

M. Kaib · B. Eisermann · E. Schoeters
J. Billen · S. Franke · W. Francke

Task-related variation of postpharyngeal and cuticular hydrocarbon compositions in the ant *Myrmicaria eumenoides*

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Abstract In the ant *Myrmicaria eumenoides* we investigated postpharyngeal and cuticular hydrocarbons. At eclosion the glands contained almost no hydrocarbons and there were no lipid inclusions in the glandular epithelium. During the first 3 weeks of adult life the amount of hydrocarbons in the gland increased until day 5, and then remained constant while the lipid content in the epithelium increased steadily. Intracolony hydrocarbon compositions were not uniform. Compositions of postpharyngeal and cuticular hydrocarbons in individual ants varied simultaneously, but in different manner depending on the tasks of the ant (brood-tenders, foragers, scouts). Variations on the cuticle were greater than in the gland, but they were strongly correlated. Independent of ants' age and task, cuticular hydrocarbon compositions were dominated by alkenes and alkadienes. Task-specific differences in cuticular compositions were mainly in the amount of alkenes (high in foragers) and alkadienes (high in brood-tenders). Variation of hydrocarbons was low in ants up to 10 weeks old. Thereafter, ants fell into two groups: (1) ants that did not change their hydrocarbons and remained in the nest, and (2) ants that changed their hydrocarbon compositions and became foragers. These results contribute to an ongoing discussion of the dynamic relationship between postpharyngeal and cuticular hydrocarbons.

Key words Gland ontogeny · Age · Task · Nestmate recognition · Colony label

Abbreviations *BT* brood-tenders · *C* cuticle · *F* foragers · *PPG* postpharyngeal gland · *Sc* scouts

Introduction

In social insects, nestmate recognition allows the cohesiveness of a colony and denies access to alien conspecifics. Nestmate recognition has been studied in a number of ant species (e.g. Hölldobler and Michener 1980; Gadagkar 1985; Breed and Bennett 1987; Vander Meer and Morel 1998). During interaction between aliens, aggressive behaviour is initiated after an actual physical contact anywhere on the antagonist's body, which suggests that nestmate recognition cues come from chemicals associated with the cuticle (Hölldobler and Michener 1980; Bradshaw and Howse 1984). The cuticular lipids include fatty acids, alcohols, aldehydes, ketones, wax esters, and hydrocarbons (Lockey 1988). Hydrocarbons are the dominant chemicals found in most social insects. Their compositions vary between species and between conspecific colonies, suggesting that cuticular hydrocarbons might play a key role in species recognition and nestmate recognition (reviewed by Vander Meer and Morel 1998). This idea is supported by numerous experimental data which demonstrate that in Hymenoptera aggressiveness between aliens is correlated with differences in hydrocarbon compositions (e.g. Bonavita-Cougourdan et al. 1987, 1989; Henderson et al. 1990; Nowbahari et al. 1990; Dahbi and Lenoir 1998a), and also with the alteration of aggressiveness after coating an insect's surface with isolated hydrocarbons or cuticular extracts from another colony (e.g. Jackson and Blomquist 1976; Bagnères et al. 1991; Meskali et al. 1995a; Ruther et al. 1998; Lahav et al. 1999). In slave-making ants, adult slave-maker workers show the same hydrocarbon composition as the slave species, apparently having acquired or mimicked the label of the slave (Habersetzer and Bonavita-Cougourdan 1993; Kaib et al. 1993; Heinze et al. 1994; Bonavita-Cougourdan et al. 1997). These observations suggest that hydrocarbons are

M. Kaib (✉) · B. Eisermann
Lehrstuhl Tierphysiologie, Universität Bayreuth,
95440 Bayreuth, Germany
e-mail: manfred.kaib@uni-bayreuth.de
Tel.: +49-921-552482; Fax: +49-921-552794

E. Schoeters · J. Billen
Zoological Institute, University of Leuven,
Leuven, Belgium

S. Franke · W. Francke
Institut für Organische Chemie,
Universität Hamburg, Hamburg, Germany

transferred between nestmates by trophallaxis and allo-grooming (Soroker et al. 1994, 1995a, b; Vienne et al. 1995), thus generating a uniform but colony-specific hydrocarbon composition. These and other observations favour the hypothesis that cuticular hydrocarbons may provide colony-specific recognition cues. However, the evidence for this is still circumstantial, based either on correlation studies or on bioassays using experimentally changed hydrocarbon compositions (Breed 1998; Lahav et al. 1999). Furthermore, lipid fractions other than hydrocarbons have often not been thoroughly investigated (see Vander Meer and Morel 1998).

Recent work shows that cuticular hydrocarbon compositions may vary seasonally in a social insect colony (e.g. Haverty et al. 1996). In the ant *Formica truncorum*, Nielsen et al. (1999) found two significantly different phenotypes of cuticular hydrocarbon compositions in spring and in summer. This might be due to a difference in age composition of ants in a colony shortly after hibernation when colonies are relatively homogeneous in respect to the age of nestmates, and in summer when the age-composition in a colony is heterogeneous. Temporal variation may be a general phenomenon, as it has also been reported for *Solenopsis invicta* (Vander Meer et al. 1989) and in *Leptothorax lichtensteini* (Provost et al. 1993). In addition, variation of cuticular hydrocarbons in an ant colony might also be task related (Wagner et al. 1998) or may depend on the reproductive status of individual ants (Peeters et al. 1999).

The postpharyngeal gland (PPG) is unique to ants (Delage-Darchen 1976; Soroker et al. 1995a). In *Cataglyphis niger* hydrocarbons are sequestered by the postpharyngeal gland and the ants apply them to their own cuticle by grooming (Soroker et al. 1998). Thus, it is not surprising to find that in an ant colony the hydrocarbon compositions in the postpharyngeal gland and on the cuticle match, suggesting an involvement of the postpharyngeal gland in nestmate recognition (Bonavita-Cougourdan et al. 1987; Soroker et al. 1995b; Hefetz et al. 1996). However, the hydrocarbon composition in the PPG may also show variation. In *C. iberica*, during adult transport at the end of hibernation, transporters have a larger amount of hydrocarbons, and of more diverse composition, than the transportees, which are probably younger (Dahbi et al. 1997, 1998). Interestingly, when callow workers are isolated, their postpharyngeal gland contents remain low, suggesting that hydrocarbons are transferred to them from mature workers (Dahbi et al. 1998). This may contribute to a uniform hydrocarbon composition in an ant colony at a given time.

The origin of hydrocarbons in the postpharyngeal gland is still debated. It is generally assumed that these hydrocarbons are not synthesised in situ but are transported into the gland. It has been proposed that this transport is due to trophallaxis and allo-grooming within a colony (e.g. Meskali et al. 1995b), but recent experiments have shown that hydrocarbons in the gland are accumulated after internal transport in the individ-

ual. It has been postulated that this mechanism enables the ants to constantly update their cuticular hydrocarbon composition (Soroker et al. 1994, 1995a, b, 1998). However, such suggestions are based on experiments in ant species, which always show uniform hydrocarbon compositions within a colony.

To investigate this matter further, we analysed in the tropical ant *Myrmecaria eumenoides* hydrocarbons in the postpharyngeal gland and on the cuticle to see how they relate to each other, and to the age and task status of individual ants.

Materials and methods

Ant colonies

Colonies of the ant *Myrmecaria eumenoides* (Formicidae, Myrmicinae) were collected in the field at Tiwi in Kwale District, Kenya in April 1993 and June 1995 and were installed under constant conditions in the laboratory in Bayreuth (25 °C, 60% RH, 12:12 L/D). Colonies were housed in transparent plastic boxes (20 cm × 10 cm × 6 cm) with the bottoms covered with a layer of plaster of Paris that was kept moist. Each nest box was connected by silicon tubing (10 mm i.d.) to a foraging arena (50 cm × 60 cm) in which tapwater and honey water were supplied ad libitum. Each colony was fed twice a week with dead cockroaches (*Periplaneta americana*). Individual ants were collected from the nest box or from the foraging arena of two laboratory colonies. One of the colonies was used as the "focus colony" for this study. To test the influence of diet on the hydrocarbon compositions, the second colony was fed with crickets (*Gryllus bimaculatus*). Cockroaches and crickets possess totally different hydrocarbons. Foragers from three field colonies were collected and brought alive to the laboratory for immediate analysis. We used a total of 194 ants (176 from the laboratory and 30 from the field) for this study. In 26 of the laboratory ants we investigated the lipid content in the postpharyngeal gland. For these ants no data are available on the composition of the glandular hydrocarbons, but the cuticular components were analysed.

To know the ages of ants, individual callow workers were colour-marked by dots of nail polish on the thorax immediately after eclosion. When returned to the colony, newly marked ants were immediately accepted by their nestmates and intensively groomed. The age of ants investigated for this study ranged from callow workers (day 1) up to 36 weeks old. Ants of mixed age were also collected from the laboratory colonies while performing specific tasks or behaviours. They were classified as: brood-tenders (BT) operating within clusters of brood in the nest at the peak of a food recruitment process; foragers (F) from the arena when no protein was available; scouts (Sc) which deposited scent marks on their way back to the nest to recruit nestmates; and motionless ants collected in the nest distant from the brood or in peripheral parts of the foraging arena distant from water supply or food. Such ants may become active to execute a task. In addition, foraging ants were collected from field colonies.

Chemical analysis

Hydrocarbons from individual ants were extracted by two different protocols. First, for extracting hydrocarbons from the postpharyngeal gland, the gland was removed from the ant's head and then extracted. Preparation was performed under water. Second, to investigate cuticular components, we used only the legs of individual ants, to avoid contamination from other glands. Constituents from tarsal glands did not influence the analyses in this study (own unpublished data). Each individual sample, of gland or legs, was extracted in 30 µl of *n*-hexane (Merck, No UN1208) for 24 h at -18 °C.

Samples were analysed by using a Hewlett Packard HP 5890 Series II GC equipped with a FID. Aliquots of 1 μl were introduced by splitless injection onto a 30 m by 0.32 mm i.d. DB1 (0.10 μm film thickness, J&W Scientific) fused-silica capillary column. Helium was the carrier gas at a constant flow rate of 1.5 ml min^{-1} . The GC was operated at splitless mode for 60 s. For the splitless time the oven was kept at 150 $^{\circ}\text{C}$ and thereafter was programmed at 3 $^{\circ}\text{C min}^{-1}$ to 180 $^{\circ}\text{C}$ and then at 2 $^{\circ}\text{C min}^{-1}$ to 300 $^{\circ}\text{C}$ with a final isothermal hold of 20 min. To confirm consistency of retention times during the course of the study, standardised samples of *n*-alkane mixtures were interspersed among the samples. For pattern analysis, peaks from different chromatograms were homologised by calculating linear retention indices based on the *n*-alkane mixtures.

Typical hydrocarbon compositions were identified by coupled gas chromatography/mass spectrometry (Blomquist et al. 1987; Nelson et al. 1980; Page et al. 1990a, b). Analysis was performed on a VG 70-250 SE mass spectrometer connected to a Hewlett Packard HP 5890 GC. Aliquots of 1–2 μl from the ant extracts were used with the column and conditions given above except a temperature programme of 80 $^{\circ}\text{C}$, 1.5 min hold, at 40 $^{\circ}\text{C min}^{-1}$ to 160 $^{\circ}\text{C}$ and then at 2.5 $^{\circ}\text{C min}^{-1}$ to 280 $^{\circ}\text{C}$. The mass spectrometer was operated in electron impact ionisation mode (70-eV, 500- μA ionisation current, 200 $^{\circ}\text{C}$ source temperature) and scanned from m/z 600 to m/z 35 at a rate of 0.7 s/decade with an inter-scan time of 0.2 s. For the determination of the double-bond positions of unsaturated hydrocarbons dimethyl disulfide (DMDs) derivatives were prepared by adding 50 μl DMDs in carbon disulfide (1:1, v:v) and 5 μl of 5% iodine in diethyl ether to the ant extracts. After heating in a sealed vial to 60 $^{\circ}\text{C}$ overnight the mixture was dissolved in 200 μl *n*-pentane. Iodine was removed by adding 20 μl of 5% aqueous sodium thiosulphate. The organic layer was separated and concentrated to about 20 μl in vacuo. Aliquots of 1 μl were then analysed by on-column injection onto a 28 m by 0.25 mm i.d. BPX-5 (0.25 μm film thickness, SGE) fused-silica capillary column with a temperature programme of 80 $^{\circ}\text{C}$, 1.5 min hold, at 40 $^{\circ}\text{C min}^{-1}$ to 180 $^{\circ}\text{C}$ and at 3 $^{\circ}\text{C min}^{-1}$ to 300 $^{\circ}\text{C}$. The mass spectrometer was scanned from m/z 700 to m/z 35 at a rate of 0.9 s/decade with an inter-scan time of 0.4 s. Other operating conditions were as given above.

Lipid analysis

Samples were fixed in 2% glutaraldehyde, buffered at pH 7.3 with 0.05 mol l^{-1} Na cacodylate and 0.15 mol l^{-1} saccharose. After postfixation in 2% osmium tetroxide, samples were dehydrated in acetone and embedded in Araldite. Thin sections for transmission EM were double stained with a LKB 2168 Ultrastainer, and examined with a Zeiss EM 900 electron microscope. For lipid histochemistry, after dehydration in ethanol samples were put into LRWhite resin, then embedded in the same resin. After polymerisation, an ethyl-gallate technique with involvement of farnesol and thymol was used for unmasking lipid.

Data analysis

Individual hydrocarbon peaks were scored and quantified by integration of the FID signal using a HP ChemStation data analysis software. Characters for our analysis consisted of 63 peaks coded as percentages of the total hydrocarbon fraction. Thus, co-eluting compounds were considered as one character for the purposes of this study. Hence, our calculated distances represent the minimum distance between hydrocarbon compositions. From the resulting data matrix for the characters we calculated the Nei distances for pairwise comparisons between each of the hydrocarbon compositions (NTSYS, Rohlf 1990). For further analysis, Nei distances were subjected to a principal components analysis in which a priori no groups of samples were formed. Results from this analysis were displayed by the first two principal components in a two-dimensional ordination (NTSYS, Rohlf 1990). These two components summarised 93.2% of the total variance. For the

plots presented in Fig. 3, we used the same ordination based on the 326 analysed hydrocarbon compositions from the focus colony, but depending on the question in focus, we highlighted positions of specific compositions. For hydrocarbons (PPG and cuticle) collected from ants performing well-defined tasks in the colony (brood-tenders and foraging or scouting ants) the four group centroids were calculated from the two first variables as mean Nei distance. These centroids are meant to serve for visual orientation. The two centroids from brood-tenders (postpharyngeal gland and cuticle) were also used as reference point for estimating hydrocarbon variations.

To estimate hydrocarbon variations the first two principle components of the ordination were used. For each "position" of a PPG hydrocarbon composition in the ordination (*x*-axis and *y*-axis) we calculated the Euclidean distance to the brood-tenders' PPG group centroid. We proceeded correspondingly for cuticular hydrocarbons. Low Euclidean distances stand for compositions similar to those observed in brood-tenders and high values for compositions similar to those in foragers or scouts. For individual ants from which we collected both hydrocarbons from PPG and cuticle we compared the variations of the two compositions by a correlation. We also correlated variations of cuticular hydrocarbons with the age of ants. Linear correlations and significance values were calculated by applying SPSS/PC+.

Results

Accumulation of hydrocarbons in the postpharyngeal gland

On the day of eclosion (day 1), the postpharyngeal gland of a worker contained almost no hydrocarbon, and no lipid inclusions were present in the glandular epithelium. Hydrocarbon accumulation in the gland correlated with age during the first four days of adult life ($r=0.85$, $P<0.001$). From day 5 onwards the total amount of hydrocarbons no longer increased ($r=0.16$, $P=0.47$) but remained more or less constant at a level of about 350 ng (Fig. 1). In contrast, number and size of lipid inclusions in the glandular epithelium steadily increased during the first 3 weeks of adult life, and increase strongly correlated with age ($r=0.90$, $P<0.001$; Fig. 1B).

Hydrocarbon compositions and their variation

Hydrocarbon compositions from postpharyngeal gland and cuticle of individual ants were analysed and compared. 63 hydrocarbons with total carbon numbers from 23 to 37 were identified belonging to the chemical classes *n*-alkanes, alkenes, alkadienes, and trace amounts of methylalkanes (Table 1). Within an ant colony, there was a large variation in the composition of postpharyngeal and cuticular hydrocarbons. To test this high intra-colonial variation, we calculated the histograms of Nei distances (pairwise comparisons) for cuticular hydrocarbon compositions of all ants analysed from the "focus colony" (Fig. 2A). Similarly, we scored the Nei distances of foragers collected from three field colonies, and from the laboratory colony reared on a different diet (Fig. 2B). This comparison showed a high intra-colonial variation

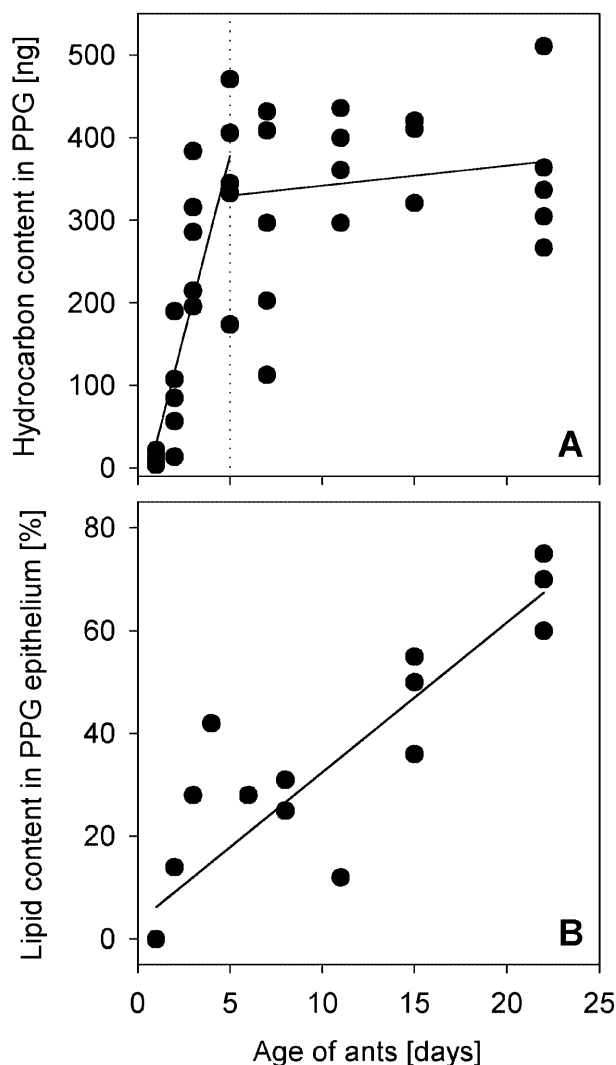


Fig. 1 Content of hydrocarbons (A) and of lipids in % of cell volume (B) in the epithelium of the postpharyngeal gland (PPG) plotted against age of *Myrmecaria eumenoides* ants. The increase of hydrocarbons and lipids do not follow the same temporal pattern. Day 1 = day of eclosion. *Solid lines* are regression lines for ants 1–5 days old ($n=20$) and 5–22 days old ($n=22$) for hydrocarbons (A) and 1–22 days old ($n=19$) for lipids (B). The *dotted line* marks the age of 5 days

of the hydrocarbons on the ants' cuticle. In contrast, inter-colonial variation was low when ants of the same task were compared.

Variation of hydrocarbons with the task of ants

The ordination of the first two principal components of hydrocarbons from the postpharyngeal gland and from the cuticle showed that firstly in brood-tenders the two compositions were more or less the same whereas in foragers or scouts the two compositions differed strongly. Furthermore, it is important to point out that in foragers or scouts the two compositions were changed in different ways: cuticular hydrocarbon variation largely followed the first axis of the ordination, whereas

that of the PPG followed the second axis (Fig. 3A). Thus, four types of hydrocarbon compositions were distinguished: type PPG/BT for postpharyngeal hydrocarbons, type C/BT for cuticular hydrocarbons in brood-tenders, type PPG/FSc for postpharyngeal hydrocarbons and type C/FSc for cuticular hydrocarbons of foraging or scouting ants. The variation of hydrocarbons was a continuous process, as demonstrated by the ordination of the data from ants for which no clearly assigned task was possible (Fig. 3B).

There was no obvious difference in chain length of the hydrocarbons between the four types. They were dominated by hydrocarbons with 31 and 33 C-atoms, mainly alkenes and alkadienes (Table 1). Except for some trace amounts (e.g. methylalkanes in type PPG/BT), the four types expressed the same set of hydrocarbons, but there were significant differences in the mean percentage of the total hydrocarbon fraction for individual compounds. In general, the difference in the composition between three types (PPG/BT, C/BT, and PPG/FSc) and the fourth type found on the cuticle of foragers or scouts (C/FSc) was mainly due to the amount of alkenes and alkadienes in the total hydrocarbon fraction. While the percentage for alkenes was low in the first three types (about 19%), it was over 45% on the cuticle of foragers or scouts. In contrast, in type C/FSc alkadienes were about 28%, compared with 67.5% to 79.4% in the other three types. The cumulative percentage of unidentified components range from 0.09% to 0.45%.

Main components of the types PPG/BT and C/BT were several hentriacontadienes and tritriacontadienes (Table 1). The tritriacontadienes were also the compounds with the highest amount (38.8%) in the gland of foragers or scouts but made up only 15.3% of the total cuticular hydrocarbons. These cuticular hydrocarbons of foragers or scouts were characterised by *n*-heptacosane and *n*-nonacosane, with 22.4% for *n*-alkanes compared with only 1% in the respective glands, and furthermore by 7-hentriacontene and 5-tritriacontene (Table 1).

To compare in individual ants the variation of the two hydrocarbon compositions (gland and cuticle), the two Euclidean distances of the two compositions to their corresponding group centroids (for brood-tenders) were calculated in the two-dimensional space of the ordination. Correlating the pairs of Euclidean distance, the result shows that for an individual ant the postpharyngeal and cuticular hydrocarbon compositions changed synchronously ($n=110$, $r=0.92$, $P<0.001$).

Variation of cuticular hydrocarbons with the age of ants

During the first 10 weeks of their adult life, *M. eumenoides* workers exclusively cared for the brood in the nest, and showed cuticular hydrocarbon compositions of the type C/BT. Older ants differentiated into two groups which performed different tasks. One group stayed in the nest, caring for the brood and the queen. Their cuticular hydrocarbons remained unchanged and

Table 1 List of hydrocarbons found in the postpharyngeal gland (PPG) and on the cuticle of *Myrmicaria eumenoides* workers, given the mean relative percentages of the total hydrocarbon fraction (mean \pm SE) for brood-tenders and foragers or scouts

Peak no.	Hydrocarbons	Brood-tenders		Foragers/Scouts	
		PPG ($n=63$)	Cuticle ($n=66$)	PPG ($n=16$)	Cuticle ($n=20$)
1	9-Tricosene	0.02 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
2	7-Tricosene	0.54 \pm 0.04	0.21 \pm 0.07	0.02 \pm 0.02	0.00 \pm 0.00
3	<i>n</i> -Tricosane	1.46 \pm 0.12	3.70 \pm 0.23	0.02 \pm 0.02	0.00 \pm 0.00
4	3-Methyltricosane	0.02 \pm 0.01	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
5	Unknown	0.01 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
6	7,21-Pentacosadiene	0.80 \pm 0.05	0.50 \pm 0.12	0.01 \pm 0.01	0.00 \pm 0.00
7	7-Pentacosene	1.98 \pm 0.14	3.21 \pm 0.35	0.04 \pm 0.02	0.00 \pm 0.00
8	Pentacosene	0.03 \pm 0.01	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
9	<i>n</i> -Pentacosane	1.90 \pm 0.12	4.96 \pm 0.26	0.07 \pm 0.03	0.44 \pm 0.10
10	3-Methylpentacosane	0.51 \pm 0.06	0.02 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
11	6,9-Heptacosadiene	3.41 \pm 0.20	3.79 \pm 0.33	0.26 \pm 0.07	0.15 \pm 0.09
12	7,21 + 7,23-Heptacosadiene	1.56 \pm 0.08	1.47 \pm 0.18	0.07 \pm 0.04	0.00 \pm 0.00
13	7-Heptacosene	1.25 \pm 0.07	0.80 \pm 0.13	0.11 \pm 0.06	0.00 \pm 0.00
14	Heptacosene	0.12 \pm 0.01	0.00 \pm 0.00	0.01 \pm 0.01	0.00 \pm 0.00
15	<i>n</i> -Heptacosane	0.86 \pm 0.10	2.12 \pm 0.20	0.57 \pm 0.08	11.72 \pm 0.55
16	7-Octacosene	0.20 \pm 0.01	0.00 \pm 0.00	0.07 \pm 0.02	0.00 \pm 0.00
17	Unknown	0.00 \pm 0.00	0.19 \pm 0.14	0.03 \pm 0.02	0.41 \pm 0.09
18	Unknown	0.01 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
19	Nonacosadienes	0.48 \pm 0.03	0.01 \pm 0.01	0.02 \pm 0.01	0.00 \pm 0.00
20	7,21 + 7,23 + 7,25-Nonacosadiene	1.73 \pm 0.08	1.18 \pm 0.12	0.47 \pm 0.01	0.00 \pm 0.00
21	Nonacosadiene	0.73 \pm 0.04	0.06 \pm 0.02	0.06 \pm 0.04	0.00 \pm 0.00
22	7-Nonacosene	9.43 \pm 0.55	9.78 \pm 0.23	7.31 \pm 0.28	3.89 \pm 0.14
23	Unknown	0.07 \pm 0.02	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
24	4 + 5-Nonacosene	0.33 \pm 0.03	0.02 \pm 0.01	0.21 \pm 0.03	0.19 \pm 0.06
25	<i>n</i> -Nonacosane	0.40 \pm 0.05	0.86 \pm 0.13	0.36 \pm 0.07	8.96 \pm 0.41
26	7,21-Triacontadiene	0.35 \pm 0.02	0.01 \pm 0.01	0.06 \pm 0.02	0.00 \pm 0.00
27	7,23-Triacontadiene	0.20 \pm 0.01	0.00 \pm 0.00	0.01 \pm 0.01	0.00 \pm 0.00
28	7-Triacontene	0.14 \pm 0.01	0.00 \pm 0.00	0.30 \pm 0.02	0.13 \pm 0.05
29	<i>n</i> -Triacontane	0.01 \pm 0.00	1.05 \pm 0.30	0.12 \pm 0.05	1.29 \pm 0.25
30	7,15-Hentriacontadiene	0.89 \pm 0.04	0.15 \pm 0.04	1.06 \pm 0.04	0.00 \pm 0.00
31	7,21 + 7,23 + 7,25-Hentriacontadiene	15.16 \pm 0.32	15.23 \pm 0.36	6.65 \pm 0.25	1.44 \pm 0.14
32	5,19 + 5,21 + 5,23-Hentriacontadiene	16.78 \pm 0.38	16.09 \pm 0.36	7.70 \pm 0.26	1.77 \pm 0.16
33	Hentriacontadiene	1.74 \pm 0.08	0.27 \pm 0.08	1.31 \pm 0.06	0.00 \pm 0.00
34	Hentriacontene	0.43 \pm 0.04	0.01 \pm 0.01	0.00 \pm 0.00	0.00 \pm 0.00
35	7-Hentriacontene	4.31 \pm 0.28	5.25 \pm 0.17	8.31 \pm 0.53	22.68 \pm 0.73
36	4 + 5-Hentriacontene	0.12 \pm 0.01	0.00 \pm 0.00	0.26 \pm 0.04	2.36 \pm 0.09
37	Hentriacontane	0.01 \pm 0.00	0.20 \pm 0.01	0.10 \pm 0.04	2.26 \pm 0.14
38	Unknown	0.04 \pm 0.01	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
39	7,21-Dotriacontadiene	0.25 \pm 0.03	0.00 \pm 0.00	0.09 \pm 0.02	0.00 \pm 0.00
40	7,23-Dotriacontadiene	0.65 \pm 0.05	0.09 \pm 0.03	0.78 \pm 0.02	0.00 \pm 0.00
41	Tritriacontadiene	0.44 \pm 0.03	0.08 \pm 0.04	0.48 \pm 0.03	0.12 \pm 0.01
42	Tritriacontadiene	0.06 \pm 0.01	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
43	7,21 + 7,23 + 7,25-Tritriacontadiene	23.30 \pm 0.13	25.00 \pm 0.24	32.99 \pm 0.43	12.71 \pm 0.56
44	5,19 + 5,21 + 5,23-Tritriacontadiene	3.13 \pm 0.13	2.48 \pm 0.20	5.83 \pm 0.27	2.61 \pm 0.16
45	5,25-Tritriacontadiene	1.15 \pm 0.05	0.42 \pm 0.09	2.29 \pm 0.04	2.07 \pm 0.12
46	7 + 8 + 10-Tritriacontene	0.28 \pm 0.03	0.02 \pm 0.02	0.17 \pm 0.09	0.00 \pm 0.00
47	4 + 5-Tritriacontene	0.25 \pm 0.03	0.03 \pm 0.02	2.03 \pm 0.22	14.16 \pm 0.67
48	<i>n</i> -Tritriacontane	0.00 \pm 0.00	0.05 \pm 0.04	0.05 \pm 0.03	0.52 \pm 0.14
49	7,21 + 7,23-Tetratriacontadiene	0.04 \pm 0.01	0.00 \pm 0.00	0.16 \pm 0.02	0.00 \pm 0.00
50	7 + 8 + 10 + 12 + 14 + 16-Tetratriacontene	0.05 \pm 0.01	0.00 \pm 0.00	0.17 \pm 0.03	0.00 \pm 0.00
51	Pentatriacontadiene	0.03 \pm 0.01	0.01 \pm 0.01	0.10 \pm 0.03	0.06 \pm 0.06
52	Pentatriacontadiene	0.13 \pm 0.02	0.00 \pm 0.00	0.51 \pm 0.03	0.00 \pm 0.00
53	7,23-Pentatriacontadiene	0.46 \pm 0.05	0.10 \pm 0.04	0.58 \pm 0.33	0.11 \pm 0.08
54	7,25-Pentatriacontadiene	1.21 \pm 0.12	0.57 \pm 0.13	9.10 \pm 0.54	4.28 \pm 0.31
55	7,27-Pentatriacontadiene	0.27 \pm 0.03	0.02 \pm 0.02	2.75 \pm 0.15	1.00 \pm 0.13
56	5,21-Pentatriacontadiene	0.11 \pm 0.02	0.00 \pm 0.00	1.87 \pm 0.11	0.30 \pm 0.14
57	Pentatriacontadiene	0.01 \pm 0.00	0.00 \pm 0.00	0.65 \pm 0.04	1.11 \pm 0.15
58	Pentatriacontene	0.00 \pm 0.00	0.00 \pm 0.00	0.15 \pm 0.05	2.44 \pm 0.28
59	<i>n</i> -Pentatriacontane	0.00 \pm 0.00	0.00 \pm 0.00	0.02 \pm 0.02	0.05 \pm 0.05
60	Unknown	0.00 \pm 0.00	0.00 \pm 0.00	0.05 \pm 0.02	0.03 \pm 0.03
61	Heptatriacontadiene	0.01 \pm 0.00	0.00 \pm 0.00	0.28 \pm 0.03	0.00 \pm 0.00
62	Heptatriacontadiene	0.01 \pm 0.00	0.00 \pm 0.00	0.52 \pm 0.03	0.00 \pm 0.00
63	Heptatriacontadiene	0.09 \pm 0.02	0.00 \pm 0.00	2.77 \pm 0.04	0.72 \pm 0.13

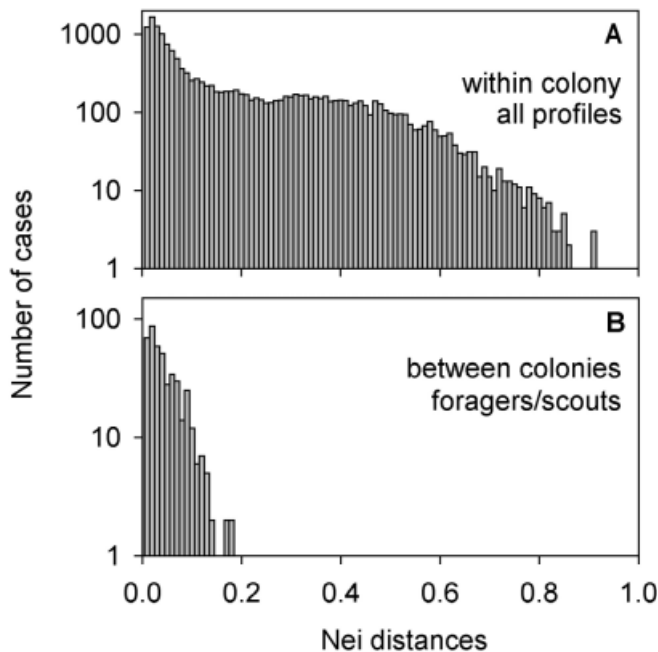


Fig. 2A, B Hydrocarbon compositions in *M. eumenoides* show a high variation. **A** Histograms of Nei distances (pairwise comparisons, $n = 15,400$) between all cuticular hydrocarbon compositions of the 176 ants collected from the “focus colony”. **B** Pairwise comparisons ($n = 435$) of cuticular hydrocarbon compositions from 30 foragers (F) collected from three different field colonies, as well as from a laboratory colony fed on crickets

thus cuticular hydrocarbon variation did not correlate with age ($r = 0.043$, $P = 0.86$, n.s.; Fig. 4). In the other group the ants become foragers, the composition of their cuticular hydrocarbons changed, and the degree of change correlated with age ($r = 0.54$, $P < 0.001$; Fig. 4). This data does not include scouts as their age was not recorded. However, scouts showed the same cuticular hydrocarbons as the foragers did.

Discussion

There is an extensive literature associating cuticular hydrocarbons with recognition cues in ants (for a recent summary see Vander Meer and Morel 1998). The majority of the papers demonstrate a uniform composition of hydrocarbons in a colony. In addition, a high similarity between the cuticular hydrocarbons and the postpharyngeal hydrocarbons was shown (e.g. Bagnères and Morgan 1991). Therefore, the postpharyngeal gland is assumed to serve as a “Gestalt” organ in which the hydrocarbons are stored, and mixed, and then distributed through allogrooming and perhaps trophallaxis on the cuticle of nestmates (Soroker et al. 1994, 1995b, 1998), thus forming a unified and colony-specific odour which would follow the “Gestalt” model (Crozier and Dix 1979). In contrast, the results of our study provide strong evidence that within colonies of *M. eumenoides* there is a high variation in the hydrocarbon composi-

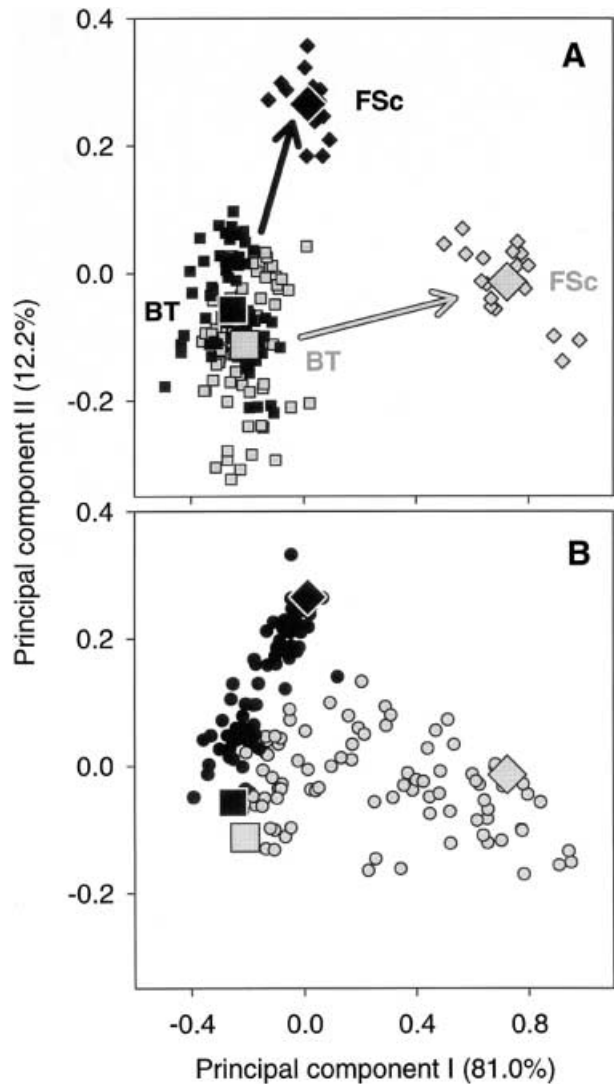


Fig. 3 Ordination of the first two principal components for Nei distances between hydrocarbon compositions in the PPG (black symbols) and on the cuticle (grey symbols) of *M. eumenoides*. **A** Brood-tenders (squares, BT) and foraging or scouting ants (diamonds, FSc). PPG of BT: $n = 63$, cuticle of BT: $n = 66$, PPG of FSc: $n = 16$, cuticle of FSc: $n = 20$. **B** Hydrocarbon compositions of ants for which task allocation could not be defined (black and grey circles). PPG: $n = 71$, cuticle: $n = 89$. The large symbols in **A** represent the mean components of the hydrocarbon compositions (centroids) collected from the PPG and the cuticles of brood-tenders and foragers or scouts. These centroids are also shown in graph **B** for visual orientation

tions both in the postpharyngeal gland and on the cuticle. Furthermore, particularly in foragers or scout ants, postpharyngeal and cuticular hydrocarbon compositions of individual ants differ greatly.

Sequestration of hydrocarbons in the postpharyngeal gland

Larval stages of ants carry no or only very small amounts of hydrocarbons on their cuticle (e.g. Arnold

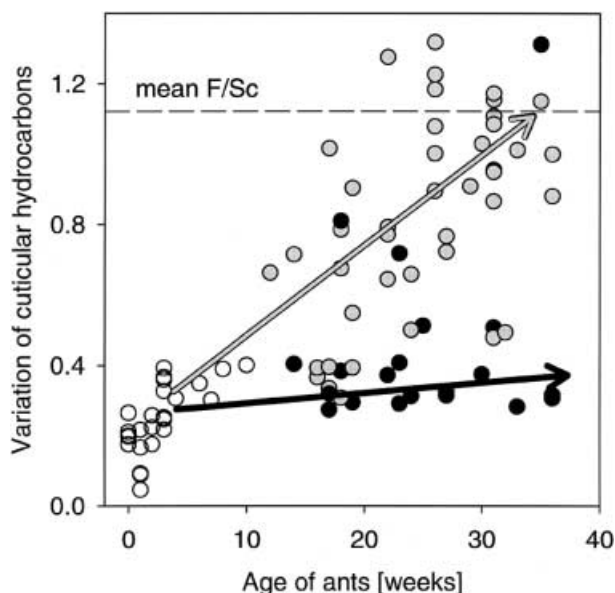


Fig. 4 Variation of cuticular hydrocarbons in *M. eumenoides* as function of the age of an ant. *Open circles* young brood-tenders ($n=25$); *black circles* ants staying in the nest ($n=20$), *grey circles* ants foraging for food ($n=42$). For the calculation of the degree of variation see text. The *dashed line* represents the mean Euclidean distance between the centroids for brood-tenders and compositions of foragers or scouts (mean FSc; for the centroid see Fig. 3A). Due to the small variation of cuticular compositions young brood-tenders group around the centroid. For these ants the calculated Euclidean distances are not zero but have small values. The *arrows* indicate the consistency of hydrocarbon compositions in the nest and the variation of the compositions in ants leaving the nest (from type C/BT to type C/FSc)

and Regnier 1975; Heinze et al. 1994). Following eclosion, during the ontogenesis of the postpharyngeal gland, hydrocarbons accumulate in the gland. This phenomenon that we observed in *M. eumenoides* has also been reported for *Cataglyphis iberica* (Dahbi et al. 1998), *Camponotus floridanus* (Morel et al. 1988), and *Cataglyphis niger* (Soroker et al. 1995b). Thus, the build-up of a chemical signature in callow workers may be a general phenomenon.

The kinetics of hydrocarbon accumulation in the postpharyngeal gland have been investigated previously by extracting postpharyngeal glands as a whole, which means the epithelial cells together with the central lumina of the glandular fingers, which form the reservoirs. The morphology of the PPG has been investigated in some ant species and lamellar structures were observed, which may be involved in lipid metabolism (see in: Soroker et al. 1995a), but no lipophilic substances were demonstrated in the epithelium. In this paper we compare an age-dependent accumulation of hydrocarbons in the whole gland with the increase of lipid inclusions in the epithelial cells. We demonstrate that accumulation of lipids in epithelial cells is slower than accumulation of hydrocarbons in the gland as a whole, including the reservoirs. This suggests that when being nursed by older ants, lipids are transferred to young ants by trophallaxis,

which allows new members of a colony to integrate quickly. In young ants, lipid material seems to be transported into the postpharyngeal gland and apparently is stored in its lumina. The muscular system controlling the opening of the pharynx allows the oesophagus to be closed (Soroker et al. 1995a).

Variation of cuticular hydrocarbon compositions

The similarity of cuticular hydrocarbon compositions of individuals within a colony is explained by an extensive reciprocal transfer of hydrocarbons among nestmates. However, in recent years there is increasing evidence that hydrocarbon compositions within ant colonies are not always uniform. In *Leptothorax lichtensteini* the relative proportion of some hydrocarbons on the cuticle of foraging workers changes with season and variations in nestmates occur in a synchronous manner (Provost et al. 1993). Similarly, non-callow adult workers of *Formica truncorum* show considerable seasonal variation in cuticular hydrocarbons (Nielsen et al. 1999). This phenomenon has also been reported for *Solenopsis invicta* (Vander Meer et al. 1989) and for a number of other social insects like the hornet *Vespa crabro* (Butts et al. 1995) and the honey bee *Apis mellifera* (Page et al. 1991). Because ants have a long life-span, one might assume that season-specific variation of cuticular hydrocarbons may be a general phenomenon caused by environmental factors. However, seasonal variation may also be due to differences in the age composition of a colony, which in temperate zones is rather homogeneous after hibernation but becomes heterogeneous later in the season (Nielsen et al. 1999). Similarly, heterogeneous hydrocarbon compositions may be expected in tropical species. In the tropical *Myrmecaria* we find high hydrocarbon variation within colonies kept under constant environmental conditions in the laboratory. Furthermore, age-dependent labour division is well documented in various ant species (Hölldobler and Wilson 1990). Wagner et al. (1998) showed that *Pogonomyrmex barbatus* foragers have a higher relative proportion of *n*-alkanes than nest maintenance workers and the difference is consistent from one colony to another.

Variation of hydrocarbon compositions in the postpharyngeal gland

Published results on the postpharyngeal hydrocarbon compositions follow a similar pattern to those obtained from the cuticle. In *C. niger* and *Manica rubida* uniform compositions within an ant colony have been reported (Soroker et al. 1994, 1995b, 1998; Vienne et al. 1995; Hefetz et al. 1996; Lahav et al. 1999). However, in *C. iberica*, season-specific, task-specific and age-specific variation have all been documented (Dahbi et al. 1997, 1998; Dahbi and Lenoir 1998b). This discrepancy in results for the postpharyngeal gland and

the cuticle may be explained by the sampling techniques used. In some cases cohorts of ants of the same age, the same behavioural context, and under similar environmental conditions were collected and similar hydrocarbon compositions were found. In other cases ants had different but known ages, were members of different task groups, or were collected in different seasons and showed variability in hydrocarbon compositions.

Variation of hydrocarbon compositions in *M. eumenoidea*

To our knowledge we present here the first study in which postpharyngeal and cuticular hydrocarbon compositions were simultaneously analysed for the same individual ants. We used ants of known age, starting from callow workers; ants with different task status; and ants which were exposed to different climatic conditions and had different diet. In all cases, hydrocarbon compositions were strongly dominated by unsaturated hydrocarbons, both alkenes and alkadienes, while branched alkanes were only found in trace amounts. This hydrocarbon composition is fairly unusual for ants, as in other species for which hydrocarbons have been chemically identified the compositions were dominated by branched hydrocarbons and to a lesser extent by *n*-alkanes (e.g. Soroker et al. 1995a; Dahbi and Lenoir 1998b; Wagner et al. 1998).

Our study strongly suggests that the observed high intra-colonial variation is not caused by environmental conditions. The majority of the results presented were derived from the “focus colony” which was kept in the laboratory under constant environmental conditions, and which had a constant diet during the course of the study. Considering field colonies as well as laboratory colonies fed on a different diet, the intra-colonial differences between nestmates from different age groups or task groups is far greater than inter-colonial variation among ants of the same task group (foragers or scouts). This suggests that changes in hydrocarbon composition are controlled by internal factors of the colony.

Following eclosion, young ants are nursed, and during this period they acquire hydrocarbons in the postpharyngeal gland and on the cuticle. Up to an age of about 10 weeks, *Myrmecaria* ants remain in the nest to nurse the brood or to maintain the nest (own unpublished observations). At this age, hydrocarbon compositions are almost uniform, and cuticular and postpharyngeal compositions are largely congruent. When ants grow older, they either stay in the nest or leave the nest to search for food. Irrespective of their age, ants staying in the nest keep their cuticular hydrocarbon composition unchanged; however, in ants that leave the nest (foragers and scouts) cuticular hydrocarbon compositions change significantly. Thus, the variation is only partly correlated with age, but strongly correlated with the task allocation of individual ants.

This variation resides in the ratios among the unsaturated hydrocarbons, the alkenes and alkadienes.

It is not known what triggers that changes in hydrocarbon compositions in individual ants. Besides environmental factors, which in *Myrmecaria* do not explain the variation of the hydrocarbons, Gordon (1996) discussed external and internal factors in individual nestmates. External stimuli for ants, such as the level of interactions between workers, may modify the biosynthesis of hormones, as shown for honeybees (Huang and Robinson 1992). Such external factors may also determine in *Myrmecaria* colonies how many ants remain in the nest and how many ants switch their task to become foragers or scouts. Once this “decision” has been taken, the change of the ant’s hydrocarbon composition may then depend on internal factors such as age. However, the role and relation of external and internal factors influencing the ant’s task status and the changes in hydrocarbon compositions still requires further studies.

Although changes in hydrocarbon compositions are strongly correlated, those in the postpharyngeal gland are less pronounced than those on the cuticle and are due to several different components. In consequence, hydrocarbon compositions in the gland and on the cuticle become significantly different in *Myrmecaria* foragers and scouts. We found a rather high abundance of *n*-alkanes on their cuticle but not in their postpharyngeal gland. Similarly high proportions of *n*-alkanes in the composition of cuticular hydrocarbons were reported for *P. barbatus* foragers (Wagner et al. 1998). In *C. niger*, adult workers of at least 1 month in age were analysed in respect of their postpharyngeal and cuticular hydrocarbon compositions (Soroker et al. 1995a). Though the authors claim that the two compositions revealed congruency, the data they presented (Table 2 in Soroker et al. 1995a) show a much higher amount of *n*-alkanes on the cuticle than in the PPG, and thus parallel our present results. These individual differences in *Myrmecaria* and *Cataglyphis* do not support the hypothesis that the postpharyngeal gland alone serves as a “Gestalt” organ for a uniform cuticular bound hydrocarbon label in a colony as supposed by Soroker et al. (1995b).

The increasing number of cases of inhomogeneous hydrocarbon compositions within an ant colony as well as for age-dependent or task-dependent differences being consistent between colonies, leads us to suggest that the total mixture of hydrocarbons should not be used to assess differences or correlations between chemistry and behaviour. Instead, one might expect to find that only certain components of the lipids serve as colony-specific recognition cues, whereas other components signal task allocation of an individual ant.

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