Sociobiology
An international journal on social insects

RESEARCH ARTICLE - ANTS

Effect of Temperature on Nestmate Recognition in the Ant *Odontomachus Chelifer*

Kleber L. Silva[1], Nathan R. Batista[2], William F. Antonialli-Junior[1]

1 - Universidade Estadual do Mato Grosso do Sul, Dourados-MS, Brazil
2 - Universidade Federal da Grande Dourados, Dourados-MS, Brazil

Article History

Edited by
Fábio Santos Nascimento, USP, Brazil
Received 09 June 2023
Initial acceptance 11 July 2023
Final acceptance 11 April 2024
Publication date 25 July 2024

Keywords
Behavior, Communication, Cuticle, Hydrocarbons, Odontomachus, Waterproofing.

Corresponding author
Kleber Luna Silva
Universidade Estadual do Mato Grosso do Sul
Rod. Dourados-Itahum, nº 12
CEP: 79804-970 – Dourados-MS, Brasil.
E-Mail: kleberluna99@gmail.com

Abstract

Cuticular hydrocarbons play multiple roles in social insects. Their primary function is to waterproof the external surface of the body of individuals to prevent desiccation. However, they also act as a chemical signature in social insects, unique to each colony, through which individuals recognize themselves as nestmates. These compounds may change due to exogenous factors, aiming to maintain the integrity of the cuticle. However, changes in cuticular chemical composition may impair recognition among nestmates. Thus, this study tested the hypothesis that nestmates of the ant *Odontomachus chelifer* may change their normal recognition pattern when submitted to different temperature conditions. To do this, groups of workers were kept under two different temperatures, 15 and 30 °C, for 24 hours and then submitted to induced encounters with workers who remained for this same period at a temperature of 25 °C. As a form of control, the same type of encounter was performed between ants that remained separated but at the same temperature and between ants from different colonies. The results show that ants that remain for 24 hours under different temperature conditions have the process of nestmate recognition disrupted, performing more aggressive behaviors and taking longer to touch themselves (antennation) than in the control condition.

Introduction

Ants (Hymenoptera: Formicidae) are social insects that live in colonies of varying size and population that may consist of only a few individuals living in a space of a few centimeters or count on millions of workers living in nests of thousands of kilometers (Wilgenburg et al., 2010; Burchill & Moreau, 2016). For such a social organization to function effectively, it is essential that the colony individuals can communicate efficiently (Turillazzi, 2013). In this sense, the most common communication among social insects is based on chemical compounds (Hölldobler & Wilson, 1990; Le Conte & Hefetz, 2008; Richard & Hunt, 2013).

Chemical communication in social insects can be mediated by two types of compounds, called pheromones: the first type is light molecules of simple chains that are secreted by exocrine glands to the exterior of animal's bodies and cause a specific reaction in individuals of the same species that capture them (Karlson & Butenandt, 1959; Karlson & Luscher, 1959; Yew & Chung, 2015). The second type has longer chains and, in general, is located on the surface of the cuticle of insects (Howard, 1993; Blomquist et al., 2020). Among the compounds present in the cuticle, the hydrocarbons stand out, especially linear alkanes, branched alkanes, alkenes, and alkadienes (Brown et al., 1991; Howard & Blomquist, 2005; Hefetz, 2007; Blomquist & Bagnères, 2010).

Cuticular hydrocarbons (CHCs) perform multiple roles on the cuticle of insects (Howard & Blomquist, 1982; Blomquist & Bagnères, 2010). Their primary function is to prevent water loss (Gibbs, 1998), a significant problem for insects due to their small body size (Gibbs et al., 1997; Singer, 1998; Kühsel et al., 2017). However, they also act as cues

or signals, which are key for communication. The mixture of CHCs found on the cuticle of social insects represents a chemical signature unique to each individual, and that varies between species, populations, colonies, and even between sexes, ages, and functions in the colony (Howard & Blomquist, 2005; Blomquist & Bagnères, 2010; Leonhardt et al., 2016).

The waterproofing ability of the CHCs layer depends on its melting point, which is directly related to its chemical composition (Gibbs, 1995; Sprenger et al., 2018). The compounds with greater aggregation and alignment between the molecules (i.e., the longer carbon chains and the linear ones) have a higher melting point (Gibbs, 1998; Gibbs & Pomonis, 1995; Menzel et al., 2018; Sprenger et al., 2018). The literature mainly highlights linear alkanes as waterproofing agents (Gibbs, 1998; Hefetz, 2007; Chung & Carroll, 2015), while branched alkanes, alkenes, and alkadienes are usually associated with communicative processes (Bonavita-Cougourdan et al., 1991; Dani et al., 2001; Hefetz, 2007; Murakami et al., 2015).

Studies have shown that insects adapt to the climate by modifying the composition of their CHCs according to the temperature of the environment where they live (Gibbs et al., 1997; Rourke & Gibbs, 1999; Wagner et al., 2001; Menzel et al., 2017, 2018; Michelutti et al., 2018; Otte et al., 2018). According to Menzel et al. (2019), critical temperatures (between 30 and 45°C) cause the melting of the compounds present in the cuticle, resulting in a change in the physical state of the lipid layer and, consequently, an increase in permeability. Menzel et al. (2018), in their study with ants of the genus *Temnothorax*, showed that even under different temperature conditions, the individuals presented similar acclimatization responses, increasing the waterproofing of the CHCs layer. Thus, the plasticity of the compounds in the cuticle is crucial to avoid desiccation.

Because of CHCs' multiple roles (Howard & Blomquist, 1982; Blomquist & Bagnères, 2010), changes in their composition in response to temperature changes may alter the recognition ability between nestmates, disrupting the colony's cohesion. Although it is known that temperature variation can, indeed, alter the cuticular composition (Michellutti et al., 2018), little is known about its effects on intracolonial recognition.

In order to investigate how behavior is affected by temperature variation, we choose *Odontomachus chelifer*. These Ponerinae ant species occur in rainforests in the neotropical zone, ranging from Mexico to Argentina (Brown, 2000). This genus is known by its mouthparts, which are specialized to close quickly when sensory bristles are triggered (Carlin & Gladstein, 1989). The colonies of *O. chelifer* vary in size and may contain some dozens of individuals to a few hundred (Fowler, 1980; Guimarães et al., 2018).

Therefore, this study aims to evaluate the effects of different temperature conditions on the nestmate recognition of the species *O. chelifer* (P.A. Latreille). We hypothesize that nestmates may change their usual pattern of recognition when subjected to different temperature conditions.

Material and Methods

Ants Sampling

Eighty foragers of the species *Odontomachus chelifer* from 5 different colonies were collected, totaling 400 ants. The samples were collected in the municipality of Dourados (22°13'24.39"S; 54°54'44.53" W) in Mato Grosso do Sul-MS, Brazil. Foragers were collected returning from their activity to the nest, thus standardizing their age (Crall, 2021). After sampling, the ants were stored in the laboratory under a constant temperature of 25 ºC, in plastic pots of 500 mL, with holes in the lids and sides to allow airflow, and containing water and honey in microtubes of 1.5 mL, as a food resource. The ants remained in these conditions for one day to get used to laboratory conditions.

Effect of Temperature Variation on the Recognition of Nestmates

After the habituation period, three groups of 10 workers and another of 50 workers were separated from each colony in 500 mL plastic pots. Two groups of 10 workers, called treatment groups, were placed in a Biochemical Oxygen Demand (BOD) chamber with a ± 2 °C variation and a light-dark cycle of 12:12 hours. One group was kept under a temperature of 15 °C for 24 hours, while another was under 30°C for the same period. The relative humidity conditions were maintained at 80% ± 10 by placing damped cotton balls in Petri dishes, and a micro tube of 1.5 mL containing honey and another one with water were provided as a food resource.

To control the effect of the different temperature conditions, the group of 50 workers was kept outside the BOD under a constant temperature of 25 °C. Also, to control the effect of the separation of the nestmate ants, another group of 10 workers was kept separated from the first one for 24 hours but under the same temperature of 25 °C. Both groups were fed the same honey and water diet offered in microtubes. This procedure was adopted as a way to control because the separation of nestmates can prevent the homogenization of the cuticular chemical compounds through social interactions, such as group cleaning (allogrooming), which may disrupt the usual pattern of nestmate recognition (Richard & Hunt, 2013). Additionally, to compare the level of aggressivity in intercolonial encounters (i.e., between ants from different colonies) with the levels of the different treatments (intracolonial), foragers from 3 other colonies were collected – being 20 individuals from each colony – to carry encounters between non-nestmates. After the sampling, the ants were kept in the laboratory under the same conditions described above for 24 hours until they acclimatized.

The temperatures under which the ants were kept in BOD were selected according to the maximum and minimum regional temperature averages for 29 years, from 1980 to 2009 (Schneider, 2014), following the methodology suggested by Michelutti et al. (2018). According to the data provided by the

meteorological station of the Empresa Brasileira de Pesquisa Agropecuária – Embrapa/Oeste, the average temperature in the region is 21 °C, with a minimum average of 16 °C and a maximum of 28 °C.

To evaluate whether the adjustment to the different temperature conditions can lead to a change in the pattern of recognition among nestmates, ten paired encounters were induced between ants subjected to the temperature of 15 °C with their nestmates kept for the same period, to 25 °C. The same number of paired encounters were induced between ants kept at 30 °C and their nestmates kept at 25 °C. To test the separation effect, the same number of induced paired encounters were performed between nestmates from two separated groups kept at 25 °C. To evaluate whether the results observed during the encounters differ from the usual interaction pattern of nestmate ants, ten induced paired encounters were performed between nestmates kept under the temperature of 25 °C and in the same pot. Finally, to compare the level of aggressivity shown during these encounters with those between non-nestmates, ten paired encounters were induced between ants from 3 different colonies. It is worth mentioning that a pair of ants were placed to interact only once, regardless of the groups to which they belonged.

To evaluate whether the results might be a consequence of the stress related to manipulation and experimental conditions and not exactly by possible alteration of cuticle composition, ten induced paired encounters were performed between an ant of the group kept under 25 °C and another ant from one of the treatment groups killed by freezing, as has already been done in other studies (Morel et al., 1988; Hernández et al., 2002; Hernández et al., 2006). To do this, we killed the ants from the experimental groups (those acclimatized to temperatures of 15 and 30 °C) right after the paired encounters and used these same ants.

To define which group of workers would start any aggressive act and the recognition difficulty, all ants kept under 25 ºC were marked with nontoxic ink in the thorax region right after the sampling. There was no marking in the encounters between ants from different colonies.

The induced paired encounters were performed in rectangular plastic pots of 500 mL called “arena”. An ant from the group kept under 25 °C was placed in the center of the arena, where it was trapped inside a 50 mL plastic cup turned upside down. Then, an ant kept in the BOD for 24 hours under one of the two temperatures or from the control group was placed in the same arena. After 1 minute, the plastic cup was removed, allowing the encounter between the two ants. Each encounter was held in an arena that had never been used before.

All encounters were recorded for 5 minutes without interruption using a camera. To minimize observer bias, the recordings were analyzed by a blind observer who recorded the occurrence and duration of each behavioral act performed by each ant and which ant started the behaviors.

To evaluate the level of aggressivity during the induced paired encounters, the behaviors displayed received a score scale concerning the level of aggressiveness (modified by Suarez et al., 1999) from 0 to 2, with 0 for touch, antennation, or ignore; 1 for escape, antennal boxing, body lifting and display of abdomen; and 2 for attempted seizure, seizure, bites and fight. Then, an aggression index (A.I.) was calculated using the following formula, adapted from Valadares et al., 2015:

$$A.I. = \frac{\textit{Total number of interactions scoring 1 or 2}}{\textit{Total number of all interactions}}$$

Thus, the A.I. will be between 0 and 1, with 0 being no aggressiveness and 1 being the highest degree of aggression.

The antennation time during each induced encounter was also counted from the first contact between the ants. This parameter can define the difficulty of recognition between the workers during the interactions. When no truly aggressive behaviors exist, the longer antennation time may indicate greater difficulty recognizing nestmates (Starks et al., 1998).

Statistical Analyses

To evaluate whether the level of recognition during the encounters between ants kept under 25 ºC and those of the other treatments is significantly different from that of encounters between ants of the same group kept under 25 °C, Kruskal-Wallis tests were applied, followed by the subsequent Student-Newman-Keuls test, using the A.I. scores given to the encounters and the observed antennation times.

To evaluate whether the level of recognition in the encounters between live ants of the 25 °C group and dead ants of the 15 and 30 °C groups differs significantly from the encounters in which the ants of both groups were alive, paired Wilcoxon tests were applied, using the antennation times during these encounters. All the analyses were performed in the R software (R Core Team, 2023).

Results

A total of 33 hours of interactions between ants from different groups were analyzed, including the experiments where the tested ants were dead and the posterior intercolonial encounters. A total of 1.903 behavioral acts were recorded, 72% categorized as aggressiveness level 0 (touch and antennation) and 28% of aggressiveness level 1 (antennal boxing and display of abdomen). Behaviors categorized as level 2 occurred only in the intercolonial experiments, corresponding to 50% of these encounters being seizures, bites, and fights. The entire dataset of this work is available in Online Resource 1.

The mean level of aggressiveness according to A.I. for encounters between nestmates kept together at 25 °C was 0.10 ± 0.15, while between nestmates kept separated at the same temperature of 25 °C was 0.12 ± 0.15; between ants from the group kept at 25 °C and their nestmates submitted to

15 °C, it was 0.16 ± 0.11; between ants from the group kept at 25 °C and their nestmates submitted to 30 °C, it was 0.19 ± 0.15; and between non-nestmates kept separated at 25 °C was 0.42 ± 0.76 (Figure 1).

The Kruskal-Wallis test shows a significant difference between the mean level of aggressiveness during the different encounters of ants ($p < 0.001$). There is no significant difference between the mean A.I. scored during the encounters between nestmates kept together at 25 °C and that from encounters between these ants with their nestmates who were separated but kept at this same temperature ($p = 0.203$). However, the mean A.I. scored during the encounters between nestmates kept at 25 °C differs significantly from that A.I. from encounters between these ants with their nestmates submitted to the temperature of 15 ºC ($p = 0.008$), as well as those submitted to a temperature of 30 °C ($p < 0.001$). Finally, the mean A.I. scored during encounters between non-nestmates is significantly different from all the other encounters (sets 5 and 1 $p < 0.001$/ sets 5 and 2 $p < 0.001$/ sets 5 and 3 $p = 0.009$), except from that between ants kept at 25 °C with your nestmates submitted to 30 °C ($p = 0.098$) (Figure 1).

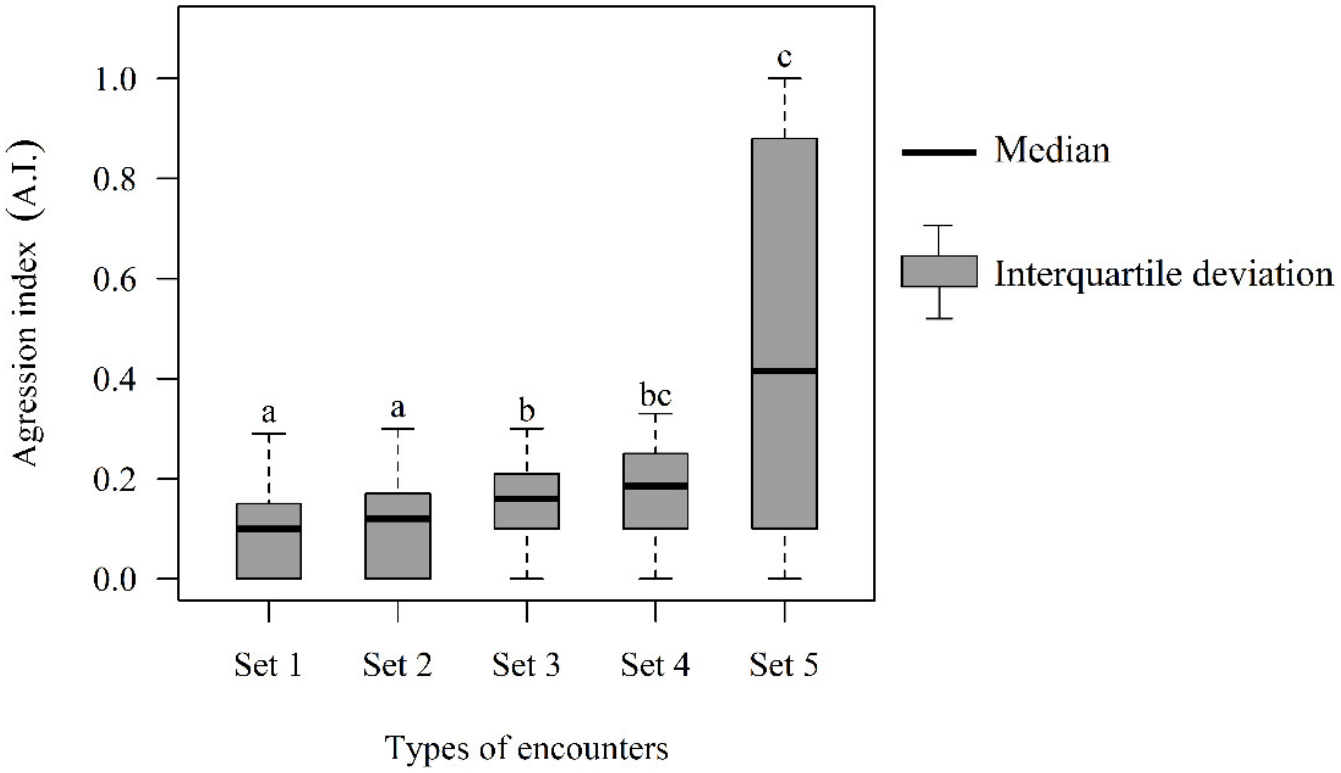

Fig 1. Average A.I. in each type of induced paired encounter. Different letters indicate significant differences according to the Kruskal-Wallis test ($p < 0.05$). Captions: Set 1 – nestmates kept together under the same temperature of 25 °C; Set 2 – nestmates kept separated but under the same temperature of 25 °C; Set 3 – Nestmates kept separated and under different temperatures (25 °C and 15 °C); Set 4 – Nestmates kept separated and under different temperatures (25 °C and 30 °C); Set 5 – Non-nestmates kept separated but under the same temperature of 25 °C.

The mean antennation time (A.T.) during the encounters between nestmates kept together at 25 °C was 24.5 ± 13 seconds; between nestmates kept separated at 25 °C was 30.5 ± 17 seconds; between ants from the group kept at 25 °C and your nestmates submitted to 15 °C, it was 47 ± 20 seconds; between ants from the group kept at 25 °C and your nestmates submitted to 30 °C, the antennation time was 45 ± 31 seconds; and between non-nestmates, the mean antennation time was 5 ± 17 seconds (Figure 2).

The Kruskal-Wallis test shows a significant difference in the antennation time between the treatments ($p < 0.001$). There is no significant difference between the antennation times seen during the encounters between nestmates kept together under 25 °C and that (A.T.) from encounters between these ants with your nestmates who were separated but kept under this same temperature ($p = 0.442$). However, the antennation times seen during the encounters between nestmates kept under 25 °C differs significantly from that A.T. from encounters between these ants with their nestmates submitted to the temperature of 15 ºC ($p < 0.001$), as well as those submitted to a temperature of 30 °C ($p < 0.001$). Finally, the antennation time seen during encounters between non-nestmates is significantly different from all the other encounters (sets 5 and 1 $p = 0.002$; sets 5 and 2 $p < 0.001$; sets 5 and 3 $p < 0.001$; sets 5 and 4 $p < 0.001$) (Figure 2).

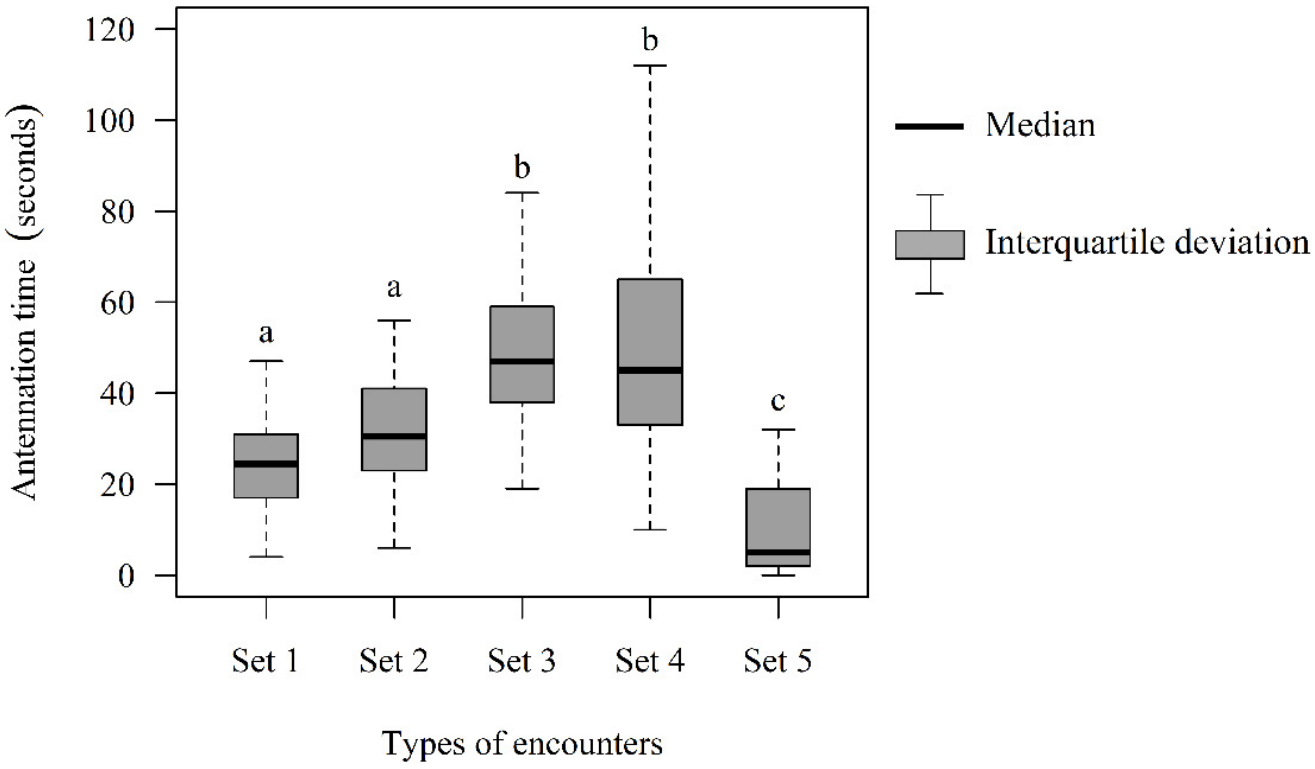

Fig 2. Average antennation time in each type of induced paired encounter. Different letters indicate significant differences according to the Kruskal-Wallis test ($p < 0.05$). Captions: Set 1 – nestmates kept together under the same temperature of 25 °C; Set 2 – nestmates kept separated but under the same temperature of 25 °C; Set 3 – Nestmates kept separated and under different temperatures (25 °C and 15 °C); Set 4 – Nestmates kept separated and under different temperatures (25 °C and 30 °C); Set 5 – Non-nestmates kept separated but under the same temperature of 25 °C.

The mean antennation time between live ants from the group kept under 25 °C and dead ants from the group submitted to 15 °C was 36.5 ± 42.3 seconds. According to the analysis, there is no significant difference between this time and that A.T. saw during interactions between live ants of both groups (Wilcoxon $p = 0.850$). The mean antennation time between live ants from the group kept under 25 °C and the dead ants of the group submitted to 30 °C was 32.5 ± 52.3 seconds, and there is also no significant difference between this time and that A.T. saw during interactions between live ants of both groups (Wilcoxon $p = 0.390$).

During the encounters, 51.5% of the behaviors, whether aggressive or not, were performed by the group kept under 25 °C, and ants of the other groups performed 48.5%.

Discussion

Our results show that nestmate ants of this species exhibit some change in their usual recognition pattern if kept under different temperature conditions for at least 24 hours. The highest level of aggressiveness observed during the encounters between nestmates was level 1, in which ants performed antennal boxing, body lifting, and abdomen display, which are common behavioral acts when ants are involved in agonistic interactions (Starks et al., 1998). On the other hand, the highest level of aggressiveness showed during the encounters between non-nestmates was 2, seizures, bites, and fighting. This makes the A.I. scores for these encounters differ from almost all the others, except for those with ants submitted to the temperature of 30 °C (Figure 1). These results suggest that temperature plays a crucial role and possibly affects the CHCs composition of individuals, which may impact how their nestmates perceive them. However, it is also evident that intercolonial variation in the chemical composition of CHC plays a more significant role in the dynamics of nestmate and non-nestmate recognition.

The possible change in the composition of CHCs suffered by the ants led to a disruption in the usual pattern of recognition between nestmates. However, more was needed to generate high aggressiveness in 90% of the encounters. This may be because the period they were subjected to different temperature conditions was not enough to cause significant changes in the cuticular composition, as suggested by the results reported by Menzel et al. (2018) on ants of the genus *Temnothorax*. Nevertheless, the statistical analysis shows that the A.I. and the antennation time seen in the encounters between nestmates kept under 25°C (separated or in the same group) differs significantly from those encounters between ants kept under 25 °C with your nestmates submitted to temperatures of 15 or 30 °C (Figures 1 and 2). Therefore, these results suggest

that ants exposed to different temperatures underwent distinct adjustments in cuticular chemical composition, which can disrupt the process of nestmate recognition

The antennation time during the encounters reinforces that the ants have the process of nestmate recognition disrupted. Starks et al. (1998) have already assessed antennation time as reliable in determining the level of recognition in a study in which the authors performed similar encounters to those of this study between wasps of the species *Polistes dominula* Christ, 1791. In this case, longer antennation times were considered indicative of a more significant disruption in the process of nestmate recognition. Our results show a significant difference between the antennation times between ants kept under the same temperature (25 °C) and the antennation times during the encounters between ants kept for 24 hours under different temperatures.

In addition, the mean antennation times and the mean A.I. shown in the encounters of ants kept together under 25 °C do not differ from those from encounters of ants kept separated from each other but under this same temperature. Social interactions like trophallaxis, allogrooming, and body contact provide an exchange of post-pharyngeal gland hydrocarbons, which contributes to the formation of the colonial chemical profile (Meskali et al., 1995; Boulay et al., 2000). In this sense, the absence of such interactions (e.g., by separation) can lead to a difference in the chemical profiles of individuals. Studies like Lenoir et al. (2001) and Ichinose and Lenoir (2009) have already shown differences in the composition and quantity of CHCs of individuals submitted to a separation regime. However, our results invalidate the possibility that the simple separation of two groups of ants would have disrupted the process of nestmate recognition.

However, the antennation time observed during the encounters between non-nestmates was significantly shorter than all the others between nestmates (Figure 2). This can be explained by ants from different colonies quickly identifying that the other ant is not a nestmate and, thus, quickly performing aggressive behaviors. This occurs differently when the ants are from the same colony, given that they are nestmates after all; in this case, the longer antennation time is considered indicative of a disruption in the usual pattern of nestmate recognition.

According to the literature, this disruption in the nestmate recognition can be explained by the alteration of the chemical cuticular composition (i.e., a change in viscosity induced by the production of new compounds) of ants that were subjected to different temperature conditions since the cuticle of insects can change to adapt to environmental pressures (Menzel et al., 2018; Otte et al., 2018). Although we have yet to analyze cuticular chemical composition to prove this hypothesis, studies such as those of Michelutti et al. (2018) and Duarte et al. (2019) reinforce our suspicions.

Temperature variation, especially higher temperatures, can increase the cuticle's permeability, causing the animal to lose water from inside (Ramsay, 1935; Wigglesworth, 1945). Thus, the survival of insects depends on the plasticity of the compounds of their cuticle, which must adapt to the new condition to reduce permeability and, consequently, the loss of water (Menzel et al., 2018). The cuticle's waterproofing ability is given by a thin lipid layer - mainly composed of hydrocarbons and located in the outer region of the epicuticle of the insects - and depends on the melting point of the compounds present in it. The melting point, in turn, increases according to the alignment and aggregation of the molecules, in addition to the length of the chain (Sprenger et al., 2018).

The melting point of the CHCs is essential because it is related to another physical property of the lipid layer: the higher this value for the cuticle compounds, the higher its viscosity. Menzel et al. (2019) refer to the cuticular chemical composition as a layer of wax covering the insect's body because the CHCs are not completely solid at room temperature and may be more fluid or more viscous. This is extremely important in waterproofing because insects lose water when molecules diffuse through the hydrocarbon layer, which occurs more easily with the lower viscosity of liquid compounds and less abundance of solids (Gibbs & Rajpurohit, 2010).

Therefore, insects can increase the waterproofing of the cuticle by producing CHCs with stronger molecular cohesion and high melting points. In this way, the lipid layer becomes more viscous, which prevents the passage of water molecules (Sprenger et al., 2018). On the other hand, CHCs only act as signals for communication if they are liquid enough to diffuse and spread through the cuticle and sensiles of another insect (Blomquist & Bagnères, 2010).

The disruption in the nestmate recognition between ants of the group kept at 25 °C and ants subjected to 15 °C may have occurred due to the viscosity of the cuticular compounds, which may also be higher under conditions of lower temperatures, as usually happens with fluids, which solidify when reaching the solidification point. Sprenger et al. (2018) and Menzel et al. (2019) observed that extracts of the cuticular profile of ants acclimatized to temperatures below 20 °C present biphasic behavior, with part of the compounds in their solid state and part in the liquid state. Menzel et al. (2019) also highlight that the viscosity of extracts of ants acclimatized to 20 °C was higher than that of extracts of ants acclimatized to 25 °C.

Considering this, subjecting the ants to different temperature conditions may have generated an environmental pressure that led to a readjustment in the compounds in the insects' cuticles. Indeed, Michelutti et al. (2018) observed that wasps subjected to conditions like this experiment had their cuticular composition significantly altered. As the adjustment of the cuticular composition happens to keep the internal conditions of the insect constant, the increase in the viscosity of the lipid layer results in the lower abundance of more fluid compounds, which are essential in the exchange of chemical signals between conspecifics. Thus, the ability to transmit these signals to a nestmate is impaired, which explains why ants spend more time antennating during encounters with individuals who have undergone different temperature regimes.

Other studies have shown that wasps and ants of different species change their cuticular profile when exposed to temperature variation (Wagner et al., 2001; Menzel et al., 2017; Michelutti et al., 2018; Sprenger et al., 2018). Duarte et al. (2019), studying the effects of temperature variation on the cuticular chemical composition of three different species of ants, concluded that the cuticle undergoes alteration of its compounds in an attempt to readjust to the new conditions. Although we have not evaluated whether our experimental conditions may have altered the composition of CHCs, one of the species studied by Duarte et al. (2019), *Odontomachus bauri* Emery, 1892, is an ant of the same genus, which supports the hypothesis that the disruption in the nestmate recognition exhibited during paired encounters may have occurred due to a change in the cuticular chemical profile of ants exposed to different temperatures.

Our results also show that the behaviors performed during the encounters were performed with relatively similar frequency, both by ants of the control group and by ants subjected to different temperature conditions to which this group was maintained. This shows that the level of disruption in the nestmate recognition displayed may, indeed, be more related to changes in the compounds that signal the odor of their colonies (Obin & Vander Meer, 1989; Leonhardt et al., 2007; Nunes et al., 2008) than a more pronounced stress condition of a group due to experimental manipulation.

Although the level of aggressiveness seen in the intracolonial encounters may have been generated by the different temperatures at which the ants were kept, we also considered that this could be explained by the fact that experimental manipulation could have led the ants to a stress condition, which would make them intolerant, even towards their nestmates (Poole, 1997). However, this hypothesis can be ruled out when it is observed that there were no significant differences between the antennation times during the encounters between live ants and dead ants and those between two live ants from the different treatments. This leads us to believe that the level of disruption in the nestmate recognition displayed is not a consequence of any stress generated by manipulation. Indeed, our results are similar to those found in some studies in which encounters between dead ants and their nestmates were induced (Morel et al., 1988; Hernández et al., 2002; Hernández et al., 2006).

The results suggest that different temperatures may affect cues that are key for nestmate recognition. Moreover, although we have not performed chemical analysis of cuticles, there is evidence in the literature that suggests our results are related to the adjustment suffered by the cuticular chemical composition under the different temperature conditions.

Acknowledgements

The authors thank Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for providing a Doctorate fellowship to NRB. The author, WFAJ (grant number 308182/2019-7), thanks for the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) research grants.

Declarations

Competing interests*:* The authors declare no competing interests.

Ethics approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Data availability: This published article (and its supplementary information files) includes all data generated and analyzed during this study.

Authors' Contributions

KLS: Conceptualiztion, methodology, investigation, formal analysis, writing-initial draft, writing-review & editing.
NRB: Conceptualiztion, methodology, investigation, supervision, writing-initial draft, writing-review & editing.
WFA-Jr: Conceptualiztion, methodology, supervision, writing-initial draft, writing-review & editing.

Eletronic Supplementary Material Caption

ESM 1 – Aggression indexes and antennation time recorded in the different types of treatments across all the induced encounters. Paired antennation time from encounters where the acclimated ant was killed by freezing.

References

Blomquist GJ, Bagnères AG (2010). Insect hydrocarbons: biology, biochemistry, and chemical ecology. Cambridge University Press, New York

Blomquist GJ, Tittiger C, Jurenka R (2020). Cuticular Hydrocarbons and Pheromones of Arthropods. In: Wilkes, H (ed) Hydrocarbons, Oils and Lipids: Diversity, Origin, Chemistry and Fate. Springer, Switzerland, pp 213-244

Bonavita-Cougourdan A, Theraulaz G, Bagnères AG, Roux M, Pratte M, Provost E, Clément JL (1991). Cuticular hydrocarbons, social organization and ovarian development in a polistine wasp: *Polistes dominulus* christ. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 100: 667-680. https://doi.org/10.1016/0305-0491(91)90272-F

Boulay R, Hefetz A, Soroker V, Lenoir A (2000). *Camponotus fellah* colony integration: worker individuality necessitates frequent hydrocarbon exchanges. Animal Behavior, 59: 1127-1133. https://doi.org/10.1006/anbe.2000.1408

Brown WL (2000). Diversity of ants. In: Agosti D, Majer JD, Alonso LE, Schultz TR (ed) Ants: standard methods for measuring and monitoring biodiversity. Smithsonian Institution, Washington, pp 45-79

Brown WV, Spradbery JP, Lacey MJ (1991). Changes in the cuticular hydrocarbon composition during development of the social wasp, *Vespula germanica* (F.) (hymenoptera: vespidae). Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 99: 553-562. https://doi.org/10.1016/0305-0491(91)90337-D

Burchill AT, Moreau CS (2016). Colony size evolution in ants: macroevolutionary trends. Insectes Sociaux, 63: 291-298. https://doi.org/10.1007/s00040-016-0465-3

Carlin NF, Gladstein DS (1989) The "bouncer" defense of *Odontomachus ruginodis* and other odontomachine ants (Hymenoptera: Formicidae). Psyche, 96: 1-19. https://doi.org/10.1155/1989/96595

Chung H, Carroll SB (2015). Wax, sex and the origin of species: Dual roles of insect cuticular hydrocarbons in adaptation and mating. BioEssays, 37: 822-830. https://doi.org/10.1002/bies.201500014

Crall J (2021). Social insects: Stochastic switches and behavioral maturation in ants. Current Biology, 31: 481-483. https://doi.org/10.1016/j.cub.2021.03.076

Dani FR, Jones GR, Destri S, Spencer SH, Turillazzi S (2001). Deciphering the recognition signature within the cuticular chemical profile of paper wasps. Animal Behavior, 62: 165-171. https://doi.org/10.1006/anbe.2001.1714

Duarte BF, Michelutti KB, Antonialli-Junior WF, Cardoso CAL (2019). Effect of temperature on survival and cuticular composition of three different ant species. Journal of Theoretical Biology, 80: 178-189. https://doi.org/10.1016/j.jtherbio.2019.02.005

Fowler HG (1980). Populations, prey capture and sharing, and foraging of the paraguayan ponerine *Odontomachus chelifer* latreille. Journal of Natural History, 14: 79-84. https://doi.org/10.1080/00222938000770081

Gibbs A (1995). Physical properties of insect cuticular hydrocarbons: Model mixtures and lipid interactions. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 112: 667-672. https://doi.org/10.1016/0305-0491(95)00119-0

Gibbs AG (1998). Water-proofing properties of cuticular lipids. American Zoologist, 38: 471-482. https://doi.org/10.1093/icb/38.3.471

Gibbs AG, Rajpurohit S (2010). Cuticular lipids and water balance. In: Blomquist GJ, Bagnères AG (ed) Insect hydrocarbons: biology, biochemistry, and chemical ecology. Cambridge University Press, New York, pp 100-120

Gibbs AG, Chippindale AK, Rose MR (1997). Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. Journal of Experimental Biology, 200: 1821-1832. https://doi.org/10.1142/9789812567222_0010

Gibbs A, Pomonis JG (1995). Physical properties of insect cuticular hydrocarbons: The effects of chain length, methyl-branching and unsaturation. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 112: 243-249. https://doi.org/10.1016/0305-0491(95)00081-X

Guimarães IDC, Pereira MC, Batista NR, Rodrigues CAP, Antonialli-Junior WF (2018). The complex nest architecture of the Ponerinae ant *Odontomachus chelifer*. PLoS One, 13: e0189896. https://doi.org/10.1371/journal.pone.0189896

Hefetz A (2007). The evolution of hydrocarbon pheromone parsimony in ants (Hymenoptera: Formicidae) – interplay of colony odor uniformity and odor idiosyncrasy. A review. Myrmecol News, 10: 59-68.

Hernández JV, Goitía W, Osio A, Cabrera A, Lopez H, Sainz C, Jaffe K (2006). Leaf-cutter ant species (Hymenoptera: *Atta*) differ in the types of cues used to differentiate between self and others. Animal Behavior, 71: 945-952. https://doi.org/10.1016/j.anbehav.2005.09.004

Hernández JV, López H, Jaffe K (2002). Nestmate recognition signals of the leaf-cutting ant *Atta laevigata*. Journal of Insect Physiology, 48: 287-295. https://doi.org/10.1016/S0022-1910(01)00173-1

Hölldobler B, Wilson EO (1990). The ants. Springer, Berlin.

Howard RW (1993) Cuticular hydrocarbons and chemical communication. In: Stanley DW, Nelson DR (ed) Insect lipids: chemistry, biochemistry and biology. University of Nebraska Press, Lincoln, pp 179-226

Howard RW, Blomquist GJ (1982). Chemical ecology and biochemistry of insect hydrocarbons. Annual Review of Entomology, 27: 49-172. https://doi.org/10.1146/annurev.en.27.010182.001053

Howard RW, Blomquist GJ (2005). Ecological, behavioral, and biochemical aspects of insect hydrocarbons. Annual Review of Entomology, 50: 371-393. https://doi.org/10.1146/annurev.ento.50.071803.130359

Ichinose K, Lenoir A (2009). Ontogeny of hydrocarbon profiles in the ant Aphaenogaster senilis and effects of social isolation. Comptes Rendus Biologies, 332: 697-703. https://doi.org/10.1016/j.crvi.2009.04.002

Karlson P, Butenandt A (1959). Pheromones (ectohormones) in insects. Annual Review of Entomology, 4: 39-58. https://doi.org/10.1146/annurev.en.04.010159.000351

Karlson P, Lüscher M (1959). 'Pheromones': a new term for a class of biologically active substances. Nature, 183: 55-56. https://doi.org/10.1038/183055a0

Kühsel S, Brückner A, Schmelzle S, Heethoff M, Blüthgen N (2017). Surface area-volume ratios in insects. Insectes Sociaux, 24: 829-841. https://doi.org/10.1111/1744-7917.12362

Le Conte Y, Hefetz A (2008). Primer pheromones in social hymenoptera. Annual Review of Entomology, 53: 523-542. https://doi.org/10.1146/annurev.ento.52.110405.091434

Lenoir A, Cuisset D, Hefetz A (2001). Effects of social isolation on hydrocarbon pattern and nestmate recognition in the ant *Aphaenogaster senilis* (Hymenoptera, Formicidae). Insectes Sociaux, 48: 101-109. https://doi.org/10.1007/PL00001751

Leonhardt SD, Brandstaetter AS, Kleineidam CJ (2007). Reformation process of the neuronal template for nestmate-recognition cues in the carpenter ant *Camponotus floridanus*. Journal of Comparative Physiology A, Neuroethology, Sensory, Neural, and Behavioral Physiology, 193: 993-1000. https://doi.org/10.1007/s00359-007-0252-8

Leonhardt SD, Menzel F, Nehring V, Schmitt T (2016). Ecology and Evolution of Communication in Social Insects. Cell, 164: 1277-1287. https://doi.org/10.1016/j.cell.2016.01.035

Menzel F, Blaimer BB, Schmitt T (2017). How do cuticular hydrocarbons evolve? Physiological constraints and climatic and biotic selection pressures act on a complex functional trait. Proceedings of the Royal Society B: Biological Sciences, 284: 17-27. https://doi.org/10.1098/rspb.2016.1727

Menzel F, Morsbach S, Martens JH, Räder P, Hadjaje S, Poizat M, Abou B (2019). Communication versus waterproofing: The physics of insect cuticular hydrocarbons. Journal of Experimental Biology, 22: 1-11. https://doi.org/10.1242/jeb.210807

Menzel F, Zumbusch M, Feldmeyer B (2018). How ants acclimate: Impact of climatic conditions on the cuticular hydrocarbon profile. Functional Ecology, 32: 657-666. https://doi.org/10.1111/1365-2435.13008

Meskali M, Bonavita-Cougourdan A, Provost E, Bagnères AG, Dusticier G, Clément JL (1995). Mechanism underlying cuticular hydrocarbon homogeneity in the ant *Camponotus vagus* (SCOP.) (Hymenoptera: Formicidae): Role of postpharyngeal glands. Journal of Chemical Ecology, 21: 1127-1148. https://doi.org/10.1007/BF02228316

Michelutti KB, Soares ERP, Sguarizi-Antonio D, Piva RC, Súarez YR, Cardoso CAL, Antonialli-Junior WF (2018). Influence of temperature on survival and cuticular chemical profile of social wasps. Journal of Theoretical Biology, 71: 221-231. https://doi.org/10.1016/j.jtherbio.2017.11.019

Morel L, Vander Meer RK, Lavine BK (1988). Ontogeny of nestmate recognition cues in the red carpenter ant (*Camponotus floridanus*). Behavioral Ecology and Sociobiology, 22: 175-183. https://doi.org/10.1007/BF00300567

Murakami ASN, Nunes TM, Desuó IC, Shima SN, Mateus S (2015). The Cuticular Hydrocarbons Profiles in the Colonial Recognition of the Neotropical Eusocial Wasp, *Mischocyttarus cassununga* (Hymenoptera, Vespidae). Sociobiology, 62: 109-115. https://doi.org/10.13102/sociobiology.v62i1.109-115

Nunes TM, Nascimento FS, Turatti IC, Lopes NP, Zucchi R (2008). Nestmate recognition in a stingless bee: does the similarity of chemical cues determine guard acceptance? Animal Behavior, 75: 1165-1171. https://doi.org/10.1016/j.anbehav.2007.08.028

Obin MS, Vander Meer RK (1989). Mechanism of template-label matching in fire ant, *Solenopsis invicta* buren, nestmate recognition. Animal Behavior, 38: 430-435. https://doi.org/10.1016/S0003-3472(89)80036-3

Otte T, Hilker M, Geiselhardt S (2018). Phenotypic Plasticity of Cuticular Hydrocarbon Profiles in Insects. Journal of Chemical Ecology, 44: 235-247. https://doi.org/10.1007/s10886-018-0934-4

Poole T (1997). Happy animals make good science. Laboratory Animals, 31: 116-124. https://doi.org/10.1258/002367797780600198

R Core Team (2023). R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Ramsay JA (1935). The evaporation of water from the cockroach. Journal of Experimental Biology, 12: 373-383. https://doi.org/10.1242/jeb.12.4.373

Richard FJ, Hunt JH (2013). Intracolony chemical communication in social insects. Insectes Sociaux, 60: 275-291. https://doi.org/10.1007/s00040-013-0306-6

Rourke BC, Gibbs AG (1999). Effects of lipid phase transitions on cuticular permeability: Model membrane and in situ studies. Journal of Experimental Biology, 202: 3255-3262. https://doi.org/10.1242/jeb.202.22.3255

Schneider H, Silva CA (2014). As Características Do Clima De Dourados/Ms E Adjacências A Partir Da Série Histórica De 1980 A 2009. Geografares, 16: 1-21. https://doi.org/10.7147/GEO16.5614

Singer TL (1998). Roles of hydrocarbons in the recognition systems of insects. American Zoologist, 38: 394-405. https://doi.org/10.1093/icb/38.2.394

Sprenger PP, Burkert LH, Abou BR, Federle W, Menzel F (2018). Coping with the climate: Cuticular hydrocarbon acclimation of ants under constant and fluctuating conditions. Journal of Experimental Biology, 221: 1-12. https://doi.org/10.1242/jeb.171488

Starks PT, Fischer DJ, Watson RE, Melikian GL, Nath SD (1998). Context-dependent nestmate discrimination in the paper wasp, *Polistes dominulus*: A critical test of the optimal acceptance threshold model. Animal Behavior, 56: 449-458. https://doi.org/10.1006/anbe.1998.0778

Suarez AV, Tsutsui ND, Holway DA, Case TJ (1999). Behavioral and genetic differentiation between native and introduced populations of the Argentine ant. Biological Invasions, 1: 43-53. https://doi.org/10.1023/A:1010038413690

Turillazzi S. (2013). The biology of hover wasps. Springer, Berlin

Valadares L, Nascimento D, Nascimento FS (2015). Foliar substrate affects cuticular hydrocarbon profiles and intraspecific aggression in the leafcutter ant *Atta sexdens*. Insects, 6: 141-151. https://doi.org/10.3390/insects6010141

Van-Wilgenburg E, Torres CW, Tsutsui ND (2010). The global expansion of a single ant supercolony. Evolutionary Applications, 3:136-143. https://doi.org/10.1111/j.1752-4571.2009.00114.x

Wagner D, Tissot M, Gordon D (2001). Task-related environment alters the cuticular hydrocarbon composition of harvester ants. Journal of Chemical Ecology, 27: 1805-1819. https://doi.org/10.1023/A:1010408725464

Wigglesworth VB (1945). Transpiration through the cuticle of insects. Journal of Experimental Biology, 21: 97-114. https://doi.org/10.1242/jeb.21.3-4.97

Yew JY, Chung H (2015). Insect pheromones: An overview of function, form, and discovery. Progress in Lipid Research, 59: 88-105. https://doi.org/10.1016/j.plipres.2015.06.001