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Honey Bee (*Apis mellifera*) Pollen Foraging Reflects Benefits Dependent on Individual Infection Status

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Abstract

Parasites often modify host foraging behavior, for example, by spurring changes to nutrient intake ratios or triggering self-medication. The gut parasite, *Nosema ceranae*, increases energy needs of the European or Western honey bee (*Apis mellifera*), but little is known about how infection affects foraging behavior. We used a combination of experiments and observations of caged and free-flying individual bees and hives to determine how *N. ceranae* affects honey bee foraging behavior. In an experiment with caged bees, we found that infected bees with access to a high-quality pollen were more likely to survive than infected bees with access to a lower quality pollen or no pollen. Non-infected bees showed no difference in survival with pollen quality. We then tested free-flying bees in an arena of artificial flowers and found that pollen foraging bees chose pollen commensurate with their infection status; twice as many infected bees selected the higher quality pollen than the lower quality pollen, while healthy bees showed no preference between pollen types. However, healthy and infected bees visited sucrose and pollen flowers in the same proportions. Among hive-level observations, we found no significant correlations between *N. ceranae* infection intensity in the hive and the proportion of bees returning with pollen. Our results indicate that *N. ceranae*-infected bees benefit from increased pollen quality and will selectively forage for higher quality while foraging for pollen, but infection status does not lead to increased pollen foraging at either the individual or hive levels.

Keywords *Apis mellifera* · Parasites · *Nosema ceranae* · Pollen preference · Foraging behavior · Hive

Introduction

Gut parasites are a common, but little understood, driver of food preferences and foraging behavior. Gut parasites can impose metabolic challenges on their hosts and increase energy requirements when they compete with the host for nutrients or trigger immune responses [1, 2]. As a result, the host's appetite, nutritional preferences, and food-seeking behavior may change [3–6]. While many dietary changes will benefit the parasite to the detriment of the host, some may also enable hosts to resist parasite infections or mitigate their effects [3, 7].

Two possible dietary responses to infection are altering nutrient intake ratios, a form of compensatory feeding [8, 9], and self-medicating [10]. Hosts may change the ratio of carbohydrates to protein consumed to effectively combat their infections [9, 11–13]. For example, virus-challenged caterpillars (*Spodoptera* spp.) increase their survival by choosing a diet with a higher protein: carbohydrate ratio [12, 13]. In contrast, locusts infected with a fungal pathogen are better off with a lower protein: carbohydrate ratio, presumably because the fungus is more efficient at exploiting protein than the host is at increasing its immune response [9]. These or other dietary changes that result in decreased parasite load and increased host fitness may be considered self-medication if the same changes would be harmful to healthy individuals [10]. For example, consumption of whole unchewed leaves by chimpanzees (*Pan troglodytes schweinfurthii*) irritates the intestines and causes diarrhea, but also reduces parasitic worm load [14]. It can be more difficult to demonstrate fitness costs in healthy individuals and therefore distinguish true self-medication from an adaptive diet choice when host diet changes are quantitative in nature rather than qualitative [10].

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Dietary changes that benefit the host can increase parasite survival in some cases [15]. For example, parasitized *Platyprepia virginalis* caterpillars increase their survival, as well as the weight of the emerging tachinid fly they have hosted, by choosing to feed on poison hemlock instead of lupine, the preferred food of non-parasitized caterpillars [16]. Likewise, well-fed *Daphnia* infected with the bacterium *Pasteuria ramosa* have increased survival but also produce more of the bacterial transmission-stage spores relative to undernourished *Daphnia* [17]. Even if the only observable effect is increased survival or longevity of the host, the parasite may benefit indirectly by having more time for reproduction and/or transmission to a new host [18].

Increased parasite transmission is a key criterion distinguishing a parasite-induced behavior from an incidental behavioral byproduct of infection [19]. In the above cases, if the tachinid fly is indeed more likely to survive to parasitize another caterpillar host, and the increased number of bacterial spores increases transmission of the bacterium to other *Daphnia*, then these are likely examples of parasite manipulated behaviors [15]. Parasite exploitation of host compensatory responses, dietary or otherwise, has only recently been hypothesized to be a form of host manipulation [7, 15, 20]. Parasites need only induce a fitness cost to induce a compensatory response in the host, and therefore theory suggests that natural selection will favor parasites that exploit such responses whenever they improve transmission [15, 20]. Thus, distinguishing parasite-induced behaviors from incidental responses to infection is important for understanding the evolution and maintenance of parasite-host relationships.

The evolution and maintenance of parasite-host relationships, and their effect on foraging behavior are particularly relevant and interesting to understand in social insects such as *Apis mellifera.* One practical reason is that their food preferences underpin their pollination service, which in turn is worth tens of billions of dollars to the global agricultural economy [21]. From an eco-evolutionary perspective, their sociality offers opportunities for further investigating behavioral responses to parasitism. Their sociality increases the pathways for, and likelihood of, parasite transmission but also provides the option of responding to infection either individually or collectively, such as with a behavioral fever [22]. Sociality also centralizes reproduction away from foraging individuals that are most likely to become parasitized and therefore forces natural selection to operate at the colony level. Thus, both individual and colony behavior need to be considered when investigating parasite-driven host behavior.

Across its global distribution, *A. mellifera* contends with dozens of parasites and pathogens [23]. One of these parasites is *Nosema ceranae*, a globally widespread gut microsporidian that decreases longevity of individuals and hives [6, 24–26]. *N. ceranae* was first described in the Asian honey bee, *A. cerana*, in 1996 [27], and was subsequently detected in *A. mellifera* in Europe and Taiwan in 2005 [28]. It has apparently been present in *A. mellifera* in many parts of the world for much longer [29–31]. *N. ceranae* infections are most prevalent in forager bees [32], which typically forage for either carbohydrate-rich nectar or protein-rich pollen. The parasite increases energetic demands of infected *A. mellifera* individuals, leading to higher carbohydrate consumption [25, 33]. If individual energetic demands dictate foraging choices, then we might predict that infected bees would be more likely to forage for nectar, which on average has a higher ratio of calorie return for the energy expended in collecting it (10:1) than pollen (8:1) [34]. However, subsequent studies have found that *N. ceranae*-infected individuals fed pollen have increased survival corresponding with pollen quality [35] and quantity [36] even as the *N. ceranae* spore load increases with these diets. All of these studies were no-choice experiments of captive individual bees. The obvious and interesting question is whether infected foraging bees would make foraging choices to maximize their immediate energy gain or their survival. To date, no studies have tested the foraging preferences of free-flying bees infected with *N. ceranae*. Hive-level effects of *N. ceranae* on foraging choices also have not been studied, despite hive-level changes in foraging causing potentially greater concerns for both hive management and pollination. For *A. mellifera*, individuals choose which specific flowers they will visit, but which resource they collect is largely determined by the needs of the hive [34]. Infected hives rarely reach 100% infection by *N. ceranae* and have wide variation in spore loads among individuals within the hive [37]. If individual and hive-level needs differ, then we predict that the needs of the hive will ultimately dictate foraging behavior.

In this study, we investigated the effects of the parasite *N. ceranae* on the foraging behavior and survival of *A. mellifera*. We specifically asked the following: (1) What is the effect of diet on infection intensity and survival of infected individual *A. mellifera*?; (2) Do foraging choices of individual free-flying bees correspond with those effects?; and (3) Is foraging behavior at the hive level associated with infection intensity? We answered these questions by experimentally infecting bees with *N. ceranae* and comparing survival rates and *N. ceranae* spore loads among bees provided only sucrose or sucrose plus a low- or high-quality pollen. Next, we presented an artificial flower array offering the same three resources to free-flying *A. mellifera* to assess floral resource choice by individual bees and its relationship to *N. ceranae* infection status. Finally, we observed foraging behavior at the hive level to investigate pollen foraging behaviors in relation to *N. ceranae* infection at the hive level.

Methods

Survival Experiment

We purified *Nosema ceranae* spores from locally collected *Apis mellifera ligustica* using the triangulation method [38]. We created an inoculum by diluting purified spores with 50% (1:1 weight: volume) sucrose solution to a concentration of 1×10^4 spores per 5 μL. Each inoculum was used within 2 days to avoid loss of spore viability [38]. Prior to use, we confirmed the presence of *N. ceranae* and the absence of sister species *N. apis* with polymerase chain reaction (PCR) (Online Resource 1).

To set up the treatment groups for the experiment, we transferred frames of capped *A. mellifera* brood from three hives into a growth chamber at 30 °C and 60% relative humidity [38]. Approximately 16 h later, we separated newly emerged bees into six separate groups of 50 and starved each group for 2 h. We individually fed bees in three randomly selected groups with 5 μL of the 50% sucrose solution containing 1×10^4 *N. ceranae* spores, and individually fed bees in the remaining three 5 μL of 50% sucrose solution without spores. We offered each treatment as a droplet from a micropipette tip. Individuals that did not consume the entire solution were discarded from the experiment. Each group was placed in a hoarding cage and randomly selected to receive one of three diets in a fully factorial design of diet x inoculation. All three diets included 50% sucrose solution ad libitum via two 10-mL syringes attached to the lids. We provided one inoculated and one non-inoculated group per replicate with ad libitum sucrose solution only. The second diet included ad libitum gamma-irradiated red gum (*Corymbia calophylla*) pollen, and the third diet included ad libitum gamma-irradiated white gum (*Eucalyptus wandoo*) pollen. Both pollens are commercially available as bee-collected pellets from Saxonbee in Western Australia. We ground the pollen with a coffee grinder and offered pollen in 5-mL weigh boats as a paste made up of 9 parts pollen: 1 part water [39]. As per pollen storage guidelines, we irradiated the pollen to remove pathogens and kept pollen frozen at − 20 °C until use to reduce nutritional loss [40]. The two pollens are closely related and are thus likely similar in structure and digestibility. Red gum pollen is a higher quality pollen than white gum, containing more crude protein and higher amounts of 18 amino acids and some minerals and fatty acids [41, 42] (Online Resource 2; Dr. Rob Manning unpublished data, 2015).

Cages were returned to the growth chamber, and daily measurements were taken to record volume of sucrose solution consumed, weight of pollen paste consumed, and mortality rates. We removed dead *A. mellifera* from cages and replaced food resources daily, and removed an additional bee per cage on days 4, 6, 8, and 10 post-inoculation to assess ongoing cage infection levels. Removed bees were not included in survival calculations. We performed eight replicates for each treatment.

Removed bees were stored at − 20 °C and individually processed by dissecting the gut and vortexing for 90 s with 1 mL of deionized water and a 2-mm steel ball-bearing. We quantified spore loads following the method described by Cantwell [43] using a hemocytometer. We sacrificed all remaining bees on the 19th day of the experiment and conducted spore counts on each individual.

Floral Resource Choice Experiments

To investigate whether individual *A. mellifera* foraging choices correspond with survival experiment outcomes, we presented an array of artificial flowers containing different resource options to bees in an apiary with four hives at James Cook University Cairns Campus in Smithfield (16.82° S, 145.68° E) and an apiary with 15 hives approximately 21 km away in Bentley Park (17.00° S, 145.73° E), both in Queensland, Australia. Each of the 16 flowers in the array was made of eight 3-cm diameter "petals" made of laminated yellow construction paper with white floral guides around a 3-cm diameter blue plastic cap from a 50-mL vial (ProSciTech Pty Ltd). Each cap was either left empty or filled with 8 mL 50% sucrose solution, 5 g gamma-irradiated red gum pollen, or 5 g gamma-irradiated white gum pollen (Saxonbee, Western Australia), such that there were four flowers for each of the four resources. To ensure ease in handling, we ground pollen for 10 s with a coffee grinder prior to experimental use. Flowers were mounted on wooden skewers and positioned in two numbered rows of eight within a polystyrene base, 10 cm apart on each side, with resources distributed randomly.

We placed the array on a metal stand at a height of 75 cm and a distance of 5 m from the nearest hive 24 h before the observation period to allow *A. mellifera* to recruit to the resource patch. Before the observation period, we re-randomized the position of flowers offering each resource. Between 10 am and 11 am on each observation day, we captured each bee that landed on a flower, recording flower position within the array and time of visitation. Captured individuals were placed in kill jars and then stored in labeled individual glassine envelopes. We then collected at least 50 bees directly from four hives in the apiary by placing an open 750-mL plastic container over the hive entrance and sealing after bees were contained. Collected bees were stored at − 20 °C. The spore load of each individual bee collected from the array was determined as described for survival experiments [38, 43]. For the hive samples, we counted spores for five groups of ten bees per hive after removing the abdomen of each bee and macerating them together in 10 mL of water. We confirmed the presence of *N. ceranae* and absence of *N. apis* in bees with spores with PCR (Online Resource 1). We

repeated the experiment at each apiary four times, with 2 weeks in between each trial.

Hive Observations

We observed bees from 14 *A. mellifera* hives across three apiaries to investigate hive-level interactions between *N. ceranae* infection and foraging. Four hives were observed at each of the apiaries used for the floral choice experiment, and six were observed at a third apiary near Mareeba (16.98° S; 145.48° E), which is 28 and 25 km away from the Smithfield and Bentley Park apiaries, respectively. Observations took place for two 15-min periods, one between 10 am and 11 am and one between 2 pm and 3 pm, on a single day every 2 weeks at each location for 2 months. During each observation period, we counted both the total number of foragers returning to the hive and number of foragers returning to the hive carrying pollen in their corbiculae. Returning drones were not counted as they do not forage.

Following each observation period, we collected the first five foragers returning to the hive using fine-tip surgical steel forceps. Bees were placed individually in kill jars initially and then stored in individual glassine envelopes at − 20 °C. We removed pollen bundles from the corbiculae using forceps and recorded weights of the collected pollen in addition to the weights of each bee. Individual spore counts were conducted following the method described previously. We also sampled 50 bees from each hive following the method described for floral resource choice experiments, counted spores in five groups of ten, and conducted PCR to confirm spore type.

Statistical Analyses

For the survival experiment, we performed statistical analyses on spore load, pollen consumption, sucrose consumption, and the proportion of bees surviving. We used two separate models to analyze pollen consumption and sucrose consumption, respectively. Inoculation treatment and diet were included as fixed effects for all three models, and cage was a random effect in both consumption models. To determine the effect of diet and *N. ceranae* infection on *A. mellifera* survival, we used a quasi-binomial generalized linear model with success/failure notation. We did not use a mixed model because preliminary analyses indicated that variance among cages was unimportant for survival. Furthermore, using quasi-binomial distribution allowed us to account for overdispersion, but the distribution is not well-suited for use in mixed models [44]. To better understand the interaction between diet and infection, we used Tukey's tests in the interaction term with the R package multcomp [45] to evaluate whether diet significantly affected survival for infected and uninfected bees separately. To ascertain whether diet influenced the spore counts of inoculated bees throughout the study, we used a generalized linear model with a negative binomial distribution. Spore count refers to the mean of the two counts per bee taken when quantifying spores. Diet and days post-inoculation were included as fixed effects. We attempted to include cage as a random effect but chose to exclude this due to convergence issues.

To investigate whether *N. ceranae* infection influences foraging decisions of *A. mellifera*, we compared individual infection status with the reward selected from the artificial flower array. Bees were considered infected when a minimum of two spores were observed during microscopic examination. We used quasi-binomial models to determine whether there was an effect of infection presence/absence on reward choice. We fit one model to analyze the effects of infection status on pollen collection (grouped red gum and white gum) compared to sucrose, and a second model to evaluate the effect of infection status on the selection of red gum versus white gum pollen. Apiary was also included as a fixed effect in each model. Observation date was initially included as a random effect but was removed because it did not improve model fit, and removing it allowed us to model with a quasi-binomial distribution that better accounted for overdispersion.

We evaluated the effect of hive-level spore loads on the total number of foragers observed and the proportion of those foragers collecting pollen. For both response variables, we used a binomial generalized linear mixed model with mean spore count (taken from five groups of ten bees) and time of day as fixed effects and hive and observation date as random effects. We included an observation-level random effect to absorb excess variation and reduce overdispersion [46].

We also analyzed the effect of infection status on resource collection (pollen/not pollen) and amount of collected pollen. We used a binomial generalized linear mixed model with success/failure notation to examine the influence of infection on pollen presence in the corbiculae (pollen baskets) and a generalized linear mixed model with Poisson distribution to analyze the effect of infection on pollen weight. For both models, infection status (infected/not infected) and time of day were included as fixed effects, and date and an observation-level effect (to account for overdispersion) were included as random effects. Hive was not included as a random effect due to model convergence issues. As with the floral array experiment, we considered a bee to be infected if we detected at least two spores.

We conducted all analyses in R i386 3.2.1 and R Studio using the lme4 package [47–49]. We conducted all statistical inference tests using likelihood ratio tests, assuming a chi-squared approximation.

Results

Survival Experiment

We detected *Nosema ceranae* spores in inoculated *A. mellifera* in 21 out of 24 inoculated groups by day 4 post-inoculation and in all inoculated groups by day 6. Bees from non-inoculated cages remained largely uninfected, with only 12 out of 1200 individuals across 11 cages presenting with spores. After 19 days, significantly more non-inoculated bees remained alive compared to inoculated bees (Fig. 1, Tables 1 and 2). Survival depended on both inoculation status and diet. The higher quality red gum pollen provided a greater survival benefit over the white gum and sucrose only treatments for inoculated groups (both $p < 0.00005$) (Fig. 1, Tables 1 and 2). Among the non-inoculated bees, there was no significant variation in survival rates among diet groups (Fig. 1). Average daily sucrose and pollen consumption were not affected by diet, inoculation status, or their interaction (Tables 1 and 2).

Spore loads of inoculated groups significantly increased with time and inclusion of either pollen type in the diet. Bees with access to pollen had increased spore loads over time, but spore load did not significantly differ by pollen type provided (Table 2).

Floral Resource Choice Experiment

Across all eight observation periods, we observed 187 bees visiting flowers and captured 184 of these. The three individuals that were not captured were excluded from analyses because of the lack of infection data. Only five bees visited the "No reward" group of flowers so these also were excluded from statistical analysis. Among the captured bees, approximately one third were infected (61 out of 184). Infection status did not significantly influence the proportion of *A. mellifera* that selected sucrose flowers compared to pollen flowers (Fig. 2, Table 2). However, infected bees that foraged for pollen were more likely to select red gum pollen than white gum pollen, whereas non-infected bees did not distinguish between the two (Fig. 2, Table 2). Spore load did not differ significantly between infected red gum and white gum pollen foragers (Table 2).

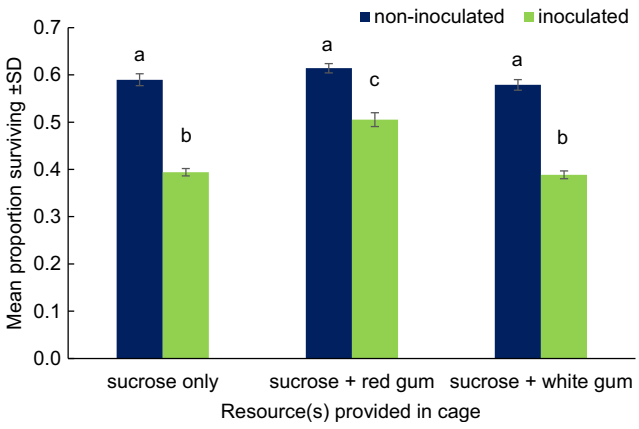

Fig. 1 Mean proportion ± SD of bees in each cage surviving the 19-day survival experiment by inoculation and diet treatments. Letters above bars indicate significant differences at $p < 0.00005$. Table 2 shows generalized linear model results

Table 1 Median and range of *N. ceranae* mortality, sucrose and pollen consumption, and infection intensity (spore load) with response variables from the survival experiment

Variables	*N*	Median	Range
Survival (percentage survival to 19 days)			
Inoculated, sucrose only	8	40.22	34.78–43.48
Inoculated, red gum pollen	8	51.09	45.65–54.35
Inoculated, white gum pollen	8	39.13	34.78–43.48
Non-inoculated, sucrose only	8	58.70	56.52–63.04
Non-inoculated, red gum pollen	8	59.78	56.52–67.39
Non-inoculated, white gum pollen	8	57.61	54.35–60.87
Mean sucrose consumption per cage (mL/bee/day)			
Inoculated	24	0.035	0.021–0.046
Non-inoculated	24	0.034	0.019–0.057
Mean pollen consumption per cage (mg/bee/day)			
Inoculated, red gum pollen	8	1.60	1.0–2.7
Non-inoculated, red gum pollen	8	1.90	1.45–3.26
Inoculated, white gum pollen	8	2.18	1.20–3.47
Non-inoculated, white gum pollen	8	1.50	1.05–4.34
Spore load			
Inoculated, sucrose only	400	9×10^5	$0–29.2 \times 10^5$
Inoculated, red gum pollen	400	10.5×10^5	$0–43.7 \times 10^5$
Inoculated, white gum pollen	400	9.5×10^5	$0–33.5 \times 10^5$
Non-inoculated, sucrose only	400	0	$0–8.3 \times 10^5$
Non-inoculated, red gum pollen	400	0	$0–6.5 \times 10^5$
Non-inoculated, white gum pollen	400	0	$0–8.8 \times 10^5$

Hive Observations

Across all of our 124 hive observations, the proportion of foragers returning with visible pollen ranged from 0.8–71% (median = 19.2%). Neither the total numbers of foragers observed returning to their hives in the 15-min observation period nor the proportion of foragers returning with pollen varied predictably with hive spore count (Fig. 3, Table 2). Total forager numbers and the proportion of foragers collecting pollen were both significantly lower in the afternoon compared to the morning observation period (Fig. 3, Table 2).

Table 2 Summary of general or generalized linear model results for different response variables in the survival and floral choice experiments and for hive observations

Response and explanatory variables	df	χ^2	p
Survival experiment			
Survival			
Inoculation	1	60.6	< 0.0001*
Diet	1	10.6	< 0.0001*
Inoculation * diet	1	3.5	< 0.0001*
Sucrose consumption			
Inoculation	1	0.04	0.84
Diet	2	2.31	0.31
Inoculation * diet	2	1.83	0.40
Pollen consumption			
Inoculation	1	0.35	0.55
Pollen type	1	1.59	0.21
Inoculation * pollen type	1	0.24	0.62
Spore load			
Diet	2	25.07	< 0.0001*
Days post-inoculation	1	18.32	< 0.0001*
Diet * days post-inoculation	2	9.44	0.0089*
Floral choice experiment			
Pollen choice (red gum vs white gum)			
Infection	1	4.23	0.0396*
Apiary	1	0.017	0.89
Infection * apiary	1	0.29	0.59
Resource choice (pollen vs sucrose)			
Infection	1	0.68	0.41
Apiary	1	0.01	0.91
Infection * apiary	1	0.59	0.44
Spore load (infected pollen foragers only)			
Pollen type	1	0.97	0.32
Apiary	1	0.02	0.89
Pollen type * apiary	1	1.44	0.23
Hive observations			
Total foragers			
Hive spore load	1	0.46	0.4954
Time of day	1	12.9	0.0003*
Hive spore * time	1	0.15	0.70
Proportion of foragers-pollen			
Hive spore load	1	2.16	0.14
Time of day	1	23.3	< 0.0001*
Individual resource choice			
Individual infection status	1	0.97	0.33
Time of day	1	9.09	0.0003*
Infection * time	1	0.51	0.48
Individual pollen load			
Individual infection status	1	0.95	0.33
Time of day	1	14.57	0.0001*
Infection * time	1	0.62	0.44

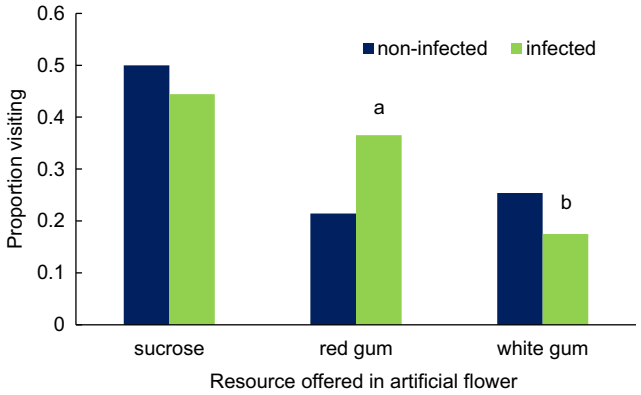

Fig. 2 Numbers of *N. ceranae*-infected and healthy *A. mellifera* visitors observed collecting different resources from the artificial flower array. Letters above the pollen bars indicate significant differences between the two pollens for infected bees. Non-infected bees were not significantly different. Table 2 shows generalized linear model results

Whether or not individual bees collected pollen and the amount pollen foragers collected both depended on the time of day but were not affected by individual infection status (Table 2). Morning foragers were more likely to collect pollen than afternoon foragers. Individual pollen loads ranged from 1 to 36.8 mg and were significantly greater in morning foragers (Table 2).

Discussion

Our results reveal that infected pollen foraging bees were more likely to forage on pollen that increased their longevity. In our first experiment, bees with access to red gum pollen survived longer than bees with access to white gum pollen or sucrose only. Our floral choice experiment revealed that infected pollen foragers were more likely to choose red gum pollen over white gum pollen. Although there have been multiple studies investigating how resource quality affects bee health [50–52], including for bees with *N. ceranae* fed pollen [35, 53, 54], we are not aware of any other studies that have shown that individual diseased *A. mellifera* differ in foraging preferences from healthy bees. Several studies have revealed associations between bumble bee floral foraging choices and infection with gut parasites [55–57]. Gut parasites may be an underappreciated influence on floral foraging choices of honey bees and other pollinators.

We stop short of characterizing the preference for red gum pollen among pollen foragers as self-medication because it only meets two of the four established criteria [10]. The red gum pollen was clearly intentionally chosen by infected free-flying bees (criterion 1), and as shown in our survival experiment, it does increase longevity of infected bees (criterion 3). However, we did not find red gum pollen to be detrimental to the parasite (criterion 2), as evidenced by the higher spore load we observed in inoculated bees with access to red gum pollen

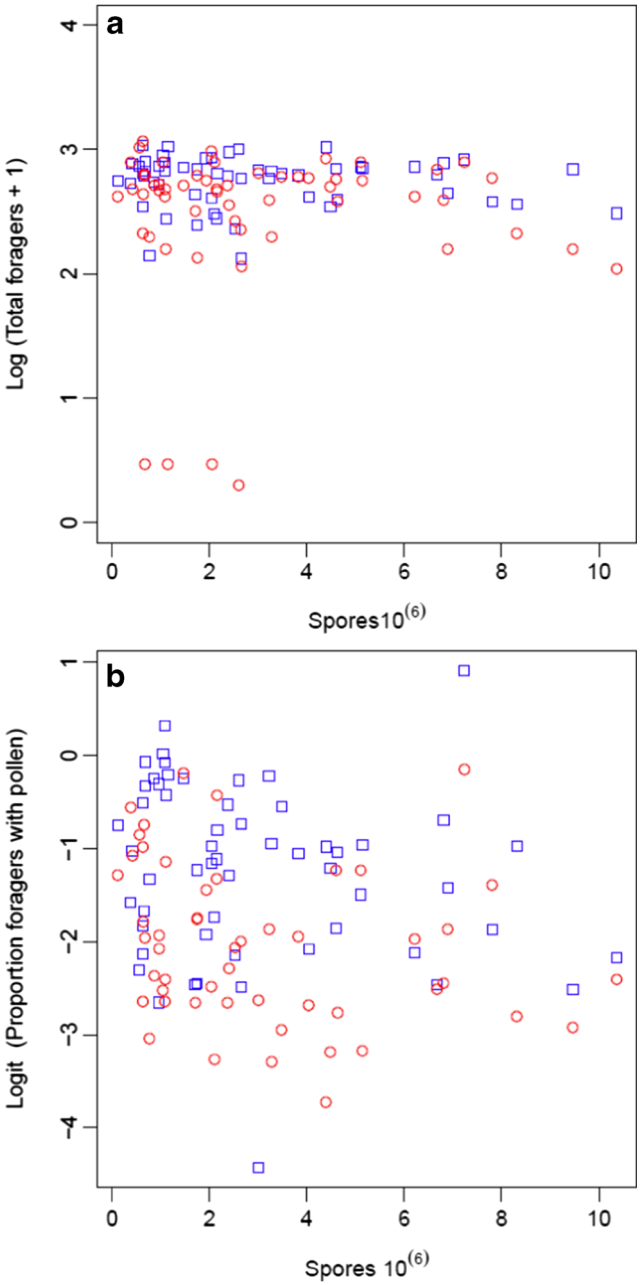

Fig. 3 The relationship between mean hive spore load and **a** total returning foragers and **b** proportion of foragers returning with pollen in the morning (blue squares) and afternoon (red circles). Each data point represents an *A. mellifera* hive. Total foragers and proportion foragers are $\log_{10}(x + 1)$ and logit transformed to match the log and logit link functions for the respective generalized linear mixed model analyses. Table 2 shows generalized linear model results

relative to sucrose. Nor was the red gum pollen detrimental to healthy consumers (criterion 4) as shown by the similar longevity of healthy bees across all diet types in the survival experiment. Two recent studies have described the responses of parasite-challenged honey bees as self-medication. Gherman and colleagues [58] found that *N. ceranae*-infected nurse bees preferentially feed on honey with higher antimicrobial activity leading to a decrease in infection intensity, and Simone-Finstrom and Spivak [59] report that chalk-brood infected colonies increase resin collection. However, neither study showed a detrimental effect of the behavior in the absence of infection. If the behavior does not have an associated cost, then it will be beneficial in all circumstances [10] and is unlikely to be in response to infection.

Despite red gum pollen increasing spore load, at least two lines of evidence indicate that it is unlikely that the parasite manipulated bees to choose red gum pollen in our floral choice experiment. First, spore load increased as much or more in bees with access to white gum pollen. Other studies have also observed an increase in *N. ceranae* spore load in bees fed pollen ([35, 36] and studies reviewed in [60]), possibly because protein leads to mid-gut expansion that facilitates spore production [60]. The protein may also increase tolerance to the pathogen [61], as evidenced by increased spore counts coinciding with increased longevity. Second, if the parasite manipulated bees to choose red gum pollen, we would expect to see more infected bees choose red gum pollen over both sucrose and white gum pollen. Instead, we saw the same proportion of infected bees choosing pollen as was observed for healthy bees, and it was only among the pollen foragers that we saw the preference for red gum over white gum among infected bees. Thus, individual bees can choose food that might benefit themselves, even if it also benefits their parasite, but they are still constrained by what we assume to be the overall nutritional colony needs.

The increased longevity of infected but not healthy bees fed red gum pollen and the preference of infected pollen foragers for red gum is likely because red gum pollen provides nutrients or quantities of nutrients that only infected bees need. Honey bee foragers are known to seek resources that complement their nutritional deficiencies [62], though the mechanistic basis for how foragers choose among pollen types remains to be elucidated [63]. We cannot surmise whether it is a particular mineral or amino or fatty acid, or the higher crude protein content of red gum or a combination of factors that benefits them. In a study of 25 different pollens and some polyfloral blends, longevity of healthy *A. mellifera* increased with pollen protein content [64]. Our findings are similar to Di Pasquale et al. [35] who found that the most protein and essential amino acid-rich pollen species and a polyfloral blend increased survival of *N. ceranae*-infected bees more than other pollen provided. In contrast to our study, Di Pasquale et al. [35] observed all four pollen species that they tested increased longevity for both infected and healthy bees, including a pollen that has protein content similar to white gum pollen. The differences in our findings may be due to differences in the initial nutritional and health status of the experimental bees. We were not able to test the nutritional quality of pollen collected by our observational hives, but we would hypothesize based on our floral choice experiment that as hive infection intensity increased, the quality of the pollen

selected would also increase, assuming the bees have a range of pollen quality available with similar accessibility. Changes in pollen preference of honey bees due to *N. ceranae* infection are important to understand as they may ultimately affect which plants are pollinated.

Consistent with the floral choice experiment, our survival experiment and hive-level observations indicate that infection does not influence the quantity of pollen consumed or collected. In the survival experiment, we observed no significant difference in pollen consumption between healthy and inoculated bees. Our hive observations revealed no significant effect of individual infection status on the weight of pollen collected. Moreover, neither total forager numbers nor the proportion of foragers returning with pollen varied significantly with hive infection intensity. Taken together, these results indicate that the quantitative demands of the hive for pollen remain consistent regardless of in-hive infection levels with *N. ceranae*. Reduced pollen collection at the hive level [65] and reduced pollen carriage for pollination [66] have both been reported for bees infected with the closely related *N. apis*.

Infection did not increase sucrose consumption in our survival experiment, nor did it increase preference for sucrose in our floral resource choice experiments, despite previous studies describing increased carbohydrate demands associated with *N. ceranae* [25, 33]. However, our survival experiment was conducted under different conditions to these studies. Most notably, in both other studies, bees were either inoculated or collected later in life compared to those in our study (5 days post emergence: [33]; foraging age: [25]), and immobilized for handling by anesthetizing with CO_2 or by chilling in a freezer. Age of inoculation and the use of CO_2 anesthesia alter *A. mellifera* survival and *N. ceranae* virulence [67], suggesting that energetic demands may also be altered by experimental conditions. Additionally, some *Nosema*-tolerant bees do not appear to suffer energetic stress when infected [68], suggesting that the source of bees used for the study plays a role in their consumption habits. If *A. mellifera* hives used in our study are somewhat tolerant to *N. ceranae* infection, this may also explain why infected individuals did not show a preference for sucrose solution over pollen within the array.

In summary, our study found evidence of behavioral modification in individual *A. mellifera* infected with *N. ceranae*, seemingly in response to a survival benefit provided by high-quality pollen. Neither the proportion of foragers returning with pollen, nor the amount they collected varied with infection, but further investigations would be useful to determine whether the decisions of infected individuals to collect higher quality pollen are reflected in the quality of pollen in the hive overall. Our work is one of very few studies investigating the behavioral effects of *N. ceranae* on foraging at both the individual and hive levels, encompassing both controlled laboratory experiments and observations of free-flying *A. mellifera*. Our findings may have important implications for the spread of *N. ceranae* on maintenance of pollination services, as *A. mellifera* pollen foraging choices change with infection status. Our work supports and advances work done to-date indicating that gut parasites may have substantial influence on pollinator foraging behavior.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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