SHORT COMMUNICATION

Exposure to nectar-realistic sugar concentrations negatively impacts the ability of the trypanosome parasite (Crithidia bombi) to infect its bumblebee host

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Abstract. 1. The ideal conditions for a parasite are typically found with its preferred host. However, prior to transmission to a naïve host and successful infection, a parasite may have to withstand extrinsic environmental conditions. Some parasites have adapted to time away from hosts, for example, by co-opting vectors or by having drought-resistant growth stages. However, other parasites may have no obvious adaptations to persist during prolonged transmission cycles. Consequently, the environment may detrimentally impact parasite fitness and ultimately epidemiology.

2. Here, we investigate the impact of nectar-realistic sugar concentrations on the ability of the trypanosome parasite Crithidia bombi, which may be transmitted between conspecifics at flowers, to infect its bumblebee host Bombus terrestris and to reproduce during the infection (parasitaemia). Our results show, following 30 min exposure to our experimental nectars that as sugar concentration increases, infection prevalence and parasitaemia decrease. This is likely due to the increased osmotic stress C. bombi experiences in high sugar, aqueous environments.

3. Consequently, if C. bombi transmission is facilitated by nectar or a high-sugar environment, it may have a negative impact on parasite fitness.

Key words. Bombus terrestris, parasitaemia, Trypanosomatidae, pollinator health, pathogen.

Introduction

Parasites are ubiquitous and may have severe impacts on host fitness (e.g., Hudson et al., 1992). However, prior to infection, parasites may have to withstand extrinsic environmental stressors. To minimise time spent in sub-optimal conditions some parasites have co-opted vectors or are resistant to drought to ensure they can persist in the environment. However, other parasites depend on population density and transmission networks to ensure that time away from a host is minimised (Otterstatter & Thomson, 2007). Consequently, such parasites may have limited adaptations for persisting outside of a host, and the environment may therefore have a detrimental impact on parasite health.

Bumblebees produce small, annual and eusocial colonies. They can be impacted by a range of microbial parasites (Schmid-Hempel, 1998), some of which depend on host transmission networks (Otterstatter & Thomson, 2007). One example is the gut trypanosome Crithidia bombi, which has a relatively high environmental prevalence (exceeding 80% in some field populations by early summer, Shykoff & Schmid-Hempel, 1991) and can reduce colony fitness by up to 40% (Brown et al., 2003). Transmission of C. bombi occurs both within and between colonies of bumblebees. Intra-colonial transmission occurs through contact networks within nests (Otterstater & Thomson, 2007, Folly et al., 2017), while inter-colonial transmission of C. bombi occurs at shared floral resources (Durrer & Schmid-Hempel, 1994). However, if C. bombi is deposited inside a flower (Figueroa et al., 2019) it may come into contact with nectar, which may impact parasite fitness (Cisarovsky & Schmid-Hempel, 2014). Here we test a range of sugar
concentrations on *C. bombi in vivo* to investigate whether exposure to a sugar-rich environment impacts the parasite’s ability to infect the buff-tailed bumblebee (*Bombus terrestris*) and, if infection is successful, whether it has any effect on parasitaemia.

**Materials and methods**

Five sugar-water concentrations, encompassing a range of ecologically relevant nectar concentrations, including higher and lower concentrations not typically found in nature, were selected for experimental testing to identify a linear relationship [10%, 20%, 40%, 60%, 73% (wt/wt)] (Baude et al., 2016). For each sugar concentration, seven workers were randomly selected from each of five *Bombus terrestris audax* (Biobest, UK Ltd.) colonies (*n* = 35 bees per treatment). To inoculate bumblebees a *C. bombi* inoculum was created by centrifuging the faeces of wild caught *B. terrestris* queens that were naturally infected with *C. bombi* (Cole, 1970) and resuspending the pellet in 100 μl of 0.9% Ringer’s solution. An aliquot of this was placed on to a Neubauer improved haemocytometer to quantify the concentration of the inoculum using a microscope. The *C. bombi* inoculant was then diluted in the five experimental sugar waters, creating five inocula. The completed five *C. bombi* inocula were left for 30 min prior to inoculation, as this is the typical time between pollinator visits at a single flower (Stout & Goulson, 2002). Each bumblebee was then fed a droplet of the relevant inoculum for their treatment, which contained 10 000 parasite cells, using a pipette. Bumblebees that consumed the inoculant were placed in individual, vented cages (130 × 100 × 50 mm) for the duration of the 14-day experiment and provided *ad libitum* Apiinvert (~39% fructose, ~31% glucose, ~30% sucrose) and 0.2 g of pollen (Biobest, UK Ltd.) after each sampling date (described below), which had been irradiated to remove any microbes.

To investigate whether infection had occurred and to monitor *C. bombi* parasitaemia, parasite counts were taken for each bumblebee at day 3, 7 and 14 following inoculation. For this, a sample of the bumblebee’s faeces was taken using a microcapillary and a parasite count was taken using a Neubauer improved haemocytometer.

To analyse the effect of treatment on *C. bombi* prevalence a binary logistic regression was created in R (http://www.R-project.org). Here infection state (0/1) was used as a response variable, with treatment as a categorical factor, day and faeces volume as co-variates and colony and bee as random factors, in addition this model included an interaction between day and treatment. To test the impact of treatment on the intensity of *C. bombi* infections over the 14-day experiment a linear mixed model was created. This model did not include uninfected bees and had *C. bombi* infection intensity (cells/μl) log transformed as a response variable, with treatment as a categorical factor and faeces volume and day as co-variates. As before, this model also included an interaction between treatment and day, and colony and bee were included as random factors.

**Results**

Treatment had a significant negative effect on *C. bombi* infection prevalence (*Z* = −1.964, *P* = 0.04), while day had a significant positive effect (*Z* = 4.22, *P* < 0.001). Essentially, as sugar concentration increased, the probability of infection decreased, while as time passed, more bees presented as infected (Fig. 1).

Treatment also had a negative effect on the intensity of *C. bombi* infections (*F* < 0.005, Fig. 2) and as before, day had a significant positive effect on *C. bombi* infection intensity (*F* = 38.59, *P* < 0.001). In essence, the bees exposed to higher sugar concentrations had lower infection intensities, but these intensities increased over time. The covariate faeces volume, had no effect on infection intensity, with bees that produced smaller volumes of faeces having higher infection intensities. Finally, there was no significant interaction between treatment and day (*F* = 0.019, *P* = 0.88).

**Discussion**

Here we show that the ability of *C. bombi* to infect its bumblebee host (*B. terrestris*) declines with exposure to increasing aqueous
sugar concentration and that prior exposure to higher sugar concentrations also results in lower intensity of infections. Aqueous environments with a high sugar content have been shown to reduce the ability of *C. bombi* to persist *in vitro* and this is believed to be due to the extreme osmotic stress that nectar may exert on microbial life (Cisarovský & Schmid-Hempel, 2014). Our results corroborate this and show that these effects result in reduced infection success and subsequent parasitaemia.

Previous work has shown that foraging worker bumblebees have a preference for nectars that contain 55% (wt/wt) sugar concentration (Bailes et al., 2018). Critically, this sugar concentration falls within the range where the greatest drop in infection prevalence and intensity occurs in our results. We inoculated each bumblebee with 10,000 cells as this is known to result in an ~75% infection rate (Ruiz-González & Brown, 2006). However, our infection prevalence, even at day 14, was consistently lower than this for sugar concentrations at or above 40% (wt/wt). In addition, our recovered *C. bombi* infection intensities at day seven, following exposure to a sugar rich environment, were 10-fold lower than those reported previously and 4-fold lower at day 14 (Logan et al., 2005). However, while the prevalence and intensity of *C. bombi* was reduced in our experimental bumblebees, our results suggest that their infection intensities after the 14-day time period were still sufficient to infect conspecifics (Ruiz-González & Brown, 2006). Consequently, for those bees where inoculation was successful, exposure to sugar solutions did not have an irreversible impact on parasite fitness.

Inter-colonial transmission of *C. bombi* is presumed to be opportunistic and occur via floral surfaces (Durrer & Schmid-Hempel, 1994; Ruiz-González et al., 2012; Adler et al., 2018; Figueroa et al., 2019). In a previous study, Cisarovský and Schmid-Hempel (2014) failed to recover *C. bombi* from sampled nectar, but they did not sample other plant parts and provided no background data on parasite presence at their study sites. Consequently, interpreting what this means for parasite transmission biology is unclear. In contrast, Figueroa et al. (2019) showed both that infected bees defecate inside corollas, where the parasite is likely to encounter nectar, and survived longer there, suggesting that interactions with nectar are likely to be a common occurrence in the transmission cycle. In addition, *C. bombi* has to pass through the crop of infected bumblebees (Gorbunov, 1987) and thus will be exposed to nectar at the same time. Together, this suggests that exposure to sugar solutions is likely to be a key aspect of inter and intra-colony transmission, and the impact of sugar concentration on parasite success needs to be factored into our understanding of epidemiology in this system.

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**Author Contributions**

AJF, MJFB, and MB-N devised the experiment. AJF provided training and MB-N undertook the experimental work. AJF analysed the data in discussion with MJFB. AJF wrote the manuscript and all authors provided feedback on the manuscript and agreed to its publication.

**Declaration of conflicts of interest**

The authors declare no conflicting interests.

**Data availability statement**

Data has been made open access and deposited on to Figshare under the title ‘Exposure to nectar-realistic sugar concentrations negatively impacts the ability of the trypanosome parasite (*Crithidia bombi*) to infect its bumblebee host’. https://doi.org/10.6084/m9.figshare.12197604.v1

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