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Environmental temperature affects the dynamics of ingestion in the nectivorous ant *Camponotus mus*

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ABSTRACT

Environmental temperature influences physiology and behavior in animals in general and is particularly determinant in ectotherms. Not least because temperature defines metabolism and body temperature, muscle activity in insects also strongly depends on this factor. Here, we analyzed how environmental temperature influences the dynamics of ingestion due to its effect on the sucking pump muscles in the nectivorous ants *Camponotus mus*. Feeding behavior and sucking pump activity during sucrose solution ingestion were first recorded in a natural environment in an urban setting throughout the day and in different seasons. Then, controlled temperature experiments were performed in the laboratory. In both situations, feeding time decreased and pumping frequency increased with temperature. However, different pumping frequencies under a same temperature were also observed in different seasons. Besides, in the laboratory, the volume of solution ingested increased with temperature. Consequently, intake rate increased when temperature rose. This change was exclusively promoted by a variation in the pumping frequency while volume taken in per pump contraction was not affected by temperature. In summary, environmental temperature modified the dynamics of ingestion and feeding behavior by directly affecting pumping frequency.

1. Introduction

It is widely known that environmental temperature is a crucial factor for the development, physiology and behavior of ectotherms. Temperature regularly changes with the time of day among days and seasons. Hence, animals adjust their behavior and physiological systems in accordance with these variations. For example, during the embryonic phase, temperature affects time of development (flies, mosquitoes, butterflies, beetles: Ratte, 1985), larval growth (blowflies: Hückesfeld et al., 2011), brain synaptic organization (honeybees: Groh et al., 2004). During adult life, it affects behavioral responses (ants: Weidenmüller et al., 2009), longevity, size and weight, fecundity (Ratte, 1985), gustatory responses (blowflies: Dethier and Arab, 1958; fruit flies: Napolitano et al., 1986), locomotion (dragonflies: May, 1981; beetles: Oertli, 1989; ants: Heinrich, 1993) and foraging preferences (bumblebees: Whitney et al., 2008), among others.

Physiological processes function optimally within a limited temperature range, which is determined by the environment where the insect has evolved and where it inhabits (Chapman, 1998). Within this optimal range, enzyme activity, tissue functions and behavior show their best performance. Temperatures above and below this optimal range can lead to death if conditions persist for a long period of time. Insects can use behavioral responses to avoid thermal injuries. Moreover, insects modify their behavior even within the optimal thermal range (Chapman, 1998) in order to fine-tune their responses in accordance to short-term environmental changes.

In ants, foraging activity almost completely depends on temperature (Hölldobler and Wilson, 1990; Heinrich, 1993). Inside the nest, conditions can be maintained relatively controlled and more stable than those of the environment (Wilson, 1971; Heinrich, 1993; Jones and Oldroyd, 2006; Bollazzi and Roces, 2010). However, once outside, ants immediately acquire a body temperature that is set by the surrounding conditions (Heinrich, 1993). Thus, environmental factors determine when and where to forage. They define the beginning and end of foraging activity (Hölldobler and Wilson, 1990; Cerdá et al., 1998; Pol and Lopez de Casenave, 2004; Bucy and Breed, 2006) and also affect other behavioral variables, such as locomotion velocity and recruitment (Hölldobler and

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Wilson, 1990; Heinrich, 1993; Cassill and Tschinkel, 2000; Azcárate et al., 2007; Hurlbert et al., 2008; Bollazzi and Roces, 2011; van Oudenhove et al., 2011). Despite the extensive list of publications on this topic, relatively few studies have considered temperature effects on variables related to the dynamics of fluid ingestion in ants (Bonser et al., 1998; Cassill and Tschinkel, 2000).

Fluid intake in insects is achieved by the sucking pump, which is composed of different sets of muscles that by rhythmical contractions dilate and adduct the pharyngeal and/or the cibarial chamber. The contraction and relaxation of the dilator muscles expands and reduces the cavity they are attached to and therefore, generate a negative pressure that drives the fluid into the chamber and then carries the fluid towards the esophagus.

Sucking pump activity has been well characterized for the nectivorous ant *Camponotus mus*. Different factors, such as nectar concentration, colony starvation level and the amine serotonin (previously ingested) affect pumping activity. The dynamics of ingestion can vary by modifying intake rate, pumping frequency, volume of solution taken in per pump contraction or total number of pump contractions (Josens et al., 2006; Falibene and Josens, 2008; Falibene et al., 2009, 2012).

It is well known that muscle activity is influenced by temperature in both invertebrates and vertebrates. Maximal force, rate of force generation, contraction, relaxation and power output of muscles are all altered by body temperature (Bennett, 1985; Heinrich, 1993). Thus, it seems likely that temperature also has an effect on the activity of the sucking pump muscles.

In the present study, we examined how environmental temperature influences sucking pump activity and, consequently, the dynamics of ingestion in nectivorous ants. We recorded the behavior of ants during sucrose solution intake in their natural environment within an urban setting first. We analyzed the relationship between environmental conditions and sucking pump activity and studied temperature-dependent changes in pumping frequency throughout the day and the influence of seasonality on its modulation. Secondly, in laboratory experiments under controlled conditions, we evaluated in detail the effect of temperature on different feeding variables and sucking pump activity.

2. Materials and methods

2.1. Feeding behavior recordings

Feeding behavior and sucking pump activity were recorded during sucrose solution intake using a non-invasive set-up as described in previous studies (Josens et al., 2006; Falibene and Josens, 2008; Falibene et al., 2009, 2012). Briefly, a wooden bridge (2 cm × 7 cm) led to the recording arena which consisted of a metallic mesh (approximately 2 cm × 2 cm) covered by a thin layer of conductor gel and a wet filter paper underneath. An Eppendorf tube (0.5 ml) was inserted in a central hole so that the open extreme of the tube levelled with the metallic mesh. The tube was completely filled with sucrose solution until a little drop was exposed on the top, making sure that there was no contact with the mesh. One electrode was fixed to the metallic mesh while the other one was in contact with the solution. When the ant stood on the mesh and contacted the solution with its mouthparts the circuit closed, which allowed to record the electrical signals generated by the ant during feeding (amplification 210×; band-pass filter 0.4–17 Hz, −3 dB; sampling rate: 200 Hz). The records were observed and stored on a computer using an analogue-to-digital converter (ADC-212, Pico Technology Limited, UK).

Pumping frequency (i.e. number of contractions of the sucking pump muscles per second, p/s) was defined as the predominant frequency (the highest peak) in the periodogram (energy × frequency) which resulted from the analysis of the entire signal (entire intake). *Feeding time* (min) represents the duration of the electrical signal and coincides with the time that the ant was in contact with the drop of sucrose solution. *Total number of pump contractions* in the entire intake was estimated by multiplying the predominant frequency by the feeding time. In laboratory experiments, every single ant was weighed before (initial weight) and after (final weight) feeding. Load mass was obtained from the difference between final and initial weights. *Volume of solution ingested* (μl) was calculated by dividing the load mass by the density of the sucrose solution obtained from tables (Wolf et al., 1984). Then, *Intake rate* (μl/min) resulted from dividing the volume of solution ingested by the feeding time. Finally, the *Volume of solution ingested per pump contraction* (nl/p) was estimated from the relation between the volume of solution ingested and the total number of pump contractions.

2.2. Field recordings

Feeding behavior of *C. mus* foragers was recorded in natural conditions in an urban area. A domiciliary colony (34°33′S, 58°33′W, Buenos Aires, Argentina) was identified and foraging behavior was observed throughout the day, for several days between September 2009 and November 2010. *Spring 2009:* 29 September (*Sep 09*), 22 October (*Oct 09*), 26 November (*Nov 09*) 2009; *summer 2010:* 9 January (*Jan 10*), 18 February (*Feb 10*); *autumn 2010:* 30 March (*Mar 10*); *spring 2010:* 5 November (*Nov 10*). Because of lack of foraging activity, no data were recorded in winter. Each recording day started approximately at 8 am local time. Foraging activity was computed by observing the presence or absence of ants in the main foraging trail. When activity was observed, a wooden stick was laid on the main foraging trail waiting for individuals to walk over. Then, individual ants were gently carried to the recording arena placed among the vegetation, near the main foraging trail. Once on the arena, ants were not disturbed and were allowed to find and drink the solution, and then leave the arena by themselves. For each ant, we recorded feeding time and sucking pump activity while drinking 30% w/w sucrose solution, as well as environmental (air) temperature and relative humidity near to the arena (within a 20 cm area around it). We did not weigh the ants before or after solution intake in these assays. Sucrose solution was frequently replaced to avoid concentration changes due to evaporation.

2.3. Laboratory experiments

In this series, we evaluated temperature effects on feeding behavior and sucking pump activity under controlled conditions. We used an adaptation of the set-up previously described that consisted of a recording arena inside a temperature-controlled chamber (Fig. 1). The chamber (20 cm × 12 cm × 12 cm) was made of a Styrofoam-box with an acrylic lid. Inside, there was the recording arena: a plastic flask with a circular base (with a diameter of 6 cm and 3 cm high fluon-painted walls) with a circular metallic mesh (2.5 cm diameter) on filter paper placed in the center. An Eppendorf tube filled with a 30% w/w sucrose solution was inserted in a central hole. Electrodes were arranged as previously described. Ants accessed the recording arena by connecting the flask where they were weighed to a lateral entrance. Chamber temperature was controlled by means of a regulated-flow pump that pushed air through a copper streamer submerged in a thermal bath. Inside the chamber, temperature and relative humidity (RH) were measured just above the recording arena for each record.