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Crithidia bombi can infect two solitary bee species while host survivorship depends on diet

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Research Article

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Abstract

Pathogens and lack of floral resources interactively impair global pollinator health. However, epidemiological and nutritional studies aimed at understanding bee declines have historically focused on social species, with limited evaluations of solitary bees. Here, we asked whether *Crithidia bombi*, a trypanosomatid gut pathogen known to infect bumble bees, could infect the solitary bees *Osmia lignaria* (females) and *Megachile rotundata* (males), and whether nutritional stress influenced infection patterns and bee survival. We found that *C. bombi* was able to infect both solitary bee species, with 59% of *O. lignaria* and 29% of *M. rotundata* bees experiencing pathogen replication 5–11 days following inoculation. Moreover, access to pollen resulted in *O. lignaria* living longer, although it did not influence *M. rotundata* survival. Access to pollen did not affect infection probability or resulting pathogen load in either species. Similarly, inoculating with the pathogen did not drive survival patterns in either species during the 5–11-day laboratory assays. Our results demonstrate that solitary bees can be hosts of a known bumble bee pathogen, and that access to pollen is an important contributing factor for bee survival, thus expanding our understanding of factors contributing to solitary bee health.

Introduction

Pathogen pressure, inadequate nutrition and the interaction between these stressors can drastically impair pollinator health (Goulson *et al.*, 2015). The relationship between nutrition and bee disease dynamics is complex, as diet quality and diversity can support immunocompetence and reduce infections in some instances, while increasing disease burdens in other cases (Koch *et al.*, 2017; Dolezal and Toth, 2018). A bee's diet is made up of nectar and pollen, both of which have primary metabolites such as sugars, amino acids and lipids, as well as secondary compounds, such as flavonoids, terpenoids and alkaloids, with the potential to decrease (Richardson *et al.*, 2015) or increase (Palmer-Young and Thursfield, 2017) infections. In a world dominated by agricultural landscapes, with low floral diversity and frequent movement of commercial bee colonies that can potentially introduce pathogens into wild solitary bee populations (Kremen *et al.*, 2002; Otterstatter and Thomson, 2008; Furst *et al.*, 2014; Alger *et al.*, 2019), understanding the interplay between nutrition and pathogen burdens is important for protecting pollinator health.

Immune defence against pathogens is energetically costly, yet the resulting physiological trade-offs between immunity and other fitness components can be compensated by changes in diet (Moret and Schmid-Hempel, 2000). When *Bombus terrestris* bees are starved of pollen, infection with *Crithidia bombi*, a trypanosomatid gut pathogen, markedly increases host mortality compared to bees with access to pollen (Brown *et al.*, 2000). However, when both *B. terrestris* and *B. impatiens* have access to pollen, they can present higher *C. bombi* loads than pollen-starved counterparts (Logan *et al.*, 2005; Conroy *et al.*, 2016). Similarly, access to pollen can increase honey bee survival, while simultaneously increasing pathogen loads of the microsporidians *Nosema apis* (Rinderer and Dell Elliott, 1977) and *Nosema ceranae* (Zheng *et al.*, 2014; Jack *et al.*, 2016). As such, disentangling the effects of nutritional stress and pathogen infection on bee survival is important for understanding pollinator health, especially for solitary bees, which contribute substantially to pollination services worldwide yet have been historically understudied (Danforth *et al.*, 2019).

Honey bees and bumble bees have been the predominant model systems for addressing questions regarding bee health, especially for nutritional and epidemiological evaluations (Schmid-Hempel, 1998). Recent advances in molecular surveillance have revealed widespread pathogen prevalence across solitary bee taxa from most bee families (Andrenidae, Apidae, Colletidae, Halictidae and Megachilidae). This includes pathogens known to infect honey bees and bumble bees (Apidae), including *Apicystis bombi*, *Ascosphaera* spp., *C. bombi*, *C. mellificae*, *N. ceranae* and numerous viruses (Singh *et al.*, 2010; Evison *et al.*, 2012; Ravoet *et al.*, 2014; Schoonvaere *et al.*, 2018; Figueroa *et al.*, 2020). However, except for single-stranded RNA viruses which allow for strand-specific PCR assays to detect viral replication, we currently cannot distinguish between transient passage through the bee gut and active infections, nor do we know if there are negative consequences for the host based solely on molecular screenings

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(Bramke *et al.*, 2019). Some existing studies that have experimentally evaluated the impacts on solitary bee health have shown increased mortality associated with infections [e.g. *Megachile rotundata* larvae infected with the fungus *Ascosphera aggregata* (James, 2005) and *Osmia bicornis* infected with the neogregarine *A. bombi* and the microsporidian *N. ceranae* (Tian *et al.*, 2018; Bramke *et al.*, 2019)], further highlighting the need to address the host range of bee pathogens and negative consequences for these understudied bee species.

Pathogens are spread between bee species *via* shared use of floral resources (Graystock *et al.*, 2015). Despite ample possible routes of indirect transmission *via* flowers at the community level (Figueroa *et al.*, 2020), the host range of many bee pathogens remains currently unknown. Infection with the trypanosomatid *C. bombi* can affect bumble bee foraging behaviour, cognitive function (Gegear *et al.*, 2005, 2006) and reproduction (Goulson *et al.*, 2018). While *C. bombi* is known to infect multiple bumble bee species (Colla *et al.*, 2006; Cordes *et al.*, 2012; Ruiz-González *et al.*, 2012), honey bees are not a known host (Ruiz-González and Brown, 2006; Graystock *et al.*, 2015), even though both groups belong to the same family. It is largely unknown whether solitary bee species, which frequently test positive for *C. bombi via* PCR-based screenings (Figueroa *et al.*, 2020; Graystock *et al.*, 2020), are actually infected by this pathogen (Ravoet *et al.*, 2014). The solitary bees *Osmia lignaria* and *M. rotundata* are cavity-nesting species that provide important pollination services for fruits and vegetables in North America and Europe (Velthuis and van Doorn, 2006; Pitts-Singer and Cane, 2011; Brittain *et al.*, 2013) and can serve as model organisms for experimentally evaluating epidemiological questions due to their commercial availability. Recent experimental work has shown that *C. bombi* collected from *B. impatiens* can infect these species (Ngor *et al.*, 2020), further highlighting the need to understand the biotic and abiotic factors, such as diet, that influence disease dynamics in species beyond honey bees and bumble bees.

To fill this critical knowledge gap, we conducted a study to understand the influence of nutritional stress on the susceptibility of solitary bees to pathogens. Specifically, we asked: (1) how frequently after exposure to *C. bombi* do the solitary bee species *O. lignaria* and *M. rotundata* become infected, (2) does pollen access influence the likelihood of infection and/or subsequent load of *C. bombi* in *O. lignaria* and *M. rotundata*, and (3) does pollen access and/or *C. bombi* exposure influence *O. lignaria* and *M. rotundata* survival?

Materials and methods

Study system

For our experiments, we used the trypanosomatid pathogen *C. bombi* (Kinetoplastea, Trypanosomatida) and two solitary bee species: *M. rotundata* (Hymenoptera, Megachilidae; Crown Bees, Woodinville, WA, USA) and *O. lignaria* (Hymenoptera, Megachilidae; Watts bees, Bothell, WA, USA). The bees were obtained as cocoons and allowed to eclose in individually marked housing units: inverted 59 mL salsa cups (Fabi-Kal® Greenware, Lancaster, PA, USA) lined with filter paper (Whatman®, Marlborough, PA, USA) that provided access to 30% sucrose solution through a small opening at the tip of a 1.5 mL microcentrifuge tube (VWR™, Radnor, PA, USA) (Fig. S1). The sex of these individuals can be easily determined visually upon eclosion. As such, only females were maintained for the experiment with *O. lignaria*. Females represented <10% of the eclosed *M. rotundata*, and we therefore conducted the experiments exclusively with males for this species in order to have sufficient sample sizes. As such, we do not make any formal species or sex comparisons in our analyses.

The *C. bombi* we used in our trials was collected from wild *Bombus impatiens* (Hymenoptera, Apidae) workers from Massachusetts, USA (GPS: 42822'17.5300N, 72835'13.5200W). The strain was sustained in laboratory bumble bee colonies (Biobest, Leamington, Ontario, Canada). The two solitary bee species are commonly used in commercial agriculture in the USA, though *O. lignaria* is native to North America while *M. rotundata* is European (Velthuis and van Doorn, 2006).

Experimental design

To investigate *C. bombi* replication and its effect on bee survival, we conducted a 2×2 factorial experiment for each bee species, contrasting nutritional stress (presence/absence of pollen) and infection status (inoculated/sham-inoculated with *C. bombi*). We prepared *C. bombi* inoculum fresh for each inoculation day by dissecting the gut of infected *B. impatiens* bees from the laboratory source colony. We homogenized the bee guts in distilled water and quantified *C. bombi* cells using a haemocytometer. We diluted the mixture to 1200 *C. bombi* cells μL^{-1}, which we then combined 1:1 with 30% sucrose solution for an inoculum of 600 cells μL^{-1}, a standard inoculum concentration for infecting bumble bees with *C. bombi* (Richardson *et al.*, 2015; Figueroa *et al.*, 2019). We used 30% sucrose without *C. bombi* as a control (sham) inoculum. The sucrose solution was coloured with blue food colouring (McCormick & Company, Baltimore, MD, USA) to facilitate confirmation of consumption and availability.

For each trial, half of the bees were inoculated with *C. bombi* and half received the sham inoculation. Each bee species was inoculated on separate dates: *O. lignaria* on 27 June 2019 ($n = 97$), 22 July 2019 ($n = 48$) and 23 July 2019 ($n = 52$), while *M. rotundata* was inoculated on 7 July 2019 ($n = 100$). The unit of replication for this study was individual bees. The lower sample size for *M. rotundata* was due to lower availability of bees. The bees had eclosed 1–2 days before being inoculated in their individual housing units. Each treatment was evenly represented on each inoculation date. Standard *C. bombi* inoculations in bumble bees are conducted by eliciting proboscis extension from the sugar in the inoculum and directly feeding the bees 10 μL of inoculum (Richardson *et al.*, 2015; Figueroa *et al.*, 2019). However, in a pilot study, we found that *O. lignaria* and *M. rotundata* bees were uncooperative and would not consume the inoculum droplets using the standardized bumble bee protocol. When we subdued the bees using CO_2, we were able to place the inoculum droplet directly on their extended proboscis, finding that this method was effective at infecting the solitary bees.

To administer the inoculum for this experiment, we exposed individual bees to CO_2 gas for 45 s, during which time most bees extended their proboscis. We then placed 5 μL of inoculum on their proboscis and on the pollen, if present, or on the side of the feeding tube for pollen-free treatments, to maximize exposure to the pathogen (10 μL on the first day of trial), *O. lignaria* received an additional 5 μL dose on the pollen/feeding tube for two consecutive days for a total of 20 μL (12 000 *C. bombi* cells) administered compared to the 10 μL (6000 *C. bombi* cells) for *M. rotundata*. The additional doses were administered to increase the likelihood of exposure to the pathogen in the larger of the two species (*O. lignaria*). Trays containing the bees were separated by treatment to avoid cross-contamination, but were all maintained on the same laboratory bench for the duration of the assay.

Half of the bees in both the inoculated and sham-inoculated treatments were provided ~36 mg balls of pollen, made from a mixture of honey bee collected poly-floral pollen (Bee Pollen Granules, CC Pollen High Desert, Phoenix AZ, USA), and all bees were provided 30% sucrose solution. The sucrose solution was replaced every 3 days and pollen balls were given to the pollen

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treatment bees every other day. We verified that the pollen had little to no pesticides by screening a pollen sample for 267 pesticides using liquid chromatography/mass spectrometry (Urbanowicz *et al.*, 2019). We detected only one pesticide, the acaricide coumaphos, at a level below the limit of quantification (<0.525 ng g^{-1}), and thus concluded that pesticide exposure would not be a primary driver of any pattern found with pollen access. The pollen was not a source of *C. bombi* as can be verified by the absence of *C. bombi* in our sham-inoculated bees fed pollen (Fig. 1). While *O. lignaria* larvae tend to develop more quickly and larger body sizes on pollen collected by members of its own species, they will nonetheless develop on honey bee-collected pollen (Levin and Haydak, 1957). Whether this translates into differences in pollen feeding by adults (both female *O. lignaria* and male *M. rotundata*) has not been tested. Though we did not quantify pollen consumption, we observed frass with pollen residues for some of the bees in the pollen-access treatments, indicating consumption. The bees were maintained in laboratory conditions at an average temperature of approximately 20°C in constant dark.

We checked bee survival daily for the duration of the trial (terminated after 11 days for *M. rotundata*, and 8, 6 and 5 days for the three *O. lignaria* trials, respectively), and recorded daily mortality for each bee. Trial lengths varied due to differences in mortality; while *M. rotundata* had low overall mortality (18% died by the end of the 11-day trial), mortality for *O. lignaria* was overall higher and varied greatly (ranged from 14 to 54% depending on the trial). We dissected any bee that had died within 24 h to screen for *C. bombi* (checked daily), as well as all bees that survived until the end of the trial. Given that our pathogen counts are based on motile cells, we expected the most accurate counts from bees that were alive. As such, we shortened the trial times for *O. lignaria* in order to have enough live bees to accurately quantify infection because observing live *C. bombi* in dead bees, while possible, is likely less accurate. For example, only 8% of recorded *C. bombi*-positive *O. lignaria* bees had died before the end of the trial and had much lower corresponding pathogen loads (mean of 71 active *C. bombi* cells μL^{-1} compared to 171 cells μL^{-1} in bees that survived the length of the trial). We cannot determine whether the lower likelihood of detection and lower counts were products of insufficient time for the pathogen to replicate or whether the pathogen had died within the host before dissection and could not be visualized. As such, infection analyses were conducted only on bees that survived until the end of the trial. Nonetheless, the shorter trial times for *O. lignaria* are within time frames relevant for *C. bombi* replication in bumble bees (Schmid-Hempel and Schmid-Hempel, 1993). We homogenized the dissected bee guts in 200 μL of distilled water, incubated the mixture for 4 h at room temperature and finally quantified motile *C. bombi* using a haemocytometer (Richardson *et al.*, 2015; Figueroa *et al.*, 2019).

For the bees that had motile *C. bombi*, we evaluated whether the values indicated active replication by the pathogen. To do this, we compared estimated whole gut counts to the value in the entirety of the inoculum provided to the bees. The *C. bombi* μL^{-1} observed for each bee was multiplied by 200 μL, the volume of water in which the gut was incubated, indicating the total number of *C. bombi* cells estimated to be in the bee gut. This is a conservative estimate as we are not including the volume of the gut itself. Values above 12 000 for *O. lignaria* and 6000 for *M. rotundata* indicate active pathogen replication in the bees as these values correspond to the maximum possible cells consumed in the inoculum. We report these numbers as 'whole gut *C. bombi* estimates' in the results.

At the end of the test periods, we measured the inter-tegular distance (ITD) of all of the bees using an Olympus SZX10 microscope and cellSens Standard software (Olympus Corporation of the Americas, Scientific Solutions Group, Waltham, Massachusetts, USA). The ITD is commonly used as a proxy for bee body size (Greenleaf *et al.*, 2007), which is a factor related to immunity in bumble bees (Otterstatter and Thomson, 2006).

Statistical analyses

Data analyses were conducted using R version 3.5.1 (R Development Core Team, 2008) with the lme4, glmmTMB and coxme packages (Bates *et al.*, 2015; Therneau and Therneau, 2015; Brooks *et al.*, 2017). We conducted analyses on *M. rotundata* and *O. lignaria* separately.

To determine the role of pollen access on *C. bombi* infection patterns in the solitary bees, we employed a manual two-step hurdle model due to zero-inflation and overdispersion in the data (Zuur *et al.*, 2009). As mentioned above, these analyses were only conducted on inoculated bees that survived until the last day of the trial to increase the accuracy of *C. bombi* counts (11 days for *M. rotundata* and 5, 6 or 8 days for the three *O. lignaria* trials). We first evaluated whether pollen access predicted the presence or absence of *C. bombi* in the gut of inoculated bees, followed by an evaluation of the *C. bombi* counts for those that were infected. For *O. lignaria*, we first constructed a generalized linear mixed model (GLMM) to evaluate whether the solitary bee species harboured *C. bombi* in their gut as the response (yes/no), predicted by access to pollen (yes/no) and bee size ($n = 59$). The model included inoculation date as the random effect and a binomial distribution (logit link). To determine significance, we employed a likelihood ratio test and compared the model to one with the same response, distribution and random-effect structure, but which excluded pollen as a predictor. We then developed a GLMM that included *C. bombi* count in *C. bombi*-positive bees as the response variable, predicted by access to pollen (presence/absence) and bee size ($n = 49$). The model included inoculation date as the random effect and fit a truncated negative binomial distribution, which is suitable for count data with overdispersion (Brooks *et al.*, 2017). Significance was similarly determined using a likelihood ratio test. We verified model assumptions using the DHARMa package (Hartig, 2017). The statistical analyses for *M. rotundata* were likewise evaluated, with the sole difference that the manual hurdle model was conducted using a Generalized Linear Model instead of a GLMM because the species was inoculated in a single day and thus did not require inoculation date as a random effect ($n = 35$ for binomial response and $n = 10$ for *C. bombi* count as a response).

To evaluate whether exposure to *C. bombi*, access to pollen and their interactions influenced solitary bee survival, we conducted survival analyses using Cox proportional hazards models ($n = 179$ and $n = 93$ for *O. lignaria* and *M. rotundata*, respectively). For *O. lignaria*, the survival analysis evaluated bee survival (death/days elapsed) as the response, *C. bombi* inoculation and access to pollen as explanatory variables, and inoculation date as the random effect. To determine the significance of the treatments (pollen and inoculation), we conducted a likelihood ratio test comparing the full model with a model that included the same random-effect structure but excluded either explanatory variable or included an additive relationship instead of an interaction. The analyses for *M. rotundata* were conducted using the *coxph* function (no random effect since all bees were inoculated on the same day) while the *coxme* function was used for *O. lignaria* (inoculation date as a random effect) (Therneau and Therneau, 2015).

Results

We found that both *O. lignaria* females and *M. rotundata* males became infected with *C. bombi* sourced from the common eastern

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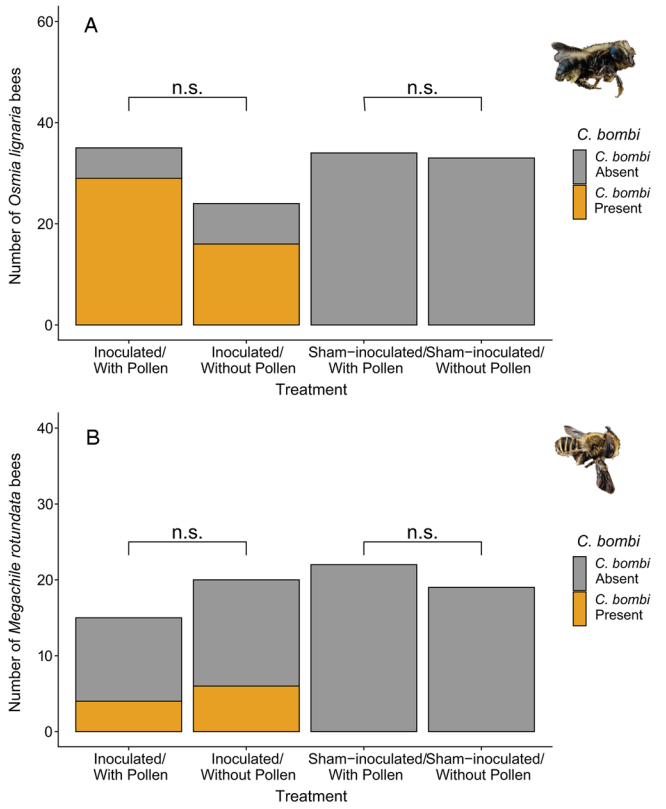

Fig. 1. Effect of pollen access on motile *C. bombi* presence in solitary bees that survived the length of the trial. Pollen access did not affect the likelihood of *C. bombi* presence in (A) *Osmia lignaria* females or (B) *Megachile rotundata* males. Differences in sample size are a product of mortality. (n.s.) indicates $P > 0.05$.

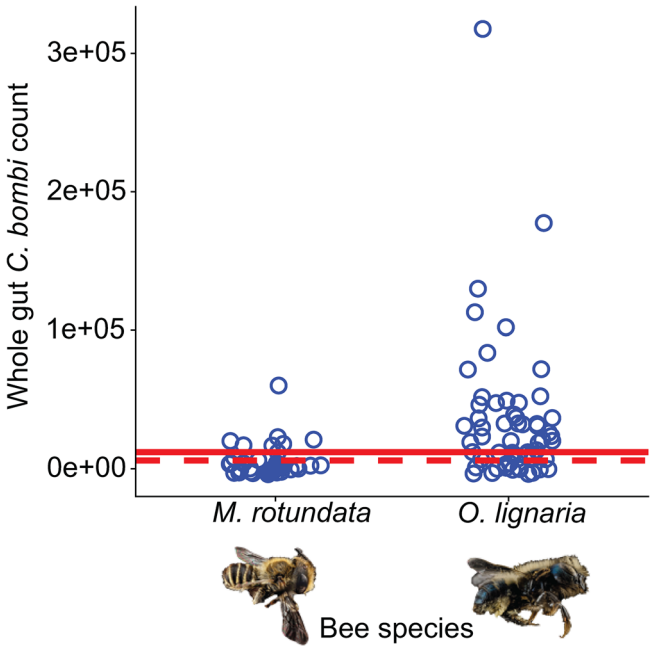

Fig. 2. Estimated whole gut counts of *C. bombi* in solitary bees 5–11 days post-inoculation. Blue circles indicate estimated whole gut counts in inoculated *M. rotundata* males and *O. lignaria* females (see Methods). The dashed red line indicates the total number of *C. bombi* cells in inoculum provided to *M. rotundata* males and the solid red line indicates the total number of *C. bombi* cells in inoculum provided to *O. lignaria* females. Values above these thresholds indicate active pathogen replication in the host bees.

bumble bee (*B. impatiens*). Specifically, 76% of inoculated *O. lignaria* and 29% of *M. rotundata* harboured active *C. bombi* in their gut 5–11 days post inoculation; we did not detect *C. bombi* in any sham-inoculated bees (Fig. 1). The median *C. bombi* load at the end of the trial for inoculated *O. lignaria* females was 100 moving cells μL^{-1} of bee gut (range: 0–1600 cells), while for *M. rotundata* males, the median was 0 cells μL^{-1} (range: 0–300 cells). The *O. lignaria* females were inoculated with 12 000 *C. bombi* cells and final counts averaged 20 000 cells (range: 0–320 000 whole gut cell estimates), indicating active replication of *C. bombi* in *O. lignaria* (Fig. 2). While the median *C. bombi* whole gut count was zero in *M. rotundata* males, the bees with the highest gut counts in this species (60 000) had more than the administered 6000 *C. bombi* cells, indicating replication of *C. bombi* in *M. rotundata* was also possible (Fig. 2). Specifically, 56% of inoculated *O. lignaria* females and 29% of *M. rotundata* males had estimated whole gut *C. bombi* counts above the inoculation concentration indicating active pathogen replication (Fig. 2).

Pollen access did not influence the likelihood of becoming infected with *C. bombi* for *O. lignaria* females or *M. rotundata* males ($\chi^2_1 = 1.86$, $P = 0.173$ and $z = 0.05$, $P = 0.962$, respectively; Fig. 1) nor subsequent load for infected bees ($\chi^2_1 = 0.05$, $P = 0.830$ and $z = 1.78$, $P = 0.076$, respectively for *O. lignaria* females and *M. rotundata* males; Fig. 3). Bee size did not explain the likelihood of infection with *C. bombi* nor subsequent load for the infected bees in *O. lignaria* females or *M. rotundata* males ($z < 0.84$ and $P > 0.402$ for all).

Access to pollen increased *O. lignaria* female bee survival rates ($\chi^2_1 = 4.13$, $P = 0.042$; Fig. 4A), though not for *M. rotundata* males ($\chi^2_1 = 1.19$, $P = 0.275$; Fig. 4C). Inoculation with *C. bombi* did not influence survival in *O. lignaria* females or *M. rotundata* males ($\chi^2_1 = 1.56$, $P = 0.212$ and $\chi^2_1 = 2.02$, $P = 0.155$, respectively; Fig. 4B and D), nor was there pollen access by inoculation interaction for survival in *O. lignaria* females ($\chi^2_1 = 2.26$, $P = 0.133$) or *M. rotundata* males ($\chi^2_1 = 1.73$, $P = 0.189$).

Discussion

In this study, we found that the solitary bee species *O. lignaria* and *M. rotundata* could become infected with *C. bombi* collected from bumble bees. We found evidence for *C. bombi* replication in 56% of inoculated *O. lignaria* females and 29% of inoculated *M. rotundata* males. Access to pollen did not affect *C. bombi* infection in *O. lignaria* females or *M. rotundata* males. However, pollen-fed *O. lignaria* females survived longer than their pollen-starved counterparts. Inoculation with *C. bombi* did not affect survival in either solitary bee species during the 5–11-day laboratory assay. Overall, our study illustrates that solitary bees can be hosts for a pathogen known to infect bumble bees and that diet can play a key role in the health of these important but historically understudied solitary bee taxa.

Most bee species within a community can be exposed to numerous pathogens when foraging at flowers, including *C. bombi* (Figueroa *et al.*, 2020; Graystock *et al.*, 2020). Our results support a growing body of literature indicating the need to assess the host range of bee pathogens, including assessments of replication and impacts on survival (Bramke *et al.*, 2019; Müller *et al.*, 2019). The presence of *C. bombi* in bee feces has been used to identify active infections in bumble bees (Imhoof and Schmid-Hempel, 1999), where it can be detected in as quickly as 5 days post-inoculation (Logan *et al.*, 2005). We detected active *C. bombi* in solitary bee guts at the end of the trials, 5–11 days post-inoculation, for a total of 76% of inoculated *O. lignaria* females and 29% of inoculated *M. rotundata* males harbouring motile cells. Moreover, 56% of the inoculated *O. lignaria* females and 29% of the inoculated *M. rotundata* males had estimated whole gut counts above the concentration of inoculum provided,

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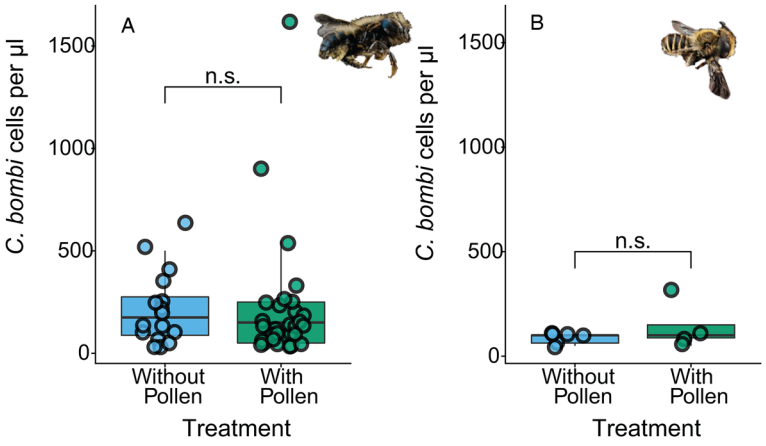

Fig. 3. Effect of pollen access on *C. bombi* load in solitary bees. Pollen access did not affect *C. bombi* load (cells μL^{-1}) in (A) *Osmia lignaria* females or (B) *Megachile rotundata* males. (n.s.) indicates $P > 0.05$. This figure represents subsequent load for inoculated bees that presented motile *C. bombi*.

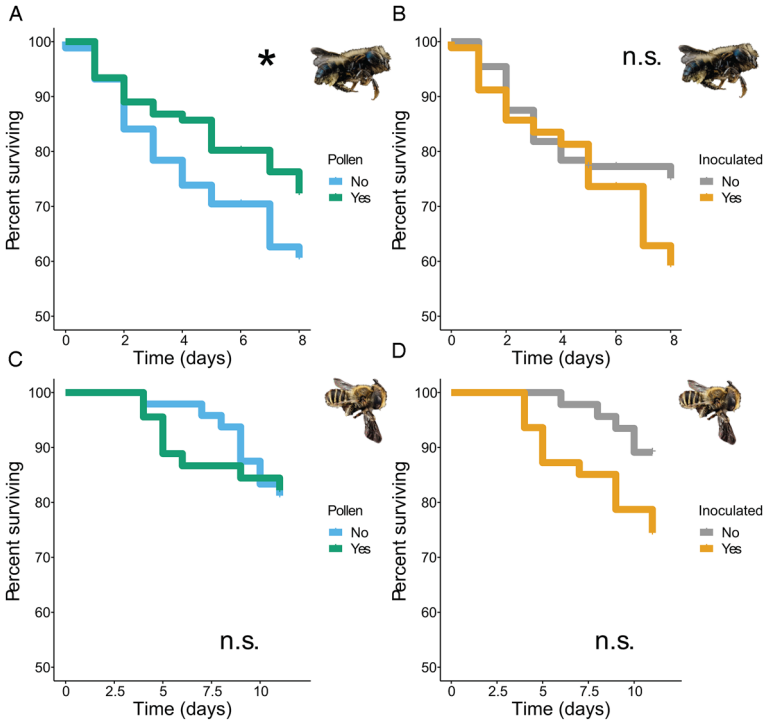

Fig. 4. Solitary bee survival across pollen access and *C. bombi* inoculation treatments. (A) *Osmia lignaria* females with access to pollen survived longer than those without access to pollen. (B). Inoculation with *C. bombi* did not influence *O. lignaria* survivorship. (C) Pollen access did not affect *Megachile rotundata* male survivorship, (D) nor did inoculation with *C. bombi*. Trial lengths varied according to general mortality, which was greater in *M. rotundata* than for *O. lignaria* (see Methods). Shaded error bands indicate 95% confidence intervals. (*) denotes $P < 0.05$ while (n.s.) indicates $P > 0.05$.

indicating active pathogen replication. These values are comparable to infection probability in bumble bees [e.g. 57% of inoculated *B. terrestris* bees presented active infections after a 7-day trial (Näpflin and Schmid-Hempel, 2018)]. Furthermore, our estimates for the percentage of bees experiencing *C. bombi* replication are conservative (i.e. low) since we assume the bee gut did not contribute to the volume of the extraction and that the bees consumed the entirety of the inoculum droplet, further highlighting the extent to which solitary bees can develop active *C. bombi* infections.

While phylogenetic distance often predicts the ability of hosts to be infected by a given pathogen (Gilbert and Webb, 2007; Streicker *et al.*, 2010), this may not be the case with *C. bombi* and its known hosts. Specifically, one recent study (Ngor *et al.*, 2020) has found that *C. bombi* does not replicate in honey bees (*Apis mellifera*), but this pathogen does replicate in bumble bees (*Bombus* spp.). Both honey bees and bumble bees are in the Apidae family, while the two evaluated solitary bees evaluated in the present study are in the Megachilidae family. The factors that enable honey bees to avoid chronic *C. bombi* infections, despite being able to transmit the pathogen *via* flowers (Ruiz-González and Brown, 2006; Graystock *et al.*, 2015) and being more phylogenetically similar to bumble bees than *O. lignaria* and *M. rotundata*, remain unknown. Future evaluations of *C. bombi* infection and health impacts on solitary and social species across additional bee families are clearly warranted.

Host nutrition can increase infection intensity by providing resources to pathogens, but may also support host tolerance of infection. Specifically, increased access to food could increase infections by (1) directly providing nutrients to the pathogen; or (2) improving host quality for the pathogen. Conversely, food availability could suppress pathogens by (3) enabling physiologically costly immune responses; and/or (4) providing antimicrobial compounds (Conroy *et al.*, 2016; Palmer-Young and Thursfield, 2017). While we did not find that pollen access directly influenced the likelihood or severity of *C. bombi* infections (Figs 1 and 3), others have found that pollen-deprivation in bumble bees can reduce *C. bombi* infections (Logan *et al.*, 2005; Conroy *et al.*, 2016). We found that pollen access increased *O. lignaria* female survival, independent of *C. bombi* infection. While we did not find an effect of pollen on *M. rotundata*, we cannot determine whether this was due to low mortality during the 11-day trial and whether patterns would differ in longer time scales. Similarly to *O. lignaria*, access to pollen can increase

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honey bee survival rates despite greater pathogen loads [e.g. *N. apis*, *N. ceranae* and numerous RNA viruses (Rinderer and Dell Elliott, 1977; Zheng *et al.*, 2014; Jack *et al.*, 2016; Zhang *et al.*, 2020)], further illustrating the importance of diet in pollinator health. Access to pollen in provisioning female solitary bees has been shown to increase the number of brood cells and proportion of offspring surviving to adulthood [e.g. in *O. bicornis* and *O. californica* (Cane, 2016; Bukovinszky *et al.*, 2017)], suggesting that differences in physiological demands between sexes could result in differential effects of pollen access. Furthermore, a recent study that experimentally inoculated *O. cornuta* with *C. mellificae*, a trypanosomatid known to infect honey bees, found that while both sexes had higher pathogen loads over time, only male survival was significantly reduced by infection (Strobl *et al.*, 2019). Future evaluations of the impact of *C. bombi* on physiology and survival in solitary bees across sexes are well justified, especially in the field, and could incorporate assessments of resource availability and bee immune responses.

Bumble bees infected with *C. bombi* (Richardson *et al.*, 2015) and honey bees infected with *N. ceranae* (Mayack and Naug, 2009) often consume more pollen, likely to compensate for the energetic cost of mounting an immune response (Moret and Schmid-Hempel, 2000). Interestingly, the role of pollen on *C. bombi* growth varies depending on whether the assessments are conducted *in vitro* or *in vivo*; for example, sunflower pollen *in vitro* increases pathogen growth (Palmer-Young and Thursfield, 2017), while *in vivo* this pollen type has a strong inhibitory effect on the pathogen when consumed by bumble bees (Giacomini *et al.*, 2018; LoCascio *et al.*, 2019). When inhibitory, secondary compounds appear to facilitate these anti-*C. bombi* interactions in bumble bees (Richardson *et al.*, 2015; Koch *et al.*, 2017). These differences suggest that pollen may interact in important ways within the host, leading to varying effects on *C. bombi* growth. Future evaluations of the role of pollen quality, quantity and diversity on susceptibility to pathogens mediated by interactions between immune responses, gut physiology and microbiota are important avenues for pollinator research (Alaux *et al.*, 2010; Cotter *et al.*, 2011; Dolezal and Toth, 2018), especially focusing on solitary bees and other understudied pollinators.

Life-history and functional traits are important mediators of disease transmission and dynamics within a host. While we did not find a role of pollen in *M. rotundata* male infection patterns, recent work has shown that adult male solitary bees (*Andrena* spp.) frequently harbour pollen in their digestive tract (Urban-Mead *et al.*, unpublished results). The importance of males in dispersing pathogens in plant–pollinator networks is not well established, despite male and female solitary bees having comparable infection rates (Müller *et al.*, 2019; Strobl *et al.*, 2019; Ngor *et al.*, 2020) and marked differences in floral preference (Roswell *et al.*, 2019). Moreover, natural rates of pathogen acquisition or deposition by solitary bees while foraging is not known (Figueroa *et al.*, 2019), including the threshold of pathogen cells necessary for solitary bees to develop active infections, all of which could strengthen disease spread models. While we report high rates of *C. bombi* infection for the two solitary species evaluated, especially for *O. lignaria* females, we did not find that experimentally exposing the solitary bees to *C. bombi* influenced host survival in the 5–11-day laboratory assay. Characterizing naturally occurring infection rates as well as the corresponding impacts on mortality and reproduction in the field, especially alongside numerous co-occurring stressors (e.g. inadequate diet, pesticide exposure and co-infections), is an important future direction. Differences in life histories across solitary bees may influence these dynamics. For example, *O. lignaria* overwinter as adults, similar to bumble bee queens [a life stage highly vulnerable to *C. bombi* (Brown *et al.*, 2003)], while *M. rotundata* overwinter as prepupae (Kemp *et al.*, 2004). Similarly, given that we know infection with *C. bombi* alters resource allocation patterns in *B. terrestris*, whereby infected bees invest more in their fat body and less in reproduction than non-infected bees (Brown *et al.*, 2000), assessing differences in resource allocation, foraging behaviour and pollen-provisioning abilities for infected solitary bees compared to uninfected counterparts could advance our understanding of pollinator health in wild bee communities.

Overall, our work supports the importance of improving knowledge concerning solitary bee species in pollinator epidemiology, including their assessment as hosts for what have traditionally been considered social bee pathogens, and considering resource availability when evaluating host–pathogen interactions. Future evaluations of the likelihood of transmission between solitary and social species on flowers would benefit from this knowledge, as transmission probability is already known to vary between bumble bee species (Ruiz-González *et al.*, 2012). Moreover, the activity period of *Osmia* spp. and *Megachile* spp. often greatly overlaps with bumble bees, further highlighting the need to understand interspecies disease transmission networks (Figueroa *et al.*, 2020). In addition, pathogen transmission between social and solitary bees could occur *via* spillover by introduced commercial colonies (Colla *et al.*, 2006; Otterstatter and Thomson, 2008; Graystock *et al.*, 2013), and could occur in various directions (Graystock *et al.*, 2016), further highlighting the need to understand host ranges and impacts of disease on pollinator communities. In order to respond to the growing dependence of pollinators for food security (Aizen *et al.*, 2019), which is in large part contributed by wild bees (Garibaldi *et al.*, 2013; Winfree *et al.*, 2018), we must expand our understanding of the role of pathogens, nutrition and other stressors on solitary bee health.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0031182020002218

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