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House flies delay fungal infection by fevering: at a cost

ROBERT D. ANDERSON, SIMON BLANFORD and MATTHEW B. THOMAS Department of Entomology and Center for Infectious Disease Dynamics, Penn State University, University Park, Pennsylvania, U.S.A.

Abstract. 1. Many ectothermic species have evolved the ability to invoke a 'behavioural fever' when infected with a pathogen. The relative costs and benefits of this response, however, have rarely been quantified.

2. The aim of this study was investigate the nature and consequences of behavioural fever in the house fly, *Musca domestica* L., in response to infection with a possible biocontrol agent, the fungal entomopathogen, *Beauveria bassiana* (Balsamo) Vuillemin.

3. It was found that infected flies preferred higher temperatures and allocated more effort to thermoregulation than uninfected flies. Flies could not overcome infection but the altered thermal behaviour allowed infected flies to extend their survival and to lay more eggs relative to infected flies maintained under constant conditions. However, flies allowed to fever had lower egg viability suggesting a possible cost.

4. Under the present experimental conditions, the putative costs and benefits fever balanced one another resulting in no net change in fitness. Fever did not, therefore, limit the control potential of the fungus. We discuss whether the costs and benefits of behavioural fever might differ in other ecological contexts.

Key words. *Beauveria bassiana*, behavioural fever, costs and benefits, fungal entomopathogens, immune response, *Musca domestica,* thermoregulation.

Introduction

Fever is a highly conserved response to infection that can be observed in many animals (Kluger *et al*., 1996; Kozak *et al*., 2000). For ectotherms, fever is generally manifested via changes in thermoregulatory behaviour and exploitation of warmer microhabitats in order to increase the body temperature above the normal thermal optimum (Bronstein & Conner, 1984; Watson *et al*., 1993; Inglis *et al*., 1996; Adamo, 1998; Blanford *et al*., 1998; Karban, 1998; Blanford & Thomas, 2001; Elliot *et al*., 2002; Campbell *et al*., 2010).

Several studies in insects have shown that temporary increases in body temperature via behavioural fever bestow fitness benefits by reducing pathogen virulence and increasing infected host survival time (Watson *et al*., 1993; Elliot *et al*., 2005). Increases in host body temperature are thought to negatively impact the infection process in at least two ways. First, the elevated fever temperature might be sub-optimal/lethal for the infecting pathogen, thereby decreasing growth and replication rates and any associated production of toxic metabolites. Several experiments with pathogenic bacteria and fungi have shown that *in vitro* growth and replication rates are reduced when cultured at temperatures comparable to those of their fevering hosts (Langeveld & Cuperus, 1980; Lenski & Bennett, 1993; Inglis *et al*., 1996; Fernandes *et al*., 2008). Second, fever may simultaneously enhance the efficiency of the host immune system, further limiting virulent effects. For example, Ouedraogo *et al.* (2003) found that haemocytes of fevering locusts were more numerous and better able to phagocytose fungal blastospores than those from non-fevering locusts. Exposure to high temperatures may also enhance humoural immune function (Wojda & Jakubowicz, 2007) and increase the rates of apoptosis, a primary defence against viruses (Granja *et al*., 2003).

In contrast to these beneficial effects, fevering can impose costs on the host. As in endothermic animals (Banet, 1986; Kluger *et al*., 1996), maintaining a body temperature above the normal thermal optimum has been shown to be metabolically expensive in ectotherms, and is associated with both increased oxygen and food consumption (Lactin & Johnson, 1995; Sherman & Stephens, 1998). These energetic costs can translate into a reduced capacity to perform basic behaviours,

Correspondence: Robert D. Anderson, Department of Entomology and Center for Infectious Disease Dynamics, Penn State University, W113-A Millennium Science Complex, University Park, PA 16802, U.S.A. E-mail: rda138@psu.edu

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including flight and mate competition and reduced growth rates (Elliot *et al*., 2005). In addition, as many terrestrial ectotherms thermoregulate by basking, fevering might increase the risk of predation (Pianka & Pianka, 1970; Knapp & Casey, 1986; Carruthers *et al*., 1992) while altering the time budgeted for other essential activities such as feeding (Adolph & Porter, 1993).

Here we present the results of a study investigating behavioural fever in house flies (*Musca domestica* L.) in response to infection with the fungal entomopathogen *Beauveria bassiana* (Balsamo) Vuillemin. One motivation for the study was to determine whether behavioural fever could impact the potential of *B. bassiana* for use in the biological control of houseflies [there is a substantial problem with houseflies in intensive animal units such as chicken houses and there is growing interest in the potential for utilizing novel fungal biopesticides in integrated fly management strategies (Barson *et al*., 1994; Watson *et al*., 1995; Kaufman *et al*., 2005; Lecuona *et al*., 2005; Malik *et al*., 2007)]. More broadly, we use the system as a model to examine costs and benefits of fever.

The study begins with an exploration of the thermal behaviour of infected and uninfected flies using simple linear gradients to determine the effect of infection on thermal set point selection. We then extend the approach to allow free-ranging thermal behaviour throughout the course of infection, enabling us to examine the impacts of fever on a range of life history traits. By quantifying fitness correlates such as fecundity and survival, we demonstrate both costs and benefits of fever, with the net effects probably dependent on the ecological context in which they are played out.

Materials and methods

Insects

Musca domestica were reared in an environmentally controlled chamber kept at 27 °C with a LD 12:12 h photoperiod. Eggs were collected by placing a small plastic cup containing larval diet (wheat bran, baker's yeast, Manno-Pro™ calf feed supplement and water) directly into stock cages for ~1 h. Roughly, 300 mg of eggs was collected and seeded into 1.5 l of the larval diet. Larvae were monitored daily until pupation, at which time pupae were collected and placed into screened stock cages (60 cm × 30 cm × 30 cm) to eclose. Adult flies were provided constant access to food, consisting of a 1 : 1 mixture of dried milk powder and granulated sugar, and water, which was replaced every 3 days.

Fungal conidia

The preparation of a fungal conidial suspension was carried out according to the protocol presented in Anderson (2011). Briefly, dry conidia of *B. bassiana* (strain GHA) were formulated in a mixture of mineral oils (20% Ondina: 80% ShelSol) and agitated for 30 s using a vortex mixer. The conidial concentration was determined using an improved Neubauer haemocytometer at 400×, and the volume of the suspension adjusted to yield 1×10^9 conidia per ml. This concentration was chosen to represent a relatively high dose likely to be used in a residual biopesticide product (e.g. see Blanford *et al*., 2011).

The formulation was sprayed onto 9-cm circles of HP ColorLaser™ paper (Hewlett-Packard Co., Palo Alto, CA) inside a 0.25 m^2 area on the back wall of a chemical fume hood at a rate of 20 ml m^{-2} using a hand-held airbrush. Care was taken during the application process to insure an even coverage of conidia on each paper substrate. The treated paper circles were fitted into the lids of 90-mm Petri dishes and left to dry overnight. Subsequent germination tests showed > 90% conidial viability.

Infecting flies with B. bassiana

Two days after the onset of eclosion, flies were removed from the stock cage using a battery-powered aspirator, briefly anaesthetized using CO_2, and placed approximately 10 cm below a block of subliming dry ice which enabled manipulation of individual flies into treatment dishes. Flies ($n = 50$) were infected by enclosing them in a Petri dish containing treated paper substrates for 4 h. Previous studies (Anderson, 2011) have shown that this exposure method results in individual flies picking up roughly 3.5–4.5 × 10^4 conidia. For each experiment, an equivalent number of flies were exposed to unsprayed filter paper to serve as controls [exposure to formulating oil has been shown to have no effect on fly mortality (Anderson, 2011), nor to lead to any change in thermal behaviour in other diptera (Blanford *et al*., 2009)].

Experiment 1: thermal preference on linear gradients

After exposure, we investigated the thermal set point selection of flies using linear thermal gradients. The thermal gradient surface comprised an aluminum sheet (60 cm long × 20 cm wide × 0.5 mm thick) with the last 10 cm of each end bent downwards at a 90° angle. One end was placed into a hot water bath (70 °C) whereas the other end was placed into an ice bath (2 °C). A 40 × 20 × 5 cm piece of polystyrene foam was placed under the aluminum sheet, between the bends, to promote a stable gradient (Fig. 1a). A plexiglass runway (15.5 cm × 33 cm × 1 cm) was constructed with 15 separate 1-cm wide channels to contain individual house flies. A 1-cm gap was left in the centre of the runway lid in order to add and remove flies from each channel. Holes (1.5 mm diameter) were drilled into the lid at 2-cm intervals along each channel to allow the escape of warm air generated by the gradient surface (Fig. 1b). A strip of tape was adhered to the long edges of each channel to prevent flies from receiving visual cues from adjacent flies. Once stable (~30 min), the temperature of the entire gradient surface was 'mapped' using a fine-wire fast-response digital thermocouple (Model HH11; Omega Engineering Inc., Stamford, CT). The relationship between temperature and position on any point on the gradient was described by the polynomial regression:

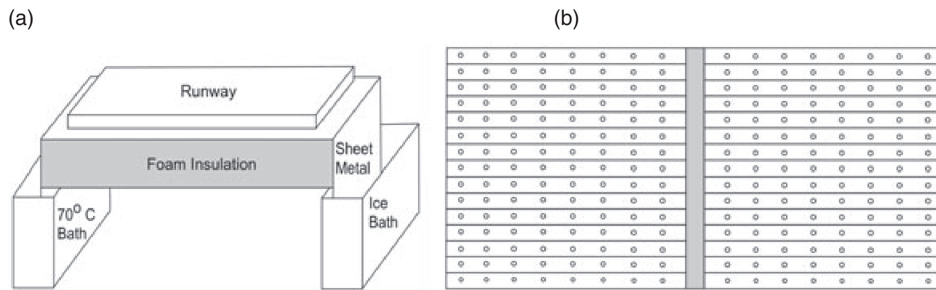

Fig. 1. Side view (a) and top view of runway (b) of the linear gradient.

$$\text{Temp} = 0.0106(x^2) - 0.6723(x) + 32.66;\ R^2 = 0.996, \text{ where 'x' is the distance from the hot edge of the gradient.} \quad (1)$$

Linear gradient assays were done on the lab benchtop at room temperature (~25 °C). Infected and uninfected flies were anaesthetized with cold and placed alternately into the runway channels. Flies were restricted to the inner 13 channels to ensure all were laterally insulated by at least 1 channel, which allowed six or seven flies from each treatment to be assayed within a single run. Flies were left to recover for 15 min, after which time their position on the gradient was recorded at 10-min intervals for 2 h using a digital camera (model A620; Canon Powershot™, Canon U.S.A., Melville, NY). The temperature selected by each fly at each time point was estimated using the polynomial regression given above. One 2-h run was completed daily for 5 days, with the gradient disinfected with 70% ethanol after each run.

Experiment 2: free-ranging thermal behaviour in gradient boxes

Linear thermal gradients provide snap-shot measures of thermal preference. In order to measure the effect of free-ranging thermoregulatory behaviour over the entire course of infection, we employed thermal gradient boxes (TGB) consisting of a wood-framed box (30 × 30 × 15 cm) enclosed on four sides with fibreglass screening. The front was sealed with a plexiglass sheet, whereas the back was sealed with a piece of sheet aluminum (0.05 cm thick) to provide the gradient surface. A metal can (12.5 cm height × 7.5 cm diameter) was adhered to the centre of the outside surface of the aluminum sheet with clear silicone sealant. A 60-W incandescent light bulb was placed into the can and packed with aluminum foil to prevent the bulb from shifting. A continuous dimmer switch was spliced into the power supply for each TGB, which allowed fine-tuning of heat output. The heat output was then adjusted to provide a temperature profile as depicted in Fig. 2. All boxes were housed in a Percival growth chamber maintained at a constant 26 °C and LD 12:12 h photoperiod.

To characterize the heat distribution of the gradient surface, the temperature at the centre point of the gradient was allowed to stabilize to 50 °C and the temperature measured in 1-cm increments to the edge of the gradient at 0, 45, 90, 135, 180, 225, 270, and 315° from the vertical. From these measurements, we derived a polynomial regression to predict temperature from any point on the gradient, as given by:

$$\text{Temperature} = -0.002x^4 + 0.0746x^3 - 0.709x^2 + 0.238 + 48.56,\ R^2 = 0.98 \quad (2)$$

During the course of the experiment, the temperature of each box was checked daily to insure that each gradient provided an equivalent pattern of heat.

To characterize the influence of infection on thermoregulatory behaviour, 125 house flies of a mixed sex were assigned individual colour codes using non-toxic enamel paint, exposed to conidia as described above and released into a TGB, while equivalent uninfected controls were housed in an identical TGB. Both groups were provided free access to food (1 : 1 mix of powdered milk and granulated sugar) and water over the course of the experiment. Starting the day after infection, TGBs were heated for 6 h per day, starting 2 h after the onset and ending 4 h prior to the end of the daily photophase. Every 10 min during this period, the position of individual flies on the gradients was recorded using the digital camera. These recordings were taken for 6 days. Images were then imported into ImageJ (version 1.43), where the position of each fly and the number of flies on the gradient were recorded.

Experiment 3: effects of thermoregulation on survival and reproduction

To complement the detailed observational study on thermal behaviour in the gradient boxes, we also examined the effects of thermoregulation on housefly survival and reproduction. Twelve replicates of 100 randomly selected house flies (sex ratio 1 : 1) were evenly split and exposed to either treated or untreated paper substrates for 4 h ($n = 50$ insects per dish, separated by sex). After exposure, flies were released directly into TGBs, half of which were supplied with heat as described for Experiment 2 above, whereas half were maintained at an ambient 26 °C. Treated and untreated flies (sex ratio 1 : 1) were split among heated and unheated gradient boxes resulting in a complete block design of three replicates per treatment.

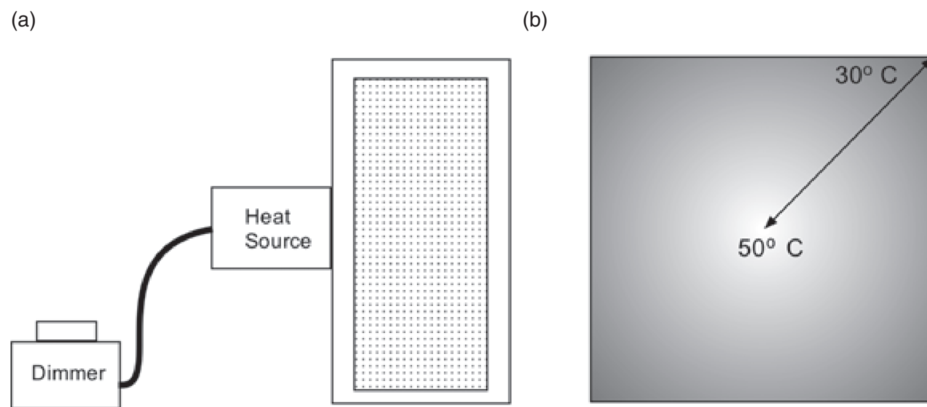

Fig. 2. Schematic of thermal gradient boxes showing a side view of the gradient box (a) and the temperature profile of the thermal gradient surface (b).

Egg collection

The day after exposure, oviposition cups were placed in each TGB to collect eggs. Oviposition cups were constructed by packing a coiled length of saturated paper toweling (~1.5 cm fold width) loosely on top of ~15 ml of larval diet in a plastic cup. One millilitre of sour milk was also applied to the paper towel to encourage oviposition.

Oviposition cups were placed into each TGB 2 h before the end of the daily photophase and removed 2 h after the onset of photophase the following day, providing ample time for oviposition. This method was found to be ideal for collecting eggs; female flies were able to insert their ovipositors between the coils of paper towel to lay their eggs, which were kept moist and viable. Eggs were easily recovered from the cups by uncoiling the towel and rinsing with room temperature tap water.

Egg counting and viability

Eggs from each collection were rinsed directly into plastic cups, which were sealed with a lid and gently shaken to break up clumps of eggs. The eggs were then poured into a Petri dish, which was placed under a dissection microscope. One hundred individual eggs were removed using a glass pipette and placed into a Petri dish containing a saturated, 90 mm circle of black filter paper (GE Healthcare Life Sciences, Piscataway, NJ). Dishes containing eggs were sealed with parafilm and maintained at 26 °C. After 48 h, empty chorions were counted and recorded. Any egg that had not hatched by this time was considered unviable.

The remainder of eggs was counted volumetrically using a modified 0.05-ml insulin syringe. The tip of the syringe was removed and covered by several layers of nylon mesh. The entire sample of eggs was drawn up into a disposable pipette, transferred into the syringe barrel, and the settled egg volume measured using the syringe graduations.

The total number of eggs was estimated using linear regression. Three replicates of 200, 400, 600, and 800 eggs were counted directly and measured using the volumetric method. The egg number to volume relationship was described by:

$$\text{Total eggs} = (146.04 \times \text{volume}) - 83.24,\ R^2 = 0.93 \quad (3)$$

Eggs that measured less than one unit (10 μl) in volume were counted individually at 30× magnification.

Fly survival

At the onset of the photoperiod each day, dead flies were removed from each gradient box, sexed, and recorded. The number of living females in each gradient box was used to estimate the daily egg production per female in each replicate.

Sporulation of cadavers

Dead flies were placed into separate cups each day according to treatment. Flies were allowed to dry for 3 days to prevent cadavers from putrefying and interfering with sporulation. A filter paper disc was saturated with water, placed with the cadavers, and the cup was sealed with a lid. After 5 days, the cadavers were examined for mycosis using a dissection microscope at 15× magnification.

Statistical analyses

House fly survival data were analyzed using Kaplan–Meier survival analysis (SPSS, software version 19) with differences in median survival time between treatments compared using the Log-rank test.

As different flies were used to test the thermal preference of flies on subsequent days post-infection, Student's *t*-tests were used to analyse preferred daily temperatures of flies on the linear gradients. A linear mixed model was used to compare the temperature preferences of marked flies in TGBs, with individual flies included as the replicate and treatment as the fixed

effect. Results of this analysis were then compared by day to identify daily differences in thermoregulatory behaviour.

The proportion of sporulating flies was analysed using a G-test goodness of fit that compared sporulation rates between infected flies housed in heated and unheated boxes. Female fecundity was measured as the total number of eggs and the total number of viable eggs produced per replicate, divided by the initial number of females present in each replicate at the start of the experiment ($n = 50$). The number of viable eggs was estimated by multiplying the daily egg production by the hatch rate of eggs collected on that day. Both total and viable eggs were log transformed (log eggs + 1), to satisfy assumptions of normality and homoscedasticity, and analysed using a general linear model (GLM) incorporating infection status and heat treatment as fixed factors. Significant interactions for all dependent variables were broken down by analysing the effect of fungal infection for each level of thermoregulation. All statistical analyses were performed in SPSS Statistics (version 19) and significance for all statistical tests was set at $P < 0.05$.

Results

Experiment 1: thermal preference on linear gradients

The daily mean preferred temperature of house flies on the linear gradient is shown in Fig. 3. Infected flies tended to select higher temperatures than control flies, with the difference significant at days 4 and 5. The overall mean temperature on these days was 40.9 ± 0.1 °C for infected flies and 38.7 ± 0.7 °C for controls. These results indicate a shift in the preferred thermal set point of infected flies, consistent with a behavioural fever response.

Experiment 2: free-ranging thermal behaviour in gradient boxes

The day after exposure, there was no difference in temperatures selected on the gradient by treated and control

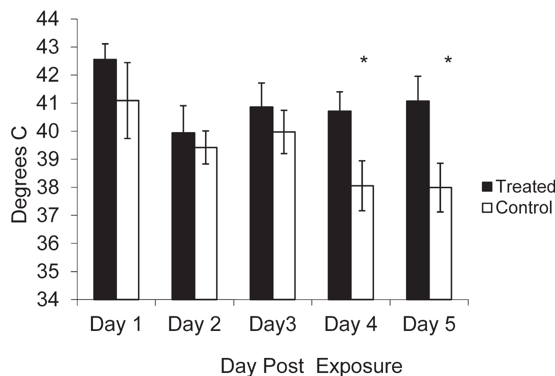

Fig. 3. Average temperature preferred by infected and uninfected house flies over a 2-h period during the course of *Beauveria bassiana* infection. Asterisks represent significant difference between treatment groups ($P < 0.05$). Bars represent ± 1 SEM.

Table 1. Median survival time (days ± 1 SEM) for male and female flies according to treatment.

Treatment	Males MST ± 1SEM	Females MST ± 1SEM
Fungus/heated	6.0 ± 0.2b	7.0 ± 0.3c
Fungus/unheated	5.0 ± 0.1a	5.0 ± 0.1a
Control/heated	14.0 ± 0.3d	*e
Control/unheated	15.0 ± 0.0d	*e

*The population did not drop below 50% survival over the 14-day period in which the study was conducted.
Different letters indicate significant differences in survival according to Log-rank test ($P < 0.05$).

flies, although a smaller proportion of treated flies were found basking on the gradient surface (Fig. 4). From day 2 onwards, a consistent pattern emerged with a greater proportion of infected flies basking on the heated gradient surface and selecting hotter temperatures when on the gradient (linear mixed model: $F_{1,583.2} = 6.91$, $P = 0.009$) than control flies (Fig. 4). These results are again consistent with a behavioural fever response as a result of infection, with not only a shift in the preferred set point temperature but also a greater investment in active thermoregulatory behaviour.

Experiment 3: effects of thermoregulation on survival and reproduction

Fly survival. Fungal infection caused significant reductions in survival (Table 1; Fig. 5). The ability to actively thermoregulate and generate fevers reduced the rate of mortality, with the survival benefit most marked in female flies. However, active thermoregulation and fever did not prevent fungal-induced mortality overall. There were no apparent effects of thermoregulation on control survival.

Cadavers from flies exposed to heated gradients sporulated at similar rates to flies maintained under ambient conditions (85.7 vs. 89.7%; G = 2.23, d.f. = 1, $P = 0.135$), indicating no ultimate fitness costs to the fungus as a result of host thermoregulation.

Female fecundity. Fungal infection caused a significant reduction in both the total number of eggs ($F_{1,8} = 48.44$, $P < 0.001$) and the number of viable of eggs ($F_{1,8} = 48.88$, $P < 0.001$) produced per female over the course of the experiment (Fig. 6). There was no main effect of thermoregulation on fecundity overall, nor any effect on fecundity of just the uninfected flies ($F_{1,8} = 1.550$, $P = 0.248$). However, there was a significant interaction between fungal infection and thermoregulation with respect to total egg production ($F_{1,8} = 11.25$, $P < 0.01$). Simple effects analysis revealed that thermoregulating, infected flies produced roughly twice as many eggs as infected flies maintained under restricted ambient conditions ($F_{1,8} = 12.239$, i = 0.008; Fig 6a).

However, infected flies that were allowed to fever showed a marked reduction in the proportion of viable eggs ($F_{1,8} = 7.03$,

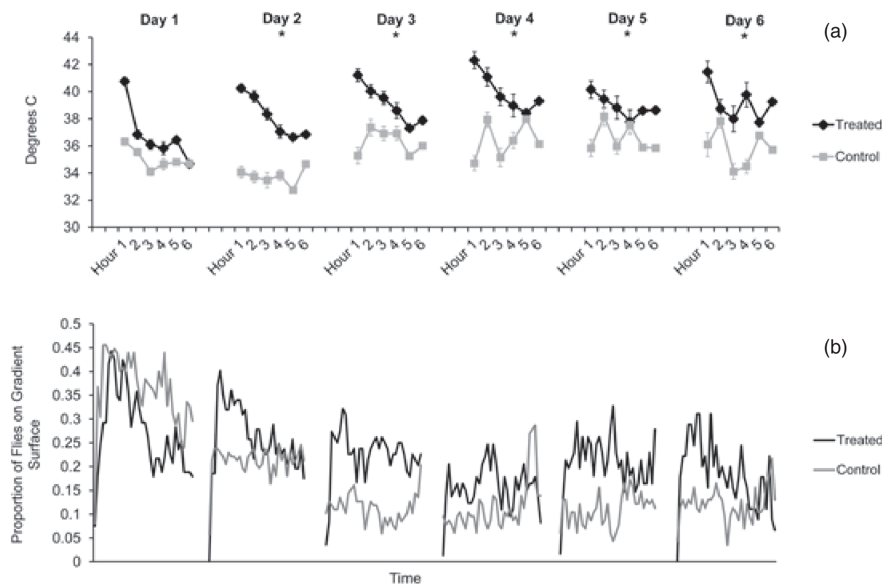

Fig. 4. (a) Preferred gradient temperatures of infected and control flies over the 6-h daily heated period. Each data point represents the mean gradient temperature preferred by flies resting on the gradient, averaged over the course of 1 h. Bars represent ± 1 SEM. (b) The proportion of flies on the gradient surface at each time point over the 6-h daily heated period. Each inflection represents the mean number of flies on the gradient surface at each 10-min interval over the 6-h daily heating period. Asterisks represent significant differences at $P < 0.05$.

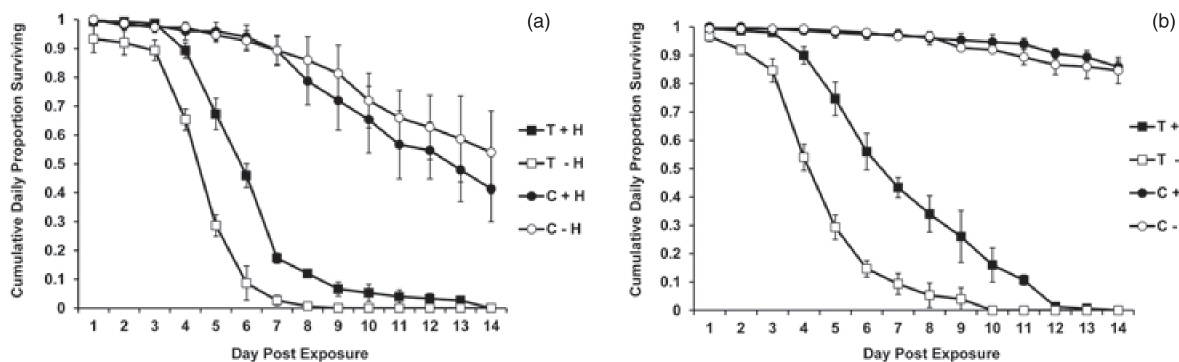

Fig. 5. Cumulative mean proportional survival of female (a) and male house flies (b) in each treatment. T + H, Infected, heated gradient; T-H, Infected, unheated gradient; C + H, Uninfected, heated gradient; C-H, Uninfected unheated gradient. Bars represent ± 1 SEM.

$P = 0.012$, Fig. 6b). This resulted in no significant interaction between heat treatment and infection with respect to the number of viable eggs produced per female ($F_{1,8} = 3.67$, $P = 0.092$, Fig. 6c) and hence, roughly equivalent numbers of viable eggs being produced by infected flies irrespective of thermal treatment.

Discussion

Our initial investigation using linear thermal gradients revealed that house flies invoke a behavioural fever when infected with *B. bassiana*, selecting temperatures around 3 °C higher than control insects 4 days after infection. Behavioural fever was confirmed in experiments using the gradient boxes, with significant differences in thermal site selection observed from day 2 after infection. Why there was some discrepancy in the onset of fever between these two methods is unclear but as it takes only 24–48 h for *B. bassiana* to germinate, penetrate the cuticle and invade the host hemolymph, it is possible that the approach of taking simple 'snapshots' of thermal site selection using linear gradients masked certain aspects of a more natural thermoregulatory behaviour possible in the gradient boxes. It is also the case that the differences in preferred temperatures in the linear gradient study appeared to be driven by a decline in temperatures selected by control flies rather more than an increase in temperature in the infected flies. It is possible that flies naturally shift their preferred temperatures as they age but as fever is quantified relative to normal preferred set points, the differences relative to the age-matched controls still qualify as fevers. Why equivalent patterns were not detected in the gradient boxes is unclear but might again reflect some limitations in the 'snapshot' approach inherent to the linear gradients.

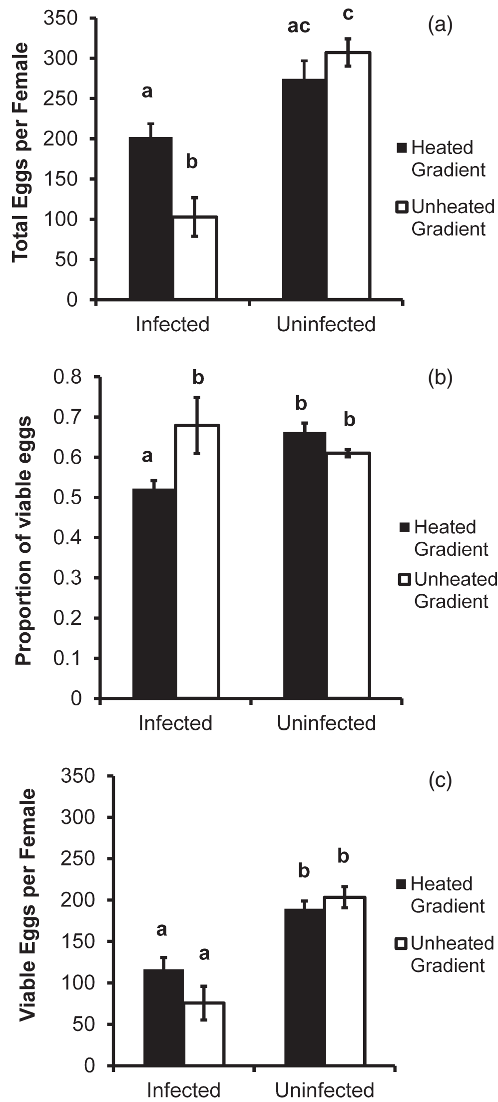

Fig. 6. Overall female fecundity in terms of (a) total, (b) proportion of hatched eggs, and (c) estimated viable eggs produced per replicate thermal gradient box from infected and uninfected females in each heat treatment. Different letters indicate significant differences at $P < 0.05$. Bars represent $\pm$ 1 SEM.

In the thermal gradient boxes, infected flies exhibited a relatively consistent daily pattern in their thermoregulatory behaviour, preferring the highest temperatures (40–42 °C) during the first hour of each daily heating period and gradually moving to cooler areas of the gradient (35–38 °C) near the end of the heating period. In comparison, while uninfected flies clearly exhibited thermoregulatory behaviour, they preferred cooler areas of the gradient (33–38 °C) during the heating period and frequented the gradient surface less often (Fig. 5).

We speculate that the general pattern of fever seen in infected flies might be explained by the influence of fever temperatures on fungal growth and/or associated metabolite production. Upon detection of the fungus by the immune system, the fly invokes behavioural fever, thereby creating a hostile thermal environment for the growing fungus and possibly enhancing immune function. As the thermal stress slows fungal activity and fever elicitors are reduced, flies return to more normal thermal behaviour. Overnight, when the fly is not able to thermoregulate, the fungus recovers and fever is exhibited once more at the next opportunity to thermoregulate. This possible explanation is consistent with the known thermal sensitivities of this fungal isolate and that fungal growth both *in vitro* and *in vivo* can respond to daily temperature fluctuations (Inglis *et al.*, 1999; Fargues & Luz, 2000; Elliot *et al.*, 2002; Devi *et al.*, 2005; Klass *et al.*, 2007a).

Behavioural fever increased the survival time of infected flies significantly, although was not sufficient to prevent ultimate fungal-induced mortality. Although sex-specific behaviours were not recorded in this experiment, infected females were able to extend their median survival time a full day longer than males by fevering. This is possibly a consequence of males spending less time basking than females, as males were observed to spend much of their time attempting to copulate. Additionally, female flies may have been better able to resist infection; it has been shown in several insect species that investment in immune function can be sexually dimorphic (Kurtz *et al.*, 2000; Nunn *et al.*, 2009; Shi & Sun, 2010; Steiger *et al.*, 2011).

Sporulation rates of infected flies were high and were unaffected by the thermal regime. These results contrast those of certain other studies (Inglis *et al.*, 1996; Elliot *et al.*, 2002) and suggest that at the relatively high conidial concentrations used in the current study, the fungus can overcome host defences and fully colonize the cadaver irrespective of fever.

Fungal infection caused a significant reduction in overall reproductive output. However, the extended survival afforded by fever enabled infected flies to lay eggs 4 days longer than equivalent flies denied the opportunity to fever, resulting in roughly twice as many eggs laid per female over their lifetime. While this at first appears to be a fitness benefit, the proportion of viable eggs produced by fevering females was significantly lower than in any other treatment, resulting in roughly equivalent numbers of viable eggs produced between each group of infected flies.

Exposing developing embryos to high temperatures, even briefly, can have teratogenic effects resulting in morphological defects and reduced natality in both vertebrates and invertebrates (Arora *et al.*, 1979; Warkany, 1986; Eberlein & Mitchell, 1987). For example, in a study investigating the effects of heat shock on developing *Drosophila* embryos it was found that exposing recently deposited eggs to 42 °C for only 15 min caused a significant reduction in eclosion rates (Eberlein, 1986). In the current study, infected female house flies were found to prefer similarly high temperatures for the first hour of each daily heating period (40–42 °C), possibly leading to developmental defects during oogenesis or while the eggs were residing in the ovaries, waiting to be deposited. If true, such an effect would be a novel direct cost of fever.

Alternatively, fungal metabolites might have negatively affected egg viability as mycosis progressed, resulting in reduced hatch rates. Fungal toxins from *Metarhizium anisopliae* (Metchnikoff) Sorokin and *B. bassiana* have been shown

to have differential toxic affects on insect tissues including the midgut epithelium and insect haemocytes (Huxham *et al*., 1989; Vey & Quiot, 1989; Hung & Boucias, 1992; Hung *et al*., 1993; Kershaw *et al*., 1999) and might, therefore, have affected oogenic tissues or eggs in gravid infected females. In theory, flies that survived infection longer via thermoregulation could have accumulated higher concentrations of these fungal metabolites over time (although this would require the toxins not to be directly linked to host death). There could also be interactions such that the effects of toxins are exacerbated at higher temperatures. While indirect, such effects would still be negative fitness costs resulting from the fever response.

These results indicate that thermoregulation provided putative fitness benefits to infected flies in the form of extended survival and greater egg production. However, thermoregulation also imposed costs in terms of reduced egg viability; roughly equivalent numbers of viable eggs were produced between groups of infected flies, regardless of their opportunity to fever. Overall, therefore, the costs of fever appeared to offset any benefits resulting in no net fitness gain. That said, our study considered just one set of conditions and it is likely that costs and benefits will be strongly context dependent. For instance, our study was partly motivated by an interest in the development of a fungal biopesticide for the control of houseflies in intensive animal houses. From work in other systems, we know behavioural fever to be important in determining the efficacy of biopesticide products (Klass *et al*., 2007a,b). In our case, it appears that fever provided little net benefit but it is possible that the relatively high, operationally relevant doses (Blanford *et al*., 2011) could have masked potential fitness benefits of the fever in response to lower fungal doses that might be experienced in nature. In addition, house flies use ephemeral, patchy resources, primarily animal excrement, in which to deposit their eggs. Competition for these resources between individual larvae is often severe; it has been shown that intra-specific competition is one of the major causes of larval house fly mortality (Lam *et al*., 2007). Thus, in field conditions the increased survival time as a result of fever might benefit female flies by enabling them to distribute more eggs over time (and in different oviposition sites), although this will undoubtedly be mediated by the quality and dispersion of larval resources, environmental conditions, and inter-specific competition (Heard & Remer, 1997).

Alternatively, it could be that there is indeed little net benefit of fever to this fungal species or strain, and that the fever responses we observed are primarily tailored to other pathogens. In nature, houseflies appear to be infected relatively rarely with *B. bassiana* but much more commonly with another fungal pathogen, *Entomophthora muscae* (Cohn) Fresen. For example, infection prevalence of *E. muscae* up to 60% was observed in house flies collected from animal units (Mullens *et al*., 1987; Steinkraus *et al*., 1993; Six & Mullens, 1996), whereas only 0.86% of flies collected from New York dairy farms were infected with *B. bassiana* at similar times of the year (Steinkraus *et al*., 1990). Flies infected with *E. muscae* have been shown to generate fevers similar to those reported here (Kalsbeek *et al*., 2001). These fevers clearly reduce the fitness of *E. muscae*, potentially clearing the vegetative stages of the infection, reducing sporulation rates, and greatly increasing infected host survival (Watson *et al*., 1993). Both *E. muscae* and *B. bassiana* possess similar cell wall components (ß -1,3 glucans) that are recognized by the insect immune system, resulting in the expression of behavioural and physiological immune defences (Latge & Beauvais, 1987; Gillespie *et al.*, 1997; Bundey *et al.*, 2003; Tartar *et al.*, 2005). However, while the immune elicitors are similar, the critical thermal limit (the point at which heat stress causes damage to developing cells) appears to be higher for both vegetative and reproductive forms of *B. bassiana* than for entomophthoralean fungi in general (e.g. see Carruthers & Haynes, 1986; Firstencel *et al*., 1990; Butt *et al*., 1994). Thus, while both fungi stimulate fever, the effectiveness of the response appears to depend (at least in part) on the relative thermal sensitivities of the infecting pathogen.

Together, these results highlight the importance of considering the specific ecology of the host and the parasite when exploring the costs and benefits of immune responses on host fitness.

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