such as flavonoids, terpenoids, and alkaloids, with the potential to decrease (Richardson *et al.*, 2015) or increase (Palmer-Young & Thursfield, 2017) infections. In a world dominated by agricultural landscapes, with low floral diversity and frequent movement of commercial bee colonies that can potentially introduce pathogens into wild solitary bee populations (Alger *et al.*, 2019; Furst *et al.*, 2014; Kremen *et al.*, 2002; Otterstatter & Thomson, 2008), understanding the interplay between nutrition and pathogen burdens is important for protecting pollinator health.

Immune defense against pathogens is energetically costly, yet the resulting physiological trade-offs between immunity and other fitness components can be compensated by changes in diet (Moret & Schmid-Hempel, 2000). When *Bombus terrestris* bees are starved of pollen, infection with *Crithidia bombi*, a trypanosomatid gut pathogen, markedly increases host mortality compared to bees with access to pollen (Brown *et al.*, 2000). However, when both *B. terrestris* and *B. impatiens* have access to pollen, they can present higher *C. bombi* loads than pollen-starved counterparts (Conroy *et al.*, 2016; Logan *et al.*, 2005). Similarly, access to pollen

can increase honey bee survival, while simultaneously increasing pathogen loads of the microsporidians *Nosema apis* (Rinderer & Dell Elliott, 1977) and *Nosema ceranae* (Jack et al., 2016; Zheng et al., 2014). As such, disentangling the effects of nutritional stress and pathogen infection on bee survival is important for understanding pollinator health, especially for solitary bees, which contribute substantially to pollination services worldwide yet have been historically understudied (Danforth *et al.*, 2019).

Honey bees and bumble bees have been the predominant model systems for addressing questions regarding bee health, especially for nutritional and epidemiological evaluations (Schmid-Hempel, 1998). Recent advances in molecular surveillance have revealed widespread pathogen prevalence across solitary bee taxa from most bee families (Andrenidae, Apidae, Colletidae, Halictidae, and Megachilidae). This includes pathogens known to infect honey bees and bumble bees (Apidae), including *Apicystis bombi*, *Ascosphaera* spp., *Crithidia bombi*, *C. mellificae*, *Nosema ceranae*, and numerous viruses (Evison *et al.*, 2012; Figueroa *et al.*, 2020; Ravoet *et al.*, 2014; Schoonvaere *et al.*, 2018; Singh *et al.*, 2010). However, except for single-stranded RNA viruses which allow for strand-specific PCR assays to detect viral replication, we currently cannot distinguish between transient passage through the bee gut and active infections, nor do we know if there are negative consequences for the host based solely on molecular screenings (Bramke *et al.*, 2019). Some existing studies that have experimentally evaluated impacts on solitary bee health have shown increased mortality associated with infections (*e.g. Megachile rotundata* larvae infected with the fungus *Ascosphera aggregata* (James, 2005) and *Osmia bicornis* infected with the neogregarine *Apicystis bombi* and the microsporidian *Nosema*

ceranae (Bramke *et al.*, 2019; Tian *et al.*, 2018)), further highlighting the need to address the host range of bee pathogens and negative consequences for these understudied bee species.

Pathogens are spread between bee species via shared use of floral resources (Graystock *et al.*, 2015). Despite ample possible routes of indirect transmission via flowers at the community level (Figueroa *et al.*, 2020), the host range of many bee pathogens remains currently unknown. Infection with the trypanosomatid *C. bombi* can affect bumble bee foraging behavior, cognitive function (Gegear *et al.*, 2005; Gegear *et al.*, 2006), and reproduction (Goulson *et al.*, 2018). While *C. bombi* is known to infect multiple bumble bee species (Colla *et al.*, 2006; Cordes *et al.*, 2012; Ruiz-González *et al.*, 2012), honey bees are not a known host (Graystock *et al.*, 2015; Ruiz-González & Brown, 2006), even though both groups belong to the same family. It is largely unknown whether solitary bee species, which frequently test positive for *C. bombi* via PCR-based screenings (Figueroa *et al.*, 2020; Graystock *et al.*, 2020a), are actually infected by this pathogen (Ravoet *et al.*, 2014). The solitary bees *Osmia lignaria* and *Megachile rotundata* are cavity-nesting species that provide important pollination services for fruits and vegetables in North America and Europe (Brittain *et al.*, 2013; Pitts-Singer & Cane, 2011; Velthuis & van Doorn, 2006) and can serve as model organisms for experimentally evaluating epidemiological questions due to their commercial availability. Recent experimental work has shown that *C. bombi* collected from *B. impatiens* can infect these species (Ngor *et al.*, 2020), further highlighting the need to understand the biotic and abiotic factors, such as diet, that influence disease dynamics in species beyond honey bees and bumble bees.

To fill this critical knowledge gap, we conducted a study to understand the influence of nutritional stress on the susceptibility of solitary bees to pathogens. Specifically, we asked: 1) how frequently after exposure to *C. bombi* do the solitary bee species *Osmia lignaria* and *Megachile rotundata* become infected, 2) does pollen access influence the likelihood of infection and/or subsequent load of *C. bombi* in *O. lignaria* and *M. rotundata*, and 3) does pollen access and/or *C. bombi* exposure influence *O. lignaria* and *M. rotundata* survival?

Materials and methods

Study system

For our experiments, we used the trypanosomatid pathogen *Crithidia bombi* (Kinetoplastea, Trypanosomatida) and two solitary bee species: *Megachile rotundata* (Hymenoptera, Megachilidae; Crown Bees, Woodinville WA, USA) and *Osmia lignaria* (Hymenoptera, Megachilidae; Watts bees, Bothell WA, USA). The bees were obtained as cocoons and allowed to eclose in individually marked housing units: inverted 59 ml salsa cups (Fabi-Kal® Greenware, Lancaster PA, USA) lined with filter paper (Whatman®, Marlborough PA, USA) that provided access to 30% sucrose solution through a small opening at the tip of a 1.5 ml microcentrifuge tube (VWR™, Radnor PA, USA) (Figure S1). The sex of these individuals can be easily determined visually upon eclosion. As such, only females were maintained for the experiment with *O. lignaria*. Females represented <10% of the eclosed *M. rotundata*, and we therefore conducted the experiments exclusively with males for this species in order to have sufficient sample sizes. As such, we do not make any formal species or sex

comparisons in our analyses. The *C. bombi* we used in our trials was collected from wild *Bombus impatiens* (Hymenoptera, Apidae) workers from Massachusetts, USA (GPS: 42822'17.5300 N, 72835'13.5200 W). The strain was sustained in laboratory bumble bee colonies (Biobest, Leamington, Ontario, Canada) and will hereafter be referred to as *Crithidia*. The two solitary bee species are commonly used in commercial agriculture in the United States, though *O. lignaria* is native to North America while *M. rotundata* is European (Velthuis & van Doorn, 2006).

Experimental design

To investigate *Crithidia* replication and its effect on bee survival, we conducted a 2x2 factorial experiment for each bee species, contrasting nutritional stress (presence/absence of pollen) and infection status (inoculated/sham-inoculated with *Crithidia*). We prepared *Crithidia* inoculum fresh for each inoculation day by dissecting the gut of infected *B. impatiens* bees from the laboratory source colony. We homogenized the bee guts in distilled water and diluted the mixture to 1,200 *Crithidia* cells μl^{-1}, which we then combined 1:1 with 30% sucrose solution for an inoculum of 600 cells μl^{-1}, a standard inoculum concentration for infecting bumble bees with *Crithidia* (Figueroa et al., 2019; Richardson et al., 2015). We used 30% sucrose without *Crithidia* as a control (sham) inoculum. The sucrose solution was colored with blue food coloring (McCormick & Company, Baltimore, MD, USA) to facilitate confirmation of consumption and availability.

For each trial, half of the bees were inoculated with *Crithidia* and half received the sham inoculation. Each bee species was inoculated on separate dates: *O. lignaria* on June 27, 2019 (*n*

= 97), July 22, 2019 (*n* = 48), and July 23, 2019 (*n* = 52), while *M. rotundata* was inoculated on July 7, 2019 (*n* = 100). The lower sample size for *M. rotundata* was due to lower availability of bees. The bees had eclosed 1–2 days before being inoculated in their individual housing units. Each treatment was evenly represented on each inoculation date. Standard *Crithidia* inoculations in bumble bees are conducted by eliciting proboscis extension from the sugar in the inoculum and directly feeding the bees 10 µl of inoculum (Figueroa et al., 2019; Richardson et al., 2015). However, in a pilot study, we found that *O. lignaria* and *M. rotundata* bees were uncooperative and would not consume the inoculum droplets using the standardized bumble bee protocol. Instead, we administered the inoculum using CO_2 to subdue each bee and elicit proboscis extension, then placed the inoculum droplet directly on their proboscis (see below). In the pilot study we found that this method resulted in 4/5 *O. lignaria* and 1/1 *O. cornifrons* becoming infected one-week post-inoculation, while no control (sham-inoculated) bees became infected (0/4 *O. lignaria* and 0/5 *O. cornifrons*), indicating that this method was effective for inoculating bees (Table S1).

To administer the inoculum for this experiment, we exposed individual bees to CO_2 gas for 45 seconds, during which time most bees extended their proboscis. We then placed 5 µl of inoculum on their proboscis and on the pollen, if present, or on the side of the feeding tube for pollen-free treatments, to maximize exposure to the pathogen (10 µl on first day of trial); *O. lignaria* received an additional 5 µl dose on the pollen/feeding tube for two consecutive days for a total of 20 µl (12,000 *Crithidia* cells) administered compared to the 10 µl (6,000 *Crithidia* cells) for *M. rotundata*. The additional doses were administered to increase the likelihood of exposure to the pathogen in the larger of the two species (*O. lignaria*). Trays containing the bees

were separated by treatment to avoid cross-contamination, but were all maintained on the same laboratory bench for the duration of the assay.

Half of the bees in both the inoculated and sham-inoculated treatments were provided ~36 mg balls of pollen, made from a mixture of honey bee collected poly-floral pollen (Bee Pollen Granules, CC Pollen High Desert, Phoenix AZ, USA), and all bees were provided 30% sucrose solution. The sucrose solution was replaced every three days and pollen balls were given to the pollen treatment bees every other day. We verified that the pollen had little to no pesticides by screening a pollen sample for 267 pesticides using liquid chromatography/mass spectrometry (Urbanowicz *et al.*, 2019). We detected only one pesticide, the acaricide coumaphos, at a level below the limit of quantification (< 0.525 ng/g), and thus concluded that pesticide exposure would not be a primary driver of any patterns found with pollen access. The pollen was not a source of *Crithidia* as can be verified by the absence of *Crithidia* in our sham-inoculated bees fed pollen (Figure 1). While *O. lignaria* larvae tend to develop more quickly and larger body sizes on pollen collected by members of its own species, they will nonetheless develop on honey bee-collected pollen (Levin & Haydak, 1957). Whether this translates into differences in pollen feeding by adults (both female *O. lignaria* and male *M. rotundata*) has not been tested. Though we did not quantify pollen consumption, we observed frass with pollen residues for some of the bees in the pollen-access treatments, indicating consumption. The bees were maintained in laboratory conditions at an average temperature of approximately 20°C in constant dark.

We checked bee survival daily for the duration of the trial (terminated after 11 days for *M. rotundata*, and 8, 6, and 5 days for the three *O. lignaria* trials, respectively), and recorded

daily mortality for each bee. Trial lengths varied due to differences in mortality; while *M. rotundata* had low overall mortality (18% died by the end of the 11-day trial), mortality for *O. lignaria* was overall higher and varied greatly (ranged from 14% – 54% depending on the trial). Each day we dissected any bee that had died within 24 h to screen for *Crithidia*, as well as all bees that survived until the end of the trial. Given that our pathogen counts are based on motile cells, we expected the most accurate counts from bees that were alive. As such, we shortened the trial times for *O. lignaria* in order to have enough live bees to accurately quantify infection because observing live *Crithidia* in dead bees, while possible, is likely less accurate. For example, only 8% of recorded *Crithidia*-positive *O. lignaria* bees had died before the end of the trial and had much lower corresponding pathogen loads (mean of 71 active *Crithidia* cells μl^{-1} compared to 171 cells μl^{-1} in bees that survived the length of the trial). We cannot determine whether the lower likelihood of detection and lower counts were products of insufficient time for the pathogen to replicate or whether the pathogen had died within the host before dissection and could not be visualized. As such, infection analyses were conducted only on bees that survived until the end of the trial. Nonetheless, the shorter trial times for *O. lignaria* are within timeframes relevant for *Crithidia* replication in bumble bees (Schmid-Hempel & Schmid-Hempel, 1993). We homogenized the dissected bee guts in 200 µl of distilled water, incubated the mixture for 4 h at room temperature, and finally quantified motile *Crithidia* using a hemocytometer (Figueroa et al., 2019; Richardson et al., 2015).

For the bees that had motile *Crithidia*, we evaluated whether the values indicated active replication by the pathogen. To do this, we compared estimated whole gut counts to the value in the entirety of the inoculum provided to the bees. The *Crithidia* μl^{-1} observed for each bee was

multiplied by 200 µl, the volume of water in which the gut was incubated, indicating the total number of *Crithidia* cells estimated to be in the bee gut. This is a conservative estimate as we are not including the volume of the gut itself. Values above 12,000 for *O. lignaria* and 6,000 for *M. rotundata* indicate active pathogen replication in the bees as these values correspond to the maximum possible cells consumed in the inoculum. We report these numbers as "whole gut *Crithidia* estimates" in the results.

At the end of the test periods, we measured the inter-tegular distance (ITD) of all of the bees using an Olympus SZX10 microscope and cellSens Standard software (Olympus Corporation of the Americas, Scientific Solutions Group, Waltham, Massachusetts, USA). The ITD is commonly used as a proxy for bee body size (Greenleaf *et al.*, 2007), which is a factor related to immunity in bumble bees (Otterstatter & Thomson, 2006).

Statistical analyses

Data analyses were conducted using R version 3.5.1 (R Development Core Team, 2008) with the lme4, glmmTMB, and coxme packages (Bates *et al.*, 2015; Brooks *et al.*, 2017; Therneau & Therneau, 2015). We conducted analyses on *M. rotundata* and *O. lignaria* separately.

To determine the role of pollen access on *Crithidia* infection patterns in the solitary bees, we employed a manual two-step hurdle model due to zero-inflation and overdispersion in the data (Zuur *et al.*, 2009). As mentioned above, these analyses were only conducted on inoculated

bees that survived until the last day of the trial to increase the accuracy of *Crithidia* counts (11 days for *M. rotundata* and 5, 6, or 8 days for the three *O. lignaria* trials). We first evaluated whether pollen access predicted the presence or absence of *Crithidia* in the gut of inoculated bees, followed by an evaluation of the *Crithidia* counts for those that were infected. For *O. lignaria*, we first constructed a generalized linear mixed model (GLMM) to evaluate whether the solitary bee species harbored *Crithidia* in their gut as the response (yes/no), predicted by access to pollen (yes/no) and bee size (ITD). The model included inoculation date as the random effect and a binomial distribution (logit link). To determine significance, we employed a likelihood ratio test and compared the model to one with the same response, distribution, and random-effect structure, but which excluded pollen as a predictor. We then developed a GLMM that included *Crithidia* count in *Crithidia*-positive bees as the response variable, predicted by access to pollen (presence/absence) and bee size (ITD). The model included inoculation date as the random effect and fit a truncated negative binomial distribution, which is suitable for count data with overdispersion (Brooks *et al.*, 2017). Significance was similarly determined using a likelihood ratio test. We verified model assumptions using the DHARMa package (Hartig, 2017). The statistical analyses for *M. rotundata* were likewise evaluated, with the sole difference that the manual hurdle model was conducted using a Generalized Linear Model instead of a GLMM because the species was inoculated in a single day and thus did not require inoculation date as a random effect.

To evaluate whether exposure to *Crithidia*, access to pollen, and their interactions influenced solitary bee survival, we conducted survival analyses using Cox proportional hazards models. For *O. lignaria*, the survival analysis evaluated bee survival (death/days elapsed) as the

response, *Crithidia* inoculation and access to pollen as explanatory variables, and inoculation date as the random effect. To determine significance of the treatments (pollen and inoculation), we conducted a likelihood ratio test comparing the full model with a model that included the same random effect structure but excluded either explanatory variable or included an additive relationship instead of an interaction. The analyses for *M. rotundata* were conducted using the *coxph* function (no random effect since all bees were inoculated on the same day) while the *coxme* function was used for *O. lignaria* (inoculation date as random effect) (Therneau & Therneau, 2015).

Results

We found that both *O. lignaria* females and *M. rotundata* males became infected with *Crithidia* sourced from the common eastern bumble bee (*Bombus impatiens*). Specifically, 76% of inoculated *O. lignaria* and 29% of *M. rotundata* harbored active *Crithidia* in their gut 5–11 days post inoculation; we did not detect *Crithidia* in any sham-inoculated bees (Figure 1). The median *Crithidia* load at the end of the trial for inoculated *O. lignaria* females was 100 moving cells μl^{-1} of bee gut (range: 0–1,600 cells), while for *M. rotundata* males the median was 0 cells μl^{-1} (range: 0–300 cells). The *O. lignaria* females were inoculated with 12,000 *Crithidia* cells and final counts averaged 20,000 cells (range: 0–320,000 whole gut cell estimates), indicating active replication of *Crithidia* in *O. lignaria* (Figure 2). While the median *Crithidia* whole gut count was zero in *M. rotundata* males, the bees with the highest gut counts in this species (60,000) had more than the administered 6,000 *Crithidia* cells, indicating replication of *Crithidia* in *M. rotundata* was also possible (Figure 2). Specifically, 56% of inoculated *O. lignaria* females

and 29% of *M. rotundata* males had estimated whole gut *Crithidia* counts above the inoculation concentration indicating active pathogen replication (Figure 2).

Pollen access did not influence likelihood of becoming infected with *Crithidia* for *O. lignaria* females or *M. rotundata* males ($\chi^2_1 = 1.86$, $p = 0.173$ and $z = 0.05$, $p = 0.962$, respectively; Figure 1) nor subsequent load for infected bees ($\chi^2_1 = 0.05$, $p = 0.830$ and $z = 1.78$, $p = 0.076$, respectively for *O. lignaria* females and *M. rotundata* males; Figure 3). Bee size did not explain likelihood of infection with *Crithidia* nor subsequent load for the infected bees in *O. lignaria* females or *M. rotundata* males ($z < 0.84$ and $p > 0.402$ for all).

Access to pollen increased *O. lignaria* female bee survival rates ($\chi^2_1 = 4.13$, $p = 0.042$; Figure 4A), though not for *M. rotundata* males ($\chi^2_1 = 1.19$, $p = 0.275$; Figure 4B). Inoculation with *Crithidia* did not influence survival in *O. lignaria* females or *M. rotundata* males ($\chi^2_1 = 1.56$, $p = 0.212$ and $\chi^2_1 = 2.02$, $p = 0.155$, respectively; Figure 4C, D), nor was there a pollen access by inoculation interaction for survival in *O. lignaria* females ($\chi^2_1 = 2.26$, $p = 0.133$) or *M. rotundata* males ($\chi^2_1 = 1.73$, $p = 0.189$).

Discussion

In this study, we found that the solitary bee species *Osmia lignaria* and *Megachile rotundata* could become infected with *Crithidia* collected from bumble bees. We found evidence for *Crithidia* replication in 56% of inoculated *O. lignaria* females and 29% of inoculated *M. rotundata* males. Access to pollen did not affect *Crithidia* infection in *O. lignaria* females or *M.*

rotundata males. However, pollen-fed *O. lignaria* females survived longer than their pollen-starved counterparts. Inoculation with *Crithidia* did not affect survival in either solitary bee species during the 5–11 day laboratory assay. Overall, our study illustrates that solitary bees can be hosts for a pathogen known to infect bumble bees and that diet can play a key role in the health of these important but historically understudied solitary bee taxa.

Most bee species within a community can be exposed to numerous pathogens when foraging at flowers, including *Crithidia* (Figueroa *et al.*, 2020; Graystock *et al.*, 2020a). Our results support a growing body of literature indicating the need to assess the host range of bee pathogens, including assessments of replication and impacts on survival (Bramke *et al.*, 2019; Müller *et al.*, 2019). The presence of *Crithidia* in bee feces has been used to identify active infections in bumble bees (Imhoof & Schmid-Hempel, 1999), where it can be detected in as quickly as five days post-inoculation (Logan *et al.*, 2005). We detected active *Crithidia* in solitary bee guts at the end of the trials, 5–11 days post-inoculation, for a total of 76% of inoculated *O. lignaria* females and 29% of inoculated *M. rotundata* males harboring motile cells. Moreover, 56% of the inoculated *O. lignaria* females and 29% of the inoculated *M. rotundata* males had estimated whole gut counts above the concentration of inoculum provided, indicating active pathogen replication. These values are comparable to infection probability in bumble bees (*e.g.* 57% of inoculated *Bombus terrestris* bees presented active infections after a 7-day trial (Näpflin & Schmid-Hempel, 2018)). Furthermore, our estimates for the percentage of bees experiencing *Crithidia* replication are conservative (*i.e.*, low) since we assume the bee gut did not contribute to the volume of the extraction and that the bees consumed the entirety of the

inoculum droplet, further highlighting the extent to which solitary bees can develop active *Crithidia* infections.

While phylogenetic distance often predicts the ability of hosts to be infected by a given pathogen (Gilbert & Webb, 2007; Streicker *et al.*, 2010), this may not be the case with *Crithidia* and its known hosts. Specifically, one recent study (Ngor *et al.*, 2020) has found that *Crithidia bombi* does not replicate in honey bees (*Apis mellifera*), but this pathogen does replicate in bumble bees (*Bombus* spp.). Both honey bees and bumble bees are in the Apidae family, while the two evaluated solitary bees evaluated in the present study are in the Megachilidae family. The factors that enable honey bees to avoid chronic *C. bombi* infections, despite being able to transmit the pathogen via flowers (Graystock *et al.*, 2015; Ruiz-González & Brown, 2006) and being more phylogenetically similar to bumble bees than *O. lignaria* and *M. rotundata*, remain unknown. Future evaluations of *Crithidia* infection and health impacts on solitary and social species across additional bee families are clearly warranted.

Host nutrition can increase infection intensity by providing resources to pathogens, but may also support host tolerance of infection. Specifically, increased access to food could increase infections by 1) directly providing nutrients to the pathogen; or 2) improving host quality for the pathogen. Conversely, food availability could suppress pathogens by 3) enabling physiologically costly immune responses; and/or 4) providing antimicrobial compounds (Conroy *et al.*, 2016; Palmer-Young & Thursfield, 2017). While we did not find that pollen access directly influenced the likelihood or severity of *Crithidia* infections (Figure 1 & 3), others have found that pollen-deprivation in bumble bees can reduce *Crithidia* infections (Conroy *et al.*,

2016; Logan *et al.*, 2005). We found that pollen access increased *O. lignaria* female survival, independent of *Crithidia* infection. While we did not find an effect of pollen on *M. rotundata*, we cannot determine whether this was due to low mortality during the 11-day trial and whether patterns would differ in longer time scales. Similarly to *O. lignaria*, access to pollen can increase honey bee survival rates despite greater pathogen loads (*e.g. Nosema apis*, *N. ceranae*, and numerous RNA viruses (Jack *et al.*, 2016; Rinderer & Dell Elliott, 1977; Zhang *et al.*, 2020; Zheng *et al.*, 2014)), further illustrating the importance of diet in pollinator health. Access to pollen in provisioning female solitary bees has been shown to increase the number of brood cells and proportion of offspring surviving to adulthood (*e.g.* in *O. bicornis* and *O. californica* (Bukovinszky *et al.*, 2017; Cane, 2016)), suggesting that differences in physiological demands between sexes could result in differential effects of pollen access. Furthermore, a recent study that experimentally inoculated *O. cornuta* with *C. mellificae*, a trypanosomatid known to infect honey bees, found that while both sexes had higher pathogen loads over time, only male survival was significantly reduced by infection (Strobl *et al.*, 2019). Future evaluations of the impact of *Crithidia* on physiology and survival in solitary bees across sexes is well justified, especially in the field, and could incorporate assessments of resource availability and bee immune responses.

Bumble bees infected with *Crithidia* (Richardson *et al.*, 2015) and honey bees infected with *Nosema ceranae* (Mayack & Naug, 2009) often consume more pollen, likely to compensate for the energetic cost of mounting an immune response (Moret & Schmid-Hempel, 2000). Interestingly, the role of pollen on *Crithidia* growth varies depending on whether the assessments are conducted *in vitro* or *in vivo*; for example, sunflower pollen *in vitro* increases pathogen growth (Palmer-Young & Thursfield, 2017), while *in vivo* this pollen type has a strong inhibitory

effect on the pathogen when consumed by bumble bees (Giacomini *et al.*, 2018; LoCascio *et al.*, 2019). When inhibitory, secondary compounds appear to facilitate these anti-*Crithidia* interactions in bumble bees (Koch *et al.*, 2017; Richardson *et al.*, 2015). These differences suggest that pollen may interact in important ways within the host, leading to varying effects on *Crithidia* growth. Future evaluations of the role of pollen quality, quantity, and diversity on susceptibility to pathogens mediated by interactions between immune responses, gut physiology, and microbiota are important avenues for pollinator research (Alaux *et al.*, 2010; Cotter *et al.*, 2011; Dolezal & Toth, 2018), especially focusing on solitary bees and other understudied pollinators.

Life-history and functional traits are important mediators of disease transmission and dynamics within a host. While we did not find a role of pollen in *M. rotundata* male infection patterns, recent work has shown that adult male solitary bees (*Andrena* spp.) frequently harbor pollen in their digestive tract (Urban-Mead *et al*., unpublished results). The importance of males in dispersing pathogens in plant-pollinator networks is not well established, despite male and female solitary bees having comparable infection rates (Müller *et al.*, 2019; Ngor *et al.*, 2020; Strobl *et al.*, 2019). Moreover, natural rates of pathogen acquisition or deposition by solitary bees while foraging is not known (Figueroa *et al.*, 2019). While we report high rates of *Crithidia* infection for the two solitary species evaluated, especially for *O. lignaria* females, we did not find that experimentally exposing the solitary bees to *Crithidia* influenced host survival in the 5–11 day laboratory assay. Characterizing infection rates as well as the corresponding impacts on mortality and reproduction in the field, especially alongside numerous co-occurring stressors (*e.g.* inadequate diet, pesticides, and co-infections), is an important future direction. Differences

in life histories across solitary bees may influence these dynamics. For example, *O. lignaria* overwinter as adults, similar to bumble bee queens (a life stage highly vulnerable to *Crithidia* (Brown et al., 2003)), while *M. rotundata* overwinter as prepupae (Kemp *et al.*, 2004). Similarly, given that we know infection with *Crithidia* alters resource allocation patterns in *Bombus terrestris*, whereby infected bees invest more in their fat body and less in reproduction than non-infected bees (Brown *et al.*, 2000), assessing differences in resource allocation, foraging behavior, and pollen-provisioning abilities for infected solitary bees compared to uninfected counterparts could advance our understanding of pollinator health in wild bee communities.

Overall, our work supports the importance of improving knowledge concerning solitary bee species in pollinator epidemiology, including their assessment as hosts for what have traditionally been considered social bee pathogens, and considering resource availability when evaluating host-pathogen interactions. Future evaluations of likelihood of transmission between solitary and social species on flowers would benefit from this knowledge, as transmission probability is already known to vary between bumble bee species (Ruiz-González *et al.*, 2012). Moreover, the activity period of *Osmia* spp. and *Megachile* spp. often greatly overlaps with bumble bees, further highlighting the need to understand interspecies disease transmission networks (Figueroa *et al.*, 2020). In addition, pathogen transmission between social and solitary bees could occur via spillover by introduced commercial colonies (Colla *et al.*, 2006; Graystock *et al.*, 2013b; Otterstatter & Thomson, 2008), and could occur in various directions (Graystock *et al.*, 2016a), further highlighting the need to understand host ranges and impacts of disease on pollinator communities. In order to respond to the growing dependence of pollinators for food security (Aizen *et al.*, 2019), which is in large part contributed by wild bees (Garibaldi *et al.*,

2013; Winfree *et al.*, 2018), we must expand our understanding of the role of pathogens, nutrition, and other stressors on solitary bee health.

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Figure 1. Effect of pollen access on motile *Crithidia* presence in solitary bees that survived the length of the trial. Pollen access did not affect the likelihood of *Crithidia* presence in (A) *Osmia lignaria* females or (B) *Megachile rotundata* males. Differences in sample size is a product of mortality. (n.s.) indicates $p > 0.05$.

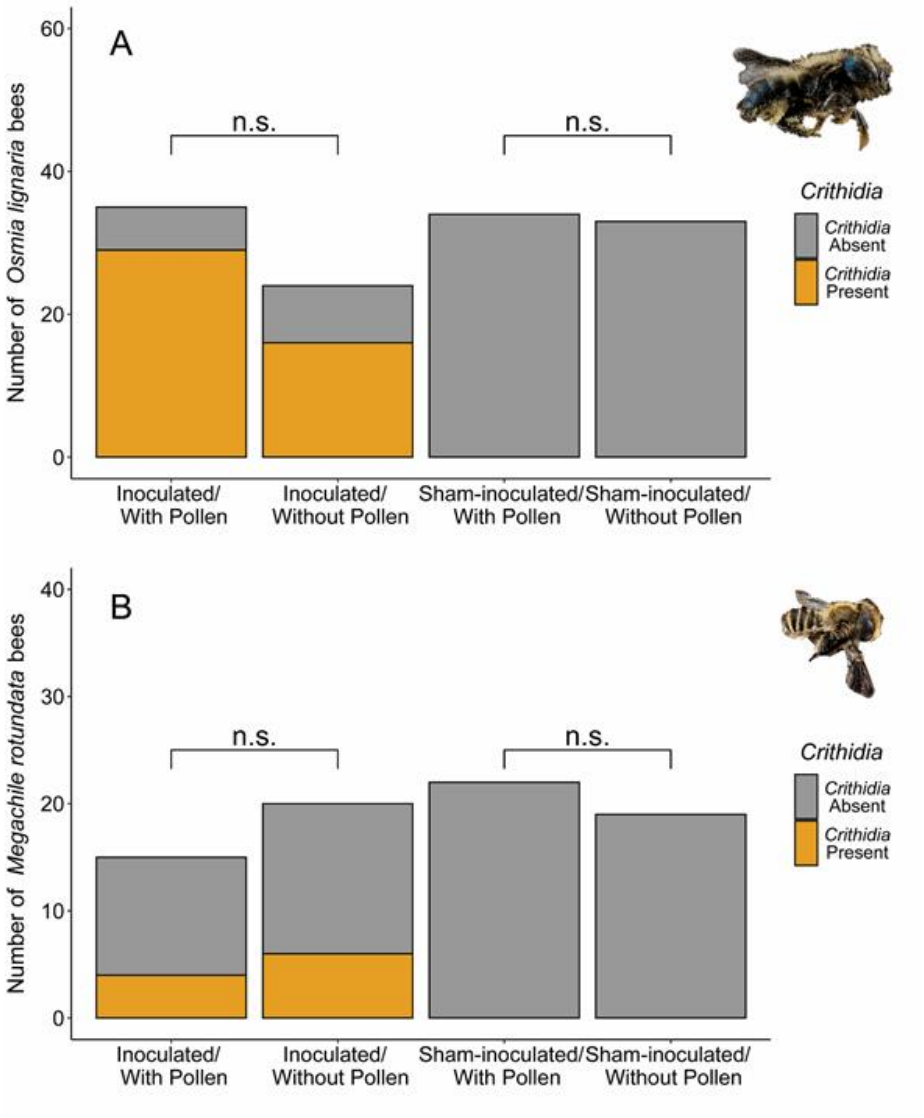


Figure 2: Estimated whole gut counts of *Crithidia* in solitary bees 5-11 days post-inoculation. Blue circles indicate estimated whole gut counts in inoculated *M. rotundata* males and *O. lignaria* females (see Methods). The dashed red line indicates the total number of *Crithidia* cells in inoculum provided to *M. rotundata* males and the solid red line indicates the total number of *Crithidia* cells in inoculum provided to *O. lignaria* females. Values above these thresholds indicate active pathogen replication in the host bees.

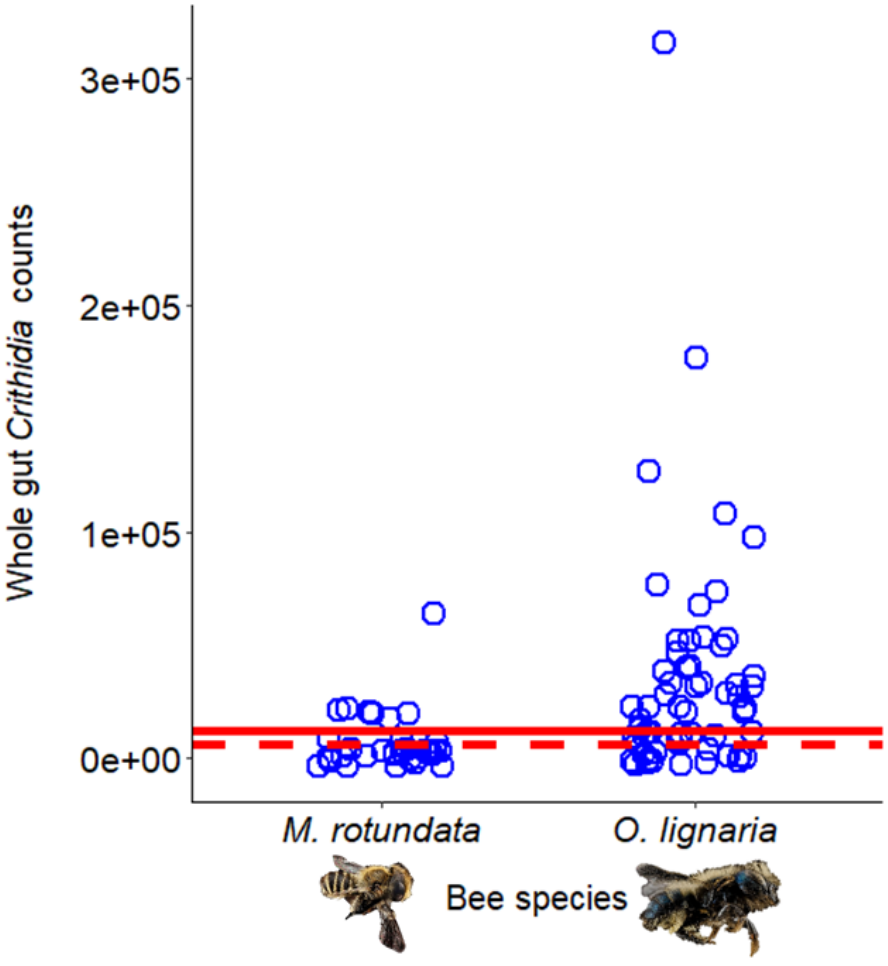


Figure 3. Effect of pollen access on *Crithidia* load in solitary bees. Pollen access did not affect *Crithidia* load (cells μl^{-1}) in (A) *Osmia lignaria* females or (B) *Megachile rotundata* males. (n.s.) indicates $p > 0.05$. This figure represents subsequent load for inoculated bees that presented motile *Crithidia*.

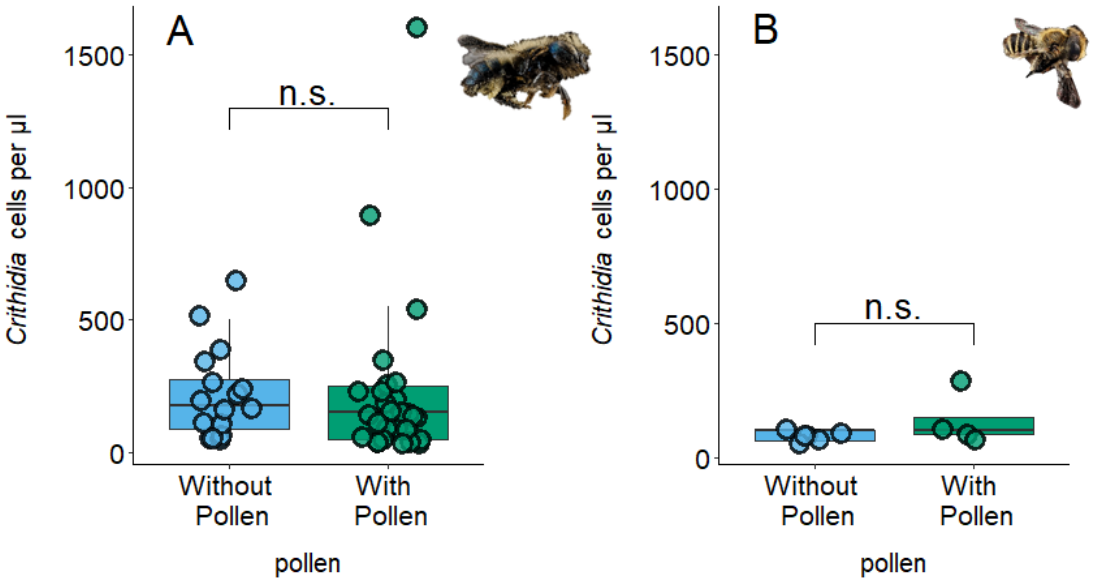
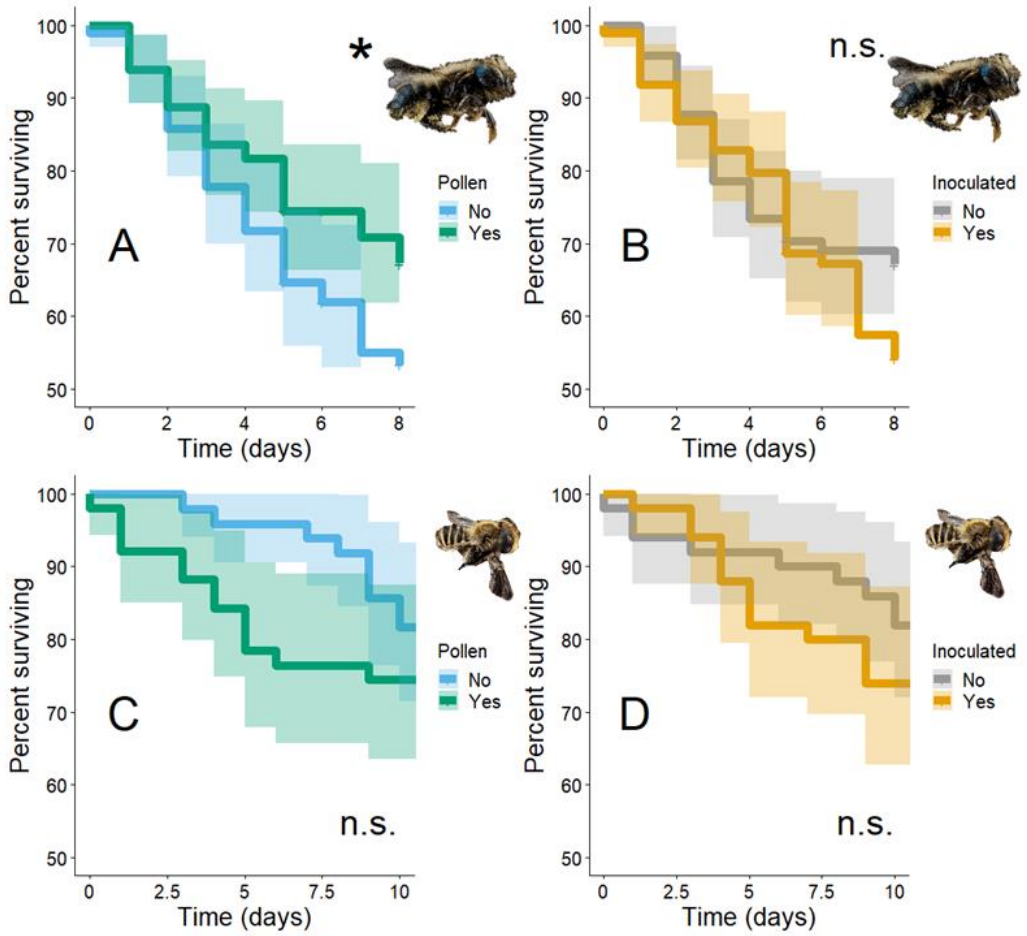


Figure 4. Solitary bee survival across pollen access and *Crithidia* inoculation treatments. (A) *Osmia lignaria* females with access to pollen survived longer than those without access to pollen. (B). Inoculation with *Crithidia* did not influence *O. lignaria* survivorship. (C) Pollen access did not affect *Megachile rotundata* male survivorship, (D) nor did inoculation with *Crithidia.* Trial lengths varied according to general mortality, which was greater in *M. rotundata* than for *O. lignaria* (see Methods). Shaded error bands indicate 95% confidence intervals. (*) denotes $p < 0.05$ while (n.s.) indicates $p > 0.05$.



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