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

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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-

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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.

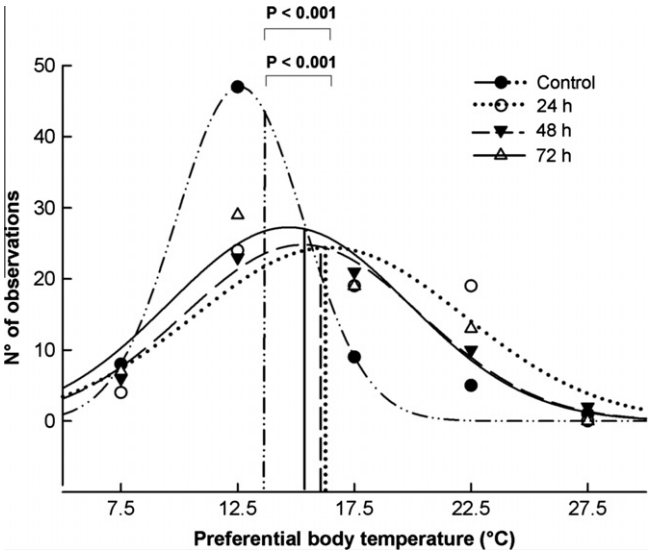

Fig. 1. Frequencies of preferential body temperature (T_p) of larvae of *T. molitor* at 0 (control), 24, 48 and 72 h after LPS injection. Twenty-four and 48 h after the immune challenge, animals presented a higher average T_p and a higher variation of T_p than after 0 h. Statistical differences are indicated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