PROCEEDINGS B

royalsocietypublishing.org/journal/rspb

Research

Cite this article: Fowler AE, Giacomini JJ, Connon SJ, Irwin RE, Adler LS. 2022 Sunflower pollen reduces a gut pathogen in the model bee species, *Bombus impatiens*, but has weaker effects in three wild congeners. *Proc. R. Soc. B* **289**: 20211909.
https://doi.org/10.1098/rspb.2021.1909

Received: 27 August 2021
Accepted: 4 January 2022

Subject Category:
Ecology

Subject Areas:
ecology

Keywords:
bee pathogen, pollinator, pollen, *Bombus*, sunflower, *Crithidia*

Author for correspondence:
Alison E. Fowler
e-mail: alisonefowler@gmail.com

†Present address: Otis Laboratory, US Department of Agriculture APHIS, Buzzards Bay, MA 02532.

Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.5800143.

THE ROYAL SOCIETY PUBLISHING

Sunflower pollen reduces a gut pathogen in the model bee species, *Bombus impatiens*, but has weaker effects in three wild congeners

Alison E. Fowler[1], Jonathan J. Giacomini[2], Sara June Connon[2,†], Rebecca E. Irwin[2] and Lynn S. Adler[1]

[1]Department of Biology, University of Massachusetts Amherst, 221 Morrill Science Center South, 611 North Pleasant Street, Amherst, MA 01003, USA
[2]Department of Applied Ecology, North Carolina State University, Raleigh, NC 27695, USA

AEF, 0000-0002-9263-2253; JJG, 0000-0002-0151-894X; SJC, 0000-0002-3275-5702; REI, 0000-0002-1394-4946; LSA, 0000-0003-2125-5582

Commercial bumblebees have become popular models to understand stressors and solutions for pollinator health, but few studies test whether results translate to other pollinators. Consuming sunflower pollen dramatically reduces infection by the gut parasite *Crithidia bombi* in commercially reared *Bombus impatiens*. We assessed the effect of sunflower pollen on infection in wild *B. impatiens*, *Bombus griseocollis*, *Bombus bimaculatus* and *Bombus vagans*. We also asked how pollen diet (50% sunflower pollen versus wildflower pollen) and infection (yes/no) affected performance in wild *B. impatiens* microcolonies. Compared to controls, sunflower pollen dramatically reduced *Crithidia* infection in commercial and wild *B. impatiens*, had similar but less dramatic effects in *B. bimaculatus* and *B. vagans*, and no effect in *B. griseocollis*. *Bombus impatiens*, *B. bimaculatus* and *B. vagans* are in the same subgenus, suggesting that responses to sunflower pollen may be phylogenetically conserved. In microcolonies, 50% sunflower pollen reduced infection compared to wildflower pollen, but also reduced reproduction. Sunflower pollen could control *Crithidia* infections in *B. impatiens* and potentially close relatives, but may hinder reproduction if other resources are scarce. We caution that research using managed bee species, such as *B. impatiens*, be interpreted carefully as findings may not relate to all bee species.

1. Background

Experimental biology often relies on model organisms that thrive in captivity and are resilient to stress. Researchers use these species to understand phenomena ranging from genetics to physiology, but insights learned from model species may not extend to wild counterparts and relatives [1]. In recent decades, commercial honeybees (*Apis mellifera*) and bumblebees (*Bombus* spp.) have become popular research models [2], but they represent a small proportion of the extraordinary variation in life history and physiology of the 20 000+ bee species worldwide. Additionally, commercial bees may differ from wild conspecifics owing to artificial selection. While honeybees have been domesticated over millennia, bumblebees have been used commercially for crop pollination since the 1980s [3]. Two species of commercial bumblebees, *Bombus impatiens* and *Bombus terrestris*, are often used as research models, but little is known about whether commercial rearing has altered their physiology or behaviour compared to wild conspecifics. Moreover, few studies have tested whether experimental findings with these model systems translate to wild congeners and other bee species, which is a particularly important knowledge gap for the conservation of wild pollinators.

Both commercial and wild bees are important pollinators, each providing billions of dollars to the global economy through pollination services [4]; however, both are threatened by pathogens [5]. A recent study compared infection intensity

of a common pathogen (*Crithidia bombi*) in wild and commercial bumblebees (*B. impatiens*) and found that wild bees were more susceptible to the pathogen than commercial conspecifics [6], suggesting that research with commercial models may underestimate important effects of pathogens on wild populations. If wild and commercial bees respond to stressors differently, their conservation and management may require different approaches. Assessing differences between wild and commercial bees and identifying limitations of research models are important first steps towards managing bee health in the wild.

Host diet can have important consequences for pathogen infection outcomes [7]. Consuming sunflower pollen (*Helianthus annuus*) dramatically reduced the gut pathogen, *C. bombi*, in commercial bumblebee (*B. impatiens*) workers [8] and queens [9]. Bumblebees in commercial colonies consume sunflower pollen when provided directly to the colony [10] and collect sunflower pollen when available in the landscape (R.L. Malfi, Q.S. McFrederick, G. Lozano, R.E. Irwin, L.S. Adler 2021, unpublished data). In both cases, increased access to sunflower pollen resulted in lower *C. bombi* infection levels, and increasing sunflower abundance on farms also increased daughter queen production, suggesting reproductive benefits of reduced infection (R.L. Malfi, Q.S. McFrederick, G. Lozano, R.E. Irwin, L.S. Adler 2021, unpublished data). However, sunflower pollen is low in protein and other essential nutrients [11] and thus could have negative trade-offs for bee health and reproduction in certain contexts. Indeed, for uninfected commercial bees, a pure sunflower pollen diet reduced survival in *B. impatiens* [12] and offspring production in *B. terrestris* microcolonies [13] compared to other pollen types. However, feeding on a 1 : 1 sunflower to wildflower pollen mixture reduced pathogen infection in commercial *B. impatiens* colonies without any reproductive trade-offs compared to a wildflower pollen lacking sunflower [10]. Sunflower mixed with wildflower pollen is therefore a potential method to manage wild bumblebee disease if results extend to wild conspecifics and other species.

We addressed two questions: (i) does sunflower pollen reduce *Crithidia* infection in wild *B. impatiens* and non-model bumblebees, including *Bombus bimaculatus*, *Bombus griseocollis* and *Bombus vagans*? and (ii) how does diet and *Crithidia* infection affect survival and reproduction in wild *B. impatiens* microcolonies? If sunflower pollen has a direct effect on *Crithidia*, we expect similar effects in different host species. Alternatively, if sunflower pollen reduces *Crithidia* indirectly by altering host behaviour or physiology, then sunflower pollen may have different effects in different host species. Given sunflower pollen's low protein content, we predict that a diet high in sunflower pollen may not provide adequate nutrients for brood production, but this could be outweighed by the benefits of pathogen reduction. The results from this study improve our understanding of the disease ecology of non-model bee species and could inform management strategies for wild and commercial bumblebees.

2. Methods

(a) Study organisms

(i) *Bombus* spp. (Apidae)

We used four bumblebee species that are common in eastern North America and not in decline: *B. impatiens*, *B. bimaculatus*, *B. vagans* (all in the subgenus *Pyrobombus*) and *B. griseocollis* (subgenus *Cullumanobombus*) [14]. These species undergo an annual life cycle; queens and males mate at the end of the season. Queens undergo solitary diapause during winter, emerge in spring, establish nests, and lay worker eggs, progressing to males and daughter queens later in summer. These four species differ in phenology (although all species overlap during peak summer months [15]), geographical range [15], floral preferences [14], prevalence of *Crithidia* infections in the wild [16,17], and immune responses [18]. Compared to the other species, *B. impatiens* produces large colonies, is easy to rear indoors, and is widely used for commercial pollination and research [19]; the other three species are not currently reared for commercial use.

We collected solitary nest-seeking queens in North Carolina and Massachusetts, USA, in spring of 2016, 2017, 2018 and 2020 (electronic supplementary material, table S1). Queens were reared in the laboratory and workers were removed individually for experiments. All queens were screened for *Crithidia* via microscopy of fecal samples, and only workers from uninfected queens were used. Additional details about queen rearing can be found in the electronic supplemental material.

(ii) *Crithidia* spp. (Trypanosomatidae)

Crithidia bombi is a common and well-studied bumblebee pathogen [20]. For some experiments, we used a *Crithidia* source that has been confirmed to be *C. bombi* and maintained for several years in commercial *B. impatiens* (referred to as 'IMP-2'; electronic supplementary material, table S2). We also used *Crithidia* derived from wild bees and maintained in wild colonies. For these *Crithidia*, species identities were not confirmed, and so we cannot rule out the possibility they are *Crithidia expoecki* [21]. We thus refer to all pathogens as *Crithidia* hereafter.

Crithidia is horizontally transmitted through contact with contaminated faeces [22,23] and primarily infects bumblebees. However, recent work has found that *Crithidia* can be detected in many solitary bee species, including the solitary leaf-cutter bee (*Megachile rotundata*), sweat bee (*Halictus ligatus*) and mason bee (*Osmia lignaria*) [24,25]. Most experimental studies on the effects of *Crithidia* infection on bee performance have been conducted with *B. impatiens* (North America) and *B. terrestris* (Europe). *Crithidia* reduced queen overwintering and colony-founding success [26], likelihood of reproduction [27], and worker survival when food-limited [28]. Infection outcomes are determined by interactions between host, parasite, and microbiota genotypes [29–31].

We maintained eight 'sources' of *Crithidia* over the course of this study, each derived from the whole gut of a single wild bee, not from a single cell, and each maintained in a live bumblebee colony. All *Crithidia* sources except for IMP-2 were used the same year they were collected. All *Crithidia* sources except for IMP-2 and IMP-3 were only used in the same species they were sourced from. See the electronic supplementary material, tables S2 and S3 for details about how *Crithidia* were maintained and used.

(iii) Pollen diets

Each experiment used some combination of the following pollen diets: 100% sunflower pollen, 100% buckwheat pollen, a wildflower pollen mix (10+ species) and a 1 : 1 (by weight) mix of sunflower pollen and wildflower pollen mix (hereafter '50% sunflower' or '50% SF'; figure 1). In the species trials, we compared bees fed 100% sunflower pollen (Changge Hauding Wax Industry, China) to bees fed a control diet. In 2016, the control diet was buckwheat pollen (*Fagopyrum esculentum*; Changge Hauding Wax Industry, China), which has a protein content comparable to sunflower [32] but results in high *Crithidia* counts in commercial *B. impatiens* [8]. For all the other species trials, the control diet was wildflower pollen mix (Koppert Biological Systems, Howell, Michigan, USA). The 2017 and 2020 trials also included a third diet of 50% sunflower. For the microcolony trials, we used 50% sunflower and wildflower mix. All pollens were collected by honeybees, ground and mixed in a 7 : 1 pollen (g): water (ml) ratio to make a paste we froze at −20°C until use.

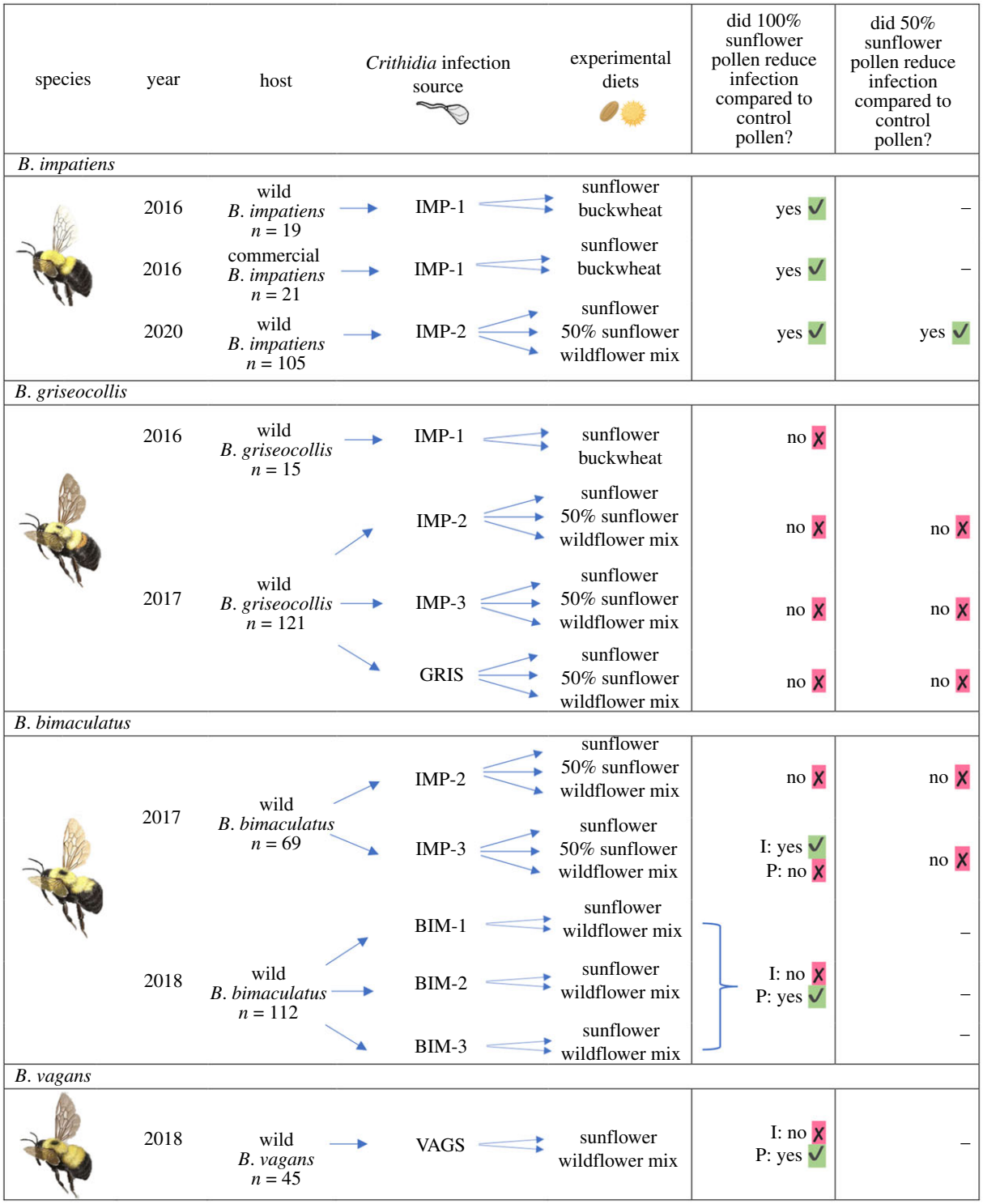

Figure 1. Experimental design for the single bee species trials. Control diet was wildflower pollen mix for all trials except in 2016, which used buckwheat pollen as the control. Experiments in 2017 and 2020 included an additional diet treatment of 50% sunflower pollen. We assessed and report below the effect of diet on both infection intensity (I) and prevalence (P). In cases where diet affected both metrics in the same way, we report one response. For 2018 *B. bimaculatus*, the top model for infection prevalence did not include *Crithidia* source as a fixed effect, so we report results with the sources pooled. Note that *Crithidia* infection sources are named for the bee species they originated from; IMP, *impatiens*; GRIS, *griseocollis*; BIM, *bimaculatus*; VAGS, *vagans*. *Bombus* illustrations by Steve Buchanan. (Online version in colour.)

(b) Experimental design

(i) Effect of sunflower pollen on *Crithidia* infection in multiple wild *Bombus* species

Experiments were conducted over four summers (figure 1) using workers from wild-caught queens, except for one group of commercially reared *B. impatiens* in 2016, which we included to directly compare to wild *B. impatiens*. Bees were inoculated with *Crithidia*, randomly assigned to sunflower or control pollen for 7 days, and dissected to count *Crithidia* cells; infection reaches a representative population size by 7 days after inoculation [33]. Bees were housed in individual containers (plastic 454 g deli cups with mesh bottoms and lids with holes) and fed 10 ml of 30% sucrose along with 0.15 g of their pollen diet, replaced every other day, and housed in an incubator in darkness at 27°C and 55–60% humidity. Pollen consumption was not measured, except for the 2020 *B. impatiens* trials.

(ii) Diet and infection effects on performance of wild *Bombus impatiens* microcolonies

We created 46 queenless *B. impatiens* microcolonies with five worker bees in each. Microcolonies were derived from one of four natal colonies, all reared from wild-caught queens in 2017 (electronic supplemental material, table S1). Using queenless microcolonies is a common technique to study bumblebee colony development [13,34]. Microcolonies were assigned to one of four treatments in a factorial design: infected with *Crithidia* or not, and fed 50% sunflower or wildflower pollen. We used 50% sunflower instead of 100% sunflower because previous work found that workers in microcolonies fed 100% sunflower suffer relatively high mortality [12,13]. All bees in the infected treatment were inoculated with 6000 *Crithidia* cells in a 10 µl drop, using the protocol described below (see 'Pathogen inoculation and assessment'). Bees in the uninfected groups received a sham inoculum made with Ringer's solution instead of homogenized guts.

We monitored microcolony activity daily, including egg laying, worker deaths and emergence of male offspring. We removed males as they emerged and measured wing marginal cell length to estimate body size. We also measured pollen consumed over 48 h by each microcolony once a week. Microcolonies were terminated either 35 days after the first eggs were laid or after all five workers had died. Remaining workers were dissected as described below to assess *Crithidia* cell counts, and all microcolonies were frozen after termination to assess the number and weight of eggs, larvae and pupae and the number of honey pots produced. Additional details about microcolony set up are described in the electronic supplementary material.

(iii) Pathogen inoculation and assessment

To prepare *Crithidia* inoculum and assess *Crithidia* cell counts, we followed established protocols [8]. We prepared a fresh batch of *Crithidia* inoculum on each trial date. Each bee was removed from its natal colony and starved for 2 h before receiving 10 µl (2016, 2017 and microcolonies) or 15 µl (2018, 2020) of inoculum containing approximately 6000 or 9000 pathogen cells, respectively; comparable to what bees would encounter in nature [35]. To measure *Crithidia* infection, the entire digestive tract of each bee was dissected out, homogenized into 300 µl of water and allowed to rest for 4 h. The number of *Crithidia* cells in a 10 µl aliquot of the supernatant from each sample was measured using a haemocytometer (Hausser Scientific) under a compound light microscope at 400× magnification. We measured *Crithidia* infection prevalence as the presence or absence of *Crithidia* cells in that sample and intensity as the number of *Crithidia* cells per 0.02 µl. We measured marginal cell length of the right forewing of each bee to estimate body size [36].

(c) Statistical analyses

(i) General points

We used the open source software R v.4.0.5 [37] for all analyses. We used emmeans [38] to extract estimated marginal means and ggplot2 [39] to produce all figures. We used generalized linear mixed models (lme4 package) [40] to analyse *Crithidia* prevalence (yes/no) and intensity (cells per 0.02 µl). We used a binomial error distribution with a logit link function to analyse infection prevalence and a negative binomial error distribution to analyse infection intensity, including counts of zero. To measure the death hazard rate, we performed a survival analysis with a Cox proportional hazards models using the coxph, coxme, survfit and Surv functions (survival and coxme packages) [41]. We used Akaike information criterion (AIC) for model selection (AICcmodavg package) [42]. The significance of terms ($\alpha = 0.05$) was tested with likelihood ratio χ^2-tests conducted with the Anova function (car package) [43], which compares relative goodness of fit between models with and without each term.

(ii) Effect of sunflower pollen on *Crithidia* infection in multiple wild *Bombus* species

For *Crithidia* prevalence, intensity and survival analyses, diet was included as a fixed effect in all models; when relevant, additional possible terms included inoculation date and natal colony as random intercept effects, and wing marginal cell length and *Crithidia* source as additional fixed effects. In 2016, commercial *B. impatiens*, wild *B. impatiens* and wild *B. griseocollis* were entered into trials together and inoculated with the same *Crithidia* source, so we can statistically compare them. We also analysed the species separately. In 2017, we used wild *B. griseocollis* (inoculated with one of three sources) and *B. bimaculatus* (two sources; figure 1). To tease apart the effects of sources and host species, we first analysed individuals from both species inoculated with the same source (IMP-2; 93 *B. griseocollis* and 45 *B. bimaculatus*) to compare species while holding source constant. We then analysed each species separately, including all sources, to compare sources within species. Initial models included source and the source by diet interaction as additional fixed effects. In 2018, we tested *B. bimaculatus* (three sources, all from wild *B. bimaculatus* queens; table 1) and *B. vagans* (one source). We analysed these two species separately. In 2020, we used wild *B. impatiens*, all infected with the same source. To analyse pollen and sucrose consumption in the 2020 experiment, we used general linear models with diet and wing marginal cell size as fixed effects and natal colony and inoculation date as random intercept effects.

(iii) Diet and infection effects on performance of wild *Bombus impatiens* microcolonies

We analysed the effect of diet, infection and their interaction on *Crithidia* cell counts in surviving workers at the end of the experiment, worker death hazard ratio for the first 36 days post egg-laying, pollen consumption, reproductive output (number and size of offspring, and number of days until first egg laid), and the number of honey pots produced (electronic supplementary material, table S8). We used the general approach described above for *Crithidia* cell counts and survival. To analyse pollen consumption, we used a generalized linear mixed model to model the grams of pollen consumed after accounting for evaporation (see methods in the electronic supplementary material). Since we measured consumption for every microcolony multiple times, we tested for temporal autocorrelation using the glmmPQL (MASS package) [44] and ACF functions (stats package) [37]. We found little evidence for autocorrelation (ACF < 0.2 for lags greater than 1), and therefore excluded the autocorrelation structure from the consumption model.

During the experiment, we discovered that five microcolonies in the 'uninfected' treatment were infected. These five were all from the same natal colony, suggesting they had a pre-existing wild infection. We compared *Crithidia* cell counts in experimentally inoculated versus wild-inoculated bees in a generalized linear model that also included pollen diet as a fixed predictor. We found no significant difference in infection intensity owing to inoculation type ($\chi^2_1 = 2.78$, $p = 0.095$), and so included all microcolonies from this natal colony in the analyses, switching these five from the 'uninfected' to the 'infected' treatment. We also conducted all analyses with these five microcolonies omitted and found qualitatively similar results. Ten microcolonies (six 50% sunflower, four wildflower) did not produce eggs after four weeks and were excluded from all analyses. Ultimately, *Crithidia* cell counts were analysed in 86 wild *B. impatiens* workers from 24 infected microcolonies.

Table 1. Model outputs (best model after selection using AIC; interaction terms included when applicable) for *Crithidia* cell counts in the single bee trials (includes bees with counts of zero). (Diet and species (when relevant) were always included in models. Other model terms that were tested for inclusion were wing marginal cell length (called 'wing size'; an estimate of bee size) and *Crithidia* source as additional fixed effects, and inoculation date and natal colony as additional random effects. 'Origin' in the 2016 *B. impatiens* experiment refers to wild versus commercial. Significant values are given in italics.)

species (year)	effect	χ^2	d.f.	*p*-value
B. impatiens and *B. griseocollis* (2016)	diet	20.917	1	*<0.0001*
	species	51.645	2	*<0.0001*
	diet * species	12.498	2	*<0.0001*
B. impatiens (2016)	diet	28.0676	1	*<0.0001*
	origin	27.1555	1	*<0.0001*
	diet * origin	2.5962	1	0.1071
B. impatiens (2020)	diet	63.851	2	*<0.0001*
B. griseocollis (2016)	diet	0.0477	1	0.8271
	wing size	4.9462	1	*0.0262*
B. bimaculatus and *B. griseocollis* (2017)	diet	2.1371	2	0.3435
	species	0.6474	1	0.421
B. griseocollis (2017)	diet	0.2202	2	0.8958
	wing size	0.9547	1	0.3285
B. bimaculatus (2017)	diet	4.2522	2	0.1193
	source	6.7759	1	*0.0092*
	diet * source	8.7924	2	*0.0123*
B. bimaculatus (2018)	diet	2.5324	1	0.1115
	source	9.7357	2	*0.0077*
	wing size	3.2667	1	0.0707
B. vagans (2018)	diet	0.8258	1	0.3635

3. Results

(a) Effect of sunflower pollen on *Crithidia* infection in multiple wild *Bombus* species

Compared to wildflower mix, sunflower pollen (both 100% and 50%) significantly reduced *Crithidia* infection intensity in commercial and wild *B. impatiens* (table 1 and figure 2) and infection prevalence in commercial (2016) and wild *B. impatiens* (2020; table 2 and figure 3). In *B. bimaculatus*, 100% but not 50% sunflower pollen reduced infection intensity of one *Crithidia* source (IMP-3 in 2017) but not the other four (table 1 and figure 2), and significantly reduced *Crithidia* prevalence in 2018, but not 2017 (table 2 and figure 3). In *B. vagans*, 100% sunflower pollen reduced prevalence (table 2 and figure 3) but not infection intensity (table 1 and figure 2). In *B. griseosollis*, sunflower pollen did not affect *Crithidia* infection prevalence or intensity (tables 1 and 2 and figures 2 and 3). In *B. griseocollis*, the only significant predictor of infection intensity was bee size; infection intensity increased by approximately 26 cells with every millimeter increase in wing size. In the 2020 *B. impatiens*, pollen diet treatment affected pollen consumption ($F_2 = 4.15$; $p = 0.02$), but not sucrose consumption ($F_2 = 0.96$; $p = 0.39$). Bees consumed more wildflower pollen than 100% or 50% sunflower. However, consumption overlapped substantially between treatments and there was no significant relationship between pollen consumed and *Crithidia* counts within treatments, suggesting that low counts in the 100% and 50% sunflower treatments were not owing to pollen starvation (electronic supplementary material, figure S3). Mortality varied from 47% (2017 *B. griseocollis*) to 2% (2018 *B. vagans*) across species and trials. However, there was no effect of pollen diet or species on mortality in any trial ($\chi^2 < 3.8$, $p > 0.13$ for all).

(b) Diet and infection effects on performance of wild *Bombus impatiens* microcolonies

Microcolonies fed the 50% sunflower diet had significantly lower *Crithidia* infection intensity than those fed the control wildflower diet ($\chi^2_1 = 5.217$, $p = 0.022$; figure 4*c*). We did not detect *Crithidia* cells in 10 of the 86 workers, all of which were fed the 50% sunflower diet. Pollen consumption was not significantly affected by diet, infection, or the infection by diet interaction ($\chi^2 < 0.8$, $p > 0.36$ for all). Microcolonies consumed more pollen overall when they had more workers, and at the peak of the experiment (number of bees: $\chi^2_1 = 7.07$, $p = 0.008$; week number: $\chi^2_1 = 18.73$, $p = 0.005$). There was a significant interaction between pollen diet and infection on the number of days until the first eggs were laid; microcolonies in the uninfected + 50% sunflower treatment laid eggs significantly sooner than the other three treatments (diet by infection: $\chi^2_1 = 4.49$, $p = 0.034$; figure 4*a*). Death hazard rates were not significantly different between pollen diets ($\chi^2_1 = 0.07$, $p = 0.79$) or infection treatments ($\chi^2_1 = 2.87$, $p = 0.09$;

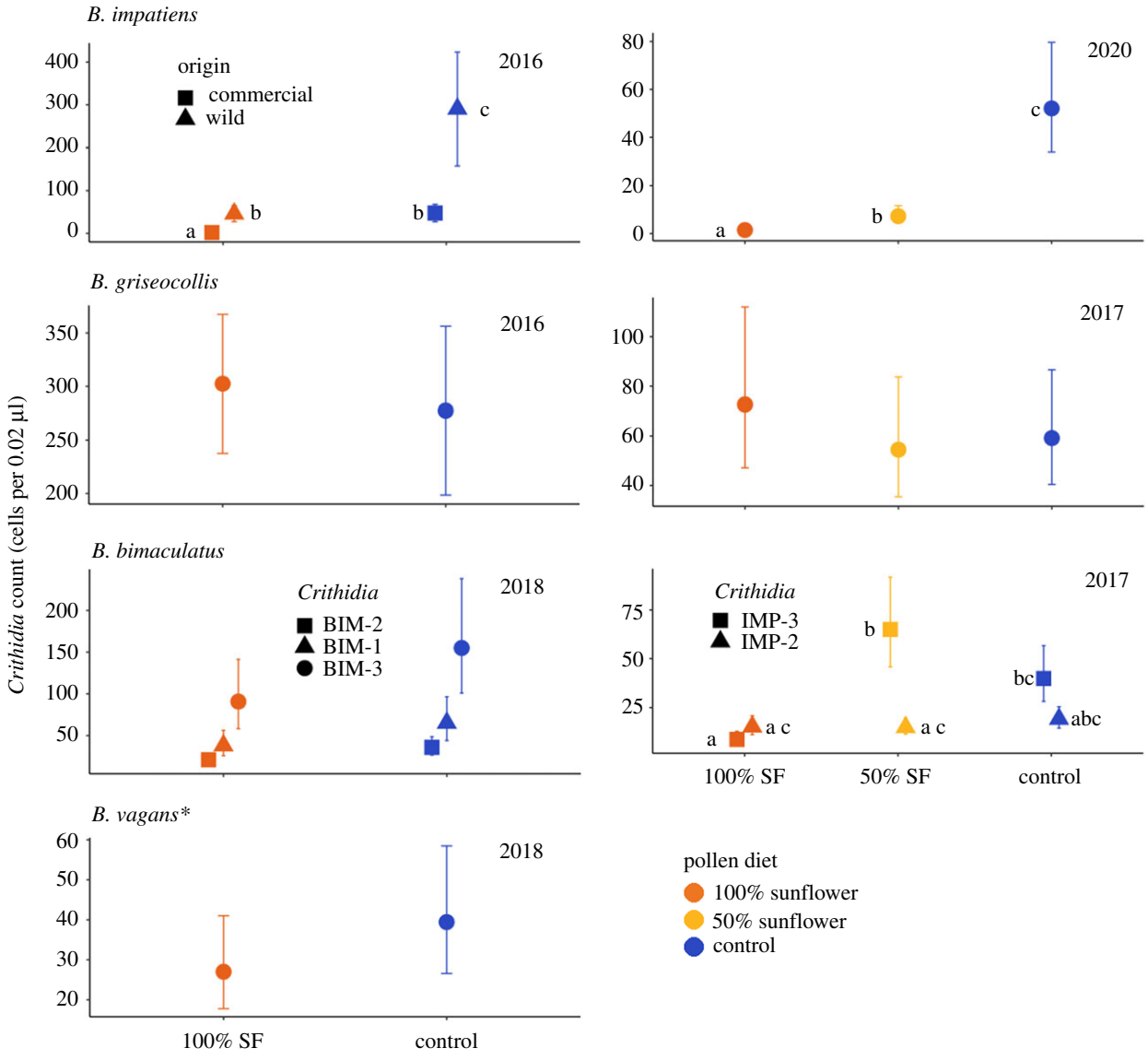

Figure 2. Results for single bee species trials. The horizontal axes are pollen diets: orange is 100% sunflower pollen, yellow is 50% sunflower pollen and blue is control pollen. The vertical axes are *Crithidia* cell counts from 0.02 µl of homogenized gut solution. Significant differences are denoted with different letters; no letters indicate no significant differences between diet treatments. We excluded letters in the *B. bimaculatus* 2018 plot because diet comparisons within sources were non-significant, although source had a significant effect on counts. The figure includes only one *Crithidia* source in *B. griseocollis* in 2017 because sample sizes for the other two sources were very low ($n = 7$ and 3). All bees were wild except for commercial 2016 *B. impatiens*. *Asterisk, results for *B. vagans* are based on workers from one natal colony. (Online version in colour.)

electronic supplementary material, figure S1). Nineteen of the 36 microcolonies (52.8%) produced offspring that survived to adulthood or microcolony termination. Microcolonies fed the 50% sunflower diet produced about half as many offspring as those fed the wildflower mix diet ($\chi^2_1 = 13.21$, $p = 0.0003$), but infection and the diet by infection interaction did not affect offspring production ($\chi^2 < 0.84$, $p > 0.36$ for both; figure 4*b*). Five microcolonies produced offspring that survived to adulthood, four of which were fed the wildflower mix. Neither diet, infection, nor their interaction affected mean larval or pupal weight (electronic supplementary material, table S8, figure S2; see Additional Results in the electronic supplementary material for discussion of a non-significant trend). Thirty of the 36 microcolonies (83.3%) produced honey pots, but production was not significantly affected by diet, infection, or their interaction (electronic supplementary material, table S8).

4. Discussion

Sunflower pollen strongly reduced *Crithidia* infection in wild and commercial *B. impatiens* (figures 1–4), consistent with previous findings [8,9,45,46], and had variable effects in the other bumblebee species we tested. In *B. bimaculatus* and *B. vagans*, sunflower pollen slightly reduced *Crithidia* infection intensity but not significantly so (figure 2), while it significantly reduced *Crithidia* prevalence in both species (figure 3), although further replication is required to verify this pattern in *B. vagans* since results are based on workers from one natal colony. In *B. griseocollis*, however, our robust sample sizes indicate no effect of pollen diet on infection intensity or prevalence (figures 2 and 3).

Our results suggest that responses to sunflower pollen may be genetic or related to other phylogenetically conserved traits. Within *B. impatiens*, we see similar results in wild and commercial bees, suggesting that such traits have not been

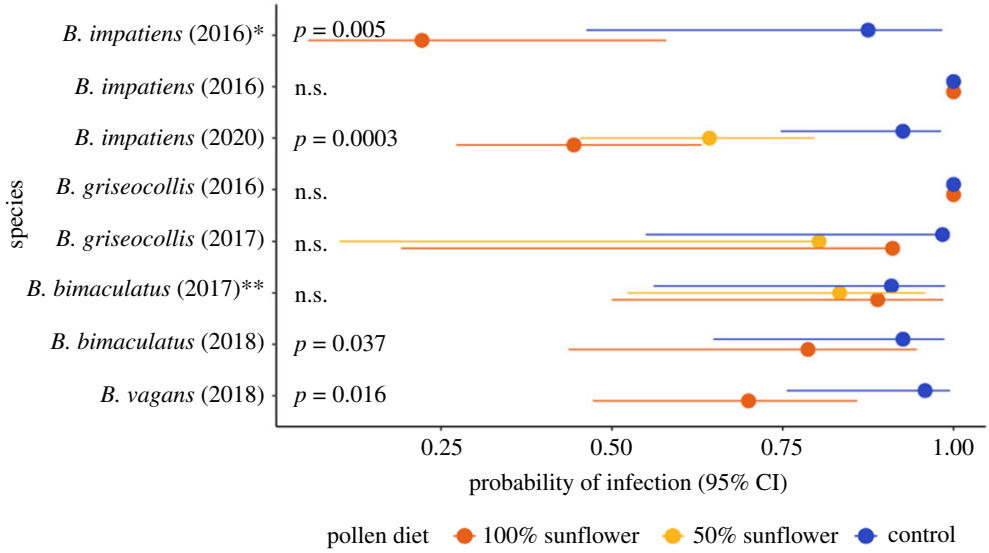

Figure 3. Probability of detecting *Crithidia* cells after 7 days of feeding on a given diet treatment. Probabilities for 2016 wild *B. impatiens* and *B. griseocollis* were not estimated (and are plotted here with a probability of 1) because all bees were infected. The effect of diet on probability of infection is displayed on the left for each species. *All bees were wild except for *B. impatiens* 2016 denoted with an asterisk, which were commercial. **2017 *B. bimaculatus* inoculated with IMP-3 *Crithidia* were all infected; therefore, changes in prevalence owing to diet are estimated from only individuals inoculated with IMP-2 *Crithidia*. (Online version in colour.)

Table 2. Model outputs for *Crithidia* presence/absence in the single bee trials. (χ^2-value for the effect of diet determined using the Anova function. Probabilities of 2016 wild *B. impatiens* and *B. griseocollis* were not estimated because all bees were infected. Additionally, 2017 *B. bimaculatus* inoculated with IMP-3 *Crithidia* were all infected, and therefore changes in prevalence owing to diet are estimated from individuals inoculated with IMP-2 *Crithidia*. Significant values are given in italics.)

species (year)	effect	χ^2	d.f.	*p*-value
B. impatiens (2016; commercial)	diet	7.945	1	*0.0048*
B. impatiens (2016; wild)	—	—	—	—
B. impatiens (2020)	diet	16.067	2	*0.0003*
B. griseocollis (2016)	—	—	—	—
B. griseocollis (2017)	diet	5.038	2	0.0805
B. bimaculatus (2017; IMP-2)	diet	0.319	2	0.853
B. bimaculatus (2018)	diet	4.108	1	*0.0365*
	wing size	1.204	1	0.9995
B. vagans (2018)	diet	5.81	1	*0.016*

significantly altered by the commercialization process. However, consistent with previous work [6], we found that wild *B. impatiens* tended to have higher overall *Crithidia* infection intensities than their commercial counterparts (table 1 and figure 2). We found that *B. bimaculatus* and *B. vagans* exhibited similar, but statistically weaker, patterns as *B. impatiens*, all of which are in the subgenus *Pyrobombus*. Conversely, infection in the more distantly-related *B. griseocollis* (subgenus *Cullumanobombus*), did not respond to pollen diet. Moreover, recent work found that sunflower pollen reduced *Crithidia* infection in *B. terrestris* (H. Koch, P.C. Stevenson 2021, unpublished data), which belongs to the subgenus *Bombus* and is more closely related to *Pyrobombus* than *Cullumanobombus* [47]. Future work assessing a broader diversity of *Bombus* species in different subgenera could determine the extent of variation in diet effect on infection in a comparative framework.

Species may differ in their resistance to *Crithidia* infection via the immune system. The bee immune system is complex and evolved to resist a variety of pathogens, including *Crithidia* [26]. To our knowledge, only one study has compared immune function between *Bombus* species, finding that *B. griseocollis* had a stronger encapsulation response and were more likely to kill parasitoid larvae than *B. impatiens* or *B. bimaculatus* [18]. Immune capacity can also be studied via immune gene presence and expression. A recent study sequenced the genomes of 17 species of bumblebees native to China and found that immune genes were fairly well conserved within the genus [48]. Recent work also found that *B. impatiens* infected with *Crithidia* and fed sunflower pollen had higher expression of multiple immune genes than those fed wildflower pollen [49], while another study found that sunflower pollen diet did not affect two measures of immunity, regardless of infection [50]. This suggests that induced immune function may be involved but is not the primary mechanism by which sunflower reduces infection. Further research would benefit from comparing immune genes and other metrics of the immune system across more clades.

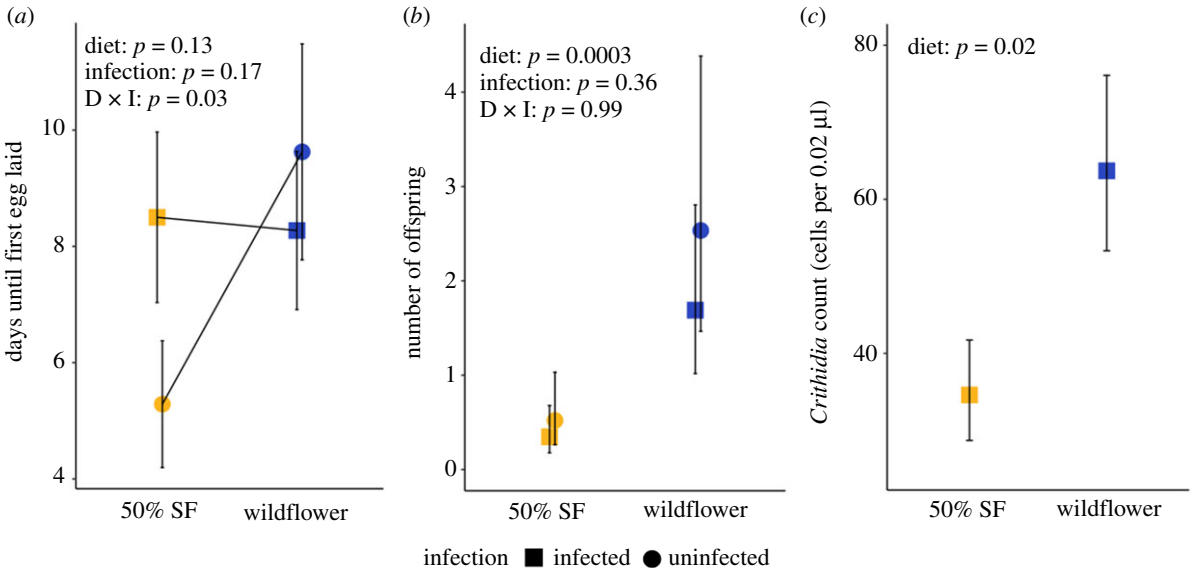

Figure 4. Effect of diet and infection on microcolony: (*a*) number of days until eggs laid, (*b*) offspring production (summed larvae, pupae and males) in wild *B. impatiens*, and (*c*) *Crithidia* count (cells per 0.02 µl). Means estimated by a generalized linear model; error bars indicate standard error back-transformed by emmeans. (Online version in colour.)

The gut microbiome is also a strong determinant of *Crithidia* infection outcomes [30] and can differ between *Bombus* species. Wild *B. impatiens*, *B. griseocollis* and *B. bimaculatus* collected from the field differed in their gut microbial communities: *B. impatiens* had 30 times more bacterial gene copies than *B. bimaculatus*, and *B. bimaculatus* had more non-core bacterial community members than *B. impatiens* and *B. griseocollis* [51]. This richer community was associated with greater *Crithidia* infection intensity [51,52]. We hypothesize that core bacterial members may mediate the interactions between *Crithidia* and sunflower pollen. Other gut properties could also influence infection dynamics; sunflower pollen increased frequency and volume of excretion in *B. impatiens* [53], which may help flush the gut and reduce infection. These changes in gut transit time may be a result of the spiny morphology of sunflower pollen; however, further research is needed to better understand this mechanism and how it may differ across bumblebee species.

Trade-offs are often an inherent and consequential component of medicine. In our microcolony study, sunflower pollen provided medicinal benefits to wild *B. impatiens* workers, but the 50% sunflower diet reduced male offspring production compared to wildflower pollen (figure 4*b*). The reduction is not surprising given that sunflower pollen has low protein content and protein is essential for larval development [11]. This is consistent with a previous study testing six single-species pollens and found that sunflower-fed microcolonies produced the smallest larvae [13]. A relative of sunflower, dandelion (*Taraxacum officinale*, Asteraceae), also reduced reproduction in *B. terrestris* microcolonies compared to control pollen [34]. However, another study found that colonies fed a diet of 50% sunflower pollen produced similar numbers of workers as colonies fed wildflower mix [10]. Thus, sunflower pollen provides a consistent benefit of reducing infection in the laboratory, but in some experiments also comes with reproductive costs.

Previous work has found that worker bumblebees prefer pollen with high rather than low protein content [54]; yet, *B. impatiens* do consume sunflower pollen in the laboratory and collect sunflower pollen in the wild, in higher proportions than available in the landscape (R.L. Malfi, Q.S. McFrederick, G. Lozano, R.E. Irwin, L.S. Adler 2021, unpublished data). All of our experiments were no-choice and so we did not assess pollen preference, but we did measure pollen consumption by *B. impatiens* and found conflicting results within our experiments. *Bombus impatiens* workers in the 2020 species trials consumed more wildflower than 100% or 50% sunflower pollen, but consumption did not differ between pollen diets in the microcolony study. This suggests that 50% sunflower pollen is still high enough quality for workers to consume and survive on since mortality did not differ between diets in any experiments. Future research should assess preferences for sunflower pollen in uninfected and infected bees using choice tests.

The low protein and high lipid content of sunflower pollen may be a poor resource for brood production, but it could provide benefits to the next generation. For example, free foraging commercial *B. impatiens* on farms with more sunflowers produced more daughter queens (Malfi RL *et al.*, unpublished data) and sunflower pollen reduced *Crithidia* infections in *B. impatiens* queens [9]. Given that daughter queens emerge in late summer when sunflowers are blooming, feeding on sunflower pollen may increase queen likelihood of surviving diapause by reducing *Crithidia* infection [55] and providing a relatively high source of lipids, an essential nutrient for diapause [56], at the end of the summer. Future studies can assess whether sunflower pollen is beneficial for queens entering diapause and if queen and worker bumblebees differ in their pollen preferences or physiological use of pollen nutrients (towards ovary development versus fat bodies, etc.).

In contrast to previous studies where *Crithidia* infection reduced production of males and queens [8,10,27,57] and worker survival [28], infection status did not affect reproduction or survival in our study (figure 4*b*; electronic supplementary material, figure S1). Therefore, we did not have the opportunity to test whether sunflower pollen rescued negative effects of infection. The negative effects of *Crithidia* on bee performance may be context-dependent and exacerbated in stressful conditions, such as food limitation [28] or exposure to pesticides [58]. We also recognize that laboratory-captive

microcolonies are a limited proxy for understanding colony dynamics; worker survival and offspring production may be affected by factors missing in a microcolony, such as queen presence and opportunities to forage.

5. Conclusion

Taken together, our results suggest that sunflower pollen does not inhibit the gut parasite, *Crithidia*, in all bumblebee species equally, although more closely related species responded more similarly. That sunflower pollen had no effect on *Crithidia* in *B. griseocollis* highlights the limitations of generalizing from only two model bumblebee species, *B. impatiens* and *B. terrestris*. Even *B. bimaculatus* and *B. vagans*, which are close relatives to *B. impatiens*, did not show the same dramatic and consistent result. Additional research is needed to elucidate processes affecting disease outcomes for non-model bee species. Assessing the generality of experimental findings beyond model bee species is particularly relevant to wild bee conservation given variation across and within bee genera in life history, morphology and physiology. Our study suggests that research with commercial *B. impatiens* may translate to close relatives in the wild, but it is not a reliable model for all bumblebee species.

Focusing research on model organisms such as *B. impatiens* has limitations, but can be valuable in cases where such species play particularly strong ecological roles. *Crithidia* spreads horizontally through the bee community through shared flower use [22], which creates distinct plant–pollinator–pathogen networks [59]. Pathogen transmission networks are largely driven by dominant species [60] and recent work has shown that *B. impatiens*, as a dominant generalist that is abundant in the wild and commonly used on farms and in greenhouses, can drive *Crithidia* prevalence in surrounding plant-pollinator communities [5,59]. Thus, pathogen dynamics in both commercial and wild *B. impatiens* may have disproportionate and profound consequences for the broader bee community. We hypothesize that even if sunflower pollen is only effective at reducing *Crithidia* in *B. impatiens*, it may reduce *Crithidia* prevalence in the plant–pollinator network, benefiting a wider range of bee species.

We suggest that sunflower pollen could be used as supplements for commercial *B. impatiens* colonies as well as in wildflower strips, provided the bees have access to other diverse and nutritious floral resources. We recommend that landowners carefully weigh the costs and benefits of sunflowers for bee health and base management decisions on region-specific ecological context and conservation goals.

Data accessibility. The datasets and code supporting this article are available here: https://github.com/aefowler/Sunflower-wild-bombus.

Authors' contributions. A.E.F.: formal analysis, investigation, methodology, validation, writing–original draft; J.J.G.: investigation, methodology, validation, writing—review and editing; S.J.C.: investigation, methodology, project administration, writing—review and editing; R.E.I.: conceptualization, funding acquisition, project administration, resources, validation, writing—review and editing; L.S.A.: conceptualization, funding acquisition, project administration, resources, validation, writing—review and editing.

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

Competing interests. We declare we have no competing interests.

Funding. Research was funded by a Lotta Crabtree Fellowship from the University of Massachusetts College of Natural Sciences (A.E.F.), National Science Foundation Graduate Research Fellowships (A.E.F. and J.J.G.), USDA-NIFA-2016-07962 and USDA-AFRI-2018-08591 (L.S.A. and R.E.I.), and USDA/CSREES NE2001 (Multi-state Hatch: L.S.A.). Any opinions, findings and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the funding agencies.

Acknowledgements. We thank N. Chevan, J. Cook, H. Giguere, G. Santiago, for research assistance, L. Aguirre for statistical advice, K. Skyrm for catching and initiating some of the queens. We also thank members of the Adler laboratory and two anonymous reviewers for feedback on the manuscript.

References

1. Phifer-Rixey M, Nachman MW. 2015 Insights into mammalian biology from the wild house mouse *Mus musculus*. *Elife* **4**, e05959. 1–13. (doi:10.7554/eLife.05959)
2. Schmid-Hempel P, Wilfert L, Schmid-Hempel R. 2019 Pollinator diseases: the *Bombus-Crithidia* system. In *Wildlife disease ecology: linking theory to data and application* (eds A Fenton, D Tompkins, K Wilson), pp. 3–31. Cambridge, UK: Cambridge University Press.
3. Velthuis HW, Van Doorn A. 2006 A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. *Apidologie* **37**, 421–451. (doi:10.1051/apido:2006019)
4. Garibaldi LA *et al.* 2013 Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science* **339**, 1608–1611. (doi:10.1126/science.1230200)
5. Colla SR, Otterstatter MC, Gegear RJ, Thomson JD. 2006 Plight of the bumble bee: pathogen spillover from commercial to wild populations. *Biol. Conserv.* **129**, 461–467. (doi:10.1016/j.biocon.2005.11.013)
6. Mockler BK, Kwong WK, Moran NA, Koch H, Drake HL. 2018 Microbiome structure influences infection by the parasite *Crithidia bombi* in bumble bees. *Appl. Environ. Microbiol.* **84**, 2335–2352. (doi:10.1128/AEM.02335-17)
7. Ponton F, Wilson K, Holmes AJ, Cotter SC, Raubenheimer D, Simpson SJ. 2013 Integrating nutrition and immunology: a new frontier. *J. Insect Physiol.* **59**, 130–137. (doi:10.1016/j.jinsphys.2012.10.011)
8. Giacomini JJ, Leslie J, Tarpy DR, Palmer-Young EC, Irwin RE, Adler LS. 2018 Medicinal value of sunflower pollen against bee pathogens. *Sci. Rep.* **8**, 14394. (doi:10.1038/s41598-018-32681-y)
9. Fowler AE, Stone EC, Irwin RE, Adler LS. 2020 Sunflower pollen reduces a gut pathogen in worker and queen but not male bumble bees. *Ecol. Entomol.* **45**, 1318–1326. (doi:10.1111/een.12915)
10. Giacomini JJ, Connon SJ, Marulanda D, Adler LS, Irwin RE. 2021 The costs and benefits of sunflower pollen diet on bumble bee colony disease and health. *Ecosphere* **12**, e03663. (doi:10.1002/ecs2.3663)
11. Nicolson SW, Human H. 2013 Chemical composition of the 'low quality' pollen of sunflower (*Helianthus annuus*, Asteraceae). *Apidologie* **44**, 144–152. (doi:10.1007/s13592-012-0166-5)
12. McAulay MK, Forrest JRK. 2019 'How do sunflower pollen mixtures affect survival of queenless microcolonies of bumblebees (*Bombus impatiens*)? *Arthropod. Plant. Interact.* **13**, 517–529. (doi:10.1007/s11829-018-9664-3)
13. Tasei J-N, Aupinel P. 2008 Nutritive value of 15 single pollens and pollen mixes tested on larvae produced by bumblebee workers (*Bombus terrestris*, Hymenoptera: Apidae). *Apidologie* **39**, 397–409. (doi:10.1051/apido:2008017)
14. Richardson LL, McFarland KP, Zahendra S, Hardy S. 2019 Bumble bee (*Bombus*) distribution and diversity in Vermont, USA: a century of change. *J. Insect Conserv.* **23**, 45–62. (doi:10.1007/s10841-018-0113-5)

15. Colla S, Richardson L, Williams P. 2012 *Bumble bees of the eastern United States*. Washington, DC: United States Forest Service.
16. Cordes N, Huang W-F, Strange JP, Cameron SA, Griswold TL, Lozier JD, Solter LF. 2012 Interspecific geographic distribution and variation of the pathogens *Nosema bombi* and *Crithidia* species in United States bumble bee populations. *J. Invertebr. Pathol.* **109**, 209–216. (doi:10.1016/j.jip.2011.11.005)
17. Averill AL, Couto AV, Andersen JC, Elkinton JS. 2021 Parasite prevalence may drive the biotic impoverishment of new England (USA) bumble bee communities. *Insects* **12**, 941, 1–11. (doi:10.3390/insects12100941)
18. Davis SE, Malfi RL, 'H Roulston T. 2015 Species differences in bumblebee immune response predict developmental success of a parasitoid fly. *Oecologia* **178**, 1017–1032.
19. Woodard SH, Lozier JD, Goulson D, Williams PH, Strange JP, Jha S. 2015 Molecular tools and bumble bees: revealing hidden details of ecology and evolution in a model system. *Mol. Ecol.* **24**, 2916–2936. (doi:10.1111/mec.13198)
20. Schmid-Hempel P. 2001 On the evolutionary ecology of host-parasite interactions: addressing the question with regard to bumblebees and their parasites. *Naturwissenschaften* **88**, 147–158. (doi:10.1007/s001140100222)
21. Schmid-Hempel R, Tognazzo M. 2010 Molecular divergence defines two distinct lineages of *Crithidia bombi* (Trypanosomatidae), parasites of bumblebees. *J. Eukaryot. Microbiol.* **57**, 337–345. (doi:10.1111/j.1550-7408.2010.00480.x)
22. Durrer S, Schmid-Hempel P. 1994 Shared use of flowers leads to horizontal pathogen transmission. *Proc. R. Soc. B* **258**, 299–302. (doi:10.1098/rspb.1994.0176)
23. Otterstatter MC, Thomson JD. 2007 Contact networks and transmission of an intestinal pathogen in bumble bee (*Bombus impatiens*) colonies. *Oecologia* **154**, 411–421. (doi:10.1007/s00442-007-0834-8)
24. Figueroa LL, Grincavitch C, McArt SH. 2021 *Crithidia bombi* can infect two solitary bee species while host survivorship depends on diet. *Parasitology* **148**, 435–442. (doi:10.1017/S0031182020002218)
25. Ngor L, Palmer-Young EC, Burciaga Nevarez R, Russell KA, Leger L, Giacomini SJ, Pinilla-Gallego MS, Irwin RE, Mcfrederick QS. 2020 Cross-infectivity of honey and bumble bee-associated parasites across three bee families. *Parasitology* **147**, 1290–1304. (doi:10.1017/S0031182020001018)
26. Brown MJF, Moret Y, Schmid-Hempel P. 2003 Activation of host constitutive immune defence by an intestinal trypanosome parasite of bumble bees. *Parasitology* **126**, 253–260. (doi:10.1017/S0031182002002755)
27. Goulson D, O'Connor S, Park KJ. 2017 The impacts of predators and parasites on wild bumblebee colonies. *Ecol. Entomol.* **43**, 168–181. (doi:10.1111/een.12482)
28. Brown MJF, Loosli R, Schmid-Hempel P. 2000 Condition-dependent expression of virulence in a trypanosome infecting bumblebees. *Oikos* **91**, 421–427. (doi:10.1034/j.1600-0706.2000.910302.x)
29. Barribeau SM, Schmid-Hempel P. 2013 Qualitatively different immune response of the bumblebee host, *Bombus terrestris*, to infection by different genotypes of the trypanosome gut parasite, *Crithidia bombi*. *Infect. Genet. Evol.* **20**, 249–256. (doi:10.1016/j.meegid.2013.09.014)
30. Koch H, Schmid-Hempel P. 2012 Gut microbiota instead of host genotype drive the specificity in the interaction of a natural host-parasite system. *Ecol. Lett.* **15**, 1095–1103. (doi:10.1111/j.1461-0248.2012.01831.x)
31. Koch H, Schmid-Hempel P. 2011 Socially transmitted gut microbiota protect bumble bees against an intestinal parasite. *Proc. Natl Acad. Sci. USA* **108**, 19 288–19 292. (doi:10.1073/pnas.1110474108)
32. Yang K, Dan W, Xingqian Y, Donghong L, Jianchu C, Peilong S. 2013 Characterization of chemical composition of bee pollen in China. *J. Agr. Food Chem.* **61**, 708–718. (doi:10.1021/jf304056b)
33. Otterstatter MC, Thomson JD. 2006 Within-host dynamics of an intestinal pathogen of bumble bees. *Parasitology* **133**, 749–761. (doi:10.1017/S003118200600120X)
34. Vanderplanck M, Gilles H, Nonclercq D, Duez P, Gerbaux P. 2020 Asteraceae paradox: chemical and mechanical protection of taraxacum pollen. *Insects* **11**, 304. (doi:10.3390/insects11050304)
35. Schmid-Hempel P, Schmid-Hempel R. 1993 Transmission of a pathogen in *Bombus terrestris*, with a note on division of labour in social insects. *Behav. Ecol. Sociobiol.* **33**, 319–327. (doi:10.1007/BF00172930)
36. Nooten SS, Rehan SM. 2020 Historical changes in bumble bee body size and range shift of declining species. *Biodivers. Conserv.* **29**, 451–467. (doi:10.1007/s10531-019-01893-7)
37. R Core Team. 2021 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
38. Lenth R. 2020 Emmeans: estimated marginal means, aka least-squares means. R package version 1.5.2-1. See https://CRAN.R-project.org/package=emmeans.
39. Wickham H. 2016 *Ggplot: elegant graphics for data analysis*. New York, NY: Springer.
40. Bates D, Maechler M, Bolker B, Walker S. 2015 Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1–48.
41. Therneau TM. 2020 Coxme: mixed effects cox models. R package version 2.2-16. See https://CRAN.R-project.org/package=coxme.
42. Mazerolle MJ. 2020 AICcmodavg: model selection and multimodel inference based on (Q)AIC(c). R package version 2.3-1. See https://cran.r-project.org/package=AICcmodavg.
43. Fox J, Weisberg S. 2019 *An {R} companion to applied regression, third edition*. Thousand Oaks, CA: Sage.
44. Venables WN, Ripley BD. 2002 *Modern applied statistics with S*. New York, NY: Springer.
45. LoCascio GM, Aguirre L, Irwin RE, Adler LS. 2019 Pollen from multiple sunflower cultivars and species reduces a common bumblebee gut pathogen. *R. Soc. Open Sci.* **6**, 190279. (doi:10.1098/rsos.190279)
46. Adler LS *et al.* 2020 Assessing chemical mechanisms underlying the effects of sunflower pollen on a gut pathogen in bumble bees. *J. Chem. Ecol.* **46**, 649–658. (doi:10.1007/s10886-020-01168-4)
47. Williams PH, Cameron SA, Hines HM, Cederberg B, Rasmont P. 2008 A simplified subgeneric classification of the bumblebees (genus *Bombus*). *Apidologie* **39**, 46–74. (doi:10.1051/apido:2007052)
48. Sun C *et al.* 2020 Genus-wide characterization of bumblebee genomes provides insights into their evolution and variation in ecological and behavioral traits. *Mol. Biol. Evol.* **38**, 486–501.
49. Giacomini JJ, Adler LS, Reading BJ, Irwin RE. 2021 Differential bumble bee gene expression associated with pathogen infection and pollen diet. Preprint. (doi:10.21203/rs.3.rs-912647/v1)
50. Fowler AE, Sadd BM, Bassingthwaite T, Irwin RE, Adler LS. In press. Consuming sunflower pollen reduced pathogen infection but did not alter measures of immunity in bumble bees. *Phil. Trans. R. Soc. B* (doi:10.1098/rstb.2021.0160)
51. Cariveau DP, Powell JE, Koch H, Winfree R, Moran NA. 2014 Variation in gut microbial communities and its association with pathogen infection in wild bumble bees (*Bombus*). *ISME J.* **8**, 2369–2379. (doi:10.1038/ismej.2014.68)
52. Näpflin K, Schmid-Hempel P. 2018 High gut microbiota diversity provides lower resistance against infection by an intestinal parasite in bumblebees. *Am. Nat.* **192**. 131–141. (doi:10.1086/698013)
53. Giacomini JJ, Moore N, Adler LS, Irwin RE. 2022 Sunflower pollen induces rapid excretion in bumble bees: implications for host-pathogen interactions. *J. Insect Physiol.* **137**, 104356. (doi.org/10.1016/j.jinsphys.2022.104356)
54. Vaudo AD, Patch HM, Mortensen DA, Tooker JF, Grozinger CM. 2016 Macronutrient ratios in pollen shape bumble bee (*Bombus impatiens*) foraging strategies and floral preferences. *Proc. Natl Acad. Sci. USA* **113**, E4035–E4042.
55. Fauser A, Sandrock C, Neumann P, Sadd BM. 2017 Neonicotinoids override a parasite exposure impact on hibernation success of a key bumblebee pollinator. *Ecol. Entomol.* **42**, 306–314. (doi:10.1111/een.12385)
56. Alford DV. 1969 A study of the hibernation of bumblebees (Hymenoptera: Bombidae) in southern England. *J. Anim. Ecol.* **38**, 149–170. (doi:10.2307/2743)
57. Regali A, Rasmont P. 1995 New bioassays to evaluate diet in *Bombus terrestris*. *Apidologie* **26**, 273–281. (doi:10.1051/apido:19950401)
58. Fauser-Misslin A, Sadd BM, Neumann P, Sandrock C. 2014 Influence of combined pesticide and parasite exposure on bumblebee colony traits in the laboratory. *J. Appl. Ecol.* **51**, 450–459. (doi:10.1111/1365-2664.12188)
59. Figueroa LL, Grab H, Ng WH, Myers CR, Graystock P, Mcfrederick QS, Mcart SH. 2020 Landscape simplification shapes pathogen prevalence in plant-pollinator networks. *Ecol. Lett.* **23**, 1212–1222. (doi:10.1111/ele.13521)
60. Keesing F *et al.* 2010 Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature* **468**, 647–652. (doi:10.1038/nature09575)