Journal of Insect Physiology 58 (2012) 1450–1455

Contents lists available at SciVerse ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Interplay between behavioural thermoregulation and immune response in mealworms

Tamara P. Catalán [a,b,*], Hermann M. Niemeyer [c], Alexis M. Kalergis [b], Francisco Bozinovic [a,b]

[a] Departamento de Ecología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago 6513677, Chile
[b] Millennium Institute on Immunology and Immunotherapy, Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas and Departamento de Reumatología, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago 6513677, Chile
[c] Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile

ARTICLE INFO

Article history:
Received 23 May 2012
Received in revised form 16 August 2012
Accepted 18 August 2012
Available online 15 September 2012

Keywords:
Ecoimmunology
Behavioural fever
Preferred body temperature
Immune response
Tenebrio molitor
Antibacterial activity
Phenoloxidase activity

ABSTRACT

Since the preferential body temperature should positively correlate with physiological performance, behavioural fever should enhance an organism's immune response under an immune challenge. Here we have studied the preferential body temperature (T_p) and its consequences on immune response performance after an immune challenge in larvae of *Tenebrio molitor*. We evaluated T_p and immune responses of larvae following a challenge with various concentrations of lipopolysaccharide (LPS), and we studied the correlation between T_p and two immune traits, namely antibacterial and phenoloxidase (PO) activities. Larvae that were immune challenged with higher LPS concentrations (C_{50} and C_{100}) preferred in average, warmer temperatures than did larvae challenged with lower concentrations (C_0 and C_{25}). T_p of C_{25}–C_{100} (challenged)-mealworms was 2.3 °C higher than of C_0 (control) larvae. At lower LPS concentration immune challenge (C_0 and C_{25}) antibacterial activity correlated positively with T_p, but at C_{50} and C_{100} correlation was lose. PO activity was higher at higher LPS concentration, but its magnitude of response did not correlate with T_p Our data suggest that behavioural fever may have a positive effect on host performance by enhancing antibacterial response under a low pathogen load situation.

1. Introduction

Under a variable environment it is important to determine and understand how animals respond to the putatively synergistic effect of key abiotic (e.g. temperature) and biotic factors (e.g. pathogens). Thus, on one hand environmental temperature exerts strong selection pressures on all organisms (Huey and Bennett, 1987; Seebacher, 2005) and represents a continuous challenge to homeostasis (Johnston and Bennett, 1996) – particularly for ectotherms whose physiological, behavioural and life-history traits are sensitive to ambient temperature and, on the other hand, pathogens are one of the most important biotic factors affecting host Darwinian fitness as well as abundance and distribution (Marcogliese and Cone, 1997; Thomas et al., 2005). Environmental temperature may significantly influence host–pathogen interactions by affecting pathogen growth rate and hence virulence (Inglis et al., 1996; Ouedraogo et al., 1997; Arthurs and Thomas, 2001), as well as host capacity to fight infection (Blanford and Thomas, 1999; Thomas and Blanford, 2003; Linder et al., 2008). In line with these processes, behavioural fever may occur, where an infection is accompanied by an increase in the preferential temperature of the host (Kluger, 1979). Behavioural fever can contribute to insect host survival in a wide range of interactions (Boorstein and Ewald, 1987; Bronstein and Conner, 1984; Bundey et al., 2003; Inglis et al., 1996; Ouedraogo et al., 2003; Karban, 1998) and, theoretically, it may enhance the host immune response (Kluger et al., 1998; Elliot et al., 2005; Adamo and Lovett, 2011). But, an increase in body temperature implies an augmentation of maintenance energy cost (Huey and Stevenson, 1979). Our previous studies have demonstrated that the energetic costs of the immune response are consequence of an increase of metabolism associated to detoxification and repair processes, which are dependent of environmental temperature (Catalán et al., 2012). Additionally, environmental temperature significantly affected the presence of the enzyme phenoloxidase and lysozyme-like enzymes (Adamo and Lovett, 2011; Fuller et al., 2011) as well as the capacity to maintain hemocytes numbers and haemolymph protein levels (Ouedraogo et al., 2003).

However, immune response may work as a "double edged sword" – i.e., immunity activation is fundamental to resist a pathogen, but can be detrimental if the response is maintained in the long term (Moret and Schmid-Hempel, 2000; Haine et al., 2008b) due to the energetic costs involved in immune response, detoxification and repair processes. These disruptions of the physiological-homeo-

* Corresponding author at: Departamento de Ecología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago 6513677, Chile. Tel.: +56 2 686 2619.
E-mail address: tpcatala@uc.cl (T.P. Catalán).

0022-1910/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jinsphys.2012.08.011

static functions lead ultimately to a reduction in fitness (Bozinovic et al., 2011). In this sense, it has been proposed that the adaptive significance of preferential body temperatures correlates with temperature values that optimise physiological performance and consequently maximise Darwinian fitness (Huey and Bennett, 1987; Angilletta et al., 2004). Albeit the fact that the direct effect of temperature on pathogens and on their ability to infect the host has been largely investigated (Inglis et al., 1996; Blanford and Thomas, 1999; Elliot et al., 2002), few studies have approached the question as to how behavioural fever can influence the immune response of the host (but see Adamo and Lovett, 2011). Thus, despite strong evidence demonstrating changes in parasitised host thermoregulatory behaviour, host benefits of behavioural fever still remain to be evaluated. Consequently, we have studied: (1) the effect of immune challenge on preferential body temperature (T_p) in an insect larva, and (2) the effects of behavioural fever on its immune response. As study model we employed larvae of the insect *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). Additionally, in a previous study using *T. molitor* we showed that environmental temperature can affect immune response performance (Catalán et al., 2012). To challenge the immune system of larvae we used *Escherichia coli* lipopolysaccharide (LPS). Although this immune elicitor is non-pathogenic and non-living, it is capable of triggering the invertebrate immune system and allows measuring the costs of deploying the immune system independently of the pathological costs associated with the parasites themselves (Fellowes et al., 2005).

We measured antimicrobial protein production using antibacterial activity, and phenoloxidase (PO) quantity as parameters of the innate immune response, two traits that have been largely used as indicators of resistance to pathogens and parasites in insects (Moret and Siva-Jothy, 2003). We hypothesised that: (1) larvae will select higher environmental temperatures according to the magnitude of the immune challenge, and (2) the preferential body temperatures will correlate positively with the immune performance. Consequently, we predicted that animals selecting higher environmental temperatures will exhibit a higher immune performance.

2. Methods

2.1. Mealworm cultures and immune challenge

Larvae of *T. molitor* were randomly selected from a stock culture maintained since the year 2000 under laboratory conditions (23 ± 2 °C and 12:12 photoperiod) and supplied with a mixture of flour (60%), oats (20%), yeast (10%) and bran (10%), and apples *ad libitum*. Insects were kept in plastic containers carefully cleaned and autoclaved; mixed dry food was heated at 70 °C for 72 h to eliminate pathogens and parasites. Larvae with similar body mass (ca. 0.12 ± 0.02 g) were employed and different sets of animals were used for each measurement to avoid any effect of previous manipulations on the data obtained. Animals used showed no signs of moulting, such as colour change or immobility.

To induce an innate immune response, animals were challenged with different concentrations of LPS (Sigma 8274). Four different concentrations of LPS were used in 4 μl of sterile pH 6.4 phosphate buffered saline solution (PBS): C_0 solution free of LPS, C_{25} (5 mM LPS), C_{50} (10 mM LPS) and C_{100} (20 mM LPS, maximal concentration used). All injections were made through the pleural membrane between the second and the third abdominal segments, using a sterilised Hamilton syringe.

2.2. Preferential body temperature (T_p)

To determine the time at which animals presented behavioural fever, measurements of T_p were made 0, 24, 48 and 72 h after an insult with C_{100} LPS solution. Seventy larvae were used in a repeated measurements model. Before T_p measurements at different times following immune insult, larvae were fasted for 1 h and then they were placed at a thermal gradient for 2 h (details of T_p measurements are below). Briefly, the thermal gradient was built using a 90-cm long, 21-cm wide aluminium plate with seven longitudinal 7.0-cm deep, 3.0-cm wide runways. The structure was painted with non-toxic black paint to achieve an opaque surface and avoid animals to imitate their own reflection, corners were rounded to avoid any angle effects and a cold light was used over the gradient to prevent the formation of a luminous gradient. The thermal gradient was obtained by heating one extreme with a heating tape controlled by a potentiometer. The cool extreme was achieved by pumping antifreeze solution from a freezer through a copper coil installed under the metal plate. The surface temperature in the thermal gradient ranged between 5 and 40 °C and was maintained during the duration of the experiment.

Before body temperature measurements larvae were fasted for 1 h and then they were placed at a thermal gradient for 2 h. Larvae were weighted in an analytical balance (±0.0001 g; JK-180, Chyo, Kyoto) and placed individually in separate runways at random position within the thermal gradient. Individuals were allowed to acclimate for 30 min before began testing. Then, we recorded body temperature every 30 min for 2 h using an infrared thermometer with ±0.5 °C precision (TempTest IR, Oakton®). T_p correspond to the average of the four "partial body temperatures" obtained for each larva through experiment.

2.3. Immune traits response

Having determined that the highest T_p was observed 24 h after immune insult (Fig. 1), this time interval was used in subsequent experiments to measure immune traits response using immune challenge with different LPS concentrations. Immediately after each series of T_p measurements, haemolymph (10 μl per animal) was collected in pre-chilled glass capillaries by puncturing the pleural membrane. Each animal ($n = 10$) was bled only once and two haemolymph immune variables were measured: antibacterial activity and haemolymph PO activity.

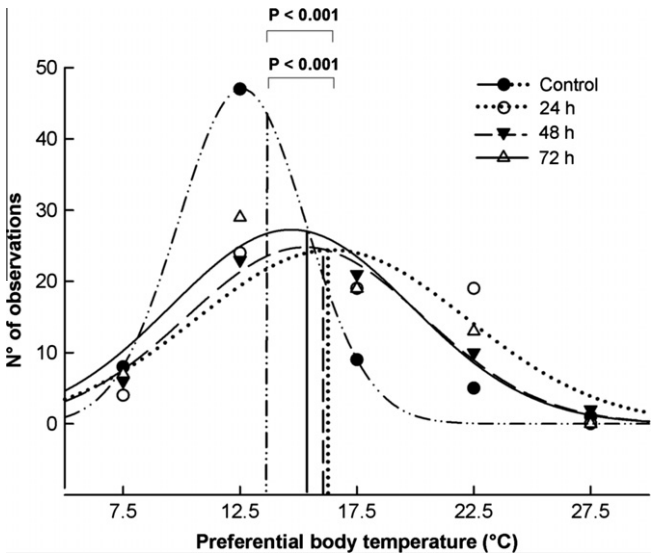

Fig. 1. Frequencies of preferential body temperature (T_p) of larvae of *T. molitor* at 0 (control), 24, 48 and 72 h after LPS injection. Twenty-four and 48 h after the immune challenge, animals presented a higher average T_p and a higher variation of T_p than after 0 h. Statistical differences are indicated ($P < 0.001$).

2.3.1. Antibacterial activity

Haemolymph (5 μL) was diluted with 24 μL PBS, and 1 μL of an overnight culture (approximately 3×10^7 CFU ml^{-1}) of streptomycin-resistant *Micrococcus luteus* was added to the solution. The mixture was incubated at 30 °C with agitation at 150 rpm for 1 h. Then, the mixture was diluted 100 times and plated on LB agar containing 5 μg ml^{-1} streptomycin (Sigma S6501). The mixture (50 and 100 μl) was spread onto two plates for each animal. Plates were incubated at 30 °C for 48 h. The number of colonies was counted on each plate and the mean number of colony-forming units (CFU) per μl of mixture spread on the two plates was used to calculate the antibacterial activity as the percentage of dead bacteria relative to control plates without larval haemolymph (modified from Cotter et al., 2004; Haine et al., 2008a; Ahmed et al., 2002).

2.3.2. Haemolymph PO quantity

Haemolymph (5 μl) was added to 200 μl of ice-cold PBS at pH 6.4 in an Eppendorf tube and vortexed. Samples were frozen at −80 °C until use. PO activity was assayed spectrophotometrically with L-dopa as substrate (Wilson et al., 2001; Cotter et al., 2004). L-Dopa (Sigma D9628, 100 μL, 20 mM) was added to 100 μL buffered haemolymph and the OD (492 nm) of the mixture was determined at 25 °C with a microplate reader (Packard Bioscience model AS 10001) at 10 min intervals during 90 min. Enzyme quantity, expressed as PO units per μl haemolymph, was obtained as described in Catalán et al. (2012).

2.4. Statistical analyses

To determine the time at which animals showed behavioural fever, repeated measurements ANOVA was used; the effects of immune response activation at different LPS-concentrations on preferential body temperature (T_p) were tested using ANOVA with fixed factor (LPS concentration: C_0, C_{25}, C_{50} and C_{100}). To test the relationship between immune performance (antibacterial and PO activities) and T_p, we used an analysis of covariance with T_p as covariable and correlations test. In the case that T_p correlated significantly with immune traits we tested the homogeneity of slopes. When slopes did not meet this assumption, a Separate-slopes model was use. When, T_p resulted no-significant as covariable, an ANOVA was used to determine the LPS concentration on immune trait. All data were tested for normality and homoscedasticity with Shapiro-Wilk and Cochran *C* tests. When necessary, data were transformed to meet statistical assumptions and covariance between body mass and all traits was tested. When differences were significant at $P < 0.05$ after the general linear model tests, *a posteriori* Tukey HSD test for multiple comparisons were used. All statistical analyses were conducted using Statistica 6.0 software (Statsoft Inc., Tulsa, OK).

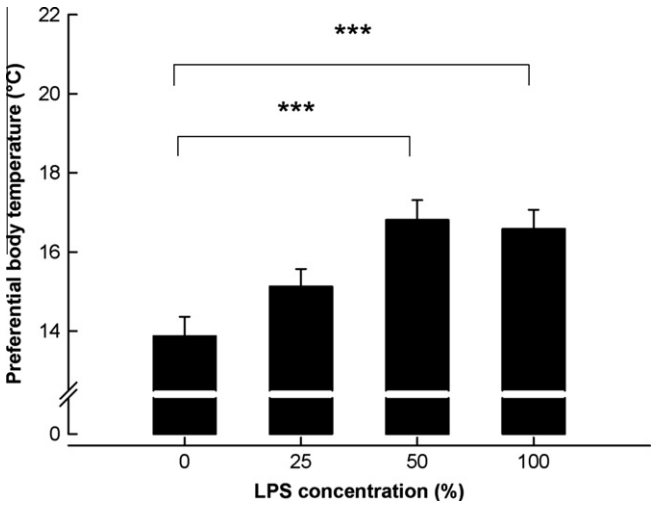

Fig. 2. Mean preferential body temperature (T_p) of *T. molitor* larvae at 24 h after LPS injection at different concentrations (C_0, C_{25}, C_{50} and C_{100}). Animals exposed to the highest LPS doses (C_{50} and C_{100}) exhibited behavioral fever, in contrast to C_0 and C_{25}-treated larvae. Statistical differences are indicated (***$P < 0.001$).

3. Results

3.1. Preferential body temperature (T_p)

The highest preferential body temperatures were observed at 24 and 48 h after the immune challenge, with average T_p of 16 ± 4.6 °C and 16 ± 4.4 °C, respectively. These values were not statistically different (Tukey HSD: $P = 0.990$) but differed significantly (Tukey HSD: $P < 0.0001$) from the group without immune insult (13.6 ± 3.8 °C) (Fig. 1). At 72 h, T_p did not differ from control (15.3 ± 4.6 °C; Tukey HSD: $P = 0.281$), indicating that behavioural fever was present only during the first 48 h after an immune challenge (Fig. 1).

3.2. Immune challenge dose

LPS concentration significantly affected T_p (ANOVA: $F_{(3,196)} = 8.17$; $P < 0.001$). Larvae that were immune challenged with the highest LPS concentrations (C_{50} and C_{100}) selected warmer temperatures than did naïve larvae (Tukey: C_0/C_{50} and C_0/C_{100}: $P < 0.001$ in both cases) (Fig. 2). Mean T_p of all LPS-challenged larvae was 16.2 (±3.4) °C as compared to 13.9 (± 3.6) °C of non-challenged larvae (Fig. 2). The warmest "partial body temperature" selected by control larvae (C_0) was 24.4 °C; for treated larvae, it reached 33.8 °C at C_{50}. The warmest average T_p was 21.8 °C for control larvae and 28.0 °C for larvae treated larvae at C_{50}. In both cases, is noteworthy that highest T_p was observed at C_{50}, and not at C_{100} as expected.

3.3. Immune traits correlation with T_p

Antibacterial activity correlated positively with T_p ($r = 0.5043$; $P < 0.0001$) and was significantly affected by LPS- concentration (Separate-slopes model: $F_{(3,52)} = 5.90$; $P = 0.002$) and by the interaction of LPS- concentration and T_p (Separate-slopes model: $F_{(4,52)} = 6.67$; $P < 0.001$). Larvae treated with LPS at C_{50} and C_{100} exhibited significantly higher antibacterial activity (99.12% and 99.14%, respectively) than larvae treated with C_{25} (74.18%) and controls (C_0) (83.91%) (Fig. 3). Meanwhile, PO quantity was not correlated with T_p ($r = -0.017$; $P = 0.88$), but was significantly affected by LPS-concentration (ANOVA: $F_{(3,76)} = 3.92$; $P = 0.012$). The amount of PO was higher only at C_{100} in contrast to C_0 (Tukey: $P = 0.006$).

Finally, standard deviations of antibacterial activity allowed us to study correlation with T_p only at C_0 and C_{25}. At C_{50} and C_{100} where antibacterial activity was higher, standard deviations where minimal (±1.14 and ±2.82, respectively) compared to those observed for the C_0 and C_{25} groups (±25.93 and ±20.75, respectively) (Fig. 3). Then, we observed a significant positive linear correlation between antibacterial activity and T_p larvae treated with C_0 and C_{25} (Table 1 and Fig. 3).

4. Discussion

Behavioural fever is a widespread phenomenon which has been described in annelids, arthropods (including insects), fishes, amphibians and reptiles. Among insects, it has been shown to occur in the cockroach *Gromphadorhina portentosa* infected with an

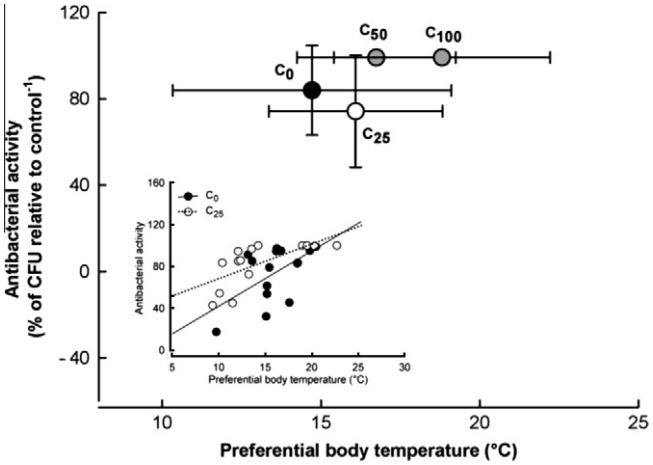

Fig. 3. Correlation between preferential body temperature (T_p) and antibacterial activity of *T. molitor* larvae at 24 h after an immune challenge. The inset shows the linear correlations for C_0 and C_{25}. Statistical details are shown in Table 1.

Table 1
Correlation analyses between preferred body temperature and immune trait responses at different LPS concentrations.

LPS concentration	Immune traits			
	Antibacterial activity		PO activity	
	r	*P*	*r*	*P*
C_0	0.56	0.0311*	0.07	0.7742
C_{25}	0.70	0.0036**	0.06	0.8075
C_{50}	0.19	0.5092	0.22	0.3551
C_{100}	0.23	0.4015	0.06	0.8121

Asteriks indicate a significant correlation.
* $P < 0.05$.
** $P < 0.001$.

E. coli suspension or an endotoxin (Bronstein and Conner, 1984), in the field cricket *Gryllus bimaculatus* and the house cricket *Acheta domesticus* infected with the lethal intracellular parasite *Rickettsiella grylli* (Louis et al., 1986; Adamo, 1998), and in the desert locust *Schistocerca gregoria* infected with the entomopathogenic fungus *Metarhizium anisopliae* (Blanford and Thomas, 1999; Wilson et al., 2002). However, even behavioural fever contribute to host survival in a wide range of interactions (Boorstein and Ewald, 1987; Bronstein and Conner, 1984; Bundey et al., 2003; Inglis et al., 1996; Ouedraogo et al., 2003; Karban, 1998), mechanisms of its benefits still remain to be established.

Here the role of environmental temperature on host immune response as mediated by host thermoregulation and behavioural fever was explored. Immune response of challenged mealworms was predicted to correlate positively with preferred body temperature, and hence environmental temperature was proposed to play a significant role on host–pathogen interaction. Our results support the notion that T_p correlates with values of environmental temperature that promote a partial increase in the insect immune response.

The highest T_p was observed 24 h after the immune challenge, which agrees with previous studies: Bundey et al. (2003) noticed in the desert locust *S. gregaria* an increase in T_p 24 h after LPS injection, and Bronstein and Conner (1984) found that the cockroach *G. portentosa* took more than 10 h after LPS injection to show a preference for warmer temperatures. Along these lines, it is known that the triggering of the processes required for an antibacterial response can take at least 1–3 h (Lavine et al., 2005) and 12–48 h to reach peak levels (Haine et al., 2008b), thus coinciding with the times at which the highest T_p was observed in this work. In larvae of *T. molitor,* antibacterial activity and PO activity was previously observed to reach maximal values 24 h after LPS injection and decayed at 72 h at 20 °C (Catalán et al., 2012) in line with the decay of preferred body temperature registered here.

Even when LPS injection generated an elevation of preferential body temperatures of 2.3 °C, average T_p of challenged larvae (16.2 °C) was inferior to 20 or 30 °C at which we previously observed the highest antibacterial and PO activities (Catalán et al., 2012). However we expected that thermal preferences should match temperatures optimal for physiological traits, in this case immune response, it is very common that thermal preferences are close to body temperatures that maximize various measures of physiological performance instead one in particular (Martin and Huey, 2008). In this sense, Murdock and collaborators (2012) reported different temperatures at which different immune traits showed their maximal expressions. In the malaria vector *Anopheles stephensi*, nitric oxide synthase expression peaked at 30 °C, cecropin expression was not significantly affected by temperature and humoral melanization, and phagocytosis and defensin expression peaked around 18 °C (Murdock et al., 2012). Then, total fitness over time might be maximized – at least in a variable environment – by centering thermal preferences at a temperature below the body temperature that maximizes physiological responses like immunity (Martin and Huey, 2008), because fitness may drop rapidly at temperatures above the optimum due to the nonlinear and highly asymmetrical shape of fitness curves of ectotherms (Huey and Stevenson, 1979).

Also worth mentioning, maximal T_p data obtained for challenged larvae of *T. molitor* in this work could be strongly influenced by acclimation to previous conditions and by the inbreeding resulting from larval culture (see Stacey et al., 2003). Larvae had been maintained for a long time under standard temperature conditions of 23 °C then, from an evolutionary perspective the current study could represent the 'genotype × environment' interaction of only one population.

Respect to LPS concentration, we observed the highest partial and average T_p at C_{50} and not at C_{100} as we expected assuming that behavioural fever should be consistent with pathogenic load. In this concern, in vertebrates, such as rats and lizards, LPS challenge can generate two different and contrasting thermoregulatory responses namely fever and hypothermia. Deen and Hutchison (2001) observed a bimodal T_p response depending on the LPS dose. Rats responded with hypothermia at high LPS doses (Romanovsky et al., 1996). In the present study a standard dose of 20 mM of LPS as maximal LPS concentration (C_{100}) was used, but further studies are required to define if immune-elicitor doses have differential effects on temperature selection by invertebrates with higher doses and different elicitors or live pathogens. Regarding the nature and concentration of the pathogen or immunity activator, results are inconclusive: high temperatures may enhance host immune defences and/or act detrimentally on pathogens, in addition behavioural fever may not to be continuous and thus may be unpredictable in its manifestations (Inglis et al., 1996). In fact, no invertebrate has been shown to exhibit fever in response to an infection by a multicellular parasite (Karban, 1998). For example, *Drosophila* did not present behavioural fever when infected with the nematode *Howardula aoronymphium* (Ballabeni et al., 1995). Behavioural fever, although clearly effective, is highly dependent of different factors such as environmental, seasonal, ontogenetic, genetic, and pathogen-derived factors (see Stacey et al., 2003).

Antimicrobial protein production was highly sensitive to environmental temperature for C_0 (naïve) and C_{25}-treated larvae, being higher at higher temperatures. In fact, C_0-larvae that preferred temperatures near 20 °C presented antibacterial activities of approximately 97%, similar to those obtained for C_{25}-treated larvae. It seems that higher environmental temperatures allows larvae infected with a low concentrations of LPS and naïve larvae

to confront a bacterial infection more effectively without the investment in immune components required at the moment of pathogenic interaction and having a prophylactic protection (Chown and Nicolson, 2004). Contrarily, C_{50}- and C_{100}-treated larvae showed no relation between body temperature preference and immune response. In fact, the variance of antibacterial activity is very small compared to C_0- and C_{25}-treated larvae. At C_{50} and C_{100}, the minimal value of antibacterial activity was 89.0% and the maximal was 100% (value shown by the 63.3% of larvae) while at low LPS concentrations, minimal antibacterial activity was 17.4% and maximal was 100% (value shown by only the 13.3% of larvae challenged with C_{25}). These results suggest that at higher LPS concentrations, antimicrobial protein production reach a threshold, as in an "all or nothing" response.

On the other hand, PO quantity did not correlate with T_p as expected. Environmental temperature has been shown to affect positively the amount of PO in *T. molitor* being higher at 30 °C than at 20 or 10 °C (Catalán et al., 2012), while PO activity in *Eurygaster integriceps* was higher and more persistent in time at 40 °C (over 72 h) than at 20 or 30 °C (Zibaee et al., 2009). Moreover, field studies have confirmed that environmental temperature is positively correlated with PO activity (Fuller et al., 2011). It seems likely that a correlation between PO presence and T_p was not obtained due to the range of preferred body temperature registered by larvae in the analysis – between 9.9 and 23.6 °C, as compared to 30 °C, the temperature at which highest enzyme quantity was previously obtained (Catalán et al., 2012). Also, it is known that PO activity is negatively correlated with antibacterial activity (Cotter et al., 2004), then the increase in one immune traits could mean the decrease in others (see Rantala and Roff, 2005). In fact, a great number of studies had demonstrated trade-offs between life-history trait and immunocompetence ensuring a cost associated to immune response (Barnes and Siva-Jothy, 2000; Ahmed et al., 2002; Armitage et al., 2003; Fedorka et al., 2004; Gwynn et al., 2005; Rantala and Roff, 2005; Elliot et al., 2005; Springate and Thomas, 2005; Rolff and Reynolds, 2009). Then a maximal response is not always the optimal. It is clear that further studies are necessary with a larger range of temperatures to define how behavioural fever affects PO activity. Indeed, questions on how persistence in time and effectiveness of immune response are affected by environmental factors such as temperature or physiological states require further investigation.

Future studies should attempt to explain how immunological traits are affected by high levels of temperature variability encountered over different habitats and conditions in a global change scenario (IPCC, 2001). Clearly, the impact of rising temperatures will depend in part on the physiological vulnerability of organisms and their capabilities to deal with infections as well as on the diversity of pathogens. Indeed, owing to climate change, the average temperature is increasing, but also extreme weather conditions are predicted to become more frequent (Parmesan et al., 2000). This expected increase in the frequency of extreme environmental conditions should have an even greater impact on interactions within ecological communities than the increase in average temperatures. Few studies have examined the effects of temperature variation or other climatic drivers on host–pathogen interactions in current host and pathogen populations. Direct investigations examining alterations in host immune function in native as well as invasive species are needed to understand the potential effect of temperature variation on pathogen resistance and diseases.

Acknowledgements

This study was funded by Comisión Nacional de Investigación Científica y Tecnológica (CONICYT) Ph.D. fellowship to TPC, FONDAP 1501-0001(Program 1) to FB, FONDECYT 1070352 to AMK, and Millennium Institute on Immunology and Immunotherapy (P-07-088-F, AMK). This article is part of the Ph.D. thesis of TPC, which was co-mentored by FB and AMK.

References

Adamo, S.A., 1998. The specificity of behavioral fever in the cricket *Acheta domesticus*. Journal of Parasitology 84, 529–533.
Adamo, S.A., Lovett, M.M.E., 2011. Some like it hot: the effects of climate change on reproduction, immune function and disease resistance in the cricket *Gryllus texensis*. Journal of Experimental Biology 214, 1997–2004.
Ahmed, A.M., Baggott, S.L., Maingon, R., Hurd, H., 2002. The costs of mounting an immune response are reflected in the reproductive fitness of the mosquito *Anopheles gambiae*. Oikos 97, 371–377.
Angilletta, M.J., Steury, T.D., Sears, M.W., 2004. Temperature, growth rate, and body size in ectotherms: fitting pieces of a life-history puzzle. Integrative and Comparative Biology 44, 498–509.
Armitage, S.A.O., Thompson, J.J.W., Rolff, J., Siva-Jothy, M.T., 2003. Examining costs of induced and constitutive immune investment in *Tenebrio molitor*. Journal of Evolutionary Biology 16, 1038–1044.
Arthurs, S., Thomas, M.B., 2001. Effect of dose, pre-mortem host incubation temperature and thermal behaviour on host mortality, mycosis and sporulation of *Metarhizium anisopliae* var. acridum in *Schistocerca gregaria*. Biocontrol Science and Technology 11, 411–420.
Ballabeni, P., Benway, H., Jaenike, J., 1995. Lack of behavioral fever in nematode-parasitized *Drosophila*. Journal of Parasitology 81, 670–674.
Barnes, A.I., Siva-Jothy, M.T., 2000. Density-dependent prophylaxis in the mealworm beetle *Tenebrio molitor* L. (Coleoptera: Tenebrionidae): cuticular melanization is an indicator of investment in immunity. Proceedings of the Royal Society of London Series B: Biological Sciences 267, 177–182.
Blanford, S., Thomas, M.B., 1999. Host thermal biology: the key to understanding host–pathogen interactions and microbial pest control? Agricultural Forest Entomology 1, 195–202.
Boorstein, S.M., Ewald, P.W., 1987. Costs and benefits of behavioral fever in *Melanoplus sanguinipes* infected by *Nosema acridophagus*. Physiological Zoology 60, 586–595.
Bozinovic, F., Calosi, P., Spicer, J.I., 2011. Physiological correlates of geographic range in animals. Annual Review of Ecology, Evolution, and Systematics 42, 155–179.
Bronstein, S.M., Conner, W.E., 1984. Endotoxin-induced behavioral fever in the Madagascar cockroach *Gromphadorhina portentosa*. Journal of Insect Physiology 30, 327–330.
Bundey, S., Raymond, S., Dean, P., Roberts, S.K., Dillon, R.J., Charnley, A.K., 2003. Eicosanoid involvement in the regulation of behavioral fever in the desert locust *Schistocerca gregoria*. Archives of Insect Biochemistry Physiology 52, 183–192.
Catalán, T.P., Wozniak, A., Niemeyer, H.M., Kalergis, A.M., Bozinovic, F., 2012. Interplay between thermal and immune ecology: effect of environmental temperature on insect immune response and energetic costs after an immune challenge. Journal of Insect Physiology 58, 310–317.
Chown, S.L., Nicolson, S.W., 2004. Insect Physiological Ecology. Oxford University Press, Oxford.
Cotter, S.C., Kruuk, L.E.B., Wilson, K., 2004. Costs of resistance: genetic correlations and potential trade-offs in an insect immune system. Journal of Evolutionary Biology 17, 421–429.
Deen, C.M., Hutchison, V.H., 2001. Effects of lipopolysaccharide and acclimation temperature on induced behavioral fever in juvenile *Iguana iguana*. Journal of Thermal Biology 26, 55–63.
Elliot, S.L., Blanford, S., Thomas, M.B., 2002. Host–pathogen interactions in a varying environment: temperature, behavioural fever and fitness. Proceedings of the Royal Society of London Series B: Biological Sciences 269, 1599–1607.
Elliot, S.L., Horton, C.M., Blanford, S., Thomas, M.B., 2005. Impacts of fever on locust life-history traits: costs or benefits? Biology Letters 1, 181–184.
Fedorka, K.M., Zuk, M., Mousseau, T.A., 2004. Immune suppression and the cost of reproduction in the ground cricket, *Allonemobious socius*. Evolution 56, 2478–2485.
Fellowes, M.D.E., Holloway, G.J., Rolff, J., 2005. Insect Evolutionary Ecology. CABI Publishing, Wallingford.
Fuller, C.A., Postava-Davignon, M.A., West, A., Rosengaus, R.B., 2011. Environmental conditions and their impact on immunocompetence and pathogen susceptibility of the Caribbean termite *Nasutitermes acajutlae*. Ecological Entomology 36, 459–470.
Gwynn, D.M., Callaghan, A., Gorham, J., Walters, K.F.A., Fellowes, M.D.E., 2005. Resistance is costly: trade-offs between immunity, fecundity and survival in the pea aphid. Proceedings of the Royal Society of London Series B: Biological Sciences 272, 1803–1808.
Haine, E.R., Moret, Y., Siva-Jothy, M.T., Rolff, J., 2008a. Antimicrobial defense and persistent infection in insects. Science 322, 1257–1259.
Haine, E.R., Pollitt, L.C., Moret, Y., Siva-Jothy, M.T., Rolff, J., 2008b. Temporal patterns in immune responses to a range of microbial insults (*Tenebrio molitor*). Journal of Insect Physiology 54, 1090–1097.
Huey, R.B., Bennett, A.F., 1987. Phylogenetic studies of coadaptation: preferred temperatures versus optimal performance temperatures of lizards. Evolution 41, 1098–1115.
Huey, R.B., Stevenson, R.D., 1979. Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. American Zoologist 19, 357–366.

Inglis, G.D., Johnson, D.L., Goettel, M.S., 1996. Effects of temperature and thermoregulation on mycosis by *Beauveria bassiana* in grasshoppers. Biological Control 7, 131–139.
IPCC, 2001. Climate Change 2001: Impacts Adaptations and Vulnerability. Cambridge University Press, Cambridge.
Johnston, I.A., Bennett, A.F., 1996. Animals and Temperature: Phenotypic and Evolutionary Adaptation. Cambridge University Press, Cambridge.
Karban, R., 1998. Caterpillar basking behaviour and nonlethal parasitism by tachinid flies. Journal of Insect Behavior 11, 713–723.
Kluger, M.J., 1979. Fever in ectotherms: evolutionary implications. American Zoologist 19, 295–304.
Kluger, M.J., Kozak, W., Conn, C.A., Leon, L.R., Soszynski, D., 1998. Role of fever in disease. Annals of the New York Academy of Sciences 856, 224–233.
Lavine, M.D., Chen, G., Strand, M.R., 2005. Immune challenge differentially affects transcript abundance of three antimicrobial peptides in hemocytes from the moth *Pseudoplusia includens*. Insect Biochemisty and Molecular Biology 35, 1335–1346.
Linder, J.E., Owers, K.A., Promislow, D.E.L., 2008. The effects of temperature on host–pathogen interactions in *D. melanogaster*: who benefits? Journal of Insect Physiology 54, 297–308.
Louis, C., Jourdan, M., Cabanac, M., 1986. Behavioral fever and therapy in a rickettsia-infected Orthoptera. American Journal of Physiology 250, R991–R995.
Marcogliese, D.J., Cone, D.K., 1997. Food webs: a plea for parasites. Trends in Ecology & Evolution 12, 320–325.
Martin, T.L., Huey, R.B., 2008. Why "suboptimal" is optimal: Jensen's inequality and ectotherm thermal preferences. The American Naturalist 171, E102–E118.
Moret, Y., Schmid-Hempel, P., 2000. Survival for immunity: the price of immune system activation for bumblebee workers. Science 290, 1166–1168.
Moret, Y., Siva-Jothy, M.T., 2003. Adaptive innate immunity? Responsive-mode prophylaxis in the mealworm beetle, *Tenebrio molitor*. Proceedings of the Royal Society of London Series B: Biological Sciences 270, 2475–2480.
Murdock, C.C., Paaijmans, K.P., Bell, A.S., King, J.G., Hillyer, J.F., Read, A.F., Thomas, M.B., 2012. Complex effects of temperature on mosquito immune function. Proceedings of the Royal Society of London Series B. http://dx.doi.org/10.1098/rspb.2012.0638.
Ouedraogo, A., Fargues, J., Goettel, M.S., Lomer, C.J., 1997. Effect of temperature on vegetative growth among isolates of *Metarhizium anisopliae* and *M. flavoviride*. Mycopathologia 137, 37–43.
Ouedraogo, R.M., Cusson, M., Goettel, M.S., Brodeur, J., 2003. Inhibition of fungal growth in thermoregulating locusts, *Locusta migratoria*, infected by the fungus *Metarhizium anisopliae* var acridum. Journal of Invertebrate Pathology 82, 103–109.
Parmesan, C., Root, T.L., Willig, M.R., 2000. Impacts of extreme weathers and climate on terrestrial biota. Bulletin of the American Meteorological Society 81, 443–450.
Rantala, M.J., Roff, D.A., 2005. An analysis of trade-offs in immune function, body size and development time in the Mediterranean Field Cricket, *Gryllus bimaculatus*. Functional Ecology 19, 323–330.
Rolff, J., Reynolds, S.E., 2009. Insect Infection and Immunity: Evolution, Ecology, and Mechanisms. Oxford University Press, Oxford, New York.
Romanovsky, A.A., Shido, O., Sakurada, S., Sugimoto, N., Nagasaka, T., 1996. Endotoxin shock: thermoregulatory mechanisms. American Journal of Physiology 270, R693–R703.
Seebacher, F., 2005. A review of thermoregulation and physiological performance in reptiles: what is the role of phenotypic flexibility? Journal of Comparative Physiology B 175, 453–461.
Springate, S., Thomas, M.B., 2005. Thermal biology of the meadow grasshopper, *Chorthippus parallelus*, and the implications for resistance to disease. Ecological Entomology 30, 724–732.
Stacey, D.A., Thomas, M.B., Blanford, S., Pell, J.K., Pugh, C., Fellowes, M.D.E., 2003. Genotype and temperature influence pea aphid resistance to a fungal entomopathogen. Physiological Entomology 28, 75–81.
Thomas, M.B., Blanford, S., 2003. Thermal biology in insect–parasite interactions. Trends in Ecology & Evolution 18, 344–350.
Thomas, F., Adamo, S., Moore, J., 2005. Parasitic manipulation: where are we and where should we go? Behavioural Processes 68, 185–199.
Wilson, K., Cotter, S.C., Reeson, A.F., Pell, J.K., 2001. Melanism and disease resistance in insects. Ecology Letters 4, 637–649.
Wilson, K., Thomas, M.B., Blanford, S., Doggett, M., Simpson, S.J., Moore, S.L., 2002. Coping with crowds: density-dependent disease resistance in desert locusts. Proceedings of the National Academy of Sciences of the United States of America 99, 5471–5475.
Zibaee, A., Bandani, A.R., Talaei-Hassanlouei, R., Malagoli, D., 2009. Temperature and Ca^{2+} ion as modulators in cellular immunity of the Sunn pest *Eurygaster integriceps* Puton (Heteroptera: Scutelleridae). Entomological Research 39, 364–371.