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Qualitative and quantitative differences in cuticular hydrocarbons between laboratory and field colonies of *Pogonomyrmex barbatus*

Madeleine Tissot[a,1], Dennis R. Nelson[b,*], Deborah M. Gordon[a]

[a] *Department of Biological Sciences, Gilbert Hall, Stanford University, Stanford, CA 94305, USA*
[b] *Biosciences Research Laboratory, Agricultural Research Service, US Department of Agriculture, State University Station, Fargo, ND 58105, USA*

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Abstract

Ants held in the laboratory and field ants of the species *Pogonomyrmex barbatus* have quantitative differences in their cuticular hydrocarbons and a qualitative difference in their methyl-branched hydrocarbons. Laboratory-held workers showed twice the hydrocarbon content as field ants. This difference was mainly due to higher amounts of straight-chain alkanes and methyl-branched alkanes in laboratory ants, whereas the proportion of the alkenes remained the same for both groups. In addition to the absence of some hydrocarbons in the field colonies, one of the methyl-branched hydrocarbons differed in amount and branching pattern between the two groups of ants. Whereas, notable peaks of 2-methylalkanes were identified in ants kept in the laboratory, these compounds could not be identified in ants living in their natural habitat. However, a trace amount of 4-methyltriacontane was found in lieu of the 2-methyltriacontane counterpart in field ants. Possible explanations for both qualitative and quantitative differences are discussed. Published by Elsevier Science Inc.

Keywords: Cuticular lipids; Harvester ants; Mass spectrometry; Methyl-branched hydrocarbons

1. Introduction

Hydrocarbons are widely studied cuticular components of insects. They are present on all insect cuticles, and can be composed of more than 100 different components (Nelson et al., 1981). This complexity in the composition of insect cuticular hydrocarbons has been used as a taxonomic character to differentiate species or to identify species at the larval stage, a method called chemotaxonomy (Jackson and Blomquist, 1976). Cuticular hydrocarbons have been studied in social insects as well, as they play an important role not only in species identification (e.g. Haverty et al., 1996; Singer, 1998) but also in colony and caste recognition (Clément and Bagnères, 1998; Singer, 1998; Lahav et al., 1999; Haverty et al., 1999; Kaib et al., 2000; Wagner et al., 2000).

Insect cuticular hydrocarbons can also differ between males and females, with sexual matura-

* Corresponding author. Tel.: +1-701-239-1286; fax: +1-701-239-1348.
E-mail address: nelsond@fargo.ars.usda.gov (D.R. Nelson).
[1] *Present address*: Silicon Genetics, 2601 Spring Street, Redwood City, CA 94063, USA.

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tion (Harris et al., 1976; Jackson and Bartelt, 1986; Trabalon et al., 1988; Chen et al., 1990), and developmental stages (de Renobales and Blomquist, 1983). They can vary between age-groups (Jackson, 1983; Jackson and Bartelt, 1986; Pomonis, 1989; Brown et al., 1992; Tissot and Gordon, in preparation), between task-specific ants (Wagner et al., 1998), and were proposed to be used as an age-determining factor (Desena et al., 1999; Sappington et al., 2000). Cuticular hydrocarbon composition has been shown to vary with time within the same ant colony (Liu et al., 1998; Nielsen et al., 1999). This variation is thought to allow social insect colonies to develop and constantly adjust their colony-specific odor (Provost et al., 1993). Cuticular hydrocarbons vary as well within the same species, whether reared in the laboratory or collected in the field. In the case of some social insects reared in the lab, these changes in hydrocarbon composition affected their response towards non-nestmates (Obin, 1986). More recent work showed that cuticular hydrocarbon composition of the harvester ants *Pogonomyrmex barbatus* changes after only 20 days under laboratory conditions (Wagner et al., in review).

This work presents further investigation on differences in cuticular lipids occurring between *P. barbatus* ants collected in the field and ants from laboratory colonies. We report a difference in the nature of a methyl-branched hydrocarbon, as well as variations in the total quantity of cuticular lipids found in laboratory and field ants, indicating a strong environmental influence on the composition and metabolism of cuticular hydrocarbons.

2. Methods

2.1. Insect collection

Two colonies of monogynous species of harvester ants, *Pogonomyrmex barbatus,* together with their queen, were collected in 1997 and 1999, respectively, near Portal, AZ, and brought back to the laboratory at Stanford University. The colonies were kept in plastic boxes, and were allowed to roam in open arenas coated with fluon to prevent them from escaping, as described previously (Gordon and Mehdiabadi, 1999). Both colonies were fed on an artificial diet made out of eggs, mealworms, sugar, vitamins and birdseed three times a week (modified diet from Keller et al., 1989). All analyses were performed during 2000.

To perform cuticular lipid analysis, 12 workers from one laboratory colony were sampled at random while performing midden work or foraging activity. Workers were freeze-killed and stored together at −20°C in a glass tube until extracted. Two additional samples of 12 ants each were collected from the same colony later on and used as replicates. One sample of 12 ants from a second laboratory colony was collected, to confirm the results from the first laboratory colony. Three samples (two of 12 ants and one of 25 ants) were taken from a larger group of ants collected from 16 colonies near Portal, AZ, in summer 1999. They were collected at random while engaged in either foraging or patrolling. Ants collected from the field were immediately freeze-killed, set on dry ice for transportation from Arizona to Stanford University, and stored in a glass tube at −80°C until hydrocarbon extraction.

2.2. Hydrocarbon extraction

Cuticular lipids were obtained by extraction of each sample (with 12–25 ants) in 3 ml pentane for 10 min. After drying, the extract was shipped overnight on dry ice to the Biosciences Research Laboratory, USDA-ARS, Fargo, ND, for mass spectral analysis. Extracts were resuspended in 50–100 μl of chloroform for analysis.

The samples were analyzed by GC-MS (gas chromatography-mass spectrometry) on a HP 5890A gas chromatograph equipped with a pressure programmable cool on-column injection port and an autoinjector (Nelson et al., 2001). The column consisted of a 1-m retention gap connected to a 12.5 m × 0.2 mm capillary column of cross-linked dimethylsilicone Ultra 1 (HP) and was coupled to a HP 5970B quadrupole mass selective detector. The carrier gas was helium. The initial column temperature was set between 150 and 200°C, then programmed to reach 320°C, at a rate of 3°C/min, and finally held at 320°C for 20–120 min. An aliquot of 1 μl in chloroform was injected. Mass spectra of the hydrocarbons were interpreted as previously described (Nelson, 1978, 1993; Blomquist et al., 1987; Bernier et al., 1998; Carlson et al., 1998).

2.3. Lipid quantification

The total amount of cuticular lipids, *n*-alkanes, methylalkanes and alkenes, were measured per ant. Total ion current data were analyzed using a computer spreadsheet in Lotus123™ in which the detector response was corrected for lack of linearity by using a standard curve described by three equations (Nelson et al., 1998). The data used to develop the equations were obtained by injection of a standard mixture of tricosanyl acetate, 3-methyltricosane, octacosane, tetracontane and tricosanyl heptadecanoate. The equations used for the ranges from 0 to 3 ng and from 100 to 1000 ng were linear, while the mid-range from 3 to 100 ng was best described by a first order polynomial equation. The equations describing the dose–response for each component of the standard mixture were calculated using Prism 2.0™ (GraphPad Software, San Diego). Per ant averages of total cuticular lipids, alkanes, methylalkanes and alkenes, for each of the three samples from the first laboratory colony (Nelson et al., 2001) were compared with the averages for each of the three samples from 16 field colonies using a Student *t*-test.

3. Results

3.1. Hydrocarbon composition

The harvester ant cuticular hydrocarbons were comprised of *n*-alkanes of 23 to 52 carbons (C23–C52), methyl-, dimethyl- and trimethyl-branched alkanes from C26 to C52, and odd-numbered alkenes from C25 to C35 (Fig. 1 and Table 1). The major hydrocarbon was pentacosane (h25, Fig. 1), but methylbranched hydrocarbons were the most abundant type of compound, e.g. methylpentacosane and methylheptacosanes (h25A, h27A and h27B, Fig. 1).

3.2. Qualitative differences in hydrocarbon composition between field and laboratory ants

Cuticular hydrocarbon composition of laboratory colony A and B were identical for all samples (Nelson et al., 2001), and similarly, hydrocarbons were consistent in all samples collected in the field. Therefore, we will simply refer to laboratory and field ants in the text. GC-MS analysis of the total cuticular surface extract showed that field ants had a similar hydrocarbon profile to those of the laboratory ants (data not shown), and that the major components were long-chain methyl-branched alkanes (Fig. 1 and Table 1). However, the methylalkanes differed between laboratory and field ants, particularly for GC-MS peaks 30A′ (terminally branched methyltriacontane), 31C (trimethylhentriacontane) and 33C (trimethyltritriacontane). Laboratory-kept colonies had obvious amounts of 2-methylalkanes, the major homologue being 2-methyltriacontane (Nelson et al., 2001). However, at the same GC-MS elution position (peak 30A′ in Table 1, Fig. 1), field colonies had only trace amounts of hydrocarbons, and the only component present in sufficient amounts to be positively identified was 4-methyltriacontane. The 2-methyl- and 4-methyl-branched isomers have almost identical elution times and similar mass spectra, and therefore, frequently, cannot be differentiated. However, in this instance, their mass spectra were clearly distinguishable. The field ant isomer had ions of diagnostic intensity at m/z 70 and 364 (M-72) (Fig. 2a) but these ions were not of increased intensity to be diagnostically significant in the laboratory ant isomer (Fig. 2b). The ions expected at m/z 365 and 421 are not evident in the field ant mass spectrum because of the low amount of this component.

3.3. Quantitative differences between cuticular hydrocarbons of field and laboratory ants

We first compared the average amount of total cuticular lipids per ant for each of the three ant samples from the first laboratory colony with each of the three ant samples from the field colonies. On average, ants from the laboratory colony had more than a two fold greater amount of cuticular hydrocarbons than did the field ants (Fig. 3). More precisely, ants from the laboratory colony had a mean total cuticular lipid of 9.3 μg $\pm$ 0.8 S.E.M. total hydrocarbons per insect, whereas field collected ants had on average only 3.9 μg $\pm$ 0.3 ($t = 6.2$, d.f. = 4, $P < 0.005$). This difference was largely due to a two-fold increase in *n*-alkanes (3.6 $\pm$ 0.4 μg in laboratory ants, vs. 1.9 $\pm$ 0.1 μg for field ants, $t = 3.9$, d.f. = 4, $P < 0.05$), and to a three-fold increase in methylalkanes (5.0 $\pm$ 0.4 μg for laboratory ants, and 1.6 $\pm$ 0.2 μg for field ants, $t = 7.2$, d.f. = 4, $P < 0.005$). Alkenes are as abundant in ants from the laboratory colony as

Table 1
Percent composition of hydrocarbons from field *P. barbatus* ants

CGC-MS peak no.[a]	Percent comp.[b]	Components[c]
20:1	T	?
21	T	?
23	3.0 ± 0.2	Tricosane
23A	T	?
23A′	T	?
24	1.2 ± 0.2	Tetracosane
24A	T	?
25:1	T	Pentacosene
25	27.5 ± 4.0	Pentacosane
25A	3.6 ± 0.7	?
25A	0.6	7-Methylpentacosane
25A	T	5-Methylpentacosane
25A′,B	0.7 ± 0.1	3-Methyl- and 7,13-dimethylpentacosane
26	2.0 ± 0.2	Hexacosane
26A	T	13-Methylhexacosane
26A	T	?
27:1	1.7 ± 0.3	Heptacosene
27	7.3 ± 1.0	Heptacosane
27A	4.9 ± 1.2	13-Methylheptacosane and t9-methylheptacosane
27A	4.7 ± 0.7	7-Methylheptacosane
27A	0.7 ± 0.1	5-Methylheptacosane
27B	0.7 ± 0.1	9,13-Dimethylheptacosane
27B	2.5 ± 0.6	7,13-Dimethylheptacosane + t3-methylheptacosane
27B′	T	5,13-Dimethylheptacosane
28	0.5 ± 0.1	Octacosane
28A	T	14- and t13-Methyloctacosanes
28A	T	8-Methyloctacosane
28A’	T	2-Methyloctacosane
29:1	1.5 ± 0.2	Nonacosene
29	2.4 ± 0.3	Nonacosane
29A	4.2 ± 0.3	15- and t13-Methylnonacosanes
29A	0.9 ± 0.8	9-Methylnonacosane
29A	1.8 ± 0.1	7-Methylnonacosane
29A	0.5 ± 0.2	5-Methylnonacosane
29B	1.0 ± 1.0	11,15-, 13,17- and t9,13-Dimethylnonacosanes
29B	1.2 ± 0.2	7,13-Dimethylnonacosane and t3-methylnonacosane
29B′	T	5,13- and 5,15-Dimethylnonacosanes
30	0.5 ± 0.1	Triacontane
30A	0.6 ± 0.1	15-, 14-, 13-, 11-, 8- and 7-Methyltriacontanes
30A	T	?
30B	T	?
30A′	T	2-Methyltriacontane
31:1	2.0 ± 0.2	Hentriacontene
31	2.0 ± 0.4	Hentriacontane
31A	4.5 ± 0.3	15-, 13-, 11- and 9-Methylhentriacontanes
31A	0.6 ± 0.1	7-Methylhentriacontane
31A	T	5-Methylhentriacontane
31B	2.0 ± 0.3	13,17-, 11,15- and 9,13-Dimethylhentriacontanes
31B	T	7,13-Dimethylhentriacontane
31A′	T	3-Methylhentriacontane
31C	T	13,15,19- and 11,??,??-Trimethylhentriacontanes
32	T	Dotriacontane
32A	0.5 ± 0.2	15-, 14- and 13-Methyldotriacontanes
32B,A′	T	2-Methyl- and 13,??- and14,18-dimethyldotriacontanes

Table 1 (*Continued*)

CGC-MS peak no.[a]	Percent comp.[b]	Components[c]
33:1	0.5 ± 0.1	Tritriacontene
33	T	Tritriacontane
33A	1.6 ± 0.1	17-, 15-, 13-, 11-, 9- and 7-Methyltritriacontanes
33B	1.7 ± 0.2	15,19-, 13,19-, 11,21- and 7,13-Dimethyltritriacontanes
33C	T	13,17,21- and 11,15,21-Trimethyltritriacontanes
33C	T	?
34A	T	15-Methyltetratriacontane
34B	T	14,20-Dimethyltetratriacontane
??	T	?
35:1	T	Pentatricontene
35	T	?
35A	T	15-, 13- and 11-Methylpentatriacontanes
35B	T	15,19-, 13,21- and 7,13-Dimethylpentatriacontanes
35C	T	13,17,23-Trimethylpentatriacontane
??	T	?
36A	T	?
37A	T	13-Methylheptatriacontane
37B	T	?
43A	T	17-, 15-* and 13-Methyltritetracontanes
43B	T	15,19-* and 13,17-Dimethyltritetracontanes
44A	T	?
44B	T	?
45A	T	15- and 13-Methylpentatetracontanes
45B	T	15,19-Dimethylpentatetracontane
46B	T	14,20-Dimethylhexatetracontane
47A	T	?
47B	T	15,21-Dimethylhepatetracontane
47C	T	15,??,??- and 13,??,??-Trimethylhepatatetracontanes
49A	T	?
49B	T	?

[a] The GC-MS peak number corresponds to the number of carbon atoms in the backbone (carbon chain) of the hydrocarbon. The letters A, B and C indicate one, two or three methyl branches, respectively. Thus, 25A has a total of 26 carbons, 25 carbons in the backbone and one methyl group. A prime symbol indicates one of the methyl branches is near the end of the carbon chain, i.e. on carbon 2, 3 or 4.

[b] Percent composition was calculated from the integrated TIC areas as described in Section 2. The amount of trace components, less than 0.1%, are indicated by a 'T'.

[c] The hydrocarbons were identified from their electron impact mass spectra. The isomers are listed in order of elution as determined by evaluating sequential scans through each GC-MS peak. If the major isomer could be estimated, it is indicated by an '*'. A 't' indicates that a trace amount of that isomer was present based on the presence of diagnostic ion(s) in the mass spectra. A '?' indicates the component(s) could not be determined with certainty from their mass spectra other than that they were an alkane. Their tentative identification is indicated by their GC-MS peak no.

in field ants ($t = 0.9$, d.f. = 4, $P = 0.4$) (Fig. 3). To test whether other laboratory colonies had similar results, a sample from a second laboratory colony was compared to the first colony. Ants from the second colony showed similar amounts of total lipids, *n*-alkanes, methylalkanes and alkenes, 8.1 μg, 3.4 μg, 3.9 μg and 2.4 μg, respectively, as did the first colony.

An additional difference distinguishing laboratory and field ants was the presence of the trimethyl-branched alkanes, GC-MS peaks 31C and 33C, in the laboratory colony, which were present only in low amounts in the field ants (Fig. 1).

4. Discussion

4.1. Qualitative differences

We report here a qualitative difference in some of the cuticular hydrocarbon compounds between *P. barbatus* ants collected in the field and colonies kept in the laboratory. This was a difference

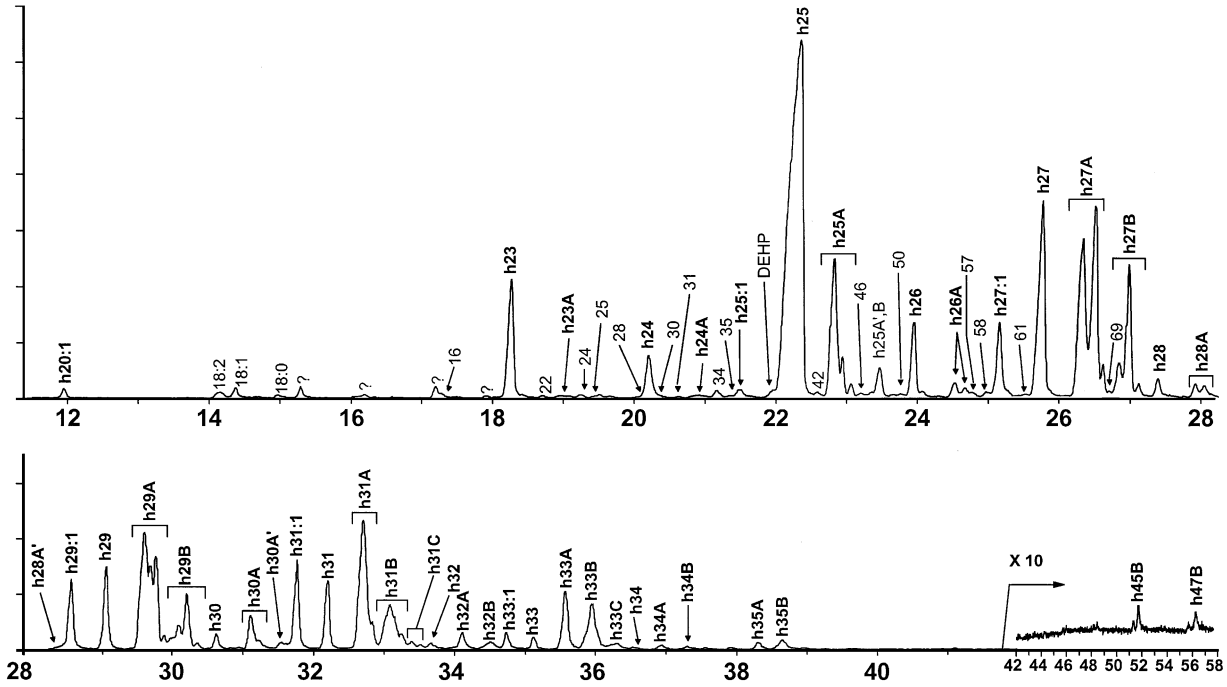

Fig. 1. Total ion plot of the cuticular surface lipids of field collected ants. The 'h' indicates a hydrocarbon, i.e. h23A′ is a monomethylalkane with 23 carbon atoms in the backbone. The prime symbol indicates the methyl branch is near the end of the molecular. The letters B and C indicate two and three methyl branches, respectively. The numbers without an 'h' prefix indicate a wax ester. These wax ester peak numbers correspond to those published in Fig. 1 and Table 2 in Nelson et al., 2001. DEHP is the common laboratory contaminant bis (2-ethylhexyl) phthalate, 18:1 etc. are free fatty acids.

observed in the nature of one type of methyl-branched hydrocarbon. We detected obvious amounts of 2-methylalkanes in laboratory ants, the major compound being 2-methyltriacontane. At the same GC-MS elution time, only 4-methyltriacontane was identified in field ant hydrocarbons. Nevertheless, we cannot rule out the presence of trace amounts of the 4-methyl isomers in laboratory ants, because the overwhelming presence of the 2-methyl isomers would mask any of the minor diagnostic mass spectral fragmentation ions from trace amounts of a 4-methyl isomer. None of these compounds were present in the pentane fraction of either rearing components or the ants' diet (Nelson et al., 2001). Therefore, a contamination from rearing equipment or diet is unlikely. To our knowledge, only one other study reported striking qualitative changes in the chemical signature of the Argentine ant, *Linepithema humile,* linked to the hydrocarbon composition of their prey (= diet) (Liang and Silverman, 2000). In this case, Argentine ants acquired the cuticular hydrocarbons of *Supella longipalpa* after being fed on this species, thereby changing the ant's hydrocarbon profile.

A clear distinction between 2- and 4-methylalkanes is often difficult to establish in practice. However, several studies identified either 2-methylalkanes or 4-methylalkanes, and sometimes both branching patterns in the same cuticular lipid profile (Bonavita-Cougourdan et al., 1987, 1993; Provost et al., 1993; Adams et al., 1995; Haverty and Nelson, 1997). In other cases, the methyl branching was specified as either a 2- or 4-methylalkane, without final determination (Brown et al., 1992; Sevala et al., 2000). The keys to discriminate between 2- and 4-methylalkanes from their mass spectra have been discussed in detail (Coudron and Nelson, 1978; Blomquist et al., 1987). In practice, they frequently elute from the gas chromatographic column together. However, the presence of both can sometimes be determined by examining sequential mass spectral scans through the chromatographic peak with a high resolution column. In this case, the 4-methylalkane elutes earlier than the 2-methylalkane,

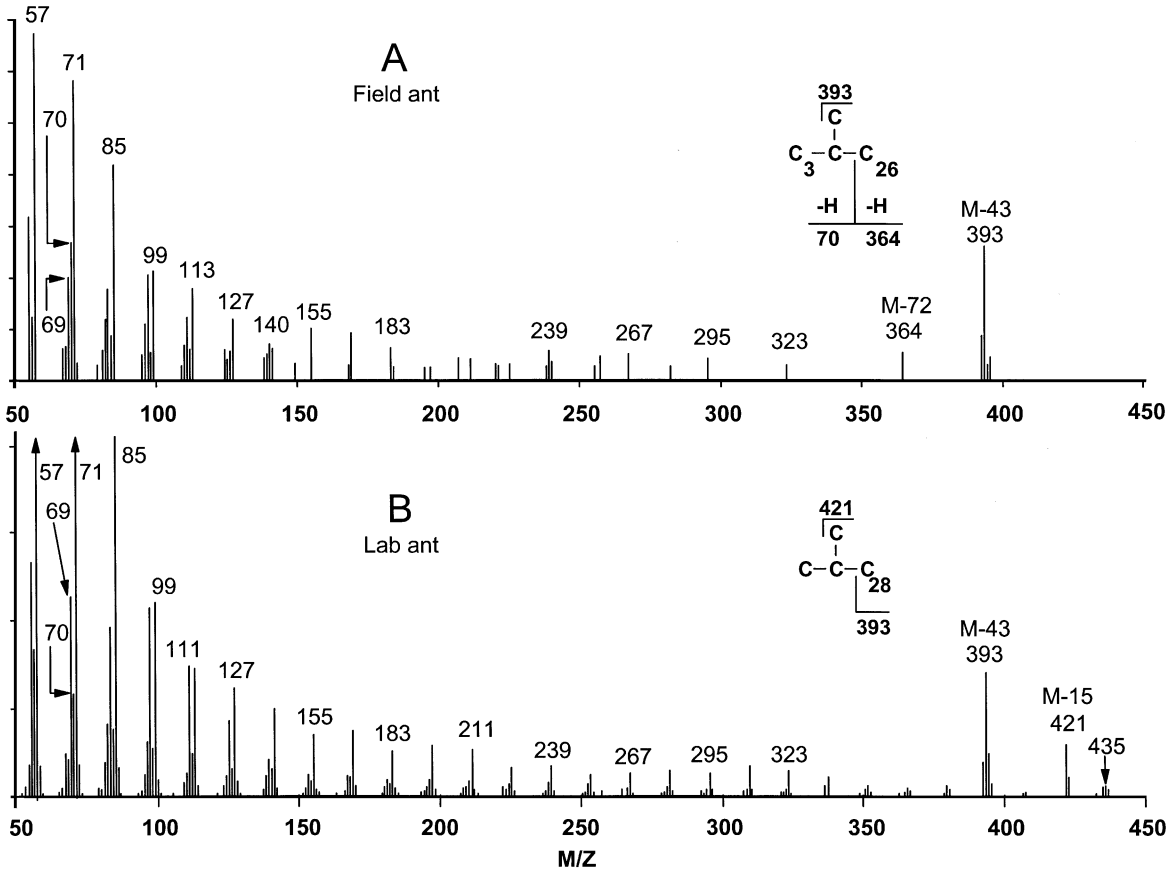

Fig. 2. (a) Mass spectrum of peak h30A′. 4-Methyltriacontane from field ants and (b) 2-methyltriacontane from laboratory-maintained ants. The ions of diagnostic intensity at m/z 70 and M-72 are characteristic of 4-methylalkanes. See text for details.

even though they may not be resolved. Both isomers form the same major diagnostic ion at m/z, m-43 (M = molecular weight, 43 being the weight of the 3 carbon unit cleaved off), and if present in sufficient amounts, both will have an ion visible in the mass spectra at m/z, m-15 due to loss of a methyl group (Fig. 2). However, a 4-methylalkane has an ion at m/z 70 in its mass spectrum of greater intensity relative to the ion at m/z 69, and equal to or greater intensity than m/z 56, than for other methylalkanes. Also, if sufficient sample is present, a pair of ions at m/z M-71:M-72 of diagnostic intensity are also present in the mass spectra of 4-methylalkanes which are not of diagnostic intensity for a 2-methylalkane. A molecular ion is frequently not visible in the mass spectra of methyl-branched alkanes. In this case, a molecular ion for the 2-methylalkane was present, but as is often the case, ions at m/z, m-1 and m-2, formed by the loss of one and two hydrogens, respectively, were of greater intensity.

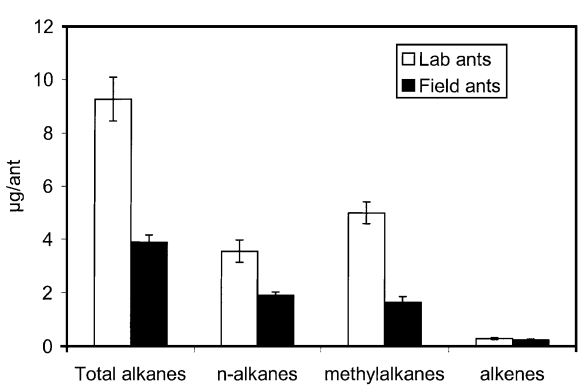

Fig. 3. Total amount of hydrocarbons and their sub-groups, per ant, in micrograms. Error bars are indicated. See text for details.

4.2. Quantitative differences

We observed strong differences in total lipid quantity between laboratory and field ants, where

laboratory ants show significantly more hydrocarbons. This difference was largely due to a higher content of *n*-alkanes and methylalkanes in laboratory ants. However, unsaturated hydrocarbons were found in the same quantity in both laboratory and field ants. Similar differences between insects reared in the laboratory and those found in the field have been reported. In the case of *Drosophila mojavensis*, laboratory-reared flies showed significantly more total epicuticular hydrocarbon than wild flies (Toolson et al., 1990). Kamhawi et al. (1992) demonstrated that the hydrocarbon profile of the dipteran *Phlebotomus argentipes* changed gradually after only five generations. However, it is not known whether the profile changes in proportion or qualitatively. In this instance, the variation occurred over several generations. In the case of *P. barbatus,* notable differences in the *n*-alkanes profile were observed only 20 days after being brought from the field into the laboratory (Wagner et al., in review). Climatic conditions may influence the composition of surface hydrocarbons, which are thought to protect the insect from desiccation (Hadley, 1984; Gibbs et al., 1991). It is possible that colonies brought to the laboratory are subject to an overall lower level of humidity, which would affect their cuticular hydrocarbon level, as well as its composition.

Diet has also been shown to influence cuticular hydrocarbon composition. For instance, feeding status of the host-specific ectoparasitoid *Cephalonomia tarsalis* seems to affect both the quantity and the relative proportion of female hydrocarbons (Howard, 1998). Similarly, when the grasshopper *Schistocerca americana* was fed on three different plants, significant differences in the quantities of some hydrocarbon chain lengths were found (Espelie et al., 1994). Francis et al. (1989) reported almost twice as much surface hydrocarbon in bees fed on both sucrose and pollen as in bees fed either sucrose or pollen alone. Interestingly, the quantity of hydrocarbons collected from bees fed with sucrose and pollen was the same as that extracted from bees from a hive (Francis et al., 1989). In the case of *P. barbatus*, it is possible that the high-protein diet fed to the laboratory ants affects their metabolism. In the field, harvester ants principally collect seeds from the Poaceae family (Gordon, 1993), which was not part of the laboratory diet. However, the absence of differences between laboratory and field ants of the species *Solenopsis invicta* in the principal component analysis suggested that diet did not have a significant influence on cuticular hydrocarbon composition in this species (Vander Meer et al., 1989). In the present study, it is not possible to rule out an influence of diet on the hydrocarbons.

Finally, quantitative changes in cuticular hydrocarbons have also been found to be influenced by the presence of endoparasites in the ant *Leptothorax nylanderi* (Trabalon et al., 2000). These authors suggested that because parasitized ants are smaller and have a higher surface-to-volume ratio than non-parasitized ants, they presented a higher concentration of hydrocarbons. This could be true for *P. barbatus* ants, because workers are somewhat smaller when eclosed in the laboratory than ants eclosed in the field. We are not aware of the presence of endoparasites that could influence the biosynthesis of hydrocarbons, either quantitatively or qualitatively. Also, it seems unlikely that differences in our study could be due to a genetic factor.

Altogether, these results show that not only quantitative, but also qualitative differences may occur within one social insect species, depending on whether the specimens are collected in the field or come from an artificial setting. The effect of laboratory conditions may be important in studies of the species-, colony- and task-specific variation in cuticular hydrocarbons.

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