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Hydrocarbons in the Ant *Lasius niger*: From the Cuticle to the Nest and Home Range Marking

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Abstract The cuticular hydrocarbons (CHCs) of the ant *Lasius niger* are described. We observe a high local colony specificity of the body cuticular profile as predicted for a monogynous and multicolonial species. The CHCs show a low geographical variation among different locations in France. The CHCs on the legs also are colony specific, but their relative quantities are slightly different from those on the main body. For the first time, we demonstrate that the inner walls of the ant nest are coated with the same hydrocarbons as those found on the cuticle but in different proportions. The high amount of inner-nest marking and its lack of colony-specificity may explain why alien ants are not rejected once they succeed in entering the nest. The cuticular hydrocarbons also are deposited in front of the nest entrance and on the foraging arena, with a progressive increase in n-alkanes relative amounts. Chemical marks laid over the substrate are colony specific only when we consider methyl-branched alkanes. Our data confirm that these "footprint hydrocarbons" are probably deposited passively by the contact of ant tarsae with the substrate. These results suggest that the CHCs chemical profiles used by ants in colony recognition are much more complex than a single template: ants have to learn and memorize odors that vary depending on their context of perception.

Keywords Hydrocarbons · Nestmate recognition · Territorial marking · Ants · Nest odor

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Introduction

Cuticular hydrocarbons in insects are essential for protection against desiccation (Gibbs 1998). In social insects, it generally is considered that they have gained a role in nestmate recognition and at least partly form the colony-specific odor: a complex set of non-volatile compounds used to discriminate aliens from nestmates [see recent reviews (Hefetz 2007; d'Ettorre and Lenoir 2009)].

It also is known that ant behavior is influenced by colony odors left in the environment. When an ant encounters an alien worker in the foraging arena, its agonistic behavior will change depending on whether it occurs on a familiar substrate or on trails (Akino and Yamaoka 2005). In the common garden ant *Lasius niger*, the exploited territory remains stable over years with nest surroundings being chemically marked by a home-range rather than a true territorial marking (Devigne and Detrain 2002). Since area marking probably originates from footprint hydrocarbons laid passively by walking ants, the level of area marking can be used by workers as a cue to assess the density and activity of nestmates at one location (Yamaoka and Akino 1994; Devigne and Detrain 2006). It

also is assumed that the level of area marking could inform the ants about their distance from the nest since in central place foragers, the density of exploring nestmates and thereby the density of home-range marks decrease with distance (Devigne and Detrain 2006). Based on the information provided by the level of home-range marking, ants fine tune their recruitment and foraging behavior. By increasing the trail intensity as well as the rate of information transfer on heavily marked areas, scouts reduce the number of foragers mobilized to less frequented areas that are potentially dangerous and promote recruitment and exploitation of food sources to better known sites (Devigne et al. 2004). The nest odor is important when the colony has to emigrate: ants will always prefer to settle in a marked nest than in an unmarked one (e.g., *Lasius niger* (Depickère et al. 2004); *Temnothorax albipennis* (Franks et al. 2007)).

In this paper, we investigated whether area marking in *Lasius niger* ant species originates from cuticular hydrocarbons and to what extent they show the same level of colony specificity as cuticular recognition cues over the ant body. First, we analyzed the cuticular hydrocarbons (CHCs) of forager workers from different localities in France to investigate geographical variations in the cuticular chemical profile of this species. Nestmate recognition in *Lasius* is highly efficient with workers also being able to discriminate nestmates from non-nestmates (Akino and Yamaoka 2005). Therefore, we predicted that the monogynous *L. niger* ant has a well differentiated colonial cuticular profile. Second, we compared the cuticular profiles of head plus thorax vs. legs alone in order to determine whether legs have a secretory role, as hypothesized for Japanese *Lasius* species (Yamaoka and Akino 1994). In this case, thorax and legs would show quite different chemical profiles. In contrast, they would be similar if there is a continuous update of the profile of the individuals due to self-grooming. Third, we analyzed the chemical profile of traces left over inner walls of ant nests, as well as those deposited by the foraging workers over the areas surrounding the nest. We predicted that the inside-nest marking would be colony-specific. Since ants behave similarly over areas that are marked by an alien or by their own colony (Devigne and Detrain 2002), we predicted that, outside the nest, there would be a less clear-cut colonial identity of traces left over the substrate of foraged areas.

Methods and Materials

The Ants The black garden ant, *Lasius niger*, is common in temperate European regions. Colonies were collected in May and June 2007 at three locations: near the cities of Tours (Azay sur Cher, 0° 48′ E, 47° 19′ N, 80 m asl, orchard, colonies T5, T6, and T7) and Pau (Sauvagnon, 0° 22′ W, 43° 25′ N, 187 m, garden, colonies S2 and S3), and in the Alps (M: Morillon, Haute-Savoie, 06° 41′ E, 46° 05′ N, 670 m, on the edge of a torrent, one colony). Within localities, colonies were separated by less than 10 m. All fragments of colonies were queenless and composed of 300 to 500 workers with brood. We checked that the ants were distinct from the close species *L. alienus* and *L. platythorax* by examining their morphological characters as well as their cuticular hydrocarbon profiles which were different (A.L. unpublished).

Colonies were kept in the laboratory (temperature 25°C, natural daylight), in artificial nests composed of two plastic containers (20×20×10 cm) connected by a bridge. The first box contained the nest made with glass tubes half filled with water and covered with black paper. The second container provided a foraging area to the colony, in which twice a week we offered *Tenebrio molitor* larvae and commercial bumblebee solution (Bee Happy®).

Chemical Analyses To study cuticular hydrocarbons, samples consisted of extracts of head plus thorax with the legs. Gaster was eliminated to prevent contamination from Dufour gland. We pooled five workers to get a concentrated sample. To determine a possible secretory role of legs, samples were made of tibias and tarsae from 20 workers. All workers were foragers. Ants were immersed in 200 µl of pentane for 5 min. Samples were removed, and 5 µl of pentane containing 50 ng of eicosane (C20) were added as an internal standard. For analyses, the solvent was evaporated until 10 µl remained. Three µl were injected into a FID gas–chromatograph (VGM250Q system, Perkin-Elmer) with a split/splitless injector and flame ionization detector that used a DB-5 fused silica capillary column. Temperature was kept at 150°C during the initial splitless 2 min, raised from 150°C to 300°C at 5°C/min, and held at 300°C for the last 10 min. The non volatile cuticular lipids were identified with the same GC coupled to a Perkin-Elmer MS operating 70 EV. A series of linear hydrocarbon standards (C20, C22, C24 … to C40) were injected regularly in order to have references. We also injected one cuticular extract into a high temperature column (DB-5HT) to 370°C to check if some hydrocarbons with high molecular weights appeared, as has been found in some ants [see (d'Ettorre and Lenoir 2009)]. As no more hydrocarbons were detected, we subsequently used the normal DB-5 column. The areas of peaks were measured by peak integration with a Perkin Elmer Turbo-Chrome Workstation, and relative proportions were calculated. The quantities of compounds were calculated by using the internal standard areas (ng per head + thorax or per six legs, i.e., one ant).

Chemical compounds deposited on the substrate by the ants were analyzed by using the SPME technique. A polydimethylsiloxane (PDMS) 7 µ (fused silica/SS) fiber

was rubbed on substrate for 5 min. The fiber was desorbed in the GC under the same conditions as for liquid extracts. Three locations were chosen to analyze deposited chemicals marks: inner nest walls, area in front of the nest entrance, and foraging area. For the inside nest marking, we gently pushed the ants outside the nest and rubbed the fiber over the inner walls of the glass tubes. To collect area marking, glass slides were washed completely with a detergent and rinsed several times with hexane. We checked with SPME that the slides were free of any detectable chemical traces. These clean slides were deposited just in front of the nest entrance and in the foraging arena. Since preliminary trials showed that slides in the foraging arena were frequently covered with refuse particles, we put them for at least 2 days on the bridge between the two containers where they were kept clean. It has been shown that the profiles obtained with SPME and classical solvent extraction are qualitatively identical (Sledge et al. 2000; Tentschert et al. 2002), but a precise quantitative analysis shows that the proportions of compounds are slightly different. So, in order to get comparable data for the cuticle and the substrate marking, we also performed a 5 min SPME on the thorax of ants killed in the refrigerator. Moreover, we deposited in five samples a standard of 100 ng of C20 on the fiber to estimate the quantities retrieved by the SPME technique.

Statistics We compared the average relative amounts of different hydrocarbon classes by using a non-parametric Kruskal–Wallis test. To analyze the grouping of samples, we performed discriminant analyses on the peaks present in more than 1% amount followed by post-hoc F tests. We did not transform the data to compensate for their non-independence, since it introduces additional background noise and gives similar results. We also analyzed the distances between groups after discriminant analyses by using the Squared Mahalanobis distances (SMD), and we compared them by using the Kruskal–Wallis non-parametric test. To compare the hydrocarbon quantities we used Mann–Whitney *U* test.

Results

Cuticular Hydrocarbons and Geographical Variations *Lasius niger* workers show a wide spectrum of hydrocarbons from 25 to 39 carbons. These are composed of saturated n-alkanes (3.67%±1.39; mean ± SD, N=46) and corresponding alkenes (1.90%±0.72), large quantities of monomethyl-alkanes (29.70%±4.73), dimethyl-alkanes (58.05% ± 4.58), and trimethyl-alkanes (6.37%±2.64) (Fig. S1 and Table S1). Twenty two peaks are present in more than 1%, the two major peaks are a mixture of methyl-C31 (peak n° 30: 9+11+13+15Me-C31; 11.41%± 3.62) and a mixture of dimethyl-C31 (peak n° 32: 9,11+ 9,13+9,15DiMe-C31; 20.83%±3.77). The total hydrocarbon quantity is estimated at 521±396 ng/head + thorax + legs (N=21).

Discriminant analysis indicates good discrimination among the samples. Among the six colonies, four are well discriminated (T6, T7, S3 and S2, with all P<0.004, *Post-hoc* test F). Colonies M (Morillon) and T5 (Tours 5), however, are not differentiated (P=0.35) (Fig. 1). In the same locality (three colonies from Tours and two colonies from Pau), colonies are clearly differentiated. The Squared Mahalanobis Distances (SMD) are different between the groups (Kruskal–Wallis H(2, N=174)=85.5, P<0.01). The intracolony variation (among individuals) is small (19.89±2.79, mean ± SD, N=35). In contrast, intercolonial SMD between neighboring colonies in the same site is high (1917.14± 939.53, N=34; P<0.001 compared to intracolonial variation). This distance is not higher when we consider two colonies from different locations (for example Tours and Pau): 1666.42±1061.21, N=105 (P=0.46 NS compared to intercolonial variation in the same habitat).

These results indicate that in *L. niger*, there is a colonial chemical identity with colonies that inhabit the same habitat, being well differentiated in distances of a few meters. Additionally, the geographical variation is weak for this species since the SMD distance between the three sites is of the same order of magnitude as those found in the same habitat (one colony from the Alps has the same profile as one from Tours).

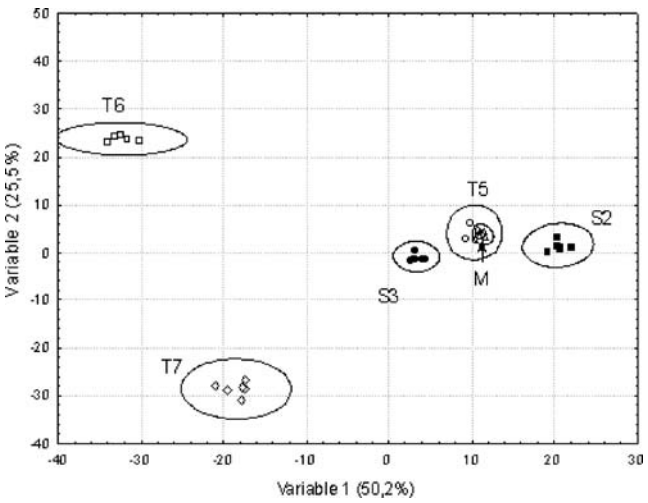

Fig. 1 Discriminant analysis of *Lasius niger* cuticular hydrocarbons. T = Tours (Azay sur Cher, colonies T5, T6, and T7); S = Sauvagnon near Pau (colonies S2 and S3); M = Morillon (Alps). Lambda Wilk< 0.001, F (120, 34)=21.68, P<0.001. *Ellipses* are 95% confidence intervals

Hydrocarbons on the Legs Legs contained the same hydrocarbons as the body cuticle (Table S1): all peaks were present and no new peak appeared. The hydrocarbon quantities were, however, much smaller on the legs (16.4 ng±10.8/worker, N=17, i.e., 3.15% of the thorax plus head cuticle content and less than 3 ng per each tibia + tarsus).

A discriminant analysis of hydrocarbons present on bodies or legs performed on the three colonies confirmed the high segregation among colonies seen in Fig. 1. It also shows that legs bear the colony profile (all P<0.001, F *post-hoc* test; see Fig. S2). By making a new analysis and using as discriminant variables the body part (head + thorax or legs) and the colony, we found that all groups are separated (all P<0.001, F *post-hoc* test) (Fig. 2). A main separation appears on the first axis (76.5% of the variance) among the three colonies. The discrimination is significant but less pronounced between the body cuticles and the legs within the same colony (12.9% of the variance). It indicates that the leg's cuticular profile is slightly different from the body one. It is characterized by an increase of the relative amount of n-alkanes (3.67%±1.39 for cuticle vs. 13.34± 6.69 for legs; H (1, n=63)=34.81, P<0.001), trimethyl-alkanes (6.37%±2.64 vs. 12.74±4.76; H=25.17, P<0.001) and alkenes (1.90%±0.72 vs. 5.73±5.10, H=4.37, P= 0.04). This increase is not due to mono-methyl-alkanes (29.70%±4.73 vs. 29.78±4.65; H=0.54, P=0.82) but to a decrease of dimethyl-alkanes (58.05%±4.58 vs. 38.41± 7.99; h=33.72, P<0.001) (see Table S1). There are no differences between the lengths of hydrocarbon chains: all n-alkanes from C25 to C31 increased and most dimethyl-alkanes decreased.

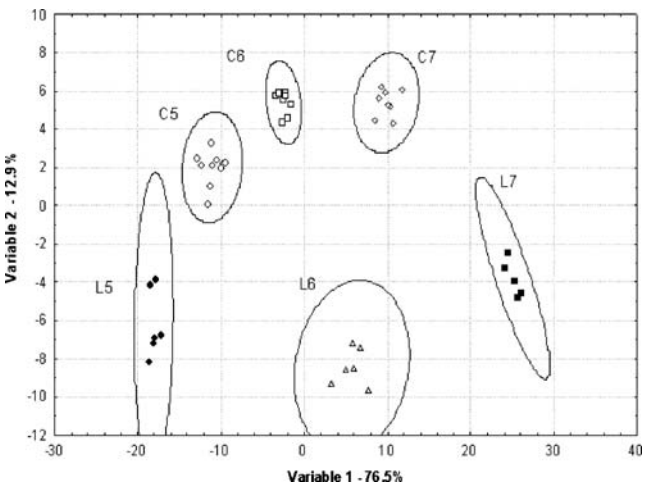

Fig. 2 Discriminant analysis of the hydrocarbons extracted from body cuticle (C) and legs (L) of colonies 5, 6, and 7. Lambda Wilk<0.001, F (130,64)=10.097, P<0.001. *Ellipses* are 95% confidence intervals

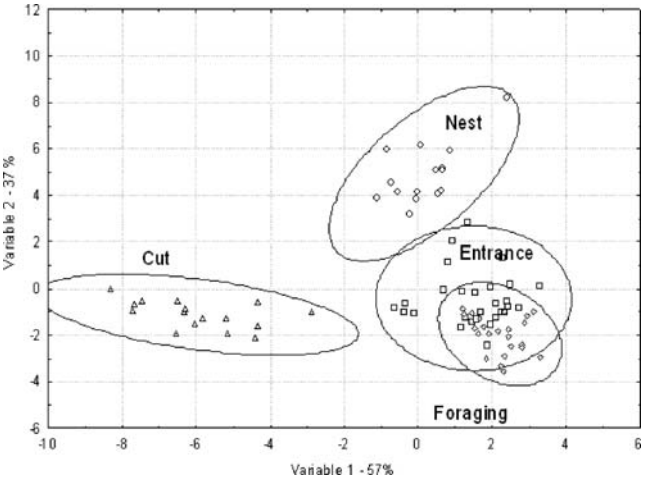

Fig. 3 Discriminant analysis of hydrocarbons obtained by SPME from cuticle (*Cut*), inner walls of the nest (*Nest*), entrance of the nest (*Entrance*) and foraging arena (*Foraging*), independently of the colonies which are not separated. Lambda Wilk 0.008, F (87, 150)= 7.450, P<0.001. *Ellipses* are 95% confidence interval

Hydrocarbons Obtained by SPME SPME and washing of the cuticle gave the same profiles, and the proportions of compounds are similar [Nei index of similarity between the two profiles = 0.96, i.e., comparable to intracolonial variation, see for example (Nowbahari et al. 1990; Lenoir et al. 2001a)]. This means that SPME and washing data can be compared directly.

In a first analysis, we compared the SPME chemical profiles of thorax cuticle, inner walls of the nest, nest entrance area and foraging arena independent of the colony (Fig. 3). The first axis separates the thorax cuticle from the deposited substances, indicating a clear distinction between them. The second axis separates the nest from the entrance and the arena. All groups are statistically different, but 8.5% of the values are not well classified in the foraging and entrance areas with Mahalanobis distances between them being much smaller (8.38 vs. all other distances >33). This indicates a clear separation between the cuticle and the substrate markings. Additionally, the marks left on the inner walls are different from the entrance and the foraging areas, the two latter being not very different. Globally, there is a gradient from the nest to the outside: the relative quantities of n-alkanes increase (from 6.58% to 29.36%), due mainly to a decrease in dimethyl-alkanes and alkenes. The proportions of methyl-alkanes and trimethyl-alkanes do not change (Fig. 4).

The amounts of hydrocarbons retrieved during 5 min of SPME rubbing are variable, but always higher when extracted from the nest walls than from the entrance, or foraging areas: 71.0 ng±80.07, N=15 for the nest, 15.56 ng ±21.45, N=28 for the entrance and 17.12 ng±14.98, N=23 for the foraging area (U Mann–Whitney N/E: P=0.005; N/ F: P=0.02 and E/F: P=0.28). These values are only

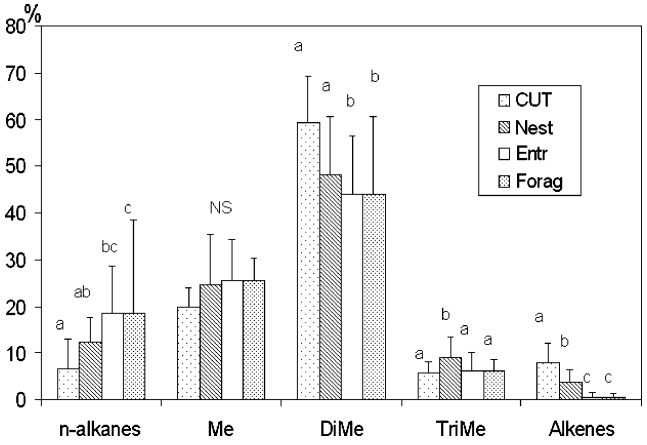

Fig. 4 Relative quantities of hydrocarbon classes present on the thorax cuticle or deposited at different locations (mean ± SD). Different letters indicate significant differences (Kruskal–Wallis test). Me = monomethyl-alkanes, DiMe = dimethyl-alkanes, TriMe = trimethyl-alkanes

comparative, and indicate that the hydrocarbons are more concentrated on the nest walls.

In a second analysis, we performed a discriminant analysis on all the SPME data for the deposited marks: inside-nest walls, entrance, and foraging areas (Fig. 5). The first axis (46.1%) showed discrimination between marking of the nest walls and those laid over areas outside the nest. The distances for inside nest marks were significant between N5/N6 (P=0.004) and N5/N7 (P=0.002) but not between N6 and N7 (P=0.30). This suggests that inside the nest, discrimination is not absolute, and errors are likely to occur. The situation also is complex concerning markings of the nest entrance and foraging arena. Colony 7 is well differentiated from colonies 5 and 6, but there is poor discrimination between its nest entrance and its foraging area (E7 / F7, P=0.04). Colonies 5 and 6 are not at all discriminated (all P>0.05, with a maximum for E6 / F6, P=0.85).

In a third step, considering that methyl branched hydrocarbons could be more important in nestmate recognition [see (Dani et al. 2001) for wasps, reviews by (Hefetz 2007; d'Ettorre and Lenoir 2009), and recent data on some species of *Formica* (Martin et al. 2008a)], we performed a discriminant analysis that used only mono-, di- and trimethyl-branched hydrocarbons. It does not change the results for nest marking: the two colonies 5 and 6 are not discriminated showing a low degree of differentiation for the inside nest marking (data not shown). By contrast, as regards entrance and foraging marks (Fig. 6), a good discrimination appears among the three colonies (only the distance in foraging arenas between colony 5 and 7 is at the significance limit with P=0.05). Nevertheless, the marks do not discriminate the nest entrance from the foraging arena of the same colony. It indicates that outside the nest, considering only the methyl-branched alkanes, the marks are colony-specific but the nest entrance and the foraging arena are not well differentiated under our experimental conditions.

Discussion

Cuticular Profile of Lasius Niger The hydrocarbon profile of *L. niger* workers is composed of a set of linear alkanes and alkenes, and many mono-, di-, and trimethyl-alkanes. The most important peaks are mixtures of monomethyl- and dimethyl-alkanes. Little is known about *L. niger* hydro-

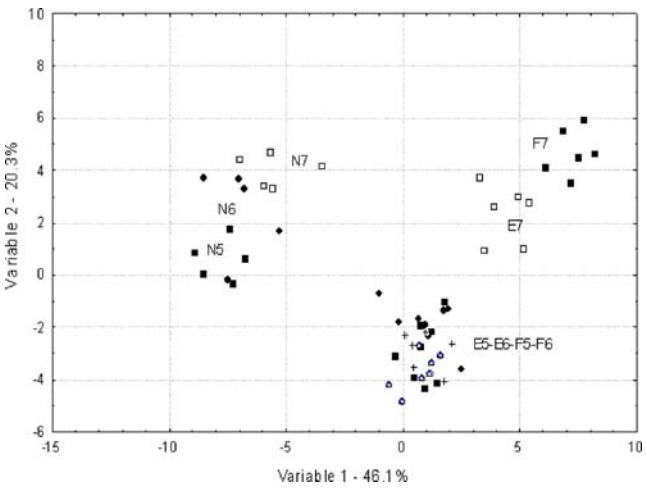

Fig. 5 Discriminant analysis of hydrocarbons obtained by SPME from inner walls of the nest (N), the entrance (E) and foraging arena (F) of colonies 5, 6, and 7. Lambda Wilk<0.001, F (240, 163)=2.92, P<0.001

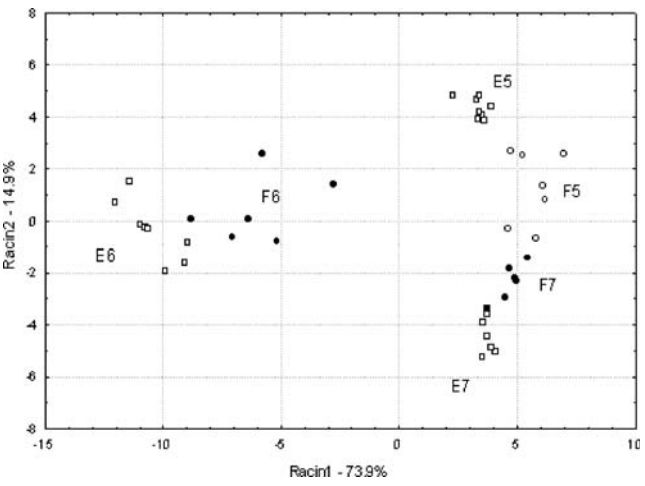

Fig. 6 Discriminant analysis of branched hydrocarbons obtained by SPME from the nest entrance (E) and foraging arena (F) of colonies T5, T6 and T7. Lambda Wilk<0.001, F (115, 73)=2.63, P<0.001. Distances not significantly different (NS): E6/F6 P=0.20; E5/F5 P=0.050; E7/F7 P=0.22 and F7/F5 P=0.23

carbons. Akino and Yamaoka described hydrocarbons of *L. niger* from Japan, which are completely different from European ones, but this species is now considered as a different twin species named *Lasius japonicus* (Akino and Yamaoka 2005). *L. niger* cuticular profiles appear to be stable in various areas in France, and also in Germany (Dinter et al. 2002), Belgium (A.L. unpublished) and Denmark (Dreier and D'Ettorre 2009). It also is the case for the invasive ant *Lasius neglectus*, which keeps the same profile in different locations (Ugelvig et al. 2008). There is no rule concerning the geographical variation of cuticular hydrocarbons in ants. Some species like *Cataglyphis cursor, C. iberica, Camponotus cruentatus*, or *Tetramorium* spp are highly variable (Nowbahari et al. 1990; Dahbi et al. 1996; Steiner et al. 2002; Boulay et al. 2007), while others like *Formica* do not change (Martin et al. 2008b).

Contrasting with the low geographical variation of chemical profiles, *L. niger* shows a strong local colony discrimination, as predicted for a monogynous and a little polyandric species [one to four copulations, see (Van der Have et al. 1988; Boomsma and Van der Have 1998; Aron et al. 2009)]. This seems to be general in monogynous species with independent founding like *Camponotus cruentatus* (Boulay et al. 2007).

The hydrocarbon quantity accounts for 0.1% of the head plus thorax mass, as is usual for ants (Lahav et al. 1998). The legs bear very small quantities of hydrocarbons that are colony specific but that have a slightly different profile from the body with an increase of linear alkanes, and tri - methyl-alkanes and alkenes in smaller proportions . Bagnères and Morgan (1991) also observed an increase in pentacosane (C25) on legs of *Myrmica rubra* queens.

Where do the leg hydrocarbons come from? Among the 20 glands described in ant legs, none seems to be involved in hydrocarbon production (Billen 2008), contrary to what was suggested by (Yamaoka and Akino 1994). Hence, hydrocarbons on the ant legs probably originate from self-grooming, but it is not clear how the difference in the profile can exist.

Are the Nest Walls Marked with Colony Odor? Nests or resting sites of many arthropods are marked and recognized by the owners. Several studies have confirmed such chemically-based nest recognition for solitary living arthropods such as tarantula spiders (Dor et al. 2008) or solitary bees (Guedot et al. 2006), for gregarious insects such as cockroaches (Ame et al. 2004) or triatomines (Lorenzo Figueiras and Lazzari 1998), and for social insects such as bumblebees (Saleh et al. 2007), honeybees (Butler et al. 1969) or ants (e.g., Depickère et al. 2004). In ants, volatile chemicals participate in nest recognition, for example in *Camponotus fellah* or *Pogonomyrmex badius* (see d'Ettorre and Lenoir 2009). Our results show for the first time that the inner walls inside the ant nest are marked with hydrocarbons comparable to those produced by the workers. This explains why many myrmecophiles obtain the colony odor by actively rubbing walls inside the nest (see reviews by Lenoir et al. 2001b; Akino 2008). Tricosane in particular is transmitted to myrmecophiles (Witte et al. 2008) agreeing with our results that show a high amount of linear alkanes inside the nest. Hydrocarbons found on the nest walls probably come from passive contacts with *L. niger* ant bodies. They also may be in the feces (Soroker and Hefetz 2000). Indeed, in the harvester ant *Messor capitatus*, the anal fluids deposited in the nest vicinity contain colony-specific cues that the ants use to recognize their nests from foraging areas (Grasso et al. 2005). We also established that the inside-nest odor is not completely colony-specific; for example, two colonies collected only a few meters apart had non discriminated odors.

The fact that ants have a colony specific cuticular odor and loose partially this specificity on the inside walls could be explained by the passive accumulation of compounds on the nest walls without permanent refreshing of the odor. Additionally, it has been observed that inside the nest, aliens generally are not rejected (Hölldobler and Wilson 1990). We thus hypothesize that the absence of alien rejection may be due not only to a higher threshold of discrimination by inner workers (Hölldobler and Wilson 1990) but also to an inside-nest colonial odor that is largely overlapping between colonies. We suggest that inside the nest, any social parasite will be considered as a fellow nestmate provided it has succeeded in "breaking the fortress" of the nest entrance, as stated by (D'Ettorre and Heinze 2001). This may explain why for instance in *Cardiocondyla elegans*, alate females are transported into alien nests by some workers, and are tolerated as soon as they have passed the nest entrance (Lenoir et al. 2006). Likewise, social parasites have a greater chance of being accepted once they succeed in entering the host nest. Such absence of alien rejection could be due to a saturation of the ant antennae receptors as hypothesized by (Ozaki et al. 2005). Our results show that the concentration of hydrocarbons actually is more important inside the nest than in the colony home-range, probably due to the high frequency of ant body contacts with the nest walls.

Are Nest Entrance and Home-Range Markings Colony Specific? Colony area marking is a widespread phenomenon in ants (see Hölldobler and Wilson 1990) and shows a diversity of glandular origin: postpharyngeal, metapleural, Dufour, cloacal glands, and also feces (Mayade et al. 1993; Cammaerts and Cammaerts 1998, 1999; Wenseleers et al. 2002; Grasso et al. 2005). In all cases, except two *Tetramorium* species (Cammaerts and Cammaerts 2000), area marking is colony-specific.

Our results on *L. niger* confirm previous behavioral results that show that the foraging area is chemically marked (Devigne and Detrain 2002, 2006; Depickère et al. 2004). It also confirms that marks consist of hydrocarbons identical to cuticular ones, as observed by (Yamaoka and Akino 1994). Beside ants, hydrocarbons left on the substrate are perceived by *L. niger* tended aphids and influence their dispersal (Oliver et al. 2007). In our experiments, the absence of active trail-laying behavior by *L. niger* indicates that marks are laid passively by walking ants, thus confirming previous observations (Depickère et al. 2004; Devigne and Detrain 2006). Footprint marks are colony-specific only when we consider the branched hydrocarbons in discriminant analyses. These footprints may act in conjunction with the trail pheromone as it was shown in the Asian species *L. japonicus* (Akino and Yamaoka 2005).

Even though the HC quantities on legs are small (less than 3 ng), the frequency of ants walking over an area may lead to a local density of marks high enough to be perceived by ants. It is well-known that social insects are highly sensitive to extremely low concentrations, for example, *Bombus* foragers rejecting flowers over which HCs doses of 10^{-12} ng/flower were deposited (Goulson et al. 2000). In *L. niger*, the perception of area marking promotes trail recruitment and food exploitation over familiar areas as compared to unexplored ones. The intensity of area marking is relevant for scouts to assess indirectly the colony occupancy at a location as well as the distance from the nest (Devigne and Detrain 2006). It is interesting to note that marks laid over the substrate have more alkanes than the cuticle, an increase in alkanes being also found on legs, which are in contact with the substrate. These higher concentrations of n-alkanes on the substrates may be due partly to differential melting points with branched hydrocarbons evaporating less rapidly than branched ones (Gibbs 1998; Hefetz 2007), and also to the fact that foragers have a larger proportion of straight-chain alkanes as compared to in-nest workers (Bonavita-Cougourdan et al. 1993; Greene and Gordon 2003). In *Formica exsecta* and *Pogonomyrmex barbatus*, this is due to exposure to high temperatures, UV, and low humidity (Wagner et al. 2001; Martin and Drijfhout 2009).

As regards the colony-specificity of area marking, similar chemical profiles are found at the nest entrance and in the foraging arena. This suggests that ants perceive the vicinity of their nest through an increased level of home-range marking rather than through qualitative changes in chemical marks. Volatile compounds also may be used by foragers to locate their nest entrance as has been shown recently on the desert ant *Cataglyphis fortis* (Steck et al. 2009).

Our main conclusion is that ants are faced with various blends of colony odors depending on their location. First, their own cuticular chemical profile varies from the head to the legs. Second, the odors of nestmates vary according to their caste (queen, workers, larvae, and males), their age, or their task performance. This indicates that, during ants' encounters, chemical cues used for nestmate discrimination are more complicated than a single bar-code. Finally, our chemical data confirm previous results that indicate that ants can perceive odors left by nestmates over substrates in their resting and foraging places (Devigne and Detrain 2006). Inside-nest odors are not colony-specific whereas area markings outside the nest are colony-specific, but at a low level. Even though discrimination may occur with branched alkanes, marks left at the nest entrance or on foraged areas are rich in linear alkanes that are considered to be poorly colony-specific.

Moreover, discrimination between colony home-range markings may be difficult to achieve due to the tiny amounts of footprint marks left over the substrate. As a consequence, in *L. niger*, defensive responses occur without a strict territorial marking but rather the ants rely on physical encounters to assess the relative force of opponent neighbors (Czechowski 1984). Area marking being poorly colony specific, rather acts as an indicator of the quality of a location. Conspecific cueing through area marking may influence the choice of a foraging space or of a nest site and thereby may promote a "shared information" strategy in *Lasius niger*. Based on the present work, one hopes that future studies will pay more attention to intracolonial sources of variability in odors, to the environmental influence on odors' colonial specificity, and to the sharing of conspecific chemical cues in insect societies.

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References

AKINO, T., 2008. Chemical strategies to deal with ants: A review of mimicry, camouflage, propaganda, and phytomimesis by ants (Hymenoptera: Formicidae) and other arthopods. *Myrmecological News* 11:173–181.

AKINO, T., and YAMAOKA, R., 2005. Trail discrimination signal of *Lasius japonicus*. *Chemoecology* 15:21–30.

AME, J.-M., RIVAULT, C., and DENEUBOURG, J.-L., 2004. Cockorach aggregation based on strain odour recognition. *Anim. Behav.* 68:793–801.

ARON, S., STEINHAUER, N., and FOURNIER, D., 2009. Influence of queen phenotype, investment and maternity apportionment on the outcome of fights in cooperative foundations of the ant Lasius niger. *Anim. Behav.* 77:1067–1074.

BAGNÈRES, A. G., and MORGAN, E. D., 1991. The postpharyngeal glands and the cuticle of Formicidae contain the same characteristic hydrocarbons. *Experientia* 47:106–111.

BILLEN, J., 2008. Occurrence and structural organization of the exocrine glands in the legs of ants. *Arthr. Struct. Develop*. 38:2–15.

BONAVITA-COUGOURDAN, A., CLÉMENT, J.-L., and LANGE, C., 1993. Functional subcaste discrimination (foragers and brood-tenders) in the ant *Camponotus vagus* Scop.: polymorphism of cuticular hydrocarbon patterns. *J. Chem. Ecol*. 19:1461–1477.

BOOMSMA, J. J., and VAN DER HAVE, T. M., 1998. Queen mating and paternity variation in the ant *Lasius niger*. *Mol. Ecol*. 7:1709-1718.

BOULAY, R., CERDÁ, X., SIMON, T., ROLDAN, M., and HEFETZ, A., 2007. Intraspecific competition in the ant *Camponotus cruentatus*: should we expect the 'dear enneny' effect? *Anim. Behav.* 74:985–993.

BUTLER, C. G., FLETCHER, D. J. C., and WALTER, D., 1969. Nest entrance marking with pheromones by the honeybee *Apis mellifera* and by the wasp *Vespula vulgaris*. *Anim. Behav.* 17:142–147.

CAMMAERTS, M.-C., and CAMMAERTS, R., 1998. Marking of nest entrance vicinity in the ant *Pheidole pallidula* (Formicidae, Myrmicinae). *Behav. Process*. 42:19–31.

CAMMAERTS, M.-C., and CAMMAERTS, R., 1999. Marking of the nest entrances and their vicinities in the *Myrmica rubra*. *Biologia* 54:553–566.

CAMMAERTS, M.-C., and CAMMAERTS, R., 2000. Foraging area in two related *Tetramorium* ant species. *J. Ins. Behav.* 13:679–698.

CZECHOWSKI, W., 1984. Tournaments and raids in Lasius niger (L.) (Hymenoptera, Formicidae). *Ann. Zool., Warszawa* 38:81–91.

DAHBI, A., CERDÁ, X., HEFETZ, A., and LENOIR, A., 1996. Social closure, aggressive behavior, and cuticular hydrocarbon profiles in the polydomous ant *Cataglyphis iberica* (Hymenoptera, Formicidae). *J. Chem. Ecol*. 22:2173–2186.

DANI, F. R., JONES, G. R., DESTRI, S., SPENCER, S. H., and TURILLAZZI, S., 2001. Deciphering the recognition signature within the cuticular chemical profile of paper wasps. *Anim. Behav.* 62:165–171.

DEPICKÈRE, S., FRESNEAU, D., DETRAIN, C., and DENEUBOURG, J.-L., 2004. Marking as a decision factor in the choice of new resting site in *Lasius niger*. *Ins. Soc*. 51:243–246.

D'ETTORRE, P., and LENOIR, A., 2009. Nestmate Recognition in Ants L. Lach, C. Parr and K. Abbott (Eds), Ant Ecology. Oxford University Press, Oxford, in press.

DEVIGNE, C., and DETRAIN, C., 2002. Collective exploitation and area marking in the ant *Lasius niger*. *Ins. Soc*. 49:357–362.

DEVIGNE, C., and DETRAIN, C., 2006. How does food distance influence foraging in the ant *Lasius niger*: The importance of home-range marking. *Ins. Soc*. 53:46–55.

DEVIGNE, C., RENON, A., and DETRAIN, C., 2004. Out of sight but not out of mind: modulation of recruitment according to home range marking in ants. *Anim. Behav.* 67:1023–1029.

DINTER, K., PAARMANN, W., PESCHKE, K., and ARNDT, E., 2002. Ecological, behavioural and chemical adaptations to ant predation in species of *Thermophilum* and *Graphipterus* (Coleoptera: Carabidae) in the Sahara desert. *J. Arid Env.* 50:267–286.

DOR, A., MACHKOUR-M'RABET, S., LEGAL, L., WILLIAMS, T., and HENAUT, Y., 2008. Chemically mediated burrow recognition in the mexican tarantula *Barchypelma vagans* female. *Naturwissenschaften* 95:1189-1193.

DREIER, S., and D'ETTORRE, P., 2009. Social context predicts recognition systems in ant queens. *J. Evol. Biol.*: 22:644–649.

FRANKS, N. R., HOOPER, J. W., DORNHAUS, A., AUKETT, P. J., HAYWARD, A. L., and BERGHOFF, S. M., 2007. Reconnaissance and latent learning in ants. *Proc. Roy. Soc. London B* 274:1505–1509.

GIBBS, A. G., 1998. Water-Proofing properties of cuticular lipids. *Amer. Zool*. 38:471–482.

GOULSON, D., STOUT, J. C., LANGLEY, J., and HUGUES, W. O. H., 2000. Identity and function of scent marks deposited by foraging bumblebess. *J. Chem. Ecol*. 26:2897–2911.

GRASSO, D. A., SLEDGE, M. F., LE MOLI, F., MORI, A., and TURILLAZZI, S., 2005. Nest-area marking with faeces: a chemical signature that allows colony-level recognition in seed-harvesting ants (Hymenoptera, Formicidae). *Ins. Soc*. 52:36-44.

GREENE, M. J., and GORDON, D. M., 2003. Cuticular hydrocarbons inform task decision. *Nature* 423:32.

GUEDOT, C., PITTS-SINGER, T., BUCKNER, J. S., BOSCH, J., and KEMP, W. P., 2006. Olfactory cues and nest recognition in the solitary bee *Osmia lignaria*. *Physiol. Entomol*. 31:110–119.

HEFETZ, A., 2007. The evolution of hydrocarbon pheromone parsimony in ants (Hymenoptera: Formicidae)—interplay of colony odor uniformity and odor idiosynchrasy. *Myrmecol News* 10:59–68.

HÖLLDOBLER, B., and WILSON, E. O., 1990. The Ants. The Belknap, Cambridge, 782

LAHAV, S., SOROKER, V., VANDER MEER, R. K., and HEFETZ, A., 1998. Nestmate recognition in the ant *Cataglyphis niger*: do queens matter? *Behav. Ecol. Sociobiol*. 43:203–212.

LENOIR, A., CUISSET, D., and HEFETZ, A., 2001a. Effects of social isolation on hydrocarbon pattern and nestmate recognition in the ant *Aphaenogaster senilis* (Hymenoptera: Formicidae). *Ins. Soc*. 48:101–109.

LENOIR, A., D'ETTORRE, P., ERRARD, C., and HEFETZ, A., 2001b. Chemical ecology and social parasitism in ants. *Annu. Rev. Entomol*. 46:573–599.

LENOIR, J.-C., SCHREMPF, A., LENOIR, A., HEINZE, J., and MERCIER, J.-L., 2006. Genetic structure and reproductive strategy in the ant *Cardiocondyla elegans*: strictly monogynous nests invaded by unrelated sexuals. *Mol. Ecol*. 16:345–354.

LORENZO FIGUEIRAS, A. N., and LAZZARI, C. R., 1998. Aggregation in the haematophagous bug *Triatoma infestans*: a novel assembling factor. *Physiol. Entomol*. 23:33–37.

MARTIN, S. J., and DRIJFHOUT, F. P., 2009. Nestmate and task cues are influenced and encoded differently within ant cuticular hydrocarbon profiles. doi:10.1007/s10886-009-9612-x.

MARTIN, S. J., HELANTERÄ, H., and DRIJFHOUT, F. P., 2008a. Evolution of species-specific cuticular hydrocarbon patterns in *Formica* ants. *Biol. J. Linn. Soc*. 95:131–140.

MARTIN, S. J., VITIKAINEN, E., HELANTERÄ, H., and DRIJFHOUT, F. P., 2008b. Chemical basis of nestmate discrimination in the ant *Formica exsecta*. *Proc. Roy. Soc. London, B* 275:1271–1278.

MAYADE, S., CAMMAERTS, M. C., and SUZZONI, J. P., 1993. Home range marking and territorial marking in *Cataglyphis cursor* (Hymenoptera: Formicidae). *Behav. Process*. 30:131–142.

NOWBAHARI, E., LENOIR, A., CLÉMENT, J. L., LANGE, C., BAGNÈRES, A. G., and JOULIE, C., 1990. Individual, geographical and experimental variation of cuticular hydrocarbons of the ant *Cataglyphis cursor* (Hymenoptera: Formicidae): their use in nest and subspecies recognition. *Biochem. Syst. Ecol*. 18:63–74.

OLIVER, T. H., MASHANOVA, A., LEATHER, S. R., COOK, J. M., and JANSEN, V. A. A., 2007. Ant semiochemicals limit apterous aphid dispersal. *Proc. Roy. Soc. London, B*.

OZAKI, M., WADA-KATSUMATA, A., FUJIKAWA, K., IWASAKI, M., YOKOHARI, F., SATOJI, Y., NISIMURA, T., and YAMAOKA, R., 2005. Ant nestmate and non-nestmate discrimination by a chemosensory sensillum. *Science* 309.

SALEH, N., SCOTT, A. G., BRYNING, G. P., and CHITTKA, L., 2007. Distinguishing signals and cues: bumblebees use general footprints to generate adaptive behaviour at flowers and nest. *Arthr.-Plant Inter.* 1:119–127.

SLEDGE, M. F., MONETI, G., PIERACCINI, G., and TURILLAZZI, S., 2000. Use of solid-phase microextraction in the investigation of chemical communication in social wasps. *J. chrom. A* 973:73–77.

SOROKER, V., and HEFETZ, A., 2000. Hydrocarbon site of synthesis and circulation in the desert ant *Cataglyphis niger*. *J. Ins. Physiol*. 46:1097–1102.

STECK, K., HANSSON, B. S., and KNADEN, M., 2009. Smells like home: desert ants, C*ataglyphis fortis*, use olfactory ladmarks to pinpoint the nest. *Front. Zool*. 6:5.

STEINER, F. M., SCHLICK-STEINER, B. C., NIKIFOROV, A., KALB, R., and MISTRIK, R., 2002. Cuticular hydrocarbons of *Tetramorium* ants from Central Europe: analysis of GC-MS data with self-organizing maps (SOM) and implications for systematics. *J. Chem. Ecol*. 28:2569–2584.

TENTSCHERT, J., BESTMANN, H.-J., and HEINZE, J., 2002. Cuticular compounds of workers and queens in two *Leptothorax* ant species—a comparison of results obtained by solvent extraction, solid sampling, and SPME. *Chemoecol*. 12:15–21.

UGELVIG, L. V., DRIJFHOUT, F. P., KRONAUER, D. J. C., BOOMSMA, J. J., PEDERSEN, J. S., and CREMER, S., 2008. The introduction history of invasive garden ants in Europe: Integrating genetic, chemical and behavioural approaches. *BMC Biol*. 6:11: doi:10.1186/1741-7007-6-11.

VAN DER HAVE, T. M., BOOMSMA, J. J., and MENKEN, S. B. J., 1988. Sex-investment ratios and relatedness in the monogynous ant *Lasius niger*. *Evol*. 42:160–172.

WAGNER, D., TISSOT, M., and GORDON, D. M., 2001. Task-related environment alters the cuticular hydrocarbon composition of harvester ants. *J. Chem. Ecol* 27:1805–1819.

WENSELEERS, T., BILLEN, J., and HEFETZ, A., 2002. Territorial marking in the desert ant *Cataglyphis niger*: does it pay to play bourgeois? *J. Ins. Behav*. 15:85–93.

WITTE, V., LEINGÄRTNER, A., SABAß, L., HASHIM, R., and FOITZIK, S., 2008. Symbiont microcosm in an ant society and the diversity of interspecific interactions. *Anim. Behav*. 76:1477–1486.

YAMAOKA, R., and AKINO, T. (1994). Ecological Importance of Cuticular Hydrocarbons Secreted from the Tarsus of Ants. Les Insectes Sociaux, Paris-Sorbonne, 21–27 août 1994, Univ. Paris Nord.

		Pentane extracts				SPME							
Peak		CUT		Legs		Cuticle		Inner Nest		outside entrance		Foraging area	
N°	Name	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	C25	0,47	0,71	1,23	0,57	0,45	0,68	2,01	2,60	2,72	2,90	4,78	3,95
2	3-MeC25	0,56	0,60	3,74	2,37	0,11	0,20	0,58	0,82	0,76	1,48	0,95	1,38
3	C26	0,14	0,18	0,50	0,35	0,20	0,47	0,67	0,89	1,45	2,18	1,26	1,29
4	10,14+8,12-DiMeC26	0,28	0,29	2,08	1,29	0,00	0,00	0,19	0,30	0,67	1,99	0,00	0,00
5	C27:1	0,07	0,06	0,42	0,58	0,23	0,30	0,28	0,45	0,01	0,05	0,01	0,03
6	C27	1,23	0,68	4,53	1,96	1,80	2,80	3,40	2,08	4,44	3,88	7,64	6,40
7	9+11+13-MeC27	0,25	0,18	0,65	1,32	0,34	0,47	0,55	0,54	2,10	3,21	3,21	3,29
8	5-MeC27	0,22	0,13	0,32	0,22	0,21	0,24	0,71	0,79	1,85	3,00	2,63	1,40
9	3-Me C27	0,14	0,10	0,33	0,53	0,36	0,46	0,89	0,90	0,81	2,36	0,37	1,09
10	5,15DiMeC27	0,53	0,55	0,62	0,68	0,07	0,18	0,93	1,28	0,84	1,58	1,78	2,12
11	C28	0,16	0,11	0,55	0,38	0,16	0,37	0,41	0,53	0,58	0,90	0,79	0,92
12	10+12+14-DiMeC28	0,10	0,07	0,20	0,55	0,11	0,15	0,24	0,50	0,62	1,64	1,35	1,89
13	10,12+10,14-DiMeC28	0,20	0,12	0,32	0,31	0,87	0,96	1,02	1,66	1,12	1,87	2,00	2,85
14	C29:1	0,25	0,17	0,77	0,76	0,00	0,00	1,46	1,49	0,18	0,48	0,05	0,18
15	C29	1,00	0,43	3,96	2,14	1,20	1,79	2,35	1,18	4,33	3,36	7,92	8,01
16	11--+15-MeC29	2,42	0,76	1,93	1,07	1,43	0,73	3,72	2,20	3,64	2,53	4,44	2,68
17	5-MeC29	1,95	0,80	2,61	1,52	1,71	1,48	3,49	2,81	1,89	1,88	1,23	1,14
18	9,11+ 9,13+9,15DiMeC29	4,65	1,66	3,18	1,36	6,16	1,05	7,50	4,68	4,71	2,67	5,25	2,98
19	3-MeC29	0,84	1,48	0,01	0,06	1,77	1,63	4,26	3,53	3,24	2,18	2,68	1,58
20	5,15-DiMeC29	5,77	1,59	3,90	1,73	6,49	2,64	6,53	2,94	2,40	2,23	0,78	0,86
21	7,11,15-TriMeC29	1,44	1,10	0,95	0,91	1,03	0,45	1,82	1,11	1,82	2,14	1,57	1,58
22	C30	0,00	0,00	0,00	0,00	1,32	0,87	0,71	0,93	0,75	1,19	0,53	0,93
23	10+12+14-MeC30	1,82	0,82	1,69	0,68	0,70	0,28	1,15	1,32	0,75	1,03	1,29	1,03
24	10,12+10,14+10,16-DiMeC3	1,40	0,47	1,39	0,74	2,66	0,58	1,25	0,98	2,12	2,41	1,62	0,78
25	8,12+8,14-DiMeC30	2,57	0,90	1,68	0,63	0,00	0,00	2,83	1,60	0,46	0,87	0,18	0,48
26	C31:1	1,23	0,48	3,64	3,08	5,84	3,57	1,25	1,26	0,33	0,57	0,13	0,40
27	4,12+4,14-DiMe C30	0,39	0,30	0,00	0,00	0,11	0,22	0,54	0,56	0,03	0,10	0,00	0,00
28	C31	0,63	0,17	2,51	2,00	0,63	0,60	1,13	3,16	1,29	1,67	3,91	3,32
29	TriMeC30	0,89	1,08	10,51	4,71	0,14	0,16	3,30	3,87	2,03	3,33	0,31	0,85
30	9+11+13+15-MeC31	11,41	3,62	10,88	3,63	9,71	3,25	4,83	4,53	4,70	4,87	6,28	4,00
31	5-MeC31	2,33	2,40	2,93	2,18	0,37	0,79	0,92	1,45	0,18	0,68	0,00	0,00
32	9,11+9,13 +9,15-DiMeC31	20,83	3,77	13,93	6,00	22,31	8,12	13,67	5,78	15,53	8,22	13,31	9,28
33	3-MeC31+5,13+5,15-DiMeC	7,92	1,34	7,01	2,42	10,31	2,80	5,36	2,61	5,98	3,39	3,95	2,52
34	7,11,13+7,9,15+9,11,13+9, 11,15-TriMeC31	1,84	0,65	0,71	0,78	1,17	1,02	2,10	2,77	0,93	1,16	0,48	0,73
35	C32	0,00	0,00	0,00	0,00	0,00	0,00	1,18	1,57	2,62	1,91	1,64	1,26
36	5,9,11-TriMeC31	4,67	0,84	2,61	1,06	3,00	1,30	1,07	1,19	0,40	0,78	0,14	0,41
37	10+12+14-MeC32	1,79	0,68	1,05	0,92	1,50	0,90	1,83	1,25	1,20	2,36	1,14	0,76
38	10,14+10,16-DiMeC32	1,90	0,96	0,72	0,61	2,29	0,72	0,92	0,95	2,35	3,45	1,16	0,92
39	C33:1	0,34	0,41	0,90	1,47	1,78	0,68	0,68	0,75	0,02	0,07	0,08	0,39
40	4,10+4,12+4,14-DiMeC32	0,15	0,17	0,00	0,00	0,18	0,22	0,19	0,22	0,07	0,27	0,10	0,36
41	C33	0,04	0,08	0,06	0,12	0,00	0,00	0,04	0,08	0,03	0,11	0,28	0,41
42	4,8,12-TriMeC32	0,85	1,39	0,25	0,40	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
43	9+11+13+15+17-MeC33	2,19	1,15	1,26	0,92	1,56	0,76	1,06	1,05	1,65	1,42	1,45	1,11
44	?	0,82	1,15	0,33	0,54	0,08	0,16	0,19	0,27	0,02	0,12	0,00	0,00
45	9,15+11,15-DiMeC33	5,43	1,95	1,58	1,10	5,37	2,14	3,25	1,90	5,51	3,99	4,94	3,92
46	5,15-DiMeC33	1,27	0,95	0,27	0,47	1,35	0,50	2,42	1,67	1,05	1,07	1,87	1,38
47	7,11,15-TriMeC33	0,54	0,78	0,00	0,00	0,35	0,33	0,61	0,83	0,91	1,92	0,42	0,55
48	C34	0,00	0,00	0,00	0,00	0,81	0,44	0,53	0,63	0,40	0,59	0,62	0,61
49	10+12-MeC34	1,12	0,88	0,31	0,46	0,00	0,00	0,00	0,00	0,30	0,48	0,19	0,27
50	10,12+12,14+10,16-DiMeC3	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,25	1,03	0,05	0,16
51	?	0,35	0,71	0,09	0,21	0,38	0,26	0,42	0,49	0,52	1,12	0,31	0,32
52	8,12+8,14-DiMeC34	0,14	0,32	0,00	0,00	0,38	0,24	0,16	0,40	0,07	0,22	0,05	0,24
53	?	0,33	0,51	0,00	0,00	0,10	0,09	0,56	1,04	0,84	2,47	0,02	0,07
54	11+13+15-MeC35	0,28	0,58	0,52	0,75	0,01	0,03	0,19	0,28	1,09	1,67	1,47	1,18
55	11,13+13,15+13,17-DiMeC3	1,17	1,09	0,35	0,69	0,63	0,53	1,19	1,95	0,34	1,09	0,17	0,57
56	?	0,30	0,46	0,00	0,00	0,12	0,08	0,05	0,21	0,59	1,83	0,06	0,20
57	?	0,07	0,16	0,00	0,00	0,00	0,00	0,00	0,00	1,00	0,00	0,00	0,00
58	?	0,07	0,17	0,00	0,00	0,09	0,09	0,78	1,29	0,18	0,94	0,00	0,00
59	?	0,07	0,14	0,00	0,00	0,04	0,09	0,40	0,83	2,21	2,55	0,13	0,24
60	13+15+17+19-MeC37	0,03	0,07	0,00	0,00	0,03	0,07	0,20	0,45	0,74	1,51	1,02	0,75
61	11,15+13,15+15,17-DiMeMe	0,24	0,46	0,00	0,00	0,00	0,00	0,04	0,13	0,37	1,47	0,00	0,00
	Total	100,00		100,00		100,00		100,00		100,00		100,00	
	n-alkanes	3,67	1,39	13,34	6,69	6,58	6,43	12,42	5,19	18,61	10,05	29,36	19,86
	Methyl-alkanes	29,70	4,73	29,78	4,65	19,90	4,21	24,62	10,70	25,52	8,79	29,69	4,93
	DiMethyl-alkanes	58,05	4,58	38,41	7,99	59,19	10,13	48,00	12,62	43,86	12,49	37,22	16,76
	TriMethyl-alkanes	6,37	2,64	12,74	4,76	5,68	2,39	8,89	4,63	6,09	3,91	2,93	2,44
	Alkenes	1,90	0,72	5,73	5,10	7,85	4,36	3,66	2,58	0,54	1,00	0,27	0,76
	unknown	0,39	0,58	0,00	0,00	0,80	0,41	2,40	2,91	5,37	4,64	0,52	0,48

Scan EI+
TIC
6.23e5

100

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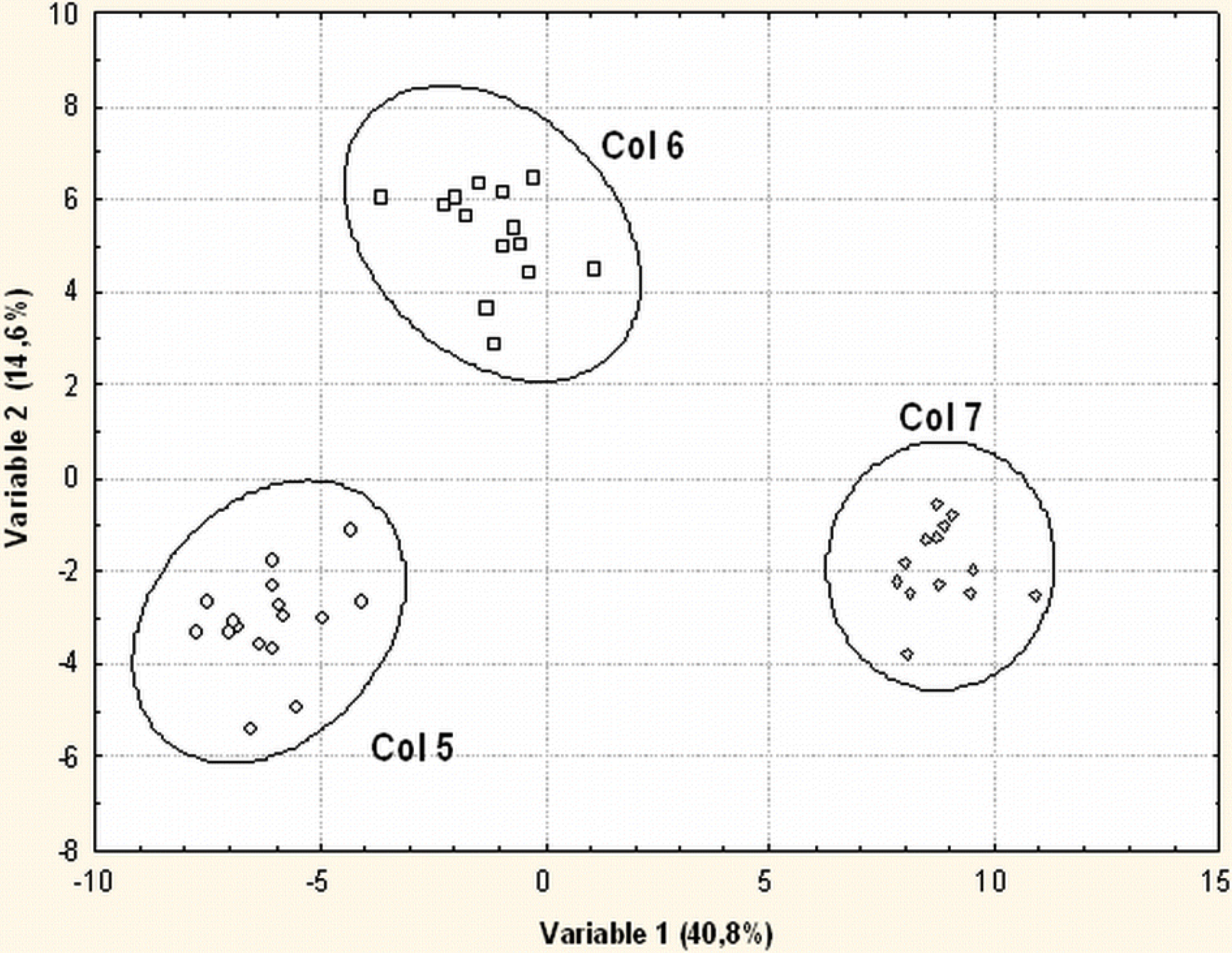
0

1 2 3 4-5 6 7 8 9 10 15 16 17 18 20 21 23 24 25 26-29 30-31 32 33 36 37 38 42-43 45 46 50-51 53 54 55 56 60 61

25.00 26.00 27.00 28.00 29.00 30.00 31.00 32.00 33.00 34.00 35.00 36.00 37.00 38.00

Time


Col 6
Col 7
Col 5
Variable 2 (14,6%)
Variable 1 (40,8%)
10
8
6
4
2
0
-2
-4
-6
-8
-10
-5
0
5
10
15