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Research

Plant–Insect Interactions

Food Limitation Affects Parasite Load and Survival of *Bombus impatiens* (Hymenoptera: Apidae) Infected With *Crithidia* (Trypanosomatida: Trypanosomatidae)

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Abstract

Bumble bees (genus *Bombus*) are globally important insect pollinators, and several species have experienced marked declines in recent years. Both nutritional limitation and pathogens may have contributed to these declines. While each of these factors may be individually important, there may also be synergisms where nutritional stress could decrease pathogen resistance. Understanding interactions between bumble bees, their parasites, and food availability may provide new insight into the causes of declines. In this study, we examined the combined impacts of pollen and nectar limitation on *Crithidia*, a common gut parasite in *Bombus impatiens* Cresson. Individual worker bees were inoculated with *Crithidia* and then assigned in a factorial design to two levels of pollen availability (pollen or no pollen) and two nectar sugar concentrations (high [30%] or low [15%] sucrose). We found that lack of pollen and low nectar sugar both reduced *Crithidia* cell counts, with the most dramatic effect from lack of pollen. Both pollen availability and nectar sugar concentration were also important for bee survival. The proportion of bees that died after seven days of infection was ~25% lower in bees with access to pollen and high nectar sugar concentration than any other treatment. Thus, nectar and pollen availability are both important for bee survival, but may come at a cost of higher parasite loads. Our results illustrate the importance of understanding environmental context, such as resource availability, when examining a host–parasite interaction.

Key words: *Bombus impatiens*, *Crithidia*, gut parasite, host quality, nutrition

The mutualistic relationships between plants and their pollinators are significant both ecologically and economically (Moeller 2004, Biesmeijer et al. 2006, Committee on the Status of Pollinators in North America 2007, Klein et al. 2007). However, these plant–pollinator interactions take place in the context of other species interactions, including those with herbivores (Strauss 1997, Singer et al. 2012), predators of pollinators (Mooney 2007), and parasites (Schmid-Hempel 2001, Gillespie and Adler 2013). Understanding relationships between multispecies interactions may shed new light on factors influencing pollinator declines. For example, bumble bees (genus *Bombus*) are globally important insect pollinators (Goulson 2009, De Luca and Vallejo-Marín 2013). Recent years have seen a marked decline in various *Bombus* species around the world (Goulson et al. 2008, Grixti et al. 2009, Colla and Ratti 2010), coinciding with declines in other bee species (Oldroyd 2007, Stokstad 2007). While no single factor has emerged as the underlying cause of these declines, pathogen spillover from commercial to wild pollinators (Colla et al. 2006), increased pesticide and herbicide use (Stevens and Jenkins 2013), loss of quality habitats (Goulson et al. 2008), and decreased genetic diversity (Cameron et al. 2011) have all been implicated as potential contributors. In addition to their individual effects, these factors have the potential to interact synergistically (Fauser-Misslin et al. 2014, Goulson et al. 2015). Consequently, bee declines may be exacerbated by the interaction between several factors acting simultaneously (Becher et al. 2013, Goulson et al. 2015).

Increased incidence of parasitism has been implicated as a significant contributor to bumble bee declines (Cameron et al. 2011, Goulson et al. 2015). *Crithidia bombi* (hereafter referred to as *Crithidia*) is a parasite of the bumble bee hindgut which is transmitted between individuals via feces deposited in the nest and on flowers (Durrer and Schmid-Hempel 1994, Cisarovsky and Schmid-Hempel 2014). Surveys of infection frequency in bumble bee populations suggest that *Crithidia* is a relatively common parasite, with some studies finding prevalence rates of up to 80% (Gillespie 2010, Malfi and Roulston 2014). Infection with *Crithidia* reduced worker ability to adapt foraging behavior to new flowers (Gegear et al. 2005) or recognize more rewarding flower choices (Gegear et al. 2006). Bumble bee queens infected with *Crithidia* experienced lower success rates founding new colonies and produced smaller colonies

than healthy counterparts. Infected colonies experienced slower initial growth, diminished production of males, and reduced fitness (Brown et al. 2003b).

Food limitation can shape both the detrimental impacts of *Crithidia* infection on hosts, and the establishment or success of the pathogen itself. In wild bee colonies, nectar and pollen gathered by foragers provides the majority of nutritional resources for individuals in the hive. Bees rely on nectar as their main source of carbohydrates, while pollen is their sole source of dietary proteins and lipids (Brodschneider and Crailsheim 2010). Complete starvation increased the mortality rate due to *Crithidia* infection by 50% in *B. terrestris* L. workers (Brown et al. 2000). Conversely, removal of pollen from *B. terrestris* worker diets reduced pathogen loads (Logan et al. 2005) and the expression of genes relating to host immune function (Brunner et al. 2014). Similarly, infected *B. terrestris* workers had shorter longevity but lower infection intensity when limited to less concentrated sugar water (Sadd, 2011). These studies suggest that both pollen and nectar limitation can reduce *Crithidia* pathogen loads, but are also detrimental to hosts.

In natural settings, flower abundance and quality can vary widely on both short-term and long-term scales (Shibata et al. 2002, Walther et al. 2002, Tuell and Isaacs 2010). Consequently, the availability of pollen and nectar resources to pollinators can be highly variable (Corbet 1978, Wolf et al. 1999, Li et al. 2014, Moisan-Deserres et al. 2014), and colonies may experience fluctuations in food availability or food shortages. Colonies are particularly vulnerable during the time of colony founding in spring. *Crithidia* infection under conditions simulating a longer, more energetically demanding hibernation reduced *B. terrestris* queen performance when founding a new colony (Heinrich 1976, Brown et al. 2003b). Analysis of pollen and honey stores in *Apis* colonies indicate that some plants are visited only for nectar rewards, indicating that different plant species provide different nutritional rewards to foraging pollinators (Sajwani et al. 2014). Thus, limited access to pollen and nectar is a realistic possibility in natural environments, particularly in agricultural settings that may have less diverse floral resources (Kennedy et al. 2013) or proximity to managed honey or bumble bees that can compete for resources (Graystock et al. 2014).

In this study, we examined the combined impacts of pollen and nectar limitation on *Crithidia* infection in workers of the common Eastern bumble bee, *Bombus impatiens* Cresson. Although the effect of nutritional stress on *Crithidia* loads, immune function, and bee performance has already been extensively studied in *B. terrestris* (Brown et al. 2000, 2003b; Logan et al. 2005; Brunner et al. 2014), susceptibility to pathogens can vary widely among bumble bee species (Cameron et al. 2011, Ruiz-González et al. 2012) and even across host and parasite genotypes (Schmid-Hempel et al. 1999, Wilfert et al. 2007, Salathe and Schmid-Hempel 2011, Barribeau et al. 2014). Furthermore, previous work assessed the role of pollen starvation (Brown et al. 2003b, Logan et al. 2005, Brunner et al. 2014), nectar limitation (Sadd 2011), or total starvation (Brown et al. 2000) on parasite loads, but no single study has compared the effects of pollen and nectar limitation simultaneously. Our study assessed how pollen limitation, nectar limitation, and their combination affected *Crithidia* pathogen loads and survival in individual *B. impatiens* workers.

Materials and Methods

Source of Experimental Bees

Four *B. impatiens* colonies (BioBest Pollination Services, Ontario, Canada) provided uninfected workers for the experiment. Colonies were given 30% (weight/volume) sucrose solution and pollen loaves ad libitum for the duration of the experiment. Pollen loaves were prepared by grinding honey bee-collected pollen (Koppert Biological Systems, Howell, MI) with 30% sucrose solution to a clay-like consistency; each colony received a cylinder ~1 by 3 cm every other day. Use of a sucrose solution in place of nectar enabled us to more precisely manipulate dietary treatments; this practice has also been used in previous experiments in our study system (Sadd 2011). Use of commercial honey bee-collected pollen, which likely contains many of the same types of pollen that would be collected by bumble bees (Heinrich 2004), is also a standard practice in studies that test the effects of diet on *Crithidia* infection in *Bombus* (Manson et al. 2010, Baracchi et al. 2015). To obtain individual workers, mature pupal clumps were periodically removed from each colony, placed in an incubator in the dark at 27 °C, and monitored for the emergence of new adults (callows). After emerging, individual bees were randomly assigned to one of the four experimental treatments and isolated in a plastic 20-ml snap-cap vial. Callow mass was recorded as an estimate of size at emergence. For the first two days after emergence, all callows were provided with 500 µl of 30% sucrose solution and 0.1–0.2 g of the pollen–sucrose mixture daily. Experimental treatments began immediately after bees were inoculated with *Crithidia* (see below). Bees were inoculated 1–2 d postemergence.

Infection of Experimental Bees

Crithidia inoculum was prepared daily from previously infected workers removed from laboratory-maintained source colonies. The source colonies were commercial *B. impatiens* colonies (Biobest Pollination Services, Ontario, Canada) infected with *Crithidia* from local wild *B. impatiens* (from 42° 23′20″ N, 72° 31′21″ W and 42° 24′31″ N, 72° 31′43″ W). A recently discovered species, *Crithidia expoeki*, can co-occur with *C. bombi* and is difficult to distinguish without molecular analysis (Schmid-Hempel & Tognazzo, 2010). Because we used visual identification of *Crithidia*, we cannot rule out the possibility that we infected bees with *C. bombi* and/or *C. expoeki*. All source bees for this experiment were infected with *Crithidia* from bees of the same source colony. Five experimental bees from each experimental colony were checked for *Crithidia* infection upon arrival, and colonies were housed separately to prevent cross-contamination.

Inoculum was freshly prepared on each day of the experiment. Ten bees were removed from a source colony and dissected with sanitized forceps. The hindgut of each bee was removed, ground in 300 µl of distilled water with a plastic pestle, vortexed, and allowed to rest for 3 h. We then counted the number of *Crithidia* cells in a 0.02-µl aliquot of a 10-µl sample of each gut solution using a hemacytometer to determine the *Crithidia* cell concentration. The three gut solutions with the highest *Crithidia* cell concentration were used to prepare the experimental inoculum, which was diluted with distilled water to reach a concentration of 1200 cells/µl. The resulting solution was combined with an equal volume of 50% sucrose solution to yield the final inoculum. Bees entering the experiment were fed a 10 µl drop of inoculum (6,000 *Crithdia* cells in 25% sucrose solution), within the range of cell concentrations in the feces of infected bees (Logan et al. 2005). Any bee that did not consume the inoculum within 30 min was excluded from the experiment.

Experimental Design

At emergence, each callow was randomly assigned to one of the four diet treatments that were imposed postinoculation. Pollen

availability (pollen vs. no pollen) and sugar concentration in artificial nectar (high-sugar vs. low-sugar) were manipulated in a 2 × 2 factorial design. Bees in the "pollen" treatment received a daily allotment of the pollen–sucrose mixture, while bees in the "no pollen" treatment did not receive any pollen postinoculation. The pollen–sucrose mixtures were formed into < 5 mm balls and dipped in beeswax (total mass ~0.1–0.2 g). Bees in the high-sugar treatment received a daily supply of 500 µl of 30% sucrose solution, while those in the low-sugar treatment received 500 µl of 15% sucrose solution. Both solutions were prepared using distilled water. These sucrose concentrations were chosen to reflect a typical range from flowering plants (Baker 1978) and because they were similar to those used in a previous experiment (12, 20, and 50% [Sadd 2011]).

Bees were transferred to clean snap-cap vials and provided with fresh food each day until dissection 1 wk postinoculation. Nectar was provided by adding sucrose solution to 2-ml microcentrifuge "feeder tubes" fitted with a dental cotton wick and inserted into a hole in the vial cap. Wax-dipped pollen balls were weighed individually and placed in the clean vial prior to the bee's transfer. After transferring the bee to the vial, the vials were stored horizontally in an incubator in darkness at 27 °C. We dissected bees 7 d postinoculation; although the trajectory of infection varies across bees, parasite levels generally reach an asymptote 7–10 d after inoculation (Schmid-Hempel and Schmid-Hempel 1993).

Response Variables

Consumption of nectar and pollen (when relevant) was recorded for each bee daily. Prior to transfer of bees to a new tube, the filled feeder and pollen allotment (if applicable) were separately weighed. After transferring the bee to the fresh vial, the old pollen and feeder were weighed, and the difference in weight was used to estimate the amount consumed during the 1-d interval. Feeder tubes were changed daily, and the differences in weights between days were used to estimate evaporation rates. To account for variability in weight measurements due to evaporation of nectar, six vials without bees were used to measure evaporation rate from lids with 15% and 30% sucrose solutions ($n = 3$ per sucrose concentration) for a subset of 24 d. Feeder tubes were changed daily, and the difference in weights each day was used to estimate evaporation rate. The evaporation rates from the three replicates were then averaged within days to estimate the daily evaporation rates for 15% and 30% sucrose solutions. These evaporation values were subtracted from the amount consumed by each bee each day. For days on which evaporation was not recorded, we calculated the mean evaporation rate of all days for each sugar concentration and used that value in place of the daily average.

Crithidia load in each bee was assessed 7 d after inoculation (Manson et al. 2010, Richardson et al. 2015). Bees were dissected and *Crithidia* cells counted as described for preparing inoculum, except that the ground gut solution was allowed to rest for 5 h. In addition, the right forewing of each experimental bee was removed and affixed to a glass microscope slide, and the length of the radial cell was measured to use as a covariate of bee size (Harder 1982). For bees that died prior to dissection, the date of death was recorded and the consumption data for that date were not recorded.

Data Analysis

We analyzed data using R version 3.1.2 (R Core Team 2014). Our initial sample sizes were 54–57 bees in each treatment combination, which were used for survival analysis. Due to differential survival, analysis of parasite load had 50 bees in the high-sugar/pollen treatment combination, and 32–36 bees in all other treatment combinations. Nectar and pollen consumption measurements varied from 39–53 replicates per treatment combination due to loss of some measurements from fecal contamination or leakage from the nectar feeder.

Parasite Load

Effects of diet on parasite load were analyzed using a penalized quasi-likelihood mixed model (function "glmmPQL" in package "MASS" [Venables and Ripley 2002]) with a log link function. Total *Crithidia* cell count in five squares of the hematocytometer grid (0.02 µl gut extract) was used as the response variable. Pollen availability, nectar sugar concentration, and their interaction were used as predictor variables. The model also included bee colony of origin as a fixed predictor, bee mass at time of emergence and number of days from emergence to inoculation as covariates, and inoculation date as a random effect to control for day-to-day variation in inoculum potency. Statistical significance of individual predictors was assessed using χ^2 tests (Fox and Weisberg 2011). Bees that died before the scheduled dissection date (7 d postinoculation) were excluded from the analysis. The final data set included 151 bees.

Survival

Death hazard rates were estimated using a Kaplan–Meier survival curve and compared with a cox proportional hazards test (Therneau 2015). A survival object consisting of bee survival time (in days) and occurrence of death before 7 d postinoculation was used as the response variable, with pollen availability, nectar sugar concentration, and their interaction as predictors. The original model included colony of origin as a fixed predictor, bee mass and number of days from emergence to inoculation as covariates, and inoculation date as a random effect. These terms were removed from the final model because they did not explain significant variation in survival ($P > 0.20$ in χ^2 tests).

Nectar and Pollen Consumption

Nectar and pollen consumption were analyzed using a mixed model analysis of variance (Bates et al. 2015 p. 4). For nectar, evaporation-corrected consumption was used as the response variable. Pollen availability, nectar sugar concentration, and their interaction were used as predictors, with colony of origin included as a fixed effect. An intercept for each bee was included as a random effect to account for repeated measures. Date of trial and number of days since inoculation were included as random effects. Bee mass and number of days from emergence to inoculation were initially included as covariates, but were removed from the final model because they did not explain significant variation in survival ($P > 0.20$ in χ^2 tests). Consumption values exceeding 500 mg before correcting for evaporation were deemed implausible and excluded *a priori* (17 out of 527 measurements). Least squares means for each diet treatment were used for graphing (Lenth and Hervé 2015). The final analysis included 510 measurements on 191 experimental bees.

The effect of sugar concentration on pollen consumption was measured on the pollen-fed subset of experimental bees using a repeated measures analysis of variance with bee mass included as a covariate. A random intercept for each bee was included as a random effect to account for repeated measures. Date of trial (i.e., date of pathogen load measurement) was included as a second random effect. Bee colony, number of days from emergence to inoculation, and number of days since inoculation were tested but excluded from the final model ($P > 0.20$ in χ^2 tests). Consumption values exceeding

100 mg were deemed implausible and excluded a priori (5 out of 147 measurements). The final analysis included 142 measurements on 82 experimental bees after exclusion of 4 outliers (standardized residual > 2.5).

Results

Parasite Load

Pollen starvation resulted in significantly lower *Crithidia* loads (adjusted mean ln(count +1): with pollen: 3.94 ± 0.14 SE, without pollen: 2.63 ± 0.34 SE). Parasite loads were also lower among bees fed low sugar nectar (high sugar: 3.46 ± 0.20 SE, low sugar: 3.10 ± 0.31 SE; Fig. 1, Table 1), but there was no significant interaction between the effects of pollen availability and sugar concentration. Parasite load varied significantly among bees from the four experimental colonies (Table 1). Parasite load was negatively correlated with the amount of time between emergence from pupation and inoculation with *Crithidia* ($\beta = -0.81 \pm 0.28$ SE).

Survival

Pollen availability and nectar sugar concentration had interactive effects on survival (Table 2). Relative to the bees with access to both pollen and high sugar nectar, rate of death was markedly increased by either pollen starvation (95% CI for odds ratio: 1.31–7.49) or a decrease in nectar sugar concentration (95% CI: 1.57–8.68), but did not decline further when both pollen and sugar were restricted simultaneously (95% CI: 1.74–9.47, Fig. 2). Accordingly, the proportion of bees surviving to 7 d postinoculation was highest in the treatment with access to high sugar and pollen (83%) and lower in treatments with low sugar nectar (57%), lacking pollen (54%), or both (63%).

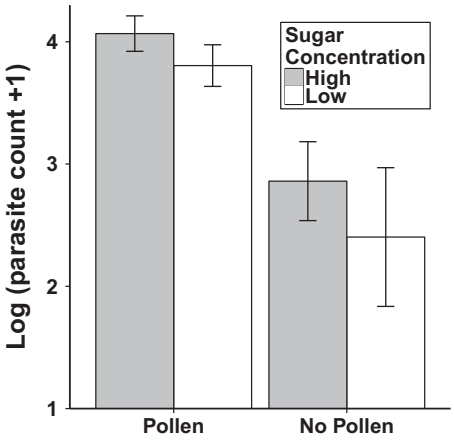

Fig. 1. Effects of pollen availability and nectar sugar concentration on *Crithidia* parasite loads in *B. impatiens*. Both pollen starvation and low nectar sugar concentration significantly reduced parasite loads. Bars show adjusted mixed model means. Error bars represent ±1 SE. Gray bars 30% (w/w) sucrose, white bars: 15% sucrose.

Table 1. Effects of pollen availability and nectar sugar concentration on *Crithidia* parasite loads in *B. impatiens*

Source	χ^2	df	*P*
Pollen	6.47	1	**0.011**
Sugar concentration	4.08	1	**0.043**
Pollen × sugar	0.10	1	0.75
Colony[a]	28.69	3	**<0.001**
Mass at emergence	2.16	1	0.14
Days to inoculation[b]	8.58	1	**0.0034**

Marginal significance of predictor variables in a generalized linear mixed model was tested using χ^2 tests. The model also included inoculation date as a random effect. Bold type indicates significant effects at $P < 0.05$.

[a] "Colony" refers to bee hive of origin.

[b] "Days to inoculation" refers to the number of days between bee emergence from pupation and inoculation with *Crithidia*.

Nectar and Pollen Consumption

Bees consumed less nectar per day when provided low compared to high sugar nectar (adjusted means—high sugar: 87.6 ± 8.6 mg SE, low sugar: 71.1 ± 8.6 mg SE; Fig. 3, Table 3A). Pollen availability did not affect nectar consumption, nor were there interactive effects of pollen availability and sugar concentration on nectar consumption (Table 3A). Colony of origin also affected nectar consumption (Table 3A). Pollen consumption was not significantly affected by nectar sugar concentration, but correlated positively with bee mass ($\beta = -0.068 \pm 0.023$ SE, Table 3B).

Discussion

Although previous work has separately assessed the role of pollen limitation (Brown et al. 2003b, Logan et al. 2005, Brunner et al. 2014), nectar limitation (Sadd 2011), or total starvation (Brown

Table 2. Effects of pollen availability and nectar sugar concentration on survival in *B. impatiens* infected with *Crithidia*

Source	χ^2	df	*P*
Pollen	3.53	1	0.060
Sugar concentration	7.22	1	**0.0072**
Pollen × sugar	3.97	1	**0.046**

Marginal significance of predictor variables in a cox proportional hazards model was tested using χ^2 tests. Bold type indicates significant effects at $P < 0.05$.

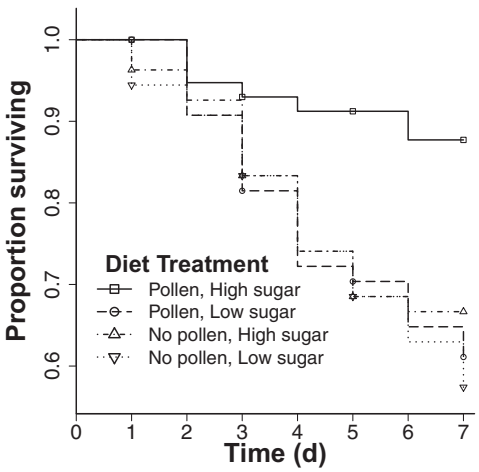

Fig. 2. Effects of pollen availability and nectar sugar concentration on survival of *B. impatiens* infected with *Crithidia*. Pollen availability and nectar sugar concentration had interactive effects on survival. Curves show survival in each diet treatment group over the 7 d between inoculation with *Crithidia* and dissection, the interval over which diet treatments were applied. Solid line with rectangle markers: pollen, 30% sucrose in nectar; dashed line with circle markers: pollen, 15% sucrose; dashed-dotted line with upright triangle markers: no pollen, 30% sucrose; dotted line with inverted triangle markers: no pollen, 15% sucrose.

et al. 2000) on *Crithidia* infection and mortality in *B. terrestris*, our research extends these prior studies by explicitly comparing the role of nectar and pollen availability individually and in combination. Furthermore, we expand the range of *Bombus* species by using *B. impatiens* rather than *B. terrestris*. We found that pollen availability and nectar sugar concentration both reduced *Crithidia* loads, with pollen starvation reducing log-transformed parasite loads by nearly a factor of 1.5 while the effect of nectar sugar was less dramatic (Fig. 1). In contrast, nectar sugar concentration and its interaction with pollen availability were most important for bee survival, with 20–29% fewer control bees dying over 7 d than in the food-limited treatments. This suggests that bees cannot compensate for low nectar sugar concentrations, even when provided with ad libitum resources (Fig. 3). Thus, limited food availability in the bumble bee diet may have different consequences for bumble bees and their pathogens.

Table 3. Effects of pollen availability and nectar sugar concentration on nectar and pollen consumption

Source	χ^2	df	*P*
A. Nectar consumption			
Pollen	0.53	1	0.47
Sugar concentration	6.94	1	**0.0084**
Pollen × sugar	0.0027	1	0.96
Colony[a]	10.26	3	**0.016**
B. Pollen consumption			
Sugar concentration	0.15	1	0.70
Mass at emergence	8.75	1	**0.0031**

(A) Nectar consumption. The model also included individual bee, number of days since inoculation, and date of trial as random effects. (B) Pollen consumption. Note that pollen consumption was assessed only on the pollen-fed subset of experimental bees. The model also included individual bee and date of trial as random effects.

Marginal significance of predictor variables in a generalized linear mixed model was tested using χ^2 tests. Bold type indicates significant effects at $P < 0.05$.

[a] "Colony" refers to bee hive of origin.

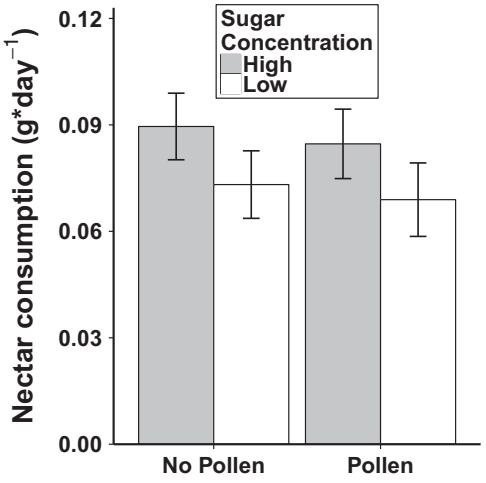

Fig. 3. Effects of pollen availability and nectar sugar concentration on nectar consumption in *B. impatiens* infected with *Crithidia*. Bees fed the high sugar nectar consumed more solution after adjusting for evaporation. Bars show adjusted mixed model means. Error bars represent ±1 SE. Gray bars: 30% (w/w) sucrose, white bars: 15% sucrose.

Our work is consistent with previous studies finding that dietary pollen strongly increased *Crithidia* loads (Brown et al. 2003b, Salathe and Schmid-Hempel 2011, Brunner et al. 2014). As an extracellular gut parasite (Schmid-Hempel 2001), *Crithidia* resides in an environment whose properties are largely dictated by the host's diet. There are several potential mechanisms by which host diets can affect gut parasites (Coop and Kyriazakis 2001, Brown et al. 2003a, Logan et al. 2005). These include alteration of 1) the direct availability of nutrients to the parasite, 2) effects of nutrients on host quality, 3) the immune response of the host, and/or 4) the presence of antiparasitic compounds from dietary sources (Coop and Kyriazakis, 2001). The first two mechanisms should result in increased parasite load with increased nutrition, while the third should decrease parasite loads with increased nutrition. The strong effect of pollen removal reducing pathogen loads suggests that pollen is an important source of dietary resources for *Crithidia*, or is important in creating a parasite-favorable environment in the host gut, rather than that pollen aids bees in mounting an effective immune response.

Increased *Crithidia* with the presence of pollen is somewhat surprising in that pollen availability should help facilitate bee immune function. Recent work has found that successful parasitism in *B. terrestris* is influenced by a pattern of up-regulation and down-regulation of several host genes related to immune function (Barribeau et al. 2014). Fat bodies function as both nutritional reserves and immune organs in invertebrates (Arrese and Soulages 2010, Azeez et al. 2014). *B. terrestris* workers completely starved while infected with *Crithidia* seem to allocate more resources to fat body development compared with their uninfected counterparts (Brown et al. 2000), and infection by *Crithidia* doubled the level of phenoloxidase activity in the hemolymph even in pollen-starved workers (Brown et al. 2003a). However, removing pollen from the diet of *B. terrestris* workers infected with *Crithidia* reduced the expression level and variability in several immune factors (Brunner et al. 2014). Studies in *Drosophila* have indicated that the fat body is involved in pathogen-specific immune responses (Leclerc and Reichhart, 2004). The findings that pollen-starved bees exhibited reduced variability of immune gene expression while preserving the general phenoloxidase response could indicate that removing pollen from the diets of bees impacts the immune system differentially, with the factors dependent on the fat body being most susceptible to dietary restriction. Due to the pattern of host immune gene expression associated with successful *Crithidia* infection, diet could potentially modulate the strong genotype-by-genotype interactions observed between host and parasite (Brown et al. 2003a, Lee et al. 2008, Barribeau et al. 2014, Brunner et al. 2014).

Our results agree with previous work demonstrating that low nectar sugar concentrations reduce *Crithidia* parasite load. A previous study that manipulated nectar sugar concentration (Sadd 2011) found that nectar sugar affected *Crithidia* loads in *B. terrestris* in a nonlinear fashion, with lowest *Crithidia* loads at the lowest sugar concentration (12%), highest at moderate sugar (20%), and intermediate at high sugar (50%). Furthermore, the effect of sugar concentration varied with *Crithidia* strain, with some being more sensitive than others (Sadd 2011). The much larger reduction in parasite loads associated with reduced pollen compared to reduced nectar sugar concentration (Fig. 1) could reflect different mechanisms of action on *Crithidia*. As the largest source of protein and lipids (Nicolson 2011), pollen in the bee's diet may also be a vital nutritional resource for *Crithidia*. The carbohydrate concentration in nectar could affect *Crithidia* indirectly, by altering the nutritional and environmental characteristics of the bee gut itself. Further

studies could investigate the nutritional requirements of *Crithidia* populations in greater depth as well as the impact these nutritional resources have on the environment in the bee's gut.

Similar to *Crithidia* success, which was dependent on both pollen availability and nectar sugar concentration (Fig. 1), bee survival was affected by nectar sugar concentration and its interaction with pollen availability (Table 2). The survival rate was highest in bees that had access to both pollen and high sugar nectar, and was decreased to a similar level in bees without access to pollen, with low sugar nectar, or both (Fig. 2). Bees were provided with ad libitum nectar resources, suggesting that when nectar is dilute, bees do not (or cannot) increase consumption sufficiently to compensate for low sugar concentrations. Indeed, surprisingly, bees consumed more of the high-sugar than low-sugar nectar. Studies of adult honey bee nutritional requirements indicate that, while pollen and nectar are both important to bee health (Haydak 1970), bees can tolerate protein deficiency for longer periods than carbohydrate deficiency due to their limited glycogen stores (Brodschneider and Crailsheim 2010). While this finding has not yet been confirmed in *Bombus* species, it does indicate the potential for different causes underlying the increased mortality seen in nutrition-limited groups from our study. Pollen availability appears to be most important for interactions with *Crithidia* infection, while nectar sugar may have a more direct effect on the health of bees due to their potentially higher carbohydrate requirements. Further work is needed to elucidate the specific requirements of bumble bee workers to assess this possibility.

Our results could have implications for the interaction of parasite infection and host dietary resources. Parasites of many animal species manipulate host behaviors to facilitate parasite development and survival, often in ways that are maladaptive for the host (Klein 2003, Grosman et al. 2008, Yanoviak et al. 2008). Although previous work has shown that infection with *Crithidia* can slow *Bombus* foraging rate (Otterstatter et al. 2005), increase time spent at individual flowers (Otterstatter and Thomson 2006), induce resource investment in fat store development (Brown et al. 2000), and impair learning (Gegear et al. 2006), whether infection influences nutritional food choices as self-medication is unknown. Bumble bees are capable of discriminating both sucrose and protein content of food options by taste (Ruedenauer et al. 2015), suggesting the potential for discriminating the relative nutritional benefits of food choices. Because foragers also avoid feeding at flowers contaminated with *Crithidia* (Fouks and Lattorf 2011), infection status and nutrition could potentially influence the behavior of individual bees, although our study did not manipulate infection and so cannot directly address this question. While we could not assess the effect of *Crithidia* infection on individual pollen intake because all bees were infected, *Crithidia* infection in a separate study of *B. impatiens* microcolonies increased pollen consumption by 24% (Richardson et al. 2015). If the changes to food consumption and resource allocation are parasite induced, this could reflect the importance of host fat availability to the establishment of successful *Crithidia* populations. Alternatively, bees may consume more pollen as a mechanism to resist or tolerate parasite infection.

Our findings are relevant to an understanding of parasitism relationships in other organisms. Dietary limitation in various forms has important effects on host–parasite responses in organisms ranging from other insects (Koella and Sørensen 2002, Logan et al. 2005, Lee et al. 2008, Cotter et al. 2011) to vertebrates (Coop and Kyriazakis 2001), including humans (Nesheim 1993, Stephenson et al. 2000, Hughes and Kelly 2006). Deficiencies in host nutrition typically alter a pathogen's population dynamics, including population size and transmission potential, in a broad variety of both invertebrate (Hall et al. 2009, Vale et al. 2013) and vertebrate hosts (Akpom and Warren 1975, Cornet et al. 2014). In mammals, malnutrition and dietary limitation often negatively affect a host's resistance to infection and the ultimate outcome of the host's infection (Eriksson et al. 1998, Coop and Kyriazakis 2001, Kristan 2007, Coutinho et al. 2008). The nutritional composition of host food resources is also significant, as certain resources often have different effects on host and parasite (Akpom and Warren 1975, Hughes and Kelly 2006, Katona and Katona-Apte 2008, Papier et al. 2014). Thus, understanding how resource availability impacts disease outcomes is a vital component of characterizing the effects of a pathogen on its host. A broad perspective that integrates the environmental, ecological, and social dynamics of disease provides necessary context for selecting the best management practices to minimize disease impact and spread.

Supplementary Data

The dataset and R scripts for this paper are freely available to the public through the Zenodo repository. The DOI for the dataset and R scripts is: http://dx.doi.org/10.5281/zenodo.58171

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