Strong context-dependent virulence in a host-parasite system: reconciling genetic evidence with theory

Mark JF Brown, Department of Zoology, Trinity College Dublin, Dublin 2, Ireland

Regula Schmid-Hempel, Ecology and Evolution, ETH-Zürich, ETH Zentrum NW, CH-8092, Zürich, Switzerland

Paul Schmid-Hempel, Ecology and Evolution, ETH-Zürich, ETH Zentrum NW, CH-8092, Zürich, Switzerland

Corresponding author: MJF Brown, Department of Zoology, Trinity College Dublin, Dublin 2, Ireland. Email: mabrown@tcd.ie Tel: +353 (0)1 608 1627 Fax: +353 (0)1 677 8094

Running headline: context-dependent virulence and host fitness

Summary

1. Parasites can have dramatic effects on the ecology of their hosts. Such strong host-parasite interactions are the result of either parasites with generally high virulence, or generally benign parasites that nevertheless express context-dependent virulence. Theoretically, one indication that an apparently benign parasite nevertheless has a large impact on its host should be the existence of strong genotypic interactions between host and parasite.

2. *Crithidia bombi* (Trypanosomatidae) is a highly prevalent but generally benign gut parasite of the bumble bee *Bombus terrestris*. The demonstration of strong genotypic interactions between *C. bombi* and *B. terrestris*, however, suggests that context-dependent virulence may have a large impact on the host population. We thus investigated the effects of *C. bombi* across the entire life-cycle of its host, including the stressful times of hibernation and colony-founding. Due to the high prevalence and rates of transmission of the parasite in field populations, we used a large-scale laboratory experiment.

3. Under stressful hibernation, infected queens lost more weight. Infection also significantly reduced colony-founding success, colony size, male production and overall fitness, by up to 40%. These findings show that strong genotypic host-parasite interactions may indeed be a reliable indicator that apparently benign and highly prevalent parasites are nevertheless exerting a dramatic impact on their host populations.

Keywords: *Bombus terrestris*, *Crithidia bombi*, Host-parasite Interaction, Trypanosome

Introduction

Theoretical and empirical work over the last two decades and in a variety of systems has demonstrated the central role that parasites play for many important aspects of host biology. For example, parasites can drive host population cycles (Anderson & May 1981; Hudson, Dobson & Newborn 1998), mediate competition among host species (Price, Westoby & Rice 1988), induce changes in host life history (Minchella 1985) and lead to the production of immunologically enhanced offspring (Moret & Schmid-Hempel 2001). While the impact of parasites may not always be so obvious, many apparently benign parasites may still have a significant effect on their host under certain specific contexts (Ewald 1983).

Recent work has identified several cases where parasites impact individual host mortality only under stressful conditions (Schaub & Loesch 1989; Jaenike, Benway & Stevens 1995; Brown, Loosli & Schmid-Hempel 2000), presumably because hosts are in such poor condition that they cannot compensate for increased parasite-related defence costs (e.g., Moret & Schmid-Hempel (2000)). Such individual-level processes may also result in population-level effects (e.g., Kraaijeveld & Godfray (1997); Ebert, Lipsitch & Mangin (2000); Stien *et al.* (2002); Telfer *et al.* (2002)). In addition to condition-dependent effects, parasites may only become virulent during host life-history stages that are linked to parasite transmission (as suggested by the theory of virulence-transmission trade-offs (e.g., Ebert & Herre (1996)), when a given host tissue is especially active (e.g., during reproduction), or due to other stage-specific situations. Therefore, parasites can exhibit context-dependent virulence either as a result of poor host condition or through a

"strategic" expression of virulence aimed at enhancing transmission. Because of such context-dependent expression of virulence, a major problem when studying the ecology of parasitism is that an important role for a given parasite is suspected, but substantial effects on host fitness are often difficult to demonstrate, especially in the natural situation (Stien *et al.* 2002; Telfer *et al.* 2002).

In contrast to this, strong parasite effects on host fitness (and, vice versa, of hosts on parasite fitness), even if they cannot be immediately verified, should leave their trace in the pattern of extant variation in the host-parasite system, particularly in the extant genotypic variation relevant for host-parasite interactions. In fact, theory predicts that where parasites exert strong selection on their hosts, rapid antagonistic co-evolution in the system can emerge. Such co-evolution in turn may maintain both host and parasite genotypic variation as well as strong genotypic interaction effects (Haldane 1949; Hamilton 1980; Hamilton, Henderson & Moran 1981; Hamilton 1993; Frank 1994; Peters & Lively 1999). Many host-parasite systems do indeed show strong components of genotypic variation in the outcome of the interaction (e.g., Wakelin (1978); Carius, Little & Ebert (2001)). If such genotypic variation is found in a host-parasite association that otherwise appears benign, theory suggests that the relevant selective situation may have been overlooked and implies the existence of context-dependent virulence. In fact, context-dependent virulence is likely to be very common (see above), and consequently understanding when and how parasites affect their individual hosts to a degree that could affect host-populations and genetics (Anderson & May 1981; Hamilton 1993) is of general ecological interest.

Crithidia bombi Lipa & Triggiani (Trypanosomatidae) is a gut parasite of bumble bees *Bombus* spp. (Lipa & Triggiani 1988). It has high prevalence [up to 80%; (Shykoff & Schmid-Hempel 1991c)] in field populations of *Bombus terrestris* L., one of the most common and abundant European bumble bees (Alford 1975). *B. terrestris*, like most bumble bees, is an annual social insect. Over-wintered queens found a colony in spring. Colonies then increase in worker number until mid-summer when sexual forms, males and gynes (the daughter queens), are produced. These leave the nest, mate and then the gynes enter diapause before emerging the following spring to found their own nest.

Previous work suggested that the virulence and impact of *C. bombi* is low, which appears to explain the generally high prevalence of the parasite in natural populations (Shykoff & Schmid-Hempel 1991c; Imhoof & Schmid-Hempel 1999). However, in contrast to these data, there is ample evidence for strong genotype-genotype host-parasite interactions in this system. For example, 1) parasite transmission is higher among related host individuals (Shykoff & Schmid-Hempel 1991a, b), 2) host genotypes vary in their ability to transmit the parasite (Schmid-Hempel & Schmid-Hempel 1993), 3) the parasite exhibits local adaptation to host populations (Imhoof & Schmid-Hempel 1998), and 4) microsatellite analyses revealed strong host-sorting of parasite strains (Schmid-Hempel *et al.* 1999). Given the theoretical requirements for the maintenance of genotypic variation the apparently benign nature of *C. bombi* is clearly at variance with the required strong host-parasite effects.

One part of this enigma was recently resolved, as *C. bombi* was found to exhibit condext-dependent virulence in food-stressed workers, increasing their mortality rate by 50% (Brown *et al.* 2000). Brown *et al.* (2000) thus suggested that parasite effects are likely to be largest during stressful stages of the bumble bee life cycle, for example, the period of colony founding, which, in social insects, is a crucial stage of the life cycle in independently founding species. Unfortunately, the impact of *C. bombi* on field populations is very difficult to ascertain *in situ*– a previous experiment which placed artificially infected and control colonies in the field was immediately confounded due to the rapid natural transmission of the parasite via flowers (Durrer & Schmid-Hempel 1994; Imhoof & Schmid-Hempel 1999). Luckily, the life cycle of bumble bees can be simulated in the laboratory.

Here, therefore, we investigate the impact of *C. bombi* across the entire life cycle of its host, from hibernation to the production of sexuals, under controlled laboratory conditions. Specifically, we ask whether parasite virulence increases under the potentially stressful host-contexts of hibernation and colony-founding. Hibernation should be stressful, as survival depends upon the utilisation of a limited resource – fat reserves. In contrast, colony-founding is likely to be stressful as queens face the problem of allocating resources to a variety of expensive activities, including the initiation of ovary activity, wax production and brooding behaviour (Alford 1975). High failure rates during colony-founding (personal observations) and energetic shortages in spring queens (Heinrich 1979) provide evidence that these life-cycle stages are indeed stressful (also see Pomeroy 1979; Bowers 1985). Our results confirmed that, under these contexts, parasite virulence

does increase and thus suggest that genotype-genotype variation in a host-parasite interaction can indeed be taken as a sign of strong parasite effects, even though these may only occur at certain restricted, but crucial stages of the host's life cycle. Conversely, the pattern of a parasite being benign and highly prevalent may be misleading with respect to the actual importance of the parasite for the ecology of its host species.

Methods

All queens used in the following experiments were taken from laboratory-reared colonies ultimately derived from wild queens of *B. terrestris* caught in the area around Zürich, Switzerland in spring 2000. All of these colonies were parasite-free, as determined by dissection and the examination of gut contents. Strains of *C. bombi* for experimental infections were taken from other colonies reared from naturally parasitised queens and unrelated to the experimental queens. Because the experimental queens came from known mother colonies, they were correspondingly grouped into matrilines (composed of sister queens), which were used as a factor in the statistical analyses. We used multiple host lines and multiple-strain inocula of the parasite (see below) to minimise the impact of any one specific host-parasite interaction on our results.

We conducted two, separate experiments, which shared mating and infection protocols (see below), but differed in the origins of queens and parasite strains and in the hibernation treatment (see below). In the first experiment we mated 52 queens from ten first-generation laboratory colonies and infected half of them with a mix of parasite strains from two naturally infected first-generation colonies. These queens were then hibernated (see below) for 3 months. In the second experiment we mated 286 queens from 15 second-generation laboratory colonies and infected half of them with parasite strains from four naturally infected second-generation laboratory colonies. These queens were then hibernated for either 2.5 or 5 months, depending upon treatment (see below).

The duration of hibernation in field populations is likely to be highly variable. Queens may enter hibernation immediately after mating in the summer (Alford 1975). However, queens can remain active in our field populations until late November (MJFB pers obs). Given that queens generally emerge from hibernation in March around northern Switzerland (MJFB, RSH, PSH unpublished data), the duration of hibernation may last anywhere between 3 and 8 months. Consequently, while our hibernation periods were chosen to ensure a sufficient sample size (hibernation survival decreases with the length of hibernation under laboratory conditions), they nevertheless represent realistic hibernation durations for our population of bumble bees. While a 2.5-month hibernation may be extremely short for Swiss populations, we note that other European populations of *B. terrestris* may regularly undergo such hibernation durations (MJFB personal observation). We used three different hibernation conditions to investigate the impact of variation in the potentially stressful context of hibernation.

Queens were mated in the laboratory in flight cages (50 5 50 5 55 cm plastic frames covered with mosquito net) to unrelated males, prior to any experimental treatment. Post-mating, queens were assigned to one of two experimental groups. “Control” queens were immediately presented with a solution of Apiinvert® sugar water. “Infected” queens were presented with an inoculum of *C. bombi* cells in a solution of Apiinvert® sugar water – queens were not provided with further sugar water until the inoculum had been imbibed. Queens were then kept for 7 days with *ad libitum* pollen and sugar water, enabling them to build up body reserves prior to hibernation. On the 8th day, queens were placed individually in cardboard matchboxes and then hibernated in a fridge at 4 ˚C. In

experiment 2, queens were weighed pre- and post-hibernation to determine relative loss of body mass due to hibernation. After hibernation, surviving queens were placed in rearing boxes (acrylic glass, 12.5 5 7.5 55.5 cm) with *ad libitum* pollen and sugar water, and allowed to start colonies. After the emergence of the fifth worker, colonies were transferred to gypsum nests (Pomeroy & Plowright 1980) and allowed to go through their natural life cycle. In order to simulate field mortality rates, the worker population of each colony was culled by 20% every week after transfer. Queens, and subsequent colonies, were kept at 30 ˚C, 60% r.h. and under constant dark or red-light conditions. All queens were checked for the presence of a *C. bombi* infection 14 days post-hibernation. Because some queens in the "Infected" treatment failed to develop an infection, only those queens whose condition corresponded with their initial parasite treatment were included in the analyses.

Queens and colonies were checked daily throughout the duration of the experiment. Observers were blind with respect to the treatment group of queens and colonies. We recorded for queens whether they a) survived hibernation, b) successfully reared at least one worker, and c) for each colony the total number of workers (colony size), and their reproductive success in males and gynes (young queens) produced. We also recorded the time lag between removal of the queen from hibernation and the production of the first worker (time to first worker), first male (time to first male), first gyne (time to first gyne), and first egg-laying by workers (time to worker eggs; visible as egg heaps of a different, tower-like shape indicating the onset of worker-queen conflict over male production). Thus, we recorded the productivity and life history data most important for bumble bee

reproductive success (Pomeroy & Plowright 1982; Duchateau & Velthuis 1988; Müller & Schmid-Hempel 1992).

Data on hibernation survival and colony-foundation were analysed using logistic regression with the forward log-likelihood procedure. The parasite and hibernation treatments were coded as indicator variables, while maternal lineage (the colony of origin for a given queen) was coded as a deviation variable. Weight loss, colony productivity and life-history data were analysed using univariate and multivariate ANOVAs. Where necessary, data were transformed to meet the assumptions of the tests. All analyses were conducted on SPSS 10 for the Macintosh.

Results

Experiment 1

Approximately 48% of the queens survived the hibernation period of 3 months (14 out of 26 Control and 11 out of 26 Infection queens). There was thus no effect of infection on hibernation survival (Fisher's exact test, $P = 0.58$).

However, in this experiment, infection with *C. bombi* completely inhibited colony founding. None of the 11 Infection queens successfully reared a worker, while six of the 14 Control queens successfully reared a colony (Fisher's exact test, $P = 0.02$). Due to the absence of colony founding by infected queens, productivity and life-history data were not collected from the Control colonies, as the entire contribution to the next generation came from only the uninfected queens.

Experiment 2

Hibernation survival

Approximately 89% of the queens survived the 2.5-month hibernation period (96 out of 106 Control and 93 out of 106 Infection queens). There was again no effect of parasite infection on hibernation survival (Fisher's exact test, $P = 0.83$). A logistic regression, with survival as the dependent variable and parasite treatment, maternal lineage (of the queen) and the interaction term as candidate variables, supported this result (none of the variables had significant predictive power).

Approximately 66% of the queens survived the 5-month hibernation treatment (25 out of 37 Control and 24 out of 37 Infection queens). Again, there was no effect of parasite infection on survival, either overall (Fisher's exact test, $P = 0.628$) or in combination with maternal lineage (logistic regression analysis; none of the variables had significant predictive power).

Loss of body mass

For each queen that survived hibernation, we calculated the relative amount of body mass lost during hibernation [calculated as (pre-hibernation mass – post-hibernation mass)/(pre-hibernation mass)]. The pre-hibernation mass of queens did not differ between Control and Infection groups (mean ± SD = 0.73 ± 0.075 g and 0.74 ± 0.086 g respectively, $t = -0.749$, $DF = 196$, $P = 0.455$). The relative loss of body mass of queens was affected both by the length of hibernation and by an interaction between hibernation treatment and parasite treatment (Figure 1). Queens that hibernated for 5 months unsurprisingly lost significantly more body mass than did queens hibernated for only 2.5 months (Figure 1). The interaction effect was due to a parasite-related increase in body mass loss in the 5 months hibernation treatment (after 5 months hibernation Infection queens lost 11% more mass than Control queens; Figure 1).

As with experiment 1, infection with *C. bombi* inhibited colony founding. We analysed the effects of infection for the two hibernation treatments separately, and then across both treatments. After a 2.5 month hibernation, Infection queens were significantly less likely

to found a colony than Control queens (31 out of 67 Infection queens produced a colony vs. 57 out of 89 Control queens; Fisher's exact test, $P = 0.034$). The logistic regression analysis correctly assigned 67.9% of cases, with both parasite treatment and maternal lineage being significant predictors of successful colony founding (Parasite: Wald-statistic = 4.410, DF = 1, $P = 0.036$, Exp(B) = 0.465; Maternal Line: Wald-statistic = 20.125, DF = 11, $P = 0.044$). After a 5 month hibernation, there was no significant difference between Infection and Control queens in the likelihood of colony founding (16 out of 20 infected queens produced a colony vs. 19 out of 24 control queens; Fisher's exact test, $P > 0.99$). Neither parasite treatment nor maternal lineage explained a significant amount of the variation in colony founding (there was no significant logistic regression model). Combining the data from both hibernation treatments in a logistic regression analysis, both hibernation treatment and parasite treatment were significant predictors of colony founding, correctly assigning 64% of cases (Parasite: Wald-statistic = 3.985, DF = 1, $P = 0.046$, Exp(B) = 0.548; Hibernation: Wald-statistic = 7.634, DF = 1, $P = 0.006$, Exp(B) = 3.116). Overall, queens hibernated for 5 months and Control queens were significantly more likely to start colonies.

Effect on offspring performance (populations)

For these analyses, all queens that survived hibernation were included, i.e. the entire population of queens. Consequently, the results are best interpreted as effects on mean queen productivity in uninfected and infected populations. Data on colony size (number of workers), male production, queen production and fitness were analysed using a 2-way MANOVA, with parasite treatment and hibernation treatment as fixed factors.

Overall, there was a significant effect of parasite treatment, but not of hibernation treatment or their interaction, on the various measures of colony performance in the MANOVA (see Figure 2 legend for statistics). On average, a queen in the infected population produced 25% fewer workers (Figure 2a), 44% fewer males (Figure 2b), 24% fewer queens (Figure 2c), and was 40% less fit than a queen in the control population (Figure 2d).

Colony-level effects

For these analyses, only those queens that successfully founded colonies were included. Thus, results should be interpreted as representing the difference between infected and uninfected colonies within the same population. Statistical analyses are as above.

Overall, there were only marginal effects of the parasite and hibernation treatments and their interaction in the MANOVA (Parasite: Wilk's λ 24 $F_{4,114} = 2.350$, $P =$ 0.058; Hibernation: Wilk's λ 32 $F_{4,114} = 2.086$, $P = 0.087$; P×H: Wilk's λ 23 $F_{4,114} = 2.392$, $P = 0.055$). Mirroring results at the population level (see above), parasitism significantly reduced colony size by 9%, male production by 30% and overall fitness by 26% (Figures 3a, b, d; see Figure Legend 3 for statistics).

In contrast to the population-level analysis, hibernation and the interaction between parasite and hibernation treatments did have significant effects on colony productivity (Figure 3 legend). Overall, in the 5-month hibernation treatment, colonies had 28% fewer

workers (Figure 3a). Significant interaction effects on colony size, male production and fitness (Figures 3a, b, d) were due to a greater effect of parasitism in the 5 month hibernation treatment.

The mean sex ratio across all colonies was heavily male-biased (calculated as males/(males+2.09(gynes)) = 0.82 ± 0.1939 (see Beekman & Van Stratum (1998) for calculation)). However, despite the difference in male production among treatment groups (see above), there were no significant effects of parasite treatment, hibernation treatment, or their interaction on mean sex ratio (2-way ANOVA – Parasite: MS = 0.0003, $F_{1,104} = 0.008$, $P = 0.931$; Hibernation: MS = 0.018, $F_{1,104} = 0.474$, $P = 0.493$; Parasite 5 Hibernation: MS = 0.049, $F_{1,104} = 1.291$, $P = 0.258$).

Life-history effects

Here we report the effects of parasitism, hibernation treatment and their interaction on the timing of life-history events. Unlike the productivity data above, the number of colonies for which data were available varied greatly across the life-history events (for example, not all colonies produced males, queens or worker-laid eggs). Consequently, sample sizes for the MANOVA analyses were greatly reduced as compared to individual ANOVA analyses. Here we report the ANOVA results so as to present as representative a picture of colony-level effects as possible.

There were no significant effects of parasite treatment on any of the life-history parameters (Table 1). Similarly, hibernation had no significant effect on time to the first

worker (Table 1) or time to the first male (Table 1). However, after a 5-month hibernation worker-queen conflict (as measured by the appearance of egg towers) and the production of new queens started significantly earlier (time to first worker-laid egg: $F_{1,84} = 14.8$, $P < 0.001$; time to first queen: $F_{1,80} = 4.6$, $P = 0.035$; Table 1).

There was a significant interaction of the Parasite and Hibernation treatments on the time to the first worker ($F_{1,117} = 8.4$, $P = 0.005$; Table 1). After 2.5 months hibernation, infected queens produced workers earlier, while after 5 months hibernation infected queens produced workers later. There were no other significant interaction effects (Table 1).

Discussion

Theory predicts that strong genotypic interactions in host-parasite systems are produced by similarly strong ecological interactions. Such predictions are seemingly at variance with the existence of prevalent and apparently benign parasites in host-parasite systems with such strong genotypic structure. Here we have shown that context-dependent virulence may resolve this enigma. *C. bombi*, an otherwise benign trypanosome parasite of the bumble bee *B. terrestris*, had strong negative effects on its host during stressful host life-history stages. These data suggest that this highly prevalent parasite may limit significantly the abundance of its host species in the field. Given that *C. bombi* is a general parasite of several *Bombus* species, it may also play a role in determining the structure of natural bumble bee assemblages.

Immediately after hibernation, surviving bumble bee queens enter the stressful colony-founding period, where resources have to be directed towards the costly activities of egg-laying, wax production, and brooding behaviour. Here, the trypanosome parasite exhibited context-dependent virulence, acting as a physiologically castrating parasite in queens by significantly lowering the probability of founding a colony. We note that this occurred despite the provision of *ad libitum* food, suggesting fundamental constraints in the acquisition and allocation of resources during this stressful life-history stage (also see Schmid-Hempel & Schmid-Hempel (1998)). This result fits earlier observations of reduced ovary sizes in infected individuals kept under similar *ad libitum* resource conditions (Shykoff & Schmid-Hempel 1991b) and could reflect a physiologically

unavoidable relative reduction in allocation of resources to ovary development in infected bees (Brown *et al.* 2000). This effect was most dramatic in the first experiment, where no infected queens founded colonies, less dramatic but still significant in the 2.5-month hibernation treatment in experiment two, and virtually absent in the 5-month hibernation treatment (but present in the overall analysis of Experiment 2). Perhaps, under longer hibernation periods weaker queens that would have been more likely to show the effects of parasitism on colony-founding were already eliminated from both the Control and Infection populations (as mortality was not affected by the infection). Nevertheless, parasitism did delay the initiation of colony founding after longer hibernation by approximately 12 days. At the host-population-level we found a parasite-induced selection differential for overall fitness (survival, colony founding, and reproduction) of around 40 %. This clearly is a significant figure that makes the theoretically anticipated antagonistic co-evolution between host and parasite likely to occur. Emphasising again the importance of context, queens that experienced the most stressful (i.e., the longest) hibernation also had the most reduced fitness.

By contrast, *C. bombi* had no impact on hibernation survival and the timing of important life-history events, such as production of the first worker, emergence of the first males and gynes, and the beginning of worker-queen conflict over male production. This latter result is surprising, given that a previous study found a correlation between infection and delayed ovary development in workers (Shykoff & Schmid-Hempel 1991d). At present, we have no explanation for this difference. With respect to hibernation, we expected that, due to the physiological diapause and the low ambient temperature that must slow all biochemical reactions, there would be no effect of parasitism on loss of body mass during

hibernation and that any effects would have a delayed-expression during colony founding. In contrast, we found that after 5 months, parasitised queens had lost 11% more mass than control queens. Perhaps the parasite absorbs resources from the host to maintain its population during hibernation, or, alternatively, infected bumble bees may up-regulate their immune system at a physiological cost (Brown, Moret and Schmid-Hempel, 2003).

Given these effects, *C. bombi* may be an important regulatory factor for populations of *B. terrestris*. There may also be wider ecological effects. Bumble bees live in multi-species assemblages and the parasite occurs in and may be transmitted among different bumble bee species (Shykoff & Schmid-Hempel 1991c; Durrer & Schmid Hempel 1995). If *C. bombi* has divergent impacts on different host species, or if levels of parasitism vary across species, then the parasite may play an important role in structuring the diversity and abundance of bumble bee assemblages (Schmid-Hempel 2001).

Many parasites appear to be relatively benign when viewed in natural field populations of hosts (Stien *et al.* 2002) or when experiments are conducted in unstressed laboratory environments (Ferguson & Read 2002). As our study shows, in such cases predictions from theory that conflict with existing empirical findings should be taken as a sign that the relevant selective situation has not yet been identified. Further studies are likely to show that context-dependent parasites, while not as obviously dramatic in their impact as more virulent parasites, nevertheless exert a pervasive and powerful influence over the ecology, life-history and population dynamics of their hosts.

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Table 1. Time-lags from the end of hibernation for four life-history events (column 1). Data shown are mean number of days since hibernation ended (±SD). The first 4 data columns show results for Hibernation treatments nested within Parasite treatments. The final 4 columns show overall results for the Parasite and Hibernation treatments. Significantly different results within treatments (the first four columns correspond to the interaction effect) are shown in bold and indicated with superscripts in the variables column (see text for statistics).

	Control		Infected		Overall Parasite		Overall Hibernation	
	2.5 months	5 months	2.5 months	5 months	Control	Infected	2.5 months	5 months
1st worker^{HxP}	**54.1 (14.21)**	**43.3 (11.26)**	**48.9 (15.17)**	**55.6 (18.08)**	51.6 (14.27)	51.2 (16.35)	52.3 (14.69)	49.3 (15.99)
1st worker-laid eggs^{H}	96.4 (17.51)	75.9 (9.24)	94.0 (20.24)	82.1 (8.84)	90.9 (18.13)	91.0 (18.67)	**95.5 (18.44)**	**78.1 (9.39)**
1st male	85.7 (22.39)	78.7 (20.44)	89.3 (25.48)	82.3 (22.31)	84.1 (22.02)	87.1 (24.49)	86.9 (23.44)	80.4 (21.00)
1st gyne^{H}	107.0 (17.69)	94.5 (15.94)	106.2 (23.41)	94.4 (18.12)	104.1 (17.96)	104.1 (22.72)	**106.7 (19.69)**	**94.5 (16.02)**

Figure legends

Figure 1

Differences in mean relative mass loss (fraction of body mass; see text) during hibernation for “Parasite” and “Hibernation” treatments. The x-axis shows the different hibernation treatments (2.5 = 2.5 months hibernation, 5 = 5 months hibernation, All = combined data). The y-axis shows the relative mass loss of queens during hibernation (see text for details). Data points are mean values ± standard error bars, with Control = ♂ and Infection = ♀. Adjacent numbers show the sample size for each treatment group (Control to the left, Infection to the right). There were significant effects of hibernation duration (2-way ANOVA, $F_{1,194} = 64.9$, $P < 0.001$) and the interaction between hibernation duration and infection status ($F_{1,194} = 5.3$, $P = 0.022$) on mass loss.

Figure 2

Differences in a) worker production, b) male production, c) gyne production, and d) fitness for queens (including all queens that survived hibernation) in experiment two. Figure design is as in Figure 1. Data are mean values ± standard error bars, with Control = ♂ and Infection = ♀. Adjacent numbers show the sample size for each treatment group (Control to the left, Infection to the right). Note the general negative effect of infection on queen productivity. There were significant overall effects of the parasite treatment (MANOVA, Wilk’s λ $F_{4,191} = 2.697$, $P = 0.032$), as well as individual effects on worker production (univariate $F_{1,194} = 4.209$, $P = 0.042$), male production (univariate $F_{1,194} = 6.532$, $P = 0.011$) and fitness (univariate $F_{1,194} = 8.686$, $P = 0.004$).

Figure 3

Differences in a) worker production, b) male production, c) gyne production, and d) fitness for queens (including only those queens that started a colony) in experiment two. Figure design is as in Figure 1. Data are mean values ± standard error bars, with Control = ᔓ and Infection = υ. Adjacent numbers show the sample size for each treatment group (Control to the left, Infection to the right). Note how the general negative effect of infection on queen productivity is seen mainly in the most stressful, 5 month hibernation treatment. There were no significant overall effects of parasite treatment, hibernation treatment or their interaction. However, there were significant effects of both treatments and their interaction on worker production (Parasite: univariate $F_{1,117} = 7.151$, $P = 0.009$; Hibernation: univariate $F_{1,117} = 5.935$, $P = 0.016$; P×H: univariate $F_{1,117} = 9.363$, $P = 0.003$), and of the parasite treatment and the interaction on male production and fitness (Male production - Parasite: univariate $F_{1,117} = 7.565$, $P = 0.007$; P×H: univariate $F_{1,117} = 5.099$, $P = 0.026$. Fitness - Parasite: univariate $F_{1,117} = 6.439$, $P = 0.012$; P×H: univariate $F_{1,117} = 6.156$, $P = 0.015$)

Figure 1

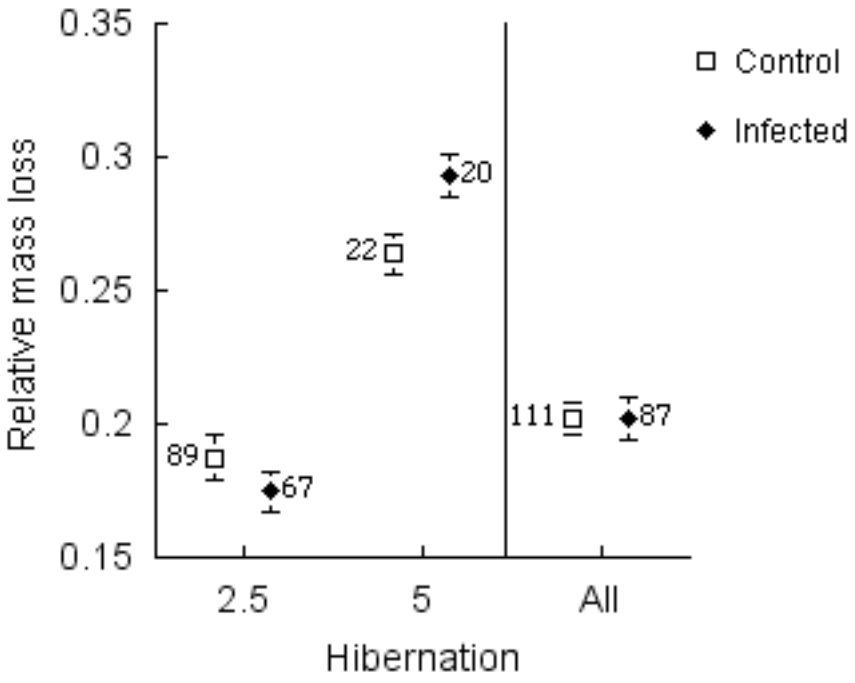

0.35
0.3
0.25
0.2
0.15
Relative mass loss
2.5
5
All
Hibernation
Control
Infected
89
67
22
20
111
87

Figure 2

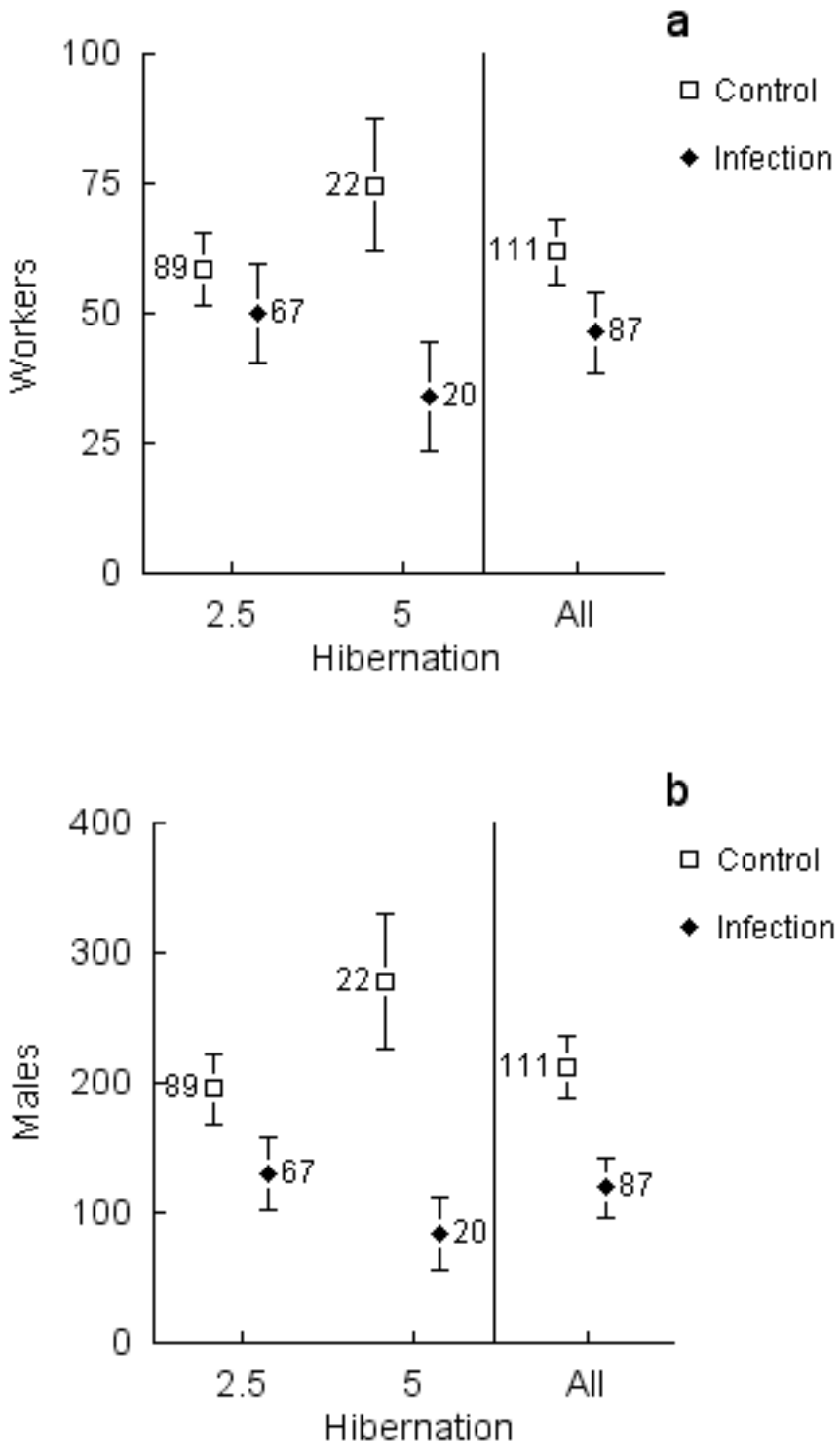
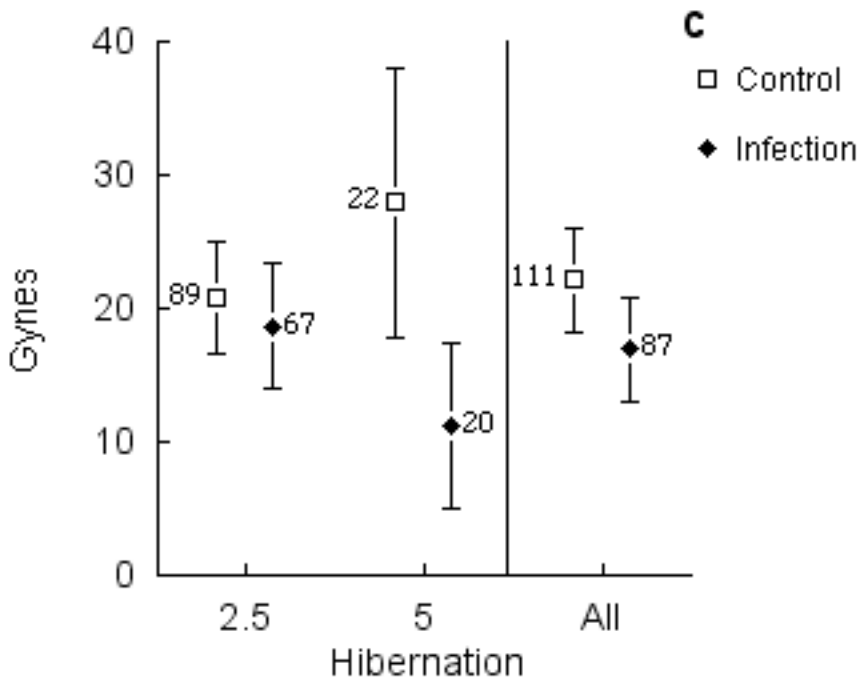

a
Control
Infection
100
75
50
25
0
Workers
89
67
22
20
111
87
2.5
5
All
Hibernation
b
Control
Infection
400
300
200
100
0
Males
89
67
22
20
111
87
2.5
5
All
Hibernation

Figure 2 / cont.



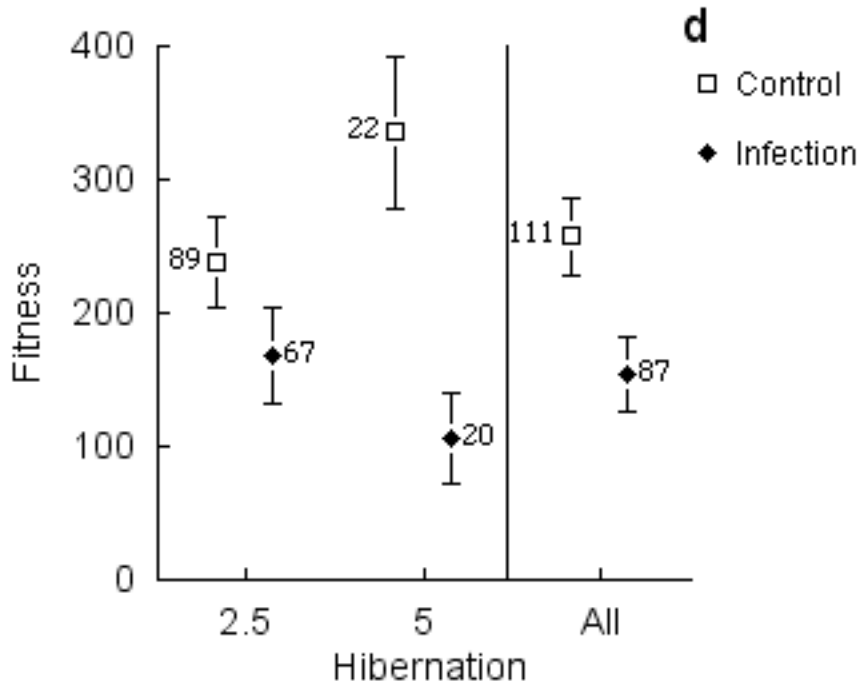


Figure 3

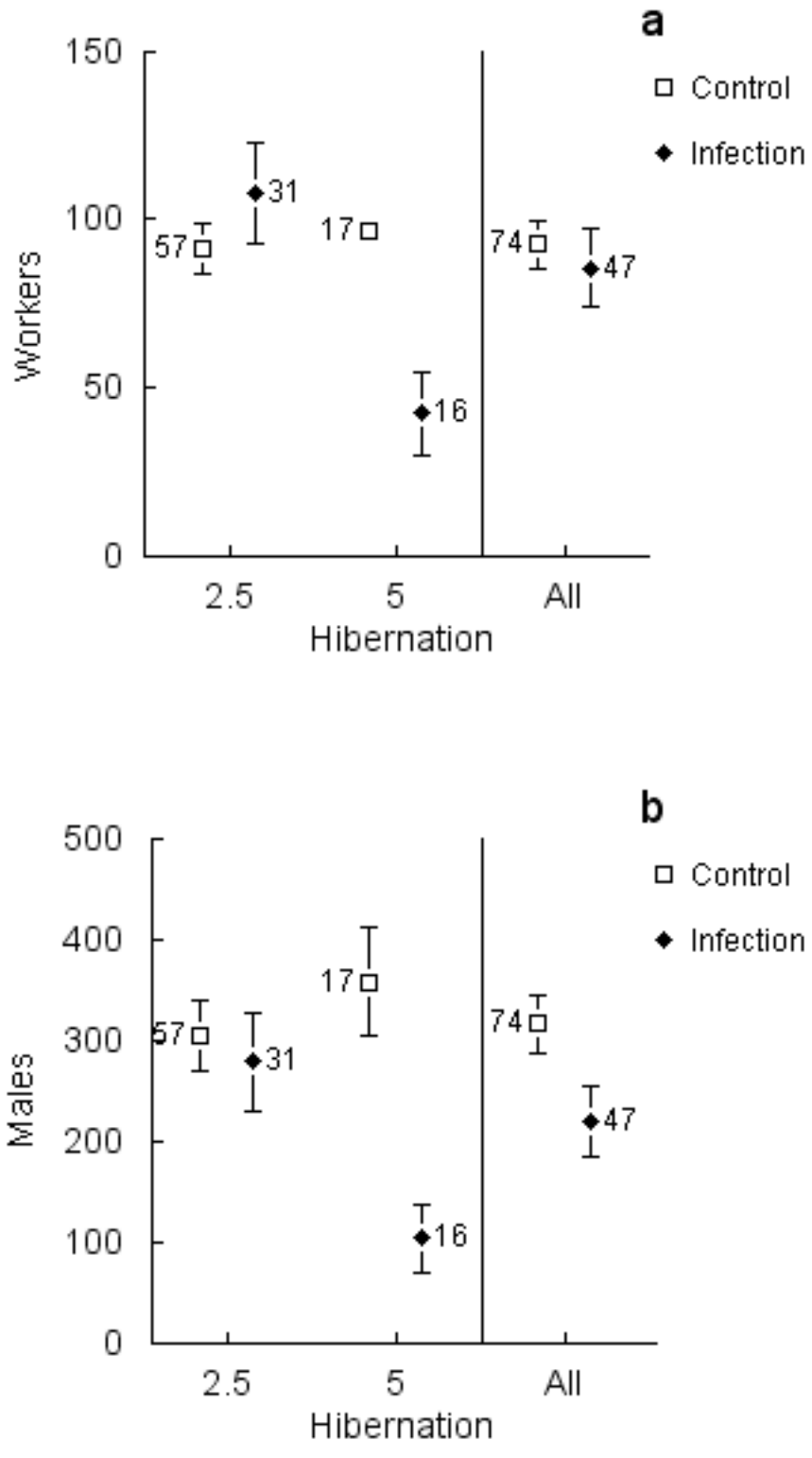

a
Control
Infection
Workers
150
100
50
0
57
31
17
16
74
47
2.5
5
All
Hibernation
b
Control
Infection
Males
500
400
300
200
100
0
57
31
17
16
74
47
2.5
5
All
Hibernation

Figure 3/cont.