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Consuming sunflower pollen reduced pathogen infection but did not alter measures of immunity in bumblebees

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Certain diets can benefit bee health by reducing pathogens, but the mechanism(s) driving these medicinal effects are largely unexplored. Recent research found that sunflower (*Helianthus annuus*) pollen reduces the gut pathogen *Crithidia bombi* in the common eastern bumblebee (*Bombus impatiens*). Here, we tested the effects of sunflower pollen and infection on two bee immune metrics to determine whether sunflower pollen diet drives changes in host immunity that can explain this medicinal effect. Bees were infected with *C. bombi* or not and given either sunflower or wildflower pollen. Subsequently, bees received a benign immune challenge or were left naive to test the induced and constitutive immune responses, respectively. We measured haemolymph phenoloxidase activity, involved in the melanization cascade, and antibacterial activity. Sunflower pollen reduced *C. bombi* infection, but we found no significant pollen diet effect on either immune measure. Phenoloxidase activity was also not affected by *C. bombi* infection status; however, uninfected bees were more likely to have measurable constitutive antibacterial activity, while infected bees had higher induced antibacterial activity. Overall, we found that sunflower pollen does not significantly affect the immune responses we measured, suggesting that the mechanisms underlying its medicinal effect do not involve these bee immune parameters.

This article is part of the theme issue 'Natural processes influencing pollinator health: from chemistry to landscapes'.

1. Introduction

Many interacting factors affect pollinator health, including nutritional and infection status of individuals and populations [1,2]. Recent concerns about bee population declines and the corresponding emergence of multiple bee pathogens [3,4] have spurred efforts to find practical ways to reduce pathogen prevalence and mitigate negative impacts. Improving access to high-quality floral resources by planting wildflower strips may enable pollinator populations to better resist and/or tolerate pathogen infections and other stressors in the wild [5], but will require careful consideration of which species to plant and in what quantity, since wildflower strips could also increase pathogen prevalence or transmission [6,7]. Identifying plant species that reduce bee pathogen prevalence and the mechanisms by which those species provide benefits is essential to inform management strategies.

The nutritional and medicinal value of forage plants is determined by their nectar and pollen composition [8]. Pollen is the primary source of protein and lipids for bees and is critical for larval development as well as adult survival and reproduction [9–14]. Other components, such as secondary metabolites

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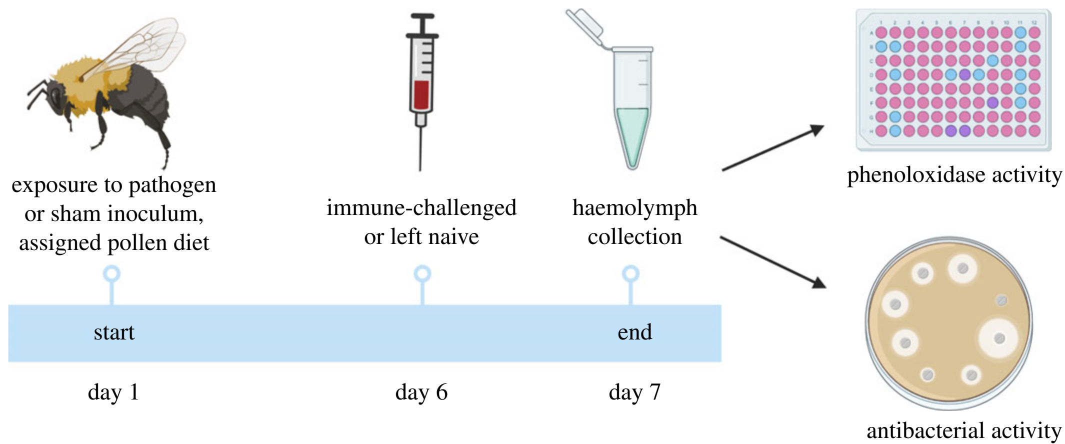

Figure 1. Experimental design. Adult bees (3–4 days post adult eclosion) were exposed to either *Crithidia bombi* or a sham inoculum and then administered a pollen diet (sunflower or wildflower mix pollen) for 7 days. Immune-challenged bees were injected with heat-killed bacteria on day 6. Haemolymph was collected and all bees were frozen on day 7. Haemolymph phenoloxidase levels, antibacterial activity and *C. bombi* infection intensities were measured at later dates. Image produced with Biorender. (Online version in colour.)

found in both pollen and nectar [15], can also impact bee survival and pathogen infection outcomes (reviewed in [16]). While much previous research has focused on the nutritional needs for honeybee or bumblebee colony growth—for example, dietary protein is positively correlated with egg production and brood growth [11,17]—the nutritional requirements of adults, particularly when under pathogen stress, are less well understood.

For adult bees, pollen consumption appears to be important for immune function. In bumblebees (*Bombus terrestris*), complete starvation led to greater survival costs of immune activation [18] and pollen starvation reduced immune gene expression following trypanosome (*Crithidia bombi*) infection [19]. Additionally, pollen starvation resulted in lower fat body mass in honeybees [20], but did not affect activity of the immune enzyme, phenoloxidase [21]. Moreover, two studies have found that bees fed pollen diets differing in protein exhibited similar levels of phenoloxidase activity [20,22], while another found that bees fed a relatively high-protein pollen (*Rubus* sp.; 22% protein) had lower phenoloxidase activity than bees fed low-protein pollen (*Erica* sp.; 14.8% protein). These studies suggest that the importance of pollen in adult immune function may be driven by components other than protein content. For example, secondary metabolites in floral rewards can be detrimental to bees [23,24], but some compounds in nectar can also reduce infections of *C. bombi* in bumblebees [25]. Studies thus far have found no clear relationships between pollen secondary metabolites and pathogen infections [26–28], but further research is needed. Overall, the role of dietary nutrients in bee immune function is key to understanding the influence of bee diet on pathogen infection.

Recent research has found that consuming pollen from sunflowers (*Helianthus annuus*) significantly reduced *C. bombi* infection in the common eastern bumblebee (*B. impatiens*) [29–31] and *Nosema ceranae* infection in honeybees [29]. Such medicinal effects against infection make sunflower a potential candidate for inclusion in pollinator wildflower habitats. Sunflower pollen, like other members of the Asteraceae family, has low protein and high lipid content [32,33]. Recent research has tested sunflower pollen chemistry against *C. bombi* and has thus far yielded inconclusive results. One study tested the effects of nine fatty acids and two secondary metabolites (the polyamine tri-*p*-coumaroyl spermidine and the flavonoids quercetin-3-O-hexoside and quercetin-3-O-(6-O-malonyl)-hexoside) on *C. bombi* infection in bumblebees, and found that none of the metabolites reduced infection [28]. Another study tested the effects of pollen chemical extracts on *C. bombi* growth *in vitro* and found that sunflower pollen extracts actually increased pathogen growth [34], suggesting sunflower pollen's effect on *C. bombi* may be mediated indirectly through the host, potentially by altering host immunity.

Here, we tested the hypothesis that sunflower pollen reduces *C. bombi* infection by altering host bumblebee (*B. impatiens*) immune function. We experimentally infected adult worker bees with *C. bombi* or not and then fed them either sunflower or wildflower mix pollen. We tested induced and constitutive immunity by assessing two commonly used insect immune metrics: activity of phenoloxidase, an immune enzyme involved in the melanization cascade, and humoral antibacterial activity of collected haemolymph (figure 1). This approach improves our understanding of the link between diet and immunity in insect pollinators, which may help inform pollinator conservation management strategies.

2. Methods

(a) Study system

(i) *Bombus impatiens* (family: Apidae)

The common eastern bumblebee is abundant in the eastern United States and is currently not in decline [4]. We purchased five *B. impatiens* colonies from Koppert Biological Systems (Howell, Michigan, USA) that were maintained in a climate-controlled room (26–29°C) and provided with a sugar solution (1 : 1 cane sugar (g): boiled tap water (ml), with 0.1% cream of tartar, McCormick and Company, Inc., Baltimore, MD, USA) and honeybee-collected wildflower mix pollen (Swarmbustin' Honey, West Grove, PA, USA). For logistical reasons, this wildflower pollen was different from the wildflower pollen used in the experimental treatment, but this also means pre-feeding with different pollen during development occurred for bees in both treatments. Colonies were confirmed to be free of common parasites, including *Crithidia* spp. and *Nosema* spp., by initial faecal screening of the queen and a subset of workers

and subsequent regular screening of dissected whole guts and faeces via microscopy.

(ii) *Crithidia bombi* (family: Trypanosomatidae)

Crithidia bombi is a gut pathogen of bumblebees that is transmitted through contact with infected faeces [35,36]. *Crithidia bombi* uses its flagellum to attach to the inner lining of the hind gut wall [37] and reduces colony fitness by affecting queens' ability to survive diapause [38], found a new colony [39] and produce new queens [40]. Additionally, infected workers have higher mortality when food-limited [41] and exhibit cognitive impairment [42]. Many interacting factors influence bee susceptibility to infection, including host and pathogen genotypes, the host microbiota and environmental context [43]. *Crithidia bombi* infection has been shown to affect bumblebee immunity: infected workers had higher levels of pro-phenoloxidase, a precursor enzyme in the melanization response, in their haemolymph [41]. Moreover, *C. bombi* strains that are less successful at establishing infections elicit higher antimicrobial peptide (AMP) gene expression than more infective strains [44] and knockdown of AMP expression leads to higher *C. bombi* infection levels [45].

(iii) *Helianthus annuus* (family: Asteraceae)

Sunflower is a native US wildflower [46] and major oilseed crop worldwide whose yield is improved by bee visitation [32]. The effect of sunflower pollen in reducing *C. bombi* infection in *B. impatiens* was consistent across *C. bombi* isolates [29] and sunflower cultivars [30]. In addition, infection in wild-caught *B. impatiens* workers was negatively correlated with acreage of sunflowers [29]. Sunflower pollen has relatively low protein content [33] and can lead to poor performance in bees that feed on it [11,47]. The low protein content is not likely responsible for reducing *C. bombi* infections since another similarly low-protein pollen diet (buckwheat, *Fagopyrum esculentum*) resulted in *C. bombi* infections that were comparatively high [29].

(b) Experimental design

(i) Overview

The experimental design is summarized in figure 1. We removed 24–48 h old adult workers from their natal colonies. We selected workers of this age cohort to control for worker age. Additionally, they will have likely been inoculated with gut microbiota from nest-mates before removal, which can influence interactions with *C. bombi* [48]. Isolated individuals were allowed to acclimate to their individual containers for 2 days before the start of the experiment (3–4 days post adult eclosion), at which time they were inoculated with either *C. bombi* or a sham inoculum and then administered either sunflower pollen or wildflower pollen mix. After a further 6 days, half of the bees in each treatment group received a benign immune challenge via an injection of heat-killed bacteria. Seven days after experiment initiation (10–11 d post adult eclosion), we collected haemolymph for the immune assays and froze each bee. We then dissected the gut and quantified *C. bombi* infection levels using qPCR. We removed the right forewing of each bee and measured the length of the marginal cell as a proxy for bee body size [49,50]. We had a total sample size of 301 bees, with 37–39 bees in each treatment category. Additional details on all methods can be found in the electronic supplemental material.

(ii) *Crithidia bombi* inoculation

We used two strains of *C. bombi* sourced from faeces of wild-caught bumblebees originally collected from Alaska in 2008 and Illinois in 2016 (cultivation methods described in the electronic supplementary material). Inoculum was prepared by mixing cultures of both *C. bombi* strains in equal amounts and diluting to 2000 cells μl^{-1}. Cells within the inoculum stock were confirmed to be live by visual inspection using a phase-contrast microscope at 400×. Cultures were then mixed with a sugar–water solution, for a final concentration of 1000 cells μl^{-1}. Each bee received 10 µl of inoculum, approximately 10 000 *C. bombi* cells, comparable to what a bee would encounter in nature [51]. Bees that did not consume the entire drop within 30 min were excluded from the experiment.

(iii) Pollen diets

Individual bees in the experiment were administered provisions (approx. 100 mg) of sunflower pollen (Changge Hauding Wax Inudstry, China Co. LTD) or wildflower pollen mix (Koppert Biological Systems, Howell Michigan, USA), which we replaced every other day for 7 d. Honeybee-collected pollen of both types was ground and mixed in a 6 : 1 pollen (g): 50% sugar water (ml) ratio to make a paste that was frozen at −20°C until use. Using a paste made it easier to form, standardize and subsequently retrieve remnants of provisions. We measured the amount of pollen consumed by each bee. We also conducted protein content assays on both pollen types, with ten replicates for each (details described in the electronic supplementary material).

(iv) Immune challenge

To stimulate the bee immune system and test an induced response in the haemolymph, we injected individuals with a mixed solution of heat-killed bacteria (*Arthrobacter globiformis* and *Escherichia coli*) on day 6 of the experiment. This method has been demonstrated to induce antimicrobial immune pathways and expression of immune genes in *B. terrestris* [52,53]. We anesthetized all bees on ice and injected those in the immune challenge treatment with 2 µl of the heat-killed bacteria solution between the 1st and 2nd abdominal tergites, using a sterile pulled glass microcapillary tube. We anesthetized and handled naive bees similarly, but did not wound or inject them, to measure constitutive immunity. We then returned bees to their individual boxes to recover. Twenty-four hours after the immune challenge, we anesthetized each bee on ice and then inserted a needle between the 5th and 6th sternites of the abdomen, puncturing the pleural membrane [53]. We then used a microcapillary tube to collect 5 µl of haemolymph, which we mixed with sodium cacodylate buffer. We flash froze the haemolymph samples and the bees in liquid nitrogen and stored them at −80°C until the immune assays and gut dissections.

(v) Immune assays

We used the haemolymph samples to measure two aspects of immunity: total phenoloxidase activity and humoral antibacterial activity. To measure phenoloxidase, we used a spectrophotometric assay [54] on samples where all phenoloxidase had been activated by the addition of chemotrypsin. To test the antibacterial activity, we assayed zones of inhibition on Petri dishes with agar seeded with *Arthrobacter globiformis* (see [55,56]). Because zone of inhibition diameter does not increase linearly with increasing antibacterial activity, we converted average zone diameters of each sample to units ($\mu g\ ml^{-1}$) of the antibiotic tetracycline based on a standard curve.

(vi) *Crithidia bombi* quantification

To quantify *C. bombi* infection, we dissected and homogenized whole guts in 100 µl of ringer saline solution (Sigma-Aldrich, St. Louis, MO, USA) and stored samples at −20°C until DNA extraction. DNA was extracted and normalized *C. bombi* infection intensities (estimated cells per bee) were determined by qPCR [57]. Previous work has found a strong relationship between gut infection levels determined by qPCR and infective

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transmitting cells in the faeces [57,58], demonstrating that this method is reliable for estimating viable infection levels.

(c) Statistical analyses

We used the open-source software R v. 4.0.5 [59] for all analyses. Figures were made using values from emmeans [60] and ggplot2 [61]. We ran full models and then used Akaike information criterion (AIC) for model selection and sequentially dropped non-significant terms (AICcmodavg package) [62]. The significance of terms was tested with *F*-tests and likelihood ratio χ^2 tests conducted with the Anova function (car package) [63]. All full models included pollen diet, infection (except for the *C. bombi* infection analysis since all bees were infected), pollen consumption (except for the analysis with consumption as a response) and wing size (estimated by marginal cell length) as fixed effects and natal colony and start date as random effects. Immune challenge treatment was included as an additional fixed effect in analyses where immune-challenged and naive bees were not separated. We always retained the pollen diet and infection terms since those were our independent variables of interest. We report results from the best-fitting models based on AIC.

To analyze *C. bombi* infection intensities, we used a generalized linear mixed model with a negative binomial distribution. To analyze phenoloxidase activity, we separated immune-challenged and naive bees, log-transformed the responses and used a linear model. To analyze average zone of inhibition, we again separated immune-challenged and naive bees. Since only 34% of bees in the naive group had any measurable zones of inhibition, they were analysed with a generalized linear mixed model with a binomial response (presence/absence of zone of inhibition). For the immune-challenged bees, we used a zero-inflated generalized linear model with a gamma distribution and log link function. For this analysis, samples below the limit of detection of 0.0598 $\mu g\ ml^{-1}$ were set at 0. However, the outcome of the analysis did not qualitatively change when these samples were left at the limit of detection value and a generalized linear model with a gamma distribution used. The zero-inflated model is presented due to its improved fit. To analyze pollen consumption, we log-transformed the response and then used a linear model with a normal distribution. We also analysed pollen consumption in infected bees only with the same model, but used infection intensity instead of infection treatment. Mortality in the experiment was very low (1.3%); therefore, we did not analyze bee survival. To analyze differences in sunflower and wildflower mix pollen protein content ($\mu g\ mg^{-1}$), we used a *t*-test.

Table 1. Linear mixed model analysis of the effect of pollen diet (sunflower/wildflower), *C. bombi* infection (yes/no), wing marginal cell length (a proxy for body size) and pollen consumption on log-transformed total phenoloxidase activity. Italicized font indicates $p < 0.05$.

effect	χ^2	d.f.	*p*-value
naive bees			
diet	1.019	1	0.313
infection	1.317	1	0.251
wing size	6.992	1	*0.008*
pollen consumption	0.022	1	0.882
immune-challenged bees			
diet	1.023	1	0.312
infection	3.954	1	*0.047*
pollen consumption	0.421	1	0.517

Table 2. Generalized linear mixed model analysis of the effect of pollen diet (sunflower/wildflower), *C. bombi* infection (yes/no), wing marginal cell length (a proxy for body size) and amount of pollen consumed on production of antibacterial zones of inhibition in naive bees and the extent of induced activity in bacterially immune-challenged bees. Italicized font indicates $p < 0.05$.

effect	χ^2	d.f.	*p*-value
naive bees			
diet	0.025	1	0.874
infection	6.14	1	*0.013*
wing size	2.269	1	0.132
pollen consumption	2.016	1	0.156
immune-challenged bees			
diet	0.121	1	0.264
infection	3.516	1	0.204
wing size	11.045	1	*0.0009*
pollen consumption	1.761	1	0.603
diet × infection	3.791	1	*0.052*
infection × pollen consumption	5.466	1	*0.019*

3. Results

All *Crithidia bombi* exposed bees showed detectable infections (range 301–560 120 748 cells per bee, mean = 28 913 875). *C. bombi* infection levels, as estimated by qPCR, were significantly affected by pollen diet treatment ($\chi^2 = 36.643$, d.f. = 1, $p < 0.0001$) and bee wing size ($\chi^2 = 6.992$, d.f. = 1, $p = 0.008$), but not pollen consumption ($\chi^2 = 1.695$, d.f. = 1, $p = 0.193$). Sunflower pollen significantly reduced *C. bombi* cell counts, by approximately 84% (electronic supplementary material, figure S1), and smaller bees had higher infection levels than larger bees.

We found no significant effect of pollen diet treatment on bee immunity (tables 1 and 2; figure 2). Phenoloxidase activity was not affected by diet, but was slightly higher in infected compared to uninfected immune-challenged bees, while infection status had no effect in naive bees (table 1, figure 2*a,b*). In naive bees, larger bees had higher phenoloxidase activity, but this pattern was not observed in the immune-challenged bees (table 1). In the naive bees, uninfected bees were more likely to produce a measurable zone of inhibition than infected bees (table 2 and figure 2*c*). In the immune-challenged bees, there was a marginally non-significant diet by infection interaction (table 2), with infected bees fed wildflower pollen having slightly higher induced antibacterial activity compared to uninfected bees fed wildflower pollen ($t = 2.96$, d.f. = 99, $p = 0.04$, figure 2*d*). Additionally, we found a significant interaction between pollen consumption and infection status in determining antibacterial activity (table 2). Zones of inhibition were larger in bees that consumed more pollen, but only in infected bees ($\beta_{uninfected} = -1.29$, $\beta_{infected} = 10.21$; figure 3).

Sunflower pollen was consumed more than the wildflower mix, and larger bees consumed more pollen than smaller bees (table 3). Sunflower pollen had lower protein content ($\mu g\ mg^{-1}$) than the wildflower mix ($t = -6.703$, d.f. = 18, $p < 0.0001$; electronic supplementary material, figure S2).

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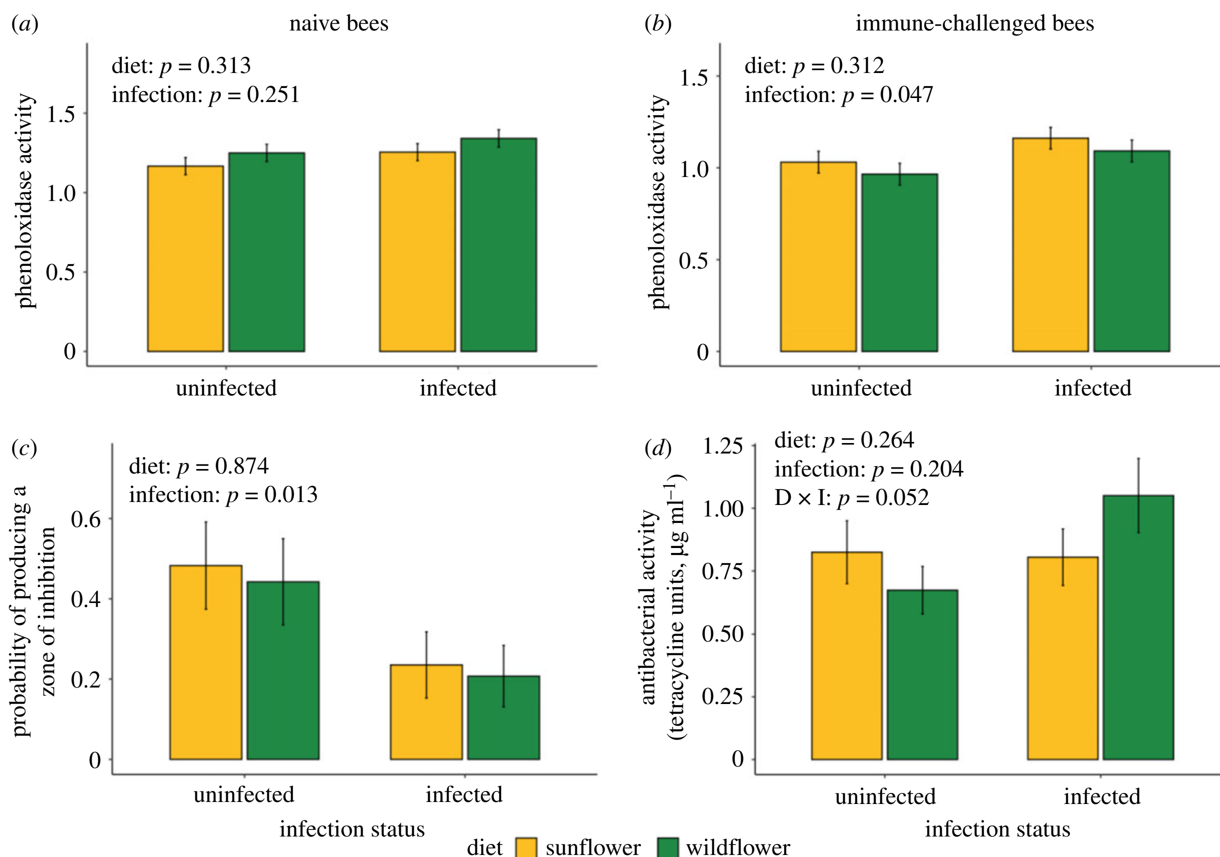

Figure 2. Haemolymph total phenoloxidase and humoral antibacterial activity in bees uninfected or infected with *C. bombi* when left naive (left panels) or bacterially immune-challenged (right panels), and fed either sunflower pollen (yellow) or the wildflower pollen mix (green). Antibacterial activity for panel (*d*) is presented in μg ml^{-1} tetracycline units; see electronic supplementary material. Diet by infection interactions were non-significant for panels (*a*, *b* and *c*) and were therefore dropped from the models. Means are back-transformed using emmeans and error bars are ± one standard error. (Online version in colour.)

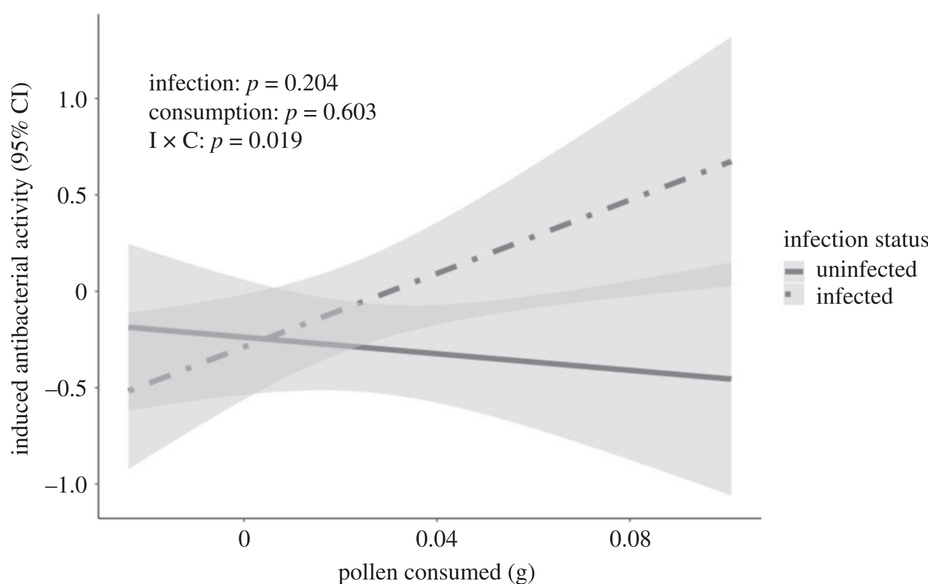

Figure 3. The effect of pollen consumption and infection status on antibacterial activity, measured by zones of inhibition and presented in μg ml^{-1} tetracycline units. The effect of pollen diet was not significant and so diets were pooled within infection group. Slopes were back-transformed using emtrends, and the shaded area represents 95% confidence intervals.

4. Discussion

Consistent with previous studies, we found that a diet of sunflower pollen significantly reduced *C. bombi* infection levels in *B. impatiens* workers [29–31]. However, our results did not support our hypothesis that sunflower pollen reduces *C. bombi* infections by increasing host immune function. We found no major differences in immune metrics between bees fed sunflower and wildflower mix pollen (figure 2).

Asteraceae pollens have relatively low protein content compared to other bee forage plants [32,33], and we confirmed that the sunflower pollen we provisioned had

Table 3. Linear mixed model analysis for the effect of pollen diet (sunflower/wildflower), *C. bombi* infection (yes/no for all bees and intensity for only infected bees) and wing marginal cell length (a proxy for body size) on pollen consumption. Pollen consumption values were log-transformed to meet model assumptions.

effect	*F*	d.f.	*p*-value
all bees			
diet	29.378	1	*<0.0001*
infection (yes/no)	0.641	1	0.424
wing size	47.09	1	*<0.0001*
infected bees			
diet	9.044	1	*0.003*
infection intensity	0.418	1	0.519
wing size	11.0	1	*0.001*

lower protein content than the wildflower pollen mix, which consisted of pollen from 10+ plant species (electronic supplementary material, figure S2). Our results are consistent with previous studies where low-protein diets did not reduce immune function in adult honeybees [20] and bumblebees [22,41]. But these findings are perhaps surprising given that dietary protein has been positively linked to immune function in caterpillars [64,65] and that pollen-starved bees had reduced immune gene expression [19]. Alternatively, nutrients other than protein may be more vital to immune function. For example, AMPs are synthesized in the fat body, which is primarily composed of lipids [66]. Lipids may be important dietary nutrients for immune function and pathogen resistance in bees, although further research is needed to test this hypothesis.

Asteraceae pollens also have low digestibility, demonstrated by poor bee larval development [11,67–69]. Whether pollen is digested similarly in larvae and adults remains an open question [9], but our results suggest that sunflower pollen is indeed digested by adult bumblebees since immune function was similar for bees fed sunflower and wildflower pollen. If bees were not digesting sunflower pollen, we would expect immune function of sunflower-fed bees to be similar to pollen-starved bees, which typically have reduced immune function, particularly antibacterial activity [19]. Additionally, we found that pollen consumption and induced antibacterial activity were positively correlated in infected bees independent of pollen diet (figure 3), suggesting that pollen was used in this response but that its effect did not differ between sunflower and wildflower pollens. Consistent with previous work, this suggests that the production of AMP and other components of the antibacterial response requires pollen consumption [19].

Our approach also allowed us to test the effect of *C. bombi* infection on bumblebee phenoloxidase and antibacterial activity. When considering immunity in unchallenged naive bees, those infected with *C. bombi* were less likely to produce antibacterial zones of inhibition (figure 2*c*), suggesting that *C. bombi* infection may be depleting the baseline humoral antibacterial activity levels in the haemolymph. This alone could be taken as evidence for a cost of maintaining immunity across separated physiological compartments of the body [70]. However, the other findings suggest that this cost is not universal. Infection had no effect on phenoloxidase activity in naive bees (figure 2*a*). This finding is in contrast with a previous study that found higher levels of pro-phenoloxidase in bees infected with *C. bombi* [41]. However, we did uncover a connection between infection and phenoloxidase activity, with higher phenoloxidase activity in infected bees following the bacterial-based immune challenge (figure 2*b*). Induced antibacterial activity was also marginally higher in infected than uninfected bees fed wildflower pollen (figure 2*d*). This could have arisen through higher *C. bombi* infections in wildflower versus sunflower-fed bees contributing to a greater antibacterial response upon immune stimulation, but the marginal effect means any result should be interpreted cautiously. However, the effects on phenoloxidase and antibacterial activity in general support the idea of cross-talk between physiological compartments of the bee body or a systemic response, with infection and/or immune stimulation in one compartment (i.e. the gut) affecting immune response in another (i.e. the body cavity, haemocoel).

While we still do not have a full picture of how sunflower pollen reduces *C. bombi* infection, our results suggest that it does not have major effects on immunity in the body cavity, relative to a wildflower pollen mix diet. We focused on two frequently used measures of insect immune function [71], but there are multiple aspects of insect immunity and the potential responses against *C. bombi* that we did not assess in our experiment. First, we measured immunity in the haemolymph, which circulates through the body cavity. As a gut pathogen, *C. bombi* cells always reside in the digestive tract and never encounter haemolymph. The immune activity of the gut could differ from activity in the body cavity. Measuring gut immunity by looking at immune gene expression in gut tissue may provide additional insights that are relevant to the response against gut pathogen infections or a particular pollen diet. Second, previous demonstrations of an immune response to *C. bombi* exposure in bumblebees measured gene expression in the gut just 18 h after exposure [19,44], whereas we tested immunity 7 days after exposure once infections were established. Important early effects of pollen diet on the response to infection may have dwindled by this time, even though the effect of sunflower pollen reducing infection persisted. Additionally, we were unable to test if the initiation of sunflower pollen feeding triggers an acute gut immune response that inhibits *C. bombi* infection shortly after inoculation.

Our study shows that despite its low protein content, sunflower pollen did not affect immunity metrics in adult bumblebee workers. Sunflower pollen provided medicinal effects in *B. impatiens* against a common pathogen, including when consumed as part of a mixed pollen diet [72], as well as adequate nutrition for adult immunity. Sunflowers could be planted as part of a diverse floral community to support bumblebee health, however, such strategies would need to carefully consider balancing the proposed medicinal benefits with potential detrimental impacts of sunflower pollen in bumblebees and other species. Compared to other monofloral pollen types, a sunflower pollen diet increased mortality in honeybees [29] and reduced the size of larvae in bumblebee microcolonies [11], while its effects on other pollinators are poorly understood. The mechanism(s) behind the medicinal effect of sunflower pollen on *C. bombi* infections remains unknown, but could be related to unmeasured aspects of immunity, pollen morphology, gut transit time, or the bee gut microbiota.

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Future research should continue to assess mechanisms behind sunflower pollen's medicinal effect and seek to identify pollen from other plant species with similar properties. Doing so will inform strategies for pollinator conservation such as planting flowering strips with plant species that improve pollinator health by providing nutritional and medicinal benefits.

Data accessibility. The datasets and code supporting this article are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.fqz612jv8 [73].

Authors' contributions. A.E.F.: Formal analysis, investigation, methodology, visualization, writing—original draft, writing—review and editing; B.M.S.: conceptualization, formal analysis, project administration, resources, software, supervision, validation, writing—review and editing; T.B.: investigation, methodology, writing—review and editing; R.E.I.: conceptualization, funding acquisition, validation, writing—review and editing; L.S.A.: conceptualization, funding acquisition, project administration, validation, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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