Within-Colony Transmission of Microsporidian and Trypanosomatid Parasites in Honey Bee and Bumble Bee Colonies

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Abstract

Parasites are commonly cited as one of the causes of population declines for both managed and wild bees. Epidemiological models sometimes assume that increasing the proportion of infected individuals in a group should increase transmission. However, social insects exhibit behaviors and traits which can dampen the link between parasite pressure and disease spread. Understanding patterns of parasite transmission within colonies of social bees has important implications for how to control diseases within those colonies, and potentially the broader pollinator community. We used bumble bees (*Bombus impatiens* Cresson) (Hymenoptera: Apidae) and western honey bees (*Apis mellifera* L.) (Hymenoptera: Apidae) infected with the gut parasites *Crithidia bombi* (Lipa & Triggiani) (Trypanosomatida: Trypanosomatidae) and *Nosema ceranae* (Fries et al.) (Dissociodihaplophasida: Nosematidae), respectively, to understand how the initial proportion of infected individuals impacts within-colony spread and intensity of infection of the parasites. In bumble bees, we found that higher initial parasite prevalence increased both the final prevalence and intensity of infection of *C. bombi*. In honey bees, higher initial prevalence increased the intensity of infection in individual bees, but not the final prevalence of *N. ceranae*. Measures that reduce the probability of workers bringing parasites back to the nest may have implications for how to control transmission and/or severity of infection and disease outbreaks, which could also have important consequences for controlling disease spread back into the broader bee community.

Key words: *Nosema ceranae*, *Crithidia bombi*, *Apis mellifera*, *Bombus impatiens*, disease

Parasites and pathogens are considered main drivers of host ecology and evolution (Ebert et al. 2000, Miura et al. 2006). They can have short-term effects on their hosts, including changes in host behavior (Moore 2002, Gegear et al. 2005) and reduced fitness (Brown et al. 2003b, Bonsall 2004), as well as long-term effects, including changes in host population cycles, genetic diversity, and life history (Kirzhner et al. 1999, Brown et al. 2003b, Bonsall 2004). One challenge for the evolution of sociality in animals is that living in close proximity with genetically related individuals increases the transmission probability of parasites and the risk of epidemic outbreaks (Schmid-Hempel 1995, Kurvers et al. 2014). However, many factors influence the outcome of the infection at the group level (Godfrey 2013, Liu et al. 2019). Transmission dynamics within social groups is of special interest for controlling emerging diseases in humans, domestic animals and wildlife populations (Hajek and Shapiro-Ilan 2018), especially ones that perform critical ecosystem services (Potts et al.

2016). Here, we studied how initial within-colony parasite prevalence influences transmission and the outcome of infection at the colony level in two eusocial bee species.

Conventional epidemiological models generally assume that interactions in social groups are random, and therefore, increasing the proportion of infected individuals in a group should expand transmission probability to susceptible hosts by increasing the interactions between infected and uninfected individuals (Anderson and May 1991, Kappeler et al. 2015). However, members within social groups often do not interact randomly, but based on a network structure, such that the potential of an individual to transmit parasites depends on their location in the social network (Naug and Camazine 2002, Godfrey 2013, Kurvers et al. 2014). For example, the social structuring of students into classes and grades strongly affected transmission of the influenza virus in an elementary school (Cauchemez et al. 2011). Although behaviors of infected

and uninfected individuals (such as changes in movement patterns, avoidance of infected individuals, and mate choice) can also affect the probability of parasite spread in social groups (Loehle 1995), understanding how parasite prevalence affects transmission in social groups has important implications for how to control disease spread in social animals and reduce the risk of epidemics.

Social Hymenoptera (ants, social wasps, and social bees) provide unique opportunities to explore the dynamics of pathogen transmission in groups (Schmid-Hempel and Schmid-Hempel 1993, Schmid-Hempel 1999). They exhibit a range of network structures (from high to low compartmentalization; Jandt and Dornhaus 2009, Baracchi and Cini 2014) and individual behaviors that can reduce the spread of parasites within the colony. For example, due to division of labor in honey bee colonies, individuals interact more with nestmates performing the same task, creating compartmentalization in the interaction network (Liu et al. 2019), which is associated with reduced parasite transmission at the colony level (Naug 2008). Other behaviors that may reduce parasite spread within the colony include sanitary behaviors, avoidance of infected individuals, self-medication, and self-exclusion of infected individuals (Cremer et al. 2007, Richardson et al. 2015, Liu et al. 2019). In these cases, transmission between colonies may be necessary to sustain epidemics at the population level (White et al. 1996). Empirical and theoretical studies of directly transmitted parasites show that when the transmission rate of the parasite is low, an infection can fade out of the host population unless there is constant immigration of infected individuals (Hudson et al. 2002).

We studied within-colony parasite transmission in the eusocial western honey bee (*Apis mellifera* L.) (Hymenoptera: Apidae) and common eastern bumble bee (*Bombus impatiens* Cresson) (Hymenoptera: Apidae), which have high ecological and economic value due to the pollination services they provide for agricultural production (Loosey and Vaughn 2006, Garibaldi et al. 2013, Schulp et al. 2014). Parasites that have been linked to the population decline of bees include the honey bee parasite *Nosema ceranae* Fries et al. (Microsporidia), and the bumble bee parasite *Crithidia bombi* Lipa & Triggiani (Trypanosomatida) (VanEngelsdorp and Meixner 2010, Meeus et al. 2011, Bianco et al. 2014). Both are gut parasites, horizontally transmitted when a host ingests parasite cells from flowers where infected bees have defecated (Smith 2012, Graystock et al. 2015). They can also be transmitted within the colony by food exchange with infected individuals, ingestion of contaminated food, or contact with feces (Graystock et al. 2015, Goblirsch 2018). In this context, social immunity (e.g., social network structure and host behaviors) can influence parasite transmission, but whether this is enough to dampen the link between initial parasite pressure and transmission dynamics are unknown. Honey bees have colonies that are several orders of magnitude bigger than bumble bee colonies (>20,000 workers) and have a perennial life cycle (Winston 1987), which means that they could harbor more parasites (Hudson et al. 2002). At the same time, honey bees have a more structured division of labor than bumble bees, which can help control parasite spread in the colony by creating compartmentalization within the interaction network (Winston 1987, Naug and Camazine 2002, Goulson 2010).

We manipulated a key epidemiological parameter, the initial percentage of infected individuals (prevalence), and measured subsequent within-colony transmission rates, severity of infection (parasite loads), and colony performance. We hypothesized that with higher initial prevalence, parasites would spread faster within the colony, suggesting that there is a limit to how much social immunity and other unknown aspects of bee biology can reduce within-colony transmission. We note that by using two different parasites, one

each in bumble bees and honey bees, any differences in transmission dynamics observed between the two could be ascribed not only to differences between the two bee species but also the biology of two different parasites. Therefore, we did not make any statistical comparison between bee species in this study, though we do discuss qualitative differences in results between the host-parasite systems. For each bee species, we asked the following questions: 1) How does the probability of an individual being infected vary with the initial prevalence of the parasite and over time? 2) Does initial parasite prevalence affect the intensity of infection per individual bee? And 3) to what degree does initial parasite prevalence affect colony growth rate and final colony productivity? Studying the dynamics of parasite spread within colonies is an important step toward a better understanding of and controlling outbreaks in commercial colonies and spillover to wild populations.

Methods

Study System

The common eastern bumble bee (*B. impatiens*) and western honey bee (*A. mellifera*) are both managed, generalist pollinators (Crane 1984, Mader et al. 2010). *Bombus impatiens* is a native, primitively eusocial species ranging from southern Canada through Florida (Colla et al. 2011). Colonies are annual and founded in the spring by single, mated queens (Goulson 2010). Because of this annual life cycle, parasites typically overwinter with infected daughter queens. Honey bees are native to Europe, Asia, and Africa. Colonies have a perennial lifecycle that allows parasites to stay within the colony year-round (Winston 1987).

We studied the parasites *Crithidia bombi* (Trypanosomatida) and *Nosema ceranae* (Microsporidia), which commonly infect bumble bees and honey bees, respectively (Cordes et al. 2012, Martín-Hernández et al. 2018), and are ecologically and economically relevant for their host (Brown et al. 2003b, Goblirsch 2018). *Crithidia bombi* usually shows a prevalence of about 30%, but in some areas, as many as 80% of flying bumble bee workers can be infected (Gillespie 2010, Cordes et al. 2012). *Crithidia bombi* reproduces in the gut lumen, and new infective cells are released in feces 5–10 d after inoculation (Schmid-Hempel and Schmid-Hempel 1993, Brown et al. 2003a). *Crithidia bombi* can reduce the number of workers, drones and new queens produced per colony (Brown et al. 2003b), impair learning of foragers (Gegeer et al. 2006), and decrease probability of infected overwintering queens to successfully start a nest in the spring (Schmid-Hempel 2001, Brown et al. 2003b). The parasite is horizontally transmitted within and among colonies when individuals ingest contaminated material either on flowers or through contact with infected nest mates or their nest mates' feces (Durrer and Schmid-Hempel 1994, Imhoof and Schmid-Hempel 1998, Smith 2012, Graystock et al. 2015). Estimates of *C. bombi* transmission rates on artificial and/or natural flowers range from 20 to 80% (Durrer and Schmid-Hempel 1994, Adler et al. 2018), suggesting variation in the number of exposed foragers returning to the colony. Within small experimental colonies (first brood workers), *C. bombi* spreads faster in colonies with dense social networks (more contacts among individuals), and workers have a higher risk of getting infected as the rate of contact with infected individuals increases (Otterstatter and Thomson 2007). Whether these transmission dynamics hold in mature colonies that are an order of magnitude larger is unknown.

The microsporidian *N. ceranae* is an obligate intracellular parasite (VanEngelsdorp and Meixner 2010, Bianco et al. 2014). Spores infect and replicate inside midgut epithelial cells and are later released to the environment in feces (Higes et al. 2007). *Nosema*

ceranae was originally described in the Asian honey bee, *Apis cerana* (Fries et al. 1996) but can also infect *A. mellifera*, and it is now the most prevalent *Nosema* species in honey bees in the United States (Higes et al. 2006, Chen et al. 2008). *Nosema ceranae* can reduce the life span of workers, decrease colony size, and cause sudden collapse of the colony (Malone et al. 1995, Higes et al. 2008). Adult bees come into contact with *N. ceranae* spores when workers rob food from an infected colony or visit contaminated flowers; within-colony transmission occurs when workers exchange food with infected workers or when they clean fecal material (oral-oral and fecal-oral route; Smith 2012, Goblirsch 2018). Larvae and the queen can also become infected when they are fed contaminated food (Rutrecht and Brown 2008, Higes et al. 2009), and an infected queen can transmit the parasite to workers when they start a new hive (Fries and Scott 2001, Higes et al. 2009). Honey bee colonies can survive *N. ceranae* infection for more than a year, but when the prevalence in nurse bees is greater than 40%, it is often followed by a sudden collapse of the colony (Higes et al. 2008). The outcome of *N. ceranae* infections can also be influenced by interactions with other stressors, such as insecticides and other pathogens (Retschnig et al. 2014, Doublet et al. 2015).

Within-Colony Transmission of *C. bombi* in *B. impatiens*

Colony Origin and Maintenance

We used 12 *B. impatiens* commercial colonies from Koppert Biological Systems (Howell, MI). We kept colonies in a dark room at approx. 27°C and 50% RH, and provided sugar-water (30% sucrose) and honey bee collected pollen ad libitum. Upon arrival, we screened colonies for *C. bombi* infection by taking a random sample of four workers per colony, collecting fecal samples and examining the samples under a compound microscope at 400 $\times$. All 12 colonies were free of *C. bombi*. Because the colonies initially had variable numbers of workers, ranging from 30 to 80, we removed workers from each colony so that each colony had 30 $\pm$ 5 workers at the start of the experiment.

Crithidia bombi can be transmitted by contact with feces (Shykoff and Schmid-Hempel 1991), and when bees are confined in the colony, feces can build up quickly. To avoid unnatural levels of parasite transmission within the colony due to excessive contact with fecal material, each colony was connected by a plastic tube to an additional container that had cat litter (PetCentral), so bees could use this container as a 'latrine', as cat litter absorbed the feces. We replaced the cat litter approx. every other week. The access to the sucrose solution was also in this additional container to encourage bees to forage for nectar outside of the colony box. Pollen was provided directly to the main colony box.

Inoculum Preparation

To prepare the inoculum, we dissected the intestines of five *B. impatiens* workers from a source colony infected with *C. bombi* isolated from Stone Soup Farm, Hadley, MA (GPS coordinates: 42.363911 N, -72.567747 W). We prepared inoculum according to a standard protocol (Richardson et al. 2015). Briefly, we homogenized each intestine in 300 μ l of dH₂O. We let the samples settle for 4 h to allow the *C. bombi* cells to swim up into the supernatant and the gut debris to sink to the bottom of the tube. We removed 200 μ l of clean supernatant from each tube and mixed them together. To determine the concentration of *C. bombi* cells, we took a 10- μ l sample and estimated the number of *C. bombi* cells per microliter in a Neubauer chamber with a compound microscope at 400 $\times$

magnification. Based on this *C. bombi* cell count, we mixed the solution with 50% sucrose to make an approx. 25% sucrose solution that contained 1,200 *C. bombi* cells per microliter. This solution was used to inoculate bees in the experimental colonies.

Experiment Set-up

To assess the effect of initial parasite prevalence on within-colony spread of *C. bombi*, we infected either 1 or 30% of the workers in the colonies (1 and 9 workers, respectively), with the 30% treatment representing a high, but realistic, percentage of workers in the colony foraging (Cartar 1992) and potentially coming into contact with *C. bombi* cells on flowers. Six colonies were randomly assigned to each treatment. On 20 February 2018, we haphazardly removed the desired number of workers from each colony and starved them for 4 h. We then gave each bee 10 μ l of the inoculum (12,000 cells per bee), making sure bees drank the entire inoculum droplet. This *C. bombi* dose is within the range that bees are exposed to when foraging in nature (Schmid-Hempel and Schmid-Hempel 1993, Otterstatter and Thomson 2006). We marked inoculated bees with a dot of indelible paint on the dorsal side of the thorax. After the paint was dry, we returned bees to their respective colonies to serve as the source of infection.

Parasite Sampling

Sampling started on 26 February 2018, 1 wk after we inoculated the colonies, given that it takes ~1 wk for *C. bombi* in *B. impatiens* to develop patent infections (Otterstatter and Thomson 2006). We collected bees from each colony twice per week for 7 wk, when colonies started to produce drones and new daughter queens (13 April 2018). On each sampling day, we haphazardly removed 10% of the workers in each colony (up to 10 workers per colony), avoiding workers marked with the paint dot and callow bees, as they would not have developed infection yet (Otterstatter and Thomson 2006). We dissected the intestines and ground each one in 500 μ l of dH₂O. We allowed samples to sit for ~4 h, after which we used a Neubauer chamber to estimate the number of cells per microliter using a 10- μ l subsample of the supernatant. As bee size can influence the intensity of *C. bombi* infection (Otterstatter and Thomson 2006), we used the length of the radial cell of the right forewing as a proxy for bee size (Müller et al. 1996). We taped the wings to microscope slides and scanned the wings. The length of the radial cell was then measured using the software ImageJ (V 1.8; Schneider et al. 2012).

Measures of Colony Growth

We recorded the initial and final weight of the colony and calculated the change in weight. Once per week, we also recorded the approximate number of workers, drones, dead workers, and new queens in each colony by visual inspection. When the experiment ended at the end of week 7 (because colonies were starting to produce sexuals), we froze the colonies at -20°C. Once colonies were frozen for at least 48 h, we dissected each colony to record the final number of adult workers, drones, daughter queens, worker pupae, queen pupae, larvae and eggs, as well as the total weight for each of those categories (to the nearest 0.01 g).

Within-Colony Transmission of *Nosema ceranae* in *Apis mellifera*

Colony Origin and Maintenance

Fifteen colonies with replicate honey bee queens were created by grafting from the genetic line MP56 in June 2017. The MP line originated from a local northeastern U.S. beekeeper. Virgin queens were

openly mated in three-frame nucleus colonies at the Dyce Lab for Honey Bee Studies, Ithaca, NY, and checked after 4 wk. On 10 July 2017, we screened all 15 colonies for *N. ceranae* by collecting 30 workers from the entrance of each colony. Bees were surface sterilized with 70% ethanol and 0.5% bleach solution. We then dissected and homogenized the intestines of bees from the same hive in 1 ml of phosphate buffered saline (PBS). After vortexing the samples, we took a 10- μ l subsample, and using a Neubauer chamber with a compound microscope at 400 $\times$ magnification, we estimated the number of spores per bee (Fries et al. 2013). It is important to note that spores of *N. ceranae* are released only after completing the intracellular cycle (3–7 d) (Higes et al. 2007), so our method could identify only sporulating infections. All but two colonies were already infected with *N. ceranae*; therefore, we used the nine colonies with the lowest *Nosema* levels (0–20,500 spores per bee) for the experiment, and the two colonies with the highest levels (58,000–74,000 spores per bee) were used as inoculum sources (Supp Table 1 [online only]). Treatments (control, 1% and 10% of workers inoculated with *N. ceranae*) were blocked by initial infection intensity of the colonies such that mean initial infection intensity was approximately equal for each treatment. Given that the foraging force of a honey bee colony can be 10–20% of the total colony population (Danka et al. 1986, Winston 1987), the 10% treatment represents a realistic percentage of foragers who could come into contact with *N. ceranae* while foraging.

After selecting the experimental colonies, we removed one frame containing workers, brood, food and the queen from each colony and placed it in an insulated five-frame nuc box, to create nine, one-frame colonies containing ~1,000 adult workers. Each colony was placed inside a 1.8 $\times$ 1.8 $\times$ 3.0-m tent that contained a feeder with 30% sucrose and an artificial pollen source filled with BeePro (Mann Lake, Ltd., Hackensack, MN). We allowed colonies to acclimate in the tents for 1 wk before starting the experiment.

Inoculum Preparation

To prepare the inoculum, we collected 100 workers from the source colonies with the highest *N. ceranae* levels. We dissected and homogenized the intestines in 2,500 μ l of phosphate buffered saline. The spore solution was then purified using the Percol triangulation method (Fries et al. 2013). We estimated the concentration of the purified spores via a hemocytometer as described above. This solution was combined with 1,500 μ l of 30% sucrose to make an inoculum containing 4.6×10^4 spores in each 5- μ l aliquot that was administered to the bees.

Experiment Set-up

After the 1-wk acclimation in the tents (22–28 July 2017), we assigned three colonies to each treatment, consisting of control (0 workers), 1% (10 workers), or 10% (100 workers) of bees in the colony inoculated with *N. ceranae*. Bees were starved for 1 h, and then given 5 μ l per bee of inoculum (4.6×10^4 spores per bee), making sure that bees drank the entire inoculum droplet. In the control colonies, the 100 workers were mock inoculated with 30% sucrose. In the 1% treatment colonies, 10 workers received the inoculum and 90 workers received only sucrose. In the 10% treatment colonies, all 100 workers received the inoculum.

Parasite Sampling

Sampling started on 29 July 2017, 1 wk after we inoculated the colonies, as *N. ceranae* start producing new spores ~6 d after inoculation (Forsgren and Fries 2010). We sampled colonies once per week

for 5 wk, haphazardly collecting 20 workers from each colony each week by opening each hive and collecting workers directly from the frames. The sampled individuals were immediately frozen and stored at –20°C until dissection and spore quantification. For dissection and quantification, individual guts from 10 bees per colony per week (approx. 1% of the colony population) were processed individually. We prepared each sample by homogenizing the gut in 500- μ l PBS and vortexed the sample for 30 s. Then, we used a Neubauer chamber to estimate the number of spores per microliter using a 10- μ l subsample from the bottom of the tube. We counted the number of spores per sample three times, vortexing the samples between counts. Bees were considered ‘infected’ if they had an average spore count ≥ 1 . To estimate the size of workers in each colony, we measured the radial cell of the right forewing for the remaining 10 workers sampled from each colony. We measured them in the same way as for bumble bees (see above).

Measures of Colony Growth

At the end of the experiment, we took a picture of both sides of the frames in each colony, and later used those photos to count the number of workers in each colony. We calculated the change in the number of workers compared to the initial 1,000 workers per colony.

Data Analysis

All statistical analyses were conducted using R version 3.5.2 (R Core Team 2018), with separate analyses for each bee-parasite combination.

1. How does the probability of an individual being infected vary with the initial prevalence of the parasite and over time?

To test how the initial parasite prevalence treatment (proportion of infected workers) affected the probability of an individual being infected, we performed logistic regression using the package ‘glm-mTMB’. The full models included the initial prevalence (treatment), time (week of sampling), and their interaction as fixed effects. We also included bee size (length of the radial cell) and the number of workers in the colony (only for *B. impatiens*) as covariates, and colony ID was included as a random effect. We evaluated the significance of terms with a likelihood ratio chi-squared test, implemented via the ‘drop1()’ function in R. We removed terms that were not significant ($P > 0.05$) and compared the fit of the reduced and full models using the AIC values and a χ^2 -test with the analysis of variance (‘anova’) function. We assessed the significance of predictor variables with likelihood ratio via the ‘Anova’ function from package ‘car’. We used Tukey’s HSD tests for post hoc pairwise comparisons using the ‘lsmeans’ package. We did not include data from the first week of sampling for either parasite because of the time required to develop and pass the infection to susceptible hosts (Schmid-Hempel and Schmid-Hempel 1993, Higes et al. 2007).

2. Does initial parasite prevalence affect the intensity of infection per individual bee?

To test how the initial parasite prevalence treatment (proportion of infected workers) affected *C. bombi* and *N. ceranae* infection intensity (*C. bombi* cells per microliter and *N. ceranae* spores per microliter), we used a generalized linear mixed model with negative binomial error distribution, because the data showed overdispersion, using the package ‘glmmTMB’. For this analysis we only included data from bees that were infected (cell count ≥ 1 for *C. bombi*, and average spore count ≥ 1 for *N. ceranae*). Initial prevalence (treatment), time (week of sampling), and their

interaction were included as fixed effects; bee size and number of workers in the colony (only for *B. impatiens*) were included as covariates, and individual bee ID nested in each experimental colony was included as a random effect. We evaluated the significance of terms and compared full and reduced models as we did in the analyses of probability of infection. Because we only included infected bees in this analysis, for both parasites, we only included data from the last three weeks of sampling, because earlier weeks resulted in too few infected bees for analysis, especially in the low initial prevalence treatments.

3. To what degree does initial parasite prevalence affect colony growth rate and final colony productivity?

To compare the effect of initial parasite prevalence on colony performance of *B. impatiens* (change in total colony weight, total number of workers produced, final number of drones, daughter queens, worker pupae, queen pupae, larvae and eggs, and the total weight for each group), we used *t*-tests with the 't.test' function. To compare the effect of treatments on *A. mellifera* colony performance (change in number of workers in the colony), we used ANOVA with the 'aov' function.

Results

1. How does the probability of an individual being infected vary with the initial prevalence of the parasite and over time?

Bombus impatiens Infection With *C. bombi*

The final best-fit model included initial parasite treatment, time (week), and their interaction, with colony ID as a random effect (Fig. 1a). The initial prevalence of the parasite had a significant effect on the probability of bees being infected ($\chi^2_1 = 35.5$, $P < 0.001$). By the end of the seventh week, the prevalence of *C. bombi* in the high treatment was on average 3.3-fold higher than in the low treatment ($t = 4.18$, $P < 0.0001$; Fig. 1a). There was also a significant effect of time on probability of infection ($\chi^2_5 = 131.4$, $P < 0.001$). In the high treatment, the probability of workers becoming infected grew markedly between weeks one and three, from zero to nearly 0.75, with a slower rate of transmission from week three to seven. In the low treatment, parasite

prevalence hovered near zero until week five, reaching a maximum of 0.25 at week seven (Supp Fig. 1a [online only]). Moreover, the maximum as well as average infection rate was higher in the high compared with low initial prevalence treatments; for example, the probability of infection increased on average 14% per week for the high treatment but only 4.5% per week for the low treatment (Supp Fig. 1a [online only]). Finally, there was no significant interaction between initial prevalence treatment and time ($\chi^2_5 = 9.4$, $P = 0.095$), suggesting that week did not alter how initial parasite prevalence affected the probability of infection.

Apis mellifera Infection With *N. ceranae*

The final best-fit model included only the main effect of time and colony ID as a random effect. Parasite treatment was not a significant term and was dropped from the model ($\chi^2_2 = 4.66$, $P = 0.097$). Time (week) had a significant effect on the probability of a bee being infected ($\chi^2_4 = 19.45$, $P < 0.001$; Fig. 1b). Although there were no significant differences from 1 wk to the next in the proportion of infected bees ($P > 0.05$ for all comparisons), at the end of the experiment, the average *N. ceranae* prevalence across all treatments had increased 2.2-fold compared with the beginning of the experiment. Additionally, although treatment was not a significant term in the model, the average prevalence of *N. ceranae* in the high-treatment colonies by the end of the experiment was 5.5-fold higher than in the control colonies (Supp Fig. 1b [online only]).

2. Does initial parasite prevalence affect the intensity of infection per individual bee?

Bombus impatiens Infection With *C. bombi*

The final best-fit model included only the main effect of treatment and colony ID as a random effect. Considering only bees that were infected, treatment had a significant effect on intensity of *C. bombi* per bee ($\chi^2_1 = 8.79$, $P = 0.003$). By the end of the experiment, the intensity of *C. bombi* infection was 1.8-fold higher in bees in the high compared with the low treatment (Fig. 2a). The lack of effect of time (week) is probably due to the fact that we only included data from the last 3 wks of the experiment (see Methods and Fig. 2a). However, in all weeks except week 5, the intensity of infection was higher in the high treatment compared with the low treatment (Fig. 2a).

Fig. 1. Distribution of infected and uninfected individuals in (A) bumble bee (*Bombus impatiens*) colonies where 1 or 30% of initial workers were experimentally infected with *Crithidia bombi* (six colonies per treatment) and (B) honey bee (*Apis mellifera*) colonies where none (control), 1 or 10% of workers were experimentally infected with *Nosema ceranae* (three colonies per treatment).

Fig. 2. Mean ($\pm$ SE) intensity of infection for (A) *Crithidia bombi* in bumble bee (*Bombus impatiens*) colonies where 1 or 30% of initial workers were experimentally infected (six colonies per treatment) and (B) *Nosema ceranae* in honey bee (*Apis mellifera*) colonies where none (control), 1 or 10% of workers were experimentally infected (three colonies per treatment). Shaded area represents the weeks that were included in the statistical models (see Methods). Error bars were jittered to improve clarity.

Apis mellifera Infection With *N. ceranae*

The final best-fit model was the full model. Treatment did not have a significant effect on the intensity of *N. ceranae* ($\chi^2_2 = 4.33$, $P = 0.11$). However, time and the interaction between treatment and time had significant effects on the intensity of infection (time: $\chi^2_2 = 27.55$, $P < 0.0001$; interaction: $\chi^2_5 = 17.44$, $P < 0.001$). Week of the experiment altered how the treatment affected the intensity of *N. ceranae* infection (Fig. 2b), but by the end of the experiment, the average *N. ceranae* concentration per bee was 1.9-fold higher in the high treatment compared with the low treatment, and it was 17-fold higher in the high treatment compared with the control (Fig. 2b). Finally, bee size had a significant effect on the intensity of infection ($X^2_1 = 5.80$, $P = 0.016$), with bigger bees having lower spore counts.

3. To what degree does initial parasite prevalence affect colony growth rate and final colony productivity?

For bumble bees, there was no effect of the treatment on any of the colony performance metrics: total number of workers, drones, queens, worker pupae, queen pupae, larvae and eggs, as well as the total weight ($P > 0.05$ in all cases; Supp Table 2 [online only]). For honey bees, there was no effect of the treatment on the change in number of workers ($F_{2,6} = 0.237$, $P = 0.79$).

Discussion

Conventional epidemiological models generally assume that increasing the proportion of infected individuals in groups should increase transmission probability to susceptible hosts. We tested this assumption in bumble bees infected with the trypanosomatid *C. bombi* and honey bees infected with the microsporidian *N. ceranae*. For *C. bombi*, we found support for our initial prediction, with higher initial *C. bombi* prevalence resulting in a faster spread of the parasite and higher intensity of infection. For *A. mellifera*, results were mixed; we found no effect of the initial parasite prevalence treatment on the spread of the parasite, but a higher initial prevalence increased the intensity of infection in infected bees.

Our results should be interpreted within the context of our controlled, laboratory experimental design. The transmission rates likely represent extreme scenarios, given the confined environment bees were in. Nonetheless, we were able to detect an effect of treatment, so the potentially high transmission rates did not obscure treatment effects. The confined laboratory nature of our experiments also means that colonies were not exposed to environmental factors that

can have synergistic negative effects on colony fitness (e.g., pesticides; Wu et al. 2012, Goulson et al. 2015), or continuous exposure to parasites from the environment. However, our approach provides valuable information as it allowed us to explore within-colony transmission of the parasites without the noise of multiple infection events or influence of external factors.

Bombus impatiens Infected With *Crithidia bombi*

In the case of the trypanosomatid *C. bombi*, it is important to note that in the high treatment, the prevalence of *C. bombi* increased from 25 to 75% from the second to the third week of the experiment. In the low treatment, the prevalence at the end of the experiment was 25%. We think it is unlikely that we would have observed a dramatic increase as in the high treatment if we had let the experiment run for more weeks because a colony is more prone to an epidemic during the growing phase, when the number of susceptible individuals is high (Naug and Camazine 2002). Our results suggest that if only one infection event occurs, or a small number of workers bring *C. bombi* back to a mature colony, within-colony parasite prevalence may never reach high levels.

Parasite prevalence at the end of the colony life cycle is also important in transmission to the next generation. New queens, produced at the end of the colony life cycle, are the only individuals that overwinter, so *C. bombi* must infect them in order to stay in its host population (Shykoff and Schmid-Hempel 1991). A lower within colony prevalence at the time of queen emergence can decrease transmission to new queens (Buechel and Schmid-Hempel 2016). Thus, we could expect that a lower parasite pressure early in the colony cycle would result in lower within-colony spread, and a smaller proportion of new queens infected with *C. bombi* in their natal nest near the end of colony life. Lower prevalence in new queens could have a positive impact on bumble bee populations, because infected queens are less likely to start a colony in the spring (Schmid-Hempel 2001, Brown et al. 2003b).

Some studies indicate that *C. bombi* can impact a bumble bee queen's fitness by reducing colony-founding success and colony size, as well as reducing the number of workers, males and new queens produced by a colony (Brown et al. 2003b). In this study, we did not find any effect of initial parasite prevalence on the different measures of colony performance. This lack of effect is something that we might expect given that colonies were kept in laboratory conditions with no exposure to other stressors or food limitation and has been reported in other studies (Shykoff and Schmid-Hempel 1991). However, in field

conditions, we note that fitness differences at the colony level have been observed with *C. bombi* infection, as the parasite can have negative synergetic effects with stressors like pesticides and malnutrition (Fausser-Misslin et al. 2014, Goulson et al. 2015).

Apis mellifera Infection With *Nosema ceranae*

Two caveats are important in the interpretation of our honey bee results. First, we imposed our treatments upon colonies with underlying *N. ceranae* infection. Although this may have obscured our ability to detect an effect of our treatments, it is rather representative of what a beekeeper would find in field conditions, with a high proportion of colonies infected with *N. ceranae* (44–100%) at low intensity levels (Traver and Fell 2011, Fernández et al. 2012, Szalanski et al. 2013). Second, we used one-frame colonies with ~1,000 workers and one queen, which is similar to a young, recently started colony. It would be important to determine whether *N. ceranae* transmission in mature colonies is any different from what we observed here, given that parasites that are transmitted by direct contact can increase in prevalence and intensity as the host group size increases (Naug and Camazine 2002). Furthermore, as the colony grows and changes in social organization occur (Michener 1998), it is possible that parasite transmission dynamics would be affected (Naug and Camazine 2002). Studies that manipulate honey bee colony size and assess transmission rates would yield additional epidemiological insight. It is also important to note that we did not homogenize the age of the workers that were inoculated at the beginning of the experiment due to practical reasons of the experimental design. The dose of *N. ceranae* we used to infect workers (46,000 spores) was at least 4–10 times higher than the ID_{50} for all ages of honey bee workers (Huang et al. 2015), so we suspect the lack of age homogenization minimally impacted our results. However, it is possible that not controlling for age increased variability within treatments.

We did not find an effect of initial parasite pressure on the within-colony prevalence of *N. ceranae*. The lack of an effect could be due to multiple nonmutually exclusive mechanisms, two of which we describe here. First, the lack of an effect could be due to the short, 5-wk duration of the experiment, which might not be enough time to detect an effect, as changes in *N. ceranae* prevalence are usually observed over seasons or years (Higes et al. 2008; Gisder et al. 2010, 2017). Second, the slow within-colony spread could be due to honey bee social network structure and behaviors such as allogrooming (Konrad et al. 2012, Guzman-novoa and Morfin 2019). Additional network and behavioral studies would be required, however, to understand how these factors affect the link between parasite pressure and within-colony prevalence.

We did find that higher initial parasite pressure increased the parasite loads per individual bee. *Nosema ceranae* reaches the highest number of spores in the gut ~15-d postinfection (Paxton et al. 2007). We started observing higher spore counts after the third week of the experiment (Fig. 2b), so it is possible that *N. ceranae* only started spreading in the colony after the group of originally inoculated bees developed high spore counts, and we observed the increase in spore counts of the secondary infections in the colony. Higher per-bee parasite loads in the colony could also facilitate the spread of *N. ceranae* during the winter, when bees remain in the colony and prevalence tends to increase (Gisder et al. 2017), possibly leading to higher chances of colony collapse (Higes et al. 2008).

Parasite Transmission and Future Directions

Taken together, our results suggest that initial parasite pressure is an important factor that can determine the outcome of an infection at

the colony level in honey bees and bumble bees. These results also seem to be consistent with the theory that social immunity helps to control the spread of parasites in honey bees up to certain point (Godfrey 2013, Liu et al. 2019). This pattern still needs to be tested in more natural conditions, where colonies are exposed to other environmental factors that can influence the host–parasite interaction, including pesticides and other parasites. In the case of honey bees, following the within-colony spread of the parasite for a longer time period would be important to fully understand the transmission dynamics and effect of parasite pressure.

Our results also indicate that measures that reduce the acquisition of parasites in the environment would help reduce within-colony transmission by reducing parasite pressure. This is also likely to have a positive feedback effect given that slower within-colony transmission would reduce the number of infected individuals that could spread parasites in the environment. The use of flowers with low parasite transmission probability could be an option to achieve lower parasite pressure of bees returning to colonies (Adler et al. 2018), as well as better monitoring and control of parasites in commercial pollinators, in order to reduce the spillover of pathogens to wild bees (Goulson and Hughes 2015). As studies find that interspecific transmission and prevalence of bee parasites can be higher than what was once thought (Gisder and Genersch 2017, Müller et al. 2019), understanding transmission dynamics and factors that affect it are critical for developing strategies that can slow the spread of parasites and diseases in commercial and wild pollinators.

Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

Data Availability Statement

Data from this study are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.qfttdz0f4> (Pinilla-Gallego et al. 2021).

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