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RESEARCH ARTICLE

Age-related pharmacodynamics in a bumblebee–microsporidian system mirror similar patterns in vertebrates

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ABSTRACT

Immune systems provide a key defence against diseases. However, they are not a panacea and so both vertebrates and invertebrates co-opt naturally occurring bioactive compounds to treat themselves against parasites and pathogens. In vertebrates, this co-option is complex, with pharmacodynamics leading to differential effects of treatment at different life stages, which may reflect age-linked differences in the immune system. However, our understanding of pharmacodynamics in invertebrates is almost non-existent. Critically, this knowledge may elucidate broad parallels across animals in regard to the requirement for the co-option of bioactive compounds to ameliorate disease. Here, we used biochanin A, an isoflavone found in the pollen of red clover (*Trifolium pratense*), to therapeutically treat *Nosema bombi* (Microsporidia) infection in bumblebee (*Bombus terrestris*) larvae and adults, and thus examine age-linked pharmacodynamics in an invertebrate. Therapeutic treatment of larvae with biochanin A did not reduce the infection intensity of *N. bombi* in adults. In contrast, therapeutic treatment of adults did reduce the infection intensity of *N. bombi*. This transition in parasite resistance to bioactive compounds mirrors the age-linked pharmacodynamics of vertebrates. Understanding how different life-history stages respond to therapeutic compounds will provide novel insights into the evolution of foraging and self-medication behaviour in natural systems more broadly.

KEY WORDS: *Bombus terrestris*, *Nosema bombi*, Pollinator health, *Trifolium pratense*, Phytochemicals, Medication

INTRODUCTION

The frequency of severe, emerging disease epidemics is increasing globally (Jones et al., 2008). While disease epidemics have a negative impact on host fitness (Hudson et al., 1992; Daszak et al., 2000), they also have wider reaching impacts, such as reducing biodiversity (Berger et al., 1998; Leopardi et al., 2011). Incidences of disease epidemics are not exclusive to vertebrates, and emerging diseases may have severe impacts on ecologically important invertebrate communities (e.g. Cameron et al., 2011). The immune system is the primary defence mechanism for metazoan life against such emerging pathogenic infection (Janeway, 2001). However, immunity is often enhanced or supplemented by medication (Haydon et al., 2006; Abbott, 2014). Both vertebrates and invertebrates can consume bioactive compounds in their diets, with these compounds acting to ameliorate disease, and therefore provide positive fitness benefits (Dias et al., 2012; Stevenson et al., 2017). Indeed, medication has become the cornerstone of health care in human populations (Bunker, 2001).

Insects, which have simpler immune systems than vertebrates, as they have no adaptive immune response (Buchmann, 2014), might be expected to gain significant benefits from the consumption of medicinal compounds. For instance, monarch butterflies oviposit onto milkweed plants which contain cardenolides, and this behaviour provides an indirect benefit to larvae infected with the protozoan parasite *Ophryocystis elektroscirrha*, as consumption of milkweed plant tissue negatively impacts parasite virulence and replication (de Roode et al., 2008; Sternberg et al., 2012). Similarly, consumption of a range of compounds by adult bumblebees is associated with a reduction in the success and intensity of infections by the trypanosome parasite *Crithidia bombi* (Manson et al., 2010; Richardson et al., 2015; Koch et al., 2019). As yet, however, these studies have focused on a single life stage, overlooking the life-history structure of natural populations. Interestingly, in vertebrates, medication may differentially affect younger and older life stages in a population. More specifically, medication may need to be adapted or optimised in younger individuals to provide the same health benefits (Swift, 1990; Russmann et al., 1997; Turnheim, 2003; Stephenson, 2005). Whether invertebrates have a similar relationship between age and parasite resistance in response to medicinal compounds is unclear.

Holometabolous insects provide an ideal model system to address this question, primarily as there are clear physiological differences between the larval instars and the adult, imago phase. Bumblebees, a genus of holometabolous insect pollinator, both consume bioactive secondary metabolites in their diet (Baker, 1977; Adler, 2000; Stevenson et al., 2017) and are impacted by a range of microbial pathogens (Schmid-Hempel, 1998). One such pathogen, *Nosema bombi* (Microsporidia) (Fantham and Porter, 1914), has relatively low environmental prevalence (Shykoff and Schmid-Hempel, 1991; Jones and Brown, 2014) but is deleterious to bumblebee populations (Otti and Schmid-Hempel, 2007; Rutrecht and Brown, 2009; Cameron et al., 2011; Brown, 2017). Infection with *N. bombi* can reduce both worker longevity and sperm count in males (Otti and Schmid-Hempel, 2007; Rutrecht and Brown, 2009). However, of greater concern is that infection can negatively impact the production of sexual castes (Otti and Schmid-Hempel, 2008; Rutrecht and Brown, 2009) and this has been linked to the range and population declines seen in some North American bumblebees (Cameron et al., 2011, 2016). Critically, for the context of this study, *N. bombi* infection persists through both larval and adult life stages of bumblebees (Rutrecht and Brown, 2008), although it largely relies on larval infection for transmission (Rutrecht et al., 2007; Rutrecht and Brown, 2008). Given that other microsporidian pathogens of bees are susceptible to biologically active plant

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metabolites found in pollen (Giacomini et al., 2018), it is likely that bumblebees may be able to enhance their resistance against *N. bombi* infection through the consumption of phytochemicals that have similar anti-fungal activity. Globally, red clover (*Trifolium pratense*) is an abundant wildflower crop (Food and Agriculture Organization of the United Nations: http://www.fao.org/statistics/en/) on which bumblebees forage (Goulson and Darvill, 2004; Pywell et al., 2011) and which some species, such as the buff-tailed bumblebee (*Bombus terrestris*), nectar rob by biting holes in floral tissue (Gurr, 1974). Here, we used the isoflavone biochanin A, which has been found in *T. pratense* pollen and floral tissue (Wu et al., 2003; Saviranta et al., 2008; Folly, 2019), as a medicinal compound to treat *N. bombi* infection in *B. terrestris* workers that were therapeutically fed as either larvae or adults, to elucidate similarities between the age-linked pharmacodynamics of vertebrates and an invertebrate. Given the previous work on vertebrate pharmacodynamics, we predict the therapeutic treatment of bumblebee adults should have a stronger impact on parasite resistance when compared with larval treatment.

MATERIALS AND METHODS

Biochanin A and bumblebee colony provenance

Eight *Bombus terrestris audax* (Harris 1776) colonies (hereafter referred to as donor colonies), each containing a queen, brood and a mean of 45 (±6.5 s.e.m.) workers, were obtained from Biobest, Belgium. Colonies were kept in a dark room at 26°C and 50% humidity (red light was used for any colony manipulation). To ensure colonies were healthy and developing normally, they were monitored for 7 days prior to use in any experimental procedures. This included randomly screening 10% of the workers every 2 days, from each colony, for common parasitic infections (*N. bombi*, *Apicystis bombi* and *Crithidia bombi*) in faeces using a phase-contrast microscope set to ×400 magnification. No infections were identified in any of the eight donor colonies.

Experimental micro-colonies were established by removing three patches of brood containing approximately 15 developing larvae (growth stage L2–3) from each of the eight donor colonies. Each of these patches of brood was placed in an individual 14×8×5.5 cm acrylic box. The micro-colonies were each provisioned with *ad libitum* pollen and sugar water (50% w/w), and 3 workers from their original donor colony to provide brood care. All pollen used throughout the experiment was irradiated to remove any microbes. Prior to being entered into the experiment, all brood-caring workers were individually marked with a coloured, numbered Opalith tag and recorded.

Artificial inoculation of *B. terrestris* larvae with *N. bombi*

Elucidating the pharmacodynamics of biochanin A under our experimental paradigm required all larvae to be inoculated with *N. bombi.* In addition, as larvae are the most susceptible life stage to *N. bombi* infection (Rutrecht and Brown, 2008), larval inoculation would probably replicate the natural transmission route into a colony. A wild *B. terrestris* queen that was naturally infected with *N. bombi* was caught from Windsor Great Park, UK (OS grid reference: SU992703) in 2016. The infected queen's gut was isolated by dissection and homogenised in 0.01 mol l^{-1} NH_4Cl. The resulting spore solution was centrifuged at 4°C for 10 min at 6800 ***g*** to isolate and purify the spore pellet as described in Rutrecht and Brown (2008). The pellet was resuspended in 0.01 mol l^{-1} NH_4Cl and the *N. bombi* concentration was calculated using a Neubauer improved haemocytometer. To confirm the presence of *N. bombi*, and to ensure the microsporidium was not *Nosema ceranae*, as these two microsporidians can be easily confused under a light microscope, a sample of the inoculum was subjected to PCR using the primers and protocol outlined in Erler et al. (2012). The inoculum was then stored at −80°C until required.

A larval *N. bombi* inoculant was prepared by combining inverted sugar water and pollen to create an artificial worker feed as outlined in Folly et al. (2017). This was then combined with the *N. bombi* inoculum to create an experimental inoculant. Prior to any larval inoculation, workers from each micro-colony were removed for an hour. Consequently, larvae had no access to food and experimental inoculation would be more likely to elicit a feeding response. Larvae were then assigned to either the larval or adult therapeutic feeding trial.

Identifying the pharmacodynamics of biochanin A in *B. terrestris* workers that were treated as larvae

The isoflavone biochanin A, which possesses antifungal activity (Weidenbörner et al., 1990), has been identified in *T. pratense* floral tissue and pollen (Wu et al., 2003; Saviranta et al., 2008; Folly, 2019). As such, biochanin A represents an excellent target compound for understanding pharmacodynamic impacts on microsporidian infections in bumblebees.

To test the therapeutic effect of biochanin A on developing larvae, 16 micro-colonies, as described above, were used. Larvae were each inoculated with 50,000 spores in 4.3 µl of experimental inoculant (see above), using a 20 µl pipette, prior to being entered into the experimental feeding regime. The spore concentration of the inoculum is within ecologically relevant values for *N. bombi* spores in faeces and has been shown to be a concentration that is infective to developing *B. terrestris* brood (Rutrecht and Brown, 2008; A.J.F., unpublished pilot work). Following inoculation, larvae were left for 30 min to consume the inoculum. Complete consumption of the inoculum was confirmed using a stereomicroscope at ×20 magnification. The inoculated larvae were returned to their respective micro-colonies with the original, marked, brood-caring workers. Each control micro-colony (n=8) was provisioned with *ad libitum* pollen and sugar water. However, in the experimental micro-colonies (n=8), *ad libitum* pollen and sugar water (50% w/w) containing biochanin A at 20 ppm was provided for 7 days. Biochanin A (Sigma-Aldrich Company Ltd, Gillingham, Dorset, UK) was added to sugar water (50% w/w) using 4 ml of 40% methanol as a solvent per litre. Control colonies also had 4 ml of 40% methanol added per litre of sugar water (50% w/w). Biochanin A has been recovered at higher concentrations in *T. pratense* floral tissue (Wu et al., 2003; Saviranta et al., 2008), so 20 ppm is likely to fall within the range of naturally occurring concentrations of biochanin A for nectar-robbing bumblebee species, such as *B. terrestris* (Gurr, 1974), that both inadvertently consume floral tissue and collect pollen.

Larvae were allowed to develop naturally and pupate in their respective micro-colonies. Once eclosed, new workers were marked using a coloured Opalith tag, recorded, and individually quarantined for 3 days in an inverted plastic cup (127×95 mm), which was modified with a hole that enabled a 15 ml Falcon tube to be inserted. The Falcon tube contained control inverted sugar water diluted with double distilled H_2O (50% w/w) that workers could feed on. A quarantine period of 3 days was used to ensure that faecal samples were not heavily contaminated with pollen grains as these can obscure parasites under a light microscope and complicate parasite quantification. At the end of the quarantine period, each worker was isolated in a 25 ml plastic vial where it provided a faecal sample, which was then collected in a 10 µl glass capillary and faecal volume (µl) was recorded. Following this, each worker's faecal

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sample was screened for *N. bombi* by microscopic examination using a phase-contrast microscope at ×400 magnification. If an infection was identified, a Neubauer improved haemocytometer was used to quantify the parasite load. In addition, each worker had its thorax width measured (mm) 3 times and averaged, as a proxy for bumblebee size, using a set of Mitutoyo™ digital callipers (Whitehorn et al., 2010). Workers were then killed and stored in a labelled Eppendorf tube at −80°C.

Identifying the pharmacodynamics of biochanin A in *B. terrestris* workers that were treated as adults

As *N. bombi* infection persists through pupation into adulthood, therapeutic foraging could indirectly improve the health of infected workers. Here, eight micro-colonies were established as described above, one for each donor colony. Brood-caring workers were removed and larvae in each micro-colony were inoculated with 50,000 spores in 4.3 µl of inoculant using a 20 µl pipette, as described above. Larvae were left, as before, to consume the inoculant before brood-caring workers were returned. The micro-colonies were provided with *ad libitum* pollen and sugar water (50% w/w) and allowed to develop normally. Once they had eclosed, new workers were individually marked and quarantined as before. All eclosed and quarantined workers were screened for *N. bombi* infection by microscopic examination of faeces using a phase-contrast microscope at ×400 magnification. Any workers that were infected had their initial parasite load counted using a Neubauer improved haemocytometer and were entered into the feeding trial.

Following quarantine, each infected worker was placed into an inverted plastic cup, as described above, which was blindly allocated to one of two feeding regimes: experimental bumblebees were provisioned with 15 ml of sugar water (50% w/w) containing biochanin A at 20 ppm and control bumblebees were given 15 ml of control sugar water (50% w/w). As before, biochanin A was added to sugar water using 4 ml of 40% methanol per litre as a solvent. Control colonies also had 4 ml of 40% methanol added per litre of sugar water. Infected workers were kept under quarantine for 7 days. Every 2 days, each worker was removed and a sample of faeces was taken using a 10 µl glass capillary tube. This sample was then measured for volume (µl) and screened for *N. bombi* parasite load using a Neubauer improved haemocytometer. After 7 days of experimental feeding, a final parasite count was taken, as described above, and thorax width measurements (mm) for each worker were taken 3 times and averaged, as a proxy for bumblebee size, using a set of Mitutoyo™ digital callipers; workers were then killed and stored in a labelled Eppendorf tube at −80°C. No pollen was provided during the therapeutic feeding trial.

Statistical analysis

All statistical analyses and graphical outputs were undertaken in R open source programming language (http://www.R-project.org/; Wickham, 2009). To analyse the therapeutic effect of biochanin A on *N. bombi* infection intensity (cells µl^{-1}) in newly eclosed workers that were fed biochanin A as larvae, a linear mixed-effects model (LMM) was constructed. The model was constructed in the R package 'lme4' (Bates et al., 2015) with the following parameters: infection intensity was used as a response variable, with treatment group, thorax width (mm) and faeces volume (µl) as designated covariates. The model also incorporated donor colony as a random effect. To analyse the effect of biochanin A feeding by infected adult workers, a second LMM model was constructed. Here, infection intensity was selected as a response variable with treatment group, days since quarantine, thorax width (mm) and faeces volume (µl) as covariates. As before, donor colony was included as a random effect. For all analyses, only bees that survived the duration of the experiment were included. Models were validated in R by visually checking the normality of residuals, and for overdispersion and collinearity of variables.

RESULTS

Does biochanin A impact *N. bombi* infection intensity in *B. terrestris* workers that were treated as larvae?

In the therapeutic larval bioassay, 116 adult workers successfully eclosed, of which 56 had *N. bombi* infections (control n=25, experimental n=31), resulting in an overall infection success of 48%. Larval treatment with biochanin A had no effect on the prevalence of infection at eclosure (χ^2=2.481, P=0.115). In addition, biochanin A treatment did not have a significant therapeutic effect on *N. bombi* infection intensity in newly eclosed workers (LMM, $F_{1,51}$=2.286, P=0.136). In addition, the covariates thorax width (LMM, $F_{1,51}$=0.049, P=0.82) and faeces volume (LMM, $F_{1,51}$=1.81, P=0.18), and the random effect colony (LMM, P=0.7) had no significant positive or negative effect on *N. bombi* infection intensity (Fig. 1).

Does biochanin A impact *N. bombi* infection intensity in *B. terrestris* workers that were treated as adults?

In the adult therapeutic investigation, 80 workers successfully eclosed, of which 34 were infected with *N. bombi*, giving an

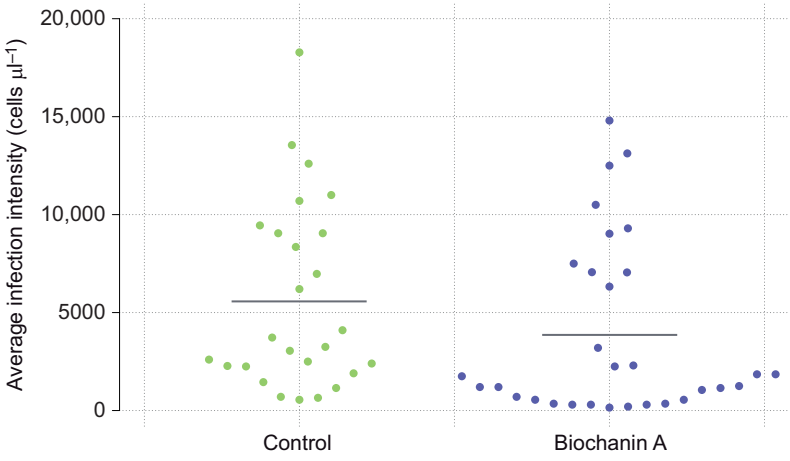

Fig. 1. Effect of biochanin A on *Nosema bombi* infection intensity in *Bombus terrestris* workers therapeutically treated as larvae. Beeswarm plot, showing the complete spread of data of *N. bombi* infection intensity in adult workers (n=56) following larval treatment, when given a control or biochanin A sugar water supplement. The sample mean is marked with a grey bar. Biochanin A consumption during the larval stage did not have a significant therapeutic effect on *N. bombi* infection intensity in adult workers (LMM, $F_{1,51}$=2.286, P=0.136).

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infection success rate of 42.5%. However, only 23 workers survived the full duration of the experiment. Both treatment group (LMM, $F_{1,78}$=12.51, P<0.001) and days since quarantine (LMM, $F_{1,78}$=71.30, P<0.001) had significant effects on *N. bombi* infection intensity, with infection intensity increasing over time, but at a significantly lower level in biochanin A-treated individuals. In addition, the random effect colony (LMM, P=0.003) also had a significant effect on *N. bombi* infection intensity. The covariates thorax width (LMM, $F_{1,78}$=1.69, P=0.196) and faeces volume (LMM, $F_{1,78}$=3.39, P=0.069) had no significant effect on *N. bombi* infection intensity (Fig. 2).

DISCUSSION

Here, we provide the first evidence that medication induces a similar age-related pattern of parasite resistance in bumblebees to that seen in vertebrates. The therapeutic treatment of *B. terrestris* larvae, equivalent to younger life stages in vertebrates, with biochanin A had no significant effect on *N. bombi* infection intensity in adult workers. In contrast, therapeutic treatment of adults significantly reduced *N. bombi* infection intensity in *B. terrestris* workers. Consequently, our results suggest that parasitic infections in bumblebees respond differently to bioactive compounds as an individual ages, and this mirrors similar patterns seen in vertebrates.

Intrinsic host–parasite physiology has been the focus of investigations into parasite resistance, with few studies examining the impact of ecological, extrinsic factors. However, in both vertebrates and invertebrates there is evidence for the co-option of bioactive compounds to improve resistance against parasites (Huffman, 2001; de Roode et al., 2008; Abbott, 2014; Gowler et al., 2015). Given that the immune response in both groups transitions over time (Müller et al., 2013), it is likely that the pharmacodynamics of bioactive compounds may also change. Our results show that under laboratory conditions, when faced with a pathogenic challenge, the effect of therapeutic medication is different in *B. terrestris* larvae and adults. Specifically, therapeutic treatment had no significant effect on *N. bombi* infection intensity in our larval treatment group. However, in adults, therapeutic treatment did significantly reduce *N. bombi* infection intensity. These findings are similar to the age-linked variation in medicated parasite resistance seen in vertebrates (Turnheim, 2003). For example, in humans, age-linked variation in the effectiveness of medication has been shown to result in higher disease prevalence in malaria in younger cohorts. More specifically, treatment with mefloquine had a significantly higher proportion of treatment failures in younger than in older participants (Nosten et al., 1991). Moreover, the age-linked variation we have identified in bumblebees may have a parallel functionality with vertebrate pharmacodynamics. For example, suppression of parasite intensity in adult bees is beneficial as it may reduce transmission to larvae, as adult bees provide food resources both directly, through feeding larvae, and indirectly, by foraging. This interaction would be analogous to the use of drugs in dogs to suppress *Toxocara canis* infections in lactating bitches to reduce transmission to puppies (Burke and Roberson, 1983). Consequently, while the vertebrate and invertebrate responses to bioactive compounds are evolving separately, our results suggest that similar selection pressures may be driving the convergent response that we have reported here. Critically, understanding how the impact of natural medicines varies across the life-history structure of wild populations may provide crucial insights into the epidemiological dynamics of both endemic and emergent diseases.

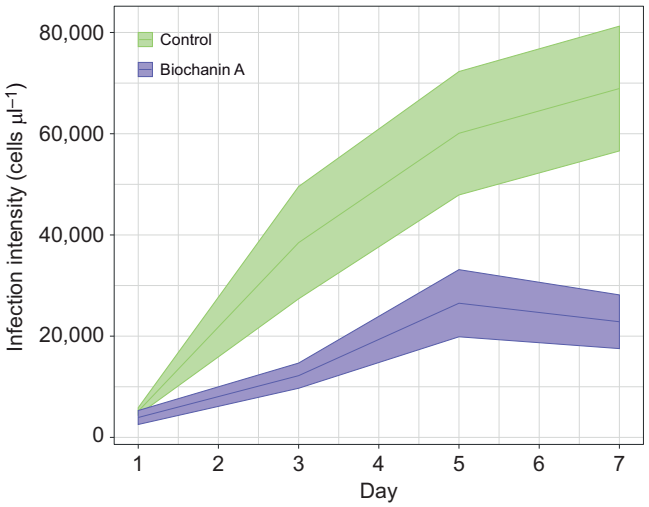

Fig. 2. Effect of biochanin A on *N. bombi* infection intensity in *B. terrestris* workers therapeutically treated as adults. Infection intensity (mean±s.e.m.) of *N. bombi* in adult workers (n=23) over a 7 day period when given a control or biochanin A sugar water supplement. The covariates treatment (LMM, $F_{1,78}$=12.51, P<0.001) and days since quarantine (LMM, $F_{1,78}$=71.30, P<0.001), and the random effect colony (LMM, P=0.003) had a significant effect on *N. bombi* infection intensity.

Nosema bombi has been implicated in rapid and catastrophic declines in the population and geographical range of a suite of bumblebee species across North America (Cameron et al., 2011, 2016). One possible explanation for the increase in prevalence and virulence of this parasite in North American bumblebee populations is that it was accidentally propagated within commercial breeding systems and then passed to wild populations (Thorp and Shepherd, 2005; Cameron et al., 2016). Our results suggest an alternative explanation: changes in the consumption of natural medicines like biochanin A, perhaps due to changes in floral availability (Samson and Knopf, 1994; Sleeter et al., 2013), could have disrupted the ability of bumblebees to control *N. bombi* naturally. Consequently, understanding how floral diversity contributes to natural disease control in wild populations, particularly for ecologically important pollinators, should be a key question for future research (McArt et al., 2014; Koch et al., 2019).

Previous work in bumblebees has shown that *in vivo* therapeutic treatment with bioactive phytochemicals can reduce the infection intensity of the prevalent gut trypanosome *C. bombi* (Manson et al., 2010; Richardson et al., 2015). Moreover, this relationship has been identified *in vitro*, in the absence of a host innate immune response (Palmer-Young et al., 2016). Consequently, the bioactivity of these compounds is presumed to have a direct negative effect on pathogen growth and development, although this has not been conclusively shown (Manson et al., 2010; but see Koch et al., 2019). In contrast, our results suggest that the antifungal efficacy of biochanin A is dependent on host life stage, and thus *in vitro* effects cannot necessarily predict *in vivo* impacts of such compounds. The antifungal activity of biochanin A is probably a function of its planar structure and methoxyl group location, which can compete for fungal cell wall receptor sites (Weidenbörner et al., 1990; Rojas et al., 2006). The reduction of *N. bombi* intensity is thus likely to be due to impacts on cellular membrane function in the parasite. However, we would note that how biochanin A interacts with host cells or how it is metabolised by bumblebees remains uninvestigated. It is likely that the contrast in the effectiveness of biochanin A, which we have identified in bumblebees, is due to

physiological differences in gut structure between adult and larval stages, which is an important site for *N. bombi* infection (Fantham and Porter, 1914). An alternative explanation might be that the impact of biochanin A on *N. bombi* is dependent on the stage of infection, if newly generated spores are more susceptible to its anti-fungal properties. Rutrecht and Brown (2008) showed that the infection intensity of *N. bombi* in *B. lucorum* did not change across the lifetime of adult bees after eclosion. The temporal increase in shed spores seen in our experiments, which given the results of Rutrecht and Brown (2008) must therefore be mirrored by a decline in within-body spore intensity, suggests that biochanin A is impacting spore production or destroying spores as they are released into the gut lumen. Further work on the mechanism behind this interaction is warranted.

Biochanin A has been recovered from the reproductive tissues and pollen of *T. pratense* (Wu et al., 2003; Saviranta et al., 2008; Folly, 2019). However, our biochanin A concentration was below that recorded from floral tissue (Wu et al., 2003; Saviranta et al., 2008). This suggests that our results may be conservative in an ecological context. Given that *B. terrestris* is a known nectar robber of *T. pratense* (Gurr, 1974), it is likely that flower-biting adults are repeatedly exposed to biochanin A at higher concentrations than we have tested here, during their daily foraging bouts, in areas of high *T. pratense* abundance (Plowright and Hartling, 1981). Our results suggest that whilst the nectar-robbing behaviour of *B. terrestris* may have a negative impact on plant reproductive success (Irwin et al., 2010), it may equally have positive health impacts for *N. bombi*-infected adult bumblebees. Any such effects will only be enhanced by the collection and consumption of pollen.

Vertebrates are viewed as having a more complex immune system than invertebrates, primarily as a result of their possession of an adaptive immune response (Buchmann, 2014). However, contrary to the established view, advances in our understanding of the invertebrate immune response have elucidated important comparisons with vertebrate immune function (Litmann et al., 2005). More specifically, immune priming (Sadd et al., 2005), adaptive behaviour (Pull et al., 2018) and the collective immune responses of social insects (Cremer et al., 2007, 2018; Otti et al., 2014) suggest that components of invertebrate immunity and the adaptive vertebrate immune responses may be functionally analogous. The development of the concept of social immunity (Cremer et al., 2007) showed that vertebrate systems can provide insight into how invertebrates manage the threat of parasites. Our results suggest that similar inspiration may be drawn from pharmacodynamics in vertebrates to understand how invertebrates, such as bumblebees, may take advantage of naturally occurring medicinal compounds. Understanding how different life-history stages respond to potentially therapeutic compounds is likely to provide novel insights into the evolution of foraging and self-medication behaviour in natural systems more broadly.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: A.J.F., P.C.S., M.J.F.B.; Methodology: A.J.F., P.C.S., M.J.F.B.; Formal analysis: A.J.F.; Data curation: A.J.F.; Writing - original draft: A.J.F.; Writing - review & editing: A.J.F., P.C.S., M.J.F.B.; Supervision: P.C.S., M.J.F.B.; Funding acquisition: P.C.S., M.J.F.B.

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Data availability
Data are available from the figshare digital repository : https://doi.org/10.6084/m9.figshare.12000615

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