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You smell different! Temperature interferes with intracolonial recognition in *Odontomachus brunneus*

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Abstract

Intracolonial recognition among social insects is performed mainly by means of cuticular hydrocarbons (CHCs) that provide chemical communication, although their primary function is the avoidance of desiccation. Therefore, the ability to adjust to climatic variation may be related to the composition of CHCs. The hypothesis adopted in this work was that workers of the ant *Odontomachus brunneus*, when exposed to higher or lower average temperatures, change the CHCs composition, as a readjustment to the new conditions, and that this, in turn, leads to a change in intraspecific recognition capacity. To test this hypothesis, colonies of *O. brunneus* reared in the laboratory were subdivided into four groups. Two groups were kept at the same temperature, in order to assess the effect of isolation itself, while one group was kept at high temperature and another was kept at low temperature. Two groups were maintained at 25 °C, with no further conditions imposed. Subsequently, encounters were induced between individuals from these groups and from the high and low temperature groups, followed by the extraction of CHCs from each individual. The results indicated significant differences in recognition time and CHC composition between the high/low temperature groups and those kept at 25 °C. Antennation time during nestmate encounters was significantly longer for the groups submitted to temperature treatments (high and low), compared to those kept at 25 °C, suggesting recognition difficulty. In order to adjust to changing temperature conditions, *O. brunneus* undergoes changes in the composition of CHCs and in intraspecific recognition capacity.

Introduction

Intraspecific recognition in social insects involves the ability of the individuals to distinguish among their nestmates. This mechanism ensures colonial cohesion, as well as protection from intruders (Oliveira & Hölldobler, 1989). The recognition is mediated by chemical compounds called surface pheromones, which are volatile compounds of relatively low molecular mass or with longer chains (Lenoir et al., 2001; Sainz-Borgo et al., 2011). The primary function of these compounds is to protect against water loss, but by virtue of their structural complexity, they have evolved to form recognition pheromones that transmit the identity of an individual to other members of the insect society (Blomquist & Bagnères, 2010).

In insect society, cuticular hydrocarbons (CHCs, surface pheromones) encode chemical recognition signals that are signatures characteristic of each species, colony, and even caste (Antonialli-Junior et al., 2007; Blomquist & Bagnères, 2010). Hence, CHCs are important mediators of intraspecific

recognition, especially in ants (Vander-Meer & Morel, 1998). These nonpolar hydrocarbons, which contain only carbon and hydrogen in their structures, may be either saturated (alkanes) or unsaturated (alkenes and alkynes, which are very common in insects) (Blomquist & Bagnères, 2010).

The formation of a colonial chemical signature arises from a complex range of cuticular chemical compounds that are homogenized among individuals by behaviors such as allogrooming, where the postpharyngeal gland (PPG) acts as a mixing organ (Boulay et al., 2000a; Lenoir et al., 2001; Soroker et al., 1994, 1998; Soroker et al., 2003). This social interaction among nestmates is extremely important for the integration of workers in the colony, forming a characteristic odor of each colony. For example, in *Camponotus fellah*, when workers were isolated individually from the mother colony for up to 20 days, their PPG and CHCs presented different characteristics, compared to the non-isolated nestmates, and they were generally attacked when they were reintroduced into the mother colony, due to their different cuticular chemical profile. However, when the period of separation was reduced (to between 3 and 10 days), the isolated workers tended to perform the allogrooming behavior with greater intensity, as a way to rapidly regain the odor of the colony (Boulay et al., 2000b).

Despite its relative uniformity, the colony odor is dynamic and there may be quantitative changes in the relative proportions of the different cuticular hydrocarbon classes (Vander-Meer et al., 1989; Provost et al., 1993; Dahbi & Lenoir 1998b; Nielsen et al., 1999). The chemical profiles of insects are strongly influenced by genetic and environmental factors (Blomquist & Bagnères, 2010). Therefore, since one of the functions of CHCs is to create a barrier against desiccation, it may be expected that the variation of physical factors, such as temperature and relative humidity, is likely to affect their composition (Gibbs et al., 1997; Boulay et al., 2017; Menzel et al., 2017; Michelutti et al., 2018). For example, species in humid climates tend to have more alkenes and fewer linear alkanes, compared to species in drier habitats, which can be explained by the different waterproofing capabilities of these compounds in the cuticle (Menzel et al., 2017). On the other hand, particular CHC compositions may also affect signaling properties during intraspecific interactions (Chung & Carroll 2015).

Several studies have discussed the dual function of CHCs (Gibbs, 2007; Chung & Carroll, 2015; Boulay et al., 2017). One mechanism that ants use to adjust to new environmental conditions involves modification of the cuticular composition (Menzel et al., 2017). Hence, the need of the insect to protect itself against desiccation may negatively influence the capacity to transmit information, and vice versa (Gibbs, 2007; Chown et al., 2011; Menzel et al., 2017). For example, there is strong evidence to suggest that compounds with intermediate melting points and volatility, such as alkenes and branched alkanes, are involved in both desiccation resistance and chemical communication (Chung & Carrol, 2015). Some species produce other compounds responsible for communication, such as unsaturated compounds or branched alkanes, which can increase the risk of dehydration. Therefore, increased permeability of the surface lipids at high ambient temperatures may also be a consequence of the need to interact with nestmates (Chown et al., 2011).

So far, however, no study has investigated the interdependence of these two CHC functions. Therefore, in the present work, our hypothesis was that exposure of *O. brunneus* worker ants to high or low temperature would lead to intraspecific non-recognition, due to changes in the CHCs composition during the attempt to adjust to new environmental conditions.

Materials and methods

Three colonies of *O. brunneus* were collected in the urban perimeter of the city of Dourados, Mato Grosso do Sul state, Brazil (22º13'16'' S; 54º48'20'' W), in September of 2016. All three colonies were collected from hollow trunks of *Caesalpinia pluviosa* (Fabaceae) using tweezers and plastic containers, and were transferred to the laboratory in artificial nests. These nests were constructed using plastic trays and plaster molds that simulated the nest chambers, and were connected to a foraging arena where food was offered to the colonies.

The colonies were kept at a constant temperature of 25 ºC during an acclimation period of seven days, and were fed *ad libitum* with water and honey on dampened cotton inside an Eppendorf tube. As a source of protein, 4 last instar larvae of *Tenebrio molitor* were offered every 4 days. This was the standard diet used during the experiment.

After the acclimation period, four groups were separated from each of the three mother colonies, according to the procedure described by Sorvari et al. (2008), with adaptations. The groups were subjected to different temperatures: **Group "A"**, composed of workers and queen, was kept at a constant temperature of 25 ± 2 ºC; **Group "B"**, composed of 50 workers, was kept at a constant temperature of 25 ± 2 ºC; **Group "C"**, composed of 50 workers, was kept at a constant temperature of 35 ± 2 ºC; and **Group "D"**, composed of 50 workers, was kept at a constant temperature of 15 ± 2 ºC (Fig 1). All the groups were maintained for 30 days, with the same standard diet, in biochemical oxygen demand (BOD) chambers (Model 347 CD, Fanem, São Paulo, Brazil), where it was possible to control the temperature of each group, at constant humidity of 65 ± 5% and 12 h photoperiod. In this way, it was possible to assess the effect of isolation of the nestmates (when exposed to the same temperature condition, in this case), as well as the effect of exposure of the nestmates to high and low temperatures.

The temperatures chosen were based on the averages for the two main seasons in the city of Dourados in the previous

year (Zavatini, 1992). Hence, it was possible to test the effect of temperature using values above the average temperature of the hot and humid season, which was 31.2 ± 3.2 ºC, and below the average temperature of the cold and dry season, which was 20.1 ± 5.0 ºC, according to data from the weather station of the Brazilian Agricultural Research Agency (EMBRAPA).

Analysis of the tolerance levels of different treatment groups during ant encounters

Soon after the colonies were collected, the cuticular compositions of 10 individuals from each colony were analyzed. This enabled identification of any changes in the odors of the colonies without any type of treatment, during the course of the experiment.

In order to evaluate whether low or high temperature, and/or isolation itself, might affect the level of intraspecific recognition and the antennation time, encounters were induced between one ant from the 25 ºC group (Group "A") and another ant from the other groups, resulting in four sets of induced behavioral encounters (Fig 1). These encounters were conducted in a Petri dish (100 mm x 20 mm), where a worker was kept trapped inside a glass container (50 mm x 20 mm) turned upside down in the center of the Petri dish, in order to minimize the stress caused by manipulation, which might influence the level of recognition. A second worker was then placed outside the glass container (Fig 2). After allowing one minute for the workers to become accustomed to the environment, the glass container was removed from the center of the Petri dish, enabling the encounter between the two workers, which were then monitored for 15 minutes without interruption.

The sets of induced behavioral encounters between the groups were organized as follows: 30 encounters between workers of Group "A" itself, used as a parameter for evaluation of the other behavioral encounters; 30 encounters between

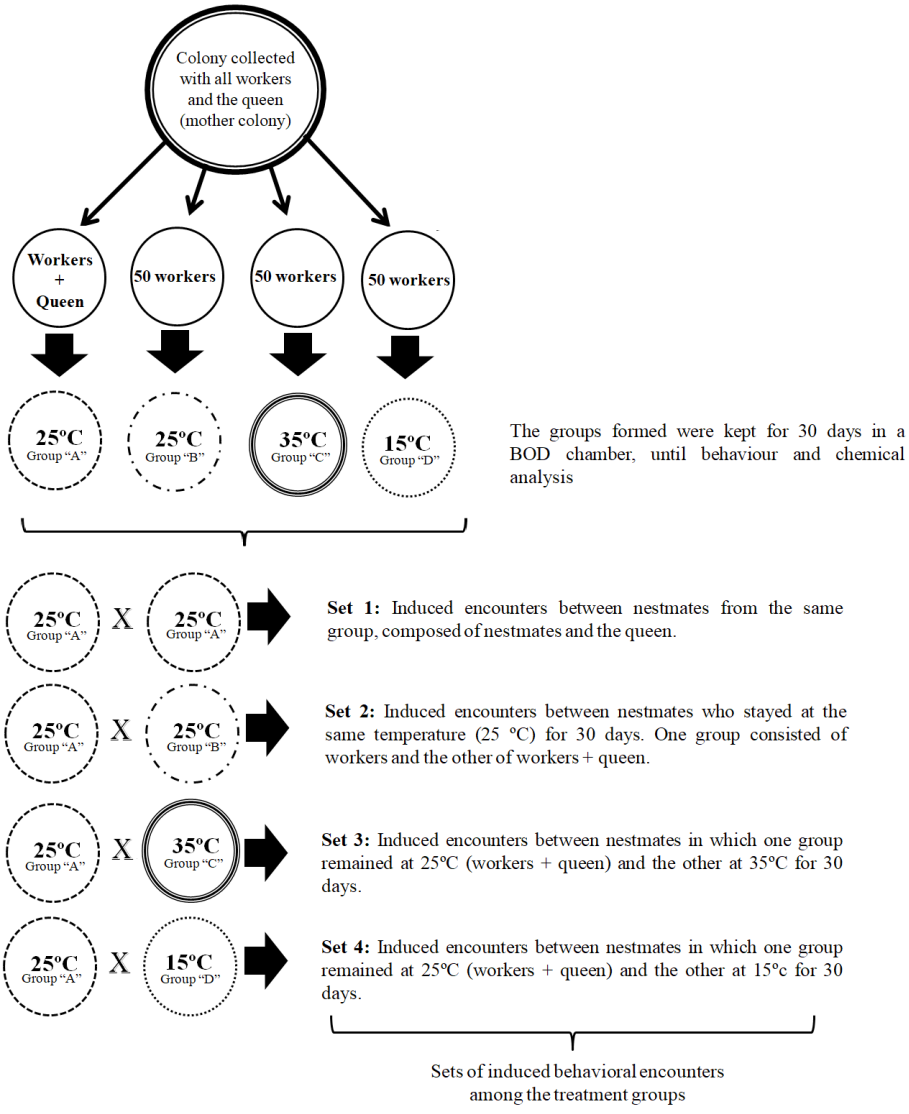

Fig 1. Sample design showing how the groups of ants were formed from the mother colony, together with the corresponding temperatures at which the workers were maintained prior to the induced encounters.

workers from Groups "A" and "B", in order to assess whether isolation could lead to increased antennation time; 30 encounters between Groups "A" and "C", in order to assess whether higher temperature, relative to Group "A", could lead to longer antennation time between workers; and 30 encounters between workers in Groups "A" and "D", in order to assess whether low temperature, relative to Group "A", could lead to longer antennation time (Fig 1). The same ant was not used more than once, and at the end of the observation period, the ants were used for subsequent chemical analyses.

Before each induced encounter, the arena was sterilized with a towel soaked in alcohol. The same ant was not used in other behavioral tests, and as soon as the tests were completed, the ants were immediately used for extraction and analysis of the CHCs. During the 15 minutes of blinded observations, the observer did not know which group the workers belonged to, hence minimizing any possible influence of the observer in evaluation of the ant behavior. The following behavioral parameters were considered: "ignore" (Tomas et al., 2004), "touch the body of another worker", "escape" (Suarez et al., 2004), "attempt to apprehend", "apprehend" (Mercier et al., 1997), "antennal boxing", "body lift, abdomen showing" (Monnin, 1999), and "fight" (Mercier et al., 1997). The execution time of each of these behaviors was counted from the first contact between the ants.

In order to evaluate the level of aggression (if present) during the encounters between ants from the different treatments, the behaviors exhibited were assigned a score from 0 to 2, based on Suarez et al. (2004), with modifications: 0 for "touch", "escape", and "ignore"; 1 for "attempt to apprehend", "apprehend", "antennal boxing", "elevation of the body", and "elevation of the abdomen"; and 2 for "fight". For each encounter, an arithmetic average of the scores related to the levels of aggression presented was compiled.

For the "touch the body of another worker" behavior, the time spent in antennation between the workers was measured, enabling calculation of the average time spent in intraspecific recognition during the induced encounters. The antennation time was used here as a parameter reflecting the degree of difficulty in the recognition of nestmates (Starks et al., 1998).

Analysis of the effect of temperature on cuticular chemical composition

For evaluation of the effects of the different treatments on the cuticular composition, the ants were sacrificed by freezing, followed by extraction of the cuticular chemical compounds from the 30 workers of each experimental set used in the behavioral encounters. Each worker was immersed for 3 min in a glass vessel containing 2 mL of hexane (HPLC grade, Tedia). The resulting extracts were dried under a fume hood and stored in a freezer (at −20 °C) for a maximum of 30 days. For chromatographic analysis, each extract was solubilized in 400 µL of hexane. The analyses employed a gas chromatograph (GC-2010 Plus, Shimadzu, Kyoto, Japan) equipped with a mass detector (Ultra 2010, Shimadzu, Kyoto, Japan). The compounds were separated using a DB-5 fused silica capillary column (J & W Scientific, Folsom, California, USA) coated with 5% phenyl-dimethylpolysiloxane (30 m length × 0.25 mm diameter × 0.25 µm film thickness). The analysis conditions were as follows: 1 µL injection, in splitless mode; heating program with initial temperature of 150 °C, ramp to 300 °C at 3 °C min^{-1}, and maintaining the final temperature for 10 min; and injector temperature of 250 °C. The temperatures of the detector and transfer line were 300 and 280 °C, respectively. The mass spectrometry parameters included electron impact ionization voltage of 70 eV, 0.3 s scan interval, and scanning in the range from m/z 45 to 600.

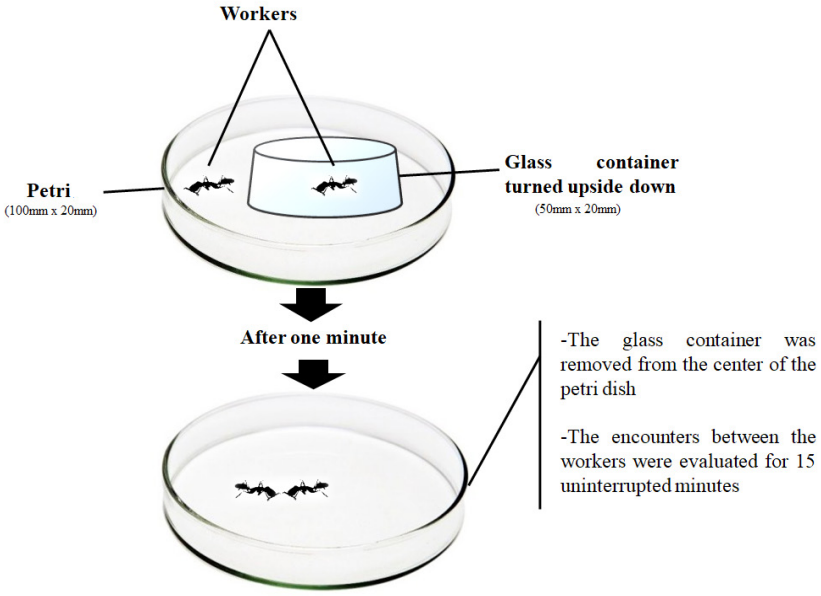

Fig 2. Scheme showing the procedure for the induced encounters between nestmate ants that had previously been maintained under different temperature conditions for 30 days.

The compounds were identified from the retention index (Bernier et al., 1998; Howard, 2001; Smith et al., 2012; Moore et al., 2014; Weiss et al., 2014; Soares et al., 2017), comparison with databases (NIST 21 and Wiley 229), and interpretation of the mass spectra. The retention index was calculated employing a mixture of linear alkanes (C_7-C_{40}, purity ≥ 95%, Sigma-Aldrich) as an external reference.

All the identified compounds were used in the statistical analysis. The major compounds for the different groups of ants were considered to be those that represented at least 10% of the total relative area.

Statistical analysis

For all the behavioral encounters, the normality of the data was evaluated using the average time of interaction between the workers of each set. After identification of non-normality of the data distribution, the Kruskal-Wallis test was applied to identify any significant differences between the median values for the time spent in antennation behavior during the encounters.

For evaluation of whether the different sets of induced behavioral encounters caused significant differences in the cuticular chemical compositions of the ants, relative to Group "A", a discriminant analysis was applied, using the relative abundances (areas) of all the compounds from the cuticles of the different ant groups. For evaluation of the effects of low and high temperatures on the chain lengths of the CHCs, discriminant analyses were applied separately for the different groups of compounds (short, intermediate, and long chains) from different ant groups, relative to Group "A". The compounds were separated into short, intermediate, and long chains based on the methodology of Menzel et al. (2017), with adaptations. The distance analysis of Mahalanobis was applied to evaluate the distance between the different groups, relative to the control, in a pairwise manner.

Results

Observations were made for a total of 30 h of interactions between the workers in the different sets of induced behavioral encounters. During all the encounters, the only behaviors observed were "touching the body of the other worker" and "ignoring", representing "0" aggression.

However, as shown in Fig 3, significant differences were observed between the average antennation times of workers during "touching the body of the other worker" behavior, when the workers were kept at different temperatures (Sets 3 and 4). No significant differences in antennation times were noted between the ants of Set 1 and of Set 2 (Fig 3).

A total of 17 chromatographic peaks were detected for the cuticle extract from the Group "A" workers (Table 1). Of these, 8 were linear alkanes, 7 were branched alkanes, and 2 were alkenes. Linear alkanes were the most representative, corresponding to 68.55% of the total area, followed by branched alkanes (18.09%) and alkenes (13.36%) (Fig 4). Three compounds were considered as major components: the linear alkanes octadecane (39.25%) and heptacosane (12.09%), and the alkene eicosene (11.39%), as shown in Table 1. The cuticular compounds varied from C_{18} to C_{32}. Compounds with short, intermediate, and long chains were considered to be those with chain lengths in the ranges C_{18} to C_{21}, C_{22} to C_{27}, and C_{28} to C_{32}, respectively.

A total of 16 compounds were identified in the extract from the Group "B" workers (Table 1), including 7 linear alkanes, 7 branched alkanes, and 2 alkenes. The linear alkanes represented 67.27% of the area, the branched alkanes 22.87%, and the alkenes 9.86% (Fig 4). The major compounds were octadecane (37.90%) and heptacosane (17.24%).

For the extract from the Group "C" workers, 19 compounds were detected (Table 1), including 10 linear alkanes, 7 branched alkanes, and 2 alkenes (Fig 4). The linear alkanes represented 65.71% of the area, the branched alkanes 26.68%, and the alkenes 7.61% (Fig 4). The major compounds were octadecane (25.50%) and heptacosane (25.32%).

For the Group "D" workers, 19 compounds were detected in the extract, including 10 linear alkanes, 7 branched alkanes, and 2 alkenes (Table 1). The linear alkanes represented 57.81% of the area, the branched alkanes 33.19%, and the alkenes 9.00% (Fig 4). The major compounds were octadecane (14.57%), heptacosane (23.75%), and 12, 16-dimethyldotriacontane (11.85%).

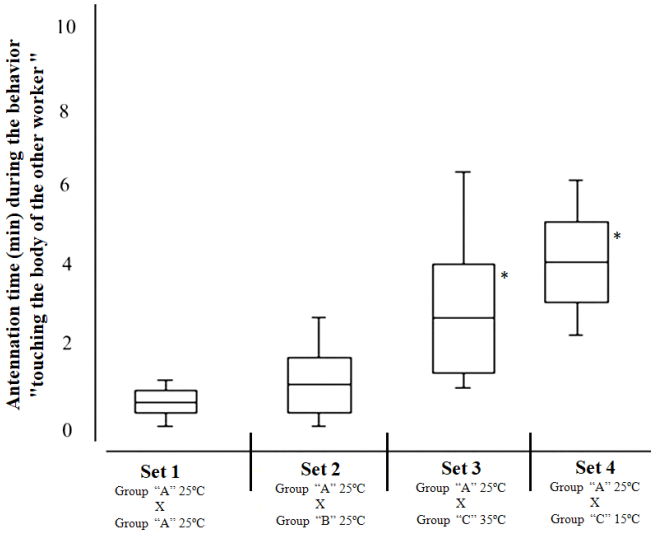

Fig 3. Median and standard deviation values for the antennation times measured during the behaviour "touching the body of the other worker", for encounters between *Odontomachus brunneus* workers previously maintained under different temperature conditions (n = 30). *Significant difference ($p < 0.05$, Kruskal-Wallis = 85.69), compared to Set 1.

Table 1. Relative abundances (peak areas) of the compounds detected in the cuticles of *Odontomachus brunneus* workers submitted to different treatments.

Time	CRI	LRI	Compound	Group "A" (25 ºC)	Group "B" (25 ºC)	Group "C" (35 ºC)	Group "D" (15 ºC)
				Abundance (% ± standard deviation)			
10.77	1800	1800	Octadecane *	39.25 ± 11.32	37.90 ± 8.77	25.50 ± 14.58	14.57 ± 6.39
13.25	1900	1900	Nonadecane	3.79 ± 5.24	1.71 ± 0.48	1.40 ± 0.73	1.50 ± 0.75
15.71	1990	1990	Eicosene *	11.39 ± 1.54	8.78 ± 1.85	6.04 ± 3.64	7.75 ± 4.62
15.88	2000	2000	Eicosane	4.27 ± 0.76	2.99 ± 0.68	2.21 ± 1.24	2.82 ± 1.62
19.01	2119	-	x-Methylheneicosane	1.67 ± 2.10	1.01 ± 0.23	0.71 ± 0.39	0.91 ± 0.50
21.12	2200	2200	Docosane	5.79 ± 1.03	4.24 ± 1.12	3.03 ± 1.84	4.54 ± 2.78
24.53	2318	-	x-Methyltricosane	1.14 ± 0.37	0.92 ± 0.18	0.53 ± 0.37	0.78 ± 0.51
26.39	2400	2400	Tetracosane	1.43 ± 0.31	0.98 ± 0.33	0.76 ± 0.58	1.35 ± 0.89
29.07	2500	2500	Pentacosane	0.50 ± 0.58	-	1.79 ± 1.97	1.19 ± 1.66
31.51	2600	2600	Hexacosane	-	-	0.73 ± 1.52	0.47 ± 0.37
33.91	2700	2700	Heptacosane *	12.09 ± 5.08	17.24 ± 5.83	23.75 ± 14.40	25.32 ± 12.72
35.05	2750	2751	5-Methylheptacosane	0.61 ± 0.38	-	-	-
36.21	2800	2800	Octacosane	-	-	0.44 ± 0.32	0.53 ± 0.26
38.45	2900	2900	Nonacosane	1.42 ± 0.57	2.21 ± 0.97	6.10 ± 4.72	5.53 ± 4.80
42.25	3070	3070	3-Methyltriacontane	3.24 ± 2.51	4.48 ± 3.37	6.43 ± 1.37	8.25 ± 2.72
42.33	3078	3078	13,17-Dimethyltriacontane	2.53 ± 2.18	2.22 ± 1.73	1.40 ± 1.63	2.02 ± 1.96
45.85	3253	3254	12,16-Dimethyldotriacontane	5.81 ± 4.98	7.56 ± 5.98	7.82 ± 3.60	11.85 ± 6.08
45.94	3259	3260	2-Methyldotriacontane	3.08 ± 2.81	3.37 ± 3.06	1.62 ± 1.18	2.88 ± 1.74
46.28	3274	3275	3-Methyldotriacontane	-	3.31 ± 3.01	8.17 ± 3.62	6.50 ± 3.41
46.31	3271	3271	Tritriacontene	1.97 ± 1.93	1.08 ± 1.18	1.57 ± 1.60	1.25 ± 1.62

* Major compounds. CRI = calculated retention index. LRI = literature retention index.

The cuticles of Groups "C" and "D" (exposed to 35 ºC and 15 ºC, respectively) presented increases in the number of linear alkanes, relative to Group "A". On the other hand, the cuticles of the workers in Groups "C" and "D" showed reductions in the abundances of linear alkanes and alkenes, but increases of branched alkanes, relative to Group "A" (Fig 4). The compounds hexacosane and octacosane only occurred in the cuticles of the ants exposed to low and high temperature, while 3-methyldotriacontane occurred in these groups and in Group "B". On the other hand, 5-methylheptacosane only occurred in the cuticles of the ants in Group "A". Pentacosane was only observed for the ants in Group "B" (Table 1, Fig 4).

Qualitative and quantitative differences were observed between the results for Group "A" and those for the different treatments. For example, the ants in Groups "C" and "D" (submitted to high and low temperatures, respectively) showed lower abundance of short-chain compounds and increase of long-chain compounds, compared to Group "A" (Table 1). Discriminant analysis revealed significant differences in cuticular chemical composition, considering all the compounds as well as when the compounds were separated into the three chain length categories (Fig 5, Table 2). The discriminant analysis F-values showed that the cuticles of the ants in Groups "C" and "D" presented significantly greater differences, compared to the ants in Group "B", relative to Group "A" (Table 2).

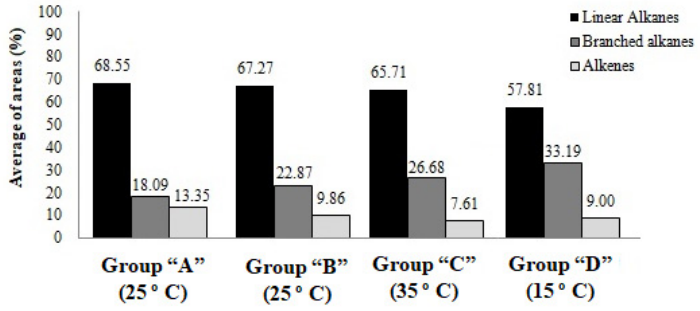

Fig 4. Abundance of compounds of each class present in the cuticles of *Odontomachus brunneus* workers submitted to different treatments.

Table 2. Values of *P* and *F* for the Mahalanobis distance analysis applied to the cuticle hydrocarbon data for *Odontomachus brunneus* workers submitted to different treatments.

	All compounds		Short-chain		Intermediate-chain		Long-chain	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Treatments								
Group "A" – Group "B"	18.655	<0.001	5.869	<0.001	26.472	<0.001	4.272	<0.001
Group "A" – Group "C"	38.537	<0.001	12.830	<0.001	34.136	<0.001	35.600	<0.001
Group "A" – Group "D"	52.150	<0.001	30.782	<0.001	41.891	<0.001	49.258	<0.001
Discriminant	**24.121**	**<0.001**	**13.547**	**<0.001**	**17.948**	**<0.001**	**19.101**	**<0.001**
Wilks' lambda	*0.004*		*0.248*		*0.105*		*0.074*	

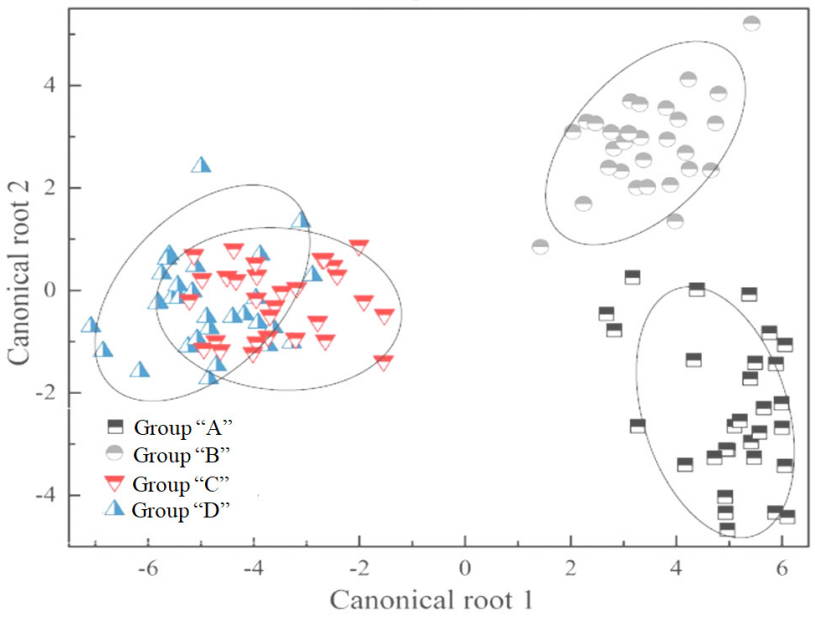

Fig 5. Scatter diagram obtained using the two canonical roots for differentiation of the compounds present in the cuticles of *Odontomachus brunneus* workers submitted to different treatments, based on the relative abundances (peak areas) of the cuticular compounds.

Discussion

The results demonstrated that the effects of exposure at different temperatures increased the difficulty in nestmates recognizing each other, probably due to the modification of cuticle chemical composition, as a function of readjustment to different temperatures. On the other hand, the ants of Set 2, which were subjected to the same temperatures, but were isolated, did not show any change in intraspecific recognition.

Aggression was absent during all the induced behavioral encounters, although longer antennation times were observed during "touch the body of another worker" behavior for Sets 3 and 4 (35 ºC and 15 ºC, respectively), compared to Set 1 (25 ºC). Antenna behavior allows these insects to capture chemical signals that identify them as members (or not) of the same colony (Hefetz, 2007). Hence, it can be inferred that a longer antennation time is related to increasing difficulty of recognition as nestmates (Starks et al., 1998), which, on the other hand, may be related to any change in what Howard and Blomquist (2005) called a chemical signature of the colony. This recognition is fundamental for social insects to ensure protection of the colony against invasion by other insects, especially members of other colonies (Hefetz, 2007).

The results of the induced behavioral encounters between workers in Set 1 and Set 2, submitted to the same temperature condition, reinforced the hypothesis outlined above, since the time of contact between them did not suggest any difficulty in recognition (Fig 3). Therefore, the results demonstrated that temperature may, in fact, be related to alteration in the level of recognition among nestmates. In addition, it is important to note that the treatment processes may have caused some stress additional to that associated with increase or decrease of the temperature, since the handling of the ants may also have led to discomfort, which could also have influenced the levels of recognition.

However, chemical analysis of the cuticles of ants subjected to temperatures of 15 ºC and 35 ºC (Groups "C" and "D") revealed significant differences, compared to the ants subjected to 25 ºC (Group "A"). Therefore, it is possible that this alteration of cuticular composition could act as a mechanism that enables ants to readjust to new temperature conditions (Menzel et al., 2017, 2018; Michelutti et al., 2018; Sprenger et al., 2018; Duarte et al., 2019).

The standard deviations of the means for the relative abundances (peak areas) of the compounds obtained from *O. brunneus* were relatively high (Table 1), which could be explained by the fact that the workers originated from different colonies. Previous studies have shown differences among the individual members of a colony (Cuvillier-Hot et al., 2001) and between members of different colonies (Wagner et al., 2001). These differences may be due to genetic factors (Wagner et al., 2001; Howard & Blomquist, 2005), as well as environmental factors (Buczkowski et al., 2005; Howard & Blomquist, 2005).

As shown in Fig 5, the effects of temperature led to significant changes in cuticular chemical composition, with both qualitative and quantitative changes being observed in this study. Qualitatively, there were increases in the numbers of linear alkanes in the ants submitted to both low and high temperatures, compared to Group "A". On the other hand, there was increased abundance of branched alkanes, but decreases of linear alkanes and alkenes. Differently, in earlier work, Wagner et al. (2001) found variation only in the relative abundances of the compounds present in the cuticle of *Pogonomyrmex barbatus*, when the ants were submitted to heat and low humidity.

The compounds hexacosane and octacosane only occurred in the cuticles of ants subjected to 35 ºC and 15 ºC (Groups "C" and "D"), while 3-methyldotriacontane was present in Groups "B", "C", and "D", and 5-methylheptacosane only occurred in Group "A". These findings suggested that the ant cuticle has the ability to respond to temperature variation, sometimes synthesizing new compounds (or failing to do so). Similarly, in other work, Michelutti et al. (2018) identified qualitative and quantitative changes in the CHCs of three species of wasps submitted to different temperatures, compared to the controls.

The literature includes studies describing the ability of the cuticle to respond to the environmental conditions in which the animal is found (Wagner et al., 2001; Menzel et al., 2017; Michelutti et al., 2018; Duarte et al., 2019). It is likely that the occurrence (or not) of certain compounds may be related to the response to changes in the environment in which the animal is located.

In insects, this ability to adjust the cuticular composition involves making the CHC layer more viscous, to avoid water loss, when average temperatures are higher and relative humidity is low. Conversely, the layer can be made more fluid, in order to facilitate the exchange of information between individuals (Menzel et al., 2017; Sprenger et al., 2018). Under low temperature conditions, the cuticle tends to become less fluid, so to compensate for this, an increase in the abundance of branched alkanes would be expected, since these compounds increase the fluidity of the layer. The resulting increase of compound mobility makes the transfer of information by the cuticle more efficient (Menzel et al., 2017). On the other hand, at high temperatures, a reduction in the abundance of alkenes would be expected, since these compounds liquefy at lower temperatures, compared to the corresponding linear alkanes (Gibbs & Pomonis, 1995).

Importantly, the trade-off between the requirements for waterproofing and communication makes the evolution and plasticity of CHC profiles an intriguing field of research, with many open questions (Sprenger et al., 2018). When *O. brunneus* responds to changes in temperature by changing the amounts of different classes of compounds, this makes recognition between nestmates more difficult. Hence, it is necessary to perform tests with specific compounds, in order to detect key compounds responsible for these changes.

Although the number of linear alkanes increased at the highest temperature, there was a reduction of their abundance, while there was increased abundance of branched alkanes. Linear alkanes are the main compounds required to avoid desiccation in social insects (Gibbs, 1998; Hefetz, 2007; Wagner et al., 2001), but the branched alkanes (mono- or dimethyl) are the main compounds required to mediate communication between nestmates (Howard & Blomquist, 2005). It is well known that methyl alkanes, and notably alkenes, liquefy at lower temperatures, compared to linear alkanes (Gibbs, 1998). That is, methyl alkanes and alkenes have lower melting points than linear alkanes (Gibbs & Pomonis, 1995). Hence, according to Menzel et al. (2017), an increasing need for waterproofing should increase the abundance of linear alkanes and reduce the abundances of branched alkanes and alkenes.

The ant groups subjected to 35 ºC and 15 ºC (Groups "C" and "D") both showed a reduction in the abundance of compounds with shorter chain length, together with increased abundance of compounds with longer chain length (Table 1). In addition, analyses considering compounds of short, intermediate, or long chain lengths indicated a significant difference between ants of both Group "C" and Group "D", compared to Group "A" (Table 2). These results were in agreement with Gibbs et al. (1997), who found that under desiccation conditions, *Drosophila melanogaster* presented CHCs with longer chain lengths. Gibbs (1998) reported that the longer chains of arthropod CHCs tended to liquefy at higher temperatures. Furthermore, Gibbs and Pomonis (1995), in experiments with synthetic hydrocarbons, found that increase of the chain length caused a corresponding increase in the melting temperature, and that the position of the alkane branching could also influence the melting temperatures of compounds present in social insects. Essentially, the presence

of compounds with longer chains may indicate a greater ability of the cuticle to withstand high temperatures. However, Menzel et al. (2017) found no differences in the chain lengths of CHCs present in ant species from different populations of the genera *Crematogaster* and *Camponotus*.

It should be noted that insect surfaces are composed of more than a single pure compound, and not only hydrocarbons (Gibbs & Pomonis, 1995). The fusion of natural lipid mixtures occurs at the melting temperature of the cuticular compounds, with interactions between saturated and unsaturated CHCs resulting in nonlinear effects (Gibbs, 1998). Furthermore, compounds with intermediate melting and volatility temperatures, such as alkenes and methyl alkanes, may have important effects in desiccation resistance and chemical communication (Chung & Carroll, 2015).

Conclusions

The findings of this work showed that the cuticles of worker ants could undergo temperature-dependent compositional changes, with some categories of compounds decreasing in abundance, while others could increase as a means of compensating for the imposed conditions. On the other hand, although isolation itself caused alteration of the cuticular chemical composition, most likely due to the absence of homogenization by means of social interactions, the alteration was not sufficient (during the time tested) to lead to significant changes in the ability to recognize nestmates. The results validated the hypothesis adopted in this work, namely that *O. brunneus* undergoes changes in the composition of CHCs, in order to adjust to changing temperature conditions, while such changes in CHC composition also have consequences in terms of alteration of intraspecific recognition capacity.

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Author contributions

Conceived and designed the experiments: LC Santos-Junior and WF Antonialli-Junior. Performed the experiments: LC Santos-Junior, EP Silva, KB Michelutti, and RC Bernardi. Analyzed the data: LC Santos-Junior, KB Michelutti, CAL Cardoso, and WF Antonialli-Junior. Wrote the paper: LC Santos-Junior and WF Antonialli-Junior.

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