Insect. Soc. 55 (2008) 86 – 90
0020-1812/08/010086-5
DOI 10.1007/s00040-007-0974-1

Insectes Sociaux

Research article

Effects of natal and novel *Crithidia bombi* (Trypanosomatidae) infections on *Bombus terrestris* hosts

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Received 2 September 2007; revised 9 November 2007; accepted 20 November 2007.
Published Online First 4 December 2007

Abstract. *Bombus terrestris* queens may contract infections of the trypanosome parasite *Crithidia bombi* from their natal nests; alternatively, the queens may also become infected after leaving their natal nests while foraging on contaminated flowers. We expected that, because *C. bombi* adapts to the natal colony during the previous generation, *C. bombi* infections from the natal colony will be more damaging to queens than a novel infection acquired from an unrelated colony. To test our prediction, we used queens exposed to three treatment groups: *natal* infection, *novel* infection, and *control* (no infection). We found that the infected queens produced fewer males and had a lower overall fitness, but we did not find any differences based on the source of the infections. We noted a strong matriline effect on the likelihood of a queen surviving hibernation and successfully founding a colony. Taken together, our results suggest that while *C. bombi* affects the fitness of *B. terrestris*, one vertical transmission event is no more damaging than randomly encountered infections. Furthermore, we found that, at least under laboratory conditions, matriline effects on fitness could override the effect of infection status.

Keywords: Host-parasite co-evolution, virulence, local adaptation, vertical transmission.

Introduction

Parasite virulence is loosely defined as the amount of damage done by a parasite to its host, usually measured in terms of host fitness loss (Poulin and Combes, 1999). It was long believed that a fully adapted parasite caused little harm to its host and thus over time all parasites would evolve towards a benign interaction with their hosts. More recently however, biologists have become aware that such an outcome is not a necessity and that the selective pressures shaping the evolution of parasites produce a large range of adaptive levels of virulence. Theoretical models have elucidated some of the general rules governing the evolution of virulence (Frank, 1996) and have identified factors that are particularly important. These can include host population density, host heterogeneity, the mode of transmission, dependence on vectors, or host background mortality (Ewald, 1983; Bull, 1994; Frank, 1996; Regoes et al., 2000).

The degree of associations with particular host (geno-) types can have lasting effects on the virulence of a parasite population, a fact exploited to produce attenuated viruses for use in vaccines (see Ebert, 1998). Relatively rapid changes in virulence can be partially attributed to the fact that some parasites produce several generations within a given host. Within the duration and through the course of an infection, parasite populations can therefore gradually adapt to the particular environment in the host (Levin et al., 1999). However, many of these adaptations will be unlikely to confer any advantage to the parasite when it transmits to its next host except when the new host presents a similar environment. This exception is most likely when the next host is genetically similar to the current host, i.e. is a relative (Hamilton, 1980; Shykoff and Schmid-Hempel, 1991b; Ebert and Hamilton, 1996). By contrast, transmitting through genetically variable hosts should make it more difficult for parasites to adapt. This is the basis for the prediction that parasite virulence should be higher in homogeneous as compared to heterogeneous populations (Ebert and Hamilton, 1996).

Yet, recent models have shown that both lower and higher overall levels of virulence can evolve due to heterogeneity in host populations (Regoes et al., 2000; Ganusov et al., 2002). The question is therefore unsettled and empirical evidence is needed.

The trypanosome *Crithidia bombi* Lipa and Triggiani (Trypanosomatidae) is a gut parasite of bumblebees such as *Bombus terrestris* L. (Lipa and Triggiani, 1988). Bumblebees are annual social insects with colonies started by a single queen in spring, after emergence from hibernation. In early summer, the prevalence of *C. bombi* in field populations can be as high as 80 % (Shykoff and Schmid-Hempel, 1991a). Spring queens and their colonies can become infected in two ways. 1) By horizontal transmission when queens or workers forage on contaminated flowers and return with infective cells to the colony (Durrer and Schmid-Hempel, 1994). Within a colony, the infection is passed on via contaminated nest material. 2) By vertical transmission when daughter queens acquire the infections in their natal nest and keep it until the following year and so infect their own incipient colony. A previous study showed that queens infected prior to hibernation lose more mass during hibernation and that infected spring queens are less successful at founding a colony, produce smaller colonies with fewer males and have a lower overall fitness when compared with uninfected queens (Brown et al., 2003b).

Vertical infections are acquired by a spring queen in her natal nest during the previous year. These should therefore be the parasites that have adapted to the worker genotypes in the natal nest. In bumble bees, the worker genotypes are statistically the same as that of their sisters that later become spring queens, because caste determination (worker or reproductive queen) is environmental rather than genetic. We therefore hypothesized that *C. bombi* from the natal nest will be more harmful to the queens than infection by random strains. To test this hypothesis of "adaptation to the family" we infected newly emerged queens with either strains obtained from their natal colony, or strains from other colonies. We then measured the fitness and various life history parameters and compared the treatments to each other and to uninfected controls.

Materials and methods

Queens used in this experiment were first generation lab-reared daughters of queens that were wild caught near Zürich, Switzerland. All of the wild-caught queens were checked for *C. bombi* and other common pathogens before including them in the experiment. The wild-caught mother queens formed colonies in the lab as outlined below for the daughter queens. Once the colonies had established themselves, multi-strain infections were initiated by collecting *C. bombi* cells from the faeces of 11 naturally infected wild-caught queens (used only as infections sources, not to produce colonies for this experiment) and mixing the cells in equal parts to form 10,000 cell doses. These doses were in turn fed to approximately 10 workers per colony. These infected workers were returned to their colonies and the infections were thus allowed to spread within and presumably adapt to the natal colonies. Once the colonies began producing daughter queens, they were removed from these infected natal colonies as pupae to prevent accidental infections (*C. bombi* only infects adults). These experimental queens were allowed to emerge and their faeces were checked for *C. bombi* and other pathogens to ensure they were not infected. Queens from ten colonies were mated with males from seven colonies such that each of the ten maternal lineages (matrilines) was mated with two to four different paternal lineages through the corresponding scheme of single matings of queens and males in laboratory flight cages. Each paternal lineage was mated with one to eight maternal lineages. Queens were then divided up such that each maternal and paternal lineage was evenly distributed among the *control*, *novel* and *natal* treatments

After mating, queens in the *control* treatment were fed approximately 1 ml of an Apiinvert® sugar water solution (Südzucker, D-97195, Ochsenfurt). Queens in the *novel* treatment were fed the same sugar water contaminated with a 10,000-cell cocktail of *C. bombi* strains. The cocktail consisted of equal parts of infective cells harvested from the faeces of a minimum of 5 workers from each of 10 infected source colonies. These source colonies were distinct from the source queens used to infect the natal colonies. The queens in the *natal* treatment were fed sugar water contaminated with 10,000 *C. bombi* cells from a minimum of 5 workers from her natal colony. The queens were not fed again until these inocula had been consumed. All queens were then given pollen and sugar water *ad libitum* for a week, allowing their infections to fully develop, before they were placed in individual cardboard matchboxes and hibernated at 4 °C for approximately 18 weeks. We weighed the queens at the beginning and end of hibernation to determine mass loss during hibernation.

Queens that survived hibernation were placed in acrylic glass boxes ($12.5 \times 7.5 \times 5$ cm) and fed pollen and sugar water *ad libitum*, and kept at 30 °C, 60 % r.h. under constant darkness or red light. After 14 days, we collected faeces samples from all the queens to confirm their *C. bombi* infection status. We checked queens daily and recorded if they had laid any eggs and later if workers had emerged. Once five workers had emerged in the acrylic boxes, we moved the queens, workers and brood to gypsum nests (Pomeroy and Plowright, 1982). We censused the colonies and culled 20 % of the workers weekly to mimic natural background mortality rates. Newly emerged males and queens were counted daily and removed from the colonies.

We compared the number of queens surviving hibernation and colony founding success of surviving queens using log-likelihood G-tests. We considered a queen to be successful at founding a colony if she reared at least one worker. To compare body mass loss due to hibernation, we first performed a linear regression of post-hibernation body mass against pre-hibernation body mass and then compared the residuals using an analysis of variance (ANOVA). Tallies of workers, males, and gynes (unmated new queens) produced by the colonies were not normally distributed and could not be adequately normalized using transformations. As such, we used Mann-Whitney U-tests to compare the *control* colonies to the pooled *novel* and *natal* treatment colonies, and then to compare the *novel* and *natal* treatment colonies alone. In addition to the counts, we compared the groups using an overall fitness measure for colonies equal to the number of males plus two times the number of gynes (to reflect both their larger mass and thus energy investment as well as the fact that they are diploid and thus carry two copies of the genome compared to the haploid males that carry only a single copy). We corrected for multiple comparisons using the stepwise Bonferroni method (Sokal and Rohlf, 1995).

Results

Out of the 229 queens put into hibernation, 87 survived (38 %). The three treatments did not differ in their likelihood of survival (log likelihood $G = 0.755$, d.f. $= 2$, $n = 229$, $p = 0.69$). However, queen survival varied strongly among matrilines ($G = 53.092$, d.f. $= 9$, $n = 229$, $p < 0.001$); note that the effect of matrilines

Table 1. Medians, interquartile ranges (IQR), and full ranges (FR) for counts of workers, males, gynes, and fitness (see text for calculation) for colonies producing one or more workers. Mann-Whitney U-tests were used to compare control (n = 7) and infected (n = 20) colonies and novel (n = 8) and natal (n = 12) treatment colonies. The U statistic and p are presented for each comparison and those marked with an asterisk are significant after a step-wise Bonferroni correction. Medians and ranges have been rounded to the nearest whole number for the sake of clarity.

	Median	IQR	FR	U	p
Workers					
control	49	4–110	4–129	41	0.108
infected	6	3–16	1–147		
novel	9	5–35	1–130	34.5	0.296
natal	5	2–15	1–147		
Males					
control	96	4–115	0–455	28	0.014*
infected	0	0–13	0–331		
novel	1	0–32	0–145	40.5	0.513
natal	0	0–5	0–331		
Gynes					
control	0	0–6	0–63	48	0.073
infected	0	0–0	0–19		
novel	0	0–0	0–0	40	0.236
natal	0	0–0	0–19		
Fitness					
control	109	4–222	0–457	27	0.010*
infected	0	0–13	0–359		
novel	0	0–32	0–145	45	0.786
natal	0	0–5	0–359		

among treatments was balanced by the experimental design. To measure the effect of the treatments on mass loss during hibernation, we used the residuals of a regression of post-hibernation with respect to pre-hibernation mass, (*post-hibernation mass*) = (*pre-hibernation mass*) × (0.782) ($F_{1,84} = 398.94$, $p < 0.001$, $R^2 = 0.831$). Note that two individuals had to be excluded from the mass analysis because their pre-hibernation mass data were lost. Unlike previous studies (Brown *et al*. 2003b), the queens in our treatments did not differ in their mass loss over the hibernation period ($F_{2,83} = 0.074$, $p = 0.971$).

Approximately 31 % (27 of 87) of the surviving queens successfully raised at least one worker, but queens in the three treatments did not differ in this respect (log likelihood $G = 0.470$, d.f. = 2, n = 87, $p = 0.80$). As with surviving hibernation, queens from different matrilines differed in their likelihood of producing a worker (log likelihood $G = 23.10$, d.f. = 7, n = 87, $p = 0.002$). In addition, infected colonies produced fewer males and suffered reduced fitness (Table 1). When outliers (± 2 standard deviations) are removed, infected colonies produced fewer workers than the uninfected colonies (results not shown but see Table 1). However, one infected colony produced more workers than any of the uninfected colonies, demonstrating the large variability in the productivity of bumblebee colonies. Of the seven matrilines that successfully started colonies, two matrilines dominated the production of males and were the only ones to produce gynes; these two matrilines accounted for 15 of the 27 successful colonies. Three other matrilines, accounting for 10 of the colonies, produced one male-producing colony each. The remaining three matrilines accounted for only 4 colonies, none of which produced sexuals.

With respect to one of our main comparisons, we found no difference in fitness between the novel and natal infections (Table 1). Fitness comparisons unfortunately suffered from low statistical power due to the fact that almost half these colonies produced no sexuals. However, this lack of difference is consistent with our comparisons of survival, mass loss, egg laying and colony founding. Although most *natal* treatment colonies produced fewer workers than the *novel* treatment colonies, one *natal* treatment colony produced more workers than both the *novel* and *control* treatment colonies, and two *natal* treatment colonies produced gynes while none were produced in any of the *novel* treatment colonies (Table 2).

Discussion

Our results did not support our main prediction that infections acquired from a queen's natal colony would be more harmful than those acquired from other colonies as measured by several fitness components. However, we did confirm earlier findings that *C. bombi* can reduce the fitness of *B. terrestris* colonies under lab conditions (Brown et al., 2003b). The high rates of attrition due to death during hibernation, and failure to achieve subsequent life history benchmarks, seem to be major sources of variation in colony fitness (Table 2). Previous studies have found that under some conditions *C. bombi* can have a negative impact on many of these factors (Brown et al., 2003b).

Although the *natal* treatment is technically vertical transmission, *C. bombi* also transmits horizontally and its fitness is not linked to that of its host therefore the lower virulence often associated with primarily vertical transmission pathways do not apply to this system. As such, we do not predict *C. bombi* to become less harmful in the *natal* treatment. It could be argued that a single generation of association may be insufficient for adaptation to occur or that such adaptation may manifest itself in forms that do not harm the host colony. However, another study found that only four transmissions of *C. bombi* infections are needed among workers from a given colony before a decrease in infection intensity can be observed in workers from other colonies (Yourth and Schmid-Hempel, 2006). Colonies only exist for a few months, but in this time the infection could be transmitted dozens of times within a colony; infected individuals begin shedding cells within

Table 2. Numbers of *B. terrestris* queens in each treatment of the experiment entering hibernation, surviving hibernation, laying eggs, producing at least one worker, founding a colony of 5 or more workers, producing at least one male and producing at least one gyne.

	Hibernation	Surviving	Eggs	Worker	Colony	Males	Gynes
control	79	27	10	7	7	6	3
novel	73	29	14	8	6	5	0
natal	77	31	15	12	7	4	2
Total	229	87	39	27	20	15	5

days, continuously infecting other workers and likely re-infecting themselves. It would be surprising if even a single generation of association did not have some effect on the infecting parasite strains.

It should be noted that some strains perform much better in some colonies than in others due to genotypic interactions (Schmid-Hempel, 2001). The *natal* treatment infections will include the subset of introduced strains that were able to successfully exploit the workers in the natal colonies. The *novel* treatment infection cocktail on the other hand represents a collection of unrelated strains that may or may not be able to exploit the colony. It is possible that in using a cocktail of strains to infect the *novel* treatment colonies we increased the likelihood of infecting them with at least one strain as harmful as those in the natal treatment by chance alone, but in the wild a colony will also encounter a variety of strains with the same risk.

Contrary to previous results, infection by *C. bombi* did not affect a queen's likelihood of hibernation survival, although the overall survival rates we report here are lower than those previously reported by Brown et al. (2003b). We did not detect a difference among our treatments in body mass loss over the hibernation period or likelihood of starting a colony. Infected colonies did differ from uninfected colonies in that they produced fewer males and thus had lower fitness. They also tended to have fewer workers, although this difference was not statistically significant unless outliers were removed. These reductions in productivity are presumably a consequence of reductions in ovary size associated with *C. bombi* infections (Shykoff and Schmid-Hempel, 1991c), and occur despite the relatively benign laboratory environment and *ad libitum* food. Under stressful conditions, the effects of *C. bombi* would likely be much more severe (see Brown et al., 2000). Since the immune system itself has costs to the bees and is stimulated by *C. bombi* (Moret and Schmid-Hempel, 2000; Brown et al., 2003a), the cost of an activated immune system could partially explain the lower fitness.

We noted that some matrilines were more likely to survive hibernation and found colonies than others. This suggests that there is a strong genetic component to the success of queens under lab conditions. The extreme differences among the matrilines at the same time precluded any meaningful statistical comparisons of their productivity. It appears that a "good" matriline is more productive than a "bad" one, even when a colony is infected. It is also noteworthy that although most colonies seem to suffer from *C. bombi* infections in terms of reduced productivity, some infected colonies are just as productive as uninfected colonies. Given that the laboratory conditions under which these colonies were raised are much less harsh than field conditions, it may be that some colonies are capable of compensating for infections. The fact that all the queens used in this experiment were from colonies that produced queens despite *C. bombi* infections may increase the probability that they possess such compensatory abilities.

In all, the interaction of *C. bombi* with its host, *B. terrestris*, is more complex than initially hypothesized. In particular, it seems that queens are not disproportionally more affected by the parasites they pick up in their natal nest as compared to those acquired anew in the field. Hence, possible adaptation by parasites during the lifetime of a colony does not pose an additional threat.

Acknowledgments

We thank R. Schmid-Hempel for comments and help. Financial support provided by the Swiss National Science Foundation, grant no. 3100–66733.01 to PSH.

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