LINK BETWEEN ELEVATED LOCOMOTOR ACTIVITY AND THE SPIKE BURSTING OF ANTENNAL THERMOSENSITIVE NEURONS IN THE CARABID BEETLE *PTEROSTICHUS OBLONGOPUNCTATUS*

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Many aspects of ectothermic life are affected by external temperature conditions. Therefore, thermosensation and thermoregulation are crucial for survival of ground dwelling carabid beetles. Research in sensory physiology with *Pterostichus oblongopunctatus* has shown change in the reaction of antennal thermoreceptor neurons at temperatures above the *P. oblongopunctatus*'s preferred temperature. From 25 °C, these neurons in dome shaped sensilla start to change from regular impulse firing to firing in bursts. We hypothesized that temperature-dependent impulse bursts are involved in *P. oblongopunctatus*'s behavioural thermoregulation. Behavioural experiments were carried out on the arena inside an environmental test chamber, and in two different temperature conditions – one with linearly increasing temperature, and the other with constant temperature. The temperature was increased from 10 °C to 40 °C linearly during 50 min on first case and with 5 degree steps on second case with duration 50 min each step. Locomotion parameters (velocity and travelled distance) were measured using an automated video tracking system. We observed both low and high activity zones in *P. oblongopunctatus*. The threshold temperature for changing between zones occurred was at 25.8 °C. This indicates a link between spike burst of antennal thermoreceptor neurons and behavioural thermoregulation in *P. oblongopunctatus*.

Key words: Coleoptera, Carabidae, behavioural thermoregulation, locomotion parameters, peripheral spike bursting.

INTRODUCTION

Abram *et al.* (2016) proposed a unified framework to generalise temperature's effects on poikilothermic animals. The effects were divided into two categories: kinetic effects (temperature effect on physiology, a result of changes in biochemical reaction rates) and integrated effects (thermal information initiating behavioural modifications via the central nervous system). Despite the importance of the latter, the first one has been much better studied. Sensitivity to heat has been demonstrated in nearly all physiological systems in insects (e.g. metabolism, respiration, endocrine dynamics, reproduction, growth, and learning- and foraging behaviour (Neven 2000, Abram *et al.* 2016). Many

ectotherms can adapt their physiology and behaviour to better tolerate their thermal environment (Stevenson 1985, Woods *et al.* 2015). Changes in behaviour are due to not only kinetic temperature effects (change in metabolism, e.g. flight muscles not working below certain temperature (Lehman 1999)), but also the integrated effects based on information gathered from the environment.

Of all the environmental abiotic factors, temperature and humidity are the most influential in governing habitat choice of carabid beetles (Coleoptera: Carabidae). Small arthropods such as carabids are vulnerable to desiccation and high temperature injury, the former being dangerous in much longer timeframes compared to the latter. In direct sunlight, due to infrared radiation, a 10 mg insect can heat up almost instantaneously by 10 °C in 10 s (Heinrich 1993). In Estonia during the summer the air temperature is 22 °C, the soil surface temperature ranges from 16 to 55 °C depending whether it is located in direct sunlight or shade (Must 2006). In carabid beetles, total heat paralysis begins at 47.4 to 51.7 °C, depending on species (Thiele 1977). In *P. oblongopunctatus*, the first indications of partial paralysis occur at 44.4 °C (Must *et al.* 2010). To survive, decisions must be made instantaneously and take into account information gathered from the environment.

Insects' external thermosensory organs (i.e. sensilla) are mostly located on antennae but can be also found on other parts of body (e.g. head, thorax, legs, ovipositor) (Altner & Loftus 1985, Altner & Prillinger 1980, Hess & Loftus 1984, Ehn and Tichy 1996, Merivee *et al.* 2003, Ruchty *et al.* 2009, Wang *et al.* 2009). A triad of neurons, one temperature-sensitive and two antagonistic bimodal hygroreceptor neurons, typically innervate these type of sensilla (Waldow 1970, Tichy 1979, Altner *et al.* 1981, Yokohari *et al.* 1982, Altner & Loftus 1985, Piersanti *et al.* 2011). The peripheral system's reaction to temperature is well studied in the carabid beetle *P. oblongopunctatus* (Nurme *et al.* 2015, Must *et al.* 2017) and the elaterid beetle *Agriotes obscurus* (Nurme *et al.* 2018). In both cases, the electrophysiological experiments with antennal dome shaped sensilla (DSS) demonstrated that, on the stressfully high temperatures (>25–30 °C), the regular firing of antennal thermo- and bimodal hygrothermoreceptor neurons are overridden by firing in bursty manner. Several parameters of spike trains are temperature dependent, and it was hypothesised that spike bursts are responsible for detecting noxiously high temperatures. Indeed, in a behavioural experiment using *A. obscurus,* threshold temperature for elevation in locomotor activity (LA) coincided with spike bursting threshold temperature (Nurme *et al.* 2018). No comparable behavioural tests have been made in other insects. In this study we hypothesise that the link between spike bursting of the antennal DSS neurons and locomotion locomotion is widespread in insects by demonstrating its existence in carabids. We expect onset for elevated locomotor activity in *P. oblongopunctatus* in the range of 25 to 30 °C.

MATERIAL AND METHODS

Study insects

The test carabid species of the study, *P. oblongopunctatus*, is common throughout the Palearctic (Löbl & Smetana 2003). We used individuals from a population in southern Estonia. This species is predominantly nocturnal (daytime activity of 30–45%) (Thiele 1977), and its preferred temperatures lay between 10 and 25 °C (Thiele 1977). *P. oblongopunctatus* is a forest generalist and tends to be euryhygric to dry-preferring (Lindroth 1986).

We collected adult beetles from their hibernation sites in late autumn 2015. Up to the time they were used in experiments, the beetles were kept at 5 °C in 20 × 30 × 10 cm plastic boxes filled with moist pieces of brown-rotted wood and moss. Two days prior to the experiments, test beetles were kept at room temperature and fed with moistened commercial cat food (Friskies Vitality, Hungary)

Test arena

The test arena was a 27 × 21 × 10 cm plastic box with moistened quartz-sand lining. The arena was located inside an Environmental Test Chamber (Sanyo MLR-351H, SANYO Electric Co., Ltd., Japan) (10 to 40 °C, RH 55%). The temperature model was double-checked with HygroPalm 3 (Rotronic AG, Switzerland). For illumination two compact fluorescent lamps OSRAM DULUX® S, 11W (OSRAM, Italy) were located 50 cm over arena and directed upwards to provide diffused illumination. Illumination on the arenas (20 lux) was measured by the Digital Light Meter TES-1335 (TES Electrical Electronic Corp., Taiwan). Video was recorded with computer-centred camera USB Webcams C120 (Logitech Inc., USA) fixed at the same height as the lamps.

Procedure

Two types of experiments were conducted: experiments with linearly changing temperature conditions and with constant temperature conditions. In the former the temperature program ran 50 min, the temperature rising linearly at 0.6 °C per min from 10 °C to 40 °C (figure 1A, B). The test beetles were introduced to the test arena 15 min before filming, when the test chamber was at 10 °C. This time allowed them settle down and become acquainted with the environment. This procedure was repeated with 12 beetles.

For the other experiments the temperature program was steady throughout the recording (50 min). Temperature was increased from 10, 15, 20, 25, 30, 35 and 40 °C. The test beetles were introduced to the arena, and video-recording began 15 min after optimal temperature was reached. Four repetitions were performed for each temperature level. The temperature program and video-recording were started simultaneously. Deput Video Capture Software (NCH Software, USA) was used, with applied frame rate of 5 frames per second.

Data management and statistical analysis

A video tracking system (EthoVision XT 11, Noldus, Netherlands) was used for gathering data on activity parameters: total distance travelled, average distance travelled per minute, and mean- and top speed. Video was analyzed at 2.5 frames per second.

Statistical analysis was carried out with R software, version 3.1.2 (R Development Core Team, Austria), and package ade4 (Dray & Dufour 2007) was used for principal component analysis.

RESULTS

Linearly changing temperature conditions

Patterns of LA of *P. oblongopunctatus*, in linearly changing temperature conditions, were characterized by low LA in the beginning of the experiment and elevated LA in the second part of the experiment, in the higher temperature zone. Activity patterns were distributed into two distinct categories. 42%
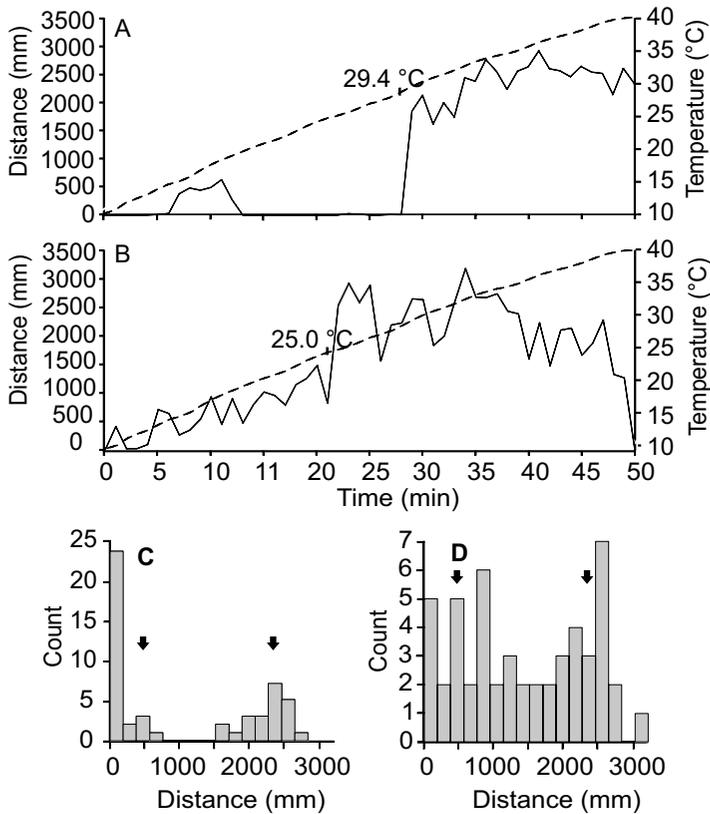

Fig. 1. Sample locomotor activity plots of *P. oblongopunctatus* in a linearly increasing temperature conditions ranged from 10–40 °C (A, B). Displayed temperatures are the threshold temperatures where the locomotor activity of the beetle changed rapidly from low to high. The travelled distance histograms (C, D) were constructed to determine the threshold temperatures. Two maxima show distinct difference in the travelled distance. Left and right maximum show low and high locomotor activity, respectively

of the data are described by pattern on sample recording on Figure 1 A, where in the first half the beetle mainly stood still and the 58% of the data are described by pattern on sample recording on figure 1B, where the beetle are active throughout the experiment, but less active in the first half, on lower temperature zone. Temperature where the change occurs was determined with the travelled distance histograms (Figs 1C, 1D). Two maxima show a distinct difference in distance travelled. Left and right maximum show low and high LA, respectively. The average threshold temperature was 25.83 °C.
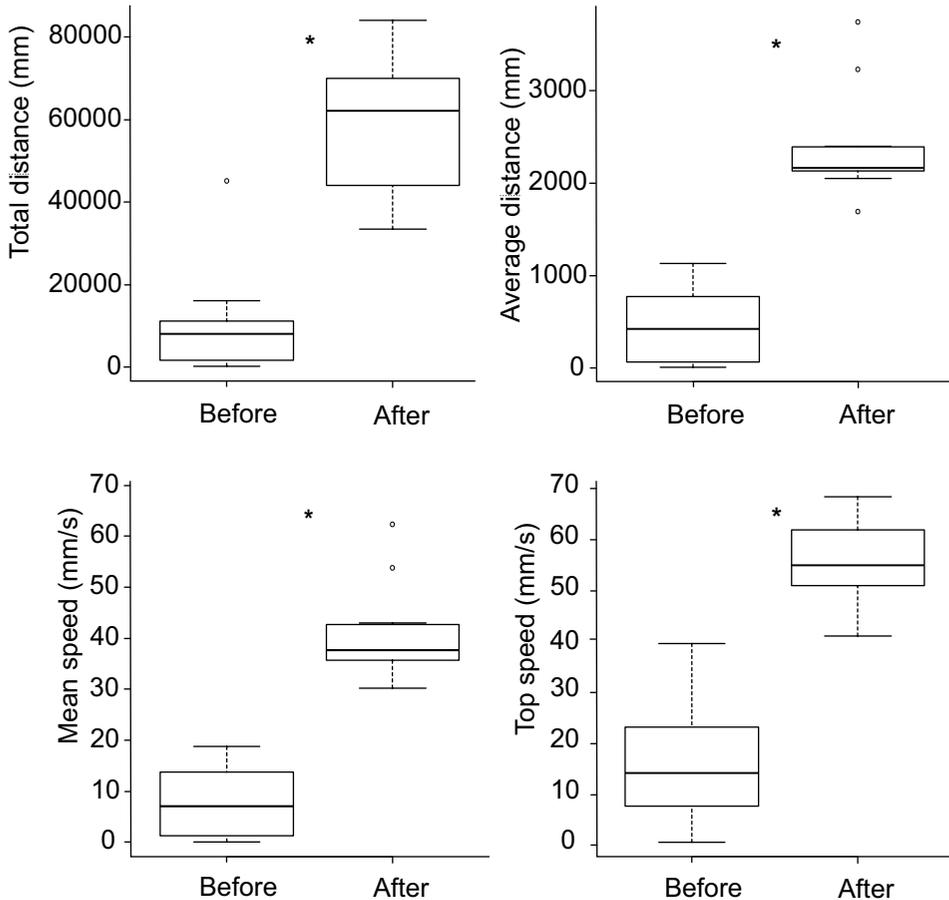

Fig. 2. Parameters describing the beetles' movement in low and high activity zones (A – D). The zones are defined by threshold temperatures, which is identified as point in temperature scale where level of activity promptly changes. The box and horizontal bar within represent the interquartile range and median of the parameters, respectively. The whiskers extend to the most extreme data point, which is no more than 1.5 times the interquartile range from the box. Dots represent outliers. Significant comparisons, as revealed in two-sided Wilcoxon rank sum test, are depicted by "*" ($p < 0.001$)

Measured LA parameters showed a distinct difference before and after threshold temperature (Wilcoxon Rand Sum Test, $p < 0.001$). Median of total distance moved per minute increased 7.75 times, being 8 009.76 mm in the low activity zone and 62 057.12 mm in the high activity zone. A 5.1-fold increase in average distance travelled, from 421.57 mm to 2 161.13 mm, was observed. In addition, both mean and top speed increased with temperature increase from 7.03 mm/s to 37.58 mm/s and from 14.25 mm/s to 54.95 mm/s, respectively, a 5.3 and 3.9-fold rise.

Different levels of steady temperature conditions

To analyse the main tendencies between activity parameters and temperature levels, a principal component analysis (PCA) was performed. Results of the PCA indicated a strong correlation between total distance and mean speed at: 10, 15, 20, and 40 °C; and 25, 30, and 35 °C. The two temperature groups were poorly correlated (Fig. 3). The first two axes explain 90.58% of the variation (84.96% and 5.62% for PC1 and PC2, respectively).
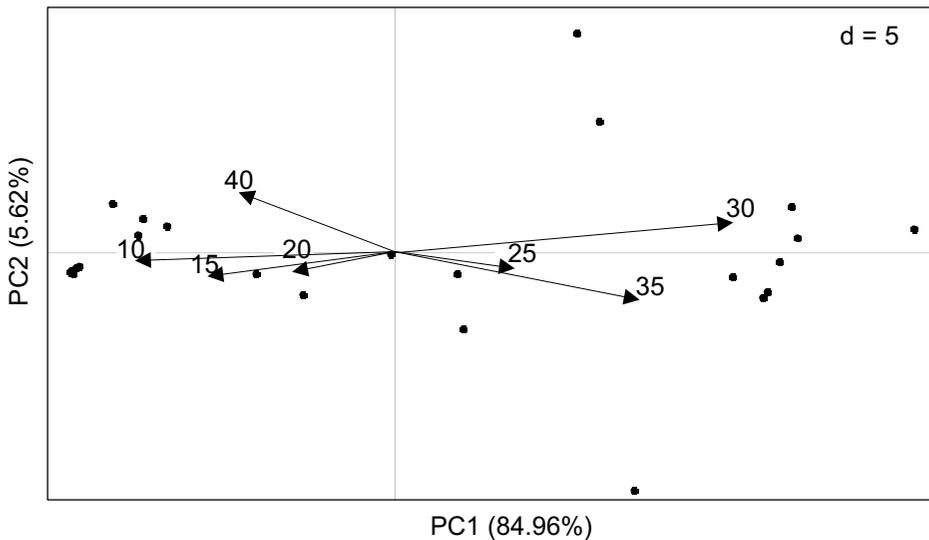

Fig. 3. Principal component analysis (PCA) of locomotion parameters data. The biplot shows the correlation between the distance travelled and mean speed (scores) as dots and temperature effect (loadings) as vectors. Vectors that are close together are highly correlated, while vectors that are orthogonal are poorly correlated. The length of a vector is proportional to the importance of the descriptor to the sites

DISCUSSION

In laboratory behavioural tests, we observed tranquil LA in the lower temperature zone and elevated LA in stressful temperature zone of *P. oblongopunctatus*, with an average threshold temperature of 25.83 °C. The threshold temperature for activity change coincided with the end of the preference temperature zone, which is in agreement with Thiele (1977). Similar results were found on *A. obscurus*, LA patterns being the same, but the threshold temperature for elevated LA was ca 2 °C higher (27.5±0.75 °C) (Nurme *et al.* 2018). The 2 °C difference could be explained with different habitat preference, *P. oblongopunctatus* being a forest species and *A. obscurus* preferring fields.

Often species have an optimal or preferred temperature zone, where the performance curve maximum lies (Esterhuizen *et al.* 2014, Kjaersgaard *et al.* 2015, Schou *et al.* 2013, Hough-Goldstein *et al.* 2016). By contrast, animal's spontaneous locomotor activity remains low at their preferred temperatures until they do not have the need to move (Bayley 2002). Our results showed that in *P. oblongopunctatus*, low spontaneous locomotion zone lay in the range of 10 to 20 °C well coinciding with its preferred temperature range of 10 to 25 °C (Thiele 1977). In the possibly dangerous temperatures they start to look for way out (avoidance behaviour), remaining active as long as possible (Kjaersgaard 2010, Stevenson 1985).

At unfavourably high temperatures (≥40 °C), we observed LA declines in *P. oblongopunctatus*. Reduced locomotion at very high temperatures has been observed in many insect species (Kjaersgaard 2010, Uyi 2017, Woods 2015, Dillon *et al.* 2012, Anderson *et al.* 2007). Kjaersgaard (2010) proposed that "a possible explanation for this could be that the exposure is so severe that any effect of thermal adaptation is effectively overridden by high thermal instability of the cells and membranes compromising the locomotor system and survival".

Behavioural regulation of body temperature is established via selection of preferred temperatures initiated by the peripheral nervous system. Each nervous signal carries on information from the environment. Previous electrophysiological experiments with *P. oblongopunctaus* have also shown that at 25–30 °C thermosensitive neurons start to switch from regular neuroimpulse firing to burst-like firing (Nurme *et al.* 2015, Must *et al.* 2017). Thus, according to our hypothesis, in *P. oblongopunctatus*, the threshold temperatures for elevated locomotion (25.83 °C) and neuronal spike bursting well coincide. Burstiness of neurons have been shown in many sensory systems, from mammalian (Dykes 1975, Sherman 2001, Gallar *et al.* 2003) to insect (Orthoptera) auditory (Marsat & Pollack 2006, Eyherabide *et al.* 2008, Triblehorn & Jager 2005) and visual systems (McMillan & Gray 2015). In the case of insects, it

has been clearly shown that bursts of the neurons initiate strong behavioural responses, while firing in a regular manner does not. In addition, the timing of the burst is a key to looming destiny, burstiness of the spike trains increases with information relevance. Therefore, the bursts are catalysts of escape behaviour – predator evasion, collision avoidance, etc. In this study, we show that the threshold temperatures for spike bursting of the thermosensitive neurons in dome shaped sensilla coincide with elevated mobility of the *P. oblongopunctatus*. This suggests that the spike bursts are responsible for encoding stressfully high ambient temperatures, inducing escape behaviour in beetles to avoid overheating.

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