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

Arran J. Folly* [1,2], Philip C. Stevenson [3,4], Mark J.F. Brown [1]

(1)Department of Biological Sciences, Royal Holloway University of London, Egham, UK (2) Animal and Plant Health Agency, Surrey, UK (3) Royal Botanic Gardens, Kew, UK (4) Natural Resources Institute, University of Greenwich, Kent, UK

*Contact: arran.folly.2010@live.rhul.ac.uk

Summary Statement: Some vertebrates co-opt natural compounds to treat disease, however their effectiveness may differ between life-history stages. Here we provide the first evidence that this variation also occurs in an invertebrate.

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 use 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 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 Jr. 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*. 2007, 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.

To address this question holometabolous insects provide an ideal model system, 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 & Porter 1914), has relatively low environmental prevalence (Shykoff & Schmid-Hempel 1991, Jones & Brown 2014) but is deleterious to bumblebee populations (Otti & Schmid-Hempel 2007, Rutrecht & Brown 2009, Cameron *et al*. 2011, Brown 2017). Infection with *N. bombi* can reduce both worker longevity and sperm count in males (Otti & Schmid-Hempel 2007, Rutrecht & Brown 2009).

However, of greater concern is that infection can negatively impact the production of sexual castes (Otti & Schmid-Hempel 2008, Rutrecht & Brown 2009) and this has been linked to the range and population declines seen in some North American bumblebees (Cameron *et al*. 2011, Cameron *et al*. 2016). Critically, for the context of this study, *N. bombi* infection persists through both larval and adult life stages of bumblebees (Rutrecht & Brown 2008), although it largely relies on larval infection for transmission (Rutrecht *et al*. 2007, Rutrecht & Brown 2008). Given that other microsporidian pathogens of bees are susceptible to biologically active plant 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 1998) on which bumblebees forage (Goulson & 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 use the isoflavone biochanin A, which has been found in *Trifolium 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 to larval treatment.

Methodology

Biochanin A and bumblebee colony provenance

Eight *Bombus terrestris audax* colonies (hereafter referred to as donor colonies), each containing a queen, brood and a mean of 45 (± 6.5 S.E.) 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 two 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 14×8×5.5cm 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 & Brown 2008), larval inoculation would likely 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 (SU992703) in 2016. The infected queen's gut was isolated by dissection and homogenized in 0.01M NH_4Cl. The resulting spore solution was centrifuged at 4°C for 10 minutes at 6800g to isolate and purify the spore pellet as described in Rutrecht & Brown (2008). The pellet was resuspended in 0.01M 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 *N. ceranae*, as these two microsporidians can be easily confused under a light microscope, a sample of the inoculum was subjected to PCR using primers and the 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 4.3µl of experimental inoculant, as described above, using a 20µl 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 & Brown 2008, AJF unpublished pilot work). Following inoculation, larvae were left for 30 minutes 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, Dorset, UK) was added to sugar water (50% w/w) using 4ml of 40% MeOH as a solvent per litre. Control colonies also had 4ml of 40% MeOH 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), consequently 20ppm 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 x 95mm), which was modified with a hole that enabled a 15ml 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 three 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 25ml 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 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) three times and averaged, as a proxy for bumblebee size, using a set of Mitutoyo^{tm} digital calipers (Whitehorn *et al*. 2011). Workers were then sacrificed 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 4.3µl of inoculant using a 20µl 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 15ml of sugar water (50% w/w) containing biochanin A at 20 ppm and control bumblebees were given 15ml of control sugar water (50% w/w). As before, biochanin A was added to sugar water using 4ml of 40% MeOH as a solvent per litre. Control colonies also had 4ml of 40% MeOH added per litre of sugar water. Infected workers were kept under quarantine as described for 7 days. Every two days each worker was removed and a sample of faeces was taken using a 10µl glass capillary tube. This sample was then measured for volume (µl) and screened for *N. bombi* parasite load using a Neubauer improved haemocytometer. After seven days of experimental feeding, a final parasite count was taken, as described above, and thorax width measurements (mm) for each worker were taken three times and averaged, as a proxy for bumblebee size, using a set of Mitutoyo^{tm} digital calipers before being sacrificed 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 (R Core Team 2019, Wickham 2009). To analyse the therapeutic effect of biochanin A on *N. bombi* infection intensity (cells/µl) 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, day 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), 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 (Figure 1.1).

Figure 1.1 Beeswarm plot, used to show the complete spread of data, of *N. bombi* infection intensities in adult *B. terrestris* workers (n = 56) that were treated therapeutically with biochanin A as larvae. The sample mean has been 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).

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 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 (Figure 1.2).

Figure 1.2 The infection intensity (shaded areas represent mean ±SEM) of *N. bombi* in adult *B. terrestris* workers (n = 23) over a seven day period when given a control or biochanin A sugar water supplement. The covariates, treatment (LMM, $F_{1,78}$ = 12.51, P < 0.001), 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.

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 respond differently to bioactive compounds as an individual ages in bumblebees, 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*. 2007, Abbott 2014, Gower *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 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 to suppress *Toxocara canis* infections in lactating bitches to reduce transmission to puppies in dogs (Burke & 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 disease.

Nosema bombi has been implicated in rapid and catastrophic declines in the populations and geographical ranges of a suite of bumblebee species across North America (Cameron *et al*. 2011,

Cameron *et al*. 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 & 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 & 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 consistently 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 likely 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 & 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 & Brown (2008) showed that the infection intensity of *N. bombi* in *B. lucorum* did not change across the lifetime of adult bees after eclosion. Given the temporal increase in shed spores seen in our experiments, which given the results of Rutrecht & Brown (2008) must therefore be mirrored by a decline in within-body spore intensity, this 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 & 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 due to 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, Otti *et al*. 2014, Cremer *et al*. 2018) 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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Acknowledgements

We would like to thank Judy Bagi and Sue Baldwin for technical support and we would like to thank Lewis Armstrong for assisting with the blind allocation of adult bumblebees. In addition we would like to thank the comments of two anonymous reviewers that helped to improve the manuscript. Finally, AJF would like to thank Sonia and Hugo for their inspirational walks.

Funding statement

This work was funded by a Biotechnology and Biological Sciences Research Council Doctoral Training Program Studentship (DTP1 BB/J014575/1).

Author contributions

AJF, PCS and MJFB devised the experiment. AJF and MJFB designed the experiment. AJF was responsible for carrying out the experimental work and writing the manuscript. All authors provided feedback on the manuscript and agreed to its publication.

Data availability

Data has been made open access and deposited on to Dryad under the title ‘Age-related pharmacodynamics in a bumblebee-microsporidian system mirror similar patterns in vertebrates’. doi:10.5061/dryad.rn1gf7k

Declaration of interests

The authors declare no competing interests.

Figure legends

Figure 1.1 Beeswarm plot, used to show the complete spread of data, of *N. bombi* infection intensities in adult *B. terrestris* workers (n = 56) that were treated therapeutically as larvae. The sample mean has been marked with a grey bar. Biochanin A did not have a significant therapeutic effect on *N. bombi* infection intensity in adult workers ((LMM, $F_{1,51} = 2.2867$, $P = 0.1366$).

Figure 1.2 The infection intensity (shaded areas represent mean ±SEM) of *N. bombi* in adult *B. terrestris* workers (n = 23) over a seven day period when given a control or biochanin A sugar water supplement. The covariates, treatment (LMM, $F_{1,78} = 12.51$, $P < 0.001$), 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.