



# 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.

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## 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 Rocas, 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

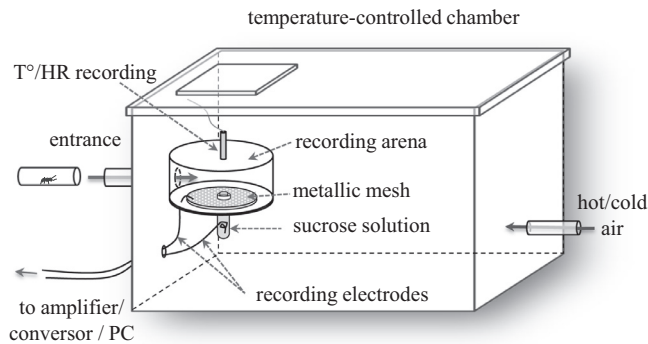
frequency (the highest peak) in the periodogram (energy × frequency) which resulted from the analysis of the entire signal (entire intake). *Feeding time* (min) represents the duration of the electrical signal and coincides with the time that the ant was in contact with the drop of sucrose solution. *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.



**Fig. 1.** Diagram of the set-up used in the laboratory to record sucking pump activity under temperature controlled conditions (see text for details).

We used three different *C. mus* colonies (each one composed of around 1000 workers and one or more queens) collected in Buenos Aires (34°32'S, 58°26'W), Argentina. Each colony was reared in an artificial nest or container which consisted of a plastic box (30 cm × 50 cm × 30 cm) with its base coated with plaster and its walls painted with fluon to prevent animals from escaping. Colonies were housed in piled acrylic plates and workers had access to fresh water, honey-water and chopped insects within the container. Nests were maintained in the laboratory for one year under natural light/dark cycles and nearly constant temperature ( $23 \pm 3^\circ\text{C}$ ). Prior to experiments, colonies were submitted to carbohydrate starvation for a period of  $10 \pm 3$  days.

Experiments were performed on several days in summer (February–March 2010). All the ants used in the experiment were foragers. To ensure that, before each assay, a group of around ten ants was allowed to feed at the experimental device and then return to the nest using a wooden stick vertically positioned inside it. These ants established a pheromone trail to the sucrose solution. Afterwards, recruited ants were individually taken from the vertical stick, gently placed on a flask (1 cm diameter, 3 cm high) and weighed (Mettler Toledo balance, resolution to the nearest 0.01 mg) in order to record the ant mass (initial weight). The tube was then connected to the recording arena. In every case, ants remained at least 2 min inside the chamber until they found the drop of sugar solution and started feeding. Then, electrical signals were recorded during solution intake at three different temperatures: 17, 24 and  $30^\circ\text{C}$ , approximately. The RH inside the controlled-temperature chamber fluctuated throughout the day with room RH. As recordings at different temperatures were made in alternate order during the day, this variation was compensated among treatments. Ants from the same colony were tested under the three temperatures but each ant was tested only once and under only one temperature. Ants were weighed again after feeding to obtain the final weight (ant mass plus load mass) and then kept separate from the rest of their nest mates until the end of the experiment in order to avoid pseudoreplication. Sucrose solution was frequently replaced to avoid concentration changes due to evaporation.

#### 2.4. Statistical analysis

Statistical analyses were performed using InfoStat statistical software (Di Rienzo et al., 2008). For field observations, we analyzed the relationship between the pumping frequency and the temperature using a Dummy Variables Regression analysis (Balzarini et al., 2008). We selected the recording date as a dummy-variable regressor (indicator variable, 7 levels). This test analyzes the relationship between both variables using a

regression model but considering pooled data (grouped by the recording day) and it also allows the study of the differences among groups by a test of slopes homogeneity.

In order to evaluate whether there were differences in the pumping frequency at a certain temperature between different dates, we calculated the confidence intervals for the pumping frequency for each day for 17, 24 and  $30^\circ\text{C}$ . For each of these temperatures, we considered only the dates in which this temperature was recorded i.e. we did not extrapolate the data.

For laboratory experiments, we compared the feeding variables between temperatures either by one-way ANOVA tests, followed by Fisher comparisons, or by Kruskal–Wallis tests, followed by Dunn comparisons, when data did not comply with normality or homogeneity of variance assumptions. The significance level was set to 5% in all cases.

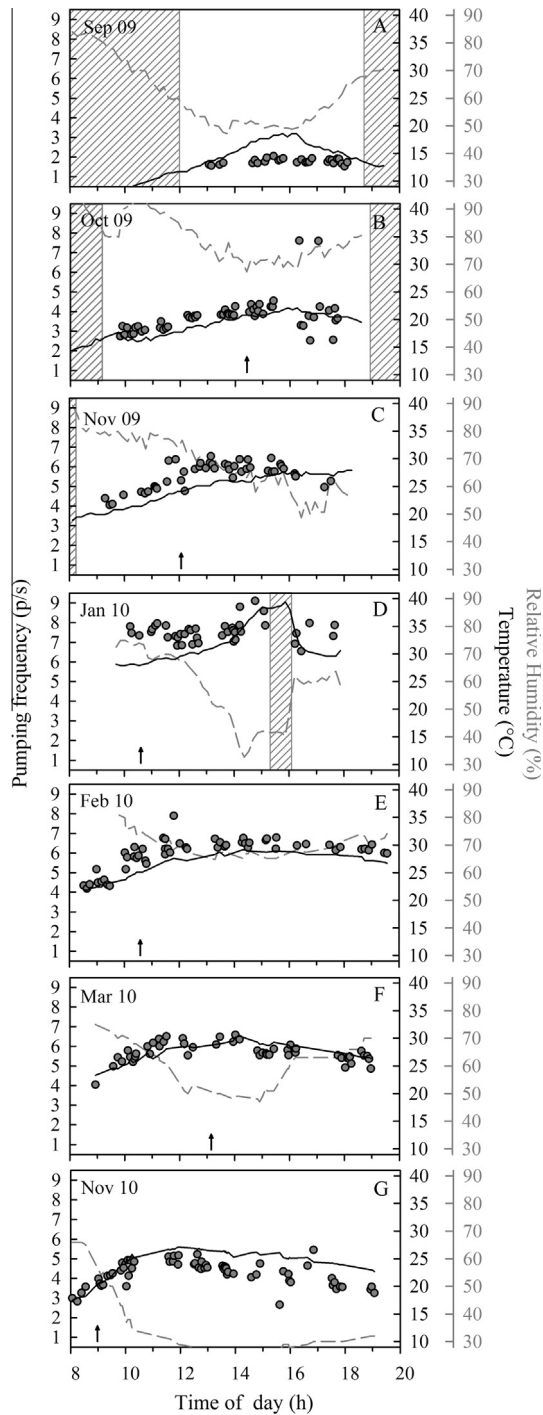
### 3. Results

#### 3.1. Field recordings

A strong relationship was observed between the environmental temperature and foraging behavior in the field. Ant activity in the foraging area varied with environmental conditions. Activity was observed only in the warmest months and within a certain temperature range. No activity was observed during the coldest months (between May and August) or when temperature reached extreme values (lower than  $16 \pm 4^\circ\text{C}$  and higher than  $37 \pm 1^\circ\text{C}$ ) during spring, summer and autumn. No foragers were observed in the main trail or in the surrounding vegetation during the morning or the afternoon of Sep 09, when temperatures were lower than  $12^\circ\text{C}$  (striped area in Fig. 2A), or during the morning or the afternoon of Oct 09, when temperatures were lower than 16 and  $20^\circ\text{C}$ , respectively (striped area in Fig. 2B). In the other extreme of the temperature range, an interruption of foraging activity was computed when values exceeded  $38^\circ\text{C}$  in the afternoon of Jan 10 (striped area in Fig. 3D) and resumed again when temperature dropped below  $36^\circ\text{C}$ . In general terms, feeding time decreased with temperature (Fig. 3.  $R^2 = 0.6$ ,  $p < 0.0001$ , pooled data, Non-Linear Regression). RH correlated negatively with environmental temperature ( $p < 0.01$  for all dates; Pearson's correlation test).

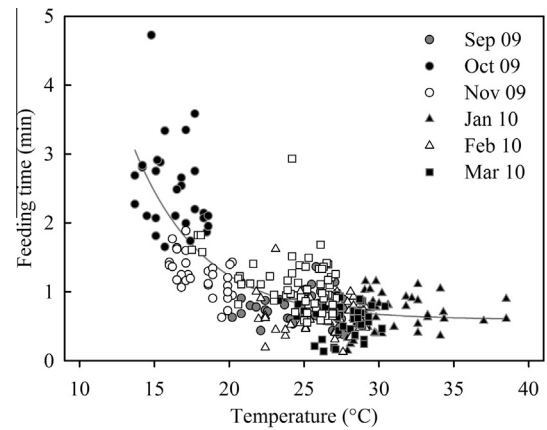
Typically, on all recording dates in the field, environmental temperature was relatively low at the beginning of the day, reached the maximum value generally between 2 and 4 pm and then dropped. Ant feeding behavior changed in accordance to this variable. Pumping frequencies seemed to follow a similar pattern as that of temperature throughout the day: they were relatively low at the beginning of the day, increased until early afternoon and then dropped (Fig. 2). A linear relationship was found between both variables (Fig. 4A.  $F_{1,323} = 88.7$ ,  $p < 0.0001$ , Dummy Variables Regression analysis): the higher the temperature, the higher the pumping frequency. However, this relationship was not constant among recording dates, i.e. slopes were not constant: an increment in one unit of temperature did not always promote the same rise in pumping frequency. The test of slope homogeneity (Table 1) displayed significant differences among dates. Slopes of both extremes (Sep 09 and Jan 10) were lower than those intermediate but similar between themselves, which is also reflected in the  $Q_{10}$  values (the change in pumping frequency for a temperature increase of  $10^\circ\text{C}$ ; Table 2). The ranges of temperatures for each day are also shown in Table 2.

We then tested if in field conditions of a given temperature ants could pump at different frequencies while drinking. We compared the pumping frequency confidence intervals for three different

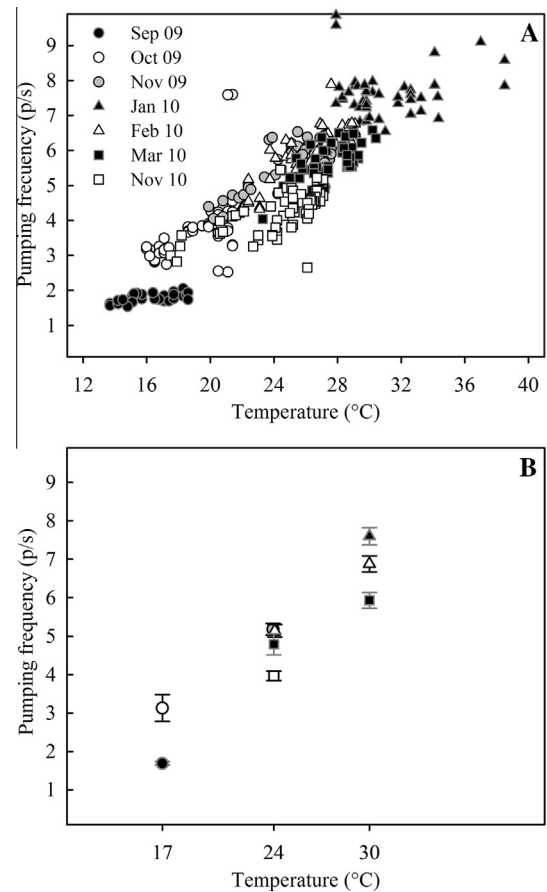


**Fig. 2.** Foraging behavior in the field. Pumping frequency during sucrose solution intake was recorded throughout the day for seven different dates in different seasons: (A) September, (B) October and (C) November 2009 (*spring*), (D) January, (E) February (*summer*), (F) March (*autumn*) and (G) November 2010 (*spring*). Every dot represents an ant; the black line indicates the ambient temperature and the gray dotted line the ambient relative humidity. The striped area indicates the absence of foraging activity (no ants in the main foraging trail) and the black arrow shows the moment when the ants started to arrive at the recording arena by themselves. In (A) no ants arrived on their own; all registered individuals were taken from the main trail and carried to the arena.

temperatures: 17, 24 and 30 °C. We effectively found significant differences among recording dates for each temperature (Fig. 4B). Therefore, pumping frequency when drinking is not only determined by temperature.



**Fig. 3.** Feeding time according to ambient temperature in field observations. Dates from each recording date are differentiated in the graph. Each symbol represents an individual ant. Curve was obtained from pooled data and adjusted to an exponential decay: feeding time =  $0.6 + 45.99(-0.21^{\text{temp}})$ .



**Fig. 4.** (A) Pumping frequency according to ambient temperature for different dates in the field. A positive relationship was found between these variables. Each symbol represents a recording. (B) Pumping frequency confidence intervals (95%) for three different temperatures. Each symbol shows the estimated pumping frequency for each temperature and lines indicate the  $\pm 95\%$  confidence limit. No overlapping lines indicate statistical differences.

### 3.2. Laboratory experiments

Laboratory recordings concurred with field observations in as much as temperature greatly affected feeding behavior. The acceptance of the solution offered in the recording arena was 84% ( $N = 44$ ), 100% ( $N = 37$ ) and 97% ( $N = 38$ ) at 17, 24 and 30 °C,



**Table 1**

Comparison of pumping frequency change according to temperature variation in different recording dates in field observations. *P* values were obtained by test of slope homogeneity (pumping frequency vs. temperature). Significant differences ( $p < 0.05$ ) are marked in bold.

Date	Oct 09	Nov 09	Jan 10	Feb 10	Mar 10	Nov 10
Sep 09	<b>0.005</b>	<b>0.02</b>	0.81	<b>0.03</b>	0.06	0.08
Oct 09		0.42	<b>&lt;0.0001</b>	0.64	0.31	0.05
Nov 09			<b>0.0001</b>	0.11	0.73	0.25
Jan 10				<b>&lt;0.0001</b>	<b>0.004</b>	<b>0.001</b>
Feb 10					0.19	0.067
Mar 10						0.58

**Table 2**

Temperature range and  $Q_{10}$  for each recording date.  $Q_{10}$  (the change in pumping frequency for a temperature increase of 10 °C) was calculated as:  $Q_{10} = (F_{T_{\max}}/F_{T_{\min}})^{10/(T_{\max}-T_{\min})}$  where  $T_{\max}$  and  $T_{\min}$  are the maximal and minimal temperature of the range, respectively; and  $F_{T_{\max}}$  and  $F_{T_{\min}}$  are the pumping frequency (estimated from the regression equation) at  $T_{\max}$  and  $T_{\min}$ .

Date	$T_{\min}^{\circ}$	$T_{\max}^{\circ}$	$Q_{10}$
Sep 09	13.7	18.6	1.3
Oct 09	16.0	21.4	2.1
Nov 09	19.9	27.6	1.5
Jan 10	27.9	38.5	1.0
Feb 10	21.8	29.1	1.7
Mar 10	23.3	30.4	1.4
Nov 10	17.5	27.1	1.6

respectively. At higher temperatures ants left the recording arena carrying a greater volume of ingested solution (Fig. 5A.  $F_{2,110} = 9.62$ ,  $p = 0.0001$ ; ANOVA. 17 vs. 24 °C:  $p = 0.06$ ; 17 vs. 30 °C:  $p < 0.0001$ ; 24 vs. 30 °C:  $p = 0.02$ ; Fisher comparisons). Furthermore, they drank for a shorter period of time (Fig. 5B.  $H_{2,N=113} = 47.42$ ,  $p < 0.0001$ ; Kruskal–Wallis). Ants at 17 °C drank for longer than ants under 24 and 30 °C (17 vs. 24 °C:  $Q = 6.18$ ,  $p < 0.001$ ; 17 vs. 30 °C:  $Q = 7.74$ ,  $p < 0.001$ ; 24 vs. 30 °C:  $Q = 2.02$ ,  $p > 0.05$ ; CV = 2.394; Dunn comparisons). Otherwise, the total number of pump contractions performed during an intake increased with temperature (Fig. 5C.  $F_{2,110} = 3.35$ ,  $p = 0.039$ ; ANOVA. 17 vs. 24 °C:  $p = 0.81$ ; 17 vs. 30 °C:  $p = 0.02$ ; 24 vs. 30 °C:  $p = 0.04$ ; Fisher comparisons). As the volume of solution ingested increased and the feeding time decreased with temperature, the intake rate significantly increased with temperature (Fig. 5D.  $H_{2,N=113} = 84.19$ ,  $p < 0.0001$ ; Kruskal–Wallis. 17 vs. 24 °C:  $Q = 4.66$ ,  $p < 0.001$ ; 17 vs. 30 °C:  $Q = 8.56$ ,  $p < 0.001$ ; 24 vs. 30 °C:  $Q = 5.41$ ,  $p < 0.001$ ; VC = 2.394; Dunn comparisons). This change was exclusively promoted by a variation in the pumping frequency. In the same way as for field recordings, the temperature experienced by ants during the intake modified pumping frequency ( $H_{2,N=113} = 99.55$ ,  $p < 0.0001$ ; Kruskal–Wallis). The higher the temperature, the higher the pumping frequency (Fig. 5E. 17 vs. 24 °C:  $Q = 4.25$ ,  $p < 0.001$ ; 17 vs. 30 °C:  $Q = 9.10$ ,  $p < 0.001$ ; 24 vs. 30 °C:  $Q = 6.87$ ,  $p < 0.001$ ; CV = 2.394; Dunn comparisons). However,  $Q_{10}$  values obtained during the laboratory experiments were higher for the 17–24 °C temperature range ( $Q_{10} = 2.64$ ) than for those obtained for the 24–30 °C range ( $Q_{10} = 1.89$ ). On the other hand, volume taken per pump contraction was not affected by temperature (Fig. 5F.  $F_{2,110} = 0.75$ ,  $p = 0.47$ ; ANOVA). The RH inside the controlled-temperature chamber did not vary with experimental temperature (17 °C:  $51.7\% \pm 1.8$ ; 24 °C:  $50.9\% \pm 1.7$ ; 30 °C:  $51.0\% \pm 1.6$ .  $F_{2,110} = 0.07$ ,  $p = 0.93$ ; ANOVA).

#### 4. Discussion

Environmental temperature promoted significant changes in feeding behavior, dynamics of nectar ingestion and sucking pump

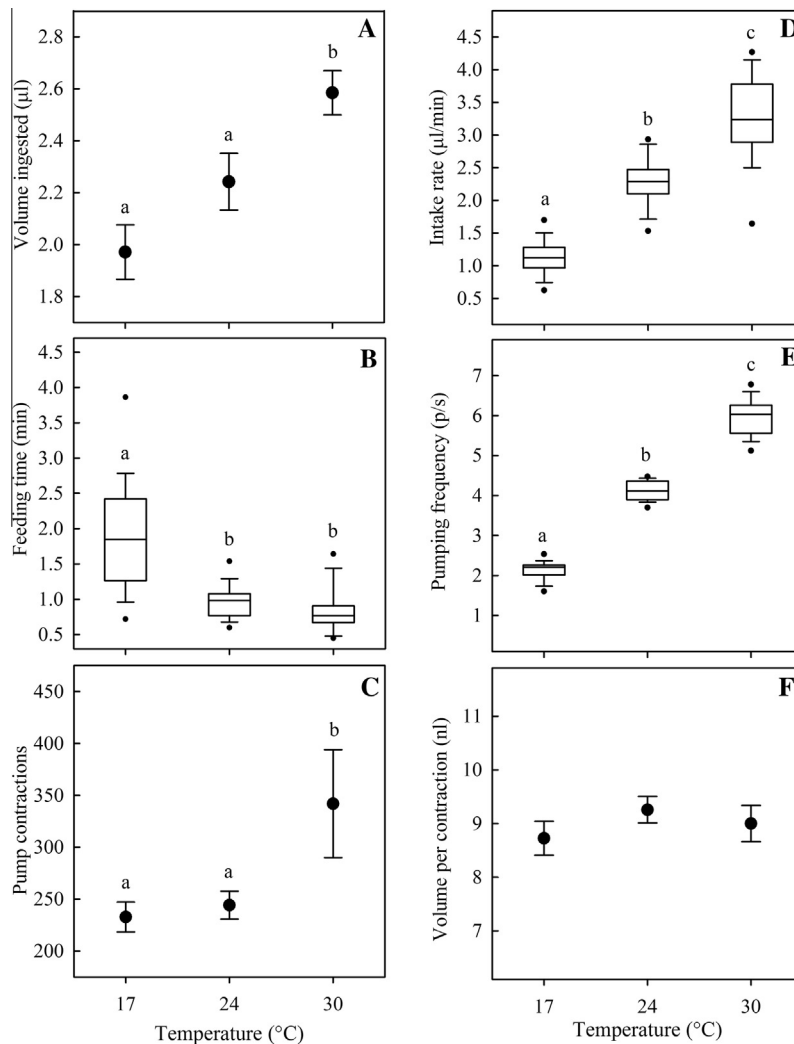
activity in *C. mus* ants. In both field and laboratory experiments we observed a reduction in feeding time and an increase in pumping frequency –and consequently in intake rate– with increasing temperature. Similar to our results, Bonser et al. (1998) observed that the nectivorous ant *Lasius niger* reduces the feeding time drastically as environmental temperature increases in the field (approx. from 9 to 20 °C). These researchers proposed that, among other reasons, an increase in temperature could (i) generate a reduction in the solution viscosity enhancing the solution ingestion and/or (ii) increase the insect metabolic rate promoting an increase in the pumping mechanism. In the butterfly *Thymelicus lineola*, recordings at 25 and 35 °C already showed a decrease of feeding time and an increase of intake rate with temperature due to both a reduction in solution viscosity and an increase in the power output of the sucking pump, proposing that muscles work faster at higher temperatures (Pivnick and McNeil, 1985). The results of our study suggest that the solution viscosity diminution due to an increase in temperature did not affect the volume of solution ingested per pump contraction (see below). *C. mus* ants present direct evidence of how temperature influences the dynamics of nectar ingestion: temperature determined pumping frequency and, consequently, intake rate.

According to Poiseuille's law of fluid dynamics, less viscous solutions flow faster. Considering that nectar viscosity diminishes with temperature, higher temperatures should lead to higher volumes of solution incorporated per pump contraction. However, temperature affects intake rate without modifying the volume taken per pump contraction. As previously mentioned, variations in temperature affected intake rate only by promoting changes in pump frequency.

What are the possible mechanisms underlying this effect? Insect body temperature is generally closely attuned to ambient temperature, so is metabolic rate. In this way, temperature affects insect general activity (Heinrich, 1993). Numerous studies show the close relationship among temperature, metabolic rate and muscular activity. Contraction and relaxation rate and maximal power of striated muscles increase with temperature in both ecto and endothermic vertebrates (Bennett, 1985) as well as in invertebrates (Neville and Weis-Fogh, 1963; Stevenson and Josephson, 1990). Even when most of the studies performed on insect muscle activity are focused on flight muscles (e.g., in moths and locusts), it is very likely that muscles involved in the sucking pump are affected in the same way.

In addition, rhythmic motor patterns, such as locomotion and feeding, are generated by central pattern generators –CPG– (Delcomyn, 1980; Pearson, 1993; Marder, 2000). These CPG are also present in the insect frontal ganglion (Ayali et al., 2002) which innervates sucking pump dilator muscles (Janet, 1905; Miles and Booker, 1998; Ayali, 2004; Davis and Hildebrand, 2006). Temperature was reported to accelerate the motor patterns of feeding in flies (Hückesfeld et al., 2011). Furthermore, the biogenic amine serotonin, which is present in some neurons of the frontal ganglion in *C. mus*, affects sucking pump activity (Falibene et al., 2012) and temperature is also believed to promote changes in the release of neurohormones by changing the electrophysiological properties of neurosecretory cells (Rössler and Bickmeyer, 1993).

Muscles, as well as all physiological systems in insects, work optimally at a given temperature range (Heinrich, 1993). Field recordings showed that the change rates of pumping frequency according to temperature (i.e. the slope between pumping frequency and temperature for each recording day) were lower on those days with extreme temperatures (see Fig. 4A, Sep 09, 13.7–18.6 °C; Jan 10, 27.9–38.5 °C) than on days with intermediate values. That is, the capacity of pumping frequency modulation at both thermal extremes was low. This fact could be related to a physiological limit of the muscle activity, i.e. these extremes might be



**Fig. 5.** Feeding behavior of ants in the laboratory. (A) Volume ingested, (B) feeding time, (C) total number of pump contractions, (D) intake rate, (E) pumping frequency and (F) volume of solution taken per pump contraction under three different temperatures: 17 °C ( $16.9 \pm 0.1$  °C,  $N = 37$ ), 24 °C ( $23.6 \pm 0.1$  °C,  $N = 37$ ) and 30 °C ( $29.6 \pm 0.1$  °C,  $N = 39$ ). (A, C and F) Dots represent the mean value of each group and lines the S.E. (B, D and E) Boxes show quartiles, horizontal lines within each box represent medians, whiskers provide the extreme values and dots indicate outliers. In all graphs, different letters indicate statistical differences.

proximal to the minimal and maximal frequency of contraction. Previous studies performed at ca. 25 °C in *C. mus* ants showed that – by manipulating the carbohydrate starvation of the colony – the sucking pump works between 4 and 7 pumps per seconds (predominant frequencies) (Falibene and Josens, 2008; Falibene et al., 2009). Here we found that by extending the temperature values within the range for which there was field foraging activity (14–36 °C), pumping frequency varied between 1.5 and 9 pumps per second.

In keeping with previous studies (Falibene and Josens, 2008; Falibene et al., 2009) we observed different pumping frequencies under a same temperature on different days, in field recordings. This seasonal variation could be due to different levels of carbohydrate reserves or requirements in the colony in relation to the food availability in the field (Kay, 2002, 2004). In this sense and in agreement with previous studies, seasonal variations could be reflecting different motivational states.

Many ants, especially urban ones, are controlled with toxics added to food, particularly in places where other methods (sprays, aerosols, liquids, emulsions or powders) are not recommended or even contraindicated. In contrast, the use of food baits has virtually

no impact on the environment and has no restrictions for its use in sensitive sites. We have already shown that acceptance and consumption of different kinds of toxic baits at an individual level depend on motivation (Sola et al., 2013). In addition, our present results may have relevance for control bait procedures because they could help to choose the most appropriate days for applying the bait, which would be more profitable. For this species, days with high temperatures not exceeding 34 °C would ensure higher crop loads, faster and shorter intakes. This would result in a greater number of foraging cycles, which would allow greater interaction of the foragers with their nest mates, which may lead to further recruitment and enhance the effectiveness of the treatment.

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