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Effect of temperature on survival and cuticular composition of three different ant species

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ABSTRACT

Climatic factors, such as temperature variation, interfere with the survival of insects. To respond to these variations, insects have some specific characteristics. These include water content of the body, thickness of the lipid layer, as well as the qualitative and quantitative characteristics of cuticular chemical components. This study hypothesizes that different ant species respond to temperature changes in different ways and that such differences may be associated with cuticle hydrocarbons (CHCs) and fatty acids. As model ant species, *Atta sexdens, Odontomachus bauri* and *Ectatomma brunneum* were used for experimental analyses. Ants were submitted to a water bath for 5 h at different temperatures, and their CHCs and fatty acids were identified and quantified, followed by correlating these chemical compounds with temperature variations and the survival. Temperatures below 30 °C did not affect the survival of the three species. *E. brunneum* had a higher percentage of survival at temperatures above 30 °C. *O. bauri* was the most sensitive species with 100% mortality at 40 °C. Survival was found to be unrelated to any of the identified fatty acids. However, CHCs underwent significant quantitative and qualitative variation, as shown by an increased percentage of CHCs with longer chain length of linear alkanes at temperatures above 30 °C. These increase enables these ants to maintain the integrity of their cuticle and survive at temperatures above 30 °C. It can be concluded that the forager ants studied respond differently to temperature variation and that changes in the conformation of CHCs are in line with the ecological characteristics of the different studied species because, they vary in terms of diurnal/nocturnal foraging and types of environments foraged. Among the three species, *E. brunneum* foragers were found to be more active under adverse conditions and more tolerant to temperature variation with the correspondingly appropriate changes in CHCs composition.

1. Introduction

Subtropical organisms are exposed to different seasonal variations, and to cope with such changes, they can acclimate themselves though changes in physiology, morphology and/or behavior (Angilletta and Angilletta, 2009). Ants, in particular, use cuticular hydrocarbons (CHCs) present on their body surface to respond to these variations. In other words, these insects can adjust the content of cuticular chemical compounds to act as a barrier against desiccation (Menzel et al., 2017).

CHCs are specifically found in the outermost layer of the cuticle (Gullan and Cranston, 2007). This structure is coated with chemical compounds that have, above all, two primordial functions. The first is to perform waterproofing, and the second is to perform chemical communication (Blomquist and Bagnères, 2010). In addition to the hydrocarbons, Lockey (1988) also highlights other compounds resident in the insect cuticle, including fatty acids, esters, alcohols, acylglycerides, phospholipids and glycolipids. In addition, Stanley-Samuelson et al. (1988) also report palmitic acid, stearic acid and monounsaturated oleic acid as standard fatty acids in insects in general (Stanley-Samuelson et al., 1988). Lipids can also act as a barrier to prevent water loss across the animal's surface (Gibbs and Pomonis, 1995). Thus, melting temperature directly correlates with the ability of an insect to maintain its water balance, i.e., allows them to withstand high temperatures (Edney, 2012).

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The ability to respond to variations in temperature depends on the exercise of different adaptations, whether behavioral, morphological or physiological (Boulay et al., 2017). Qualitative and quantitative variations of certain classes of chemical cuticular compounds, as well as different chain lengths, may also determine the ability of an insect to respond to environmental changes, such as at different temperatures (Gibbs et al., 1997; Hefetz, 2007; Menzel et al., 2017; Michelutti et al., 2018). The linear alkanes, for example, act mainly to waterproof the cuticle (Gibbs, 1998; Hefetz, 2007), while branched alkanes and alkenes mediate communication (Hefetz, 2007; Lorenzi et al., 1997). Thus, straight chain alkanes have higher melting temperatures than branched alkanes of comparable carbon chain length: the higher the branching, the lower the melting temperature (Hefetz, 2007). However, Menzel et al. (2017) report that linear alkanes and methyl alkanes are important waterproofing compounds.

Both compound class and length of carbon chain are important factors that affect waterproofing the cuticle (Gibbs and Pomonis, 1995; Gibbs et al., 1997; Gibbs, 1998). Menzel et al. (2018) studied the effect of temperature on the chain length of linear alkanes in the cuticles of the ants *Temnothorax longispinosus* and *Temnothorax ambiguus* and detected a strong correlation between chain length and increasing temperature. Michelutti et al. (2018) reported on the effect(s) of temperature variation on the CHCs of three wasp species and identified a significant change in compounds with longer chain length, thus supporting these compounds as “first responders” to environmental changes.

Different species and individuals within species possess specific lipid compositions, making each lipid layer unique. This means that different individuals exhibit more or less water loss at a given temperature, in turn, resulting in a specific critical temperature (Gibbs, 1998). In general, the composition of fatty acids is similar for all insects, and quantitative differences are more related to differences in taxonomic groups (Thompson, 1973). In addition, these fatty acid-related differences may still be affected by factors, such as order, family, species, flight activity, life stage, reproductive stage, as well as environmental factors that include photoperiod, temperature, and diet (Rosumek et al., 2017; Stanley-Samuelson et al., 1988; Thompson, 1973). Studying *Formica fusca* and *Myrmica rubra* ants, Rosumek et al. (2017) found that individual fatty acid profiles change as a function of diet and that these changes become more pronounced over time. In addition, the fatty acids appear to be involved in the formation of CHCs, as highlighted by Blomquist and Bagnères (2010).

Odontomachus bauri lives especially in forest areas; therefore, they use treetops for orientation in foraging since this represents cover with particularly restrictive lighting conditions characteristic of tropical forests (Oliveira and Hölldobler, 1989). Foraging activity occurs during the day and at night in nests and under the wood and serrapilhiera, exposing them to the little variation of climatic conditions (Ehmer and Hölldobler, 1996). *Ectatomma brunneum* nests in the soil and is abundant in areas of open vegetation or altered environments, either by anthropic or natural activity, close to human habitations, such as plantations, grasses, grasslands, roads and forest clearings (Overal, 1986; Vasconcelos, 1999). This species forages during the day and night avoiding only the hottest times of the day (Overal, 1986). The foraging activity of *Atta sexdens* is limited by the availability of resources and microenvironmental factors. During the drier months, foraging shifts to a sharp nocturnal pattern, and in the summer, foraging may also change to nighttime to avoid extremely high temperatures.

Based on the foraging behavior of ant species detailed above, this study hypothesizes that different ant species respond to temperature changes in different ways and that such differences may be associated with cuticle hydrocarbons (CHCs) and fatty acids. As model ant species, *A. sexdens, O. bauri* and *E. brunneum* were used for experimental analyses.

2. Material and methods

2.1. Collection and acquisition of the ants

Ants of three species were collected in the municipality of Dourados (22° 13′24.39″S; 54° 54′44.53″W) and Ponta Porã (22° 32′10″S; 55° 43′32″W), in the state of Mato Grosso do Sul - MS, Brazil, in December 2017. In addition, since the composition of CHCs in these ants may vary according to the ant function within their colony, only foragers (exterior workers) were collected. These foragers are most affected by temperature variation in their environment (Menzel et al., 2018; Wagner et al., 2001).

Collections were carried out during early morning and later afternoon since these are the best times to find foraging ants. For each species, approximately 300 ants were collected and divided into 7 subgroups containing 30 workers each for experiments, including a control group of 10 ants.

After collection, control ants were weighed and immediately frozen for extraction of cuticular compounds, thus avoiding any effects of experimental manipulation. Foragers collected for temperature experiments were taken to the laboratory where they were acclimated for a maximum period of 7 days under a constant temperature of 26 °C, making use of an air conditioner. A thermometer was used to monitor the ants, and the mean temperature was that recorded for the month prior to the experiment. They were kept in plastic pots of 500 mL containing water and honey in Eppendorf tubes as a food resource.

According to Zavatini (1992), Mato Grosso do Sul state has two main annual seasons, one hot and humid and the other cold and dry. The temperatures selected for the study were chosen according to the average temperature of the two main weather observation station (Empresa Brasileira de Pesquisa Agropecuária–Embrapa/Oeste) for the City of Dourados-MS during 2017. Our model ants are well acclimated to these temperature shifts. The cold and dry season presented a mean temperature of 21.6 °C ± 4.1 with a minimum temperature of 16.4 °C ± 4.5. The hot and humid season had an average temperature of 25.3 °C ± 2.4 with an average maximum of 32.0 °C ± 3.4 Thus, the experimental temperatures were selected about five degrees up and down from the average minimum and maximum, respectively, for that year.

2.2. Temperature variation experiments

The experiment was carried out following the model described by Bouchebti et al. (2015) with modifications. Ants were individually inserted into an Eppendorf tube containing a piece of moist cotton attached to its cap to humidify the air. For each temperature tested, a group of 30 foragers of each species were submitted to a digital ultrathermal water bath for 5 h. The tubes containing the ants were placed in the temperature maintenance system under different temperatures for five hours. The temperatures tested for the experiment were 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, 35 °C and 40 °C.

In accordance with Bouchebti et al. (2015), the ants were monitored every 30 min in order to monitor their survival, and the ants that did not support this time interval were removed. The experiment lasted a total of 5 h. As a control after collection, ten individuals of each species were sacrificed by freezing, followed by chemical analyses. Following chemical assessment, all ants of the three species submitted to the temperature experiment were removed from the temperature maintenance system, and those that were not dead were sacrificed by freezing for 10 min for further extraction of the cuticular compounds of ten ants from each treatment. Ants that died and survived to the end of the experiment, were submitted to chemical analyses of cuticular compounds. Control ants were sacrificed by freezing, as described for the other samples.

The CHCs of 10 foragers were extracted from each exposure group at each temperature and control. Each ant was then dipped in a glass vessel containing 2 mL hexane (Tedia, HPLC grade) for a period of 3 min. Flasks containing extract were dried at room temperature and then cooled (-4 °C) for a maximum of 7 days for further solubilization and analysis using gas chromatography coupled to mass spectrometry (GC-MS). After extraction of CHCs extraction, all foragers were subjected to extraction of their lipid contents.

After analyzing the hydrocarbon content of ants, they were then subjected to lipid extraction. Lipid extraction was performed following the methodology described by Bazazi et al. (2016) with modifications. Ants were inserted into glass vials, and 2 mL of chloroform (synth 100% m/m) were added to each flask. After 24 h, this solvent was removed, and 2 mL of chloroform were added again. This procedure was performed once again for a total of three extractions. After extraction and drying of the chloroform, each sample was esterified by adding 2 mL of hexane and 2 mL of potassium hydroxide (85% m/v) in methanol (Vetec 99.8% w/w) 2 mol/L to the sample. This mixture was vortexed for 5 min, after which the two-phase separation could be observed. The upper phase consisted of the solvent composed of the esters of fatty acids, and the lower phase was aqueous. The upper phase, which was used in the analysis of lipid compounds, was dried in an exhaust hood and stored for analysis using GC-MS.

2.3. Chromatographic analysis

2.3.1. Analysis of the hexane fraction

For analysis using gas chromatography coupled to mass spectrometry (GC-MS), the dried samples were solubilized by vortexing in 200 µL of hexane and then transferred to vials. The samples were analyzed using a gas chromatograph (GC-2010 Plus, Shimadzu, Kyoto, Japan) coupled to a mass spectrometer (GC-MS Ultra 2010, Shimadzu, Kyoto, Japan) using a DB-5 fused silica capillary (J and W, Folsom, California, USA) with 5% of phenyl dimethylpolysiloxane on capillary fused silica (30 m long × 0.25 mm internal diameter × 0.25 µm film thickness). The conditions of analysis were heating ramp with initial temperature of 150 °C, reaching 280 °C at 3 °C/min and remaining at the final temperature for 10 min. Helium (99.999%) was used as drag gas (1 mL/min), and injections were 1 µL in splitless mode. The injector, detector and transfer line temperatures were 250 °C, 250 °C and 290 °C, respectively. Scanning parameters of the mass spectrometer included electron beam ionization voltage of 70 eV, with *m/z* 40–600 and scanning range of 0.3 s.

Compound identifications were performed using the calculated retention index (IRC) (Van den Dool and Kratz, 1963) and the linear alkane (C_7–C_{40}, Sigma Aldrich purity ≥ 98%) standard, along with comparisons of the IRC with indexes found in the literature (Bonavita-Cougourdan et al., 1991; Brophy et al., 1983; Michelutti et al., 2017; Moore et al., 2014; Smith et al., 2016; Yusuf et al., 2010), associated with interpretation of mass spectra obtained from the samples and compared with the databases (NIST21 and WILEY229). Compounds containing less than 0.1% were not shown in the tables. Major compounds were those that represented at least 3% of the total relative percentage area of the CHCs and 10% of the fatty acids. For comparison of characteristic CHCs of each species, analyses of CHCs from the control group were performed. Control foragers were those ants without any experimental manipulation.

2.3.2. Analysis of the chloroform fraction

Samples extracted with chloroform and esterified were solubilized by adding 500 µL of hexane and analyzed using gas chromatograph coupled with mass detector (GC-MS Ultra 2010, Shimadzu Kyoto Japan). Chromatographic separation was performed on a fused silica column RTx-5MS (5% Phenyl- 95% Polydimethylsiloxane; 30 m × 0.25 mm ID, 0.25 µm) (Restek, Bellefonte, PA, USA). Helium gas (purity 99.995%) was used as the carrier at a constant flow rate of 5 mL/min. The temperature was set to start at 130 °C and was maintained for 1 min when it was raised to 170 °C at 6.5 °C/minute. Subsequently, another elevation of 170–215 °C was performed at 2.75 °C/min, and the temperature was maintained for 12 min. Finally, a final elevation was performed from 215 °C to 230 °C at 40 °C/minute. The temperatures of the injector, interface, and detector were 270, 280 and 280 °C, respectively. The 1 µL samples were injected in Split mode (1:20). Identification of the fatty acid methyl esters was performed by comparison with the fatty acid retention times of standards obtained from Sigma-Aldrich (dodecanoic acid ≥ 98%, myristic acid > 99%, palmitic acid > 99%, palmitolytic acid > 98.5%, stearic acid ≥ 98.5% and oleic acid > 99%) eluted under the same conditions.

2.4. Statistical analysis

From the spreadsheets obtained in the analysis of the chemical compounds extracted at each temperature, a nonparametric permutation analysis (PERMANOVA) was used to test the existence of significant differences among the temperatures. Euclidean distance was used to construct the similarity matrix, and significance of comparisons was calculated from the randomization of the original matrix (9999 permutations). Only area values greater than 1% were considered. All analyses were performed using the R platform (R Core Team, 2017).

3. Results

3.1. Thermotolerance

From 35 °C, 96.67% of *A. sexdens* workers and 86.67% of *O. bauri* workers survived during the experiment, while all *E. brunneum* workers survived. At 35 °C, a decrease in the number of survivors began to occur for *O. bauri* and *A. sexdens*. At 40 °C, 10% of *A. sexdens* workers and 56.67% of *E. brunneum* workers survived during the experiment. At 40 °C, a decrease in the number of survivors for all three species was observed, During the 100-min experiment, 100% of *O. bauri's* foragers had already died (Fig. 1). Ants of all the three species survived at temperatures from 10 °C to 30 °C, and for this reason, are not reported in the Fig. 1.

3.2. Analysis of cuticular chemical composition: hydrocarbons

A total of 24, 22 and 23 peaks were detected in the cuticle samples of *A. sexdens* (Table 1), *O. bauri* (Table 2) and *E. brunneum* (Table 3) samples, respectively. Compounds consisted of linear alkanes, branched alkanes and alkenes, with carbon chains varying from C_{15} to C_{32}. The Permanova analysis showed significant differences in the cuticular compounds of the three species of ants submitted to the different temperatures (Supplementary Table 1–3). Number of compounds, relative percentage area of the three different classes of compounds, and relative percentage area according to chain size were calculated, and the values are respectively presented in Figs. 2–4.

3.3. Analysis of cuticular chemical composition: fatty acids

Independent of exposure to different temperatures, six different fatty acids were identified in the samples of the three ant species studied, including lauric acid, myristic acid, palmitic acid, palmitoleic acid, stearic acid and oleic acid. Their relative percentage areas in the samples submitted to the different treatments and control are found in Tables 4–6. PERMANOVA analysis did not present statistical difference in the fatty acids, either in samples submitted to treatments or control, and the results are as follows: *A. sexdens*, Pseudo-$F_{(7,136)}$ = 0.231; p = 0.998; *O. bauri*, Pseudo-$F_{(7,136)}$ = 0.400; p = 0.986; and *E. brunneum*, Pseudo-$F_{(7,136)}$ = 0.598; p = 0.779.

4. Discussion

The CHCs identified in the cuticle of the three ant species (Tables

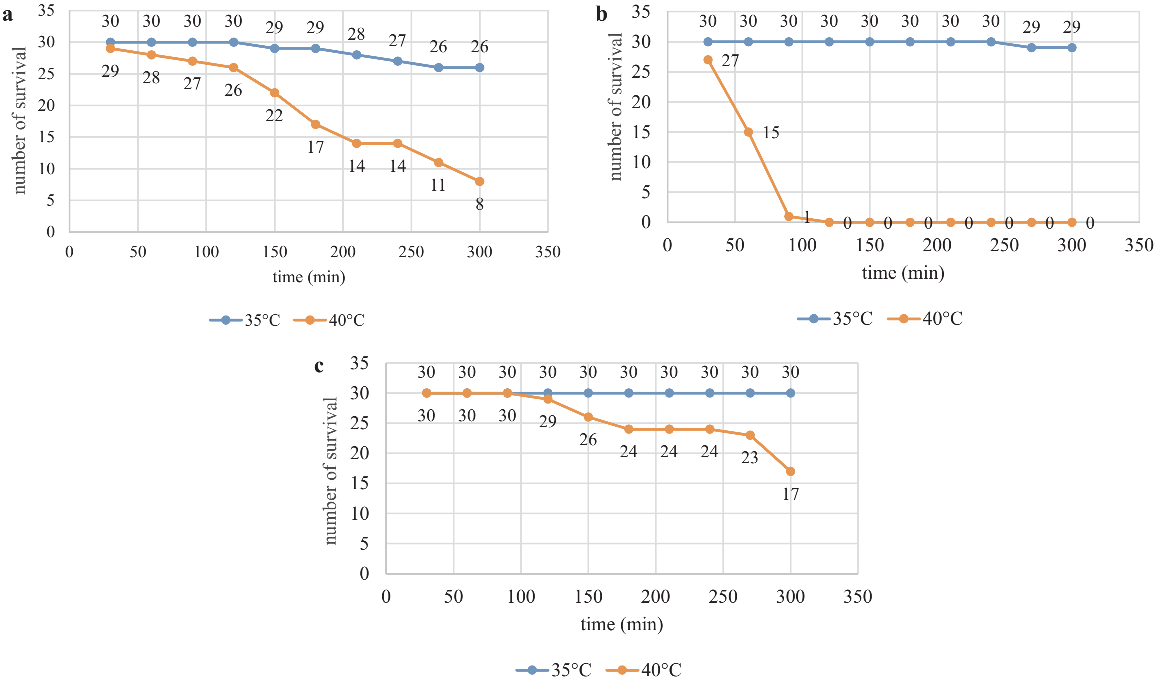

Fig. 1. Probability of survival of workers of a) *Atta sexdens*, b) *Odontomachus bauri* and c) *Ectatomma brunneum* under different temperature.

1–3) included the linear alkanes, branched alkanes and alkenes. Studies in the literature also confirm that these are the main classes of CHCs typically identified in the cuticle of insects (Blomquist and Begnères, 2010; Howard and Blomquist, 2005; Martin et al., 2004; Zhu et al., 2006). Linear alkanes had a higher number and percentage of compounds among the three species (Figs. 2 and 3). Since we studied only worker/foragers, the linear alkanes with longer chains may be linked waterproofing to protect from desiccation (Gibbs, 1998; Menzel et al., 2017; Wagner et al., 2001) since workers are the most exposed to the elements. In number of compounds, the second most representative class in the *A. sexdens* and *E. brunneum* samples was the alkenes and, finally, the branched alkanes, whereas in the *O. bauri* samples, branched alkanes was the most representative class. However, the second most representative class was alkenes in *A. sexdens* and *E. brunneum*, whereas in *O. bauri*, the second most representative class was branched alkanes. These variations in CHCs are inherent to species (Akino et al., 2002; Martin et al., 2008); therefore, CHCs function as a complementary taxonomic tool addition to species-specific variation (Kather and Martin, 2012), and they can be affected by environmental and genetic factors (Blomquist and Bagnères, 2010; Hefetz, 2007; Wagner et al., 2001).

When temperatures rose above 35 °C, a temperature when *A. sexdens* workers began to die, we can see an increase in the production of linear alkanes in an attempt to meet the need for waterproofing. Linear alkanes have a higher waterproofing capacity since these compounds increase the viscosity of the cuticle (Gibbs, 1998; Hefetz, 2007; Menzel et al., 2017; Wagner et al., 2001). At temperatures above 35 °C, the alkenes suffered a reduction in their percentage area, while the branched alkanes showed an increase relative to the control group (Fig. 3). However, these branched alkanes and alkenes increase the fluidity of the cuticle (Menzel et al., 2017), and as a result, these compounds are important for the communication (Gibbs, 1998; Hefetz, 2007; Le Conte and Hefetz, 2008; Lorenzi et al., 1997). However, branched alkanes and alkenes also have a lower melting point than their respective linear alkanes (Gibbs and Pomonis, 1995); therefore, it would not redound to the benefit of the ant to increase the percentage of this class of compounds when exposed to temperature variation, especially at relatively higher temperatures.

Table 2 shows that one of the major compounds of *O. bauri* is a dimethyl alkane (12,16-, 13,17-, 14,18- Dimethyldotriacontane). This feature does not benefit insects in the sense of avoiding waterproofing at high temperatures since, according to Gibbs and Pomonis (1995), the insertion of branches and instaurations decrease the melting temperature of the compounds, which may, in turn, contribute to the lower tolerance of this species at temperatures above 30 °C.

At temperatures below 25 °C, branched alkanes and alkenes increased for *A. sexdens* and *O. bauri*. The increase of branched alkanes and alkenes at low temperatures is expected since these compounds increase the fluidity of the cuticle which is necessary, especially under low temperatures, for the homogenization and transmission of the compounds throughout the cuticle (Menzel et al., 2017). In *E. brunneum*, this behavior was very similar, except at 10 °C when the number of alkenes decreased.

At temperatures of 35 and 40 °C, branched alkanes increased in the relative percentage area of *E. brunneum* in relation to control (Fig. 3). Although branched alkanes are considered to be important in mediating communication, all these compounds may actually perform dual functions, i.e., waterproofing and communicating (Boulay et al., 2017; Chown et al., 2011; Chung and Carroll, 2015; Gibbs, 2007). Menzel et al. (2007), in fact, also suggest that branched alkanes provide such dual functions.

Intermediate chain compounds increased in relation to relative percentage area and length of the carbonic chain when exposed to temperatures below 25 °C. At temperatures above 30 °C, intermediate and long-chain compounds increased, while short-chain compounds were reduced relative to control. These results were expected since the melting temperature of compounds increases with increasing chain length (Gibbs and Pomonis, 1995). Long-chain CHCs tend to fuse only at higher temperatures; therefore, as the melting temperature of a compound increases, the loss of water through the cuticle decreases (Gibbs et al., 1997; Gibbs, 1998). In fact, Gibbs et al. (1997) have identified the presence of CHCs with longer chain lengths in *Drosophila melanogaster* flies under desiccation conditions. In a study with the ants *T. longispinosus* and *T. ambiguus*, Menzel et al. (2018) identified significant differences in chain length of linear alkanes in foragers which perform tasks outside the colony and similarity between nurses and

Table 1

Relative percentage area of compounds of *Atta sexdens* cuticle for samples exposed to different treatments and control.

Compound	**ECL**	**Control** M ± SD	**10 °C** M ± SD	**15 °C** M ± SD	**20 °C** M ± SD	**25 °C** M ± SD	**30 °C** M ± SD	**35 °C** M ± SD	**40 °C** M ± SD
Pentadecane[a]	1500	9.93 ± 0.61	29.75 ± 10.40	30.31 ± 4.85	14.61 ± 6.58	10.58 ± 3.82	13.64 ± 5.85	24.00 ± 5.31	13.84 ± 3.74
Hexadecane	1600	15.56 ± 2.18[a]	9.90 ± 3.40[a]	2.09 ± 0.92	0.81 ± 1.42	15.65 ± 3.76[a]	14.65 ± 3.15[a]	0.69 ± 1.95	1.31 ± 2.53
Heptadecane[a]	1700	10.66 ± 0.38	7.53 ± 5.28	16.73 ± 3.65	8.75 ± 4.23	12.25 ± 3.21	13.12 ± 5.83	9.48 ± 4.24	9.05 ± 2.78
5-Methylheptadecane	1750	–	–	–	–	–	9.32 ± 8.39[a]	0.70 ± 2.21	–
Octadecane	1800	33.78 ± 1.30[a]	–	–	–	40.68 ± 6.33[a]	23.0 ± 7.28[a]	–	2.96 ± 9.36
9-Nonadecene	1874	3.77 ± 0.20[a]	0.21 ± 0.35	–	0.62 ± 0.47	2.60 ± 1.80	0.96 ± 1.24	–	–
Nonadecane	1900	2.57 ± 0.17	1.38 ± 2.22	2.98 ± 5.86	2.49 ± 2.21	2.50 ± 0.24	2.01 ± 0.67	0.21 ± 0.46	0.70 ± 1.10
1-Eicosene	1994	3.30 ± 0.13[a]	2.88 ± 4.64	7.70 ± 1.30[a]	3.99 ± 2.89[a]	3.00 ± 1.69[a]	3.40 ± 0.73[a]	0.66 ± 1.08	1.99 ± 2.32
Eicosane	2000	1.12 ± 0.08	3.82 ± 2.69[a]	4.18 ± 1.63[a]	1.93 ± 1.32	0.91 ± 0.68	0.87 ± 0.47	–	0.63 ± 1.03
Heneicosane	2100	–	0.67 ± 0.66	0.52 ± 0.31	0.80 ± 0.16	0.10 ± 0.01	0.15 ± 0.11	0.47 ± 0.26	0.51 ± 0.52
11-Methylheneicosane	2136	1.10 ± 0.08	0.40 ± 0.63	3.56 ± 4.35[a]	1.19 ± 0.90	1.13 ± 0.12	3.13 ± 0.58[a]	2.16 ± 2.66	2.00 ± 0.64
1-Docosene	2195	1.15 ± 0.05	7.21 ± 4.60[a]	6.93 ± 1.07[a]	3.78 ± 0.97[a]	0.84 ± 0.53	1.40 ± 0.28	0.85 ± 1.07	1.32 ± 0.95
11-Tricosene	2273	0.64 ± 0.03	0.20 ± 0.64	0.34 ± 0.56	5.50 ± 4.18[a]	0.61 ± 0.46	0.77 ± 1.00	1.64 ± 3.48	–
Tricosane	2300	–	1.71 ± 0.70	0.55 ± 0.27	1.72 ± 0.15	0.06 ± 0.13	0.28 ± 0.51	1.62 ± 0.61	1.69 ± 0.44
11-Methyltricosane	2333	0.71 ± 0.09	0.83 ± 0.58	1.74 ± 0.14	1.18 ± 0.38	0.65 ± 0.07	0.81 ± 0.41	1.23 ± 0.20	1.42 ± 0.36
1-Tetracosene	2396	–	0.78 ± 0.45	0.65 ± 0.19	0.42 ± 0.24	–	0.09 ± 0.07	–	0.04 ± 0.09
Tetracosane	2400	–	3.39 ± 2.17[a]	1.06 ± 0.11	15.34 ± 2.16[a]	–	0.01 ± 0.01	1.44 ± 1.73	17.15 ± 5.13[a]
Pentacosane[a]	2500	13.12 ± 1.88	15.76 ± 5.23	12.64 ± 5.27	19.95 ± 6.07	6.36 ± 2.13	7.94 ± 1.39	31.69 ± 6.74	25.23 ± 8.83
Hexacosane	2600	–	1.02 ± 0.36	0.49 ± 0.39	2.80 ± 1.18	–	0.10 ± 1.11	2.82 ± 2.58	3.45 ± 0.96[a]
Heptacosane	2700	1.71 ± 0.12	8.52 ± 3.76[a]	4.29 ± 0.89[a]	9.53 ± 2.00[a]	1.44 ± 0.54	2.45 ± 0.60	14.87 ± 3.62[a]	11.09 ± 3.88[a]
Octacosane	2800	–	–	–	1.05 ± 0.11	–	–	0.85 ± 1.28	1.13 ± 0.38
Nonacosane	2900	–	–	–	1.59 ± 0.35	–	–	0.99 ± 1.31	0.60 ± 0.69
x.y-Dimethylltriacontane	3060	–	3.02 ± 0.67[a]	0.34 ± 0.76	1.13 ± 0.84	–	0.88 ± 0.66	3.03 ± 1.60[a]	3.13 ± 1.87[a]
NI	1906	0.88 ± 0.05	0.98 ± 0.91	2.87 ± 0.53	0.78 ± 0.61	0.64 ± 0.45	1.06 ± 0.41	0.58 ± 0.79	0.74 ± 0.92
Percentage of identified compounds:		***99.12***	***99.02***	***97.13***	***99.22***	***99.46***	***99.04***	***98.41***	***99.26***

#Control not subjected to temperature change. ECL = Equivalent Chain Length.

NI: Not identified.

n = 10.

[a] Major compounds for different temperatures.

Table 2

Relative percentage area of compounds of *Odontomachus bauri* cuticle for samples exposed to different treatments and control.

Compound	ECL	**Control** M ± DP	**10 °C** M ± SD	**15 °C** M ± SD	**20 °C** M ± SD	**25 °C** M ± SD	**30 °C** M ± SD	**35 °C** M ± SD	**40 °C** M ± SD
Pentadecane[a]	1500	14.18 ± 1.60	17.14 ± 16.19	21.21 ± 8.23	20.79 ± 11.21	11.78 ± 4.08	13.5 ± 10.56	12.32 ± 3.23	7.12 ± 3.25
Hexadecane	1600	15.07 ± 0.84[a]	6.36 ± 6.30[a]	5.87 ± 2.82[a]	0.99 ± 1.26	16.68 ± 3.61[a]	14.9 ± 8.20[a]	0.20 ± 0.45	0.50 ± 1.33
Heptadecane[a]	1700	13.88 ± 1.11	15.81 ± 21.7	11.21 ± 5.71	10.75 ± 6.61	11.97 ± 4.21	12.29 ± 8.11	5.23 ± 2.31	9.09 ± 13.07
Octadecane	1800	34.29 ± 2.30	–	–	1.90 ± 3.14	38.52 ± 9.01[a]	34.94 ± 18.47	–	–
Nonadecane	1900	2.06 ± 0.43	0.54 ± 0.95	0.81 ± 0.94	0.60 ± 0.80	2.09 ± 0.66	1.06 ± 0.90	0.06 ± 0.16	0.27 ± 0.50
1-eicosene	1994	3.53 ± 0.21	4.32 ± 4.51[a]	4.28 ± 3.43[a]	2.49 ± 2.71	3.25 ± 1.96[a]	4.07 ± 2.22[a]	1.03 ± 1.02	1.08 ± 1.87
Eicosane	2000	1.71 ± 0.21	1.68 ± 2.02	1.86 ± 2.49	0.71 ± 0.89	1.01 ± 0.78	1.46 ± 0.79	0.26 ± 0.36	0.37 ± 0.71
11-Methyl heneicosane	2136	1.39 ± 0.21	0.85 ± 0.78	1.67 ± 0.53	0.90 ± 0.77	1.39 ± 0.21	1.2 ± 0.23	1.12 ± 0.44	0.91 ± 0.3
1-Docosene	2195	1.32 ± 0.19	2.15 ± 2.08	2.43 ± 1.99	2.15 ± 2.64	1.04 ± 0.57	1.27 ± 0.69	0.76 ± 0.47	0.54 ± 0.28
11-Methyltricosane	2333	0.81 ± 0.14	0.49 ± 0.65	1.24 ± 0.36	0.69 ± 0.59	0.81 ± 0.13	0.55 ± 0.20	0.86 ± 0.19	0.71 ± 0.27
Tetracosane	2400	–	–	0.24 ± 0.40	–	–	–	1.04 ± 0.28	6.22 ± 2.48
Pentacosane	2500	–	–	–	–	–	–	1.98 ± 0.45	0.96 ± 0.40
Hexacosane	2600	–	–	–	–	–	–	2.33 ± 0.55	1.03 ± 0.41
Heptacosane	2700	1.94 ± 0.17	5.46 ± 4.19[a]	7.85 ± 3.18[a]	13.11 ± 5.78[a]	1.78 ± 0.84	1.76 ± 0.66	16.69 ± 3.38[a]	6.39 ± 2.60[a]
Octacosane	2800	–	–	–	–	–	–	1.45 ± 0.60	0.51 ± 0.26
4-Methyloctacosane	2849	–	–	–	–	–	–	–	0.12 ± 0.38
Nonacosane	2900	–	–	0.28 ± 0.63	2.46 ± 1.41	–	0.4 ± 0.46	3.42 ± 0.85[a]	1.26 ± 0.72
Hentriacontene	3094	2.20 ± 0.92	7.86 ± 5.23[a]	6.53 ± 1.86[a]	6.72 ± 2.56[a]	2.19 ± 0.91	2.21 ± 1.30	10.22 ± 1.64[a]	9.86 ± 2.57[a]
12.16-; 13.17-; 14.18-Dimethyldotriacontane[a]	3248	7.61 ± 0.63	24.11 ± 15.31	22.44 ± 5.86	28.47 ± 9.40	7.3 ± 2.74	7.37 ± 2.35	32.29 ± 3.15	32.44 ± 10.25
x.y –Dimethyldotriacontane	3259	–	6.10 ± 4.88[a]	4.39 ± 2.06[a]	5.42 ± 3.22[a]	0.11 ± 0.37	2.06 ± 1.30	7.59 ± 2.06[a]	10.98 ± 2.90[a]
NI	1906	–	1.83 ± 1.09	0.81 ± 0.98	1.35 ± 0.70	–	0.85 ± 0.89	1.07 ± 0.24	0.71 ± 0.30
Percentage of identified compounds:		***100***	***98.17***	***99.18***	***98.64***	***100***	***99.14***	***98.93***	***99.28***

Control. not subjected to temperature change. ECL = Equivalent Chain Length.

NI: Not identified.

n = 10.

[a] Major compounds for different temperatures.

Table 3

Relative percentage area of compounds of substitute for *Ectatomma brunneum* cuticle for samples exposed to different treatments and control.

Compound	ECL	**Control** M ± SD	**10 °C** M ± SD	**15 °C** M ± SD	**20 °C** M ± SD	**25 °C** M ± SD	**30 °C** M ± SD	**35 °C** M ± SD	**40 °C** M ± SD
Pentadecane[a]	1500	13.41 ± 1.26	29.56 ± 13.04	7.61 ± 1.40	10.76 ± 2.08	11.9 ± 3.46	47.15 ± 32.24	10.96 ± 6.99	12.80 ± 4.04
Hexadecane[a]	1600	12.42 ± 0.68	29.83 ± 6.14	10.97 ± 2.04	6.76 ± 3.62	12.71 ± 1.83	9.14 ± 13.4	10.67 ± 3.89	10.21 ± 2.94
Heptadecane	1700	9.73 ± 0.67[a]	2.30 ± 2.49	9.12 ± 1.17[a]	6.66 ± 1.94[a]	9.34 ± 1.27[a]	2.35 ± 4.07	5.80 ± 3.59[a]	8.21 ± 1.06[a]
Octadecane[a]	1800	41.02 ± 2.40	14.97 ± 13.53	36.3 ± 2.58	29.58 ± 3.29	41.95 ± 2.24	10.32 ± 17.35	36.46 ± 7.06	24.15 ± 2.92
9-nonadecene	1874	11.01 ± 1.52	16.24 ± 10.60[a]	4.54 ± 3.10[a]	2.43 ± 4.21	12.89 ± 5.40[a]	12.02 ± 17.09[a]	3.23 ± 2.16[a]	2.86 ± 2.43
Nonadecane	1900	3.02 ± 0.08[a]	0.50 ± 0.60	2.33 ± 0.25	1.96 ± 0.21	2.56 ± 0.40	16.03 ± 30.42[a]	2.57 ± 0.39	2.39 ± 1.25
1-eicosene	1994	3.93 ± 0.36[a]	1.07 ± 1.20	4.00 ± 0.29[a]	3.70 ± 0.51[a]	4.42 ± 0.64[a]	1.27 ± 2.24	5.63 ± 0.79[a]	3.14 ± 0.66[a]
Eicosane	2000	1.33 ± 0.17	0.61 ± 0.45	1.12 ± 0.04	1.39 ± 0.28	1.39 ± 0.13	0.42 ± 0.78	1.9 ± 0.76	1.08 ± 0.18
1-Docosene	2195	0.96 ± 0.06	–	0.67 ± 0.12	0.41 ± 0.09	0.61 ± 0.28	0.07 ± 0.17	0.83 ± 0.37	0.22 ± 0.29
11-tricosene	2195	–	–	0.46 ± 0.22	0.22 ± 0.12	–	–	–	0.58 ± 0.31
Tricosane	2300		0.10 ± 0.21	0.10 ± 0.09	0.15 ± 0.33	–	–	–	0.04 ± 0.12
2-methyldocosane	2328	0.55 ± 0.13	0.93 ± 0.31	0.52 ± 0.04	3.16 ± 5.33[a]	0.41 ± 0.16	0.55 ± 0.80	0.53 ± 0.17	0.45 ± 0.29
Tetracosane	2400	–	0.01 ± 0.03	7.03 ± 0.64[a]	9.73 ± 6.19[a]	–	–	1.18 ± 0.66	14.16 ± 5.80[a]
Pentacosane	2500	–	2.99 ± 5.24	0.02 ± 0.03	0.04 ± 0.12	–	–	–	–
Heptacosane	2700	–	–	0.47 ± 0.28	1.18 ± 1.25	–	–	0.82 ± 0.56	–
Octacosane	2800	–	–	0.02 ± 0.03	0.08 ± 0.10	–	–	–	–
Nonacosane	2900	1.57 ± 0.28	–	4.48 ± 2.67[a]	4.98 ± 5.01[a]	0.78 ± 0.44	–	6.91 ± 3.87[a]	2.64 ± 1.50
5.11-dimethylnonacosane	2979	–	–	2.69 ± 1.49	6.62 ± 8.29[a]	–	–	6.6 ± 4.93[a]	3.78 ± 3.79[a]
Hentriacontane	3100	–	–	0.50 ± 0.36	0.47 ± 0.72	–	–	0.64 ± 0.51	0.83 ± 0.87
5-methylhentriacontane	3152	–	–	0.56 ± 0.41	0.88 ± 0.96	–	–	2.42 ± 2.27	–
NI	1924	–	–	4.98 ± 0.56[a]	6.78 ± 1.38[a]	–	–	1.76 ± 0.46	10.13 ± 2.26[a]
NI	2112	1.02 ± 0.06	0.87 ± 0.52	0.88 ± 0.10	1.13 ± 0.22	0.98 ± 0.04	0.64 ± 0.78	0.94 ± 0.18	0.93 ± 0.23
NI	2125	–	–	0.66 ± 0.11	0.87 ± 0.18	–	–	0.11 ± 0.13	1.24 ± 0.26
Percentage of compounds identified:		***98.98***	***99.13***	***93.48***	***91.21***	***99.02***	***99.35***	***97.18***	***87.66***

#Control not subjected to temperature change. ECL = Equivalent Chain Length.

n = 10.

NI: Not identified.

[a] Major compounds for different temperatures.

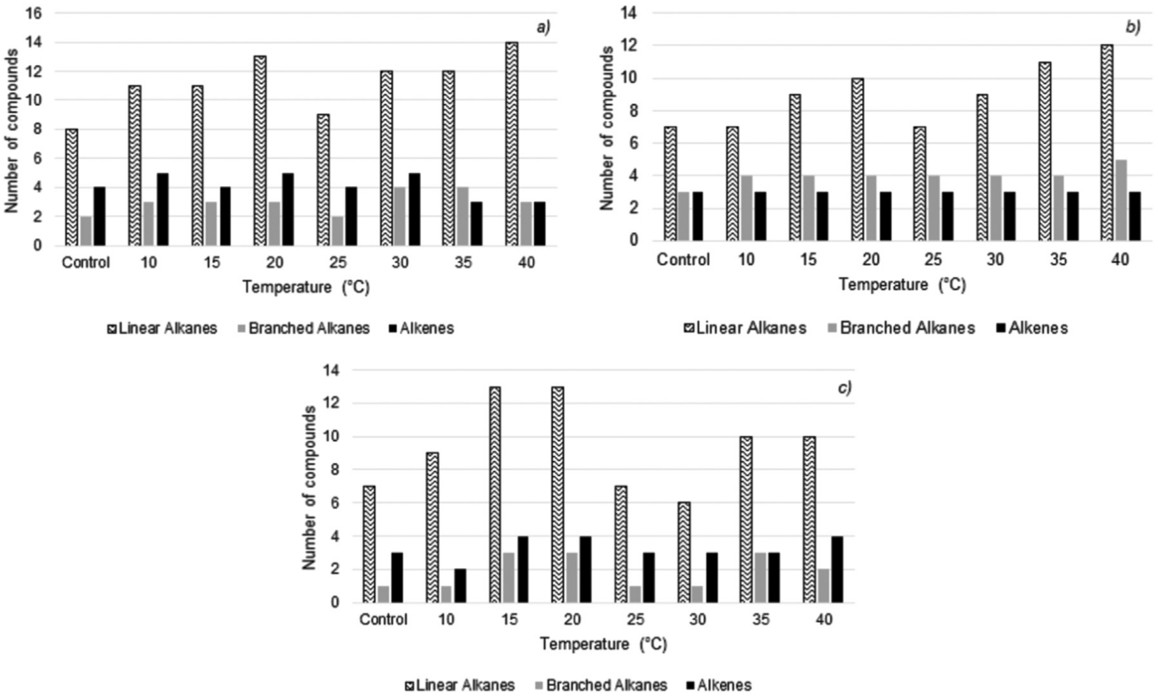

Fig. 2. Number of compounds in compound classes present in the cuticle of a) *Atta sexdens*, b) *Odontomachus bauri* and c) *Ectatomma brunneum* foragers exposed to different temperatures and control.

queens, who perform tasks within the colony. The authors then suggested that the determining factor of cuticular chemical composition would be the climatic physical factors to which these castes and subcastes are subjected. This relationship between temperature and chain length variation of cuticular compounds was also evaluated by Michelutti et al. (2018) in three species of social wasps.

Still comparing the CHC of the ants exposed to temperature variation relative to control ants, it was possible to evaluate differences in the number and relative percentage of compounds. These results add further support to the affirmation that ants can respond to temperature variation by adjustment of CHCs, as previously reported by Gibbs et al. (1997) *with D. melanogaster*, Menzel et al. (2018) with *T. longispinosus* and *T. ambiguous*, and Michelutti et al. (2018) with the wasps *Polybia paulista, Polybia ignobilis* and *Polybia versicolor*. In addition, a detailed review of temperature-dependent CHC variation in different insect species can be seen in Otte et al. (2018).

Survival tests showed that ants exposed to a temperature of 35 °C began to die, whereas in analyses of the number of compounds, relative

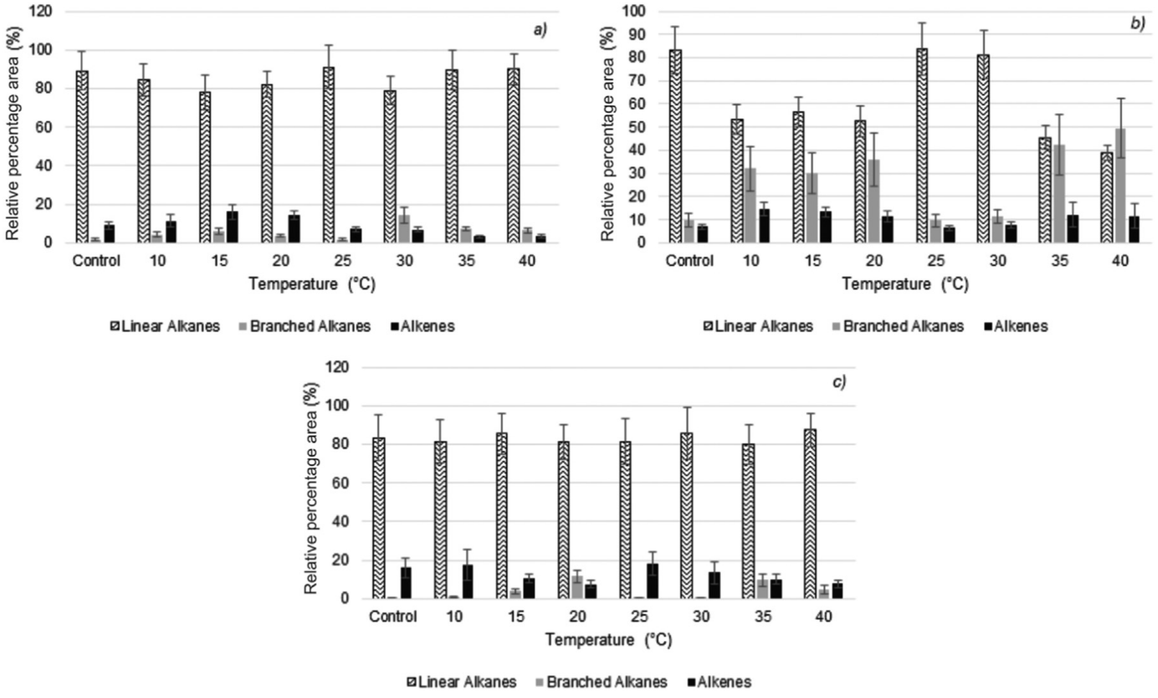

Fig. 3. Relative percentage area of the three compound classes present in the cuticle of a) *Atta sexdens*, b) *Odontomachus bauri* and c) *Ectatomma brunneum* foragers exposed to different temperatures and control.

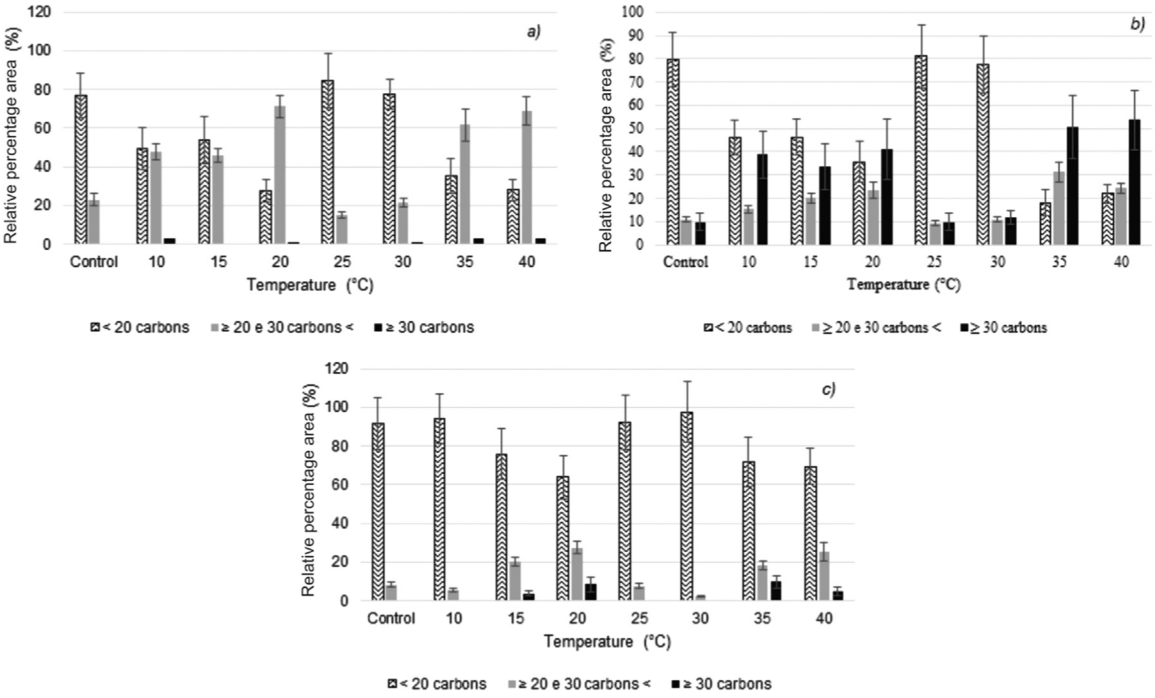

Fig. 4. Relative percentage area of the compounds present in the cuticle of a) *Atta sexdens*, b) *Odontomachus bauri* and c) *Ectatomma brunneum*, according to chain size, upon exposure to different temperatures and control.

Table 4
Relative percentage area of fatty acids presents in the cuticle of *Atta sexdens* submitted to different temperatures.

Compound	**Control** M ± SD	**10 °C** M ± SD	**15 °C** M ± SD	**20 °C** M ± SD	**25 °C** M ± SD	**30 °C** M ± SD	**35 °C** M ± SD	**40 °C** M ± SD
Lauric acid	0.52 ± 0.01	0.52 ± 0.01	0.51 ± 0.01	0.52 ± 0.01	0.52 ± 0.01	0.52 ± 0.01	0.52 ± 0.01	0.52 ± 0.01
Myristic acid	2.34 ± 0.01	2.34 ± 0.01	2.34 ± 0.01	2.34 ± 0.02	2.35 ± 0.01	2.34 ± 0.01	2.35 ± 0.01	2.35 ± 0.02
Palmitic acid[b]	34.64 ± 0.04	34.61 ± 0.08	34.63 ± 0.05	34.69 ± 0.07	34.60 ± 0.08	34.61 ± 0.08	34.65 ± 0.03	34.62 ± 0.05
Palmitoleic acid	3.59 ± 0.02	3.57 ± 0.01	3.60 ± 0.01	3.59 ± 0.02	3.59 ± 0.06	3.58 ± 0.02	3.6 ± 0.01	3.58 ± 0.01
Stearic acid	4.21 ± 0.01	4.21 ± 0.02	4.21 ± 0.01	4.2 ± 0.03	4.21 ± 0.04	4.2 ± 0.01	4.2 ± 0.01	4.18 ± 0.01
Oleic acid[b]	53.95 ± 0.01	53.96 ± 0.06	53.90 ± 0.02	53.95 ± 0.16	53.92 ± 0.12	53.95 ± 0.12	53.98 ± 0.07	53.96 ± 0.15

n = 3; Control not subject to temperature variation.
[b] Major compounds for different temperatures > 10%.

Table 5
Relative percentage area of fatty acids present in the cuticle of *Odontomachus bauri* submitted to different temperatures.

Compound	**Control** M ± SD	**10 °C** M ± SD	**15 °C** M ± SD	**20 °C** M ± SD	**25 °C** M ± SD	**30 °C** M ± SD	**35 °C** M ± SD	**40 °C** M ± SD
Lauric acid	0.51 ± 0.02	0.50 ± 0.02	0.50 ± 0.01	0.51 ± 0.01	0.50 ± 0.01	0.51 ± 0.01	0.50 ± 0.01	0.50 ± 0.01
Myristic acid	2.40 ± 0.05	2.42 ± 0.02	2.34 ± 0.01	2.42 ± 0.04	2.43 ± 0.01	2.43 ± 0.02	2.43 ± 0.02	2.42 ± 0.03
Palmitic acid[b]	33.85 ± 0.08	33.76 ± 0.12	33.90 ± 0.11	33.82 ± 0.06	33.84 ± 0.13	33.85 ± 0.14	33.79 ± 0.06	33.83 ± 0.17
Palmitoleic acid	3.74 ± 0.01	3.74 ± 0.04	3.73 ± 0.03	3.75 ± 0.01	3.73 ± 0.04	3.75 ± 0.03	3.74 ± 0.01	3.73 ± 0.04
Stearic acid	4.02 ± 0.03	4.00 ± 0.02	4.00 ± 0.02	3.99 ± 0.02	4.03 ± 0.04	4.01 ± 0.06	4.01 ± 0.02	3.98 ± 0.05
Oleic acid[b]	54.6 ± 0.03	54.62 ± 0.06	54.61 ± 0.08	54.57 ± 0.14	54.56 ± 0.09	54.53 ± 0.15	54.6 ± 0.12	54.55 ± 0.15

n = 3; Control not subject to temperature variation.
[b] Major compounds for different temperatures > 10%.

percentage area, and chain length showed that greater differentiation in structural properties of the compounds began to occur at this temperature. Thus, it is possible to affirm that such extreme temperature thresholds militate against the well-being of foraging ants since it damages cuticular composition in response to the temperature increase. On the other hand, Ramsay (1935), studying the cockroach *Periplaneta americana*, reported a marked increase water loss at temperatures above 30 °C. Wigglesworth (1945) found similar results in a study of *Blattella germanica*. These authors highlight the conformational change of the compounds that causes the rupture of the cuticle and causes water loss. Thus, a cause of structural cuticular change in temperatures above 30 °C can be attributed to the attempt of ants to match, i.e., balance, the composition of chemical constituents of their cuticle to the outside environment in order to avoid water loss.

From these results, we learn that ants tend to forage at temperatures that will result in the lowest expenditure of energy and that such temperature will, in turn, affect the foraging behavior of ants (Robinson and Fowler, 1982; Nielsen, 1986; Hölldobler and Wilson, 1990). This explains why ants will always seek a range ideal for foraging. Accordingly, Lima and Antonialli-Junior (2013) evaluated *Ectatomma vizottoi*

Table 6
Relative percentage area of fatty acids present in the cuticle of *Ectatomma brunneum* submitted to different temperatures.

Compound	Control M ± SD	10 °C M ± SD	15 °C M ± SD	20 °C M ± SD	25 °C M ± SD	30 °C M ± SD	35 °C M ± SD	40 °C M ± SD
Lauric acid	0.50 ± 0.02	0.50 ± 0.01	0.49 ± 0.01	0.49 ± 0.01	0.49 ± 0.01	0.50 ± 0.01	0.49 ± 0.01	0.51 ± 0.01
Myristic acid	2.38 ± 0.01	2.38 ± 0.01	2.37 ± 0.01	2.38 ± 0.02	2.40 ± 0.02	2.39 ± 0.01	2.39 ± 0.02	2.39 ± 0.01
Palmitic acid[b]	34.68 ± 0.03	34.65 ± 0.01	34.67 ± 0.02	34.66 ± 0.04	34.68 ± 0.02	34.67 ± 0.02	34.66 ± 0.05	34.70 ± 0.02
Palmitoleic acid	3.59 ± 0.01	3.58 ± 0.01	3.60 ± 0.01	3.59 ± 0.02	3.59 ± 0.01	3.60 ± 0.01	3.60 ± 0.01	3.62 ± 0.02
Stearic acid	4.27 ± 0.01	4.26 ± 0.03	4.27 ± 0.03	4.28 ± 0.01	4.27 ± 0.02	4.29 ± 0.01	4.25 ± 0.04	4.25 ± 0.03
Oleic acid[b]	54.34 ± 0.10	54.58 ± 0.56	54.30 ± 0.32	54.32 ± 0.31	54.32 ± 0.21	54.22 ± 0.12	54.18 ± 0.02	54.24 ± 0.05

n = 3; Control not subject to temperature variation.
[b] Major compounds for different temperatures > 10%.

foragers and found that they are active in temperatures ranging from 14 °C to 32 °C, showing that temperature is a crucial factor in foraging behavior in this species.

Thus, quite in line with the hypothesis driving the present work, different ant species exert different strategies in response to changes in environmental temperature (Bouchebti et al., 2015; Boulay et al., 2017), and changes in the conformation of CHCs demonstrate this (Gibbs et al., 1997; Menzel et al., 2017, 2018; Michelutti et al., 2018; Wagner et al., 2001). In addition, Menzel et al. (2018) showed that the survival of a species with changing climatic conditions will depend less on its current profile and more on the phenotypic plasticity of the CHC profile. In this sense, Otte et al. (2018) emphasize that it is necessary that the insect have the capacity to express phenotypic response to changes in the environment and that such response be fast enough to accompany these environmental changes.

On the other hand, the ecological habits of different species can affect the capacity of hydrocarbons to respond to temperature changes and, consequently, the ability of ants to survive. For instance, *O. bauri* presented the least tolerance to temperature change in the survival test. Foragers of this species tend to forage in places protected from wide temperature swings, such as wood and serrapilheira, as also reported by Ehmer and Hölldobler (1996), *A. sexdens*, an ant species that forages according to the availability of resources within particular microenvironments (Fowler and Robinson, 1979), presented an intermediate tolerance level to temperature variation compared to the other two species. *E. brunneum* was the most tolerant and *de facto* forages in open field areas during both day and night. It also presented the best results in the survival test (Overal, 1986). The tolerance level of the three species also seems to reflect an ability to respond to environmental changes by altering the chemical composition of their cuticles (Fig. 1 and Tables 1–3), which is likely also a reflection of their different foraging habits.

From this perspective, the chemical compound constituents of the insect's cuticle must remain in constant "balance" in order to maintain control over body temperature and communicate with nestmates (Blomquist and Bagnères, 2010), and it is well known that temperature variations and relative humidity may affect the cuticular chemical composition of insects (Hefetz, 2007; Wagner et al., 2001).

Six fatty acids were identified in the samples of the three species. These acids exhibited neither qualitative nor quantitative differences among the species, and the major acids for the three species were palmitic and oleic. Comparing the composition of fatty acids of seven orders of insects, including Hymenoptera, Thompson (1973) detected quantitative difference between insects, and oleic and palmitoleic acids were the most frequently identified fatty acids in Hymenoptera.

The similarity of composition found in the fatty acids of the three species of ants studied agrees with the findings of Lok et al. (1975) who reported on adult ants of the species *Solenopsis invicta* and *Solenopsis richteri* and demonstrated that the composition of fatty acids of adults was similar between the two species. Rosumek et al. (2017) also found similarity in the fatty acids present in the foragers of ants of the species *F. fusca* and *M. rubra*.

Palmitic acid has already been identified in the nest material of two species of wasps, *Polistes annularis* (Espelie and Hermann, 1990) and *Mischocyttarus consimilis* (Michelutti et al., 2017); stearic acid was also identified in the nest of *P. annularis* (Espelie and Hermann, 1990). Up to now, it was believed that these acids could act as an ant-repellent substance (Espelie and Hermann, 1990; Michelutti et al., 2017); however, since these compounds are found in three species of ants, perhaps the function of these compounds is also related to the maintenance of the waterproofing structure.

5. Conclusion

Based on our results, it can be concluded that the forager ants studied respond differently to temperature variation. Across species, we saw the increase of compounds with longer chains and an abundance of linear alkanes, results that could be expected based on reports in the literature showing how important these compounds are for waterproofing. Changes in the conformation of CHCs are in line with the ecological characteristics of the different species because, as reported, they vary in terms of diurnal/nocturnal foraging and types of environments foraged. Thus, among the three species, *E. brunneum* foragers were more active under adverse conditions and more tolerant to temperature variation with the correspondingly appropriate changes in CHCs composition.

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Conflicts of interest

All authors report no conflicts of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jtherbio.2019.02.005.

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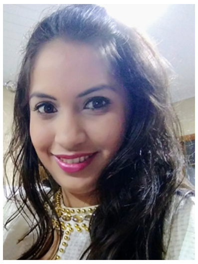

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