SHORT COMMUNICATION

Context dependency and generality of fever in insects

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Abstract Fever can reduce mortality in infected animals. Yet, despite its fitness-enhancing qualities, fever often varies among animals. We used several approaches to examine this variation in insects. Texas field crickets (*Gryllus texensis*) exhibited a modest fever (1 °C increase in preferred body temperature, T_{pref}) after injection of prostaglandin, which putatively mediates fever in both vertebrates and invertebrates, but they did not exhibit fever during chronic exposure to heat-killed bacteria. Further, chronic food limitation and mating status did not affect T_{pref} or the expression of behavioural fever, suggesting limited context dependency of fever in *G. texensis*. Our meta-analysis of behavioural fever studies indicated that behavioural fever occurs in many insects, but it is not ubiquitous. Thus, both empirical and meta-analytical results suggest that the fever response in insects 'is widespread, although certainly not inevitable' (Moore 2002). We highlight the need for future work focusing on standardizing an experimental protocol to measure behavioural fever, understanding the specific mechanism(s) underlying fever in insects, and examining whether ecological or physiological costs often outweigh the benefits of fever and can explain the sporadic nature of fever in insects.

Introduction

Fever, the behavioural or physiological ability to maintain an elevated body temperature (T_{body}) in response to pathogens or associated components (e.g. bacterial cell wall), exists in a range of animal taxa—from arthropods to mammals (Kluger 1990). Elevated T_{body} can enhance survival after infection (Kluger 1990; Adamo 1998; but see Müller and Schmidt-Hempel 1993) and, therefore, can boost fitness. Yet, the presence and magnitude of a fever response varies within and between species (Adamo 1998; Moore 2002). We used empirical and meta-analytical approaches to address two hypotheses that may explain this variation in insects, including:

1. Fever is context dependent. Ectothermic animals alter their preferred body temperature (T_{pref}) under different circumstances—e.g. by exhibiting a lower T_{pref} during food limitation (Angilletta 2009) or a higher T_{pref} during reproduction (Hedrick et al. 2002). If the increase in T_{pref} in some insects is a modest 1–3 °C (the same magnitude as fever in mammals; Kluger et al. 1998), fever may be particularly difficult to detect if fever is context dependent.
2. Fever is rare in insects. Though well established in some taxa (e.g. mammals), fever may be the 'exception' rather than the 'rule' in insects.

Materials and methods

For the first study (described below), we used long-winged adult *Gryllus texensis* that were part of a long-term colony, which has been described previously (Adamo and Lovett 2011). Briefly, we supplied crickets with water ad libitum and housed crickets in a room maintained at 26±1 °C and a 12:12 light/dark cycle except during T_{pref} trials. All studies were approved by the Animal Care Committee of Dalhousie University (#I9-026) and are in accordance with the Canadian Council on Animal Care.

To measure T_{pref}, we used a thermal gradient experimental design modified from one described previously (Adamo 1998) (see Electronic Supplementary Material (ESM): Preferred temperature trials). To ensure that *G. texensis* was

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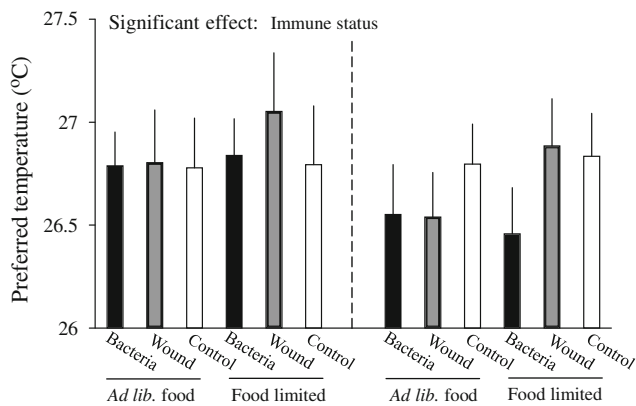e-mail: zrs@dal.ca

Fig. 1 Effects of treatment on the preferred body temperature of female *Gryllus texensis* that experienced varying access to food (ad libitum or intermittent) and were exposed to a bacterial challenge, a wound, or no immune challenge (unhandled control) before and after mating (to the *left* and the *right side* of the *dashed vertical line*, respectively). See text for details about dietary and immune-challenge treatments. Values are displayed as mean±s.e.m

capable of fever using our study system, we examined T_{pref} after the administration of a pharmacological dose of prostaglandin E_2 (PGE_2) as a positive control (see ESM: Effects of prostaglandin E_2 on T_{pref}). Prostaglandins are eicosanoids associated with immune function that rapidly induce fever in vertebrates and invertebrates (Kluger 1990).

Effects of chronic food limitation, immune challenge, and adult stage on T_{pref}

For the first study, we used a repeated 2×3 design to manipulate food availability and immune status before and after mating in a factorial fashion ($n=15–21$ for each of six treatment groups). Over the span of 4 months, we isolated
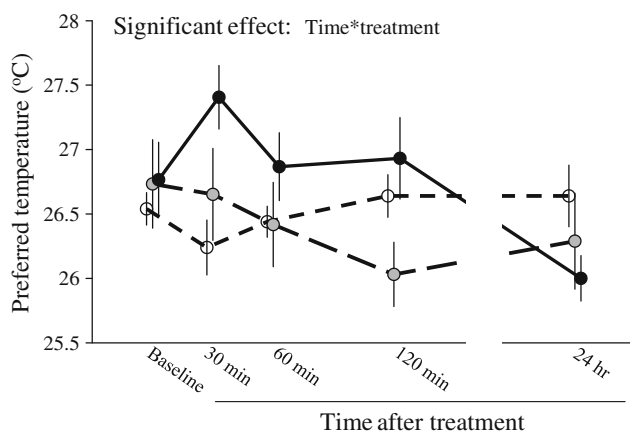

Fig. 2 Preferred body temperature of female *Gryllus texensis* that experienced one of three treatments: 'PGE_2' (*black symbols*: injection of 100 μg of PGE_2 in vehicle, 2 μl of a 1:1 solution of ethanol and water), 'vehicle control' (*grey symbols*: injection of vehicle alone), and 'unhandled control' (*white symbols*: no injection). Values are displayed as mean±s.e.m

22 cohorts of newly moulted (≤1 day post-adult moult) females and housed them individually in transparent 2,000-ml plastic containers. As described previously (Stahlschmidt et al. 2013), we manipulated body condition by manipulating each female's access to food: 'ad lib' (ad libitum food access as above) or 'intermittent' (access to cat food for 3 h every 3 days, which results in *G. texensis* with similar levels of body fat as those found in the field; Adamo et al. 2012). We also manipulated the females' immune status: 'bacterial challenge' treatment (injection with heat-killed bacteria (*Serratia marcescens*) every 3 days), 'wounded' treatment (pierced with sterile dissection pin every 3 days), or 'control' treatment (not handled). Elevated T_{body} confers a survival advantage to *G. texensis* upon exposure to live *S. marcescens* (Adamo and Lovett 2011). For details, see ESM: Immune status manipulation.

On the evening of 11 days post-adult moult, we mixed females with sexually mature males at a ratio of 1 female per 1–2 males to elicit overnight mating, and we excluded from analyses females that did not mate (mating status was determined by the presence of spermatophore-filled spermatheca at 17 days post-adult moult). We performed T_{pref} trials on each female at 8 and 15 days post-adult moult to determine the effect of adult stage on T_{pref} while controlling for individual-level effects.

We performed analyses with SPSS (version 19, IBM Corp.). We determined two-tailed significance at $\alpha<0.05$ and display all values as mean±s.e.m. All data met the assumptions of parametric statistics or were transformed as necessary. We used linear mixed models to determine the main and interactive effects of treatments on T_{pref} in the first study. We included cohort and individual as random effects. We included treatments (e.g. immune status) as fixed effects, and we included femur length (a proxy for body size) and the high temperature of each individual's experimental lane as covariates.

Generality of fever in insects: a meta-analysis approach

For the second study, we performed a literature search on empirical studies of behavioural fever in insects. Due to a lack of statistical reporting in most studies, we used Fisher's method of meta-analysis to determine the generality of behavioural fever in insects for studies that reported a specific P value ($n=31$ host–pyrogen combinations).

Results

Effects of chronic food limitation, immune challenge, and adult stage on T_{pref}

Immune status significantly influenced the mean T_{pref} of female *G. texensis*. Yet, bacterially challenged females did

Table 1 Previous research on fever in insects used in our meta-analysis, including information on insect taxon and associated pyrogen (fever-inducing agent), whether fever was detected, the magnitude of the fever response (change in T_{pref}, preferred body temperature) when it was detected and recorded, the timing after treatment at which T_{pref} was measured, and the corresponding reference

Taxon	Pyrogen type	Fever?	Change in T_{pref} °C	Time of measurement(s)	Reference
Cockroach (*Blatta orientalis*)	Acanthocephalan worm (*Moniliformis moniliformis*)	No		1–9 weeks	Moore and Freehling (2002)
Cockroach (*Supella longipalpa*)	Acanthocephalan worm (*Moniliformis moniliformis*)	No		1–9 weeks	Moore and Freehling (2002)
Cricket (*Acheta domesticus*)	Larvae of parasitoid fly (*Ormia ochracea*)	No		1–7 days	Adamo (1998)
Cricket (*Acheta domesticus*)	Bacterium (*Serratia marcescens*)	No		1–2 days	Adamo (1998)
Cricket (*Acheta domesticus*)	Intracellular prokaryote parasite (*Rickettsiella grylli*)	Yes	6	10–11 days	Adamo (1998)
Cricket (*Acheta domesticus*)	Gregarines (gut protozoans)	No		1–10 days	Adamo (1998)
Cricket (*Gryllus bimaculatus*)	Intracellular prokaryote parasite (*Rickettsiella grylli*)	Yes	6	10 days	Louis et al. (1986)
Cricket (*Gryllus texensis*)	Larvae of parasitoid fly (*Ormia ochracea*)	No		1–7 days	Adamo (1998)
Fly (*Drosophila falleni*)	Nematode (*Howardula aoronymphium*)	No		3 days	Ballabeni et al. (1995)
Fly (*Drosophila neotestacea*)	Nematode (*Howardula aoronymphium*)	No		3 days	Ballabeni et al. (1995)
Fly (*Drosophila putrida*)	Nematode (*Howardula aoronymphium*)	No		3 days	Ballabeni et al. (1995)
Grasshopper (*Chorthippus parallelus*)	Fungus (*Metarhizium flavoviride*)	No		2–7 days	Springate and Thomas (2005)
Grasshopper (*Chorthippus parallelus*)	Fungus (*Beauveria bassiana*)	No		2–7 days	Springate and Thomas (2005)
Grasshopper (*Melanoplus sanguinipes*)	Microsporidian protozoan (*Nosema acridophagus*)	Yes	6	6–9 days	Boorstein and Ewald (1987)
Grasshopper (*Oedaleus senegalensis*)	Fungus (*Metarhizium flavoviride*)	Yes	2–3	3–6 days	Blanford et al. (1998)
Grasshopper (*Zonocerus variegatus*)	Fungus (*Metarhizium anisopliae*)	No		1–6 days	Blanford et al. (2000)
Grasshopper (*Melanoplus sanguinipes*)	Fungus (*Beauveria bassiana*)	Yes		3 days	Inglis et al. (1996)
Honey bee (*Apis mellifera*)	Microsporidian fungus (*Nosema ceranae*)	Yes	2.47	7 days	Campbell et al. (2010)
Honey bee (*Apis mellifera*)	Fungus (*Ascosphaera apis*)	Yes	0.56	0–7 days	Starks et al. (2000)
Larval mealworm (*Tenebrio molitor*)	Lipopolysaccharide (bacterial cell wall component)	Yes	2.3	24–48 h	Catalan et al. (2012)
Locust (*Schistocerca gregaria*)	Fungus (*Metarhizium flavoviride*)	Yes	5	2–4 days	Bundey et al. (2003)
Locust (*Schistocerca gregaria*)	Bacterium (*Serratia marcescens*)	Yes	2–3	1–3 days	Bundey et al. (2003)
Locust (*Schistocerca gregaria*)	Beta-1,3-glucans (microbial cell wall component)	Yes	2	24 h	Bundey et al. (2003)
Locust (*Schistocerca gregaria*)	Lipopolysaccharide (bacterial cell wall component)	Yes	3–4	1–2 days	Bundey et al. (2003)

Table 1 (continued)

Taxon	Pyrogen type	Time of measurement(s)	Change in T_{pref} °C	Fever?	Reference
Locust (*Schistocerca gregaria*)	Fungus (*Metarhizium flavoviride*)	5–6 days		Yes	Elliot et al. (2002)
Locust (*Schistocerca gregaria*)	Destruxin A, toxin produced by fungus (*Metarhizium robertsii*)	1–4 days		No	Hunt and Charnley (2011)
Mosquito (*Anopheles stephensi*)	Fungus (*Beauveria bassiana*)	2–6 days		No	Blanford et al. (2009)
Mosquito (*Anopheles stephensi*)	Fungus (*Metarhizium anisopliae*)	2–6 days		No	Blanford et al. (2009)
Mosquito (*Anopheles stephensi*)	Apicomplexan parasite (*Plasmodium yoelii*)	0–4 days		No	Blanford et al. (2009)
Tenebrionid beetle (*Onymacris plana*)	Lipopolysaccharide (bacterial cell wall component)	1–12 h	1–4	Yes	McClain et al. (1988)
Tent caterpillar (*Malacosoma californicum*)	Nucleopolyhedrovirus	4 days		No	Frid and Myers (2002)

not exhibit higher T_{pref} than unhandled control females (Fig. 1, Table S1). However, PGE$_2$ did induce fever in *G. texensis* because T_{pref} was affected by a time×treatment effect ($F_{6,67}$=11, P<0.001) with T_{pref} peaking 30 min post-injection of PGE$_2$ (Fig. 2).

We did not detect independent effects of time ($F_{3,67}$=2.2, P=0.11) or treatment ($F_{2,24}$=0.12, P=0.89) on T_{pref} due to the administration of PGE$_2$ (Fig. 2).

Generality of fever in insects: a meta-analysis approach

Studies used various pyrogens (e.g. fungi, live bacteria, and bacterial components) to induce fever in 18 species of insects (Table 1). Although it was dose dependent or not found in many studies (Table 1), fever was a general response in insects (X^2=83, P=0.039). When fever was induced (n=14 host–pyrogen combinations), it began 3.6±0.9 days after pyrogen exposure, ended 1.5±0.5 days thereafter, and resulted in a 3.4±0.5 °C increase in T_{body} when it was recorded.

Discussion

Although their immune function and survival to live *S. marcescens* increases with temperature (Adamo and Lovett 2011), *G. texensis* did not exhibit fever in response to heat-killed *S. marcescens* regardless of food availability or mating status (Fig. 1), which does not support our first hypothesis (fever is context dependent in insects). Yet, crickets were capable of a modest fever in response to a pharmacological dose of PGE$_2$ (Fig. 2), suggesting that the 'context' that most influences fever involves the type of immune response activated.

Different pathogens activate different types of immune responses (Gillespie et al. 1997), and not all pathogens elicit behavioural fever. Typically macroparasites do not elicit fever in insects (Adamo 1998; Moore 2002). However, whether or not a pathogen induces behavioural fever is not entirely dependent on the pathogen type (e.g. bacterial or fungal species, see Table 1); therefore, activating behavioural fever likely relies on a more complex mechanism than simply which pathogens induce prostaglandin release. For example, the house cricket (*Acheta domesticus*) exhibits fever in response to an intracellular prokaryote parasite (*Rickettsiella grylli*), which confers a survival advantage to *A. domesticus* during *R. grylli* infection (Adamo 1998). However, *A. domesticus* does not exhibit fever in response to pathogens when fever does not confer a survival advantage, such as during infection by *S. marcescens* (bacterium) and *Ormia ochracea* (parasitoid fly) (Adamo 1998); thus, pyrogen specificity of insect fever may be adaptive. Yet, the mechanism(s) that would allow for this specificity remain a mystery.

Our meta-analysis provided weak support for our second hypothesis (fever is rare in insects). Most studies reported a negative result (absence of fever; Table 1) despite a general publication bias toward those with positive results over those with negative results across disciplines (Dirnagl and Lauritzen 2010). Nevertheless, behavioural fever occurs in many insect species, although significant variation existed with regard to the timing of T_{pref} measurement, the specific pyrogens used, and the presence of fever across studies (Table 1). For example, one orthopteran insect (desert locust, *Schistocerca gregaria*) exhibits a 2–3 °C increase in T_{pref} for 1–2 days after exposure to live *S. marcescens* (Bundey et al. 2003), but another orthopteran (*A. domesticus*) does not prefer warmer temperatures after exposure to the same pathogen (Adamo 1998). Further, the concentration of pyrogens to which animals were exposed varied across studies, and it influenced the fever response in studies that used varying dosages of pyrogen. For example, two tenebrionid beetle species exhibited fever in response to high (but not low) dosages of lipopolysaccharide (Table 1). Thus, the literature indicated that although fever is widespread in insects, its expression is sporadic.

We posit that the sporadicity of insect fever may be driven by cost–benefit trade-offs where fever in some insects entails more costs than benefits. Although the survival benefits of fever are clear in some cases (Adamo 1998; Blanford et al. 1998), there may be appreciable ecological or physiological costs associated with increased T_{pref} (Elliot et al. 2005). Other crickets (*Gryllus campestris*) that spend more time basking outside their burrows tend to be predated more frequently (Otti et al. 2012). Further, fever is estimated to increase energetic expenditure by 75 % due to the additive effects of immune activation (estimated 25 % increase in metabolic rate; Ardia et al. 2012) and a 3.4 °C increase in T_{body}, which is the average magnitude of insect fever (Table 1) and is expected to result in a 37 % increase in metabolic rate (Nespolo et al. 2003). Thus, research should continue to examine the benefits and costs of fever in insects.

In sum, we show that fever is widespread in insects, but sporadically expressed. Previous researchers have posited that fever in insects may be ubiquitous and rely upon the same fever-inducing mechanism (eicosanoids) as mammals (Bundey et al. 2003). Though we induced fever in *G. texensis* with a pharmacological dose of an eicosanoid (PGE$_2$), our meta-analysis results suggest that eicosanoid release due to immune recognition and activation is not sufficient for fever induction in insects. Future work should examine the effects of specific immune pathways on a suite of proteins (e.g. eicosanoids) to better understand their role in behavioural fever. We also suggest a standardized experimental protocol that includes exposure to several concentrations of non-infective pathogens (e.g. 10^2, 10^4, and 10^8

cells of heat-killed bacteria, to preclude any parasitic manipulation of host thermoregulatory behaviour) and fixed time points of T_{pref} measurement (e.g. 1 h prior to treatment followed by measurements 30 min, 60 min, 120 min, 24 h, and 48 h after treatment) or real-time T_{pref} measurement (e.g. Hunt et al. 2011). Studying the expression of insect fever has informed pest management strategy (e.g. the limited effectiveness of fungal biocontrol measures to eradicate locusts; Blanford et al. 1998). It can also provide insight into how global climate change may alter their disease resistance (e.g. Adamo and Lovett 2011; Adamo et al. 2012).

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