Journal of Insect Physiology 98 (2017) 55–58

Contents lists available at ScienceDirect

Journal of Insect Physiology

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

Developmental study of the Proboscis Extension Response to heat in *Rhodnius prolixus* along the life cycle

Carole Labrousse, Claudio R. Lazzari, Nadine Fresquet *

Institut de Recherche sur la Biologie de l'Insecte, UMR CNRS 7261, Université François Rabelais, Tours, France

ARTICLE INFO

Article history:
Received 13 July 2016
Received in revised form 13 October 2016
Accepted 18 November 2016
Available online 24 November 2016

Keywords:
Triatominae
Heat responsiveness
Life cycle
Vectorial competence

ABSTRACT

Triatominae are blood-sucking insects that localise their hosts using a range of sensory signals to find food, and among them, the heat emitted by the hosts. Heat is one of the main short-range cues in vertebrate hosts, able to trigger alone the Proboscis Extension Response (PER) that precedes the bite. Previous studies demonstrated that heat responsiveness of fifth-instar nymphs is maximum to moderate temperatures (30–35°C) compatible with those of their vertebrate host's body surface. This study investigated whether this thermal preference for biting is maintained along the life cycle of *R. prolixus*, from the first larval instar to male and female adults. The results showed that PER rates were at maximum around 30–35°C and decreased for a warmer temperature. The same thermal preference was maintained all along the life cycle, despite the increase in the size of the antennae linked to the growth. Interestingly, a decreased thermal responsiveness was stated in males as compared with larval instars and females. This decrease might reveal a lower motivation for host-seeking and might have an impact on males's vectorial competence.

1. Introduction

Triatomine insects are vectors of the American trypanosomiasis (Chagas disease) through their blood-feeding behaviour. All nymphal and adult stages are hematophagous and so, potential vectors of the disease. It is important to know if all developmental stages and adults have an equal vectorial competence, that should not be restricted to the quantity of the infectious factor (Loza-Murguia and Noireau, 2010; Pizarro et al., 2007) but rather estimated through a large variety of physiological and behavioural traits and among them, the feeding-related behaviours.

The present study is aimed at searching for possible differences in the responsiveness to a major host signal (heat) across the development in *Rhodnius prolixus* (Guerenstein and Lazzari, 2009). The study of the response to thermal stimuli might reveal differences in host-preference and/or motivation to feed and so, might influence to some extent the epidemiologic risk along the life cycle. Besides, it can provide relevant information on the evolution of the sensory equipment along the development of the bugs. Differences in antennal ultrastructure, in sensilla number and distribution have been reported during the nymphal development in several species of Triatomae (Bernard, 1974; Català and Schofield, 1994; Silva et al., 2002). These data are relevant because the thermoreceptors are mostly located on the antennae (Insausti et al., 1999; Ferreira et al., 2007) and changes could induce differences in the responsiveness to host signals across instars. Adults and old nymphs of triatomines exhibit a highly developed thermal sense and they respond to objects displaying temperatures close to the host's, provided that they are hoter than the environment (Fresquet and Lazzari, 2011). Besides, one previous study showed a higher attractiveness of heat and of a combination of heat and chemicals in fifth instar nymphs compared to adult males (Milne et al., 2009) in *Triatoma dimidiata* and *Rhodnius prolixus*.

Rhodnius prolixus is a well-documented triatomine model in relation to its general biology (Lazzari et al., 2013), to its host-seeking behaviour (Lazzari, 2009) and more precisely to its response to heat (Fresquet and Lazzari, 2011; 2014; Guerenstein and Lazzari, 2009). The duration of nymphal stages is among the shortest in triatomines (Buxton, 1930), making nymphs needing only one *ad repletion* bloodmeal to molt to the following instar whereas adults take several blood meals for developing different activities, including locomotion, flight and, in females, egg-production. The analysis of their thermal preference for biting has revealed that fifth-instar nymphs respond to objects which temperature roughly matches the normal temperature of the

---

* Corresponding author at: Institut de Recherche sur la Biologie de l'Insecte, Avenue Monge, Parc Grandmont, 37200 Tours, France.

E-mail address: nadine.fresquet@univ-tours.fr (N. Fresquet).

http://dx.doi.org/10.1016/j.jinsphys.2016.11.010
0022-1910/

vertebrate host's body-surface (30–35°C). This preference is independent of the temperature of the surroundings, provided that a positive difference exists between the temperature of the object and that of the insect (Fresquet and Lazzari, 2011). In physical terms, a thermal stimulus consists in an exchange of thermal energy, by conduction, convection and/or radiation (*i.e.* the three physical mechanisms for heat exchange) that warms up specialized receptor mostly located on the antennae.

A simple model, based on bimodal integration has been proposed some years ago (Lazzari, 2009) for explaining how the temperature, distance and size of an object could be discriminated by bugs. The amount of energy arriving to the insect is absorbed by the antennae and their cuticular sensory structures to eventually reach specific cells, called warm- and cold-cells, that inform the brain about the amount of heat received. The amount of energy absorbed by a receptor depends on its mass and this variable must be taken into account in any estimation of heat stimulation (*e.g.* Hartline, 1974; Dussembery, 1988). A major question remains about whether or not bugs are able to keep constant their ability for evaluating thermal sources, even though their sensory system grows.

The present experimental work is aimed at testing whether or not the preference for the temperature of objects to bite remains the same along the post-embryonic growth. Two hypotheses can be raised: either bugs preference varies as a function of the physical properties of their receptor organs (*i.e.* number of sensilla and size of the antennae) or they are able to compensate these ontogenetic changes and their heat responsiveness matches with the same potential hosts.

2. Materials and methods

2.1. Insects

Nymphs and adults of *Rhodnius prolixus* (Origin Colombia, obtained from FIOCRUZ, Brasil) reared in our laboratory were used for the experiments. The insects were maintained under a 12 h light / 12 h dark regime at 26°C and 60 to 70% relative humidity. They were fed on heparin-treated sheep blood, through an artificial feeder (Núñez and Lazzari, 1990) until ecdysis. Newly emerged insects were collected every morning (*i.e.* at most only a few hours after emergence) and different starvation durations were used before the behavioural assays in accordance with the duration of the developmental stages: 1 week of starvation for the 1^{st} and 2^{nd} instar nymphs (out of 15–20 days of stage duration), 2 weeks of starvation for the 3^{rd}, 4^{th} and 5^{th} instar nymphs (out of 25 to 35 days of stage duration), 3 weeks of starvation for adults of both sexes (out of more than 60 days of life span). Experimental insects were maintained in a reverse light-cycle (dark from 08:00 to 20:00, local time) so that experiments could be performed during the first hours of the scotophase during which the responsiveness to heat is at its maximum (Fresquet and Lazzari, 2014).

2.2. Behavioural tests

The Proboscis Extension Response (PER) to heat was measured in a large incubator thermostatised at 25°C, under dim white light (intensity 0.34 μWatt/cm^2). The experimental device used to test the PER to heat is described in Fresquet and Lazzari (2011). Insects were attached by the dorsal thorax to a steel support with double-sided adhesive tape, ensuring a tarsal contact to a freely rotating polystyrene sphere. So immobilised, all the insects were placed at the same distance from the heat source (about 5 mm), a 4 cm-side square Peltier element. Cold water circulating within an aluminum block ensures a rapid change (within 5 s) of the temperature of the Peltier element. Insects were monitored with a video camera equipped with a macro lens permanently focused on the animal's head and thorax.

The tethered animal was positioned in the experimental device and three minutes elapsed before the behavioural test began. During this familiarization period and during the inter-trial intervals, we maintained the Peltier element at a "neutral" temperature of 25°C which does not trigger any PER since it is equal to that of the experimental environment (Fresquet and Lazzari, 2011).

The behavioural test consisted in three consecutive two-minutes trials. Each trial included 30 s of thermal stimulation followed by 90 s inter-trial interval without stimulation. For each larval instar (1^{st} to the 5^{th}), as well as for adults, three experimental groups of 15 bugs each were made according to the stimulation temperature: 30, 35 or 40°C. Each experimental animal was tested once, *i.e.* for one stage at one stimulation temperature, making at the end 45 bugs by stage and 315 bugs as a total.

Binary data were collected during the 30 s of thermal stimulation, as follows: 1 = one or more PER; 0 = no PER. Thus, at the end of the three trials, the individual score varying between 0 and 3 were the data computed for the statistical analysis.

2.3. Statistical analysis

The homogeneity of variances was ascertained by carrying out the Levene test. Shapiro-Wilk test ascertained the normality of residues (Cornillon et al., 2008). Once normality and homoscedasticity assessed, individual scores were then analyzed in a two-way ANOVA with the stage of development and the stimulation temperature as independant variables. Tukey (HSD) post hoc comparisons tests were used to reveal significant differences, between and within the stages of development.

3. Results

Fig. 1 depicts the mean (+ S.E.M.) individual scores recorded for each stimulation temperature in each stage of development. A two-way ANOVA for independent variables showed a significant effect of the stage of development ($F = 3.52$; $df = 6$; $p < 0.01$) and of the stimulation temperature ($F = 39.55$; $df = 2$; $p < 0.0001$). The interaction between these two factors was not significant. A posteriori comparisons (Tukey tests) between the stages of development (whatever the stimulation temperature) showed that the 2^{nd} and 4^{th} instar larvae displayed the highest rates of responses whereas the adult males displayed significantly the lowest ($p < 0.05$ in both cases). A posteriori comparisons (Tukey tests) between the

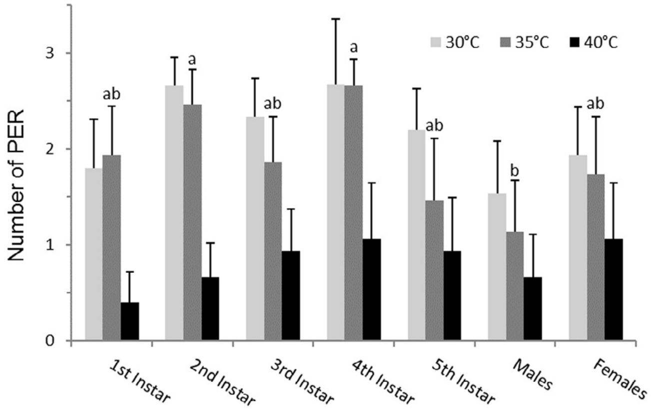

Fig. 1. Mean (+S.E.M.) number of PER recorded for each stage of development to each stimulation temperature: 30°C (white bars), 35°C (grey bars) and 40°C (black bars). Different letters indicate significant differences ($p < 0.05$).